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Lignin as Feedstock for Nanoparticles Production

Ana Lourenço and Jorge Gominho

Abstract

Lignin is an interesting natural polymer with characteristics that contribute for the development and growth of plants. Lignin presents high variability associated with the diversity of plants, which presents great challenges for its recovery after delignification (*technical lignin*), because lignin is prone to irreversible degradation, producing recalcitrant condensed structures that are difficult to disassemble afterward. Although researchers have made efforts to obtain lignin in high yields and with good characteristics for specific uses, this is not an easy task. The mind-set has changed and new biorefinery concepts are emerging, where lignin is the primary goal to achieve, and the so-called *lignin-first* approach has arisen. Lignin can be obtained firstly to prevent structural degradations, enabling an efficient and highly selectivity of the lignin monomers. Therefore, this concept places lignin and its valorization at the head of the biorefinery. However, lignin valorization is still a challenge, and to overcome this, lignin nanoparticles (LNPs) production presents a good way to achieve this goal. This chapter presents a resume of the several techniques to attain lignin, how to produce LNPs, and their possible applications (from pharmaceutical to the automobile and polymer industries).

Keywords: biomass composition, lignin isolation, *lignin-first* biorefining, lignin applications, nanoparticles production

1. Introduction

Lignin has been studied by different researchers over the years; the first output was published in 1908, and to date, a total of 74,191 documents mentioning “lignin” have been published, according to the Web of Science database (WOS). **Figure 1** presents a treemap with the published documents distributed by the WOS categories (from 1908 to 2023): Biotechnology Applied Microbiology (9440), Energy fuels (9362), Materials Science Paper Wood (9256), Chemistry Multidisciplinary (8935), Chemical Engineering (8564), to mention just a few.

A search for the word “lignin-first” revealed a smaller number of documents, totaling 127. But what was quite impressive was the date of the first document, in 1997 (**Figure 2**). The main articles were published in the last 6 years, reaching a total of 122. This fact reveals the increasing importance of the “lignin-first” approach within the research community.

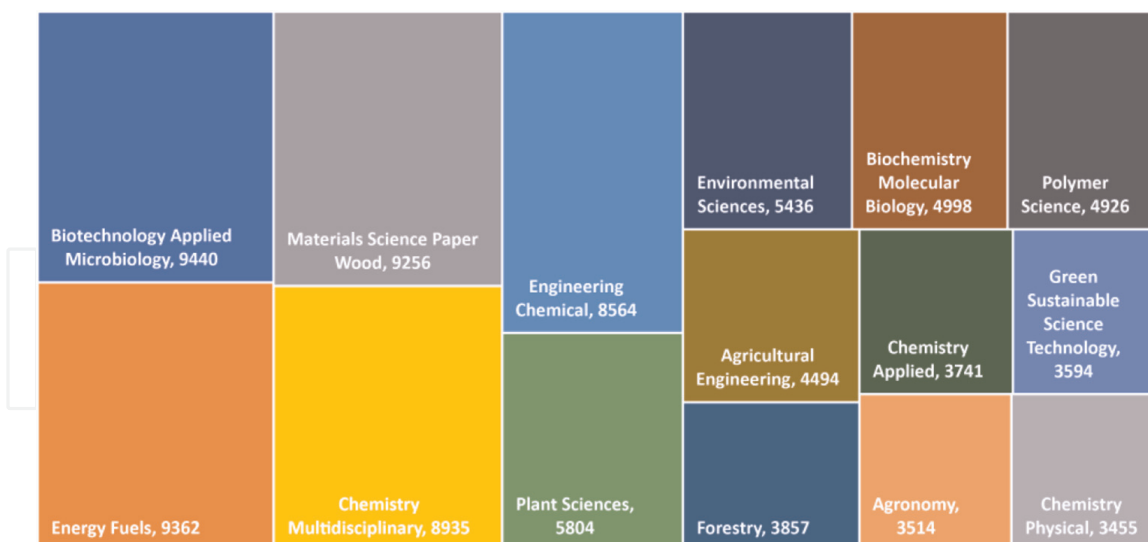


Figure 1. TreeMap chart adapted from WOS searching results using the word “lignin” (www.webofscience.com; accessed on September 30, 2022).

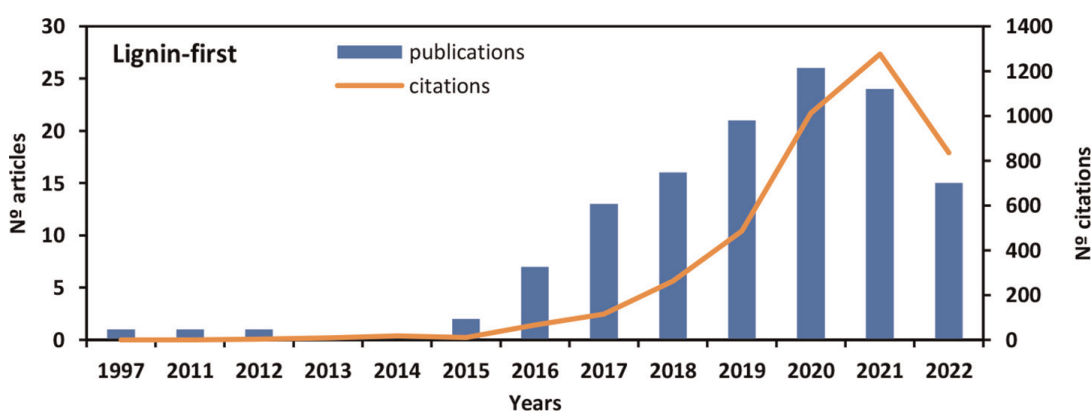


Figure 2. The number of publications that mention the word “lignin-first” and their citations over the years. Adapted from WOS (www.webofscience.com; accessed on September 30, 2022).

The topic “lignin nanoparticles” is also an increasing one (**Figure 3**), and the search provided a total of 373 documents during the period from 2008 to 2022 (September). The most highly cited papers number 10, with 332 articles and 32 review papers recorded. The distribution between the WOS categories was as follows: Chemistry Multidisciplinary (123), Polymer Science (81), Materials Science Multidisciplinary (59), and Biochemistry Molecular Biology (51). Although the year 2022 has not ended, it is possible to see the growing tendency for the number of publications and citations of this topic (**Figure 3**).

According to these results, it is clear that lignin is still an exciting research topic. To reinforce this, at the moment, a European Cooperation in Science & Technology is being developed within this theme, called “*LignoCost - Pan-European Network on the Sustainable Valorisation of Lignin*” (COST Action CA17128, <https://lignocost.eu/>), to establish a network between different researchers having a focus on lignin valorization and its sustainable industrial application. One of the Working Groups is devoted to making a Wikilignin database where relevant works are being compiled.

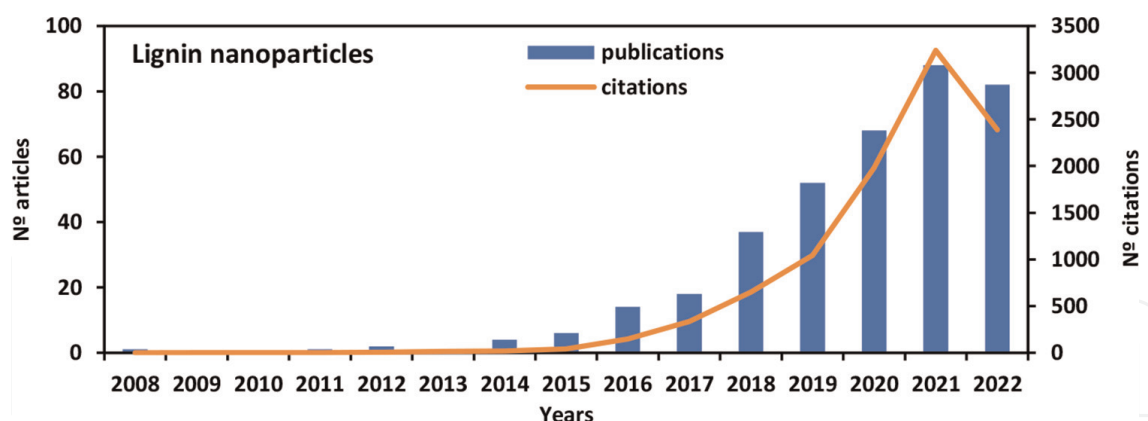


Figure 3. The number of publications that mention “lignin nanoparticles” and their citations over time. Adapted from WOS. (www.webofscience.com; accessed on September 30, 2022).

