Tutor/s

Dr. Javier Fernández González
Ciència de Materials i Química Física

Dr. José Antonio Padilla Sánchez
Ciència de Materials i Química Física

Treball Final de Grau

Extraction and characterization of nanocellulose from bamboo.

Extracción y caracterización de nanocelulosa a partir de bambú.

Raúl Adad Ruesca Martín

June 2020

Aquesta obra està subjecta a la llicència de:
Reconeixement–NoComercial-SenseObraDerivada

http://creativecommons.org/licenses/by-nc-nd/3.0/es/

The bamboo that bends is stronger than the oak that resists.

Japanese proverb.

Quisiera agradecer, en primer lugar, a mis tutores el Dr. Javier Fernández González y el Dr. José Antonio Padilla Sánchez por su tiempo y dedicación revisando y supervisando este proyecto. Por otra parte, me gustaría dar las gracias a mi familia por ser un pilar fundamental a lo largo de estos años y, por último, a mis amigos y a mi pareja por el apoyo en estos últimos momentos.

.

REPORT

CONTENTS

1. SUMMARY

There is a need for developing renewables materials due to the increasing demand of alternatives to the unrenewable petroleum supplies. Nanocrystalline cellulose (NCC) or cellulose nanocrystals (CNs), which derives from cellulose, the most abundant biopolymer, is one of the most promising materials.

Cellulose is the most abundant organic polymer on earth which mainly provides structural reinforcement to the plants cell walls. Cellulose consists of a chain of thousands of bonded glucose units with β (1→4) links. It is mainly obtained from lignocellulosic biomass (plant dry biomass) to produce paperboard and paper. That lignocellulosic biomass is mostly composed by cellulose, which by strong hydrogen bonding networks forms microfibrils, hemicellulose, which binds together those microfibrils and lignin, which have various purposes like provide stiffness and cover the cellulose microfibrils. As will be seen later, several methods can be applied to extract the cellulose from the lignocellulose biomass. That cellulose contains crystalline and amorphous regions, which have different resistance to chemical attacks (crystalline regions have higher resistance while amorphous regions have lower), through different methods, which mainly involves sulfuric and hydrochloric acids, the amorphous regions are hydrolysed leaving the crystalline regions intact, those crystals that have a diameter between 5-70 nm and a length between 100-250 nm are called nanocrystalline cellulose. CNs are a renewable material with good mechanical properties and a nano-scaled dimension which opens a wide range of possible applications.

CNs can be obtained from almost any source of cellulose, from plants to bacteria. This report is part of a collaboration with the University of Guayaquil in Ecuador, the possibility of using bamboo as a source of cellulose to obtain CNs material has been studied since it’s a material of great importance, widely used for its mechanical properties and with incredibly high growth rates.

This report provides an overview of the CNs, an emerging nanomaterial, the different ways to isolate the cellulose from which the CNs are extracted, the process required to obtain those CNs and different techniques to characterize it.

Keywords: Cellulose, Design of Experiments, Nanocrystalline Cellulose**.**

2. RESUM

Existe la necesidad de desarrollar nuevos materiales renovables debido a la creciente demanda de alternativas a los suministros de petróleo no renovables. La celulosa nanocristalina (nanocrystalline cellulose (NCC)) o nanocristales de celulosa (cellulose nanocrystals (CNs)) que deriva de la celulosa, el biopolímero más abundante, es uno de los materiales más prometedores.

La celulosa es el polímero orgánico más abundante en la tierra y proporciona principalmente refuerzo estructural a las paredes celulares de las plantas. La celulosa consiste en una cadena de miles de unidades de glucosa unidas con enlaces β (1 → 4). Se obtiene principalmente de la biomasa lignocelulósica (biomasa de plantas secas) y se suele usar para producir cartón y papel. Esa biomasa lignocelulósica está compuesta principalmente por celulosa que mediante fuertes redes de enlaces de hidrógeno forma microfibras, hemicelulosa que une esas microfibras y lignina que tienen varios propósitos como proporcionar rigidez y cubrir las microfibras de celulosa. Como se explica a continuación se pueden aplicar varios métodos para extraer la celulosa de la biomasa. La celulosa contiene regiones cristalinas y amorfas que tienen diferente resistencia a los ataques químicos (las regiones cristalinas tienen mayor resistencia mientras que las regiones amorfas tienen menos), a través de diferentes métodos, que implican principalmente ácido sulfúrico o ácido clorhídrico, las regiones amorfas se hidrolizan dejando intactas las regiones cristalinas, los cristales resultantes que tienen un diámetro entre 5-70 nm y una longitud entre 100-250 nm se denominan nanocristales de celulosa (CNs). Los CNs son un material renovable con buenas propiedades mecánicas y una nanoescala que abre una amplia gama de posibles aplicaciones.

los NC se pueden obtener a partir de casi cualquier fuente de celulosa, des de plantas hasta bacterias, debido a que este trabajo se enmarca en una colaboración con la Universidad de Guayaquil en Ecuador, se investigará la posibilidad de usar el bambú como material de partida para la extracción de los nanocristales de cellulosa ya que allí es un material de gran importancia, muy usado por sus propiedades mecánicas y con unos ratios de crecimiento increíblemente altos.

Este informe proporciona una visión general de los CNs, un nanomaterial emergente, las diferentes formas de aislar la celulosa de la que se extraen, el proceso requerido para obtenerlos y diferentes técnicas para caracterizarlos.

Palabras clave: Celulosa, diseño de experimentos, celulosa nanocristalina.

3. INTRODUCTION

The use of synthetic fibers, which are made from non-renewable resources for the reinforcement of materials it’s an environmental problem whose solutions are sought. The use of natural fibers are considered a good echo friendly alternative to the synthetic fibers, because of their biodegradable nature and the fact that are derived from renewable sources among others. Those natural fibers are mainly composed of cellulose, which is the most abundant renewable material on earth.

3.1. CELLULOSE

Cellulose is a linear organic polymer composed of repeated units of cellobiose, two β-D-glucopyranose linked together with 1,4-glycosidic bonds, each β-glucose molecule is inverted 180º from the previous one which keep cellulose chains from coiling resulting in a long straight chain, ideal for the formation of strong fibers (Figure 1) [1][2]. The usual number of units or the degree of polymerization (DP) is 10,000 for wood cellulose and 15,000 for plant cellulose [1], but can reach up to 20,000 units [3]. The single cellulose chain has a hemi-acetal group at one end which may adopt an open-chain aldehyde form, and act as a reducing agent, while the other end, the non-reducing end, lacks those properties.

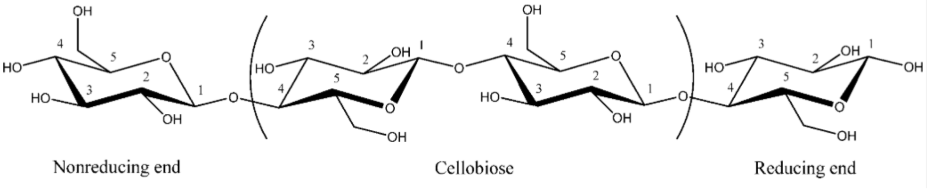

Figure 1: Chemical structure of cellulose Dimer of cellulose, cellobiose.

All β-D-glucopyranose rings adopt a $^4C_1$ chair conformation, where the letter C stands for 'chair' and the numbers indicate if the carbon atoms are located above or below the reference plane of the chair. In this conformation the hydroxyl groups are positioned equatorial to the plane, while the hydrogen atoms are in the axial position.

3.1.1. Cellulose crystalline allomorphs

Native cellulose, natural cellulose or cellulose (I) is the crystalline form of cellulose that is found in nature. It has a strong intramolecular hydrogen bond network (Figure 2) [4], which goes from the O(3)-H hydroxyl to the O(5) oxygen of the next unit and from the O(2)-H hydroxyl to the O(6) hydroxyl of the next unit, it also has an intermolecular hydrogen bond from the O(6)–H to the O(3) of the next cellulose fibril, that forms cellulose microfibrils [5][4]. The strong hydrogen bonding makes cellulose decompose at high temperatures, have good mechanical properties and makes highly concentrated solutions very viscous. Those intramolecular networks form sheets which are connected with each other by van der Waals bonds forming that crystalline form called cellulose (I) [6][7].

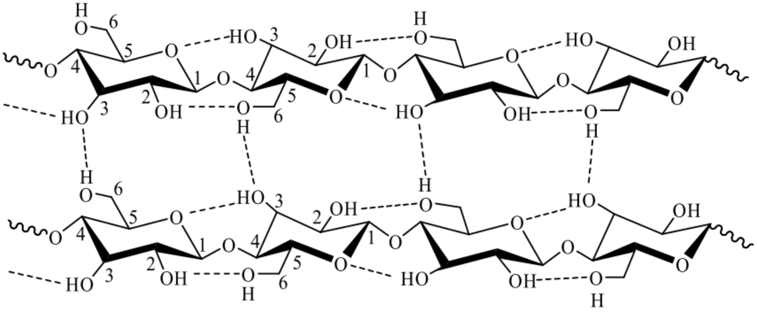

Figure 2: Intramolecular hydrogen-bonding network in a representative cellulose I_β structure.

The hydrogen-bonding network and molecular orientation of cellulose can vary, those variations are known as cellulose allomorphs. Six interconvertible allomorphs of cellulose have been identified named from cellulose I to VI. Cellulose I or native cellulose is the crystalline cellulose that is naturally produced by a variety of organisms and is the one in which the report is focused on [8]. Cellulose I have two different structures (Figure 3) [8], I_α and I_β, both coexisting with each other in nature, being I_α predominant in algae and microbes and I_β in plants.

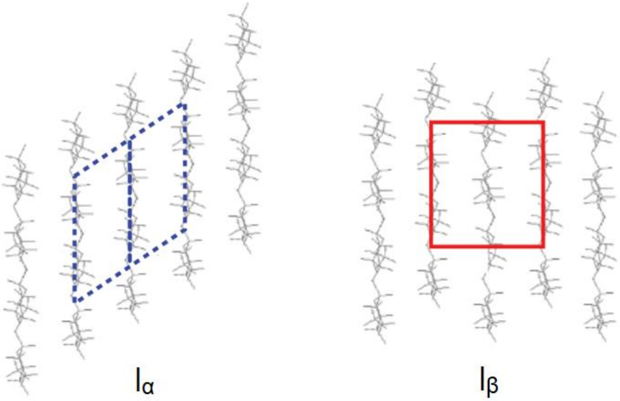

Figure 3: Iα and Iβ cellulose structures.

3.1.2. Cellulose sources

Cellulose is a fibrous, water-insoluble substance which is the main constituent that maintains the structure of plant cell walls, although it can be obtained from other sources such as bacteria, several species of algae and the only animal known to produce cellulose, tunicate [8].

In plants cellulose does not only form long chains, it forms a hierarchical structures which provide better mechanical properties , the variations in that hierarchical microstructure are one of the causes of mechanical properties differences between types of plants [9]. The single cellulose chains are linked together by hydrogen bonds forming elementary fibrils or microfibrils (consisting of tens of single cellulose chains), this microfrils form macrofibrils, which are a pack of elementary fibrils held together by hemicellulose, and form cellulose fibers (a group of macrofibrils) [10] (Figure 4 A) [10]. The crystalline regions that microfibrils form are called cellulose nanocrystals (CNs) and as it will be seen later are very resistant to degradation, unlike the amorphous parts, which are easier to degrade. The orientation of the microfibrils in the cell wall has a strong effect on the structural properties. This characteristic orientation called microfibril angles (referring to the angle formed between the microfibril and the fiber axis) and changes depending on the cell wall layer and the type of plant [11] (Figure 4 B) [12].

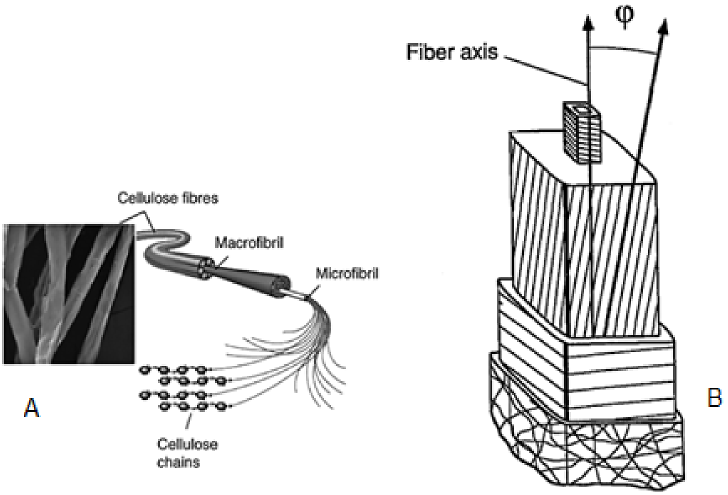

Figure 4: A) Hierarchical morphology of a cellulose fiber. B) Representation of the microfibril angles.

Lignin and hemicelluloses are also a major component of the plant fibers. Hemicelluloses bind cellulose microfibers to each other, are composed of different polysaccharides and, unlike cellulose, have a random, amorphous structure and are easily hydrolysed. Lignin is another amorphous polymer, which provides rigidity and covers the microfibrils, it also have other biological functions such as conducting water and provide resistance against microbial attacks [13][14][15].

