

Dr. Mònica Martínez López  
*Departament de Ciència de Materials i  
Química Física*

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



# Treball Final de Grau

**Bibliographic research for the extraction of lignin from Bamboo by-products.**

Yana Tronina

*June 2020*



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



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



*“Nothing in life is to be feared, it is only to be understood. Now is the time to understand more, so that we may fear less”*

Marie Curie

I would like to show mi best gratitude to my tutors for supporting me though the development of this project with their time and knowledge.



**REPORT**





# CONTENTS

<b>1. SUMMARY</b>	3
<b>2. RESUM</b>	5
<b>3. INTRODUCTION</b>	6
3.1. WHAT IS AN ADHESIVE?	6
3.1.1. Classification by Origin	8
3.1.1.1. Natural Adhesives	8
3.1.1.2. Synthetic adhesives	9
3.1.2. Classification by Chemical Bonding	9
3.2. BAMBOO	11
3.2.1. Morphology	11
3.2.2. Growth, Harvesting and Post-harvesting Treatments	12
3.2.3. Chemical Composition	14
3.2.4. Applications of bamboo	18
3.2.5. Bamboo in Ecuador	19
<b>4. OBJECTIVES</b>	20
<b>5. METHODS</b>	21
<b>6. RESULTS AND DISCUSSION</b>	24
6.1. EXTRACTION METHODS	25
6.1.1. Industrial Extraction methods	26
6.1.1.1. Comparison of industrial Methods	28
6.1.2. Laboratory Scale Methods	30
6.1.2.1. Comparison of laboratory scale methods	33
6.1.3. Post-treatment Lignin Purification	33
6.1.4. Bamboo Pulping and Fiber Residue	35
6.2. CHEMICAL CHARACTERIZATION	35
6.2.1. Fourier Transform Infrared (FT-IR)	35

---

6.2.2. Ultraviolet-Visible (UV) Absorption	37
6.2.3. Nuclear Magnetic Resonance (NMR)	38
6.2.4. Gel Permeation Chromatography (GPC)	41
6.2.5. Thermal Analysis	41
6.3. ADHESIVE TESTING	42
6.3.1. Standard Test Methods	42
6.3.2. Non-Standard Test Methods	44
<b>7. EXPERIMENTAL PROPOSAL</b>	<b>45</b>
7.1. BAMBOO PRE-TREATMENT	45
7.2. EXTRACTION METHOD	46
7.3. CHEMICAL CHARACTERIZATION	47
7.4. EVALUATION OF ADHESIVE PROPERTIES	48
<b>8. CONCLUSIONS</b>	<b>51</b>
<b>9. REFERENCES AND NOTES</b>	<b>53</b>
<b>10. ACRONYMS</b>	<b>60</b>

# 1. SUMMARY

Bamboo is a renewable raw material used in many applications from building materials to value-added chemicals. It is chemically rich in lignocellulosic biomass, principally lignin, which is a natural glue in cell walls. This compound makes bamboo by-products, generated from its processing, to be used as potential raw material for developing lignin-based adhesives. Even though, there are no described industrial extraction processes of lignin from bamboo, it has been reported the use of several different methods on a laboratory scale research which showed great results. In addition, lignin is an amorphous natural polymer structurally dependant on the extraction method, thus, it must be characterized to evaluate its subsequent use by different characterization techniques (e.g. FT-IR, NMR, UV, GPC, TGA, DSC). Also, adhesive testing is necessary for evaluating adhesion properties onto different materials.

**Keywords:** Bamboo, renewable, lignocellulosic biomass, lignin, extraction methods, lignin-based adhesives, FT-IR, NMR, UV, GPC



## 2. RESUM

El bambú és un material renovable que s'utilitza en múltiples aplicacions, des de materials de construcció fins a productes químics. És un material ric en biomassa lignocel·lulòsica, principalment lignina, la qual actua com un adhesiu natural. Aquest compost fa que els subproductes de bambú, generats en el seu processament, puguin ser utilitzats com matèria primera per el desenvolupament d'adhesius basats en lignina. Tot i que encara no s'han descrit processos industrials d'extracció de la lignina, en bambú, sí s'han provat a escala laboratori diferents mètodes els quals han donat bons resultats. Per altre banda, la lignina és un polímer amorf amb una estructura dependent del mètode d'extracció, i per tant, s'ha de caracteritzar per poder avaluar la seva aplicabilitat per diferents tècniques de caracterització (per exemple, FT-IR, NMR, UV-GPC, TGA, DSC). Així mateix, són necessàries proves que avaluin les seves propietats adhesives en diferents materials.

**Paraules clau:** Bambú, renovable, biomassa lignocel·lulòsica, lignina, mètodes d'extracció, adhesius basats en lignina, FT-IR, NMR, UV, GPC

### 3. INTRODUCTION

The use of natural adhesives is not a distant fact but closely to the humankind history. Indeed, Egyptians used crude animal and casein glues to laminate wood for bows and furniture [1]. As science and technology were developing through history, in the middle of 20<sup>th</sup> century, it appeared the first partially synthetic adhesive, nitrocellulose [2]. Since then, there has been a huge development on synthetic adhesives within a wide range application (e.g. from simple stuff, as bottle labelling or wood panelling). Indeed, their main advantages are that they can be used for joining any kind of materials (e.g. paper, ceramics, metals, etc.) and also, they are really light weighted.

Even though synthetic adhesives show high performance properties, most of them are petroleum-based polymers which cause a high environmental impact [3]. So, nowadays there is an urgent need on replacing petroleum by renewable raw materials [4][5], for instance Bamboo.

This project is going to take a close look on how to get natural adhesives from renewable raw materials, in particular, lignin (natural glue) from bamboo by-products. Several extraction methods (industrially and laboratory scale) for bamboo will be discussed from the chemical and environmental point of view.

As well as a chemical and thermal characterization on the extracted lignin, as it is an amorphous polymer whose structure depends on the extraction method.

In addition, adhesive testing will be discussed to evaluate the adhesion properties (e.g. viscosity, peel strength) of lignin.

#### 3.1. WHAT IS AN ADHESIVE?

An adhesive may be defined as any substance, natural or synthetic, capable of holding materials together and resist separation [6]. Besides, the materials joined together are named adherends or substrates.

As there is not a universal theory of adhesion which can explain exhaustively how an adhesive works, there are several theories which, together, can give a great approach on the adhesive mechanism. They are briefly described below based on [7][8][9][10]:

- **Mechanical.** Adhesion takes place by penetrating the adhesive inside pores, cavities and other surface irregularities of the adherend by a process called “mechanical interlocking”. It is useful for porous materials as wood, textile and paper. It can be increased by large superficial contact areas and clean surfaces.
- **Electrostatic.** It states that the adhesive forces are created in form of an electrical double layer formed between the adhesive-adherend interface. Even though, in many cases, the electrostatic forces are not as relevant as van der Waals forces, they play an important role in biological functions. Also, it is useful for explaining polymer-metal adhesion.
- **Diffusion.** The adhesion is developed by the interdiffusion of molecules between adhesive and adherend molecules. It is applicable when the two materials are polymeric, soluble and mobile.
- **Wetting (or Adsorption).** Adhesion takes place as a result from molecular contact between two materials and the surface forces that develop between them.
- **Chemical Bonding.** An adhesion bond is formed by intermolecular van der Waals forces, covalent or ionic bonding between the adhesive and adherend.
- **Weal-Boundary Layer.** Explains the absence of adhesion in the interface adhesive-adherend, due to excessive superficial treatment or the presence of dirtiness.

In addition to that theories, it important to understand what is happening between the adhesive-adherent atoms and which forces bind them together. The main forces can be sorted in cohesive forces (i.e. which hold materials together) and adhesive forces (i.e. which hold adjacent molecules of a single material together) [7].

Talking about adhesive properties and what makes them sticky is an intriguing and, also extended subject. So, if anyone wants to know more about it, “*Handbook of Adhesives and Sealants*” by Edward M. Petrie is a great option [11].

The following sections are focused on the classification and description of the main characteristics one can find about adhesives. Even though, there are several ways to sort them out [12], this project focuses its classification by origin and chemical bonding.

### 3.1.1. Classification by Origin

Adhesives may be classified based on their origin as natural or synthetic.

#### 3.1.1.1. *Natural Adhesives*

Often referred as bio-adhesives, they are made from natural sources such as vegetable (e.g. wood, plants) or animal (e.g. casein from cow milk) [11]. They act as natural polymers consisting of proteins, lignin, polysaccharides and natural rubber. In general, they are inexpensive and environmentally friendly, as they came from renewable materials. Although, they have low strength properties, poor water-heat resistance and limited freedom in processing [13], thus, they need additives to improve their properties. For instance, xylan, a polysaccharide, with low bonding performance, shows improvements with the addition of dispersants such as polyvinyl alcohol [14].

General aspects and some examples of naturally made adhesives are discussed below:

- **Polysaccharide-based adhesives.** Carbohydrates are considered to have adhesive properties because they are usually high weighted macromolecules with polar functional groups that allow the formation of bonds between adhesive and adherent molecules or atoms [15]. The most common polysaccharide-based adhesives are starch [16], chitosan [17], dextrin [18] and Arabic gum [19].
- **Protein-based adhesives.** Maybe, they could be the most ancient described bio-based adhesives. They are widely reported for soybean [20], blood and casein [21]. Indeed, there are multiple reviews about soybean-based adhesives, even combined with lignin biomass, with the objective to improve their water resistance [22].
- **Natural rubber-based adhesives.** It is classified into latex adhesives and solution adhesives [23]. Principally, they consist of isoprene monomer units which are sensible to heat and oxidation, due to the presence of double bonds. Latex adhesives are used for textile, leather and paper, but the solution adhesive types are used for pressure-sensitive adhesive tapes. In addition, bio-based latex adhesives combined with starch nanoparticles, are a possible substitute for replacing petroleum based monomers on reference [24].
- **Lignin-based adhesives.** Lignin is an amorphous three-dimensional natural phenolic polymer which acts as kind of a glue between hemicelluloses and cellulose fibres in cell walls [25]. Its phenolic structure makes it a potential substitute of phenol in phenol-formaldehyde (PF) resins, typically used for wood adhesive [26].



From all natural-based adhesive described above, this project is focused on lignin extracted from bamboo. The reasons are that, in one hand, lignin-based adhesives have been reported in several reviews [25][27][28] and, on the other, biomass (e.g. Bamboo, wood) contains about 15-30% on lignin content. In general terms, lignin is a potential precursor for the development of bio-based adhesives.

### 3.1.1.2. Synthetic Adhesives

Synthetic adhesives came in hand with the plastic industry, thus, most of them are petroleum-based. They are considered to have a better bonding performance and water-heat resistance than the natural ones [29], but due to their negative impact on the environment, as they contribute to greenhouse gas emissions (e.g. carbon dioxide), there is an actual need for developing bio-based and eco-friendly materials [30].

On the following section, there are exposed some examples of synthetic and natural adhesives based on their chemical bonding performance as thermoplastic, thermosetting, elastomeric or hybrid adhesives.

### 3.1.2. Classification by Chemical Bonding

Even though, classification of adhesives as natural or synthetic is intriguing, it is not accurate enough. For instance, sorting by chemical bonding performance can give a great information about properties and their further application as adhesives.

Hence, adhesives can be classified into thermosetting, thermoplastic, elastomeric and hybrid adhesives [12] whose properties are briefly described below. In addition, some examples are described on Table 1 by chemical bonding and origin.

- **Thermosetting.** Set of polymers which cross-link together by an irreversible chemical reaction [31]. These materials cannot be heated and softened back, instead they will deteriorate. Even so, it is an advantage for structural purpose adhesives.
- **Thermoplastic.** The chains are joined by intermolecular forces or Van der Waals forces, forming linear or branched structures with high molecular weighted structures. Besides, they are rigid below glass transition temperature ( $T_g$ ) but get softer above this. The cycle of cooling and heating back is reversible but limited. Each time the structure is heated and cooled after, it changes, thus, its physicochemical properties too [32]. They can be

dissolved in solvents as water, latex and emulsions, making them more flowable for their application.

- **Elastomeric.** They can be either thermosetting or thermoplastic adhesives, formulated for a wide range of applications because of their great toughness and elastic elongation. Commonly, they are used as sealants because of their high degree of flexibility and superior peel strength [12]. In addition, they may be divided on soft structural, hot-melts and pressure-sensitive adhesives [33].
- **Hybrid.** As the name indicates, they are a combination of thermosetting, thermoplastic or elastomeric adhesives in one single formulation. They are emphasized on the most useful properties of each type [12]. For instance, high temperature resistance resins are combined with flexible and tough elastomers.

Table 1. Some examples of natural and synthetic adhesives according to their chemical properties [12][34].

Type	Origin		Application
	Natural	Synthetic	
Thermosetting	Animal glues Soybean Vegetable proteins Casein	Epoxy Phenolic Polyester Polyaromatic	Structural purposes.
Thermoplastic	Rosin Shellac Oleo-resin Mineral waxes	Polyamide Vinyl polymers Acrylic polymers Cellulose derivatives	Non-metallic materials (e.g. wood, leather, paper). Not useful for structural purposes.
Elastomeric	Natural rubber	Nitrile rubber Polyurethane Silicone Neoprene	Rubber, fabric, foil, paper, leather, plastics, films, tapes.
Hybrid	Soybean-lignin based	Epoxy-Phenolic Epoxy-Sulfide Epoxy-Nylon Nitrile-Phenolic	Metals, ceramics, glass.

## 3.2. BAMBOO

Bamboo belongs to the subfamily Bambusoideae of the grass family Poaceae [35]. There are over 1,400 species described, classified in approximately 119 genera and grouped in three tribes: Arundinarieae (temperate woody bamboos), Bambuseae (tropical woody bamboos) and Olyreae (herbaceous bamboos) [36]. In general, bamboo is considered to be a forest grass which mainly grows in the tropical and subtropical belt, where the major sources are located in Asia, South America and Africa. Besides, China and India have the largest bamboo forests with 7 and 9 million hectares, respectively.