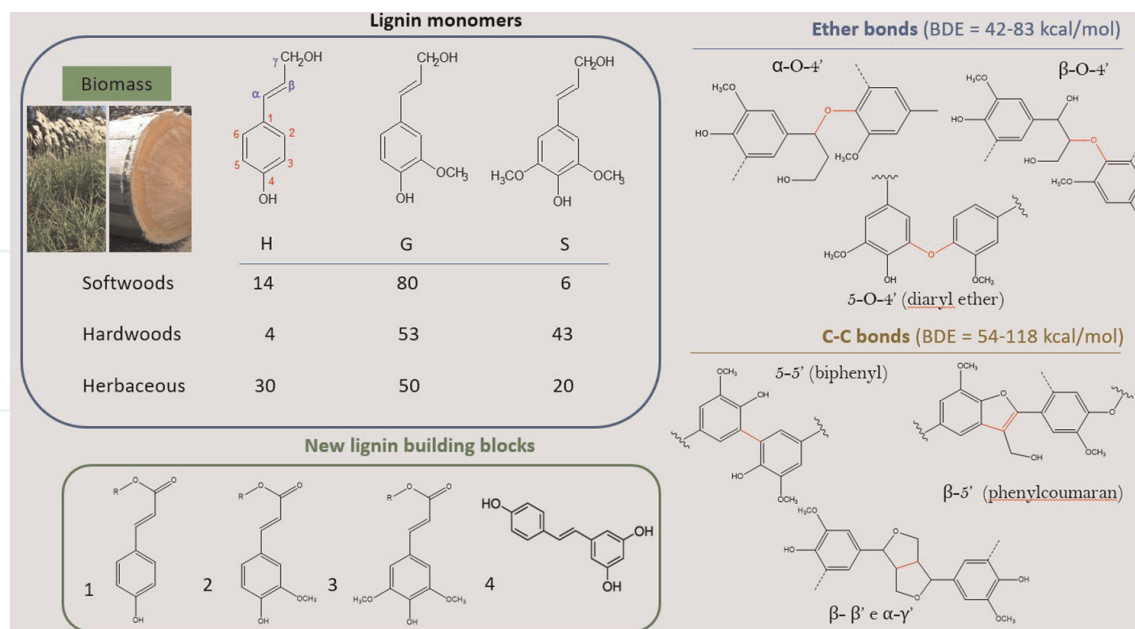
2. Lignin structure and composition in biomass

2.1 What is lignin?

Lignin is a complex polymer present in vascular plants, particularly in woody tissues. It is the second most abundant natural polymer, representing 15–30% dry weight of lignocellulosic materials (e.g., wood and bark). Its natural production on earth ranges between 5 and 36×10^8 tons annually [1]. Lignin is mainly deposited in the secondary cell walls of the supporting and water-conducting tissues, but it is also present in the middle lamella between cells and cell corners. Besides xylem cells, lignification can also occur in sclereids cells (phloem cells), roots, and seeds [2].

2.2 What is lignin made of?

Lignin is a heterogeneous aromatic polymer constituted by three phenylpropane precursors: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol that present a hydroxyl group linked to C4 and substitutions with one or two methoxyl groups in C3 and C5. If the aromatic ring is unmethoxylated, then it is called *p*-hydroxyphenyl (H), and if it has one methoxyl group in C3, it is called guaiacyl, or with two methoxyl groups linked with C3 and C5, it is a syringyl unit (S, **Figure 4**) [3]. Therefore, lignin is a highly complex phenolic polymer whose content and composition vary depending on many factors, for example, species, specimen age, plant part, environmental growth conditions, and harvesting time [2–7]. In hardwoods, lignin typically represents between 20 and 25% of the wood, with a monomeric composition mainly of syringyl and guaiacyl but few *p*-hydroxyphenyl units. In comparison, softwoods are richer in lignin (25–35%), where guaiacyl units prevail, fewer *p*-hydroxyphenyl units are found, and almost no syringyl units are present [3]. In herbaceous species, the lignin content ranges from 15 to 24% [8] and has a monomeric composition where no specific lignin units predominate [3]. It was found that other monomers are also included in the lignin polymer, such as triclin flavones, hydroxycinnamaldehydes, hydroxystilbenes, and xenobiotics [9–15]. The monomers are linked by ether bonds and carbon-to-carbon bonds in different proportions. The ether bonds prevail (e.g., β -O-4' and α -O-4'), representing between 35 and 60% in softwoods and from 50 to 80% in hardwoods [16]. Generally, C—O linkages have a bond-dissociation

**Figure 4.**

Lignin monomeric composition distributed by softwood, hardwood, and herbaceous. The new lignin building block: 1—coumaric acid, 2—ferulic acid, 3—sinapic acid, if R=H; 4—resveratrol (hydroxystilbene).

enthalpy (BDE) of 42–83 kcal/mol (they are easier to break); while C—C linkages (e.g. β — β' , β —5') have BDE values between 54 and 118 kcal/mol, so they are harder to break down, presenting more considerable challenges for lignin deconstruction [17]. Slightly different values were compiled by Liu et al. [18] and are presented in **Table 1**.

Component	Type	Total amount (%)			Bond dissociation enthalpy (BDE) (kcal/mol)	
		Softwood	Hardwood	Grass		
Monolignol	H	<5	0–8	5–33	—	
	G	>95	25–50	33–80	—	
	S	0	46–75	20–54	—	
Linkages	C—O—C	β -O-4'	43–50	50–65	74–84	56.54–72.30
		α -O-4' *	5–7	<1	n.d.	48.45–57.28
		4-O-5'	4	6–7	n.d.	77.74–82.54
	C—C	5-5	5–7	<1	n.d.	114.9–118.4
β - β		2–6	3–12	1–7	—	
β -5		9–12	3–11	5–11	125.2–127.6	
β -1		1–9	1–7	n.d.	64.7–165.8	
Others		16	7–8	n.d.	—	

*Only present in the dibenzodioxocin moieties (β -O-4' + α -O-4' + 5-5).

Table 1.

General characteristics of lignin from softwood, hardwood, and herbaceous species. Adapted from Liu et al. [18].

2.3 What is the role of lignin in plants?

Lignin deposition in plant cells: (i) provides rigidity and mechanical strength, allowing the plants to stand; (ii) gives hydrophobicity to the cell walls; (iii) promotes the transportation of water and minerals between cells, (iv) hinders the degradation of polysaccharides; and (v) is a barrier against pathogens and pests, playing an essential role in plant development [19–21].

Lignin has another active role in the plant response to several abiotic and biotic stresses, by reinforcing the cell walls in the neighborhood of infected or wounded tissue (the so-called *defense lignin*, which contrasts with the *developmental lignin* presented before) [22–24]. Plants have suffered different types of abiotic (*e.g.*, drought, mineral deficiencies, and low or high temperatures) and biotic stresses (*e.g.*, insects, bacteria, or fungi attacks) over the years. Lignin is vital for plant growth and its environmental adaptability, that is, the plant can produce different amounts and types of lignins (possessing other physical properties) depending on the growth conditions. Moura and coworkers [25] reported a review explaining the influence of different stresses, such as the increase of cold, drought, or light upon the plants; for example, maize under water deficit reduced the production of ferulic acid but increased *p*-coumaric and caffeic acids in the xylem regions. However, the roots increased their lignin content, stiffening the cell walls and reducing their expansion [25]. More recently, a review was presented focused on the biosynthesis, content, and accumulation of lignin as a plant response to abiotic stresses; during drought, *E. urograndis* increased the lignin content in its roots and leaves and reduced the S/G ratio [26]. In *P. trichocarpa*, the lignin content did not change in young shoots or mature stems, but the S/G ratio decreased significantly in the young shoots. Plants under drought stress may increase the lignin content to reduce water penetration and transpiration from the cell wall, which helps to maintain cell osmotic balance [21].

Plants under a rise in light induced a lignin accumulation, as a way to adapt to the environment [26]. Also, the lignin composition in angiosperm and gymnosperms species under stress revealed a higher amount of condensed linkages (C—C bonds) and more H-units as a response to the environmental conditions, including high nitrogen fertilization, mechanical injuries, or ozone [2, 23, 24].

Therefore, researchers have tried to understand plant adaptability, but it is difficult to generalize the behavior of plants regarding particular stress because each species behaves differently. Also, not only the lignin content or its composition is essential to understand plant behavior, since the production of lignin comprises a complex genetic network with the involvement of multiple enzymes that have different responses to the abiotic and biotic factors [24]. This will not be developed here, but readers are encouraged to consult many of the works on this topic [27–30] and a review about lignin biosynthesis [31].

The diversity of lignins (content and composition) and their recalcitrant behavior to degradation is a challenge for researchers who wish to learn more about the lignin structure and biosynthesis, but mainly for those who work in lignin valorization. Thereby, we discuss below some procedures to isolate the lignin.

