Chapter 1
Properties, Chemical Characteristics and Application of Lignin and Its Derivatives

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1.1 Occurrence of Lignin in Biomass

1.1.1 Source, Monolignol Constituents and Sub-unit Structures

The term 'lignin' is used to describe complicated and undefined phenolic biopolymers that bind together with cellulose and hemicelluloses to form plant cell wall structures [1]. As one of the three major constituents in lignocellulosic biomass, lignin makes up between 15 and 40 % of dry mass fraction in natural woody plants [2, 3]. With high molecular weight in the range of 100 kDa, lignin is a three-dimensional heterogeneous macromolecule containing many phenylpropanoid units that are the oxidative polymerization of three types of hydroxycinnamyl alcohol sub-units (monolignols) [3–5]. The monolignols are the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) phenylpropanoid units with structural differences in the extent of methoxylation at the 3′ or 3′–5′ position of phenolic rings (Fig. 1.1). The complex inter-molecular structure of lignin is due to the combination of different

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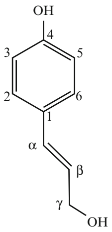
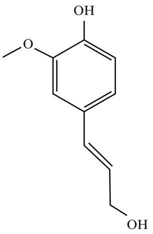
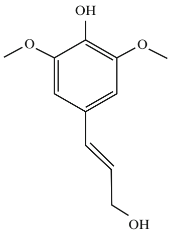
	Structure of monolignols		
	4-hydroxyphenyl (H)	Guaiacyl (G)	Syringyl (S)
			
Source	**Content (%, *w/w*) [8]**		
Softwood lignin	-	90-95	5-10
Hardwood lignin	-	50	50
Grass lignin	5	75	25

Fig. 1.1 Three sub-units of lignin and their relative content in lignocellulosic biomass

amounts of monolignols and distinct substitution patterns on their phenylpropanoid units [6, 7].

Lignin biopolymers contain a variety of ether and carbon-carbon inter-molecular linkages or bonds, such as β-O-4, 5-O-4, β-5, β-1, β-β, and 5–5 (Fig. 1.2) [4, 5, 8]. The predominant β-O-4 ether linkage type (also called arylglycerol-β-aryl) has proportions of 40–60 % among all inter-unit linkages in lignin [9]. Therefore, they commonly act as the major targets for tracking structural changes that take place during lignin fractionation or de-polymerization. It has been proposed that lignin biopolymers are not random, but have a helical structure characteristic of naturally synthesized molecules [10]. Various inter-molecular linkages between different phenylpropane sub-units contribute to the heterogeneous feature of the three-dimensional network structure of lignin.

1.1.2 Distribution, Content and Chemical Structures of Lignin Sub-units

In softwoods, the average content of lignin varies between 25 and 30 % (*w/w*) [11]. Softwood lignin is predominantly composed of a large proportion of guaiacyl (G) as well as some un-methoxylated p-hydroxyphenyl (H) sub-units (Fig. 1.3). Hardwood lignin content ranges from 22 to 27 % (*w/w*) [12] and is formed from co-polymerization of G and S sub-units [4, 5] (Fig. 1.4). In grass, lignin can be

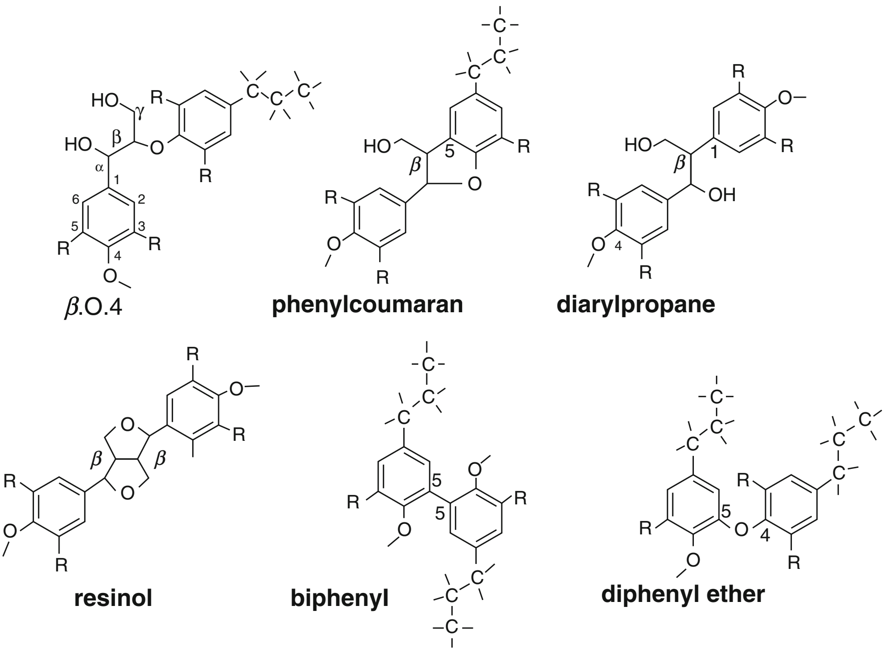


Fig. 1.2 Principal linkages between lignin sub-units R=H in hydroxyphenyl; R=OMe at C-3 and R=H at C-5 in guaiacyl; R=OMe in syringyl; Phenolic groups at C-4 may be free or etherified (Reproduced with copyright permission from Ref. [8]. Copyright © 1993 American Society of Agronomy, Crop Science Society of America, Soil Science Society of America)