3.1.3. Cellulose production and applications

Cellulose is the most abundant renewable organic material on the earth, 7.5×10^{10} t of cellulose are estimated to be produced every year [16]. Cellulose is mainly obtained from wood pulp and cotton to manufacture paperboard and paper, and it is transformed into a wide variety products [17], in general wood contain between 40% and 50% of cellulose [18] while cotton contains nearly 90% of cellulose [19].

The Pulp production has improved over the years by 24% worldwide, 82% (from 520,000 t to 950,000t) in Europe, 27% in Asia and 19% in Africa, which makes cellulose a material with an increasing relevance [17].

Cellulose have a wide variety of applications, the main application, which is the paper industry, and others ranging from pharmaceutical [20] or medical purposes [21] to the preparation of membranes [22], the treatment of water [23] or the extraction of nanocellulose which will be explained below.

3.2. NANOCELLULOSE (NC)

When a material has at least one dimension less than or equal to 100 nm it can be referred as a nanomaterial. The word nanocellulose generally refers to cellulosic materials with one dimension in the nanometer range but the other dimensions of the nanocellulose particles can vary depending on the source material used [24][25][26] and the experimental process made [27][28], going from a few nanometers to several microns.

Nanocelluloses can be classified in three main subcategories [29]:

- Microfibrillated cellulose (MFC) or nanofibrillated cellulose (NFC): Mostly extracted from plant sources. Is obtained using mainly physical treatments to the source material. It has a diameter of 5–60 nm and a length of several micrometers.
- Nanocrystalline cellulose (NCC) also known as cellulose nanocrystals (CNs or CNC) or cellulose whiskers: Extracted mainly from plant sources, although it can be obtained from algae and bacteria as well as from tunicate, it is mainly prepared with acid hydrolysis and has a diameter of 5-70 nm and a length of 100-250 nm (from plant cellulose) and 100 to several micrometers (from cellulose of tunicates, algae and bacteria).
- Bacterial nanocellulose (BNC): It is synthesised by bacteria from sugars and alcohols and has a diameter of 20-100 nm and different types of nanofiber networks.

Nanocelluloses physical properties are very dependent on the removal of non-cellulosic materials, and the amount of amorphous regions of cellulose. The degree of crystallinity and the crystal structure, which are factors that are dependent on the treatment used and the lignocellulosic source material, also change the mechanical properties of the nanocellulose [30]. In the case of nanocrystalline cellulose the amorphous regions of cellulose are eliminated by hydrolysis, that's why nanocrystalline cellulose have superior properties [31][32].

CNs are every year the subject of more research efforts due to their good mechanical properties [8], their low cost, availability and renewability [33] and their application as a reinforcement material [34], on optical and electronic devices [35] and medical applications [36] among others.

A material which cellulose can be compared with is Kevlar, a strong lightweight synthetic fiber known for its mechanical properties and its application in bulletproof vests [37]. Cellulose nanocrystals have better axial elastic modulus than Kevlar with similar density and its mechanical properties are similar to other reinforcement materials (Table 1).

Table 1: different physical characteristics of crystalline cellulose compared with Kevlar-49 fiber [8]

Material	**ρ (g cm^{-3})**	**σ_f (GPa)**	**E_A (GPa)**	**E_T (GPa)**
Kevlar-49 fiber	1.4	3.5	124-130	2.5
CNs	1.6	7.5-7.7	110-220	10-50

ρ = density, σ_f = tensile strength, E_A = elastic modulus in axial direction, E_T = elastic modulus in transverse direction.

3.3. BAMBOO

As has been seen, Nanocellulose can be prepared from any cellulose source material, but considering the current climate situation, availability and sustainability must be considered to choose one, that’s why one attractive source is bamboo as it will be seen below.

Bamboo is a type of flowering plant that belongs to the subfamily Bambusoideae of the grass family Poaceae (it is a grass not a tree). There are almost 1,500 species of bamboo, which can grow on different conditions, but mostly on tropical climate. They are the only group of grasses that create forests and demonstrate enormous adaptability to different soils and environments.

3.3.1. Morphology and physiology

Bamboo has a unique structure, mainly composed of rhizomes, roots, culms, branches, leaves and flowers (Figure 5) [38].

Rhizomes are the underground part of bamboo stem. They grow horizontally and help bamboo expand into new territories. However, rhizomes are not roots. Rhizomes collect and store nutrients for growth and can produce new bamboo shoots which will grow forming an interconnected system of plants [39].

Roots are formed from the rhizomes and are no longer than a few centimetres, their function is to secure the plant to the ground stabilizing it so it can grow more and resist severe weather. Even though isn’t its primary function, roots can also store nutrients.

Culms are the woody ringed steams, which are usually hollowed between rings, they grow from rhizomes and can vary in size, shape, colour and even smell [40].

Branches are grown from each bamboo buds, even though no tall species can grow multiple branches.

Leaves are present in the rhizomes as well as on the branches and culms, leaves provide protection to the rhizome as it grows through the soil and provides energy through photosynthesis when it’s in the branches and culms.

Bamboo flowers are rarely seen because of its long flowering interval, which can be as long as 130 years, even though some species do flower annually [41].

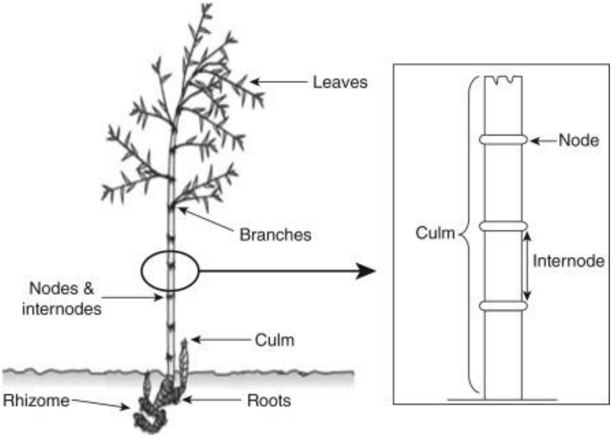

Figure 5: Bamboo morphology.

3.3.2. Growth and pruning

Bamboo is one of the fastest growing plants, it can grow around 8 cm per day in its growth period [42], but there are varieties such as Guadua or Moso that can reach a growing rate of 25 cm per day being the fastest growing plants on the planet [43]. The final height of the plant it’s reached in just 6 months, and in the following years the woody structure of the canes develops and finishes maturing. Bamboo height depends on the species, largest species of bamboo can reach 30 m in height. It can grow as a tall plant or as a shorter plant without woody stems.

The greatest advantage of using this giant grass instead of the intensive use of trees is that in just 3 years its ready to be used as a structural material having higher yield and lower cost than most woods [42].

3.3.3. Chemical composition

Cellulose is the main component of bamboo being approximately 40-55% of it, which is within the normal cellulose content for softwoods (40-52%) and hardwoods (38-56%) making it suitable for processes designed for this type of lignocellulosic biomass. Lignin constitutes between 20-25% of the bamboo dry weight and hemicelluloses between 20-23% of it.

The cellulose content of bamboo culms is higher in the upper parts of the plant and in the outer layer of the plant [44].

Bamboo also has a natural bioagent which gives bamboo a natural resistant to bacteria and fungus, this allow bamboo to easily grow without chemical pesticides [45].

3.3.4. Applications

One of the uses that is given to bamboo, mainly in the places where is produced, is as a construction material due to its high compressive strength, which is better than wood, brick, or concrete and his tensile strength that rivals steel [46]. Bamboo is also used for the production of biofuel [47], in the textile industry and for making furniture [48] as well as being food for the giant and red pandas among others.

3.3.5. Distribution and environmental impact

It is present in a wide number of countries, covering three major regions: America, sub-Saharan Africa and a large area of Asia (Figure 6) [49]. It is believed that bamboo was present in Europe, fossils of bamboo leaves and pollen where found there, but there aren't any that remain there today.

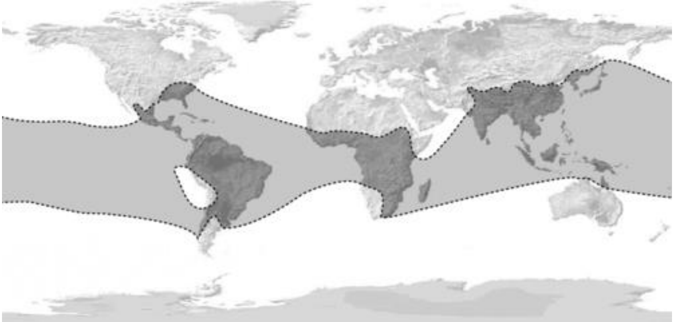

Figure 6: 3/05/2020 Flora distribution map of the Poaceae subfamily Bambusoideae.

Bamboo forests are a very good CO_2 removals (Table 2), generally speaking, the bigger the plant, the more CO_2 it absorbs, bamboo isn't bigger than most of the trees, but the growth rate plays an important role on how many CO_2 can the plant absorb too, and as it has already been said, bamboo is one of the fastest growing plants. Some bamboo species can fix more than 100 t per hectare in each life cycle [43].

Table 2: Some of the bamboo enviromental characteristics [43]

Material	**CO_2 Fication (t/ha/year)**	**Age for estructural use (year)**	**Biomass Production (t/ha/year)**
Bamboo	9	4/6	90
Conifers (Pine)	3.5	15/25	11

3.3.6. Bamboo in Ecuador

The review is part of a collaboration with Sr. Bismark Osmany Torres Ruilova a professor from the University of Guayaquil in Ecuador, a country where bamboo is of great importance and where one of the most promising bamboo species is found, Guadua Angustifolia.

Bamboo has been an underrated resource and most people don't even associate the term bamboo with those south American regions where bamboo has always been present, but in the last several years there has been a growing interest in this material not only as construction material but also as a resource for other purposes, among them the extraction of CNs [50].

Latin America is the richest region of the Americas in terms of the diversity and number of bamboo species. Ecuador specifically possesses an impressive diversity of woody bamboos considering its size, there are 42 species of bamboo. It is estimated that bamboo covers about 600,000 hectares, or 2%, of Ecuador's land being responsible for 12% of the employment in the agricultural sector [51], most of the bamboo plants are found at an altitude of 2,500 to 3,500 m with 74% of the total species being found in the eastern cordillera. Among all bamboo species Guadua Angustifolia is the most economically important specie having a culm production of 25.86 million of green culms per year [52].

Guadua Angustifolia is one of the biggest types of bamboo, it can reach more than 30 meters of height and 22 cm of diameter and has a fast-growing rate, obtaining its final height in just 6 months. Most of the mechanical properties of that type of bamboo have higher values of strength for 3 and 4 year old culms, which seems to indicate that the mature age of Guadua culms is reached between 3 to 4 years [53], being a very suitable cellulose source for the extraction of CNc among other uses.

4. OBJECTIVES

Carry out a bibliographic search on cellulose, cellulose nanocrystals and bamboo and find where they are obtained, their structure, properties, uses and characteristics.

Give a general view about what cellulose and CNs are.

Give the necessary information to understand the different techniques and processes that can be used to extract nanocellulose and what types of nanocelluloses exist.

Finding methods to extract CNs emphasizing on the environmentally friendly methods and its extraction from Guadua Angustifolia, a type of bamboo.

Compare the different methods that can be use to extract CNs.

5. METHODS

A bibliographic review has been done in order to collect the current available data about the extraction of nanocellulose, to achieve this, several databases have been consulted in search of different articles related with the topic. Databases such as SciFinder®, Scopus®, Google Scholar® and Science Direct® have been used.

The research has been started with several reviews with general information about the different methods of extraction to then focus on specific articles for each method, as well as for the introduction, always focusing the research on articles from the last twenty years.

Because the review is part of a collaboration with Sr. Bismark Osmany Torres Ruilova a professor from the University of Guayaquil in Ecuador, the research has focused on a plant of great importance there, Guadua Angustifolia, a type of bamboo. For each possible valid extraction method different articles that uses bamboo as a resource has been searched.

As seen in the introduction, nanocelluloses and specifically CNs are materials in which there is a growing interest due to their mechanical properties and for being a clean and renewable resource. These materials began to be studied recently (2002 for cellulose nanocrystals and 2005 for nanocellulose) and the growth of interest in this materials is reflected in the number of publications with the topics cellulose nanocrystals and nanocellulose showed in Figure 7 and Figure 8 in which can be seen the exponential growth on the number of publications during the years.

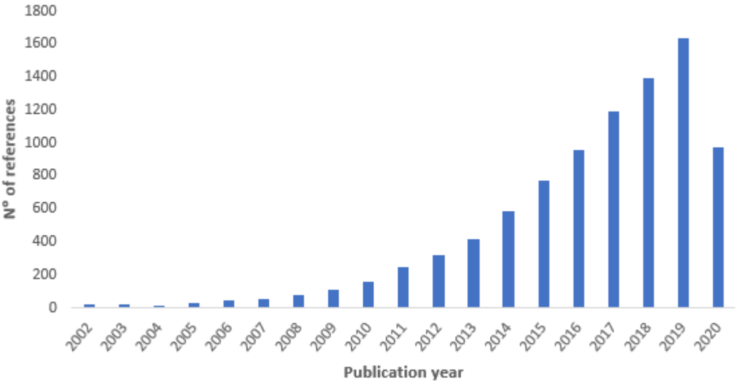

Figure 7: Number of documents with the topic "cellulose nanocrystals" published each year on Scifinder®. 27/06/2020.