3. Lignin isolation procedures and *lignin-first* concept

Most often, lignin valorization starts with its isolation from the lignocellulosic material (biomass) with two perspectives: (i) to gain insights into lignin's

chemical and structural characterization, which involves methods such as Björkman, dioxane, and cellulolytic enzyme lignin [32–34], where the obtained lignin presents a structure close to the lignin in the cell wall matrix (*native lignin*); or (ii) aiming to obtain lignin in a high yield, in which case the biomass is delignified (pulp process), obtaining a solid fraction rich in cellulose and a liquid one mainly constituted by lignin and hemicelluloses. Afterward, the lignin is isolated by precipitation from the spent liquor (Kraft, organosolv, or lignosulfonate) in yields dependent on the precipitation agent, pH, etc. In these cases, the pulping process leads to an extensive chemical change in the lignin, such as the cleavage of β —O—4' linkages and the formation of recalcitrant C—C bonds, which results in difficult conditions for lignin depolymerization. This lignin presents different characteristics (related to the isolation process) from those of *native lignin* and is called *technical lignin* [35].

There are several pulping processes from which different technical lignins can be obtained [8, 36–38]. The conditions applied depend on the biomass chemical characteristics, particularly the lignin content and composition, but these issues will not be discussed here. Instead, a brief description of the processes is presented in the following lines.

Soda pulping started in 1853 using non-woody biomass such as straw or flax, but now the process has been applied to soft and hardwoods. The delignification is done with sodium hydroxide (NaOH) liquor under temperatures around 160°C. Lignin depolymerization is promoted, starting with the cleavage of β —O—4' linkages, which allows the ionization of phenolic groups, but condensation reactions also occur that are negative for the further lignin valorization. Besides, the lignin-carbohydrate complexes (LCCs) are also broken [18].

Kraft pulping was developed later than soda pulping (around 1890) and has become the most common process involving the delignification of either softwood or hardwood, but also non-woody species, with a white liquor containing sodium hydroxide (NaOH) and sodium hydrosulfide (Na₂S) at temperatures around 170°C [39, 40]. Delignification occurs by the hydroxyl groups (OH⁻) but also by hydrogen sulfide (HS⁻), which, due to its nucleophilic character, enhances delignification. Lignin has been extracted industrially from the black liquor by *LignoBoost* or *LignoForce* technology [41]. It is the most commonly used market process to produce technical lignins [18]. The isolated lignin presents sulfur as thiol groups (which make lignin valorization difficult), is highly condensed, and contains a low amount of ether bonds [8].

Sulfite pulping started in 1930. Biomass is pulped under the attack of sulfite or bisulfite salt of sodium, ammonium, or magnesium at temperatures ranging from 140 to 170°C. The sulfonic groups are incorporated in the aliphatic chain of the lignin monomers, and water-soluble lignosulfonate salt is formed. The *technical lignin* produced is called lignosulfonate, representing 90% of the commercialized lignin [34]. Still, since Kraft pulping is more efficient, the sulfite process has decreased (<5%) [8]. The lignosulfonate: (i) can be isolated by ultrafiltration, extraction, or precipitation; attained as a salt (Na⁺, NH₃⁺, Mg⁺, Ca₂⁺); (ii) presents a large amount of sulfur (4–8% wt compared with Kraft lignin), and has a high number of degraded oligomers and a low amount of β —O—4' [8].

Organosolv pulping appeared in the late twentieth century as a promising process to selectively attain lignin using, for example, methanol or ethanol. The lignin extraction is done by solvolysis with or without the presence of acids (*Brønsted*, e.g., H₃PO₄,

H₂SO₄, AcOH, CF₃SO₃H; or *Lewis acids* such as FeCl₂, ZnCl₂). If the acid is not used, the pH will decrease from 7 to 4 due to the hemicelluloses deacetylation; on the contrary, if the acid is present, the pH can decrease to between 4 and 2; thus, the solvolysis of the biomass is accelerated, and both hemicelluloses and lignin are removed [17].

Ionic liquids (IL) have been used for lignin extraction due to their characteristics (e.g., green and recyclable). Overejo-Pérez and coworkers [42] used the protic ionic liquid 1-methylimidazolium chloride to isolate lignin from *E. globulus* under different conditions to study the treatment severity. More severe conditions promoted: (i) the cleavage of ether linkages, (ii) lignin fractions with higher molecular weight (MW) and more stable due to condensation reactions, and (iii) preferential degradation of S-units, contrasting to mild conditions where the degradation is of G-units.

Deep eutectic solvents (DESs) are promising alternative solvents for IL because they can also be recycled, are biodegradable, easy to produce, and reuse several times without losing strength, depending on the chosen system. Lignin from corncob residues was extracted by DES and used to make LNP by self-assembly [43].

Therefore, there is a great diversity of the *technical lignins* obtained due to the intrinsic variability of the biomasses used and the different extraction processes. As a result, the heterogeneity of the *technical lignins* is a negative point for their valorization. As an example, **Table 2** presents the characteristics of Kraft lignin and lignosulfonates.

Consequently, upgrading solutions must be found so that the concepts of biorefinery, the circular economy, and the zero-waste philosophy can be fully applied. Having in mind these aspects, researchers have treated biomass under mild conditions to attain more uniform lignin products in the so-called *lignin-first* approach (also known as reductive catalytic fractionation (RCF) or catalytic upstream biorefining (CUB)). Under this new biorefinery concept, the lignin is firstly removed from the biomass (instead of the carbohydrates fraction), and the cellulose and hemicellulose fractions are almost intact [44, 45]. Abu-Omar and coworkers [7] present the concept: “*The lignin-first biorefining is not a synonym for lignin valorization, but rather an integral approach that derives value from both lignin and polysaccharides, towards an atom-efficient and more sustainable utilization of lignocellulosic biomass*”. This approach is more selective for lignin; hence, it prevents undesirable and irreversible condensation reactions, eliminates the need for purification steps, and reduces production costs [45]. The traditional methods promote the cleavage of the β—O—4' linkages and the formation of C—C bonds,

Properties	Kraft lignin	Lignosulfonates
Functional groups	Many phenolic OH, carboxyl, catechol groups	Fewer phenolic OH, carboxyl, catechol groups
Organic sulfur (%)	1–1.5	4–8
Sulfonated groups (m _{eq} /g)	0	1.25–2.5
Solubility	Alkali, acetone, DMF, methyl cellosolve™	Water

Table 2. Properties of Kraft lignin and lignosulfonates. Data from Bozell et al. [34].

producing a condensed lignin that is harder to depolymerize in successive steps (**Figure 4**, fragments E and F). To overcome these negative aspects, the *lignin-first* approach involves the application of mild conditions to stabilize the β -O-4' linkages and the low lignin MW products (e.g., monomers, dimers, and short oligomers) formed during the biomass fractionation, preventing lignin condensation [46].

In summary, the *lignin-first* approach has three steps: (i) the lignin is extracted from the biomass by an organic solvent through solvolysis or acid-catalyzed reactions (similarly to organosolv pretreatment); (ii) the intermediates formed are stabilized to prevent condensation, and (iii) the lignin can be further depolymerized during the stabilization stage [7]. The stabilization approaches deliver the target chemical molecules without requiring further chemical modifications [47].

Some biomass fractionation methods applied to obtain uncondensed lignin include (i) ammonium-based fractionation—which can solubilize the lignin at room temperature under a pressure of 7–10 bars [48]; (ii) mild organosolv—using organic solvents (e.g., ethanol, methanol, acetic and formic acids) that are recycled during the recovery of the lignin [49]; (iii) γ -valerolactone-assisted hydrolysis—using γ -valerolactone (GVL, a green solvent that can be produced from glucose) and water (cosolvent), for example, pine wood fractionated by 80% γ -valerolactone at 140–180°C, yields lignin of 33% [50]; or (iv) ionic-liquid-assisted fractionation [51].