Not only it is considered to be the fastest-growing and most versatile plant on Earth but also to have a really high social, economic and environmental benefit [37]. Being socio-economic benefits such as employment, infrastructures and a livelihood for rural communities. Moreover, bamboo plantations show a reduction on the environmental effects of climate change. For instance, in terms of sequestering carbon dioxide, they show to be more efficient than other crops [38].

It is used for construction, paper, food and, it is a lignocellulosic material, being a potential raw material for lignin-based adhesives [39].

To get a better approach about what is bamboo and its properties, this section is going to describe its general aspects such as morphology, growth, chemical composition and applications.

In addition, *“Bamboo. The multipurpose plant”* [40] and *“Bamboo. The plant and its uses”* [35] are two great books to get a deep knowledge on bamboo.

### 3.2.1. Morphology

There are many species of bamboo, but morphologically, meaning its outward appearance, they all have in common a culm, rhizome, roots, sheaths, leaves and branches [41][42][43]. These main characteristic parts are described below. In addition, schematic morphological parts of bamboo are shown on Figure 1.

- **Culm.** It can be seen as the stem of the bamboo. It has a cylindrical appearance with intermediate joints called nodes. Nodes are the ones responsible for water and nutrient storage. Also, they give strength to the plant. The in-between nodes are called the internodes. Culms have different dimensions, colours and smells depending on the species.

Interesting fact, bamboo species have the world record on being the fastest growing plants on earth. Indeed, culms may grow up, in just one day, 91 cm [44].

- **Rhizomes.** It is the subterranean organ responsible of the structure, nutrient storage, stability and territory colonization. They produce the roots.
- **Roots.** Located at the rhizome, they anchor the bamboo to the ground. Also, roots do some nutrient storage and transportation.
- **Sheaths.** They are considered to be like a modified leaf. There are three types, culm, rhizome and leaf sheath.
- **Leaves and branches.** They grow from the node. Indeed, branches are also segmented with nodes and internodes. Leaves are thinner than sheaths.

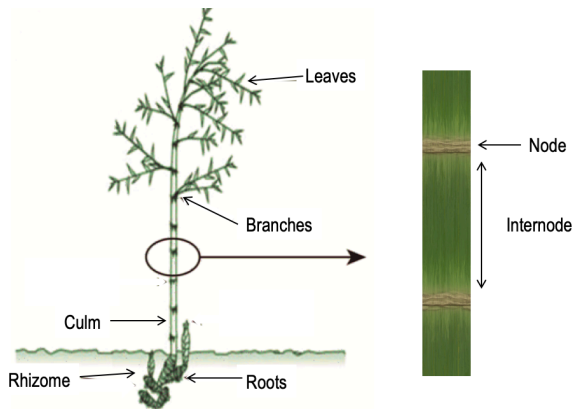


Figure 1. Bamboo morphology adapted from [43].

### 3.2.2. Growth, Harvesting and Post-harvesting Treatments

Even though it has a unique self-growing mechanism and, one can get lost on details, these sections are going to provide a general look of bamboo growth, harvesting and post-harvesting treatments.

#### Growth and Harvesting:

Bamboo is a self-regenerating plant that can be propagated by seedling, culm cuttings and rhizomes [45]. Not only the method of propagation affects its growth but also, it depends on the specie and environment. For example, bamboo does not well on alkaline soils.

Culm aging takes an important role on further properties and uses [46]. Hence, culm growth and elongation take place after rainy seasons and gathered in the internodes. At the beginning, the elongation is slow, but after the first two weeks, it gradually speeds up until its optimum size.

Different culm ages are useful for different applications in the nowadays industries. For instance, mature culms, the ones about 3-5 years old, are the most useful for construction, as the sugar content is low and they have a better defence from insects attacks [47]. On the contrary, culms between 1 and 2-year-old are useless for construction due to their high-water content. Besides, older culms, more than 5-year-old, tend to be harder and its subsequent treatment is more difficult.

In addition, some of the largest growing species are *Phyllostachys pubescens* (also named, Moso Bamboo), *Guadua Angustifolia* and *Dendrocalamus Asper* which may take up to 25 meters long and 22 cm of diameter [44].

### **Post-harvesting Treatments:**

After harvesting, bamboo is exposed to pests, fungi and insect attacks, so it needs treatment and protection from deterioration, even more, compared to wood [48].

Many treatment methods are described, which are mainly based on the posterior application and industrial purpose. The actual methods to prevent bamboo raw materials from deterioration are chemical (i.e. preservatives) and non-chemical methods (i.e. waterlogging) [49]. Non-chemical methods have been applied in rural areas since ages, considering that they are a low-costed treatment because there is no need for technical equipment. Although chemical methods are great for expanding the durability of culms, they are more expensive.

Also, some preservatives may be applied against bacteria. These are chosen by the type of material and application and usually used for the enhancement of bamboo properties and protection.

Furthermore, the most critical parameter is the moisture content, and it cannot go over 12% [48]. For achieving this water content, air-drying and kiln-drying are the best methods. On one hand, air drying is cheap, but culms are more likely to be attacked and infected by fungi. On the other hand, kiln-drying is more expensive but also, more efficient than air-drying. Besides, it is recommended for large-scale productions.

### 3.2.3. Chemical Composition

One of the most important aspects about bamboo for this project is that it is a lignocellulosic material [35][40]. In fact, its chemical composition is about 90% of lignin, hemicellulose and celluloses. The other 10% left corresponds to waxes, resins and inorganic salts.

General bamboo's chemical composition is described below, even though focused more on lignin, as it is a potential natural glue for the development of bio-based adhesives [25].

#### Cellulose:

Bamboo has a higher content (40-60%) of cellulose than hardwoods. Indeed, cellulose is the most abundant linear natural polymer formed by repeated dimer units of cellobiose, as it shows on Figure 2. Two  $\beta$ -D-glucopyranose units are linked by stable  $\beta$ -1,4-glycosidic bonds [50][51]. Van der Waals interactions and hydrogen bonds formation between oxygen and hydrogen atoms, inter- and intramolecular interaction, promote parallel stacking of multiple linear cellulose chains forming fibrils which aggregate forming fibres [52]. These fibres, from cellulose chain interactions, are responsible for high tensile strength and insolubility.

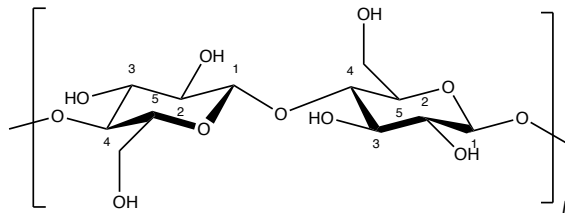


Figure 2. Cellulose structural unit. "n" number of cellobiose units.  
Structure adapted from [51].

Without getting into detail, there are four polymorphs of cellulose which are briefly described on Table 2.

Table 2. Brief description of cellulose polymorphs [50][52].

Cellulose	General description:
I	Naturally occurring cellulose, found in trees, plants, bacteria, etc.
II	It is the most thermodynamically stable type.
III	It can be obtained from cellulose I or II by amine or ammonia exposure.
IV	It is obtained after heating cellulose III with glycerol.

Since many centuries cellulose has been used as a construction material, in the paper and board industry, as well as versatile material for chemical modification.

### Hemicellulose:

Along with cellulose, hemicellulose is a branched hetero-polysaccharide with complex structure which may contain xylose, glucose, arabinose, galactose, mannose, fucose, glucuronic acid and galacturonic acid. Actually, hemicelluloses from bamboo contain about 90% of xylan (accounting for the 25% of cell wall material) which is a short linear polymer of  $\beta(1\rightarrow4)$ -D-xylopyranose linked to 4-O-methyl-D-glucuronic acid, L-arabinose and acetyl groups [35]), as shown on Figure 3.

Hemicelluloses can be extracted by water or alkali media from plant tissues [53] and can be characterized by Fourier Transform Infrared (FTIR) and Nuclear Magnetic Resonance (NMR) spectroscopy techniques.

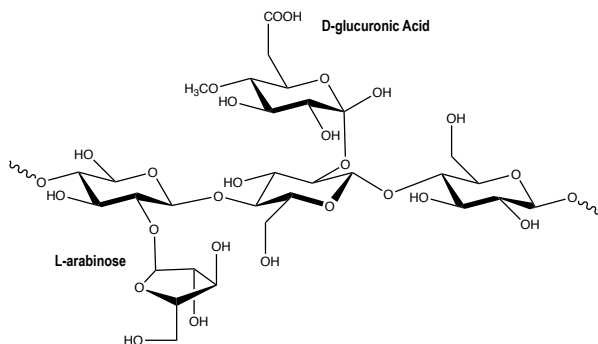


Figure 3. A portion of hemicellulose extraction showing D-glucuronic acid and L-arabinose. Adapted from [53].

### Lignin:

Lignin is one of the most abundant natural renewable polymers, being the second after cellulose [54]. In case of bamboo, specifically on culms, it ranges between 20-26% on lignin content [40]. It is the glue that binds cellulose and hemicellulose together giving strength to cell walls [55]. It forms an amorphous three-dimensional phenolic polymer formed by radical coupling polymerization of alcohol monomer precursors which are p-coumaryl (hydroxyphenyl, H-unit), coniferyl (guaiacyl, G-unit) and sinapyl (syringyl, S-unit) alcohol. The structures of these

precursors are shown in Figure 4. The amount of G, S, H units depends on plant growth, climate and nutrition [56]. Softwood, hardwood and herbaceous plants, are mainly made of G-units, G-H-units and G-H-S units, respectively. Actually, Bamboo belongs to the grass family and consists of the three unit monolignol types (G, H and S) [57].

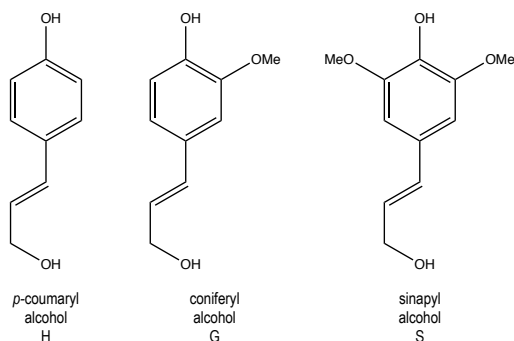


Figure 4. Alcohol monomer precursors for lignin polymerization.  
Adapted from [56].

The main elements of lignin structure are carbon, oxygen and hydrogen. Indeed, lignin has a higher carbon content than carbohydrates. The main functional groups are hydroxyl (aliphatic hydroxyl and phenolic) and carbonyl (aldehyde, ketone and carboxyl) groups. The hydroxyl groups have an important role for functional materials by lignin modifications. Carbonyl group can be conjugated (i.e.  $\alpha$ -carbon in ketones) and non-conjugated (i.e. aldehydes). The presence of phenolic hydroxyl makes it soluble in alkali solutions [54].

Lignin structure is built up with phenylpropane units connected by ether ( $\alpha$ -O-4, 5-O-4,  $\beta$ -O-4) and condensed linkages ( $\beta$ - $\beta$ , 5-5',  $\beta$ -5 and  $\beta$ -1). The most predominate one is the ether linkage  $\beta$ -O-4. An approximated structure is shown on Figure 5, in addition some linkages and alcohol monomers too. It needs to be pointed out that lignin is an amorphous polymer, and structurally dependant on the vegetable specie and chemical extraction method [56], further discussed on section 6.

In addition, for the amorphous lignin structure, potential structural analysis like FT-IR and NMR can give exhaustive linkage and bonding information.



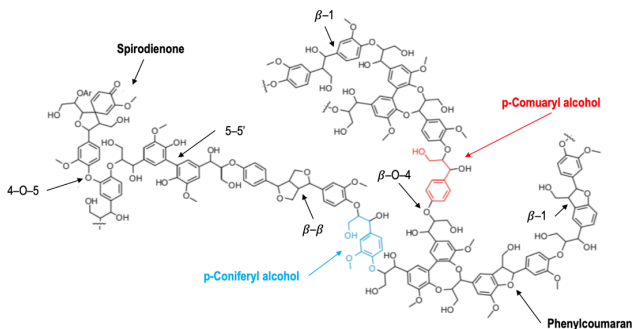


Figure 5. An approximated lignin structure showing the main linkages. Adapted from [107].

As the methods of extraction depend strongly on the structure, a wide range of physical properties can be described.

- For starters, the molecular weights are wide ranged depending on the degree of lignin association.
- The solubility depends on the type of lignin, some are soluble in water (i.e. lignosulfonates), other just in organic solvents.
- Lastly, lignin acts as a thermoplastic amorphous polymer and has a, glass transition temperature,  $T_g$ , that goes between 127-227 °C [56].

The latter physicochemical properties can be studied by Gel Permeation Chromatography (GPC) and Thermogravimetric Analysis (TGA), respectively. In addition, differential scanning calorimetric analysis (DSC) allows, for instance, to determine the  $T_g$  of lignins.

It is important to point out that lignin is a sustainable and renewable compound with reactive functional groups making it a potential chemical feedstock for biochemicals, for instance, bio-adhesives [25].

### Other components.

Apart from lignocellulosic biomass, about 10% of the overall culm mass corresponds to small quantities of soluble polysaccharides, proteins, resins, waxes, tannins and ash (1-5%) [35]. Ash is mainly composed of calcium, inorganic minerals, silica and potassium constituents [40].

### 3.2.4. Applications of Bamboo

Bamboo is a multipurpose plant and for many centuries it has been used in the construction industries [39][58]. Apart from construction purposes it is applied for biofuels, paper, textile, food and medicine.

As it is shown on Figure 6, there are multiple applications of bamboo, from which, the most common is the building industry. In fact, bamboo has great compression and bending behaviour and it is also lightweight material [58], so it can be used for walls, flooring, roofing, furniture and many other applications.