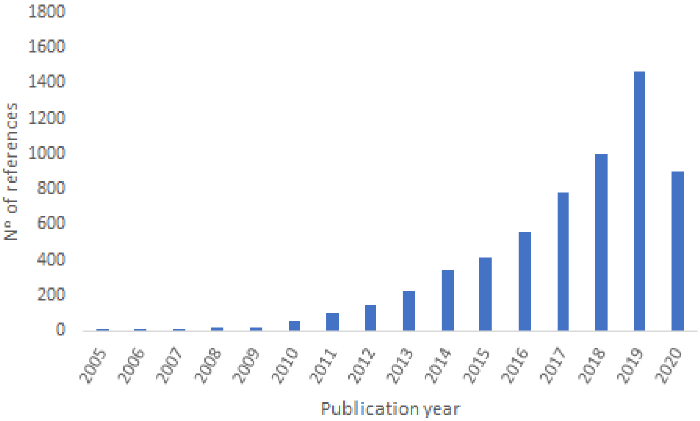

Figure 8: : Number of documents with the topic "Nanocellulose" published each year on Scifinder 07/07/2020.

Several articles have been searched to obtain information on the different parts of the report, the following table (Table 3) shows the words used in the search of this information as well as characteristics of the information found for each of those parts of the report.

Table 3: Words used in the search for information

Bamboo	**Cellulose**	**Cellulose nanocrystals**	**Type of documents found**
Guadua Angustifolia, cellulose content, growth rate.	Delignification, pre-treatment, nano-sized, amorphous regions, degree of polymerization, hydrogen bonding.	Crystallinity index, chemical treatment, hydrolysis, mechanical properties.	Articles and reviews from the past 20 years.

6. RESULTS AND DISCUSSION

As a result of the research done it has found that the NCs isolation from a lignocellulosic biomass can be divided in three steps: The lignocellulosic biomass size reduction, the pre-treatment in which the cellulose is isolated, and the treatment where NCs are obtained.

Even though the lignocellulosic biomass size reduction is usually classified as a physical pre-treatment, it hasn't been classified here as one because it's rarely used by itself as a pre-treatment. It is used to improve the pre-treatment or treatment yield and it consists of the reduction of the particle size in order to increase the surface area.

The second one is the pre-treatment of the source material. For wood and plants it consists of the removal of non-cellulosic material, mainly hemicelluloses and lignin, the most abundant compounds in lignocellulosic biomass after cellulose, and the isolation of the cellulosic fibers.

The third one is a controlled chemical treatment (generally hydrolysis) to remove the disordered regions of the cellulose, leaving intact the crystalline parts, called NCs.

6.1. SIZE REDUCTION

That step involves the reduction of the biomass size by milling or grinding among others and the dehydration (if necessary). The aim of this size reduction is not only the particle size reduction, but also the improvement of flow properties, and surface area. Increasing the surface area increases the number of contact points for chemical reactions which improves the pre-treatment and treatment results [54].

That step is usually repeated until the desired particle size is obtained. After each time, the particles are generally smaller and more uniform in diameter (Figure 9) [27]. However, the particle size reduction have a downside, decreasing the size too much could damage the crystalline cellulose decreasing the crystallinity percentage and increase the cost [27].

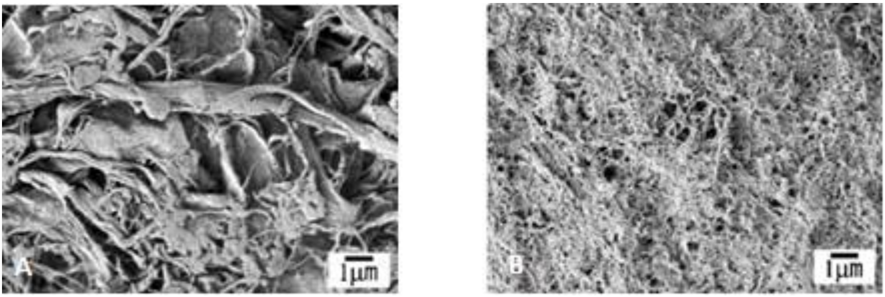

Figure 9: FE-SEM pictures of the fibrillated pulp fibers treated at up to five passes through the grinder. (A) one pass and (B) five passes.

One of the reasons why this step hasn’t been considered a pre-treatment step is that the final particle size is dependent on the energy used and that energy is too high to be cost-competitive and be useful in a full-scale process if it’s the only pre-treatment used and the adequate particle size to proceed with the treatment is wanted [15][55]. However, it is used before other pre-treatments for comfort and to improve the yield of the delignification.

There are different mechanical size reduction methods that could be used depending on the particle size wanted. The ideal particle size depends on the biomass source used and the subsequent pre-treatment method (Table 4). It is known that reducing the particle size below 0.4 mm has little effect on the hydrolysis yield but increases the energy required [56]. In the case of bamboo, since it’s a plant, the methods for reducing its size are similar.

Table 4: Particle size reductions depending on the pre-treatment method used

Source Material	**Pre-treatment method used**	**Particle size (mm)**	**Reference**
Corn stover	Biological	5, 10, 15	[57]
Citrus sinensis Bagasse	Chemical	0.85 - 1.15	[58]
Coconut husk fiber	Chemical	1.0	[59]
Corncobs	Physicochemical	0.19	[60]
Bamboo Source Material	**Pre-treatment method used**	**Particle size (mm)**	**Reference**
Phyllostachys pubescence	Biological	0.9	[61]
Phyllostachys pubescence	Chemical	0.567	[62]
Bambusa Blumeana	Chemical	0.5	[63]

As seen in the Table 4 chemical pre-treatments are done with particle sizes around 0.5 and 1 mm while biological usually use larger sizes, physicochemical pre-treatment particle sizes are variable depending on the method. Some studies show that milling can be performed after the chemical pre-treatment reducing the energy consumption and maintaining the hydrolysis yield of the treatment [64][65].

6.2. PRE-TREATMENT

The main objective of the pre-treatment steps is the elimination of the hemicelluloses and lignin (Figure 10) [15] to enhance the reactivity of cellulose on the treatment step [15][31][66].

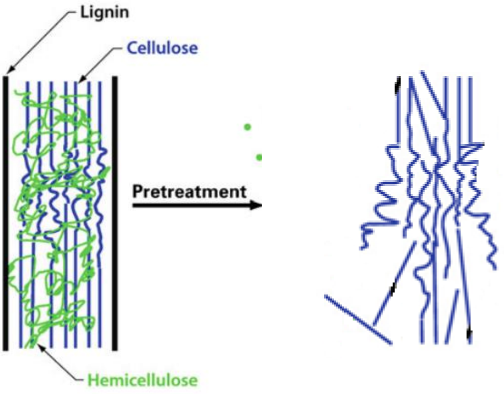

Figure 10: Pre-tratment effect on a lignocellulosic source.

The characteristics that researchers are currently looking for in pre-treatment methods are three: Avoiding the cellulose degradation, reducing any inhibitor for the subsequent treatment and to be able to recover the lignin and hemicelluloses for other uses [15]. That step also involves about 20% of the total cos of the process, that's why it is desirable to reduce the energy required too [10].

Pre-treatment methods can be divided into three different categories: chemical, physicochemical and biological being both chemical and physicochemical the most cost competitive. Physical pre-treatment, consisting on the particle size reduction, is usually classified as the fourth method [15], but it hasn't been considered here as one because of the reasons mentioned before.

Several methods will be reviewed giving a brief process description, and a discussion of the technology’s advantages and disadvantages.

6.2.1. Chemical pre-treatments

Alkaline pre-treatment

The alkaline pre-treatment uses alkaline reagents such as sodium hydroxide (Table 5), calcium hydroxide or aqueous ammonia, being sodium hydroxide the most studied and used. However, calcium hydroxide provides good results, it’s cheaper and could be recovered from the reaction solution [66]. Those reagents degrade lignin by cleaving the ether linkages within the lignin (Figure 11) adapted from [67] and the glycosidic bonds in the hemicelluloses disrupting the structure of lignin and hemicelluloses [68].

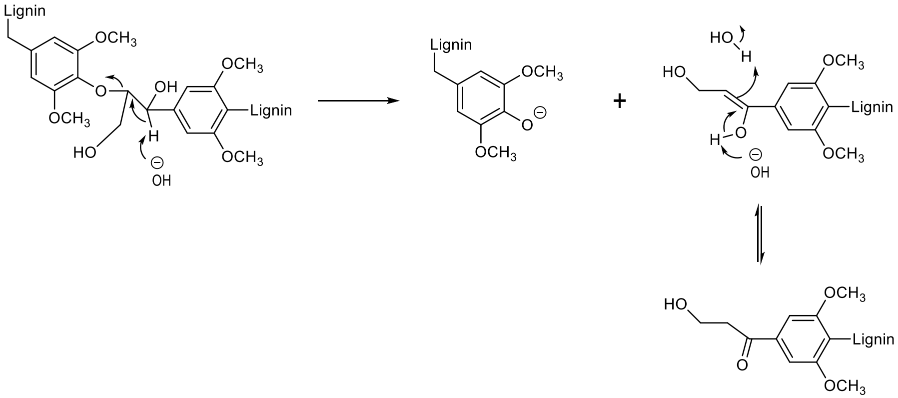

Figure 11: Lignin depolymerization by alkaline pre-treatment.

This treatment provides an efficient removal of lignin even thought it could alter its structure [69]. That pre-treatment uses lower temperatures and pressures than other technologies and cause less sugar degradation, but pre-treatment times are long (from hours to days) [66].

The alkali pre-treatment is often combined with sodium chlorite or hydrogen peroxide in order to improve the lignin and hemicellulose removal (oxidative delignification), which is improved even more in the case of hydrogen peroxide with the presence of transition metal complexes such as [Cu(bpy)] [70]. It is believed that the oxidative delignification works by oxidising the C-O- weaker linkages producing CO_2 and large molecules which are further oxidised, resulting on small water-soluble molecules [71][72].

Table 5: Alkaline pre-treatment reaction conditions

Source Material	Temperature (°C)	Time (min)	Liquid/Solid ratio (ml/g)	NaOH concentration	Reference
Reed straw	105	10	-	2 %wt	[73]
Sorghum straw	120	30	15:1	50 g/L	[74]
Sugar cane bagasse	115	38	10:1	0.34 M	[75]
Bamboo Source	**Temperature (°C)**	**Time (min)**	**Liquid/Solid ratio (ml/g)**	**NaOH concentration**	**Reference**
(Neosinocalamus afinis)	60	240	20:1	0-35 %wt	[76]
-	100	30-180	10:1	4-10 %wt	[77]
(Phyllostachys edulis)	120	60	8:1	0-7 %wt	[78]

Acid pre-treatment

This chemical pre-treatment uses concentrated or diluted acids with temperatures between 130 °C and 210 °C and constant mixing during minutes or hours depending on the conditions used to break the structure of the lignocellulosic material (Table 6) [15].

Concentrated acids are toxic, corrosive, hazardous require having the adequate material and higher energy investment which makes acid pre-treatment methods cost to be usually higher than others, even when the acid is recovered after the reaction [79].

Concentrated acids have the advantage that in some cases the subsequent treatment step is not required, as the acid pre-treatment itself hydrolyses the amorphous regions of the cellulose leaving the crystalline cellulose intact. As will be seen later, this concentrated acid method is used in the treatment step to hydrolyse the amorphous part of the cellulose. The process also destroys the hemicellulose sugars, transforming them into toxic compounds [80], that's why acids at concentrations below 4% are the most studied [66]. Diluted acid pre-treatment provides high solubilization of hemicelluloses with minimal degradation and a partial lignin solubilization too. However, requires higher temperatures and longer reaction times that the concentrated method. The most common type of dilute acid is sulfuric acid. However, nitric acid, hydrochloric acid, phosphoric acid, and other acids have also been investigated [65].

Table 6: Acid pre-treatment reaction conditions

Source Material	Temperature (°C)	Time (min)	Liquid/Solid ratio (ml/g)	Acid	Concentration %wt	Reference
Gelidium amansii	121	30	5:1	H_2SO_4	1	[81]
Wheat bran	100	60-480	15:1	H_3PO_4	1-2	[82]
Corn stover	120	40	10:1	HCl	1	[83]
Bamboo (Dendrocalamus Asper)	120, 140	30-90	10:1	H_2SO_4	0.6-1.2	[84]
Moso Bamboo	100	0-150	10:1	H_3PO_4	2-6	[85]

Ionic liquid (green solvents) pre-treatment

Ionic liquids (IL) are salts which have low vapor pressures, good thermal stability, are liquids at low temperatures and can dissolve a wide variety of biomass types. These salts are normally composed of a small anion and a large organic cation, which can be tuned to generate different liquids to dissolve different biomass types (Table 7) [86]. Those ionic liquids are also called green solvents due to their non toxicity and the fact that can be fully recovered after a large amount of pre-treatment steps because of its low vapor pressure [15].