Other methods are more focused on stabilizing the lignin monomers; the most studied one is catalytic hydrogenolysis, which combines lignin depolymerization with solvolytic extraction under the reductive stabilization of the intermediates. The hydrogenolysis cleaves the C—O bond at the β -O-4' moieties, generating lignin monomers; the reaction requires a solvent that will be responsible for the lignin

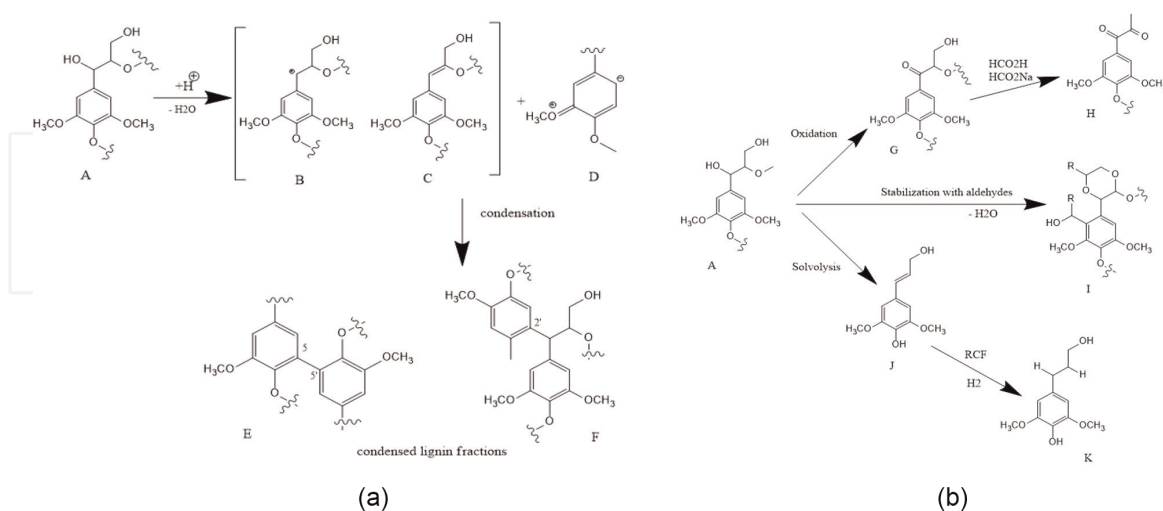


Figure 5.

Lignin depolymerization by acid hydrolysis: (a) hydrolysis and dehydration of the β -O-4' lignin fragment A, leads to fragments B (benzylic carbocation intermediate), C (alkene products from the acid hydrolysis). All these fragments can react, and condensation reactions occur, leading to products E (includes an α -2' linkage) and F (includes a 5-5' linkage). (b) The β -O-4' lignin fragment can be involved in the following reactions: (i) oxidation leading to fragment G (oxidized lignin fragment) that can form fragment H (oxidized monomer), (ii) solvolysis leads to products that can be transformed by reductive catalytic fractionation into fragment K (a reduced monomer), and (iii) stabilization with aldehydes forms the acetal-stabilized lignin fragment (I). Adapt from Questello-Santiago et al. [17].

depolymerization (e.g., methanol or formic acid that will be the H₂ donor), and a metal-containing catalyst such as Pt, Ru, Pd, Ni, or Rh, to prevent lignin condensation [17].

Shuai et al. [52] and other researchers proposed another *lignin-first* strategy to preserve the C_{aryl}-O ether bonds, particularly the β—O—4' moieties, to prevent the formation of a Benzylic carbocation intermediate (**Figure 5a**, fragment B) due to its condensation ability. During lignin extraction, the β—O—4' linkage is stabilized with an aldehyde (e.g., formaldehyde—CH₂O or acetaldehyde—MeCHO) to form the stable acetals, that is, cyclic 1,3-dioxanes (**Figure 5b**, fragment I) that entrap the diol, preventing its dehydration and degradation. This method permits the dissociation between the biomass fractionation from the subsequent depolymerization step, enabling: (i) a broader range of depolymerization methods; and (ii) the optimization of the fractionation and depolymerization steps since they are independent. The advantage is that this produces insignificant condensation reactions, a high yield of monomers, and high selectivity. However, the method presents some limitations for large-scale implementation: (i) it is challenging to find the right balance between the removal of lignin from the biomass and the preservation of the carbohydrates in the pulp; (ii) the separation of the catalyst from the pulp. Both points are seen as a downside of this method. Questello et al. [17] attained a lignin monomeric yield of 42–50%, but it was structurally more complex (**Figure 5b**, fragment G).

Other critical reviews were published in 2020 focused on developments in the *lignin-first* approach [18, 35, 44, 53, 54], in 2021 [7, 38] and in 2022 [45, 55]. One of them emphasizes the fundamental catalytic reactions of the extraction and depolymerization of lignin and posterior stabilization of the phenolic units; the authors also present a brief overview of the possible modifications of the lignin-derived phenols and monolignols, focused on added-value chemicals, polymers, and other developments [53]. Gigli and Crestine [35] present different methodologies for

Fractionation methods	Method	Possible procedure
pH	Alkali soluble lignin: change pH > 12 to 1	<ol style="list-style-type: none"> 1. Sequential lignin precipitation by H₂SO₄ or HCl (room temperature) 2. Precipitation with acid and separation of the insoluble part 3. Separation by pressure at high temperature
Solvent/water extraction	Partial solubility in different solutions (organic solvents/water)	<ol style="list-style-type: none"> 1. Ethanol/water 2. Acetone/water 3. Tetrahydrofuran (THF)/water 4. Acetic acid (ACOH)/water (room temperature) 5. AcOH/water (high temperature & pressure) 6. γ-valerolactone (GVL)/water
Solvent extraction	Partial solubility in organic solvents	Alcohols, chloroform, ether, dimethyl sulfoxide (DMSO), dichloromethane (DCM), THF, hexane, ketones
Ultrafiltration/membrane	Physical filtration	<ol style="list-style-type: none"> 1. Ceramic filters 2. Polymeric filters

Table 3. Methods for the fractionation of lignin. Adapted from Sadeghifar and Ragasukas [56].

lignin fractionation by (i) solvents (most common)—using solvent mixtures or under a sequence of solvents; (ii) membranes—based on ultra- and nanofiltration using ceramic or polymeric membranes; (iii) precipitation of the re-dissolved lignin in a binary solvent system; and (iv) pH-mediated gradient precipitation (**Table 3**). The lignin-membrane-based approach makes the separation into cuts of lignin with a defined molecular weight (MW) and narrow polydispersity, while the goal of the solvent-based approach is to obtain fractions with distinctive physico-chemical properties. However, both parameters (MW and structure/functionality) appear to be closely interdependent, since low MW fractions: (i) are typically soluble in polar solvents; (ii) possess high phenolic content and condensed structures; and (iii) have increased antioxidant activity. On the other hand, the higher MW fractions present fewer modified lignins but have a lower amount of aromatic hydroxyl groups and condensing units. The authors also highlight the applications of fractionated lignin, keeping in mind their characteristics. For example, lignin with high MW attained from *P. radiata* Kraft black liquor was successfully used to produce phenol-formaldehyde resins after methylation and demethylation (to increase the lignin reactivity) [57].

The main lignin applications studied so far are: (i) polymers production such as composites (17%), thermosets (8%) and thermoplastics (4%); (ii) properties improvement involving antioxidant activity (15%), standards generation (6%), antimicrobial activity (4%) and color reduction (2%); (iii) micro and nano-structures such as fibers (12%) and particles (6%); (iv) others (14%) and finally (v) structure modification (12%) [35].

A state of the art on lignin valorization strategy was presented by Liu et al. [18], who discuss the production of *technical lignins* (giving a resumé of the fractionation methods such as Kraft or organosolv delignification), describe conventional methods for lignin catalytic depolymerization (e.g., pyrolysis, solvolysis, catalytic oxidative and reductive depolymerizations), plus the emerging strategy of the *lignin-first* approach, and the methods to improve the yield of the lignin phenolic products. Karlsson et al. [54] present a study where green solvents are used to obtain high yields of the fraction's hemicelluloses lignin and cellulose. The biomass was treated by supercritical water extraction to remove the hemicelluloses (liquid fraction, characterized by a partial hydrolysate of the glycosidic bonds, but preserving the native structure of the oligomers). Then the solid fraction was treated with aqueous ethanol supercritical extraction catalyzed with H₂SO₄ at 160°C to remove the lignin (liquid) and the solid rich in cellulose (with crystallinity preserved after both treatments). Overall, the proposed method combines active stabilization with physical lignin protection, minimizing the lignin condensation reactions. The potential uses of each fraction were discussed: hemicelluloses and cellulose could be used for ethanol production or hydrolyzed to monomeric sugars for chemical applications; the lignin could be used directly for polymers production or be further depolymerized to the monomeric platform [54]. Abu-Omar and coworkers [7] discuss the importance of biomass diversity and the analytical methods used for its characterization (e.g., analytical pyrolysis, NMR), but also present the big picture of the *lignin-first* approach, from feedstock to reactor design, the importance of the catalyst, the mass balance and yields, and finish with an outlook on the development of *lignin-first* biorefinery [7]. Korányi et al. [44] presented an extensive list of the works done under the *lignin-first* approach from 2018 to 2020, focusing also on the studies on wood digestion/conversion from 1940 to 2014, mentioning the different feedstock, conditions, and products obtained. As mentioned before, the