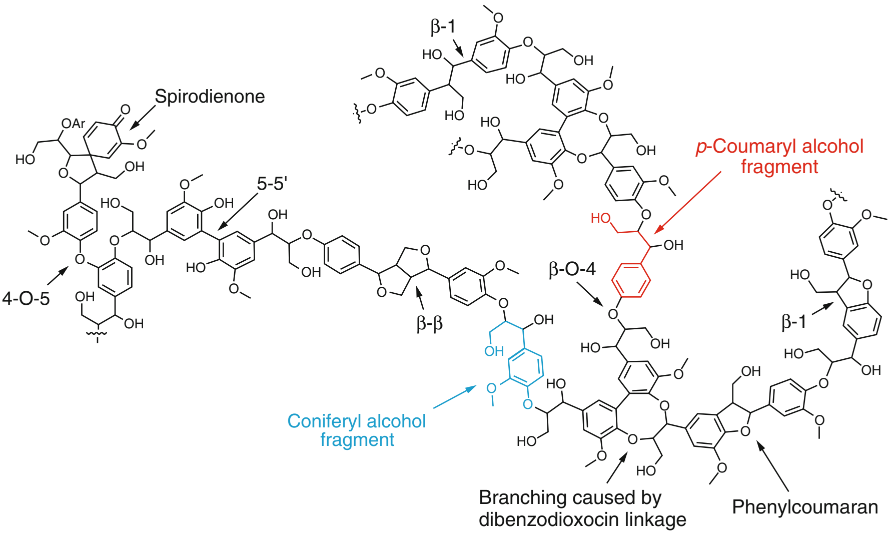


Fig. 1.3 Schematic representation of a softwood lignin structure (Reproduced with copyright permission from Ref. [19]. Copyright © 2010, American Chemical Society)

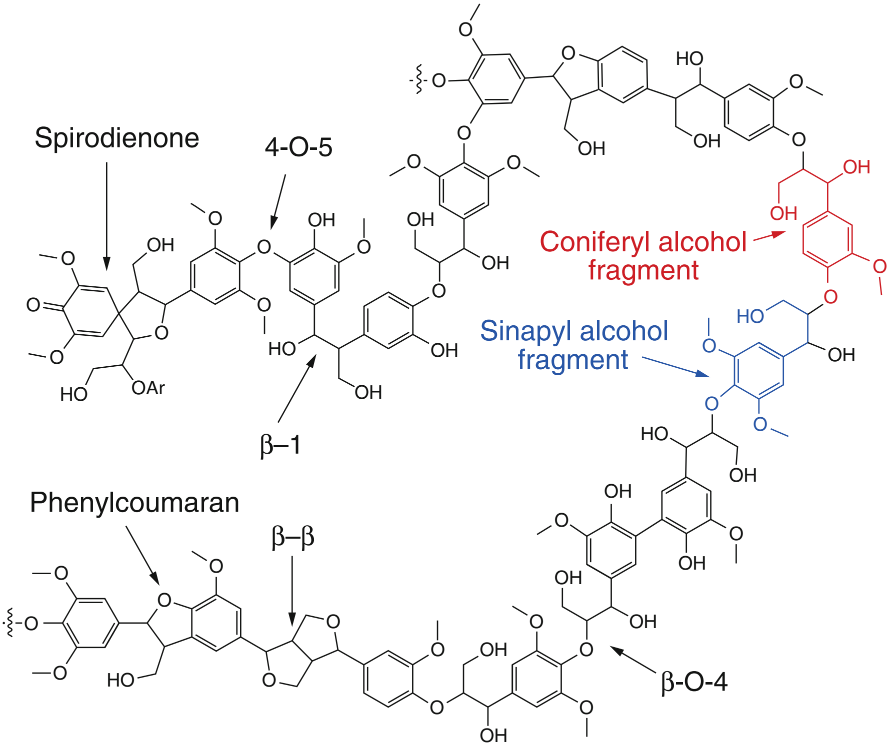


Fig. 1.4 Schematic representation of a hardwood lignin structure (Reproduced with copyright permission from Ref. [19]. Copyright © 2010 American Chemical Society)

composed of all H, G, and S sub-units and its content may vary from 1 to 19 % (*w/w*) of the total dry matter depending on plant species or growth stages [13, 14]. The chemical structure of softwood lignin does not vary much between plant species [15, 16], while hardwood lignin structures vary greatly from one plant species to another. The major inter-species difference in hardwood lignins is the S/G ratio, which influences structural features, such as amount of *β*-O-4 linkages, degree of condensation, or methoxyl content [12]. The differences between softwood and hardwood lignin also impact the application of lignin and its derivatives. For example, hardwood lignins contain more methoxyl groups than softwood lignins. The presence of methoxy groups helps to release more phenolics, methanol and CH_4 from hardwood lignin than softwood lignins in thermochemical processes [17]. Moreover, high methoxyl group content of hardwood lignins tend to give less condensed structures after pyrolysis than softwood lignins [18].

For condensed structures caused by higher proportion of H sub-units in lignin with *β*-5, *β*-1, *β*-*β*, 5–5, and 5-O-4 inter-molecular linkages, softwood has stronger recalcitrant resistance against degrading or decomposing attacks than other

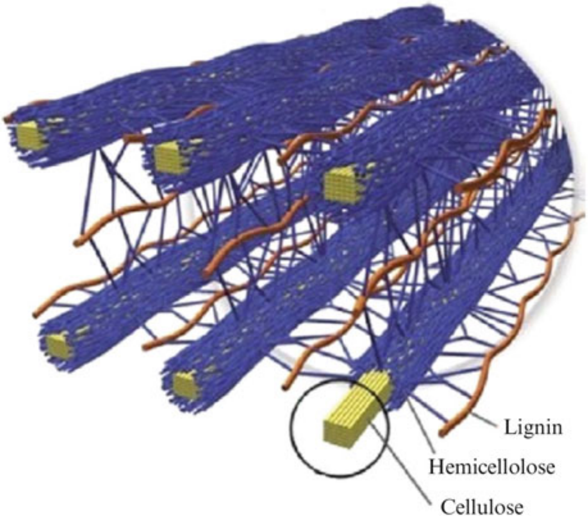


Fig. 1.5 Cellulose strands surrounded by hemicellulose and lignin (Reproduced with copyright permission from Ref. [26]. Copyright © 2010 Elsevier B.V.)

lignocellulosic biomass [8, 20, 21]. Therefore, usual pre-treatment techniques (such as, ammonia fiber explosion and dilute-acid pre-treatment methods) that work efficiently on de-structuring the hardwood or herbal biomass for subsequent enzymatic saccharification do not perform well on softwood due to its recalcitrant resistance [22, 23]. Besides the plant species, the sub-unit composition and linkage patterns in lignin vary depending on the seasons, habitat, and growth stage of the plants, as well as location of lignin in the cell wall [24]. Among these factors, the location of lignin may play a universal role. For example, wood at the top of a mature conifer tends to have higher lignin content compared with other parts of the plant [11].