Table 7: Ionic liquid pre-treatment reaction conditions

Source Material	Temperature (°C)	Time (h)	IL	IL / biomass ratio (w/w)	Reference
Sugarcane	100	0.5-8	[BMIM]Cl, [EMIM]oAc and [EMIM]DEP	25:1 (w/v)	[75]
Wheat straw	120	1-6	[emim][CH3COO]	20:1 (w/w)	[87]
Rice straw, sugarcane bagasse, eucalyptus, and pine	90	12	[Ch][Arg]	15:1 (w/w)	[88]

IL dissolving process is carried out at ambient pressures, temperatures between 90 °C and 130 °C during hours or days and the solution can be reprecipitated adding water [89]. IL can be tuned to dissolve any of the three main components of the lignocellulosic biomass (cellulose, hemicelluloses or lignin) without altering them [15] and it can even remove amorphous region of cellulose selectively.

Deep eutectic solvent (DES) pre-treatment

Deep eutectic solvents (DESs) are a mixture of substances that can form a eutectic mixture. That mixture melts or solidifies at a lower temperature than the melting point of any of its constituents. DES are chemicals that are almost equivalent to ILs (DESs are even regarded as a subcategory of ILs according to some sources) and exhibit similar properties, such as good solvent capacity and low vapor pressure. DES systems consisting of choline chloride and organic acids can be used as acidic hydrolytic solvents (Table 8). Compared with IL, DES can be obtained easily by heating the mixture around 100 °C and can be synthesized on larger scale [90].

Table 8: Deep eutectic pre-treatment reaction conditions

Source Material	Temperature (°C)	Time (min)	DES	Others	IL / biomass ratio (w/w)	Reference
Gossypium hirsutum L	70-100	3-5	Choline chloride and oxalic acid dihydrate	Microwave radiation (800 W)	40:1	[91]
Softwood pulp	100	360	Choline chloride and oxalic acid dihydrate	-	100:1	[92]

Ozonolysis pre-treatment

Ozone gas, a powerful oxidant and soluble in water, could be used in order to breakdown lignin and hemicelluloses. The degradation is mainly limited to lignin. Hemicellulose is slightly affected, but cellulose is hardly affected. Reactions are carried out at room temperature and normal pressure and that processes can be designed to minimize environmental pollution. Different reaction mechanisms have been purposed, it is believed that ozone preferentially reacts with olefinic, aromatic and phenolic compounds because of their electron density. The reactivity and the route of reaction of these compounds change depending on the substituents [93]. A drawback of ozonolysis is that a large amount of ozone is required, which can make the process expensive [55].

Chemical pre-treatment summary

Table 9 shows the summary of the advantages and disadvantages of the chemical pre-treatment methods.

Table 9: Chemical pre-treatments advantages and disadvantages

Method	Advantages	Disadvantages
Alkaline pre-treatment	Mild conditions, low cellulose degradation, can be combined with oxidizing agents to improve the yield, is the most used and studied pre-treatment.	Requires long pre-treatment times, not all bases can be recovered after the process which makes this method less environmentally friendly.
Acid pre-treatment	Lower reaction times than alkaline methods, solubilizes high percentage of hemicellulose,	Concentrated acids prevent the recuperation of lignin and hemicellulose and can damage cellulose, also need special equipment.
IL / DES	It solubilises both lignin and hemicelluloses, can dissolve lignin, hemicelluloses or cellulose selectively, is used in mild conditions and is environmentally friendly.	Solvents have a high cost and need to be recovered and recycled.
Ozonolysis pre-treatment	Selective lignin degradation, carried out at ambient temperature and pressure, residual ozone can be decomposed reducing the environmental pollution.	Flammable, corrosive, toxic and highly reactive, requires special equipment.

6.2.2. Physicochemical pre-treatments

This category involves conditions and compounds that affect both physical and chemical properties of the lignocellulosic biomass.

Steam explosion (SP)

Steam explosion is the most extensively studied and commonly applied physicochemical biomass pre-treatment. In this method the biomass is treated with steam at 160-240 °C under pressures between 0.7 and 4.8 MPa (Table 10) [65], the steam saturates the dry material, the pressure is held to help hydrolyse hemicellulose (the hydrolyzation could be improved with diluted acid) and then released to cause the rupture of the material and exposing the cellulose [94].

Hemicellulose is the predominant solubilized compound in the liquid phase, while the lignin is transformed as result of the high temperature. However, cellulose could be degraded if the conditions are too severe.

Hydrolysis of hemicellulose is thought to be mediated by the acetic acid generated from acetyl groups associated with hemicellulose and other acids released during pre-treatment, that may catalyse the hemicellulose hydrolysis.

SP can be effectively enhanced by addition of H_2SO_4, or SO_2 as a catalyst. The use of acid catalyst not only increases the solubilization of hemicellulose, but also decreases the production of inhibitors and improves the NCC yield [60] .

The low chemical quantity and the low energy requirements makes this method very attractive. However, lignin can’t be recovered because of its degradation which, if it’s not complete, could generate soluble lignin components resulting in the destruction of a portion of the hemicelluloses [65].

Table 10: SP pre-treatment reaction conditions

Source Material	Temperature (°C)	Time (min)	Pressure (MPa)	Other chemicals used	Reference
Pinus pinaster	190-210	4-8	4.12	-	[95]
Spruce	184-235	2.5-20	1.1-3.1	-	[96]
Bamboo (Bambusa blumeana) Bamboo (Bambusa pervariabilis)	-	4	2	Sodium chlorite / acetica acid solution	[97]
Bamboo (Phyllostachys edulis)	224	4	-	Alkaline / Peroxide solution	[98]

Ammonia Fibre/Freeze Explosion (AFEX)

AFEX Is an alkaline pre-treatment method that use liquid ammonia to pre-treat biomass, the lignocellulosic material is exposed to ammonia at a given temperature and high pressure, similarly to SP method, and causes swelling and phase change in cellulose crystallinity of biomass in addition to the alteration and removal of lignin (Table 11). After holding the target temperature for about 5 min, the vent valve is opened rapidly to explosively relieve the pressure. The chemical effect of ammonia under pressure causes the cellulosic biomass to swell, this results in a phase change in the crystal structure of cellulose I to cellulose III. The combined physical and chemical changes markedly increase the susceptibility of the pre-treated lignocellulosic biomass to subsequent hydrolysis.

Table 11: AFEX pre-treatment reaction conditions

Source Material	Temperature (°C)	Time	Ammonia (g/g of biomass)	Water (g/g of biomass)	Reference
Alamo CIR switchgrass	80-200	5-30	0.4-2	0.4-2	[99]
Sugarcane bagasse	100-140	30	1	2	[100]
Moso bamboo	70-190	1-30	0.5-5	0.2-2	[101]

CO_2 explosion pre-treatment

CO_2 explosion uses supercritical CO_2 on high pressures and temperatures for several minutes which once dissolved in water forms carbonic acid that helps in the hydrolysis of the hemicelluloses, the pressurized gas is released disrupting the biomass source similarly to AFEX or SP methods (Table 12) [65]. This method effectively increases the yield of the treatment with no generation of toxins. Increasing the pressure improves the penetration of CO_2 into the biomass structure [102]. Compared to steam and ammonia explosion the yields are lower but the overall pre-treatment is more cost-effective [79].

Table 12: CO_2 explosion pre-treatment reaction conditions

Source Material	Temperature (°C)	Time	Pressure	H_2O content	Reference
Sugarcane	120-180	1 h	20.6 MPa	80%	[103]

Physicochemical pre-treatment summary

Table 13 shows the summary of the advantages and disadvantages of the physicochemical pre-treatment methods.

Table 13: Physicochemical pre-treatment advantages and disadvantages

Method	Advantages	Disadvantages
Steam explosion	It requires little pre-treatment times, it doesn't need a large prior particle reduction since the same process decreases the biomass particle size,	It requires more complicated assembly and machinery than in chemical methods and diluted acids need to be added if high yield is wanted which requires special equipment and increases the cost.
Ammonia Fibre/Freeze Explosion	It solubilises a high amount of lignin and hemicelluloses without affecting cellulose,	It requires complicated assembly and has a high cost.
CO_2 explosion pre-treatment	Low temperatures are used which prevents the degradation of lignin and hemicelluloses.	It is a process that has not yet been fully investigated and its price is too high to be viable on a large scale.

6.2.3. Biological pre-tratments

The biological or microbial pre-treatment involves the use of microorganisms or enzymes (mainly fungi) to degrade lignin and hemicelluloses but leaving the cellulose intact. In biological pre-treatments, particle size, moisture content, resident time, and temperature, besides the microbial agents used, could affect lignin degradation rate [57].

For tunicate the pre-treatment involves the isolation of the mantel from the animal and the isolation of individual cellulose fibrils with the removal of the protein matrix [104].

Pre-treatments for algal cellulose sources typically involve culturing methods, and then purifying steps for removal of algal wall matrix material [105].

Bacterial cellulose pre-treatments focus on culturing methods for cellulose microfibrillar growth. Under special culturing conditions the bacteria secrete cellulose microfibrils, producing a thick gel [8]. In bacterial cellulose production another important factor is the carbon source, some studies [106] show that when the carbon source is arabitol or mannitol, the production of cellulose is 6.2 and 3.8 times better than glucose, respectively.

Fungal pre-treatment is mainly focused on fungi that selectively degrade lignin and hemicelluloses, while utilizing little cellulose. The pre-treatment process is significantly affected by the particle size and moisture content of the material, as well as the time and temperature of the microbial pre-treatment [57]. Many microorganisms are capable of degrading and utilizing hemicelluloses as carbon and energy sources. However, a much smaller group of filamentous fungi has evolved with the ability to break down lignin, these are known as white-rot fungi [107].

The advantages of biological pre-treatment include low energy requirement and mild environmental conditions. However, the rate of hydrolysis in most biological pre-treatment processes is very low [79], others disadvantages of that method include long time process, large space requirement, and the need for continuous monitoring of microorganism growth [108][109]. that’s the reason why the current efforts in biological pre-treatments are in combining this technology with other pre-treatments and in developing novel microorganisms for rapid hydrolysis [15].

Biological pre-treatment summary

Table 14 shows the summary of the advantages and disadvantages of the biological pre-treatment methods.

Table 14: Biological pre-treatment advantages and disadvantages

Advantages	Disadvantages
Very low energy requirements, mild conditions and environmentally friendly.	Low yield and very long pre-treatment times.

6.3. CELLULOSE TREATMENT

The last step is obtaining the CNs from the isolated cellulose in the pre-treatment. To extract the CNs, the amorphous regions of the cellulose must be removed, the fact that those regions are not well organized makes them more susceptible to chemical attacks while the crystalline regions have higher resistance (Figure 12) [3][8][110].

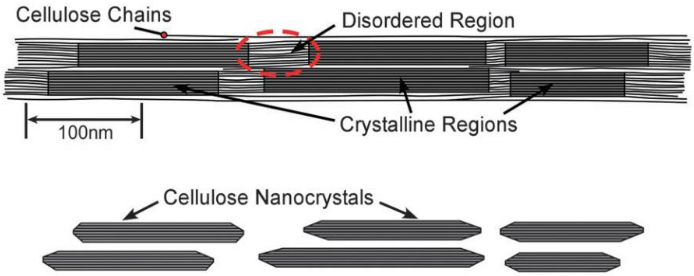

Figure 12: Cellulose crystalline and non-crystalline regions.

This step can be done in several ways, the most common is the acid hydrolysis, but there are many other methods that have been investigated to extract NCs or to improve the acid hydrolysis method as it will be seen below.

6.3.1. Acid hydrolysis

The most commonly used method to extract NCs from cellulose fibers is acid hydrolysis. It consists of the hydrolysation (Figure 13) adapted from [111] of the amorphous regions of cellulose (Figure 12) with a strong acid under controlled conditions of temperature, time and agitation (Table 15). The type of acid used, and the acid-to-cellulose ratio are parameters that must be considered too. That acid attack leads to rod-like nanocrystals.

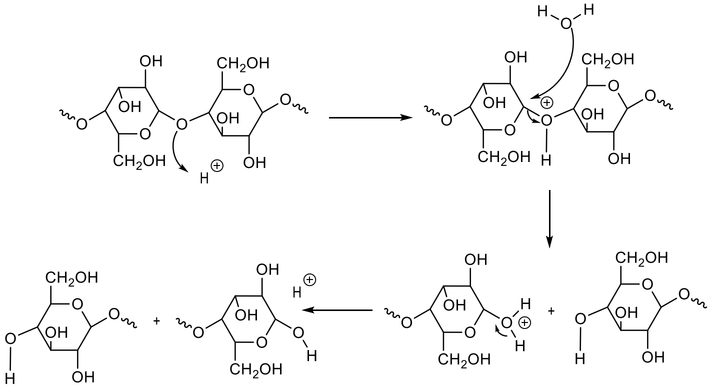

Figure 13: Cellulose acid hydrolysis.

Table 15: Acid hydrolisis reaction conditions

Source Material	**Temperature (°C)**	**Time (min)**	**Liquid/Solid ratio(ml/g)**	**Method**	**Concentration %wt**	**Reference**
Cotton cellulose (filter paper)	45	10-300	9:1 (v/w)	H_2SO_4	64-66	[112]
Pine wood flour	44	90	10:1 (v/w)	H_2SO_4	62	[113]
Bamboo Source	**Temperature (°C)**	**Time (min)**	**Liquid/Solid ratio(ml/g)**	**Method**	**Concentration %wt**	**Reference**
Bamboo pulp	40-60	90-210	30:1 (w/w)	H_3PO_4	77-85	[46]
Bambusa vulgaris	50-60	12-40	10:1-15:1 (v/w)	H_2SO_4	64	[114]
Phyllostachys heterocycla	60	120	25:1 (v/w)	H_2SO_4	68	[42]

The final product is washed to stop the acid attack, is usually centrifugated several times and dialysed with distilled water in order to remove any acid molecules. The hydrolysis and washing conditions depend on the source of cellulose [1].