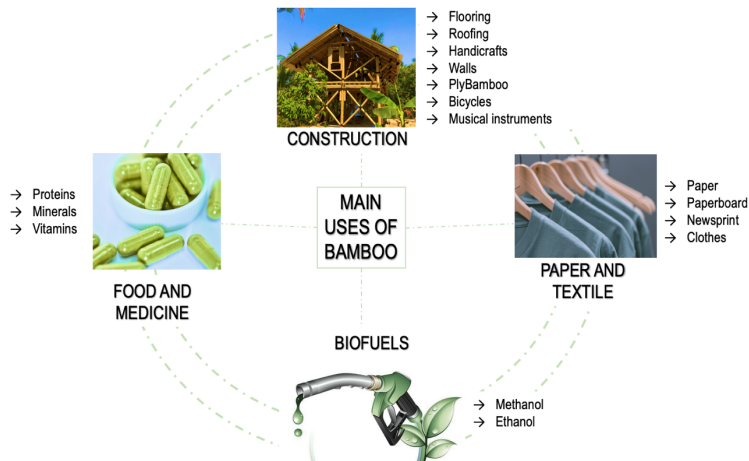


Figure 6. Main uses of bamboo. Adapted from [39].

The actual need for renewable resources to replace fossil fuel burning showed that bamboo is a potential feedstock for biofuel production, such as charcoal, pellets, ethanol and methane gas [59].

Furthermore, in China paper was handmade from bamboo from years ago [39]. Indeed, in the nowadays pulping industries bamboo showed a better resistance to bleaching than wood.

Finally, it is interesting to point out that most bamboo species are edible and contain vitamins, carbohydrates, proteins and minerals. Hence, it plays an important role on daily diets in Asian countries as Thailand and China [60].

### 3.2.5. Bamboo in Ecuador

This project has been carried out in collaboration with Bismark Osmany Torres Ruilova, professor in the University of Guayaquil in Ecuador, with the aim on researching and developing the use of bio-based adhesives from bamboo by-products, specifically from *Guadua Angustifolia* [35].

Indeed, *Guadua* is the most important specie of Ecuador because of its physical and mechanical properties. It is a fast-growing specie and has a life cycle of about 5 years. Physically, culms are large (they reach up to 30 m high and 20 cm of diameter). They tend to be dark green with white band at nodes. Also, it has an excellent use for construction, furniture, pulping and laminates [40].

The use of bamboo in Ecuador is reported from 10,000 years ago. Nowadays, there are about 600,000 hectares of bamboo plantations, representing the 2% of the whole country. It has a great economic and social impact on the country because it represents the 12% of employment that gives a job to more than 500,000 people [61].

Environmentally it has beneficial aspects such as CO<sub>2</sub> capturing, wild life preservation and regulation of hydraulic sources.

## 4. OBJECTIVES

1. Bibliographic research on adhesives from natural and synthetic sources. Emphasising on environmental impact and bio-based alternatives.
2. Bibliographic research on Bamboo. Information about its morphology, growth, special properties and further applications.
3. Take advantage of Bamboo as a raw material for the production of lignin-based adhesives.
4. Proposal of development and optimization of lignin extraction methods from Bamboo.
5. Proposal of development of chemical characterization methodology of bamboo lignin extracts.
6. Proposal development of adhesive properties of lignin extracts with standard and non-standard test methods.

## 5. METHODS

Bibliographic search on this project was subjected to an exhaustive research on Bamboo and bio-based adhesives from three main data bases including Science Direct®, Scifinder® and Google Scholar® available on the Learning and Research Resources Centre (CRAI) Universitat de Barcelona. The search criteria were based on keyword (e.g. lignin, adhesive, etc.), type of document (e.g. journals, article, etc.) and year of publishing, which may be consulted on Table 3 and Table 4. In addition, this bibliographic research was performed during the spring semester of 2020.

For the last 40 years it has been published more than 67,900 documents about bamboo. Besides, the exponential increase on these publications was not 20 years ago, until the highest peak at 2018, as shows Figure 7. If one takes a close look and compares the individual years with the total amount of documents, will see that around 10-13% of the documents correspond the years between 2017-2018. This shows that bamboo generated a great impact on the research fields of different industries, universities and so on.

### Bamboo Publications Evolution

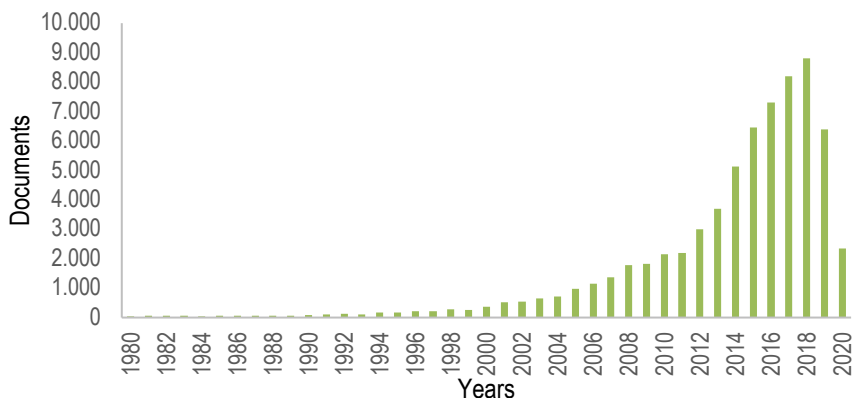


Figure 7. Published bamboo documents from 1980 to 2020. Adapted from Scifinder®.

According to Scifinder® the first two publications about bio-based adhesives took place in 1997 and, not since, the beginning of 2004 there was an increase of reported documents, as shows Figure 8. It is intriguing how increased the interest between 2014 and 2019, and still 2020 not ended yet seems that will be continuing to increase. The main reason, which could be responsible for this, is the social sensitivity about the environment and the development of materials from natural resources

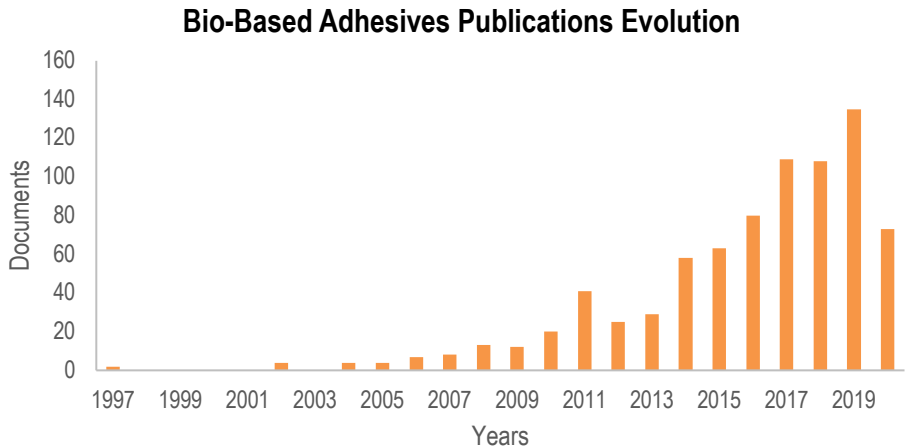


Figure 8. Published bio-based adhesive documents from 1997 to 2020. Adapted from Scifinder ®.

### Bamboo Bibliographic Research:

Bamboo is the renewable raw material of this project and several information is required for a better understanding of the principal aspects of this vegetative specie as morphology, growth, chemical properties and applications. The specifics of this research are described on Table 3.

Table 3. Bibliographic research specifics for Bamboo.

Key words for:		Types of documents	Years of publishing
Bamboo	Lignin		
Morphology, growth, harvesting, lignin, hemicellulose, properties, Ecuador, Applications, <i>Guadua Angustifolia</i>	Isolation, extraction, properties, lignocellulose, adhesives, characterization	Book, review, research article, encyclopedia, handbook	All years

**Bio-based Adhesive Bibliographic Research:**

Bibliographic research on bio-based adhesives was firstly performed on adhesive general information, but highlighting publications on natural adhesives, and more specifically on lignin-based adhesives. The specifics of this research are described on Table 4.

Table 4. Bibliographic research specifics for Bio-based adhesives.

<b>Key words for Adhesives</b>	<b>Types of documents</b>	<b>Years of publishing</b>
Definition, natural, synthetic, bio-based, standards tests, lignin-based	Book, review, research article, encyclopedia, handbook	All years

## 6. RESULTS AND DISCUSSION

Bamboo, as any other building raw material, generates wastes during manufacturing processes, for example, bamboo cut pieces, bamboo skin and semi grounded bamboo dust [62], as shows Figure 9.



Figure 9. Waste by-product from bamboo. Adapted from [62].

These wastes are in the spotlight of this project because, as previously mentioned, bamboo has a rich chemistry on lignocellulosic material, specifically on lignin, which was reported to be a potential source for the development of bio-based adhesives [25].

Even though, most of the commercially available lignin comes from the pulping and paper making industry of wood [63], there have been reported some extraction methods applied on bamboo too. This section is going to take a close look on general aspects of the most important pulping methods as Kraft, Sulfite, Organosolv and Soda pulping, and how bamboo responds to those at a laboratory scale. In addition, some other chemical and physicochemical extraction methods applied to bamboo will be briefly discussed.

In addition, the residual bamboo fiber left, after the chemical treatment, cannot be forgotten, so a brief description on possible application of these will be discussed.



After seeing the most characteristic aspects of different extraction methods, it is important to structurally characterize those extracted lignins due to the fact that lignin structure depends on the extraction method [56]. FTIR, UV and NMR spectroscopy will be further discussed, along with GPC and TGA.

Finally, standard and non-standard methods will be discussed for the proper evaluation of the adhesive properties of extracted lignins.

## 6.1. Extraction Methods

As previously mentioned, bamboo is a lignocellulosic material, meaning it contains lignin, cellulose and hemicellulose. Lignin is the glue that binds hemicelluloses and celluloses together [41], as shows Figure 10. Therefore, after pre-treating the sample (e.g. cutting, debarking) the first step on extracting any of the lignocellulosic materials must be the breakage of intermolecular linkages (e.g. by heating, pressure or chemical treatments), followed by a chemical recovery (e.g. acid precipitation for lignin recovery) [64].

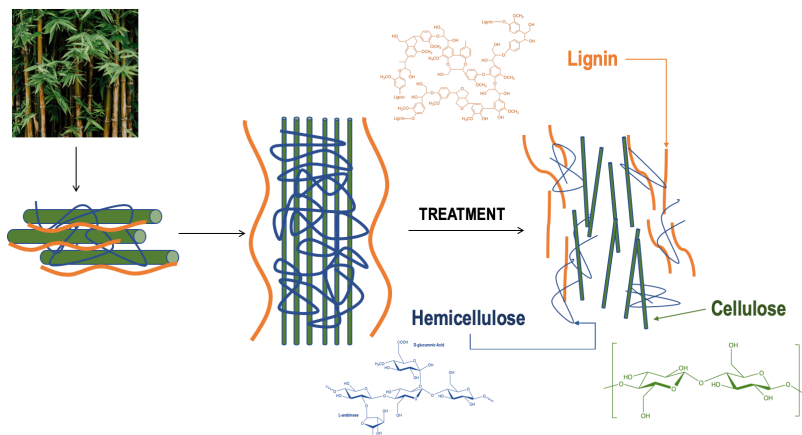


Figure 10. Schematic lignocellulosic material before and after treatment. Adapted from [64].

Before digging on extraction methods of lignin from bamboo, firstly, it will be discussed the industrial chemical methods applied in the pulping industry of wood. Hence, wood lignins are the main source of the commercially available lignin in the nowadays industry [25]. Furthermore, the methods applied to wood lignins also were applied, at a laboratory scale, on bamboo.

In addition to industrial methods, some physical and chemical pre-treatments, at a laboratory scale, applied to bamboo will be mentioned.

### 6.1.1. Industrial Extraction Methods

Currently, the major sources of commercially available lignins are from the pulping and paper making industry from wood [63]. The main objective of this processes is to obtain paper, but during processing there is a step, pulping, where lignin is extracted to avoid problems during bleaching. This residual liquor, with high lignin content, is usually used for power and heat generation of the pulping plant, and just a small amount goes to chemical applications.

Without getting into detail, these papermaking processes are briefly described below, based on reference [65].

- **Raw material preparation.** It is based on debarking and chipping wood to reduce the material sizes.
- **Pulping.** Lignin removal step by chemical methods (e.g. Kraft pulping) or mechanical methods (e.g. hydrothermal treatment).
- **Chemical Recovery.** The chemical reactants used in pulping is recovered for economic and environmental reasons.
- **Bleaching.** Chemical treatment for increasing the brightness, softness and whiter pulps. This step requires a low content on lignin because it complicates bleaching.
- **Papermaking.** Several steps give the final finish to paper production.

There are four main processes reported in literature: Kraft, Sulfite, Organosolv and Soda/Soda-Anthraquinone pulping. Even though, these are industrially described for wood, there has been some laboratory scale reports applied to bamboo too. The latter will be explained in the next section. Next, the four main industrial methods, focused on pulping, will be explained and compared based on their chemical reactants, chemical conditions, environmental impact and lignin impurities. In addition, on Table 5 there is a brief summary on their advantages and disadvantages.

#### **Kraft Lignin:**

Kraft pulping is considered to be the dominant source of lignin production that is obtained from lignocellulosic biomass [63]. It consists, mainly, on treating wood with an aqueous sodium hydroxide with sodium sulfide solutions (sometimes, called “white liquor”) at temperatures between 150-180 °C for two hours at high pH (basic) conditions. As lignocellulosic biomass is

released, the initial “white liquor” takes a brownish color because of the lignin release, which then takes the name of “black liquor”.

Several processes take place during treatment such as breakage of lignin-carbohydrates bonds and lignin depolymerization. Phenolic hydroxyl groups of lignin hydrolyze at high pH being soluble in alkali environments [66].

The dissolved lignin can be recovered by acid precipitation (e.g. with carbon dioxide or sodium sulfide), then washed and filtered. On average, the recovered lignin contains low content on sulfur (i.e. presence of thiol groups) and carbohydrates. Kraft lignins are soluble in N,N-dimethylformamide, 2-methoxyethanol and alkali media [67].