In typical lignocellulosic biomass, especially woody biomass, lignin mostly deposits or condenses in cell walls, especially in the mature xylem cell walls and can form rising layers that differ in cellulose composition [25] and act as a skeleton with hemicellulose for a matrix to tightly pack the cellulose microfibrils to form ordered polymer chains (Fig. 1.5) [26]. The covalent bonds linked between lignin and carbohydrate polymers are reported as benzyl ethers and phenyl glycosides [27–29].

1.1.3 Biological Functions

It is not easy to decompose natural lignin with a single chemical, enzyme or microbiological method due to its non-regular macromolecular structure as well as the various linkage types. This feature of lignin helps it to have highly protective capacity against degradation from mechanical, chemical and biological forces in nature. In plants, lignin functions not only as structural support but also to aid in transport of moisture and nutrients [2]. Lignin contributes to the compressive strength and hydrophobicity of cell walls of xylem in woody biomass, which are considered of importance to the physiological processes of water transport, binding and encrusting. These functions are likely to be affected by the variation in lignin localization, content and sub-unit constituents [2, 3].

1.1.4 Sources of Technical Lignin and Their Promise in Bio-refining Process

As a renewable resource, lignin and lignin derivatives have potential for producing advanced chemicals or lignin-based materials in a biorefinery. When used as raw material, lignin with or without chemical modification has several distinct advantages in industrial processes as described next. Firstly, there is wide availability of technical lignin from pulping and biofuels industries. For instance, the annual production of Kraft lignin from global pulp mills is 50 million tons approximately [1]. The cellulosic ethanol industry that uses lignocellulosic feedstock is another large producer of enzymatic lignin by-product. About 0.5–1.5 kg lignin from the enzymatically-hydrolyzed residuals is co-generated per liter of ethanol produced [1]. In the USA, 126.3 and 537.7 million liters of cellulosic ethanol were produced in 2014 and 2015, respectively [30]. An increase in the output of cellulosic ethanol will also lead to an increase in the production of enzymatic lignin. Secondly, technical lignin has advanced physicochemical features for further processing or conversion [31], such as (i) good stability and mechanical strength, mainly as the results of the presence of aromatic rings; (ii) the possibility of a broad range of chemical transformations, such as with increased phenolic OH, reduced aliphatic OH and methoxyl groups, condensed polymer fragments, or multiple polydispersity of molecular weights [32]; (iii) good reactivity for graft copolymers because of existing many reacting site on the phenolic rings (phenoxy radicals), or functional groups, such as phenolic hydroxyl and carboxyl groups [33, 34]; (iv) good solubility and compatibility with a wide range of organic solvents (e.g., alcohols, acetone, formic acid and acetic acid) for homogeneous conversions with high efficiency; (v) good distributability for blending with other materials because of the small particle size and hydrophobicity; and (vi) good rheological properties and film-forming ability for a structural component in composite materials. Thirdly, use of lignin has been demonstrated to have economic benefits on an industry scale. For example, lignin can serve directly as a substitute material additive for value-added chemicals, such as phenolic and aromatic compounds, or it can be combusted as a fuel or converted through pyrolysis to generate heat or gas.

1.2 Techniques for Determining Structural and Chemical Features of Lignin

1.2.1 Importance of Lignin Chemistry

Knowledge of the chemical structure of lignin structure and its chemistry is fundamental for developing technology for its processing and refining. Understanding lignin structure allows one to (i) determine the key time points of operation during de-lignification or lignin modification processes; (ii) develop strategies of decomposing targeted lignin structure or bonds for lignin reuse by determining changes in linkages and structures in the lignin polymers; (iii) build a gene regulation mechanism and to develop relationships between lignin structural organization and certain wood properties in plant physiology and molecular biology by screening the lignin formation and distribution during the growth of the plant; (iv) elucidate mechanisms in lignin chemistry as well as develop new characterization methods.

Nowadays, both traditional and multi-disciplinary methods are used to investigate lignin structures. Due to the complexity of lignin's heterogeneous structure, there is a continual need for suitable methods of characterization of the many types of lignin polymers. Methods should be selective, quantitative, and capable of being applied directly to the sample without destroying it [35]. With current methods, lignin can be qualitatively or quantitatively determined in situ, or in an isolated form in terms of with or without derivatization. The derivatization of lignin samples prior to analysis uses mechanical, chemical, physiochemical and biological treatment, or even their combination. However, the isolation or derivatization techniques generally cause changes in the structure of native lignin samples depending on the severity of the method employed. Changes in chemical linkages and structural representations after treatment should be considered with the proper corresponding reports [1].

1.2.2 Lignin Content

1.2.2.1 Wet Chemistry Methods

Wet chemistry methods are widely used for lignin content determination. A standard NREL analytical procedure [36] uses concentrated (72 %, *w/v*) sulphuric acid solution and its further dilution (4 %, *w/v*) to dissolve and hydrolyze cellulose and hemicellulose in wood biomass. The content of acid-insoluble lignin remaining after acid hydrolysis is determined gravimetrically by excluding the incinerated ash residual. As a low proportion of the total lignin dissolves in the acid, the content of the trace acid-soluble lignin (ASL) in the neutralized hydrolysate can be spectrophotometrically measured at 320 nm or 205 nm using literature extinction coefficients [35, 37]. This method is generally applied to lignocellulosic biomass. Similarly, "Klason lignin" is defined as a wood or pulp constituent specifically insoluble in 72 % (*w/w*) sulfuric acid (TAPPI T222). Determination of the content of Klason lignin can be performed following an equivalent procedure according to TAPPI standards.

1.2.2.2 Spectroscopic Methods

X-ray photoelectron spectroscopy (XPS) is an effective technique to semi-quantitatively determine the content of lignin distributed on the surface of biomass[38–40]. This surface specific method detects about 5–10 nm deep into the biomass. Lignin content can be estimated based on oxygen-to-carbon atomic ratios and aliphatic carbon component acquired by XPS analysis [41]. Fourier transform infrared (FT-IR) spectroscopy coupled to chemometrics is also useful for quantitative analysis of lignin content in wood samples with proper models. Given the rapid prediction of the content of wood components, this method is suitable for on-line use during wood processing [42, 43].