The most commonly used acid is sulfuric acid followed by hydrochloric acid, although there are many other acids that have been used to extract CNs such as phosphoric and hydrobromic.

The use of sulfuric acid has one major advantage, it forms negatively charged sulphate esters which generates electrostatic repulsion between nanocrystals and form stable aqueous suspensions [115][116], while the use of hydrochloric acid produces CN's with little suspension in water due to the hydrogen bonds formed [116]. However, those electrostatic repulsions groups introduced into the outer surface of the cellulose crystals can decrease its thermal stability [117].

Other factor to consider is that the degree of polymerization (DP), which is the number of monomeric units in the polymer, decreases rapidly until it reaches the level-off DP (LODP) from which the DP remains relatively constant [118]. Because the amorphous or para-crystalline regions are usually evenly distributed along the microfibers the LODP can be correlated with the crystal sizes obtained before the acid hydrolysis[3][118]. The value of LODP depends on the cellulose source, and the DP obtained is different for every source, even at the LODP. However, the acid hydrolysis of some sources that have no regular distribution of the amorphous regions and can result in a wide variety of molecular weight polymers without reaching the LODP [3].

Even though sulfuric acid treatment it's the usual method due to the introduction of groups that help stabilizing the cellulose nanocrystals in solution, the use of sulfuric acid and other mineral acids has a number of drawbacks such as corrosivity, environmental incompatibility, the possible degradation of cellulose, being time-consuming and the relatively low yield.

6.3.2. Cation exchange hydrolysis

The use of cation exchange resin hydrolysis to produce CNs is one effective alternative method, ion-exchange resins can act as a solid catalyst in many reactions which are normally catalysed by acids (Table 16) [119], those resins have several advantages such as being more environmental-friendly, not needing special equipment, being easier to handle and being able to be recycled [120]. There are just a few examples of this method producing CNs but some studies show that this method could be a good method for manufacturing CNs in an environmental-friendly way [120].

Table 16: Cation exchange reaction conditions

Source Material	Temperature (°C)	Time (min)	Liquid/Solid ratio (ml/g)	Reference
MCC	50	180	7.5:1	[120]

6.3.3. Ionic Liquids

As said before ionic liquids (IL) are liquid salts that can dissolve a wide variety of biomass types. Some of those ionic liquids can remove a large percentage of lignin, almost all hemicelluloses and even selectively hydrolyse cellulose amorphous regions (Table 17) [121]. The reason for this selectivity could be due to ionic liquid entering the space between cellulose chains in the amorphous region and hydrolysing the cellulose molecules while leaving the crystalline parts intact [122]. Some reports [123] demonstrate that pre-treatment step could be removed by adding a catalytic amount of sulfuric acid, removing the need to do two steps to extract the CNs. However, that makes the ionic liquid much harder to recuperate.

Table 17: Ionic liquids reaction conditions

Source Material	Temperature (°C)	Time (min)	Liquid/Solid ratio (ml/g)	Reference
Angelim vermelho	100	60	20:1 (w/w)	[121]
MCC	70-100	90	10:1 (w/w)	[124]
MCC (Avicel PH-101)	90	60	10:1	[122]

6.3.4. Ultrasonic treatment

Sonication is the application of sound energy to physical or chemical systems; the sound waves introduce pressure variations that grow and collapse. The sonication process is normally used after acid hydrolysis to disperse cellulose nanocrystals. Some studies have developed purely physical methods of high-intensity ultrasonication to overcome the drawbacks of acid hydrolysis with good results (Table 18) [125]. However, the high intensity ultrasonic treatment usually have non-selective effect and brakes the intermolecular bonds even on the crystalline parts of the NCC which reduces the crystallinity percentage [126]. Other application to the ultrasonic treatment is the addition of low-intensity ultrasonic assistant to the acid hydrolysis which improve the yield of NC's obtained without changing the morphology (Table 18). The use of that ultrasonic treatment breaks down the interaction force between cellulose microfibrils, facilitating the disintegration of cellulosic fibers into nanofibers improving the yield [126][127].

Table 18: Ultrasonic treatment reaction conditions

Source Material	Temperature (°C)	Time (min)	Liquid/Solid ratio(ml/g)	Method	Concentration (%wt) / Conditions	Reference
Micro-crystalline cellulose	50	90	10:1	Ultrasonic-assisted sulfuric acid hydrolysis	59-67	[126]
Bacterial Cellulose (Acetobacter xylinum)	Ice bath	15-75	-	Ultrasonic treatment	200 W, 20 kHz	[125]
Wood sawdust	60	60	10:1	Ultrasonic-assisted sulfuric acid hydrolysis	60	[128]

6.3.5. Chemical treatment summary

Table 19 shows the summary of the advantages and disadvantages of the chemical treatment methods.

Table 19: Chemical treatment advantages and disadvantages

Method	Advantages	Disadvantages
Acid hydrolysis	Is the most used treatment to produce CNs, has high yield and can be combined with other methods	Requires special equipment and is not environmentally friendly
Cation exchange hydrolysis	Environmentally friendly, easy to handle, it's not dangerous and do not cause equipment corrosion	Needs to be regenerated and very little research is available.
Ionic liquids	Environmentally friendly, it's not dangerous, if combined with acid the pre-treatment step can be avoided.	Needs to be regenerated and can be expensive
Ultrasonic treatment	It can improve the yield is combined with other methods	By itself it causes a decrease in crystallinity

6.4. CHARACTERIZATION

Different characterization techniques are used for different purposes:

6.4.1. Shape

As mentioned above, once obtained, cellulose nanocrystals have the characteristic of not containing amorphous zones as well as having a specific shape and size. One of the ways to know if the nanocrystals extraction process has been successful is through techniques capable of providing information on the morphology of the product such as Field Emission Scanning Electron Microscopy (FE-SEM), Atomic Force Microscopy (AFM) or Transmission Electron Microscopy (TEM). On those characterization techniques cellulose nanocrystals show always the same rod-like shape with widths and lengths between 5-70 nm and 100-250 nm respectively [120][126][129][130].

6.4.2. Structure

As already explained in previous sections, the composition of the initial lignocellulosic biomass changes as the different processes for the extraction of nanocrystals are applied, a good way to study those changes in the chemical functionality is by Infrared Spectroscopy (IR). Three different possible spectra can be differentiated, one of the initial lignocellulosic biomass, another of the pre-treatment product and another of the final CNs.

The main changes observed between the initial source and the pre-treated product are the disappearance of the bands at 1,730 cm^{-1} and 1,512 cm^{-1} (Table 20) and the intensity reduction of the band at 1,250 cm^{-1} [126][129]. However, the spectra of the pre-treatment product and the final cellulose nanocrystals are very similar, both spectra have characteristic common bands that appear approximately at 3,340 cm^{-1}, 2,900 cm^{-1}, 1,640 cm^{-1}, 1,120 cm^{-1}, and 1030 cm^{-1} (Table 20)[131][132][133].

Table 20: IR bands

Band frequency (cm^{-1})	Band description
3,340	Stretching of O-H groups
2,900	C-H symmetric vibrations
1,730	C-O stretching vibration for the acetyl and ester linkages
1,640	Absorbed water
1,512	Associated to the aromatic rings of lignin
1,250	C-O stretching of aryl group from lignin
1,120	Stretching of the intermolecular ester bond

6.4.3. Crystallinity

Another important property to determine is the crystallinity content, this can be characterized by various methods.

In IR the absorbances at 1,430 cm^{-1} and 897 cm^{-1} are sensitive to the crystal structure of the cellulosic material and the ratio of A_{1430}/A_{897}, which is known as crystallinity index or lateral order index (LOI), is used to calculate the crystallinity, being bigger the more crystallinity there is [134].

X-ray diffraction (XRD) is another method used to determine the crystallinity. XRD provides strong and pointed signals from the crystalline fraction of the cellulose while the non-cellulose structure have broader and less clearly defined signals [135], finally the degree of crystallinity (X_c) (Figure 13) can be determined by knowing the intensity of the amorphous (I_a) and crystalline (I_c) peaks [136][137].

6.4.4. Differential thermal analysis

The thermostability of the is another characterization method that is usually used to study the response of the material to a change in temperature. An initial small weight loss can be observed always at a low temperature (100 °C) that corresponds to the water absorbed. The lignocellulosic biomass thermal degradation starts earlier (∼270 °C) than the pre-treated cellulose (∼290 °C) due to the presence of lignin and hemicelluloses which decomposes at lower temperature than cellulose. The thermal degradation of CNs is initiated at lower temperatures (∼235 °C) due to the greater number of free ends caused by the crystalline cellulose nano-sizes which gives them lower thermal stability. In the case of the CNs obtained by sulfuric acid hydrolysis, the sulphate esters introduced by that method decrease the thermal stability, the elimination of those groups require low energy, and the sulfuric acid released can facilitate the decomposition or depolymerisation of cellulose [126][138][129].

7. INITIAL EXPERIMENTAL PROCEDURE

This report was originally focused on optimizing an experimental method to extract cellulose nanocrystals from Guadua Angustifolia, but due to the circumstances, it was reoriented as a bibliographic report. The proposed method for extracting cellulose nanocrystals from Guadua Angustifolia was as follows.

7.1. SIZE REDUCTION

The Guadua Angustifolia leaves from Ecuador were cut with scissors to a size of a few centimetres to be able to dry them in an oven at 100 ºC. Once the leaves were dry, they were passed through a Retsch RS100 ring mill from the Faculty of Earth Sciences. Once milled, two types of sample could be observed: fibers a few centimeters long and a brown dust. Both samples were sieved, leaving 3 different sizes, one of less than 125 microns, another between 125 and 250 microns and another of more than 250 microns. The smallest sizes were mixed due to lack of sample quantity, only the samples smaller than 250 microns were used for the extraction process.

7.1. ALKALINE PRE-TREATMENT

The initial approach was to use a combination of an alkaline method, based on sodium hydroxide, together with an oxidative delignification using hydrogen peroxide as a pre-treatment. To be able to evaluate the influence of the different parameters that could affect the yield of the pre-treatment, temperature, concentration and reaction time, an experimental design was made (Table 21). Once the replicates for the experiment design started, they could not be completed due to Covid-19 confinement.

After having completed the bibliographic search of the report, it can be concluded that the range chosen for the parameters of the design of the experiment, despite the fact that none was out of the normal, in the case of the NaOH concentration was slightly above what is normally used in this type of treatment.

The following steps of the method for extracting cellulose nanocrystals could not be carried out, nor was it possible to propose an experimental design for the cellulose hydrolysis steps.

Table 21: Design of experiment for the pre-treatment step

Method	Run	Temperature (ºC)	Concentration (%wt)	Time (h)
17	1	55.00	17.50	2.00
1	2	40.00	10.00	1.00
18	3	55.00	17.50	2.00
10	4	80.00	17.50	2.00
11	5	55.00	5.00	2.00
19	6	55.00	17.50	2.00
12	7	55.00	30.00	2.00
7	8	40.00	25.00	3.00
5	9	40.00	10.00	3.00
2	10	70.00	10.00	1.00
9	11	30.00	17.50	2.00
3	12	40.00	25.00	1.00
20	13	55.00	17.50	2.00
6	14	70.00	10.00	3.00
15	15	55.00	17.50	2.00
16	16	55.00	17.50	2.00
4	17	70.00	25.00	1.00
14	18	55.00	17.50	3.68
13	19	55.00	17.50	0.32
8	20	70.00	25.00	3.00

8. CONCLUSIONS

In the bibliographic analysis, an exponential growth of interest in nanocellulose and nanocrystalline cellulose has been observed.

Bibliography related to the extraction of cellulose nanocrystals from bamboo has been found, but not from Guada Angustifolia.

Many pre-treatment methods are used to produce other products apart from the CNs (mainly paper and bioethanol), so there is more information and have been more studied.

Bamboo is a very good source from which to extract these CNs due to its high growth rate. In addition, the extraction methods are applicable for this plant in the same way as for any other plant or wood.

Reducing the size of the starting material is something that is often seen as a type of pre-treatment and not as the first step to do before the pre-treatment in the process of extraction despite being of such importance.

Pre-treatment step usually combines several methods to obtain the highest possible yield. In the case of chemical pre-treatments alkaline methods are usually combined with bleaching processes using hydrogen peroxide or sodium chlorite, while physicochemical processes such as steam explosion are usually combined with acids. This trend is also observed in the case of bamboo.

In the CNs extraction process, there seems to be room for improvement, the method based on sulfuric acid has been extensively investigated despite having several drawbacks such as cellulose degradation or environmental incompatibility, but ways to increase the yield of that acid hydrolysis, like ultrasonication, have been investigated, and greener ways to extract CNs have been discovered such as ionic liquids, that are selectively capable of hydrolysing the amorphous areas of cellulose and are reusable, or cation exchange resins that despite having a generally lower yield higher crystallinity indices can be obtained than with the sulfuric acid method.