### **Sulfite lignin:**

Sulfite pulping has been the major source of commercially available lignin for about 80 years, but due to the higher efficiency and quality of Kraft lignin production, the nowadays sulfite lignin has decreased significantly [66].

The process consists on treating wood by an aqueous solution of sulphite or bisulphite salts (sodium, ammonium, magnesium or calcium) at temperatures between 140-170 °C. The pH range goes from 2 to 12 depending on the cationic composition of the salts. The treatment causes lignin-carbohydrate linkages to split up. The phenolic and non-phenolic units of lignins are sulfonated, receiving the name of lignosulfonates.

These lignosulfonates have higher polydispersity and higher molecular weight than Kraft lignins, making them more impure and contaminated with cations. Generally, sulfonated lignins behave as inorganic and ionic polymers (polyelectrolyte polymers, [63]) which are soluble in water at any range of pH, but also in some organic solvents and amines.

### **Organosolv lignin:**

Organosolv pulping, mainly consist on treating lignocellulosic biomass with organic solvents (i.e. methanol) at elevated temperatures, 140-220 °C [67]. Also, acids or alkali substances are applied to enhance the pulping rates. Besides, methanol and ethanol with 50% water content are the most popular solvents to be used in this process [68].

Organosolv lignin is practically insoluble in water with pHs ranging from 2 to 7, but it can be dissolved in alkali media and polar organic solvents. Even though, it is a high cost operation system, due to the handling and recovery of organic solvents, the resultant lignin structure is

practically unaltered, low weighted and impurity free, meaning that it can be used for value-added chemicals [69].

In fact, it is more environmentally friendly than Kraft and Sulfite lignins. Moreover, it is a promising method for future extraction method of lignins due to its potential on recovery of separate fraction of cellulose, hemicellulose and lignin [70].

### **Soda and Soda-Anthraquinone Lignin:**

Soda pulping was firstly industrialized in 1853, after Kraft pulping processes [66]. It is not quite popular in the paper making industry due to its applicability that is based for non-wood fibers such as straw and sugarcane.

The material is treated with an aqueous solution of sodium hydroxide and provokes the cleavage of lignin-carbohydrate linkages. Indeed, the chemical processes are similar to Kraft lignin. It is a sulfur-free processes making it non-harmful for the environment. The resulting lignins are low weighted due to its low sugar content. Also, Soda lignins are soluble in water.

Actually, the soda-anthraquinone lignin shows higher yields of lignin extraction than soda lignin [71]. Indeed, the presence of anthraquinone serves as a catalyst for the sodium pulping process.

#### *6.1.1.1. Comparison of Industrial Methods:*

Summarizing the principal aspects of Table 5, the Kraft and Sulfite processes are not attractive from the environmental point of view due to their content on sulfur. Even though, lignosulfonates are used in the chemical industry as dispersants in cements [72].

On one hand, Organosolv lignins are practically pure but it is a high costed process due to organic solvents. On the other hand, Soda lignin is low costed but contains more impurities of carbohydrates and ashes due to alkali metals, but still the most sustainable option [73].

Comparing the different methods, if one of them could be used for the bamboo lignin extraction, considering the economic and environmental point of view, the best option would be Soda pulping.

Table 5. General aspects of the main industrial methods highlighting its advantages and disadvantages.

Aspects/Methods	Kraft	Sulfite	Organosolv	Soda / Soda-Anthraquinone
Reactant Agents	Mixture of Sodium hydroxide and Sodium sulfide, Lignin recovered by acid precipitation	Aqueous solution of sulfite or bisulfite salts (calcium, magnesium, ammonium or sodium)	Organic solvents, (mixtures with organic or inorganic acids as catalysts)	Aqueous solution of sodium hydroxide, Lignin recovered by acid precipitation
Chemical Conditions	Temperatures: 150-180 °C Basic pH	Temperature: 140-170 °C pH dependent on the salt	Temperature: 140-220 °C	Temperature: 120 °C
Advantages	Economic (due to chemical recovery efficiency)	Greater quantity of lignin is removed	Sulfur free, relatively pure lignin recovery	Sulfur free, low cost and low environmental impact.
Disadvantages	Sulfur presence	High sulfur presence (sulfonated lignin)	High cost, solvent recycling needed	Low yield pulping
Impurities	Ash: 0.5-3% Sulfur: 1-3% Carbohydrates: 1-2%	Ash: 3.5-8% Sulfur: 3.5-8% Carbohydrates: NA	Ash: 1.7% Sulfur: 0% Carbohydrates: 1-3%	Ash: 0.7-2.3% Sulfur: 0% Carbohydrates: 1.5-3.0%
Lignin	Great yield of extracted lignin.	Lignosulfonates, sulfonation of lignins.	High purity lignins	Needs purification steps.
Performed on the following Bamboo species:	<i>Phyllostachys heterocycla</i>	<i>Phyllostachys heterocycla</i> <i>Bambusa blumeana</i> <i>Bambusa pervariabilis</i>	<i>Phyllostachys acuta</i>	<i>Gigantochloa scortechinii</i> <i>Gigantochloa levis</i>
References	[74][75][76]	[77][76][72]	[78][75][76]	[79][75][80]

### 6.1.2. Laboratory Scale Methods.

The next lignin extraction methods of bamboo presented below have been reported on a laboratory scale. It should be pointed out that some of these following methods mention the use of dewaxed bamboo. This bamboo was pre-treated with toluene/ethanol (1:2, v/v), usually using a Soxhlet apparatus for 6h [81]. Even though, sometimes it not necessary for its dewaxing to proceed with the extraction, but they may alter further characterization. Therefore, a study about the effect of dewaxed and non-dewaxed bamboo may be performed.

Important to point out that, no reported lignin extraction, on *Guadua Angustifolia* was found, but the following methods applied to other bamboo spices may be extrapolated for Guadua.

#### **Industrial methods applied on a laboratory scale:**

Laboratory scale methods using industrial methods, previously mentioned above, have been reported for different species of bamboo:

- **Kraft.** *Phyllostachys heterocycle* was subjected under Kraft pulping pre-treatment with sodium sulfide and sodium hydroxide solution [74] and showed that most of the lignin content was removed.
- **Sulfite.** Three bamboo species from Guangxi (China): *Phyllostachys heterocycla*, *Bambusa blumeana*, *Bambusa pervariabilis* were subjected to sulfite pre-treatment with sulfuric acid and sodium sulfite [77]. The results showed that delignification was effective at higher acid and, as sodium sulfite conditions increased.
- **Organosolv.** Bamboo, *Phyllostachys acuta* was put thorough formic acid pulping and show great yield and pure high-quality lignins [78]. In addition, there was studied the effect of adding hydrochloric acid and hydrogen peroxide to evaluate the delignification capacity.
- **Soda/Soda-Anthraquinone.** *Gigantochloa scortechinii* and *Gigantochloa levis*, two Malaysian species were subjected to soda pulping extraction of lignin [79].

#### **Other laboratory scale methods:**

The following extraction methods are based on a laboratory scale lignin extraction from bamboo.

### Hot water, Alkali and Alkali-Ethanol lignin extraction:

Dewaxed bamboo can be subjected to different treatments, for lignin extraction, highlighting hot water, alkali and aqueous alkali-ethanol solution. The starting point for each treatment depends on the type of reactants used. Hot water is carried out with distilled water at high temperatures (80-120 °C), aqueous alkali media may be used with NaOH (or KOH, LiOH, etc.) at 50 °C for 3 hours and finally, aqueous alkali-ethanol solutions with ethanol and NaOH at 50 °C for 3 hours, too. The following steps after treatment are based on acidification to pH 5.5 with HCl and, hemicellulose precipitation, by 95% ethanol solutions. Lignin is recovered by acidifying the media to pH 1.5-2. A flowchart, based on the previous methods, is described on Figure 11.

Hot water treatment on *Dedrocalamus brandisii* [82], showed lower molecular weights of lignin (1350-1490 g/mol) and higher content of hemicelluloses (9-22%) than aqueous alkali-ethanol solutions treatments, which showed, for the same species, lower hemicellulose content and higher molecular weighted lignins (2830-3170 g/mol).

Alkali treatment showed for *Bambusa Rigida* [83] an important role on cleavage of lignin-carbohydrate linkages. The different alkali reactants had an important effect on the hemicelluloses presenting different chemical compositions. Recovered lignins by alkali treatment of *Neosinocalamus affinis* [84], showed molecular weights between 1400-1680 g/mL, low carbohydrate content (0.22-0.94%) and polydispersity around 1.95-2.22.

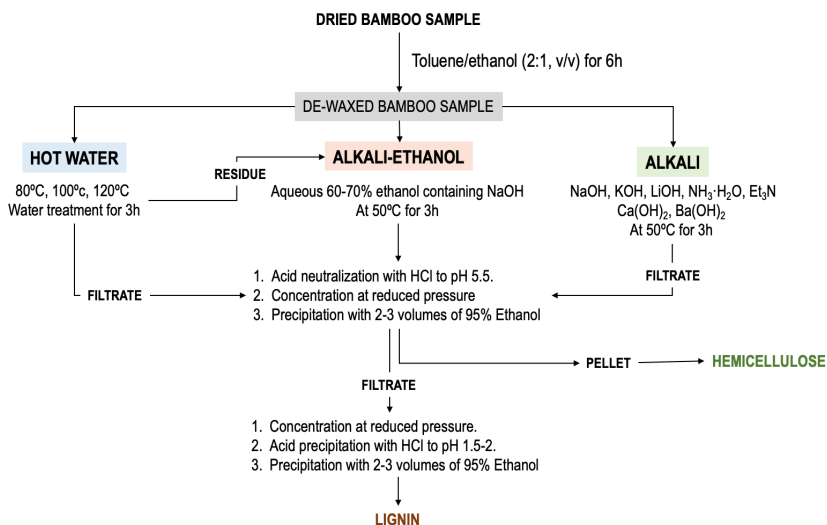


Figure 11. Scheme of hot water, alkali and aqueous alkali-ethanol extraction methods of lignin and hemicellulose.

**Ionic liquid (IL) Extraction:**

Lignin extraction with ionic liquids such as 1-allyl-3-methylimidazolium chloride (AMICl) which is considered a “green solvent”, can dissolve lignin and carbohydrates simultaneously [85], showing a great yield on lignin recovery [76]. Lignin can be recovered by precipitation and the ionic liquid, recycled, but nowadays is still not applied on industrial scale manufacturing.

*Phyllostachys sulphurea* was extracted with 1-allyl-3-methylimidazolium chloride where lignins were slightly degraded by the following treatment [86].

**Steam-exploded Pre-treatment:**

Steam exploded lignin is a thermochemical process based on the breakdown of lignocellulosic biomass starting with a steam impregnation under pressure (200-500 psig) followed by a rapid decompression with temperatures around 180-230 °C [85]. These lignins are low weighted and soluble in organic solvents, even more than Kraft lignins [25]. .

Actually, it is an impressive pre-treatment method which there is no other chemical than the material itself, allowing the autohydrolysis of ether bonds between lignin and carbohydrates. Besides, due to low pulping yields it is not the most commercially available lignin in the market, but it is being a subject of interest because it is an environmentally friendly process [87].

Indeed, *Phyllostachys pubescen* was subjected under stem-explosion pre-treatment and then, an alkali-ethanol extraction, showing that there was a bigger release of lignin from cell walls [87]. In addition, the extracted lignins were low weighted, thus, with high phenolic hydroxyl content.

**Microwave-assisted organic acid extraction:**

It is a promising separation technique of lignocellulosic material which uses microwave energy, under high pressure and high temperature parameters.

Reported *Phyllostachys Sulphurea* has been subjected under microwave extraction with formic and acetic acids with small amounts of hydrochloric acid [88], showing an increase of phenolic hydroxyl content and decrease on carbohydrates bonded, thus, low weighted lignin structures.

**Ultrasound extraction:**

Ultrasound extraction with proper solvents and acoustic energy showed potential on extracting vegetative materials. In addition, the ultrasonic irradiance enhances the mass transfer



in plant tissue. For instance, *Neosinocalamus affinis* was extracted with ethanol by ultrasound assisted extraction [89]. The extracted lignin showed great yields and higher molecular weight as the irradiance increased.

#### 6.1.2.1. Comparison of Laboratory Scale Methods

The seven environmental extraction methods applied on bamboo and grouped on Table 6, may be divided in two: the ones who require an especial equipment (i.e. Microwave extractor) and the ones which do not need any.

The first four methods (hot water, alkali, alkali-ethanol and IL) are, mainly, differentiated on the chemical reactants used. Indeed, the most economical is hot water extraction method because it just need water and temperature for extracting lignin. Even though, the latter is low costed and, one could say, simple, compared with the other three it is not the most efficient because it is the one who contains most impurities of carbohydrates. Therefore, some purification steps will be required. In case it is needed a high degree of purity, the best method will be alkali-ethanol which showed the lower carbohydrate content [82] from the other three.

The Steam exploded, Microwave- and Ultrasound-assisted methods require special material to carry out the extraction. But, setting aside the equipment and focusing on the yields of lignin extraction there are quite similar. From these three methods, it should be noted the fact that ultrasound assisted was carried out fully with ethanol and no other chemical as alkaline or acid treatment.

#### 6.1.3. Post-Treatment Lignin Purification

As previously described for industrial and laboratory scale lignin extraction methods, the obtained lignins may contain residual impurities of carbohydrates and other inorganic contaminants. Therefore, before any future application (e.g. for bio-based adhesives) lignin needs to be purified.

There has been reported few lignin purification methods of which some are briefly mentioned below:

- For Extracted lignin from Soda and Organosolv pulping, on reference [90], the purification was conducted with green solvents, water and 1,3-dimethylimidazolium methylsulfate. The purification of Soda lignins was more difficult than for Organosolv lignins.