Lignin chemistry	Possible procedure
Base-catalyzed reactions	<ol style="list-style-type: none"> Alkaline conditions enable: (i) cleavage of lignin-carbohydrate (LCC) bonds; (ii) cleavage of ether bonds \Rightarrow lignin fragmentation; Distinctive reactivity of $\beta-O-4'$ bonds, cleaved on: (i) phenolic units (free phenolic OH groups) \Rightarrow easily cleaving to depolymerize the lignin (the unit is transformed into a quinone methide (if the $C\alpha$ has a suitable leaving group like $-OH$ or $-OR$). Quinone methide can undergo nucleophilic attack by HS^- (Kraft pulping), with the prevailing pathway of $\beta-O-4'$ cleavage and formation of an episulfide intermediates (can undergo some reactions forming coniferyl alcohol and others \Rightarrow prone to degrade and repolymerize); (ii) non-phenolic units (etherified phenolic OH groups) \Rightarrow $\beta-O-4'$ bonds are slowly cleaved and involve the formation of an epoxide intermediate; Third pathway involves the elimination of the $C\gamma H_2-OH$ group (retro-aldol reaction) \Rightarrow formation of an enol ether structure (alkali stable) and formaldehyde (C_2HO, during soda pulping). In this case, ether bonds are cleaved but with less efficiency. C_2HO induces repolymerization via formaldehyde-phenol type of condensation. <p>Disadvantage: possible lignin degradation and repolymerization.</p>

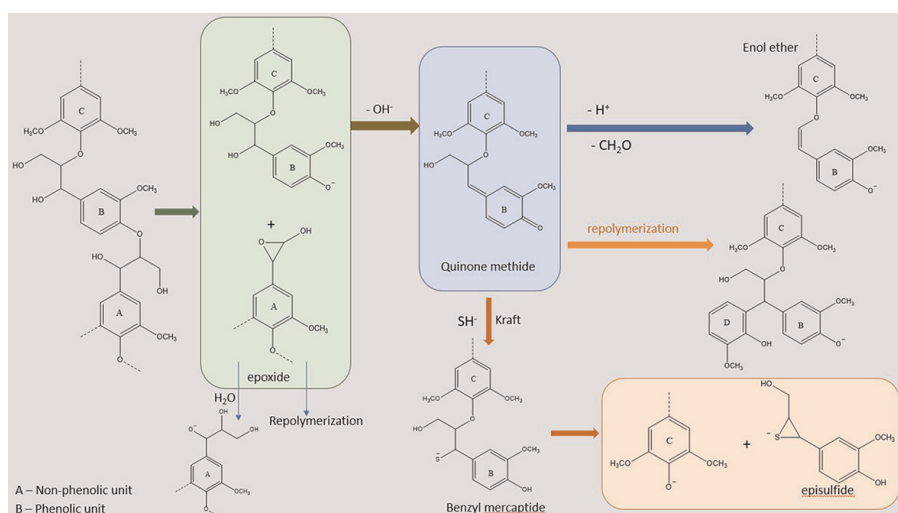
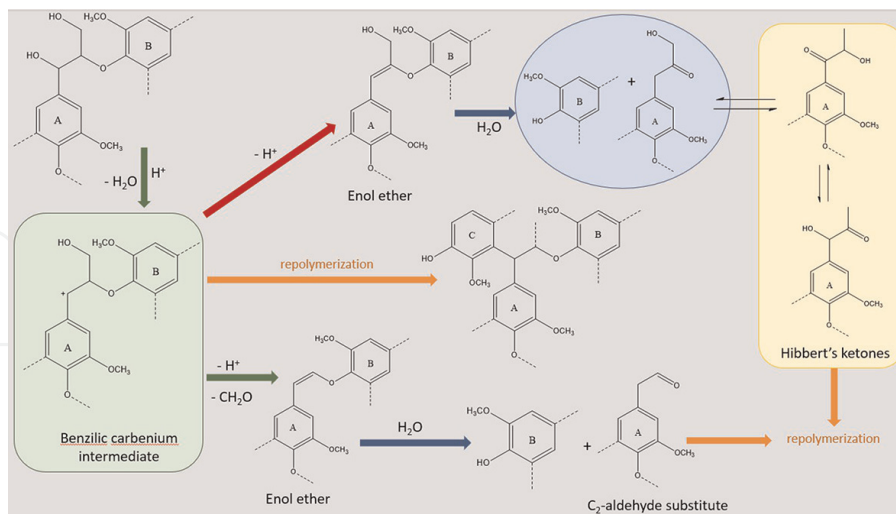


Table 4. Possible chemical reactions during lignin fractionation by base solvents. Adapted from Schutyser et al. [8].

characteristics of the technical lignins depend on the methods used to isolate or depolymerize the lignin. Therefore, **Tables 4–6** present a resumé of the lignin chemistry during the different processes.

Lignin chemistry	Possible procedure
Acid-catalyzed reactions	<ol style="list-style-type: none"> Promote hydrolysis of ether bonds in cellulose and hemicelluloses, but also the acidolysis of lignin \Rightarrow depolymerization and repolymerization reactions; Cleavage of ether bonds ($\beta-O-4'$) \Rightarrow removal of OH in $C\alpha$, formation of a benzylic carbenium ion that can be transformed into two enol-structures with or without the cleavage of $C\beta-C\gamma$ bonds and concurrent formation of formaldehyde; Using H_2SO_4 promotes enol-structure without the bond $C\beta-C\gamma$; using HCl or HBr \Rightarrow enol-structures formation; The hydrolysis of the enol-structures leads to C2-aldehyde-substitutes or C3-ketone-substituted phenolics (Hibbert's ketones). <p>Disadvantage: Hibbert's ketones structures and carbenium ions can produce condensed structures \Rightarrow lignin condensation.</p>

Lignin chemistry Possible procedure



Reductive reactions

1. Redox catalyst & combination with H₂ or H-donors ⇒ targets: cleavage of ether bonds (β-O-4' and α-O-4') and side chain OH groups;
2. Other pathways result in: (i) hydrogenolysis of ether bonds; (ii) removal of benzylic OH-groups in C_α and possibly in C_γ ⇒ formation of substituted methoxyphenols and small fragments (e.g. phenol). Secondary reactions can occur, such as hydrogenation/hydrogenolysis ⇒ production of cyclohexanols and cycloalkanes.

Advantage: prevents the formation of reactive functional groups ⇒ avoiding condensations or repolymerization.

Disadvantage: inability to cleave C—C bonds ⇒ degree of depolymerization is linked to the amount of ether bonds that can be cleaved.

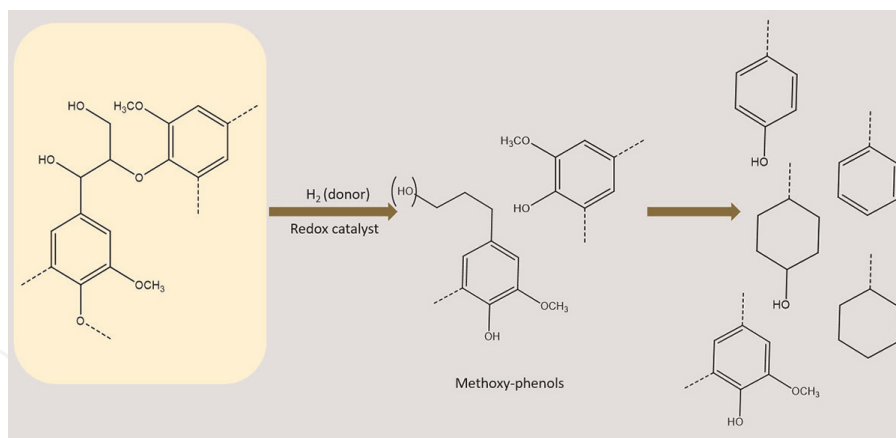


Table 5.

Possible chemical reactions during lignin fractionation by acid solvents and reductive reactions. Adapted from Schutyser et al. [8].

Despite all the work developed so far, there are still some issues to be overcome for the large-scale implementation of the *lignin-first* approach, such as that (i) the lignin fractions are correlated with the native lignin, that is, depend on the biomass used, so obtaining fractions with high reproducibility is an issue; (ii) the fractioning process is usually done in a batch, but a continuous process is more favorable for industrialization and to use the black liquor directly from the pulping industry [35].