9. REFERENCES AND NOTES

[1] A. Blanco, M. C. Monte, C. Campano, A. Balea, N. Merayo, and C. Negro, *Nanocellulose for industrial use: Cellulose nanofibers (CNF), cellulose nanocrystals (CNC), and bacterial cellulose (BC)*. Madrid: Elsevier Inc., 2018.

[2] "Starch and Cellulose." [Online]. Available: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Smith)/Chapter_05%3A_Stereochemistry/5.01_Starch_and_Cellulose. [Accessed: 06-Jul-2020].

[3] Y. Habibi, L. A. Lucia, and O. J. Rojas, "Cellulose nanocrystals: Chemistry, self-assembly, and applications," *Chem. Rev.*, vol. 110, no. 6, pp. 3479–3500, 2010, doi: 10.1021/cr900339w.

[4] B. Lu, A. Xu, and J. Wang, "Cation does matter: How cationic structure affects the dissolution of cellulose in ionic liquids," *Green Chem.*, vol. 16, no. 3, pp. 1326–1335, 2014, doi: 10.1039/c3gc41733f.

[5] K. H. Gardner and J. Blackwell, "The hydrogen bonding in native cellulose," *BBA - Gen. Subj.*, vol. 343, no. 1, pp. 232–237, 1974, doi: 10.1016/0304-4165(74)90256-6.

[6] E. Kontturi, "Cellulose: Structure, morphology, and crystalline forms," 2015.

[7] A. Chami Khazraji and S. Robert, "Self-assembly and intermolecular forces when cellulose and water interact using molecular modeling," *J. Nanomater.*, vol. 2013, pp. 1–12, 2013, doi: 10.1155/2013/745979.

[8] R. J. Moon, A. Martini, J. Nairn, J. Simonsen, and J. Youngblood, "Cellulose nanomaterials review: Structure, properties and nanocomposites," *Chem. Soc. Rev.*, vol. 40, no. 7, pp. 3941–3994, 2011, doi: 10.1039/c0cs00108b.

[9] L. J. Gibson, "The hierarchical structure and mechanics of plant materials," *J. R. Soc. Interface*, vol. 9, no. 76, pp. 2749–2766, 2012, doi: 10.1098/rsif.2012.0341.

[10] X. Yuan and G. Cheng, "From cellulose fibrils to single chains: Understanding cellulose dissolution in ionic liquids," *Phys. Chem. Chem. Phys.*, vol. 17, no. 47, pp. 31592–31607, 2015, doi: 10.1039/c5cp05744b.

[11] H. P. S. Abdul Khalil, A. H. Bhat, and A. F. Ireana Yusra, "Green composites from sustainable cellulose nanofibrils: A review," *Carbohydr. Polym.*, vol. 87, no. 2, pp. 963–979, 2012, doi: 10.1016/j.carbpol.2011.08.078.

[12] C. Ye, M. O. Sundström, and K. Remes, "Microscopic transmission ellipsometry: measurement of the fibril angle and the relative phase retardation of single, intact wood pulp fibers," *Appl. Opt.*, vol. 33, no. 28, p. 6626, 1994, doi: 10.1364/ao.33.006626.

[13] H. V. Scheller and P. Ulvskov, "Hemicelluloses," *Annu. Rev. Plant Biol.*, vol. 61, no. 1, pp. 263–289, 2010, doi: 10.1146/annurev-arplant-042809-112315.

[14] Q. Liu, L. Luo, and L. Zheng, "Lignins: Biosynthesis and biological functions in plants," *Int. J. Mol. Sci.*, vol. 19, no. 2, pp. 1–16, 2018, doi: 10.3390/ijms19020335.

[15] G. Brodeur, E. Yau, K. Badal, J. Collier, K. B. Ramachandran, and S. Ramakrishnan,

"Chemical and physicochemical pretreatment of lignocellulosic biomass: A review," *Enzyme Res.*, vol. 2011, no. 1, pp. 1–17, 2011, doi: 10.4061/2011/787532.

[16] A. A. Sundarraj and T. V. Ranganathan, "A review on cellulose and its utilization from agro-industrial waste," *Drug Invent. Today*, vol. 10, no. 1, pp. 89–94, 2018.

[17] D. R. Bogati, "Cellulose Based Biochemicals and Their," Saimaa University of Applied Sciences, 2011.

[18] R. Rowell, R. Rowell, R. Pettersen, and M. Tshabalala, *Cell Wall Chemistry*. London: CRC Press, 2012.

[19] W.-H. W. Chen and F. K. Cameron, "Cellulose Content of Cotton and Southern Woods," *Ind. Eng. Chem.*, vol. 34, no. 2, pp. 224–225, 1942, doi: 10.1021/ie50386a018.

[20] Javad Shokri and Khosro Adibkia, "Application of Cellulose and Cellulose Derivatives in Pharmaceutical Industries," in *Intech*, Tabriz, 2016, pp. 47–66.

[21] S. Khattak *et al.*, "Applications of cellulose and chitin/chitosan derivatives and composites as antibacterial materials: current state and perspectives," *Appl. Microbiol. Biotechnol.*, vol. 103, no. 5, pp. 1989–2006, 2019, doi: 10.1007/s00253-018-09602-0.

[22] A. W. T. H. Williamson, "Cellulose and some of its industrial uses.," vol. 6, no. 1, pp. 16–20, 1927.

[23] Y. Y. Li, B. Wang, M. G. Ma, and B. Wang, "Review of Recent Development on Preparation, Properties, and Applications of Cellulose-Based Functional Materials," *Int. J. Polym. Sci.*, vol. 2018, 2018, doi: 10.1155/2018/8973643.

[24] R. Malcolm Brown Jr, "The Biosynthesis of Cellulose," *J. Macromol. Sci. Part A Pure Appl. Chem. Publ.*, vol. 33:10, no. March 2013, pp. 1345–1373, 1996.

[25] S. Elazzouzi-Hafraoui, Y. Nishiyama, J. L. Putaux, L. Heux, F. Dubreuil, and C. Rochas, "The shape and size distribution of crystalline nanoparticles prepared by acid hydrolysis of native cellulose," *Biomacromolecules*, vol. 9, no. 1, pp. 57–65, 2008, doi: 10.1021/bm700769p.

[26] H. Yamamoto, F. Horii, and A. Hirai, "In situ crystallization of bacterial cellulose II. Influences of different polymeric additives on the formation of celluloses Iα and Iβ at the early stage of incubation," *Cellulose*, vol. 3, no. 1, pp. 229–242, 1996, doi: 10.1007/bf02228804.

[27] S. Iwamoto, A. N. Nakagaito, and H. Yano, "Nano-fibrillation of pulp fibers for the processing of transparent nanocomposites," *Appl. Phys. A Mater. Sci. Process.*, vol. 89, no. 2, pp. 461–466, 2007, doi: 10.1007/s00339-007-4175-6.

[28] A. Dufresne, J. Cavaillé, and M. R. Vignon, "Mechanical behavior of sheets prepared from sugar beet cellulose microfibrils," *J. Appl. Polym. Sci.*, vol. 64, no. 6, pp. 1185–1194, 1997, doi: 10.1002/(sici)1097-4628(19970509)64:6<1185::aid-app19>3.3.co;2-2.

[29] D. Klemm *et al.*, "Nanocelluloses: A new family of nature-based materials," *Angew. Chemie - Int. Ed.*, vol. 50, no. 24, pp. 5438–5466, 2011, doi: 10.1002/anie.201001273.

[30] B. Deepa *et al.*, "Utilization of various lignocellulosic biomass for the production of nanocellulose: a comparative study," *Cellulose*, vol. 22, no. 2, pp. 1075–1090, 2015, doi: 10.1007/s10570-015-0554-x.

[31] L. Brinchi, F. Cotana, E. Fortunati, and J. M. Kenny, "Production of nanocrystalline cellulose from lignocellulosic biomass: Technology and applications," *Carbohydr. Polym.*, vol. 94, no. 1, pp. 154–169, 2013, doi: 10.1016/j.carbpol.2013.01.033.

[32] S. Mondal, "Preparation, properties and applications of nanocellulosic materials,"

Carbohydr. Polym., vol. 163, pp. 301–316, 2017, doi: 10.1016/j.carbpol.2016.12.050.

[33] M. Rahimi Kord Sofla, R. J. Brown, T. Tsuzuki, and T. J. Rainey, “A comparison of cellulose nanocrystals and cellulose nanofibres extracted from bagasse using acid and ball milling methods,” *Adv. Nat. Sci. Nanosci. Nanotechnol.*, vol. 7, no. 3, 2016, doi: 10.1088/2043-6262/7/3/035004.

[34] P. Lu and Y. Lo Hsieh, “Preparation and properties of cellulose nanocrystals: Rods, spheres, and network,” *Carbohydr. Polym.*, vol. 82, no. 2, pp. 329–336, 2010, doi: 10.1016/j.carbpol.2010.04.073.

[35] J. D. Mendez and C. Weder, “Synthesis, electrical properties, and nanocomposites of poly(3,4-ethylenedioxythiophene) nanorods,” *Polym. Chem.*, vol. 1, no. 8, pp. 1237–1244, 2010, doi: 10.1039/c0py00118j.

[36] A. B. Seabra, J. S. Bernardes, W. J. Fávaro, A. J. Paula, and N. Durán, “Cellulose nanocrystals as carriers in medicine and their toxicities: A review,” *Carbohydr. Polym.*, vol. 181, pp. 514–527, 2018, doi: 10.1016/j.carbpol.2017.12.014.

[37] R. Stopforth and S. Adali, “Experimental study of bullet-proofing capabilities of Kevlar, of different weights and number of layers, with 9 mm projectiles,” *Def. Technol.*, vol. 15, no. 2, pp. 186–192, 2019, doi: 10.1016/j.dt.2018.08.006.

[38] H. P. S. Abdul Khalil *et al.*, “The use of bamboo fibres as reinforcements in composites,” in *Biofiber Reinforcements in Composite Materials*, 2015, pp. 488–524.

[39] “Types of Bamboo Rhizomes.” [Online]. Available: https://bit.ly/38VbGxc. [Accessed: 07-Jul-2020].

[40] E. Britannica, “Bamboo,” *Encyclopædia Britannica, inc.*, 2018. [Online]. Available: https://bit.ly/3j4rnqh. [Accessed: 07-Jul-2020].

[41] “Complete Bamboo - Bamboo Plant Information Resource.” .

[42] G. Editorial, “Membrane reactors – Part I,” *Technology*, no. 17, pp. 686–695, 2009, doi: 10.1002/apj.

[43] “Bambusa.” [Online]. Available: https://bit.ly/2C3VcqE. [Accessed: 05-Jun-2020].

[44] X. Li, “Physical, chemical, and mechanical properties of bamboo and its utilization potential for fiberboard manufacturing,” *Agric. Mech. Coll.*, vol. Master of, p. 76, 2004.

[45] et al. Darma R, Purnamasari IM, Agustina D, Pramudito TE, Sugiharti M, “A Strong Antifungal-producing Bacteria from Bamboo Powder for Biocontrol of Sclerotium rolfsii in Melon (Cucumis melo var. amanta),” *J. Plant Pathol. Microbiol.*, vol. 07, no. 02, p. 334, 2016, doi: 10.4172/2157-7471.1000334.

[46] Q. Lu, W. Lin, L. Tang, S. Wang, X. Chen, and B. Huang, “A mechanochemical approach to manufacturing bamboo cellulose nanocrystals,” *J. Mater. Sci.*, vol. 50, no. 2, pp. 611–619, 2014, doi: 10.1007/s10853-014-8620-6.

[47] S. Zhang, Y. Su, D. Xu, S. Zhu, H. Zhang, and X. Liu, “Assessment of hydrothermal carbonization and coupling washing with torrefaction of bamboo sawdust for biofuels production,” *Bioresour. Technol.*, vol. 258, pp. 111–118, 2018, doi: 10.1016/j.biortech.2018.02.127.

[48] Michelle Nijhuis, “Bamboo Boom,” *J. Chem. Inf. Model.*, vol. 53, no. 9, pp. 1689–1699, 2013, doi: 10.1017/CBO9781107415324.004.

[49] “Bambu World map.” [Online]. Available: https://bit.ly/2Oscujy.

[50] James J. Parsons, “Giant American Bamboo in the Vernacular Architecture of Colombia and Ecuador,” *Hilos Tensados*, vol. 81, no. 2, pp. 131–152, 1991, doi:

10.1017/CBO9781107415324.004.

[51] INBAR, "Roadmap of ecuador's plans for the bamboo industry published." [Online]. Available: https://bit.ly/2AZlBVO. [Accessed: 07-Jul-2020].

[52] X. Londoño, "Evaluation of bamboo resources in latin america," 2001.

[53] D. J. F. Correal and C. Juliana Arbeláez, "Influence of age and height position on colombian Guadua angustifolia bambo mechanical properties," *Maderas Cienc. y Tecnol.*, vol. 12, no. 2, pp. 105–113, 2010, doi: 10.4067/S0718-221X2010000200005.

[54] H. A. Ruiz, D. S. Ruzene, D. P. Silva, M. A. C. Quintas, A. A. Vicente, and J. A. Teixeira, "Evaluation of a hydrothermal process for pretreatment of wheat straw-effect of particle size and process conditions," *J. Chem. Technol. Biotechnol.*, vol. 86, no. 1, pp. 88–94, 2011, doi: 10.1002/jctb.2518.