Table 6. General aspects of laboratory scale extraction methods. M<sub>LV</sub>: Lignin Molecular Weight

Chemical Method	Chemical Conditions and Main Reactants	M <sub>LV</sub> (g·mol <sup>-1</sup> )	Advantages	Disadvantages	Bamboo species
Hot Water [82]	Carried out with distilled water Temperatures: 80-120°C Time: 3h	1340 – 1490	Extractant: Water. Low cost process.	High carbohydrate impurities.	<i>Dedrocalamus brandisii</i>
Alkali [84]	Aqueous solution of alkali hydroxides (i.e NaOH, KOH, Ca(OH) <sub>2</sub> or Ba(OH) <sub>2</sub> ), NH <sub>3</sub> ·H <sub>2</sub> O or Et <sub>3</sub> N Temperature: 50°C Time: 3h	1400-1680	Low carbohydrate impurities.	Inorganic impurities.	<i>Neosinocalamus affinis</i>
Alkali-Ethanol [82]	Aqueous mixed solution with: NaOH and Ethanol 60-80% Temperature: 50°C Time: 3h	2830-3170	Low carbohydrate impurities.	Inorganic impurities.	<i>Dedrocalamus brandisii</i>
IL [86]	Ionic liquid pre-treatment: 1-allyl-3-methylimidazolium chloride Followed by alkaline extraction (NaOH). Acid water recovery of Lignin	2100-2830	Use of green solvents.	High cost of solvents.	<i>Phyllostachys sulphurea</i>
Steam-Explosion [86]	Steam explosion pre-treatment. Followed by Alkali/Alkali-ethanol extraction	860-1190	Pre-treatment facilitates lignin release from cell walls.	Special equipment required.	<i>Phyllostachys pubescen</i>
Microwave-assisted [87]	Microwave-assisted treatment with organic acids. Water precipitation of lignin.	6070	Successful extraction. Low carbohydrate impurities.	Special equipment required.	<i>Phyllostachys Sulphure</i>
Ultrasonic-assisted [89]	Ultrasound irradiance with ethanol. Time of irradiance: 0-50 min Lignin recovered in ethanol.	1470-1650	No alkaline nor acid treatment.	Special equipment required.	<i>Neosinocalamus affinis</i>

- Kraft lignin impurities are basically: sulfur, carbohydrates and inorganics. These can be reduced by several treatments as ultrasonic (UE) or Soxhlet extraction with organic solvents (e.g. Acetone/water), ash removal by dialysis and hot water treatment in a microwave reactor [91]. Even though these are great for carbohydrate and inorganic removal, sulfur content is still a challenge.

#### **6.1.4. Bamboo pulping and Fiber Residues**

After extracting the lignocellulosic materials of bamboo, pulp and fiber residues are left which mainly are used for paper and paperboard manufacturing. Indeed, as the lignin and carbohydrates are extracted via different methods mentioned before, there is no need for bleaching, a technique used for removing remaining lignin due to its chromophore content that gives color to paper [40].

Furthermore, there has been reported bamboo-based membranes for water treatment from residual fibers left, after lignin and hemicelluloses extraction by green solvents [92]. They were comparable to cellulose-based ones, or even had better performance properties.

## **6.2. CHEMICAL CHARACTERIZATION**

After lignin is extracted it is important to chemically characterize its structure, due to the fact that its amorphous and structurally dependent on the method of extraction.

Combining FT-IR and NMR, two of the most powerful spectroscopic techniques for structural determination, allow to determinate the most prominent linkages and presence of carbohydrate impurities of lignin. In addition, UV spectroscopy is useful for lignin purity determination as it has a high amount of chromophores groups.

Complementary, techniques such as TGA, DSC and GPC are great techniques for purity analysis and molecular weight determination, respectively.

Important to point out, before getting into detailed characterization, that there was not found reported documents on chemical characterization for *Guadua Angustifolia*. Although, several reported documents on other bamboo species have been found and may give useful information and serve as a reference for *Guadua*.

### **6.2.1. Fourier Transform Infrared (FT-IR)**

FT-IR is an intriguing characterization technique which allows to identify organic, polymeric, and also, inorganic materials. The most relevant spectroscopic bands will be discussed for hemicelluloses and lignin.

Even though, the main aim of this project is for lignin characterization it needs to be considered that it is almost impossible to obtain pure lignin, and in many cases, some impurities may be found, for instance small quantities of hemicelluloses attached to lignin. Therefore, hemicellulose characteristic FT-IR bands are mentioned too.

### Lignin:

FT-IR analysis was performed on *Dendrocalamus sinicus* [81] and *Phyllostachys sulphurea* [93], extracted with dioxane and Organosolv methods, respectively. The reported bands are on Table 7, showing a strong band around 3400  $\text{cm}^{-1}$  corresponding to -OH stretching and the aromatic skeleton vibrations of lignin at 1596-1420  $\text{cm}^{-1}$ .

Table 7. Reported FT-IR lignin vibration bands obtained for *Dendrocalamus sinicus* (BaF<sub>2</sub> pellet) and *Phyllostachys sulphurea*.

Bands	<i>Dendrocalamus sinicus</i> ( $\text{cm}^{-1}$ )	<i>Phyllostachys sulphurea</i> ( $\text{cm}^{-1}$ )
OH- stretch	3423	3430
CH stretch (on CH <sub>2</sub> )	2924	2940
CH stretch (on CH <sub>3</sub> )	2939	2845
Unconjugated carbonyl stretch	1708	-
Conjugated carbonyl stretch	1655	1160
Aromatic skeleton vibration	1590, 1501, 1420	1596, 1502, 1420
Methoxy C-H deformation and aromatic ring vibration	1456	1454
Aliphatic C-H stretch on CH <sub>3</sub>	1362	-
Syringyl ring stretching	1325	1321, 1226, 1114
Guaiacyl ring stretching	1224	1267, 1029, 917
p-hydroxy phenylpropane	1119	-
Guaiacyl	832	831
Syringyl	1158	-
Formic ester groups	-	1715

### Hemicellulose:

FT-IR spectra bands, showed on Table 8, have been described for hemicellulose obtained from *Neosinocalamus affinis* [94] and *Bambusa rigida* [83] species, treated with aqueous alkali-ethanol solution and alkali solutions, respectively. The region between 1200-800  $\text{cm}^{-1}$  gives information about polysaccharides types. The  $\beta$ -glycosylic linkages between xylose and hemicellulose units takes place at 895  $\text{cm}^{-1}$ . The aromatic skeletal vibrations are found around 1500-1520  $\text{cm}^{-1}$  (principally, due to lignin presence, being the intensity proportional to lignin content).

Table 8. Reported FT-IR bands for hemicellulose for *Neosinocalamus Affinis* and *Bambusa rigida*. The samples were prepared with a KBr disk with 1%w sample.

Bands	<i>Neosinocalamus Affinis</i> ( $\text{cm}^{-1}$ )	<i>Bambusa rigida</i> ( $\text{cm}^{-1}$ )
-OH stretching	3384	3421
C-H stretching (methyl groups)	2930	2920
Water presence	1642	1645
Aromatic skeletal vibrations	1506	1515
C-H bending	1296	-
C-H wagging	1327	-
O-H bending	1431	-
Polysaccharide type region	1200 – 800	
Arabinoxylans	1152 and 995	-
Acetyl groups	-	1735 and 1247
Xylans (C-O,C-O,C-O-C)	-	1043
$\alpha$ -glycosidic linkages	-	834
$\beta$ -glycosidic linkages	896	898
Arabinosyl side chains	-	1166 and 985
Xylans	-	1420 and 1051
Glucans	-	1079 1113

#### 6.2.2. Ultraviolet-visible (UV) Absorption

Ultraviolet-visible absorption spectroscopy is a characterization technique which is, principally, used for quantitative and qualitative analysis for analytes containing chromophore groups. Lignin, being a three-dimensional phenolic structure containing aromatic skeletal is an

intriguing subject for applying this technique. A semi-qualitative analysis can give information about its purity, after its chemical extraction [95].

Bamboo UV spectra and absorption bands were described for *Dendrocalamus brandisii* [95], extracted with hot water and alkali method, and *Phyllostachys sulphurea* [86], extracted with alkali and IL methods. An absorption spectrum was analyzed from 250 to 380 nm showing some characteristic bands shown in Table 9.

Bands at 280 nm are originated from non-conjugated phenolic groups and it is an indicator of guaiacyl and syringyl rich lignins. At 318 nm there is the conjugated phenolic groups absorption due to p-coumaric and ferulic acids.

Table 9. Lignin absorption bands of UV absorption for *Dendrocalamus brandisii* (dioxane/water solvent) and *Phyllostachys sulphurea* (green solvent) in  $\text{cm}^{-1}$  units.

Absorption bands	<i>Dendrocalamus brandisii</i> ( $\text{cm}^{-1}$ )	<i>Phyllostachys sulphurea</i> ( $\text{cm}^{-1}$ )
Non-Conjugated phenolic groups	279-280	280
Conjugated phenolic groups	316-320	318

### 6.2.3. Nuclear Magnetic Resonance (NMR):

Nuclear magnetic resonance for proton,  $^1\text{H-NMR}$ , carbon,  $^{13}\text{C-NMR}$  and two-dimensional, 2D-NMR is reported in practically every article about lignin characterization because of its exhaustive ability for structural characterization [96][97][98].

- **$^1\text{H-NMR}$ .** The region between 6.00-8.00 ppm gives information about the aromatic protons on S, G and H units.  $\beta\text{-O-}4'$  linkages are found on 4.85 and 4.09 ppm. The characteristic signals for *Neosinocalamus affinis* [94], extracted with an water/ethanol mixture, are shown on Table 10.
- **$^{13}\text{C-NMR}$ .** The spectra shows the typical polysaccharide signals between 57 and 103 ppm. S and G units have multiple signals, from 104.3 to 152.1 ppm and 111.2 to 149.7 ppm, respectively. H units are found in the region of 129.0 ppm. Around 86.0 ppm there is  $\beta\text{-O-}4'$  linkage. On Table 12 there are described the characteristic lignin displacement signals for *Dedrocalamus Brandisii*, *Neosinocalamus affinis* and *Phyllostachys sulphurea*.

→ **2D-HSQC.** Two dimensional heteronuclear single-quantum correlation (2D-HSQC) bands, described on Table 11, allow to see the correlation between directly bonded  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR giving significant structural information.

*Dedrocalamus Brandisii*, *Neosinocalamus affinis* and *Phyllostachys sulphurea* were chemically extracted by hot water and alkali, water-ethanol mixture and Organosolv methods, respectively, before  $^{13}\text{C}$ -NMR and 2D-NMR analysis.

Table 10. Lignin  $^1\text{H}$ -NMR signals for *Neosinocalamus affinis* ( $\text{D}_2\text{O}$  solvent) [94].

$^1\text{H}$ -NMR	<i>Neosinocalamus affinis</i> ( $\delta$ ppm)
$\beta$ -O-4'	4.85, 4.09
Aromatic protons in syringyl, guaiacyl, p-hydroxyphenyl, p-coumarate and ferulate	Region from 6.00 to 8.00
p-coumarate	6.30, 6.27, 6.81, 6.83, 7.45, 7.47, 7.49
ferulate	6.34, 6.37
syringylpropane	6.69
guaiacylpropane	6.81
benzyl aryl ethers	5.58
$\text{CH}_3$	0.8
$\text{CH}_2$	1.6

Table 11. Lignin 2D-HSQC of  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR for *Dedrocalamus Brandisii* (in  $\text{DMSO-d}_6$ ) [95], *Neosinocalamus affinis* (in  $\text{D}_2\text{O}$ ) [94] and *Phyllostachys sulphurea* (in  $\text{CDCl}_3$ ) [86].

2D-HSQC NMR spectroscopy	<i>Dendrocalamus Brandisii</i> ( $\delta\text{C}/\delta\text{H}$ )	<i>Neosinocalamus affinis</i> ( $\delta\text{C}/\delta\text{H}$ )	<i>Phyllostachys sulphurea</i> ( $\delta\text{C}/\delta\text{H}$ )
Aliphatic	-	0-50/0-2.5	-
Side chain	40-100/2.5-6.0	50-100/2.5-6.5	50-103/2.6-6.0
Aromatic	100-150/6.0-8.5	100-160/5.5-9.0	103-145/6.0-8.0

Table 12. Lignin  $^{13}\text{C}$ -NMR signals ( $\delta$  ppm) for *Dedrocalamus Brandisii* (in  $\text{DMSO-d}_6$ ) [95], *Neosinocalamus affinis* ( $\text{D}_2\text{O}$ ) [94] and *Phyllostachys sulphurea* ( $\text{CDCl}_3$ ) [86].

$^{13}\text{C}$ -NMR	<i>Dendrocalamus Brandisii</i> ( $\delta$ ppm)	<i>Neosinocalamus affinis</i> ( $\delta$ ppm)	<i>Phyllostachys sulphurea</i> ( $\delta$ ppm)
Polysaccharide signals	57 and 103	-	90 and 103
$\alpha$ -CHO cinnamaldehyde	191.2	-	-
Carbonyl resonance (uronic acids and esters)	174	-	-
Aliphatic acids (-COOH)	171.4	-	-
Aromatic moiety of lignin	104-168	-	-
Syringyl residues	152.2, 138.2, 134.8, 134.3, 133.0, 106.8, 104.3	152.1, 149.7, 147.1, 138.1, 134.2, 106.8, 104.3	152.3, 147.6, 147.2, 138.3, 135.0, 134.4, 133.1, 104.4
Guayacyl residues	149.7, 149.2, 147.3, 147.1, 145.4, 134.8, 134.3, 133.0, 119.4, 114.8, 111.4	149.7, 245.3, 134.2, 133.0, 119.2, 115.8, 114.7, 111.1	149.3, 147.6, 147.2, 145.5, 135.0, 134.4, 133.1, 132.7, 119.5, 114.9, 111.2
p-Hydroxyphenyl residues	129.9, 12.1	128.1	
Esterified p-coumaric ester	168.7, 159.7, 144.7, 130.4, 125.9, 125.4, 115.9, 115.4	167.9, 159.8, 145.3, 130.0, 125.1, 115.8	159.7, 130.3, 129.9, 125.4, 115.4
Ferulic acids	168.1, 122.4, 122.9	122.2, 167.9, 144.2	168.2, 122.4
$\gamma$ -methyl	14.3	13.9	-
Syringyl and Guayacyl -OCH <sub>3</sub>	56.0	55.9	56.0
$\beta$ -O-4' nits	86.2, 72.3, 60.2	72.2, 86.0, 59.6	86.2, 84.9, 72.4, 71.6, 60.2
b- $\beta$	71.6, 52.3	-	84.6, 71.1, 53.8
$\beta$ -5	86.9, 52.3, 62.7	-	87.1, 52.3



#### 6.2.4. Gel Permeation Chromatography (GPC):

GPC is a size exclusion chromatography for non-polar and high molecular weighted analytes, for instance lignin. Hence, it is used with an organic solvent and hydrophobic packing. The latter usually is made of porous particles from 5 to 10  $\mu\text{m}$  and porous size, 40-2500  $\text{\AA}$ . The technique separates molecules by their size, being the biggest the first ones to come out and the smallest more retained, therefore to be the latest to leave the column.