1.2.3 Distribution of Lignin

1.2.3.1 Scanning Electron Microscopy and Atomic Force Microscopy Methods

To determine the deposited lignin on a material's surface after treatment, such as in Kraft pulping, dilute acid or hydrothermal pre-treatment, scanning electron microscopy (SEM) and atomic force microscopy (AFM) can be applied to directly observe the surface dispersion patterns of lignin [44–47]. Through SEM, the 3-D images of the lignin allow efficient identification of lignin shapes, like droplets, crystalline particles, flocks or regular globules that tend to have a size range from about 0.05–2 mm as precipitates on the surface of biomass [48–51]. For observing the detailed ultrastructure of lignin particles, field emission scanning electron microscopy (FESEM) is used to provide high resolution of the fractures and small openings on the lignin droplets and patches [52].

AFM imaging is a common, but efficient technique, for characterizing the topography and supra-molecular structure of solid materials. It can be used solely or even combined with other observation methods [53, 54]. Through scanning across the biomass surface with a sharp probe on a vibrating cantilever driven by multiple voltages, the height, amplitude and phase images can be captured using tapping mode under certain resonant frequencies [50]. The phase contrast images of the lignin fragments can give information on lignin distribution patterns and the proportion of particle sizes [44, 50, 55–57].

1.2.3.2 Spectroscopy and Other Microscopy Methods

With exception of the phase contract images of AFM, SEM-supplemented energy dispersive X-ray (EDX) spectra can be of help to locate the distribution of lignin based on the differences in elemental composition [58]. Hyperspectral stimulated Raman scattering microscope can be used for monitoring lignin deposition on plant cell walls by mapping the aromatic rings of lignin groups with 9 cm^{-1} spectral resolution and sub-micrometer spatial resolution. This technique allows determination of a spatially distinct distribution of functional groups such as aldehyde and alcohol groups [59].

As lignin is a predominantly ultraviolet (UV)-absorbing component, UV microscopy determination methods are sensitive and rapid for locating and for determining semi- quantitative changes in lignin composition in biomass. Under UV illumination, lignin components can be distinguished by strong and unique fluorescence. Fluorescence analysis, on the other hand, is of limited use due to the present of many unrelated fluorescencing compounds or by-products in biomass [60]. Other techniques, as confocal and regular optical microscopy may provide information on lignin particle shape and size as well as the distribution patterns on transparent surfaces of single fiber or thin fiber layers. The observed lignin particle size should be restricted to be above the limit of resolution that is practically 200 nm [51, 61].

1.2.4 *Molecular Weight and Polydispersity*

The molecular weight of lignin is commonly evaluated by gel permeation chromatography (GPC) [62–65]. Both the weight-average molecular weight $(\overline{Mw})$ and number-average molecular weight $(\overline{Mn})$ can be obtained but $(\overline{Mw})$ is more popular, as it better describes the mass-related physical property of lignin. The polydispersity Index d $(\overline{Mw}/\overline{Mn})$ is often used for characterizing the distribution of the molar masses of lignin fragments. Smaller d values indicate a narrower mass diversity of the lignin fragments. Lignin with a high stabilization for use as additives with polymers usually possess a low $\overline{Mw}$ and narrow d [66]. GPC method requires lignin to be dissolved into a solvent for analysis. Dilute NaOH solution or THF, DMF or chloroform organic solvents are commonly used as the mobile phase depending on the properties of the column stationary phase [67, 68]. Sometimes, due to poor solubility of the most technical lignins in organic mobile phases, lignin needs to undergo acetylation or methylation pre-treatment to improve its solubility by introducing hydrogen bonds [69]. The effluent is generally monitored by a UV detector with the wavelength being between 254 and 270 nm according to typical procedures [70].

1.2.5 Functional Side-Chain Groups

In lignin, hydroxyl groups including phenolic hydroxyl and aliphatic hydroxyl, as well as methoxyl functional groups widely exist on which the linking or derivatization reactions occur that also affect aqueous solubility. These terminal functional groups serve as the candidate sites to connect with other reacting substrate through covalent bonds [71]. Quantification of the functional groups requires extensive analysis.

1.2.5.1 Nuclear Magnetic Resonance Methods

Among the available methods, nuclear magnetic resonance (NMR) spectroscopy, mostly ^{1}H NMR and quantitative ^{31}P NMR spectroscopy are efficient for characterizing the content of functional groups [72–75]. In most of NMR spectroscopy determinations, lignin has to be dissolved or derivatized in an NMR solvent as a homogeneous solution. For example, in ^{1}H NMR analysis, chloroform ($CDCl_3$) or deuterated water (D_2O) is commonly used for dissolving lignin with tetramethylsilane or *p*-nitrobenzaldehyde as the internal standard. To ensure the solubility of lignin in NMR solvent, the lignin must be acetylated [72, 73, 76]. In ^{31}P NMR analysis, the hydroxyl groups of lignin are selectively derivatized with organic phosphoric reagent, such as 2-chloro-4, 4, 5, 5-tetramethyl-1, 3, 2-dioxaphospholane (TMDP). The derived lignin solution can be subsequently analyzed with internal standards, such as cyclohexanol [70, 77–80]. Quantitatively estimating the hydroxyl and methoxyl functional groups refers to the intensity ratios of the integrated signals of the specific protons versus the proton signals from the internal standards. Content of phenolic hydroxyl group can also be specifically determined using modified ^{1}H NMR spectroscopy methods based on distinct integrated intensities between protons in lignin and lignin with phenolic protons exchanged by D_2O. The differences are proportional to the phenolic proton content [81].

1.2.5.2 UV and GC-FID Methods

The UV method can be applied to estimate the amount of phenolic hydroxyl groups in either milled wood lignin or Kraft lignin. In terms of the spectroscopic properties of the phenolic units carrying ionized (in alkaline solvent) and the non-ionized aromatic (in neutral solvent) hydroxyl groups, UV measures the differences in the maximum adsorption ($\Delta\varepsilon$) between the alkali solution and the neutral solvent at wavelengths ranging from 300 to 350 nm [81]. A GC-FID method can be employed to quantitatively estimate the content of methoxyl groups. In this method, the derived lignin sample is reacted with concentrated sulfuric acid under reflux. The methanol generated is then distilled off from the mixture and quantified by GC-FID. The amount of methoxyl groups in the lignin sample is considered equivalent to the methanol produced [69, 82].