[55] M. Michelin, H. A. Ruiz, D. P. Silva, D. S. Ruzene, J. A. Teixeira, and M. L. T. M, "Cellulose from Lignocellulosic Waste Michele," *Springer Int. Publ. Switz.*, vol. 52, no. 1, pp. 1–33, 2014, doi: 10.1007/978-3-319-03751-6.

[56] V. S. Chang, B. Burr, and M. T. Holtzapple, "Lime Pretreatment of Switchgrass," *Biotechnol. Fuels Chem.*, vol. 63, no. 4, pp. 3–19, 1997, doi: 10.1007/978-1-4612-2312-2_2.

[57] C. Wan and Y. Li, "Microbial pretreatment of corn stover with Ceriporiopsis subvermispora for enzymatic hydrolysis and ethanol production," *Bioresour. Technol.*, vol. 101, no. 16, pp. 6398–6403, 2010, doi: 10.1016/j.biortech.2010.03.070.

[58] M. Mariño, L. L. Da Silva, N. Durán, and L. Tasic, "Enhanced materials from nature: Nanocellulose from citrus waste," *Molecules*, vol. 20, no. 4, pp. 5908–5923, 2015, doi: 10.3390/molecules20045908.

[59] D. M. Nascimento *et al.*, "A novel green approach for the preparation of cellulose nanowhiskers from white coir," *Carbohydr. Polym.*, vol. 110, pp. 456–463, 2014, doi: 10.1016/j.carbpol.2014.04.053.

[60] W. Yang, T. Cheng, Y. Feng, J. Qu, H. He, and X. Yu, "Isolating cellulose nanofibers from steam-explosion pretreated corncobs using mild mechanochemical treatments," *BioResources*, vol. 12, no. 4, pp. 9183–9197, 2017, doi: 10.15376/biores.12.4.9183-9197.

[61] X. Zhang, H. Yu, H. Huang, and Y. Liu, "Evaluation of biological pretreatment with white rot fungi for the enzymatic hydrolysis of bamboo culms," *Int. Biodeterior. Biodegrad.*, vol. 60, no. 3, pp. 159–164, 2007, doi: 10.1016/j.ibiod.2007.02.003.

[62] J. Xie, C. Y. Hse, C. F. De Hoop, T. Hu, J. Qi, and T. F. Shupe, "Isolation and characterization of cellulose nanofibers from bamboo using microwave liquefaction combined with chemical treatment and ultrasonication," *Carbohydr. Polym.*, vol. 151, pp. 725–734, 2016, doi: 10.1016/j.carbpol.2016.06.011.

[63] H. D. Nguyen, T. T. Thuy Mai, N. B. Nguyen, T. D. Dang, M. L. Phung Le, and T. T. Dang, "A novel method for preparing microfibrillated cellulose from bamboo fibers," *Adv. Nat. Sci. Nanosci. Nanotechnol.*, vol. 4, no. 1, 2013, doi: 10.1088/2043-6262/4/1/015016.

[64] J. Y. Zhu and X. J. Pan, "Woody biomass pretreatment for cellulosic ethanol production: Technology and energy consumption evaluation," *Bioresour. Technol.*, vol. 101, no. 13, pp. 4992–5002, 2010, doi: 10.1016/j.biortech.2009.11.007.

[65] V. B. Agbor, N. Cicek, R. Sparling, A. Berlin, and D. B. Levin, "Biomass pretreatment: Fundamentals toward application," *Biotechnol. Adv.*, vol. 29, no. 6, pp. 675–685, 2011,

doi: 10.1016/j.biotechadv.2011.05.005.

[66] P. Kumar, D. M. Barrett, M. J. Delwiche, and P. Stroeve, "Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production," *Ind. Eng. Chem. Res.*, vol. 48, no. 8, pp. 3713–3729, 2009, doi: 10.1021/ie801542g.

[67] J. Li, J. Zhang, S. Zhang, Q. Gao, J. Li, and W. Zhang, "Alkali lignin depolymerization under eco-friendly and cost-effective NaOH/urea aqueous solution for fast curing bio-based phenolic resin," *Ind. Crops Prod.*, vol. 120, no. 1, pp. 25–33, 2018, doi: 10.1016/j.indcrop.2018.04.027.

[68] S. McIntosh and T. Vancov, "Enhanced enzyme saccharification of Sorghum bicolor straw using dilute alkali pretreatment," *Bioresour. Technol.*, vol. 101, no. 17, pp. 6718–6727, 2010, doi: 10.1016/j.biortech.2010.03.116.

[69] R. Sun, J. M. Lawther, and W. B. Banks, "Influence of alkaline pre-treatments on the cell wall components of wheat straw," *Ind. Crops Prod.*, vol. 4, no. 2, pp. 127–145, 1995, doi: 10.1016/0926-6690(95)00025-8.

[70] N. Bansal *et al.*, "Cell wall-associated transition metals improve alkaline-oxidative pretreatment in diverse hardwoods," *Green Chem.*, vol. 18, no. 5, pp. 1405–1415, 2016, doi: 10.1039/c5gc01748c.

[71] K. Miura, K. Mae, H. Okutsu, and N. A. Mizutani, "New oxidative degradation method for producing fatty acids in high yields and high selectivity from low-rank coals," *Energy and Fuels*, vol. 10, no. 6, pp. 1196–1201, 1996, doi: 10.1021/ef960051a.

[72] L. Doskočil, L. Grasset, D. Válková, and M. Pekař, "Hydrogen peroxide oxidation of humic acids and lignite," *Fuel*, vol. 134, pp. 406–413, 2014, doi: 10.1016/j.fuel.2014.06.011.

[73] D. N. Barman *et al.*, "Effect of mild alkali pretreatment on structural changes of reed (Phragmites communis Trinius) straw," *Environ. Technol.*, vol. 35, no. 2, pp. 232–241, 2014, doi: 10.1080/09593330.2013.824009.

[74] W. S. Cardoso *et al.*, "Use of sorghum straw (sorghum bicolor) for second generation ethanol production: Pretreatment and enzymatic hydrolysis," *Quim. Nova*, vol. 36, no. 5, pp. 623–627, 2013, doi: 10.1590/S0100-40422013000500002.

[75] L. W. Yoon, G. C. Ngoh, A. S. May Chua, and M. A. Hashim, "Comparison of ionic liquid, acid and alkali pretreatments for sugarcane bagasse enzymatic saccharification," *J. Chem. Technol. Biotechnol.*, vol. 86, no. 10, pp. 1342–1348, 2011, doi: 10.1002/jctb.2651.

[76] J. H. Chen, J. K. Xu, P. L. Huang, and R. C. Sun, "Effect of alkaline pretreatment on the preparation of regenerated lignocellulose fibers from bamboo stem," *Cellulose*, vol. 23, no. 4, pp. 2727–2739, 2016, doi: 10.1007/s10570-016-0983-1.

[77] Z. Yuan, Y. Wen, and N. S. Kapu, "Ethanol production from bamboo using mild alkaline pre-extraction followed by alkaline hydrogen peroxide pretreatment," *Bioresour. Technol.*, vol. 247, pp. 242–249, 2018, doi: 10.1016/j.biortech.2017.09.080.

[78] C. L. Tri, L. D. Khuong, and I. Kamei, "The improvement of sodium hydroxide pretreatment in bioethanol production from Japanese bamboo Phyllostachys edulis using the white rot fungus Phlebia sp. MG-60," *Int. Biodeterior. Biodegrad.*, vol. 133, pp. 86–92, 2018, doi: 10.1016/j.ibiod.2018.06.010.

[79] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresour. Technol.*, vol. 83, no. 1, pp. 1–11, 2002, doi: 10.1016/S0960-

8524(01)00212-7.

[80] A. Lorenci *et al.*, "Bioresource Technology Lignocellulosic biomass : Acid and alkaline pretreatments and their effects on biomass recalcitrance – Conventional processing and recent advances," *Bioresour. Technol.*, vol. 304, p. 122848, 2020, doi: 10.1016/j.biortech.2020.122848.

[81] P. Sivagurunathan and G. Kumar, "Effects of various dilute acid pretreatments on the biochemical hydrogen production potential of marine macroalgal biomass," *Int. J. Hydrogen Energy*, vol. 42, no. 45, pp. 27600–27606, 2017, doi: 10.1016/j.ijhydene.2017.05.106.

[82] R. B. Nair, M. Kalif, J. A. Ferreira, M. J. Taherzadeh, and P. R. Lennartsson, "Bioresource Technology Mild-temperature dilute acid pretreatment for integration of fi rst and second generation ethanol processes," *Bioresour. Technol.*, vol. 245, pp. 145–151, 2017, doi: 10.1016/j.biortech.2017.08.125.

[83] S. An *et al.*, "Bioresource Technology Combined dilute hydrochloric acid and alkaline wet oxidation pretreatment to improve sugar recovery of corn stover," *Bioresour. Technol.*, vol. 271, pp. 283–288, 2019, doi: 10.1016/j.biortech.2018.09.126.

[84] W. Leenakul and N. Tippayawong, "Dilute Acid Pretreatment of Bamboo for Fermentable Sugar Production," *J. Sustain. Energy Environ.*, vol. 1, pp. 117–120, 2010.

[85] L. W. and X. G. Bo Hong, Guoxin Xue, "Pretreatment of moso bamboo with dilute phosphoric acid," *bioresources*, vol. 7, no. 4, pp. 4902–4913, 2012.

[86] S. H. Lee, T. V. Doherty, R. J. Linhardt, and J. S. Dordick, "Ionic liquid-mediated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis," *Biotechnol. Bioeng.*, vol. 102, no. 5, pp. 1368–1376, 2009, doi: 10.1002/bit.22179.

[87] A. M. da Costa Lopes *et al.*, "Pre-treatment of lignocellulosic biomass using ionic liquids: Wheat straw fractionation," *Bioresour. Technol.*, vol. 142, pp. 198–208, 2013, doi: 10.1016/j.biortech.2013.05.032.

[88] Y. X. An, M. H. Zong, H. Wu, and N. Li, "Pretreatment of lignocellulosic biomass with renewable cholinium ionic liquids: Biomass fractionation, enzymatic digestion and ionic liquid reuse," *Bioresour. Technol.*, vol. 192, pp. 165–171, 2015, doi: 10.1016/j.biortech.2015.05.064.

[89] I. P. Samayam and C. A. Schall, "Saccharification of ionic liquid pretreated biomass with commercial enzyme mixtures," *Bioresour. Technol.*, vol. 101, no. 10, pp. 3561–3566, 2010, doi: 10.1016/j.biortech.2009.12.066.

[90] J. A. Sirviö, M. Visanko, and H. Liimatainen, "Acidic Deep Eutectic Solvents As Hydrolytic Media for Cellulose Nanocrystal Production," *Biomacromolecules*, vol. 17, no. 9, pp. 3025–3032, 2016, doi: 10.1021/acs.biomac.6b00910.

[91] Y. Liu *et al.*, "Efficient Cleavage of Strong Hydrogen Bonds in Cotton by Deep Eutectic Solvents and Facile Fabrication of Cellulose Nanocrystals in High Yields," *ACS Sustain. Chem. Eng.*, vol. 5, no. 9, pp. 7623–7631, 2017, doi: 10.1021/acssuschemeng.7b00954.

[92] O. Laitinen, J. Ojala, J. A. Sirviö, and H. Liimatainen, "Sustainable stabilization of oil in water emulsions by cellulose nanocrystals synthesized from deep eutectic solvents," *Cellulose*, vol. 24, no. 4, pp. 1679–1689, 2017, doi: 10.1007/s10570-017-1226-9.

[93] R. Travaini, J. Martín-juárez, A. Lorenzo-hernando, and S. Bolado-rodríguez, "Bioresource Technology Ozonolysis : An advantageous pretreatment for lignocellulosic biomass revisited," *Bioresour. Technol.*, vol. 199, pp. 2–12, 2016, doi:

10.1016/j.biortech.2015.08.143.

[94] B. M. Cherian, A. L. Leão, S. F. de Souza, S. Thomas, L. A. Pothan, and M. Kottaisamy, "Isolation of nanocellulose from pineapple leaf fibres by steam explosion," *Carbohydr. Polym.*, vol. 81, no. 3, pp. 720–725, 2010, doi: 10.1016/j.carbpol.2010.03.046.

[95] M. J. Negro, P. Manzanares, J. M. Oliva, I. Ballesteros, and M. Ballesteros, "Changes in various physical/chemical parameters of Pinus pinaster wood after steam explosion pretreatment," *Biomass and Bioenergy*, vol. 25, no. 3, pp. 301–308, 2003, doi: 10.1016/S0961-9534(03)00017-5.

[96] T. Pielhop, J. Amgarten, P. R. Von Rohr, and M. H. Studer, "Steam explosion pretreatment of softwood: The effect of the explosive decompression on enzymatic digestibility," *Biotechnol. Biofuels*, vol. 9, no. 1, pp. 1–13, 2016, doi: 10.1186/s13068-016-0567-1.

[97] Z. Li, B. Fei, and Z. Jiang, "Effect of steam explosion pretreatment on bamboo for enzymatic hydrolysis and ethanol fermentation," *BioResources*, vol. 10, no. 1, pp. 1037–1047, 2015, doi: 10.15376/biores.10.1.1037-1047.