As previously mentioned, lignin structure is affected by the chemical treatments of extraction, so different molecular weighted lignins can be obtained. For instance, in *Dendrocalamus Brandisii* (Bamboo specie) [95] after a hot water treatment, the lignin molecular weights were ranging from 1350 to 1490 g/mol. Although, the lignin obtained from aqueous alkali-ethanol solutions weighted between 2830 and 3170 g/mol. In addition, the solvent for GPC was tetrahydrofuran (THF)

Also, this technique allows to calculate de degree of polydispersity, variation degree of molecular weights, of the lignin polymers.

#### 6.2.5. Thermal Analysis:

Thermal analysis allows chemical characterization and thermal stability evaluation on lignin samples.

The TGA diagrams for the bamboo species, *Dendrocalamus Brandisii* [95] and *Phyllostachys sulphurea* [93], showed three stages:

- First stage (Temperatures lower than 200 °C). There is a weight loss due to dehydration and volatilization of moisture present in lignin structure.
- Second stage (Temperatures between 200 and 500 °C). Lignin degradation by fragmentation of internal linkages between lignins.
- Third stage (Temperatures higher than 500 °C). Decomposition, pyrolytic degradation and condensation of aromatic rings of lignins.

In addition to TGA analysis, DSC allows to determine the glass transition temperature,  $T_g$ , of the lignin, which will be different according to the bamboo specie and method of extraction. Indeed, lignin, as it was mentioned, is a thermoplastic polymer with maximum endothermic peak at temperatures between 127 to 193 °C.

For instance, *Phyllostachys pubescens* (bamboo) showed an endothermic peak between 156-169 °C [99].

### 6.3. ADHESIVE TESTING:

Evaluate the adhesive properties such as strength could be challenging, but at the same time, intriguing. Indeed, testing adhesive properties is quiet important because of the fact that one cannot rely solely on the chemical characteristics of the adhesive or substrate [100].

The following section is going to discuss the several parameters (i.e. viscosity, durability, cure rate, etc.) which can be evaluated by standard methods. These are detailed performing specifics test, for instance, the American Society for Testing and Materials (ASTM).

In addition, industries or research laboratories make their own test, also denominated non-standard test methods. These are faster, and maybe cheaper, ways to determinate approximated adhesive properties without the need of using standard tests which require more time and, maybe, specific materials. Besides, this latter are fast properties check outs during procedure, so anyways, at the end, it would be required to apply standard test methods.

#### 6.3.1. Standard Test Methods:

Standard Test methods are usually made for comparing properties, determining parameters or effectiveness of materials [93], in this particular case, adhesives. A brief description of evaluable adhesive parameters is on Table 13. In addition, some examples of ASTM tests are described on Table 14.

Table 13. Brief description on some evaluable adhesive properties.

Property	General description
<b>Shear strength</b>	Measure of the maximum shear stress that may be sustained before the rupture of the adhesive bonding.
<b>Viscosity</b>	Measure of the resistance to flow. It could give information about the how easy an adhesive may be spread onto a surface.
<b>Shelf life</b>	Measure of the time an adhesive may be stored without any alteration.
<b>Working life</b>	Measure of the adhesive pot life.
<b>Aging</b>	Measure of adhesive degradation through time
<b>Cure rate</b>	Measure of the time required for an adhesive to go from liquid to solid. Sometimes, heat, pressure or catalysts may be required.
<b>Peel Strength</b>	Measure of the resistance to separate two materials joined by an adhesive.

Table 14. Examples of ASTM for evaluate adhesive properties. Table adapted from [100][101]

<b>VISCOSITY</b>
<p>ASTM D1084: Viscosity of adhesive</p> <p>ASTM D2556: Apparent viscosity of adhesives having shear-rate-dependent flow properties using rotational viscometry</p> <p>ASTM D3236: Viscosity of hot melt adhesives and coating materials</p>
<b>SHELF LIFE/DURABILITY</b>
<p>ASTM D1151: Effect of moisture and temperature on adhesive bonds</p> <p>ASTM D1828: Atmospheric exposure of adhesive- bonded joints and structures</p> <p>ASTM D2918: Determining durability of adhesive joints stressed in peel</p> <p>ASTM D2919: Determining durability of adhesive joints stressed in shear by tension loading</p>
<b>WORKING LIFE</b>
<p>ASTM D1338: Working life of liquid or paste adhesive by consistency and bond strength</p> <p>ASTM D1337: Storage life of adhesives by viscosity and bond strength</p>
<b>TACK</b>
<p>ASTM D2979: Pressure sensitive tack of adhesives using an inverted probe machine</p> <p>ASTM D3121: Tack of pressure-sensitive adhesives by rolling ball</p>
<b>AGING</b>
<p>D 1183: Resistance of Adhesives to Cyclic Aging Conditions</p> <p>D 1581: Bonding Permanency of Water- or Solvent-Soluble Liquid Adhesives for Labeling Glass Bottles</p> <p>D 1713: Bonding Permanency of Water- or Solvent-Soluble Liquid Adhesives for Automatic Machine Sealing Top Flaps of Fiber Specimens</p> <p>D 1877: Permanence of Adhesive-Bonded Joints in Plywood under Mold Conditions</p> <p>D 3632: Accelerated Aging of Adhesive Joints by the Oxygen-Pressure Method</p>
<b>PEEL STRENGHT</b>
<p>D 3807: Properties of Adhesives in Cleavage Peel by Tension Loading (Engineering Plastics-to-Engineering Plastics)</p> <p>D 903: Peel or Stripping Strength of Adhesive Bonds</p> <p>D 1781: Climbing Drum Peel Test for Adhesives</p> <p>D 1876: Peel Resistance of Adhesives (T-Peel Test)</p> <p>D 2558: Evaluating Peel Strength of Shoe Sole Attaching Adhesives</p> <p>D 2918: Determining Durability of Adhesive Joints Stressed in Peel</p> <p>D 3167: Floating Roller Peel Resistance</p>

The following are some examples of the previously described properties applied on lignin-based adhesives:

- Measurements of shear strength on three plywood panels were performed with different lignin-based phenol formaldehyde adhesives [102]. Mixing lignin with phenol formaldehyde showed better bonding performance than pure Phenol-formaldehyde resins.
- Viscosity measurements with Brookfield viscometer were performed on soy flour-based adhesives improved by lignin-based resins [103].
- Lignin phenol formaldehyde adhesives were tested by ASTM D1337 [104]. Shelf life was measured by viscosity and bond strength measurements for six weeks.
- Curing behavior was studied for lignin-based PF adhesives with high pressure DSC at different heat rates [105].

### **6.3.2. Non-Standard Test Methods:**

Non-standard methods, in this case for adhesives, are established by researchers and industries for a rapid evaluation and monitoring of properties. Even though, for a proper evaluation of adhesion it would be required the use of Standard Test Methods.

For this project it was described an adhesive proposal for testing the adhesion capacity of extracted lignins which can be found on the next section, 7.

## 7. EXPERIMENTAL PROPOSAL

Before emergency state alarm, due to COVID-19 pandemic, this project was thought to be carried out as an experimental procedure. Indeed, bamboo sample pre-treatment and a Design of experiment (DOE) was developed. So, regardless the situation this project wanted to expose what was done and what could have been done, considering the exhaustive bibliographic research, if there was the possibility of a laboratory experimentation.

### 7.1. BAMBOO PRE-TREATMENT:

Before lignin extraction methods, Bamboo by-products, from Ecuador, were subjected to cutting and trituration. First, the goal with cutting was to obtain small pieces from Bamboo boards, as the Figure 12, and left oven dried. Then, these small pieces were trituated with a Retsch® Vibratory Disc Mill RS100 (Material disc: Special Steel) to obtain smaller fibers and powder of Bamboo.

This latter procedure was done in the Service of Thin Film at Earth Science Faculty of Universitat de Barcelona.



Figure 12. Small pieces of cut Bamboo.

## 7.2. EXTRACTION METHOD:

After taking a look at several papers describing extraction methods for lignin, the most simple and which already has been proved to give adhesive products, was based on boiling Bamboo in an aqueous solution of 2.5 M sodium hydroxide (NaOH) and 0.4M sodium sulphite ( $\text{Na}_2\text{SO}_3$ ) for 7 hours reported on [106].

For the experimental procedure it was decided to do several experiments modifying three factors (temperature, boiling time and % of  $\text{Na}_2\text{SO}_3$ , in relation with NaOH) and maintaining constant the amount of solid.

The effect of working with different variables in an experiment is often analysed individually modifying one single variable for each experiment. This procedure does not allow us to verify if there is any synergistic effect between the variables or which components may have a major effect, neither optimize the number of experiments. So, that is the reason of using a Design of Experiment (DoE), a mathematic tool which combines different statistic parameter for planning, conducting and analysing an experiment.

A design of experiment was carried out with Design-Experts (Software Version 7.0 form I.State-Ease) for developing an extraction methodology of lignin from bamboo. The purpose of DoE was to determine the best conditions which could get the greatest amount of lignin using three variables (temperature, time and % $\text{Na}_2\text{SO}_3$ ). The statistic approach was a surface response design, specifically, the Central Composite in order to perform an optimization process. In addition, the order of experiments was randomized in order to minimize systematic and accumulative errors on the results. As well, several replicates would be done for evaluating the standard deviation. The experimental parameters are described in Table 15.

After the exhaustive bibliographic research for the development of this written project and taking a close look on lignin extraction methods, it is concluded that the NaOH/ $\text{Na}_2\text{SO}_3$  proposed extraction methods is not the best option. The main reason is that these chemicals correspond to the sulfite process, which it was described, produces liginosulfonates (i.e. sulfonated lignins). Therefore, several purification steps, to eliminate impurities, would be required.

If this project would be experimentally developed, in the future, on lignin extraction from bamboo, the best options could be an extraction by an aqueous solution of NaOH/Ethanol mixture. This method gives great yields and low impurity content. In addition, a DoE could be

developed modifying boiling time, temperature and % of NaOH (or Ethanol) of the aqueous mixture.

Table 15. Design of experiment

ORDER	Temperature (°C)	Time (hours)	% Na <sub>2</sub> SO <sub>3</sub>
6	58.00	8.00	100.00
18	40.00	1.00	100.00
20	40.00	1.00	0.00
3	40.00	1.00	0.00
7	40.00	5.19	59.61
1	70.00	1.00	60.06
17	90.00	1.00	0.00
2	90.00	3.53	100.00
14	90.00	3.63	36.91
8	40.00	8.00	0.00
4	40.00	1.00	100.00
10	70.00	5.16	0.00
19	70.00	1.00	60.06
12	71.00	5.38	60.26
5	90.00	1.00	0.00
13	52.00	3.26	25.73
11	90.00	8.00	100.00
9	90.00	8.00	34.78
15	59.00	3.63	100.00
16	40.00	8.00	0.00

These experimental proposals were not able to be done due to the COVID-19 situation.

Once the extraction was done, the obtained lignin could be directly chemically characterized and/or purified before characterization.

### 7.3. CHEMICAL CHARACTERIZATION

According to the available laboratory equipment of the DIOPMA research group for the characterization of lignin are: FTIR, TGA and DSC. These techniques would give information about:

- FTIR: Structural information.
- TGA: Structural information as well as thermal stability.
- DSC: Glass transition temperature.

## 7.4. EVALUATION OF ADHESIVE PROPERTIES:

The adhesive testing proposal would be applied for studying viscosity and peel strength with the following examples:

### Viscosity

Following the standard ASTM D1084 (Standard Test Method for Viscosity of Adhesives) the viscosity may be measured using a Brookfield viscosimeter, or an equivalent instrument.

### Peel Strength

Following the standard ASTM D 1876 (T-Peel Test), it will be able to determine the relative peel resistance of adhesive bonds between two adherends.

### Non-Standard Test Method:

The non-standard method proposal for the evaluation of adhesive properties of extracted lignins is based on the following general procedure, with Figure 13 as schematic support:

1. The extracted lignin is spread at one end of a tongue depressor.
2. Another tongue depressor is hooked from the other end as shows
3. The two tongue depressors are hold together.



Figure 13. Schematic representation on how would be performed the methods.

The latter is a general procedure where several parameters may be considered:

- **Material.** Initially this is thought to be carried through tongue depressors because they may simulate as it was gluing bamboo.
- **Amount of lignin.** The amount of lignin spread onto the surface may be measured by the difference between the material with and without lignin.
- **Thickness.** This part could be tricky because it depends on the viscosity of the material. If its two flowable or liquid, it could be difficult to be measured.
- **Length.** It could be related to the amount of spread lignin. Besides, the length occupied by lignin could give an idea on how lignin behaves into large and small surfaces.



→ **Pressure.** Changing the amount of pressure in step three may give information of how it affects on adhesive strength.