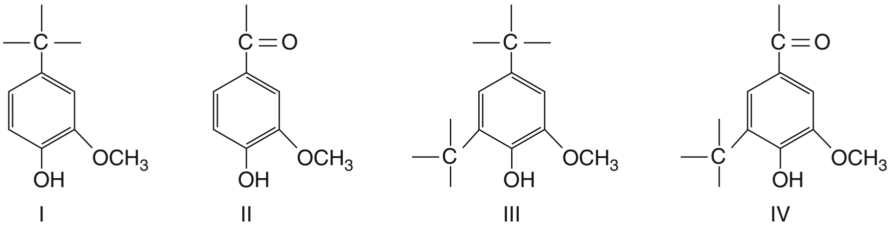


Fig. 1.6 Types of phenolic structures determined in different lignins (Reproduced with copyright permission from Ref. [69]. Copyright © 2005 Elsevier B.V.)

1.2.6 Content of Phenolic Units of Lignin

The content of different phenolic units of lignin can be estimated by the $\Delta\varepsilon$ method [69, 83]. Based on the unique maximum absorbing wavelengths between phenolic units dissolved in neutral and alkaline solvents, the content of phenolic units can be quantitatively evaluated by comparing the $\Delta\varepsilon$ values at certain wavelengths with those of the respective model types of I, II, III, and IV shown in Fig. 1.6 [69, 83].

Detailed quantitative analysis of lignin monomer compositions can be performed via pyrolysis-gas chromatography (Py-GC) method using acetylated lignin samples [84]. In the pyrolysis of the acetylated lignin, the secondary polymerization of terminal alcohol groups is prevented. On the basis of the characteristic pyrograms, lignin monomer composition can be determined with high resolution. This method works well for extractive-free plant samples [84].

1.2.7 Content of Inter-molecular Linkages

The β-O-4 ester bonds are the most frequent inter-molecular linkages present in lignin polymers. Cleavage of the β-O-4 linkages occurs more easily than other types of chemical bonds and acts an important mechanism for chemical isolation and depolymerization of lignin [70]. Elucidating the content of the β-O-4 bonds by mild, selective, and efficient methods is an important target for understanding the structural features of lignin.

1.2.7.1 ^{13}C- and ^{31}P NMR Methods

Quantitative ^{13}C NMR spectroscopy is commonly used in analysis of the bonding type for lignin dissolved in DMSO-d_6 [85–87]. To improve the sensitivity of ^{13}C NMR, two-dimensional heteronuclear single quantum coherence (HSQC) NMR analysis is used that correlates analysis of the ^{13}C and ^{1}H NMR spectra. The efficacy and usefulness of the HSQC NMR method have been well demonstrated in the characterization of lignin structures over other NMR methods [88–90]. Monitoring

Table 1.1 Spectral ranges and peak assignments of ^{13}C NMR spectral analysis of the chemical structure of woody lignin [70, 74, 85]

Range of δ (ppm)	Assignment
178.0–167.5	Unconjugated – CO_2H
167.5–162.5	Conjugated – CO_2H
154.0–140.0	C_3, C_4 aromatic ether or hydroxyl
140.0–127.0	C_1, aromatic C–C bond
127.0–123.0	C_5, aromatic C–C bond
123.0–117.0	C_6, aromatic C–H bond
117.0–114.0	C_5, aromatic C–H bond
114.0–106.0	C_2, aromatic C–H bond
90.0–78.0	Aliphatic C–O bond, C_β in β-O-4, C_α in β-5 and β-β
79.0–67.0	Aliphatic C–O bond, C_α in β-O-4
65.0–61.5	Aliphatic COR
61.5–57.5	Aliphatic C–O C_γ in β-O-4
57.5–54.0	Methoxyl-OCH_3
54.0–52.0	C_β in β-β and C_β in β-5
51.0–48.0	β-1 bond
0–49	Aliphatic C–C bond

the linkages and group changes present in lignin by ^{31}P NMR is another approach for elucidating the structure of lignin after selective derivatization. Advanced ^{31}P NMR methodology can distinguish some subtle differences in the fine structures of lignins by providing an improved resolution in NMR spectrum [91]. The principle of ^{13}C-NMR and ^{31}P-NMR analysis is the integration of chemical shift (δ) and intensity of the peaks forms to give both quantitative and qualitative information on the linkages in lignin (Tables 1.1 and 1.2) [70, 74, 80, 85, 92].

1.2.7.2 FT-IR Spectroscopy Method

Beside NMR methods, FT-IR spectroscopy is commonly used to determine changes that occur in chemical linkages and major constituents in lignin. Through FT-IR spectra, the transformed resonant absorbance at different wavenumbers assignable to various carbon linkages of the lignin skeleton can be observed (Tables 1.3 and 1.4). Because the relative content of the chemical bonds given by the intensities are comparable, changes in the lignin structure can be quantitatively inferred [8, 92, 93].