Lignin chemistry	Possible procedure
Oxidative lignin chemistry	<p>1. Pathway: (i) fragmentation of the side chain (cleavage of Cα—Cβ of Cα—C4\Rightarrow unstable structures are formed under alkaline conditions, thus can be converted to aliphatic carboxylic acids and the aromaticity is retained; (ii) the aromatic ring is cleaved \Rightarrow yields aliphatic carboxylic acids.</p> <p>Advantage: cleavage of C—C bonds (instead of ether bonds, as in the other reactions mentioned).</p> <p>Disadvantage: the reaction follows a radical mechanism, so radical coupling of lignin fragments takes place \Rightarrow producing biphenyl structures (condensed structures).</p>

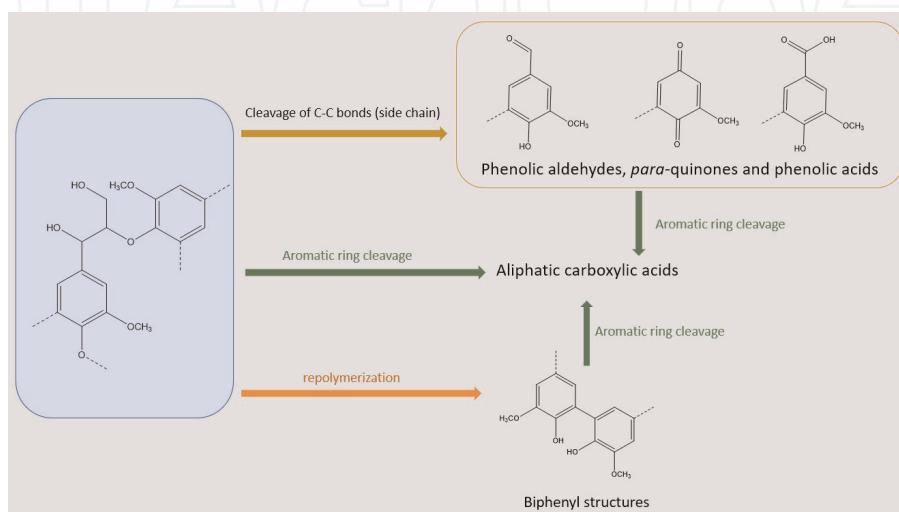


Table 6.
 Possible chemical reactions during lignin fractionation by oxidative reactions. Adapted from Schutyser et al. [8].

4. Lignin applications: focus on nanoparticles production

Lignin is an exciting polymer due to its nontoxicity and bio-based renewable nature, making it a sustainable feedstock that does not compete with food chain production [58], and recent technologies to recover lignin and its conversion into valuable compounds have been reviewed [59]. However, lignin applications on a large scale present some limitations due to lignin's poor solubility in water and complex macromolecule structure [35].

However, many efforts have been made; for example, the *Borregaard company* commercializes tailor-made lignin that can be used in wide-ranging industries, as a binding, dispersing agent, but also as an emulsion stabilizer (<https://www.borregaard.com/product-areas/lignin-biopolymers/>). The *Bloom company* uses aldehyde-assisted fractionation to obtain a stabilized lignin, delivering lignin as a biopolymer with known characteristics that can be used for the production of nutraceuticals, food additives, or even cosmetics (<https://www.bloombiorenewables.com/>).

Despite these good examples of lignin valorization, in both cases, the lignin is tailor-made, but what about the other lignins produced on a laboratory scale but with high diversity? They have not yet reached the market, in particular because the lignin is obtained in low yields, with structural diversity, and some processes also use dangerous solvents. So, the target of many researchers has been to modify the lignin, or to develop lignin nanoparticles (LNPs), that is, to obtain lignin in the form of aqueous nanoparticles with uniform size and shape, showing better characteristics

such as a higher surface area and stability in an aqueous medium, that are non-toxic (in reasonable concentrations), biodegradable (either by bacteria but also fungi), and present bioactive properties, which can replace the commercial lignin [60].

Furthermore, LNP has functional groups that can be chemically modified, increasing their applicability, and can be applied in a wide range of applications [61–92]: (i) encapsulation with biocides or anticancer drugs [62–68], (ii) incorporated in coatings for wood UV protection [69], or in polymeric matrices to enhance mechanical properties (e.g., strength and toughness), barrier properties and thermal stability [70, 71], and (iii) in the agriculture field [72]. **Table 7** presents some LNP applications based on the starting technical lignin.

Still, some limitations have to be overcome, and LNP application at the industrial level has not yet been achieved due to diverse factors. The *first* one is about lignin's variability in nature that is quite heterogeneous (as already mentioned in this chapter); therefore, its isolation will produce a mixture of products, apart from being a challenging task and less economically feasible to purify all the different compounds for further applications [45, 71]. The *second* point is related to the lignin fractionation methodologies that yield complex condensed lignin, which limits its high-value applicability [71, 94], difficulties in attaining lignin in high yield and with good chemical and physical properties (e.g., molecular weight distribution, solubility, reactivity and number of functional groups), and with quality (in relation to the inter-units linkages, condensed lignin is not attractive) [43, 95]. The *third* point is associated with LNP production and their adequate stabilization for different applications [96]. This last point will be developed in the following paragraphs.

Technical lignin nanoparticles	Applications	Ref
Soda lignin	Packaging, agriculture	[73]
	Wastewater treatment	[74]
	Biomedicine	[75, 76]
	Coatings	[77]
	Cosmetics	[78]
Alkali lignin	Cosmetics	[79]
	Wastewater treatment	[80, 81]
	Biomedicine	[82, 83]
	Coatings	[84]
Kraft lignin	Emulsion stabilization & biocatalyst	[85, 86]
	Biomedicine	[62, 63, 87, 88]
	Wastewater treatment	[89]
	Dispersants	[90]
Organosolv	Nanocomposites	[91, 92]
	Cosmetics	[78]
Hydrolytic lignin	Paint and coating	[69]
	Enzyme immobilization	[93]

Table 7.

Possible applications of the different nanoparticles produced from technical lignins. Adapted from Ekielski and Mishra [38].

4.1 Techniques used for the synthesis of LNP

Kumar et al. [58] present an interesting resumé of the several strategies used to produce lignin micro and nanoparticles. Diverse techniques can be used and include: shifting methods (by pH or solvents) [97], a template-based synthesis technique (or polymerization), aerosol process, electrospinning, supercritical fluid processes, solvent antisolvent precipitation [98], or acoustic cavitation. A resumé of techniques to attain LNP is presented below.

Self-assembly—is the most common method to produce LNP; it starts by dissolving the lignin in an organic solvent and by adding the antisolvent (usually water) or, during dialysis, the particles start to form in a self-assembling process.

Antisolvent precipitation—first the lignin is dissolved in a solvent, such as: acetone solution (9:1, v/v) [99]; tetrahydrofuran (THF) [62]; dimethylsulfoxide (DMSO) [92]; dimethylformamide (DMF) [100]; and aqueous sodium *p*-toluenesulfonate (APS) [101]; then this solution is added slowly to an antisolvent (usually water, where the lignin is insoluble) to form the LNP. It is important to choose properly the solvents and antisolvents for the formation of LNP; good miscibility between the lignin and the solvent is the best choice. Solvents with good miscibility and a low boiling point lead to the formation of smaller size LNP and with a spherical shape, but if the solvent has poor miscibility and the antisolvent has a higher boiling point, the resulting LNPs do not have a uniform shape and are larger [58]. But other parameters such as the lignin concentration in the solvent, antisolvent temperature, and stirring rate are also important [58].

pH shifting—this technique has similarities with the posterior technique; but here the pH is changed from acid to base or vice versa. Lignin solubility changes with the pH of the solution, and precipitates. As examples of these methods: (i) the lignin is dissolved in NaOH at pH 12 and by HNO₃ the LNPs precipitate [102]; (ii) the lignin is dissolved in ethylene glycol and the LNPs are precipitated by adding hydrochloric acid [95]. The use of an acid to precipitate the LNP makes the process more expensive and dangerous, but the acid can be recovered, and the method has the advantage of attaining well-defined LNP with a good shape and stability [58].

Solvent exchange or solvent shifting—this technique involves a dual system of solvents, an organic solvent (where the lignin is dissolved), and water (in excess allowing the formation of LNP due to the decrease of lignin solubility in water at pH neutral). The LNP size ranges from nanometer to hundreds of micrometers with spherical-shaped particles [58]. Suitable solvents are: THF, acetone, acetone/water, and DMSO [58]. This is similar to the antisolvent precipitation methods, but it is the excess of water that makes the LNP; in the other method, it was the pH shifting. For the solvent exchange method, the dissolved lignin is put in dialysis bags immersed in water and the LNPs are formed during the dialysis process. LNPs were produced by dialysis of a mixture of lignin solution and oleic acid coated with Fe₃O₄ [62]. The need for dialysis bags increases the costs of this method (**Table 8**).

Supercritical fluid (SCF)—this is a technique widely used in the pharmaceutical industry. Generally, the supercritical fluid used is CO₂ due to its diverse advantages (abundance, nontoxicity, low cost, and not flammable). This technique has different names depending on the conditions and the fluids used: supercritical antisolvent (SAS), supercritical gas antisolvent (GAS) are just two examples; LNPs were prepared by SAS, using acetone (solvent) and CO₂ (antisolvent) from poplar organosolv lignin; because the solubility was enhanced, the LNP formed (0.144 μm) presented higher antioxidant capacities compared with the starting lignin [103].