→ **Heat.** Trials with heat may reveal if the adhesive changes, thus, its thermos sensitive.

This proposal is intended to be a fast and low-cost method to evaluate adhesive properties of extracted lignins.



## 8. CONCLUSIONS

- The amount of documents published about bamboo and bio-based adhesives, for the last 20 years, provided a successful bibliographic research for development of this project.
- Besides, pre-treatment (e.g. cutting and debarking) should be considered to facilitate lignin release and subsequent removal, which is greatly reported too.
- Industrial methods applied to the extraction of lignin from woods could be used for extraction from bamboo. Soda pulping seemed the more environmentally friendly with great yield on lignin extractions.
- There is several reported information about lignin extractions, at a laboratory scale, which were performed on different bamboo species. The alkali-ethanol treatment is the most useful extraction process, showing great yield and low carbohydrate impurities.
- Even though there was not found any reports on *Guadua Angustifolia* the given information for other species of bamboo, as chemical extractions and characterization seem useful for future implementation on *Guadua Angustifolia*.
- The bibliographic information on characterization techniques reported on FTIR and NMR analysis is quite exhaustive for lignin, even for bamboo lignin extractives.
- To evaluate adhesive properties (e.g. viscosity, peel strength, etc) of lignin-based adhesives there are specific standard test methods (e.g. ASTM).
- The initial experimental proposal was not ideal from the structural and environmental point of view because the resulted lignins would be sulfonated and would require an exhaustive purification process.
- Even though, the experimental proposal was not the best option, this method was developed applying a Design of Experiment (DoE), which is a useful tool for evaluate the factor's effect and their interactions. Despite, its advantages, there were almost non-reported articles using it.



## 9. REFERENCES AND NOTES

- [1] K. L. Pizzi, A. Mittal, *Handbook of Adhesive Technology*. Taylor & Francis Group, LLC, 2003.
- [2] R. A. Pike, "Adhesive," *Encyclopaedia Britannica*. p. 7.
- [3] T. Prioleau, "Environmental Impact of the Petroleum Industry," *Hazard. Substances Reserch Cent.*, pp. 1–4, 2003.
- [4] D.-R. Christine, "Renewable Raw Materials for the Chemical Industry," *ESSAI*, vol. 10, no. 15, pp. 1–3, 2013.
- [5] A. Pizzi, "Recent developments in eco-efficient bio-based adhesives for wood bonding: Opportunities and issues," *J. Adhes. Sci. Technol.*, vol. 20, no. 8, pp. 829–846, 2006, doi: 10.1163/156856106777638635.
- [6] L. B. Hare, "Introduction," in *Adhesion and Adhesives: Science and Technology*, vol. 3, no. 2, 1987, pp. 1–17.
- [7] E. M. Petrie, "Theories of adhesion," in *Handbook of Adhesion: Second Edition*, 2005, pp. 49–91.
- [8] S. Ebnesaajad and A. Landrock, "Introduction and Adhesion Theories," in *Adhesives Technology Handbook*, Norwich, NY: William Andre INC, 2009, pp. 1–19.
- [9] E. M. Petrie, "Adhesive bonding of textiles: Principles, types of adhesive and methods of use," in *Joining Textiles: Principles and Applications*, 2013, pp. 225–274.
- [10] G. Wypych, "Mechanisms of Adhesion," in *Adhesion and Adhesives: Science and Technology*, Springer Netherlands, 1987, pp. 56–100.
- [11] E. M. Petrie, *Handbook of Adhesives and Sealants*. 2000.
- [12] E. M. Petrie, "Adhesive Classifications," in *Handbook of Adhesives and Sealants*, 2000, pp. 279–317.
- [13] J. D. Mathias, M. Grédiac, and P. Michaud, "Bio-based adhesives," in *Biopolymers and Biotech Admixtures for Eco-Efficient Construction Materials*, vol. 2012, Elsevier Ltd, 2016, pp. 369–385.
- [14] E. Norström, L. Fogelström, P. Nordqvist, F. Khabbaz, and E. Malmström, "Xylan - A green binder for wood adhesives," *Eur. Polym. J.*, vol. 67, pp. 483–493, 2015, doi: 10.1016/j.eurpolymj.2015.02.021.
- [15] A. K. Patel, J. D. Mathias, and P. Michaud, "Polysaccharides as adhesives: A critical review," *Rev. Adhes. Adhes.*, vol. 1, no. 3, pp. 312–345, 2013, doi: 10.7569/RAA.2013.097310.
- [16] A. J. F. Carvalho, "Starch: Major sources, properties and applications as thermoplastic materials," *Monomers, Polym. Compos. from Renew. Resour.*, pp. 321–342, 2008, doi: 10.1016/B978-0-08-045316-3.00015-6.

- [17] A. K. Patel, P. Michaud, E. Petit, H. De Baynast, M. Grédiac, and J. D. Mathias, "Development of a chitosan-based adhesive. Application to wood bonding," *J. Appl. Polym. Sci.*, vol. 127, no. 6, pp. 5014–5021, 2013, doi: 10.1002/app.38097.
- [18] H. M. Kennedy and A. C. Fischer, "Starch and Dextrins in Prepared Adhesives," in *Starch: Chemistry and Technology*, 2nd ed., Illinois: Academic Press, 1984, pp. 593–610.
- [19] S. Parija, M. Misra, and A. K. Mohanty, "Studies of natural gum adhesive extracts: An overview," *J. Macromol. Sci. - Rev. Macromol. Chem. Phys.*, vol. 41, no. 3, pp. 175–197, 2001, doi: 10.1081/MC-100107775.
- [20] X. Ren and M. Soucek, "Soya-based coatings and adhesives," *ACS Symp. Ser.*, vol. 1178, pp. 207–254, 2014, doi: 10.1021/bk-2014-1178.ch010.
- [21] A. Pizzi, K. Mittal, and A. Lambuthy, "Protein Adhesives for Wood," *Handb. Adhes. Technol. Revis. Expand.*, 2003, doi: 10.1201/9780203912225.ch20.
- [22] S. Pradyawong, G. Qi, N. Li, X. S. Sun, and D. Wang, "Adhesion properties of soy protein adhesives enhanced by biomass lignin," *Int. J. Adhes. Adhes.*, vol. 75, no. February, pp. 66–73, 2017, doi: 10.1016/j.ijadhadh.2017.02.017.
- [23] S. K. De, "Natural Rubber-Based Adhesives," in *Handbook of Adhesive Technology*, Kharagpur: Taylor & Francis Group, LLC, 2003, pp. 23–26.
- [24] Y. Zhang, M. F. Cunningham, N. M. B. Smeets, and M. A. Dubé, "Starch nanoparticle incorporation in latex-based adhesives," *Eur. Polym. J.*, vol. 106, no. June, pp. 128–138, 2018, doi: 10.1016/j.eurpolymj.2018.07.014.
- [25] H. Khan, T. A.; Lee, J.; Kim, "Lignin-Based Adhesives and Coatings," in *Lignocellulose for Future Bioeconomy*, 2019, pp. 153–206.
- [26] C. Xu and F. Ferdosian, "Lignin-Based Phenol-Formaldehyde (LPF) Resins/Adhesives," in *Conversion of Lignin into Bio-Based Chemicals and Materials*, 2017, p. 164.
- [27] R. V. Gadhawe, P. S. Kasbe, P. A. Mahanwar, and P. T. Gadekar, "Synthesis and characterization of lignin-polyurethane based wood adhesive," *Int. J. Adhes. Adhes.*, vol. 95, no. October 2018, p. 102427, 2019, doi: 10.1016/j.ijadhadh.2019.102427.
- [28] A. Nasiri, J. Wearing, and M. A. Dubé, "The use of lignin in emulsion-based pressure-sensitive adhesives," *Int. J. Adhes. Adhes.*, vol. 100, no. March, 2020, doi: 10.1016/j.ijadhadh.2020.102598.
- [29] A. H. Conner and M. S. H. Bhuiyan, "Wood: Adhesives," in *Reference Module in Materials Science and Materials Engineering*, no. December 2015, Elsevier, 2017, pp. 1–18.
- [30] L. A. Hamilton, S. Feit, C. Muffett, and M. Kelso, "Plastic & Climate: The hidden costs of plastic planet," *Cent. Int. Environ. Law*, pp. 1–108, 2019.
- [31] T. Engels, "Thermoset adhesives," in *Thermosets: Structure, Properties, and Applications.*, 2nd ed., Elsevier Ltd., 2018, pp. 341–368.
- [32] A. Bîrca, O. Gherasim, V. Grumezescu, and A. M. Grumezescu, "Introduction in thermoplastic and thermosetting polymers," in *Materials for Biomedical Engineering: Thermoset and Thermoplastic Polymers*, Elsevier Inc., 2019, pp. 1–28.

- [33] W. Harrington, "Elastomeric Adhesives," *Encyclopedia of Materials: Science and Technology*. Elsevier Ltd., pp. 2451–2456, 2001, doi: 10.1201/9780203912225.ch24.
- [34] J. Shields, "Adhesive materials and properties," in *Adhesives Handbook*, 1984, pp. 30–86.
- [35] W. Liese and M. Kohl, *Bamboo. The Plant and its Uses*. Springer International Publishing Switzerland, 2015.
- [36] L. G. Clark, X. Londoño, and E. Ruiz-Sanchez, "Bamboo Taxonomy and Habitat," in *Bamboo. The plant and its Uses*, vol. 10, 2015, pp. 1–30.
- [37] Y. Goh, S. P. Yap, and T. Y. Tong, "Bamboo: The Emerging Renewable Material for Sustainable Construction," *Encyclopedia of Renewable and Sustainable Materials*, vol. 2. Elsevier Ltd., pp. 365–376, 2020, doi: 10.1016/b978-0-12-803581-8.10748-9.
- [38] D. R. Akwada and E. T. Akinlabi, "Economic, Social and Environmental Assessment of Bamboo for Infrastructure Development," *Int. Conf. Infrastruct. Dev. Africa*, pp. 1–15, 2016.
- [39] W. Liese, J. Welling, and Thi Kim Hong Tang, "Utilization of Bamboo," in *Bamboo. The plant and its Uses*, vol. 10, 2015, pp. 299–346.
- [40] E. T. Akinlabi, K. Anane-fenin, D. R. Akwada, and T. M. Plant, *Bamboo. The Multipurpose Plant*. Springer International Publishing AG, 2017.
- [41] E. T. Akinlabi, K. Anane-Fenin, and D. R. Akwada, "Properties of Bamboo," in *Bamboo: The Multipurpose Plant*, no. Banik 2015, 2017, pp. 87–147.
- [42] L. G. Clark, X. Londoño, and E. Ruiz-Sanchez, "Morphology and Growth," in *Bamboo. The plant and its Uses*, vol. 10, 2015, pp. 43–89.
- [43] H. P. S. Abdul Khalil et al., "The use of bamboo fibres as reinforcements in composites," in *Biofiber Reinforcements in Composite Materials*, Elsevier Ltd, 2015, pp. 488–524.
- [44] P. Van Der Lugt, "Por qué el bambu gigante es un recurso tan promete dor Corre en la familia," 2020. [Online]. Available: [https://blog.moso-bamboo.com/es/por-que-el-bambu-gigante-es-un-recurso-tan-prometedor?lang\\_selected=true](https://blog.moso-bamboo.com/es/por-que-el-bambu-gigante-es-un-recurso-tan-prometedor?lang_selected=true). [Accessed: 18-Jun-2020].
- [45] E. T. Akinlabi, K. Anane-Fenin, and D. R. Akwada, "Regeneration, Cultivation, and Sustenance of Bamboo," in *Bamboo: The Multipurpose Plant*, 2017, pp. 39–86.
- [46] W. Liese and G. Weiner, "Ageing of bamboo culms. A review," *Wood Sci. Technol.*, vol. 30, no. 2, pp. 77–89, 1996, doi: 10.1007/BF00224958.
- [47] R. L. Banik, "Harvesting Techniques," in *Bamboo. The plant and its Uses*, vol. 10, 2015, pp. 193–226.
- [48] W. Liese and T. K. Ho. Tang, "Preservation and Drying of Bamboo," in *Bamboo. The plant and its Uses*, vol. 10, 2015, pp. 257–297.
- [49] W. Liese, "Preservation of Bamboo in Service," pp. 1–5, 2002.
- [50] C. F. Liu and R. C. Sun, "Cellulose," in *Cereal Straw as a Resource for Sustainable Biomaterials and Biofuels*, 1st ed., Elsevier, 2010, pp. 131–167.
- [51] 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.