1.2.8 Lignin-Lignin Linkages and Macromolecular Assembly

Strategies of integrating selective or random de-polymerization of lignin with further quantitative or qualitative analysis methods are commonly used to characterize the macromolecular structure of lignin. On this basis, linkage breakdown

Table 1.2 Spectral ranges and peak assignments of ^{31}P NMR spectral analysis of the chemical structure of woody lignin [74]

Range of δ (ppm)	Assignment
150.0–145.5	Aliphatic OH
144.7–145.5	Cyclohexanol (internal standard)
136.6–144.7	Phenols
137.3–140.0	Combined *p*-OH and guaiacyl
140.0–144.7	C_5 substituted “condensed”
139.0–140.0	Guaiacyl
138.2–139.0	Catechol
137.3–138.2	*p*-Hydroxyl-phenyl
133.6–136.6	Carboxylic acid OH

Table 1.3 FT-IR absorbance of typical lignin component in biomass [92, 94]

Wavenumber (cm^{-1})	Assignment/functional group	Component
1035	C–O, C=C, and C–C–O stretching	Cellulose, hemicellulose, lignin
1215	C–C + C–O stretching	Lignin
1270	Aromatic ring vibration	Guaicyl lignin
1327	C–O stretching of syringyl ring	Lignin
1335	C–H vibration, O–H in-plane bending	Cellulose, hemicellulose, lignin
1380	C–H bending	Cellulose, hemicellulose, lignin
1425	C–H in-plane deformation	Lignin
1440	O–H in-plane bending	Cellulose, hemicellulose, lignin
1465	C–H deformation	Lignin
1500	Aromatic ring vibration	Lignin
1595	Aromatic ring vibration + C=O stretching	Lignin
1682	C=O stretching (unconjugated)	Lignin
2840, 2937	C–H stretching	Lignin
3421	O–H stretching	Lignin

usually occurs through chemical or thermal treatment, which has the advantage of being high selectivity or efficient. Chemical and thermal treatments can be applied together. Products can then be analyzed with chromatographic mass analysis, such as GPC, GC- FID, GC-MS or NMR, to identify different functional groups [96–100].

Table 1.4 FT-IR absorbance brand and assignment for Kraft lignin from hardwood and softwood [95]

Absorbance band (cm^{-1})		
Hardwood lignin	Softwood lignin	Assignment
3421	3349	O–H stretching
2937	2934	C–H stretching
2840	2840	C–H stretching
1682	1704	C=O stretching (unconjugated)
1603	1594	Aromatic skeletal vibration + C=O stretching
1514	1513	Aromatic skeletal vibration
1462	1463	C–H deformation (methyl and methylene)
1425	1427	C–H in-plane deformation with aromatic ring stretching
1327	–	C–O stretching of the syringyl ring
1269	1269	C–O stretching of the guaiacyl ring
1215	1214	C–C + C–O stretch
1151	1150	Aromatic C–H in-plane deformation in the guaiacyl ring
1116	–	Aromatic C–H deformation in the syringyl ring
–	1081	C–O deformations of secondary alcohols and aliphatic ethers
1033	1031	Aromatic C–H in-plane deformation (G > S)

1.2.8.1 Chemical Oxidation and GC-MS/FID Method

In chemo-GC-MS/FID analysis, thioacidolysis selectively cleaves aryl ether bonds to chemically degrade lignin that allows determination of the composition and portions of the uncondensed alkyl aryl ether structures. The evidence of aryl glycerol aryl ether structures in lignin can be confirmed by the characterized C_6C_3 trithioethyl phenylpropane compounds after de-polymerization [101, 102]. Alternatively, a method called, derivatization followed by reductive cleavage (DFRC), cleaves the alpha- and β- ethers in lignin, but leaves the γ-esters intact. This method is highly efficient for cleanly and completely breaking the abundant β-O-4 ether linkages existing in lignin [101, 102]. Characterization of the mono-, dimer- and trimer-lignol derivatives through GC- MS/FID can provide sufficient structural information about the polymer, especially in locating and quantifying the β-ether linkages, as well as quantifying the types of linkages at sites of the lignol γ-esters. Research shows that this method works well on both lignin model compounds and technical lignin samples [103–106].

1.2.8.2 Pyrolysis Degradation and GC-MS/FID Method

In thermo-degradation of lignin, pyrolysis, hydrothermal and organosolv treatment and are three commonly-used methods [107, 108]. Among these methods, analytical pyrolysis combined with GC-FID/MS (Py-GC-FID/MS) is a powerful analytical tool for structural characterization of lignin and for determining monomeric proportions of S, G and H sub-units [98, 109–112]. The de-polymerization of lignin occurs at pyrolytic temperatures from 100 to 900 ° C through dehydration, depolymerization, hydrolysis, oxidation and decarboxylation reactions that produce compounds with unsaturated side chains and low molecular mass species with phenolic OH-groups [113, 114]. Generally, there are three portions, such as coke, liquid and gas generated from the pyrolysis of lignin. By directly coupling the pyrolyzer to on-line GC-FID/MS, analysis of the compounds in the gas phase and liquid phase can be performed simultaneously [8, 99, 115–117]. Due to the complex constituents in the liquid pyrolysate, only a limited number of compounds can be quantified by the GC-MS/FID method. Use of comprehensive two-dimensional gas chromatography and time-of-flight mass spectrometers (GC × GC-TOFMS)/FID can allow characterization of the complex liquid fractions [118].

1.2.8.3 Chemo-Thermo Degradation Method

The disadvantage of the Py-GC-MS/FID technique is the loss of structural information caused by extensive fragmentation as well as limited detection capacity for separation and determination of polar functional groups. The combination of pyrolysis with chemical derivatization overcomes these issues. For example, with *in situ* methylation using tetramethylammonium hydroxide (TMAH) [119], lignin fragments containing any of the carboxylic acids, alcohols or phenols can be methylated to form methyl ethers after the cleavage [96]. Another example is that, by introducing the preliminary acetylation of lignin, prevention of secondary formation of cinnamaldehydes from the corresponding alcohols is possible [84]. In this case, the lignin monomer derivatives formed can contain intact side chains that sufficiently reflect the structure of the lignin.

1.2.8.4 Enzymatic Oxidization and Resonance Raman Spectroscopy Method

As a sensitive and selective method, enzymatic probing treatments of lignin in conjunction with resonance Raman (RR) spectroscopy, combined with Kerr gated fluorescence rejection in the time domain, can be used for elucidating lignin polymer structures. After treatment of lignin through oxidation by laccases + ABTS [2,2’-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt] or *p*- benzoquinone adsorption, spectra of fluorescent lignin polymers that reflect the redox potential can be obtained by light laser excitation with a specific wavelength. Basic structural information, such as syringyl lignin groups can be implied. This method requires selection of the proper wavelengths for fluorescence excitation to produce satisfactory results and must be compared within certain sources of lignin [61].