Method	Advantages & disadvantages
Antisolvent precipitation	Advantages: good lignin dissolution; low-cost solvents; LNP synthesis with adequate size and morphology. Disadvantages: some solvents are expensive, and others are toxic or difficult to recover.
Solvent exchange or shifting	Advantages: high lignin dissolution; some solvents can be recovered (in a rotavapor almost 95% of acetone can be recovered), but others are more difficult (e.g. DMSO, high boiling point (189°C)); high yield of LNP production; use of water as antisolvent ⇒ low cost. Disadvantages: high consumption of solvent ⇒ high cost; choosing the best affordable solvent is essential. Recovery is difficult; dialysis bags are expensive. Low yield of LNP, and difficulty in controlling their morphology.
Supercritical fluid	Advantages: industrial scale-up; able to control the size and shape of the nanoparticles easily (by changing the parameters); high yields. Disadvantages: lignin solubility in supercritical CO ₂ ⇒ add methanol or ethanol to overcome this; need expertise to run with the equipment, sophisticated laboratory facilities; expensive maintenance.
Ultrasonication	Advantages: fast, easy and green synthesis of LNP, low requirements of equipment ⇒ low cost; is harmless to the environment. Disadvantages: low yield of LNP, particles with irregular morphology and shape.
Interfacial crosslinking, Polymerization and Nanoprecipitation	Advantages: good lignin dissolution; LNP produced with desired morphology and sizes. Disadvantages: use expensive and toxic solvents (epichlorohydrin, hydrogen peroxide); removability of the templates is an issue.
Aerosol flow	Advantages: one step, high yields and no need for antisolvent. Disadvantages: use expensive solvents and some are toxic.
Electrospinning	Disadvantages: associated with physicochemical properties and the solubility of lignin.
Biological pathway	Advantages: simple and low-cost production of LNP; use of green solvents. Disadvantages: low yield of LNP production and with irregular morphology and shape (usual macro).

Table 8.

Some advantages and disadvantages of the methods used for the synthesis of LNP. Adapted from Schneider et al. [60] and Kumar et al. [58].

Aerosol process—this is a single-step method for the production of nanoparticles applied in biomedical applications and was first presented by Eerikäinen et al. [104]; it consists of an atomizer that works continuously to generate an aerosol. This aerosol passes through a heated tube connected to a low-pressure impactor where the particles formed are collected. Researchers were successfully able to produce LNPs with sizes ranging from 30 to 2000 nm [105].

Ultrasonication or acoustic cavitation—the lignin is an aqueous solution and this suspension is sonicated by changing the intensity, time, and temperature; then the sample is dried under mild conditions [106]. These authors used two commercial alkali lignins suspensions to produce efficiently nanosized particles (spherical shapes

and homogeneous). If this technique is used alone, the LNPs formed are not uniform in terms of size or the particle size distribution, which are dependent on the ultrasound conditions. Some researchers have overcome this by producing the LNP by solvent exchange combined with ultrasound. This combination is advantageous because the LNPs are rapidly formed and easily detached by centrifugation.

Electrospinning—this is a popular technique for the production of nanomaterials; the lignin is dissolved in a suitable solvent and the mixture is put in a syringe, pumped through a nozzle with reduced size (diameter of around 100 μm) that jets the liquid into a collector plate, creating a nanofiber matrix; an electric field is used with voltage of 100–500 kV/m to create the nanofibers (10–25 cm). Technical lignins (softwood Kraft, hardwood Kraft, and sulfonated Kraft lignins) were mixed with PEO (polyethylene oxide) at different percentages (1–5 wt%) and obtained fibers by electrospinning [107]; the incorporation of PEO improved the fibers produced (more uniformity) at lower viscosities. However, this technique has limitations associated with the lignin properties and it was suggested that electrospraying would be more favorable for nanoparticles production [58].

Interfacial crosslinking—this is not a method for LNP, but is better for their stabilization, generating particles and capsules. The process involves lignin emulsification, usually in an oil-water phase, where the addition of a cross-linking agent improves the lignin cross-link. First, the lignin is dissolved in an alkaline solution that will activate the OH chains and generate a dispersed phase; or it will be dissolved in a solvent with a surfactant such as Span80[®], 1-pentanol, or Tween 80[®]. An oil/water microemulsion is prepared and emulsified to the solubilized lignin, creating the lignin cross-linkages with the water/oil interface. The next step is to add the cross-linking agent, which can be toluene diisocyanate, thiol, or epichlorohydrin [60].

Polymerization—this is similar to the cross-linking method. The lignin has to be modified or some groups activated so that they can be grafted onto a surface that has an affinity to the lignin. As examples of the application of this technique: lignin nanotubes were produced in an alumina membrane that was dissolved in phosphoric acid [108]; lignin nanoparticles were produced by lignin being grafted onto PDMAEMA (2-(dimethylamino)ethyl-methacrylate) and PDEAEMA (2-(diethylamino)ethyl methacrylate) [109, 110].

Biological pathway—this is not a common technique for LNP synthesis, so far. LNPs are produced by microorganisms or by enzymes (formed by either bacteria or fungi). Examples: (i) enzymes were used to break the linkages between lignin and cellulose in *Luffa* fibers, producing lignin-derived particles with cuboidal shape and size around 20–100 nm [111]; (ii) lignin was extracted by coconut fibers (soda pulping), the lignin obtained was hydrolyzed using *Aspergillus* sp. Nanolignin was synthesized by different techniques at distinct yields: homogenization (81%), ultrasonication (64.3%), and microbial (58.4%) [76]; (iii) the colloidal stability of LNP was improved using fungi species [87].

Table 8 presents some of the methods mentioned and their strengths, as well as some of their weaknesses. The comparison was made regarding the type of solvents used (if environmentally-safe, cost), but also regarding the process time, LNP morphology, and size. Generally, solvents such as acetone, dioxane, THF, and DMSO are used in medium volume, purchased, and are relatively affordable. The use of hazardous reagents is a negative point. Some studies include green solvents such as levulinic acid that had high efficiency in dissolving Kraft lignin (up to 40%) compared with other carboxylic acids, and the lignin dissolved can be regenerated by adding an

Method	Method resume	Morphology & size	Potential applications	Ref
Antioxidants				
Antisolvent	<ul style="list-style-type: none"> Lignin dissolved in acetone: water (9:1, v/v) Dissolved lignin precipitate in water 	Spheres 104 nm	Properties: antioxidant and UV-protection ⇒ food, pharmaceutical, cosmetic industries	[73]
Supercritical antisolvent (SAS)	<ul style="list-style-type: none"> Organosolv lignin used to prepare nanoscale lignin by antisolvent precipitation using acetone (solvent) and supercritical carbon dioxide (antisolvent) SAS did not degrade the lignin, but increased its solubility (12.4 times) ⇒ enhanced the antioxidant properties and free radical scavenging activities 	Spheres 0.144 μm	Nanoscale lignin presented higher antioxidant activities than starting lignin ⇒ animal husbandry, food processing and pharmaceutical industries	[103]
Antisolvent	<ul style="list-style-type: none"> Lignin dissolved in ethylene glycol Precipitation in HCl 	181 nm	Reinforcing agent in polymers and nanocomposites	[119]
Nanocomposites				
Self-assembly	<ul style="list-style-type: none"> Alkali lignin (100 g) dissolved in dioxane (1 L) Adding cyclohexane (100 mL) ⇒ flocculation and precipitation of lignin (formation of lignin reverse micelles—LRM, separated and dried by vacuum) 	Micelles 120 nm to micrometers	Properties: UV-absorbance and good miscibility with plastics such as high-density polyethylene (HDPE) ⇒ suitable for blends with HDPE, improving mechanical properties	[120]
Aerosol flow reactor	<ul style="list-style-type: none"> Different lignins: Kraft (KL), organosolv (OS) and alkali lignin (AL) AL dissolved in water, KL and OS dissolved in dimethylformamide (DMF) – concentrations 0.5–2% Drops were generated in a Collision-type jet atomizer with nitrogen gas, carried to a heated laminar flow reactor, collected as solid particles and dried Solid particles were fractionated with a Berner-type low-pressure impactor ⇒ size fractionation (30 nm to 2 μm) 	Spheres 30 nm–2 μm	Stabilizes oil-in-water Pickering emulsions	[105]
Electrospinning	<ul style="list-style-type: none"> Preparation of polyethylene oxide solution with acetonitrile (10% vol) and sodium hydroxide (0.1 N) stirring overnight; then addition of soy protein 	Nanofibers 124–400 nm	Composite development	[121]