- [52] P. Bajpai, "Structure and Properties of Cellulose and Nanocellulose," *Pulp Pap. Ind.*, pp. 27–40, 2017, doi: 10.1016/b978-0-12-811101-7.00003-4.
- [53] J. L. Ren and R. C. Sun, *Hemicelluloses*, 1st ed. Elsevier, 2010.
- [54] C. Xu and F. Ferdosian, *Conversion of Lignin into Bio-Based Chemicals and Materials*. 2017.
- [55] X. Bai and K. H. Kim, *Biofuels and Chemicals from Lignin Based on Pyrolysis*. 2016.
- [56] D. Structures and S. Structures, "Structure and Characteristics of Lignin," *Lignin Chem. Appl.*, pp. 25–50, 2019, doi: 10.1016/b978-0-12-813941-7.00002-3.
- [57] W. Liese and M. Kohl, *Bamboo. The plant and its Uses*. Hamburg: Springer International Publishing Switzerland, 2015.
- [58] E. T. Akinlabi, K. Anane-Fenin, and D. R. Akwada, "Applications of Bamboo," in *Bamboo: The Multipurpose Plant*, Springer International Publishing AG, 2017, pp. 179–219.
- [59] E. T. Akinlabi, K. Anane-Fenin, and D. R. Akwada, "Bamboo as fuel," in *Bamboo: The Multipurpose Plant*, 2017, pp. 149–178.
- [60] S. Satya, L. M. Bal, P. Singhal, and S. N. Naik, "Bamboo shoot processing: food quality and safety aspect (a review)," *Trends Food Sci. Technol.*, vol. 21, no. 4, pp. 181–189, 2010, doi: 10.1016/j.tifs.2009.11.002.
- [61] "El Bambú en Ecuador." [Online]. Available: [www.inbar.int](http://www.inbar.int). [Accessed: 20-Apr-2020].
- [62] N. Banik, "Manufacturing of bamboo composite in the industrial aspect," *Mater. Today Proc.*, pp. 2214–7853, 2020, doi: 10.1016/j.matpr.2020.02.596.
- [63] C. Xu and F. Ferdosian, "Structure and Properties of Lignin," in *Conversion of Lignin into Bio-Based Chemicals and Materials*, 2017, pp. 1–12.
- [64] B. Ahmed et al., "Improvement of anaerobic digestion of lignocellulosic biomass by hydrothermal pretreatment," *Appl. Sci.*, vol. 9, no. 18, pp. 1–17, 2019, doi: 10.3390/app9183853.
- [65] P. Bajpai, "Basic Overview of Pulp and Paper Manufacturing Process.," in *Green Chemistry and Sustainability in Pulp and Paper Industry*, 2015, pp. 1–258.
- [66] J. H. Lora, "Industrial commercial lignins: Sources, properties and applications," in *Monomers, Polymers and Composites from Renewable Resources*, 2008, pp. 225–241.
- [67] J. D. Holladay JE, Bozell JJ, White JF, Top value added chemicals from biomass volume I — Results of screening for potential candidates from sugars and synthesis gas, vol. II, no. October. 2004.
- [68] J. Fernández-Rodríguez, X. Erdocia, F. Hernández-Ramos, M. G. Alriols, and J. Labid, "Lignin separation and fractionation by ultrafiltration," in *Separation of Functional Molecules in Food by Membrane Technology*, 2019, pp. 229–265.
- [69] E. Ahmad and K. K. Pant, "Lignin Conversion- A Key to the Concept of Lignocellulosic Biomass-Based Integrated Biorefinery," in *Waste Biorefinery*, New Delhi: Elsevier B.V., 2018, pp. 409–444.
- [70] D. Zhao, Xuebing; Li, Siming; Liu, "Organosolv fractionating pre-treatment of lignocellulosic biomass for efficient enzymatic saccharification: chemistry, kinetics, and substrate structures," p. 24, 2017, doi: 10.1002/bbb.1768.



- [71] M. N. Mohmad, M. Y. Nor Nadiah, and H. Azian, "Comparison Studies Between Soda Lignin and Soda-anthraquinone Lignin in Terms of Physico-chemical Properties and Structural Features," *J. Appl. Sci.*, vol. 2, no. 6, pp. 292–296, 3006.
- [72] T. Aro and P. Fatehi, "Production and Application of Lignosulfonates and Sulfonated Lignin," *ChemSusChem*, vol. 10, no. 9, pp. 1861–1877, 2017, doi: 10.1002/cssc.201700082.
- [73] J. C. Carvajal, Á. Gómez, and C. A. Cardona, "Comparison of lignin extraction processes: Economic and environmental assessment," *Bioresour. Technol.*, vol. 214, pp. 468–476, 2016, doi: 10.1016/j.biortech.2016.04.103.
- [74] C. Huang et al., "Effect of kraft pulping pretreatment on the chemical composition, enzymatic digestibility, and sugar release of moso bamboo residues," *BioResources*, vol. 10, no. 1, pp. 240–255, 2015, doi: 10.15376/biores.10.1.240-255.
- [75] E. Fortunati, F. Luzi, D. Puglia, and L. Torre, "Extraction of Lignocellulosic Materials From Waste Products," in *Multifunctional Polymeric Nanocomposites Based on Cellulosic Reinforcements*, Terni: Elsevier Inc., 2016, pp. 1–38.
- [76] A. Vishtal and A. Kraslawski, "Challenges in industrial applications of technical lignins," *BioResources*, vol. 6, no. 3, pp. 3547–3568, 2011, doi: 10.15376/biores.6.3.3547-3568.
- [77] Z. Li, Z.; F. B.; Jiang, "Study of sulfite pretreatment to prepare bamboo for enzymatic hydrolysis and ethanol fermentation," *Chem. Technol. fuels Oils*, vol. 50, no. 3, pp. 189–196, 2014, doi: 10.1007/s10553-015-0548-2.
- [78] M. F. Li, S. N. Sun, F. Xu, and R. C. Sun, "Formic acid based organosolv pulping of bamboo (*Phyllostachys acuta*): Comparative characterization of the dissolved lignins with milled wood lignin," *Chem. Eng. J.*, vol. 179, pp. 80–89, 2012, doi: 10.1016/j.cej.2011.10.060.
- [79] S. Osman and M. Ahmad, "Chemical and thermal characterization of Malaysian bamboo lignin (Beting & Semantan) extracted via soda pulping method," *AIP Conf. Proc.*, vol. 1985, no. Nspm, pp. 1–7, 2018, doi: 10.1063/1.5047196.
- [80] G. H. Kim and B. H. Um, "Fractionation and characterization of lignins from *Miscanthus* via organosolv and soda pulping for biorefinery applications," *Int. J. Biol. Macromol.*, vol. 158, pp. 443–451, 2020, doi: 10.1016/j.ijbiomac.2020.04.229.
- [81] G. Xu et al., "Structural characterization of lignin and its carbohydrate complexes isolated from bamboo (*Dendrocalamus sinicus*)," *Int. J. Biol. Macromol.*, vol. 126, pp. 376–384, 2019, doi: 10.1016/j.ijbiomac.2018.12.234.
- [82] Z. Yang, H. Peng, W. Wang, and T. Liu, "Physicochemical Characterization of Lignin Fractions Sequentially Isolated from Bamboo (*Dendrocalamus brandisii*) with Hot Water and Alkaline Ethanol Solution," *J. Appl. Polym. Sci.*, vol. 116, no. 5, pp. 2658–2667, 2010, doi: 10.1002/app.
- [83] J. L. Wen et al., "Comparative study of alkali-soluble hemicelluloses isolated from bamboo (*Bambusa rigida*)," *Carbohydr. Res.*, vol. 346, no. 1, pp. 111–120, 2011, doi: 10.1016/j.carres.2010.10.006.
- [84] S. N. Sun, M. F. Li, T. Q. Yuan, F. Xu, and R. C. Sun, "Sequential extractions and structural characterization of lignin with ethanol and alkali from bamboo (*Neosinocalamus affinis*)," *Ind. Crops Prod.*, vol. 37, no. 1, pp. 51–60, 2012, doi: 10.1016/j.indcrop.2011.11.033.

- [85] A. Tribot et al., "Wood-lignin: Supply, extraction processes and use as bio-based material," *Eur. Polym. J.*, vol. 112, no. October 2018, pp. 228–240, 2019, doi: 10.1016/j.eurpolymj.2019.01.007.
- [86] D. Yang, L. X. Zhong, T. Q. Yuan, X. W. Peng, and R. C. Sun, "Studies on the structural characterization of lignin, hemicelluloses and cellulose fractionated by ionic liquid followed by alkaline extraction from bamboo," *Ind. Crops Prod.*, vol. 43, no. 1, pp. 141–149, 2013, doi: 10.1016/j.indcrop.2012.07.024.
- [87] S. L. Sun, J. L. Wen, M. G. Ma, R. C. Sun, and G. L. Jones, "Structural features and antioxidant activities of degraded lignins from steam exploded bamboo stem," *Ind. Crops Prod.*, vol. 56, pp. 128–136, 2014, doi: 10.1016/j.indcrop.2014.02.031.
- [88] M. F. Li, S. N. Sun, F. Xu, and R. C. Sun, "Microwave-assisted organic acid extraction of lignin from bamboo: Structure and antioxidant activity investigation," *Food Chem.*, vol. 134, no. 3, pp. 1392–1398, 2012, doi: 10.1016/j.foodchem.2012.03.037.
- [89] M. F. Li, S. N. Sun, F. Xu, and R. C. Sun, "Ultrasound-enhanced extraction of lignin from bamboo (*Neosinocalamus affinis*): Characterization of the ethanol-soluble fractions," *Ultrason. Sonochem.*, vol. 19, no. 2, pp. 243–249, 2012, doi: 10.1016/j.ultsonch.2011.06.018.
- [90] R. Prado, X. Erdocia, L. Serrano, and J. Labidi, "Lignin purification with green solvents," *Cellul. Chem. Technol.*, vol. 46, no. 3–4, pp. 221–225, 2012.
- [91] W. Fang, M. Alekhina, O. Ershova, S. Heikkinen, and H. Sixta, "Purification and characterization of kraft lignin," *Holzforschung*, vol. 69, no. 8, pp. 943–950, 2015, doi: 10.1515/hf-2014-0200.
- [92] M. R. Esfahani et al., "Sustainable Novel Bamboo-Based Membranes for Water Treatment Fabricated by Regeneration of Bamboo Waste Fibers," *ACS Sustain. Chem. Eng.*, vol. 8, no. 10, pp. 4225–4235, 2020, doi: 10.1021/acssuschemeng.9b07438.
- [93] M. F. Li, S. N. Sun, F. Xu, and R. C. Sun, "Sequential solvent fractionation of heterogeneous bamboo organosolv lignin for value-added application," *Sep. Purif. Technol.*, vol. 101, pp. 18–25, 2012, doi: 10.1016/j.seppur.2012.09.013.
- [94] T. Q. Sun, S. N.; Yuan, "Structural Characterization of Hemicelluloses from Bamboo Culms (*Neosinocalamus affinis*)," *Cellul. Chem. Technol.*, p. 12, 2011.
- [95] Z. J. Shi, L. P. Xiao, Jia-Deng, F. Xu, and R. C. Sun, "Physicochemical characterization of lignin fractions sequentially isolated from bamboo (*Dendrocalamus brandisii*) with hot water and alkaline ethanol solution," *Journal of Applied Polymer Science*, vol. 125, no. 4. pp. 3290–3301, 2012, doi: 10.1002/app.36580.
- [96] T. Q. Yuan, S. N. Sun, F. Xu, and R. C. Sun, "Characterization of lignin structures and lignin-carbohydrate complex (LCC) linkages by quantitative <sup>13</sup>C and 2D HSQC NMR spectroscopy," *J. Agric. Food Chem.*, vol. 59, no. 19, pp. 10604–10614, 2011, doi: 10.1021/jf2031549.
- [97] M. Balakshin, E. Capanema, H. Gracz, H. min Chang, and H. Jameel, "Quantification of lignin-carbohydrate linkages with high-resolution NMR spectroscopy," *Planta*, vol. 233, no. 6, pp. 1097–1110, 2011, doi: 10.1007/s00425-011-1359-2.
- [98] S. Ralph, J. Ralph, L. Landucci, U. F. Service, and J. Ralph, "NMR Database of Lignin and Cell Wall Model Compounds," 2009.

- [99] S. Shao, Z. Jin, G. Wen, and K. Iiyama, "Thermo characteristics of steam-exploded bamboo (*Phyllostachys pubescens*) lignin," *Wood Sci. Technol.*, vol. 43, no. 7–8, pp. 643–652, 2009, doi: 10.1007/s00226-009-0252-7.
- [100] S. Ebnesajjad and A. H. Landrock, "Testing of Adhesive Bonds," in *Adhesives Technology Handbook*, Elsevier Inc., 2015, pp. 339–352.
- [101] E. M. Petrie, "Standard Test Methods," in *Handbook of Adhesives and Sealants*, 2008, pp. 127–175.
- [102] M. Mittal and C. B. Sharma, "Studies on lignin-based adhesives for plywood panels," *Polym. Int.*, vol. 29, no. 1, pp. 7–8, 1992, doi: 10.1002/pi.4990290103.
- [103] Y. Shang, Xiaochun; Zhu, Yuding; Yu, "Improve Performance of Soy Flour-Based Adhesives with lignin-based Resin."
- [104] M. A. Khan, S. M. Ashraf, and V. P. Malhotra, "Development and characterization of a wood adhesive using bagasse lignin," *Int. J. Adhes. Adhes.*, vol. 24, no. 6, pp. 485–493, 2004, doi: 10.1016/j.ijadhadh.2004.01.003.
- [105] Z. Liu, Y. Zhang, X. Wang, and D. Rodrigue, "Reinforcement of Lignin-Based Phenol-Formaldehyde Adhesive with Nano-Crystalline Cellulose (NCC): Curing Behavior and Bonding Property of Plywood," *Mater. Sci. Appl.*, vol. 06, no. 06, pp. 567–575, 2015, doi: 10.4236/msa.2015.66060.
- [106] J. Song et al., "Processing bulk natural wood into a high-performance structural material," *Nature*, vol. 554, no. 7691, pp. 224–228, 2018, doi: 10.1038/nature25476.
- [107] J. Zakzeski, P. C. A. Bruijninx, A. L. Jongerius, and B. M. Weckhuysen, "The catalytic valorization of lignin for the production of renewable chemicals," *Chem. Rev.*, vol. 110, no. 6, pp. 3552–3599, 2010, doi: 10.1021/cr900354u.

## 10. ACRONYMS

American Society for Testing and Materials	ASTM
Carbon-13 Nuclear Magnetic Resonance	$^{13}\text{C}$ -NMR
Design of Experiment	DoE
Differential Scanning Calorimetry	DSC
Fourier Transform Infrared Spectroscopy	FT-IR
Gel Permeation Chromatography	GPC
Glass Transition Temperature	$T_g$
Guaiacyl alcohol	G-unit
Lignin Molecular weight	$M_{Lw}$
Nuclear Magnetic Resonance	NMR
p-Coumaryl Alcohol	H-unit
Phenol-Formaldehyde	PF
Proton Nuclear Magnetic Resonance	$^1\text{H}$ -NMR
Syringyl Alcohol	S-unit
Thermogravimetric Analysis	TGA
Two-dimensional Nuclear Magnetic Resonance	2D-NMR
Ultrasonic Extraction	UE