1.3 Derivatization and End-Use of Lignin and Lignin Derivatives

1.3.1 Sources of Lignocellulosic Biomass for Technical Lignin Derivatives

Depending on the isolation approaches, common technical lignin produced on a large scale include Kraft or alkali lignin [120, 121], lignosulfonate [122, 123], soda lignin [31, 124], organosolv lignin [73, 125], cellulase isolated-lignin [126, 127], and lignin residuals after acid hydrolysis [126, 128]. Similar isolating mechanisms, i.e., acid-catalyzed hydrolysis (HCl or HBr), oxidation (ligninolytic enzymes, HF, CF_3COOH, $Na_3H_2IO_6$, Cu $(NH_4)_4$ $(OH)_2$)), and extraction (acetone, phenol, dioxane or ionic liquids), some amounts of technical lignin, such as ionic liquid-extracted lignin [129], ball-milled lignin [130, 131] and lignozyme(fungal)-degraded lignin [132, 133], are prepared for the purpose of lab-scale investigations.

1.3.2 Application of Lignin and Lignin Derivatives

Typically, Kraft and organosolv lignin as well as cellulase isolated-lignin obtained from pulping and biofuels industries, respectively, represent a significant opportunity in the market for upgrading to value-added chemicals, such as fuels and performance products of materials. Figure 1.7 shows that a wide range of renewable chemicals and materials can be produced from technical lignin [134]. As it is a challenge to identify all potential materials and chemical products from lignin due to its complex nature [135], selected examples that are representative of end-uses of technical lignin or lignin derivatives are discussed in the next section, while other extensive applications and detailed information are available by referring to reviews and books on the subject [136–140].

1.3.2.1 Energy

Due to its high-energy content, lignin that largely exists as black liquor in industry is commonly combusted for heat recovery or used as an alternative fuel [107, 141]. Burning lignin constitutes the largest source of energy derived from an industrial by-product in North America, especially in the USA [142]. Through thermochemical approaches, the black liquor rich in lignin can be separate into three products, namely, biogas, bio-oil containing low-molecular-weight compounds, and brown tar containing high-molecular-weight compounds [143]. Processing aqueous black liquor by means of catalytic gasification can produce combustible biogas [144–147] or produce hydrogen [148] through electrolysis. Fast pyrolysis lignin can yield bio-oil to allow the production of either fuel substitutes or phenolic platform

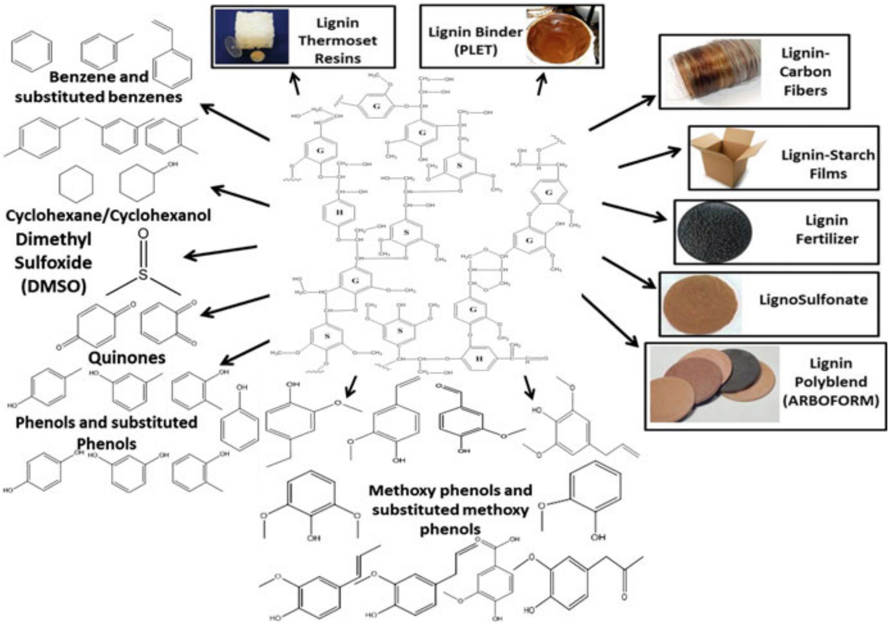


Fig. 1.7 Schematic routes to convert lignin into renewable materials and chemicals (Adapted from Ref. [134], under the terms of the Creative Commons Attribution License http://creativecommons.org/licenses/by/4.0/legalcode)

molecules [149, 150]. Oxygen-blown-pressurized thermal conversion of lignin in black liquor or causticization of lignin solid can produce methanol directly as an important material for biodiesel production [151].

1.3.2.2 Renewable Chemicals

Besides being used as an energy source, lignin is increasingly being applied as a starting material for producing chemicals. Several common technical lignins, i.e., lignosulfonate, Kraft, soda-anthraquinone, enzymatic organosolv and alcoholysis lignin, can act as suitable feedstocks for producing renewable monomeric aromatic compounds that have relatively high value as renewable raw commodity chemicals for direct use or for building specific polymers.

Thermal degradation of lignin for producing chemicals has received much interest. Catalytic thermal-cracking, hydrolysis, reduction or oxidation using temperatures between 250 and 600 °C can lead to low-molecular-weight chemical compounds as commodities or as chemical fragments for further processing [19, 141]. These techniques have been widely employed to obtain phenols or aromatics from lignin, such as guaiacols, syringols, alkyl phenols, catechols [118], C_1-C_2 alkyl-substituted phenols, meth-oxyphenols and C_3-C_4 alkyl-substituted phenols through catalytic or non-catalytic pyrolysis [152]; or 2-methoxyphenol, 4-hydroxy-3-methoxy- benzaldehyde, 2,6-dimethoxyphenol, and 1-(4-hydroxy-3-methoxyphenyl) ethanone through alkaline de-polymerization [153]; or polyols [154] through lignin hydrolysis; or phenols [155, 156] cresols [157], 4-propyl-guaiacol, dihydroconiferyl alcohol [158], alkylphenols, xylenols, guaiacol [156, 159], catechol, syringols [156], phenyl methyl ethers [160], as well as possibly benzene, toluene, and xylene through catalytic hydrogenation or hydrodeoxygenation [161]; or vanillin [162, 163] syringic/vanillic acid [162, 164], syringaldehyde [162] through catalytic oxidation. Generally, lignin-reductive catalytic systems produce bulk chemicals with reduced functionality, whereas lignin-oxidative catalytic systems produce fine chemicals with increased functionality [19].