Method	Method resume	Morphology & size	Potential applications	Ref
	<p>under stirring (4 h) until complete dissolution; only then Kraft lignin in powder was added slowly; stirring further for 4 h</p> <ul style="list-style-type: none"> • Polyethylene oxide (coadjutant) was used to facilitate the formation of nanofibers with high quality 			
Delivery systems/encapsulation				
Antisolvent	<ul style="list-style-type: none"> • Nanoparticles (NP) production by two methods: • Low-sulfonated lignin (Indulin® AT, IAT, 0.28 g) dissolved in ethylene glycol (ET, 50 mL) and precipitation by adding diluted HCl (8 mL, 0.025 M) ⇒ yield NP stable at wide range of pH (1–9). NP stability due to the stacking of uncharged lignin in ET upon precipitation • IAT dissolved in high-pH aqueous solution (NaOH, 1 N, pH 12) and acidic precipitation with HNO₃ (0.25 M, 900 µL) ⇒ NP stable at low pH (dissolve at pH > 5) 	100 nm to micrometers	NP highly porous ⇒ promising vehicles for drug delivery, stabilizers of cosmetics and pharmaceutical preparations, and sorbents for heavy metal ions and other pollutants	[98]
Antisolvent	<ul style="list-style-type: none"> • Indulin AT lignin dissolved in ethylene glycol and precipitation in HCl • LNP infused with silver ions and coated with poly-(diallyldimethylammonium-chloride- PDAC) to boost the adhesion to microbial cell membranes 		LNP infused with silver ions and coated with cationic polyelectrolyte (PDAC) layer ⇒ synthesis of biodegradable cores that have high antimicrobial activity	[102]
Self-assembly	<ul style="list-style-type: none"> • Alkali lignin was acetylated, dissolved in tetrahydrofuran (THF) • LNP formation by adding water, then the THF was evaporated in rotavapor to attain colloidal spheres 	Colloidal lignin spheres	LNP for drug delivery, microencapsulation of pesticides	[122]
Interfacial crosslinking	<ul style="list-style-type: none"> • Lignin dissolved with sodium chloride in water, mixed with cyclohexane containing polyglycerol polyricinoleate (surfactant); • Ultrasonification to produce an emulsion by adding toluene diisocyanate (TDI) 	Hollow nanocapsules 150–200 nm	LNP encapsulated with hydrophilic compounds (e.g. fertilizers, pesticides and drugs), that can be released by enzymatic degradation ⇒ LNP suitable for agricultural purposes	[123]

Method	Method resume	Morphology & size	Potential applications	Ref
Interfacial crosslinking	<ul style="list-style-type: none"> • Creation of an emulsion of water, oil and lignin • Ultrasonic technology to create cross-linkages between lignin and the water/oil interface 	Sphere lignin micro (LMC) and nanocapsules (LNC) 300 nm to 1 μ m	Lignin capsules (LMC/LNC) encapsulated with oil \Rightarrow potential use for biomedical purposes	[66]
Aerosol assisted self-assembly	<ul style="list-style-type: none"> • Alkali lignin was dissolved in dioxane, dried in rotavapor (after removal of insoluble fraction), and dissolved in DMSO at different concentrations • DMSO solutions were sprayed by ultrasonic nebulizer dispersed in liquid nitrogen upon a cooled Cu-plate and collected as frozen drops 	Hollow solid lignin colloidal nanoparticles without chemical modifications 96–193 nm	LNP have UV absorbing ability \Rightarrow sunscreen cosmetics; drug delivery application	[84]
Polymerization	<ul style="list-style-type: none"> • Lignins isolated by different methods: sulfuric acid (Klason lignin), thioglycolic acid, phosphoric acid, sodium hydroxide • Lignins reacted with amine groups on the nanopores surface of the alumina membrane template \Rightarrow dehydrogenation of lignin • Deposition of multiple layers of lignin monomers onto base layer • Dissolution of alumina templates in phosphoric acid to obtain lignin nanotubes • LNT can be tailored according to the application 	Lignin nanotubes (LNT)	DNA can adsorb to LNT \Rightarrow useful for genetic delivery into human cells. LNT from NaOH-extracted lignin were quite interesting (short in length, nucleus easily penetrated \Rightarrow enhance the delivery of DNA)	[108]
Self-assembly	<ul style="list-style-type: none"> • Kraft lignin (KL, 1 g) dissolved in ethanol (ET, 50 mL) and then ultrasound to accelerate the dissolution (2–3 min), site for 72 h \Rightarrow insoluble separated by centrifugation, supernatant filtered by 0.45 μm syringe • LNC formed by adding water to the solution KL/ET 	KL nanocapsules (KLC)	KLC can be applied in a wide range of applications	[124]
Biological pathway	<ul style="list-style-type: none"> • Dried fruit of <i>Luffa cylindrica</i> was extracted with acetone, ethanol and toluene (1:1:4) for 6 h in soxhlet apparatus • Extractive-free fibers were milled and soaked in acetate 	LNP cuboidal shape 20–100 nm	LNP potential applications for wide range of fields from pharmaceutical, automobile and polymer industries	[111]

Method	Method resume	Morphology & size	Potential applications	Ref
	buffer (pH 4.5, 1–2 h) and were tripled enzymatic treated with MultifectCX GC Cellulase (10%), Multifect Pectinase FE (15%), Optimase CX 255 L (15%) to produce cuboidal lignin nanoparticles			
Antisolvent	<ul style="list-style-type: none"> Wheat straw alkali lignin, without any modification was dissolved by ionic liquids (e.g. [Emim][Ac] at concentration 5– 200 mg/mL, stirred at room temperature) Water added (speed 2–6 mL/min to 80%) to produce lignin nanospheres stable at pH between 4 and 10 LNS were freeze-dried and stored; LNS were stable in water without aggregation within 60 days (stability attributed to an electrical double layer formed by the phenolic OH and possible carboxylic groups that repulse nanospheres from each other) 	Lignin nanospheres (LNS)	LNS with potential for coatings, drug delivery and nanocomposites production	[117]
Self-assembly	<ul style="list-style-type: none"> Isolation of lignin from corncob residues by choline chloride/lactic acid-based deep eutectic solvent (DES) Lignin was dissolved in acetone/water solution (4:1, v/v), filtrated to remove undissolved lignin Water was added (antisolvent) under moderate stirring ⇒ LNP production with high stability 	LNP sphere structure, with small size, well-defined shape 48.66–183.7 nm		[43]

Table 9.
 Compilation of some potential uses and methods for lignin nanoparticles production.

excess of water, maintaining the morphological characteristics and the thermal stability of the lignin [112].

Researchers are able to produce LNP with a range of shapes, such as nanoparticles [62–64], nanotubes [108], nanospheres [113], nanofibers [114], nanocapsules [115], nanomicelles [85], nanofilms [116]; and sizes (micrometers and nanometers). This high diversity of shapes and sizes contrasts with a high demand for uniformity for

specific applications, which requires reproducibility at industrial-scale production [58]. According to Zhang et al. [43], within a certain lignin concentration range, the particle size increased with increasing lignin concentration. A higher concentration of lignin in the system means more lignin available for the growth of the nanospheres (LNS); a high stirring rate of aqueous phase and ionic liquids produces smaller particles. Also, the dropping speed of water affects the diameters of the LNS, where the increase of the water dropping speed from 2 to 6 mL/s decreased the LNS diameter [117]. Besides these aspects, the feasibility of LNP production depends on the scale-up, manufacturing costs, and applications. Having this in mind, Assis and coworkers [118] carried out a techno-economic study to evaluate the costs of lignin production, the costs of LNP synthesis, and their applications. **Table 9** presents a list of LNPs produced by different methods and compiled by their possible applications.

5. Conclusions

This chapter includes an overview on lignin and lignin nanoparticles. Lignin is a complex natural polymer with characteristics that contribute to the development and growth of plants. Lignin provides rigidity, hydrophobicity, and protection against biotic agents and plays an important role in the plant's adaptability to the environment. Plants can produce different monomers as a response to stress (e.g., drought, high or low temperature, and light).

As a natural polyphenol polymer, lignin presents high variability associated with the diversity of plants, which creates great challenges for its recovery and valorization.

There are several methods to deconstruct the biomass and obtain the so-called *technical lignin*. These approaches present some advantages, the major one being to obtain a pulp (a valuable product). However, from the lignin point of view, there are several disadvantages: lignin is highly condensed, difficult to recover and process for subsequent valorization. Aiming to change this, a new perspective has been used, the *lignin-first* approach. Here, lignin is obtained first to prevent adverse reactions and be easier to valorize under the scope of the biorefinery. The *lignin-first* approach includes different methods also due to lignin's natural diversity, and it is still hard to attain lignin both in high yield and with good characteristics for different purposes.

The production of lignin nanoparticles (LNPs) started, at the beginning of 2000, because the nano-size improves the lignin characteristics (e.g., increased antioxidant activity). LNPs can be produced by different methods, where a diverse range of sizes and shapes can be obtained and tested for multiple applications. However, there are some aspects that still have to be improved so that LNP can be scaled up (e.g., costs of lignin isolation and LNP production, use of more green solvents).

Undoubtedly, lignin is an unfinished theme for research due to its chemical characteristics and endless potential applications.

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Conflict of interest

The authors declare no conflict of interest.

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