[98] Y. Xing, H. Yu, L. Zhu, and J. Jiang, "Efficient enzymatic hydrolysis of bamboo by pretreatment with steam explosion and alkaline peroxide," *BioResources*, vol. 8, no. 4, pp. 5392–5408, 2013, doi: 10.15376/biores.8.4.5392-5408.

[99] B. Bals, C. Rogers, M. Jin, V. Balan, and B. Dale, "Evaluation of ammonia fibre expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations," *Biotechnol. Biofuels*, vol. 3, pp. 1–11, 2010, doi: 10.1186/1754-6834-3-1.

[100] C. Krishnan, L. da Costa Sousa, M. Jin, L. Chang, B. E. Dale, and V. Balan, "Alkali-based AFEX pretreatment for the conversion of sugarcane bagasse and cane leaf residues to ethanol," *Biotechnol. Bioeng.*, vol. 107, no. 3, pp. 441–450, 2010, doi: 10.1002/bit.22824.

[101] Q. Shao, C. Cheng, R. G. Ong, L. Zhu, and C. Zhao, "Hydrogen peroxide presoaking of bamboo prior to AFEX pretreatment and impact on enzymatic conversion to fermentable sugars," *Bioresour. Technol.*, vol. 142, pp. 26–31, 2013, doi: 10.1016/j.biortech.2013.05.011.

[102] Y. Zheng, H. M. Lin, and G. T. Tsao, "Pretreatment for cellulose hydrolysis by carbon dioxide explosion," *Biotechnol. Prog.*, vol. 14, no. 6, pp. 890–896, 1998, doi: 10.1021/bp980087g.

[103] D. T. Phan and C. S. Tan, "Innovative pretreatment of sugarcane bagasse using supercritical CO2 followed by alkaline hydrogen peroxide," *Bioresour. Technol.*, vol. 167, pp. 192–197, 2014, doi: 10.1016/j.biortech.2014.06.006.

[104] M. Neus Angles and A. Dufresne, "Plasticized starch/tuniein whiskers nanocomposites. 1. Structural analysis," *Macromolecules*, vol. 33, no. 22, pp. 8344–8353, 2000, doi: 10.1021/ma0008701.

[105] M. H. and K. Y. Kazuhide Okuda, Kazuyuki Oka, Ayumu Onda, Koji Kajiyoshi, "Hydrothermal fractional pretreatment of sea algae and its enhanced enzymatic hydrolysis," *J. Chem. Technol. Biotechnol.*, vol. 83, pp. 836–841, 2008, doi: 10.1002/jctb.

[106] R. Jonas and L. F. Farah, "Production and application of microbial cellulose," *Polym. Degrad. Stab.*, vol. 59, no. 1–3, pp. 101–106, 1998, doi: 10.1016/S0141-3910(97)00197-3.

[107] C. Sánchez, "Lignocellulosic residues: Biodegradation and bioconversion by fungi,"

Biotechnol. Adv., vol. 27, no. 2, pp. 185–194, 2009, doi: 10.1016/j.biotechadv.2008.11.001.

[108] M. J. Taherzadeh and K. Karimi, "Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review," *Int. J. Mol. Sci.*, vol. 9, pp. 1621–1651, 2008, doi: 10.3390/ijms9091621.

[109] C. E. Wyman, B. E. Dale, R. T. Elander, M. Holtzapple, M. R. Ladisch, and Y. Y. Lee, "Coordinated development of leading biomass pretreatment technologies," *Bioresour. Technol.*, vol. 96, pp. 1959–1966, 2005, doi: 10.1016/j.biortech.2005.01.010.

[110] W. L. Lim, A. A. N. Gunny, and F. H. Kasim, "Overview of cellulose nanocrystals: Extraction, physicochemical properties and applications," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 670, p. 012058, 2019, doi: 10.1088/1757-899X/670/1/012058.

[111] N. Lelekakis, J. Wijaya, D. Martin, and D. Susa, "The effect of acid accumulation in power-transformer oil on the aging rate of paper insulation," *IEEE Electr. Insul. Mag.*, vol. 30, no. 3, pp. 19–26, 2014, doi: 10.1109/MEI.2014.6804738.

[112] S. Pirani and R. Hashaikeh, "Nanocrystalline cellulose extraction process and utilization of the byproduct for biofuels production," *Carbohydr. Polym.*, vol. 93, no. 1, pp. 357–363, 2013, doi: 10.1016/j.carbpol.2012.06.063.

[113] F. I. Ditzel, E. Prestes, B. M. Carvalho, I. M. Demiate, and L. A. Pinheiro, "Nanocrystalline cellulose extracted from pine wood and corncob," *Carbohydr. Polym.*, vol. 157, pp. 1577–1585, 2017, doi: 10.1016/j.carbpol.2016.11.036.

[114] B. S. L. Brito, F. V. Pereira, J. L. Putaux, and B. Jean, "Preparation, morphology and structure of cellulose nanocrystals from bamboo fibers," *Cellulose*, vol. 19, no. 5, pp. 1527–1536, 2012, doi: 10.1007/s10570-012-9738-9.

[115] T. Abitbol, E. Kloser, and D. G. Gray, "Estimation of the surface sulfur content of cellulose nanocrystals prepared by sulfuric acid hydrolysis," *Cellulose*, vol. 20, no. 2, pp. 785–794, 2013, doi: 10.1007/s10570-013-9871-0.

[116] M. A. Henrique *et al.*, "Kinetic study of the thermal decomposition of cellulose nanocrystals with different polymorphs, cellulose I and II, extracted from different sources and using different types of acids," *Ind. Crops Prod.*, vol. 76, pp. 128–140, 2015, doi: 10.1016/j.indcrop.2015.06.048.

[117] E. de Morais Teixeira, A. C. Corrêa, A. Manzoli, F. de Lima Leite, C. de Ribeiro Oliveira, and L. H. C. Mattoso, "Cellulose nanofibers from white and naturally colored cotton fibers," *Cellulose*, vol. 17, no. 3, pp. 595–606, 2010, doi: 10.1007/s10570-010-9403-0.

[118] O. A. Battista, S. Coppick, J. A. Howsmon, F. F. Morehead, and W. A. Sisson, "Level-Off Degree of Polymerization," *Ind. Eng. Chem.*, vol. 48, no. 2, pp. 333–335, 1956, doi: 10.1021/ie50554a046.

[119] K. Rajamani, S. C. Shenoy, M. S. Rao, and M. G. Rao, "Kinetics of the Hydrolysis of Ethyl Acetate Catalysed By Cation-Exchange Resin.," *J Appl Chem Biotechnol*, vol. 28, no. 11, pp. 699–708, 1978, doi: 10.1002/jctb.5700281102.

[120] L. rong Tang, B. Huang, W. Ou, X. rong Chen, and Y. dan Chen, "Manufacture of cellulose nanocrystals by cation exchange resin-catalyzed hydrolysis of cellulose," *Bioresour. Technol.*, vol. 102, no. 23, pp. 10973–10977, 2011, doi: 10.1016/j.biortech.2011.09.070.

[121] H. Abushammala, I. Krossing, and M. P. Laborie, "Ionic liquid-mediated technology to produce cellulose nanocrystals directly from wood," *Carbohydr. Polym.*, vol. 134, pp.

609–616, 2015, doi: 10.1016/j.carbpol.2015.07.079.

[122] Y. T. Xiao, W. L. Chin, and S. B. Abd Hamid, "Facile Preparation of Highly Crystalline Nanocellulose by Using Ionic Liquid," *Adv. Mater. Res.*, vol. 1087, pp. 106–110, 2015, doi: 10.4028/www.scientific.net/amr.1087.106.

[123] C. Li and Z. K. Zhao, "Efficient acid-catalyzed hydrolysis of cellulose in ionic liquid," *Adv. Synth. Catal.*, vol. 349, no. 11–12, pp. 1847–1850, 2007, doi: 10.1002/adsc.200700259.

[124] X. Y. Tan, S. B. Abd Hamid, and C. W. Lai, "Preparation of high crystallinity cellulose nanocrystals (CNCs) by ionic liquid solvolysis," *Biomass and Bioenergy*, vol. 81, pp. 584–591, 2015, doi: 10.1016/j.biombioe.2015.08.016.

[125] P. C. S. F. Tischer, M. R. Sierakowski, H. Westfahl, and C. A. Tischer, "Nanostructural reorganization of bacterial cellulose by ultrasonic treatment," *Biomacromolecules*, vol. 11, no. 5, pp. 1217–1224, 2010, doi: 10.1021/bm901383a.

[126] Y. Tang, S. Yang, N. Zhang, and J. Zhang, "Preparation and characterization of nanocrystalline cellulose via low-intensity ultrasonic-assisted sulfuric acid hydrolysis," *Cellulose*, vol. 21, no. 1, pp. 335–346, 2014, doi: 10.1007/s10570-013-0158-2.

[127] P. B. Filson and B. E. Dawson-Andoh, "Sono-chemical preparation of cellulose nanocrystals from lignocellulose derived materials," *Bioresour. Technol.*, vol. 100, no. 7, pp. 2259–2264, 2009, doi: 10.1016/j.biortech.2008.09.062.

[128] T. I. Shaheen and H. E. Emam, "Sono-chemical synthesis of cellulose nanocrystals from wood sawdust using Acid hydrolysis," *Int. J. Biol. Macromol.*, vol. 107, pp. 1599–1606, 2018, doi: 10.1016/j.ijbiomac.2017.10.028.

[129] A. Kumar, Y. S. Negi, V. Choudhary, and N. K. Bhardwaj, "Characterization of Cellulose Nanocrystals Produced by Acid-Hydrolysis from Sugarcane Bagasse as Agro-Waste," *J. Mater. Phys. Chem.*, vol. 2, no. 1, pp. 1–8, 2014, doi: 10.12691/jmpc-2-1-1.

[130] Z. Man, N. Muhammad, A. Sarwono, M. A. Bustam, M. V. Kumar, and S. Rafiq, "Preparation of Cellulose Nanocrystals Using an Ionic Liquid," *J. Polym. Environ.*, vol. 19, no. 3, pp. 726–731, 2011, doi: 10.1007/s10924-011-0323-3.

[131] Q. Yang, X. Pan, F. Huang, and K. Li, "Synthesis and characterization of cellulose fibers grafted with hyperbranched poly(3-methyl-3-oxetanemethanol)," *Cellulose*, vol. 18, no. 6, pp. 1611–1621, 2011, doi: 10.1007/s10570-011-9587-y.

[132] M. S. Jahan, A. Saeed, Z. He, and Y. Ni, "Jute as raw material for the preparation of microcrystalline cellulose," *Cellulose*, vol. 18, no. 2, pp. 451–459, 2011, doi: 10.1007/s10570-010-9481-z.

[133] R. Cha, Z. He, and Y. Ni, "Preparation and characterization of thermal/pH-sensitive hydrogel from carboxylated nanocrystalline cellulose," *Carbohydr. Polym.*, vol. 88, no. 2, pp. 713–718, 2012, doi: 10.1016/j.carbpol.2012.01.026.

[134] I. Spiridon, C.-A. Teacă, and R. Bodîrlău, "Structural changes evidenced by FTIP spectroscopy in cellulosic materials after pre-tratment with ionic liquid and enzymatic hydrolysis.," *BioResources*, vol. 6, no. 1, pp. 400–413, 2011.

[135] N. Terinte, R. Ibbett, and K. C. Schuster, "Overview on Native Cellulose and Microcrystalline Cellulose I Structure Studied By X-Ray Diffraction (Waxd): Comparison Between Measurement Techniques," *Lenzinger Berichte*, vol. 89, pp. 118–131, 2011, doi: 10.1163/156856198X00740.

[136] J. P. Patel and P. H. Parsania, *Characterization, testing, and reinforcing materials of biodegradable composites*. Rajkot: Elsevier Ltd, 2017.

[137] G. H. D. Tonoli *et al.*, “Cellulose micro/nanofibres from Eucalyptus kraft pulp: Preparation and properties,” *Carbohydr. Polym.*, vol. 89, no. 1, pp. 80–88, 2012, doi: 10.1016/j.carbpol.2012.02.052.

[138] H. Yu, Z. Qin, B. Liang, N. Liu, Z. Zhou, and L. Chen, “Facile extraction of thermally stable cellulose nanocrystals with a high yield of 93% through hydrochloric acid hydrolysis under hydrothermal conditions,” *J. Mater. Chem. A*, vol. 1, no. 12, pp. 3938–3944, 2013, doi: 10.1039/c3ta01150j.

10. ACRONYMS

Acronyms	Meaning
AFEX	Ammonia Fibre/Freeze Explosion
AFM	Atomic Force Microscopy
CMC	Carboxymethylcellulose
CNs or CNC	Cellulose nanocrystals
DES	Deep eutectic solvent
DOE	Design of experiment
DP	Degree of polymerization
ρ	Density
E_A	Elastic modulus in axial direction
E_T	Elastic modulus in transverse direction
FE-SEM	Field Emission Scanning Electron Microscopy
IR	Infrared spectroscopy
IL	Ionic liquids
LODP	Level-off degree of polymerization
MFC	Microfibrillated cellulose
NCC	Nanocrystalline cellulose
NFC	Nanofibrillated cellulose
SEM	Scanning Electron Microscopy
SP	Steam explosión
σ_f	Tensile strength
TEM	Transmission Electron Microscopi
XRD	X-ray diffraction

APPENDICES