Chemicals can also be produced from lignin or lignin derivatives through combined catalytic thermo-treating methods. For example, an integrated approach that combines hydrogenation with dihydroxylation catalyzed by zeolites has been applied to efficiently process water-soluble pyrolysis oils for olefins and aromatic hydrocarbons [165]. The hydrogenation produces polyols and alcohols by increasing the intrinsic hydrogen content in the pyrolysis oil. The subsequent conversion of the hydrogenated products with zeolite catalyst leads to a remarkable yield of light olefins and aromatic hydrocarbons (Fig. 1.8).

Alkylbenzenes, which are potential liquid fuels containing C_7–C_{10} components, can be produced from lignin through a two-stage pyrolysis approach [166]. The lignin is firstly decomposed into phenolic compounds and then reformed into the oxygenated products (Fig. 1.9). Moreover, pyrolysis of lignin in fast-fluidized bed with a subsequent catalytic dihydroxylation of the pyrolytic phenolic fraction mainly yields cycloalkanes and alkanes, as well as cyclohexanols that could act as oxygenates in engine fuels [118].

Lignin polymer fragments or bio-oils can be upgraded to more chemically-stable or less-reactive products by using thermo-treating methods, like reductive thermo de-polymerization Kraft lignin with hydrogen or hydrogen donating sources [167]. Nowadays, techniques have been developed for releasing compounds from lignin with alternative reaction media. Through an ionic liquid-based process using 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]), Kraft lignin and low sulfonate alkali lignin fractions can be depolymerized and converted into a variety of renewable chemicals, including phenols, guaiacols, syringols, eugenol, catechols and their oxidized products, such as vanillin, vanillic acid, syringaldehyde, or derivatized hydrocarbons, such as benzene, toluene, xylene, styrene, biphenyls and cyclohexane [69]. Using protic ionic liquids, e.g. triethylammonium methanesulfonate, the alkali lignin can be depolymerized into low molecular weight compounds through electro-catalytic oxidative cleavage, that include guaiacol, vanillic acid, vanillin, acetovanillone, syringols, syringaldehyde, and syringic acid [168].

Integrating bioprocesses with traditional chemical methods can be an efficient strategy to expand the number of available molecules for lignin upgrading. For example, applying gene-modified bacteria *Pseudomonas putida* Trevisan KT2440 in biochemical separations, and transformation of lignin-derived materials into *cis*, *cis*-muconic acid can be chemically converted to adipic acid and further to the most prevalent dicarboxylic acid with catalytic hydrogenation [169].

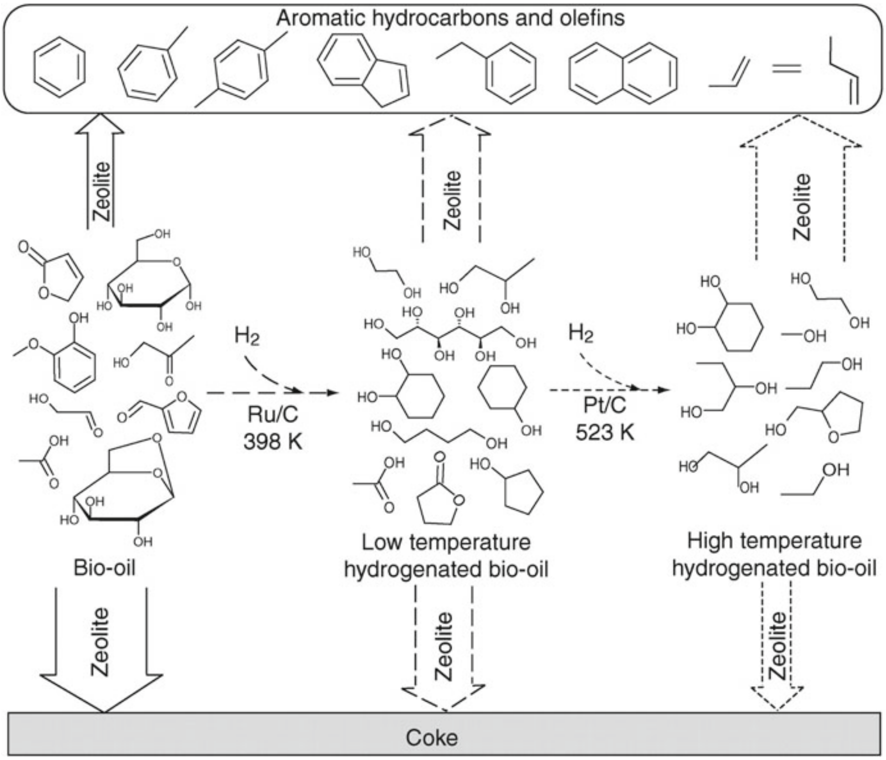


Fig. 1.8 Reaction schematic for integrated hydroprocessing and zeolite upgrading of pyrolysis oil (The width of the vertical arrows represents the product carbon yield from a particular field. Reproduced with copyright permission from Ref. [165]. Copyright © 2010 The American Association for the Advancement of Science)

1.3.2.3 Materials and Additives

Due to the presence of phenolic groups in the lignin structure, the phenolic compound from lignin derivatization can be used for partly replacing petroleum-based phenol substitutes of phenol in preparing bio-based phenol-formaldehyde resol resins. The introduction of lignin in the resin formula decreases the thermal stability of the resin, leading to a lower decomposition temperature and a reduced amount of carbon residue at elevated temperatures. It is applicable if the portion of replaced phenol with lignin is controlled to be below 50 % (*w/w*). The thermal stability can be further improved by using purified lignin with cellulose and hemicellulose contaminants removed [170]. Replacing bisphenol-A with the depolymerized lignin in the epoxy resin synthesis also performs well. Under optimum synthesis conditions, a high product yield (99 %) and high epoxy equivalent of up to 8 can be achieved [171, 172]. The epoxy resin has good dielectric, mechanical and adhesive properties, and can be further used in the electronics industry [173]. Moreover, lignin can

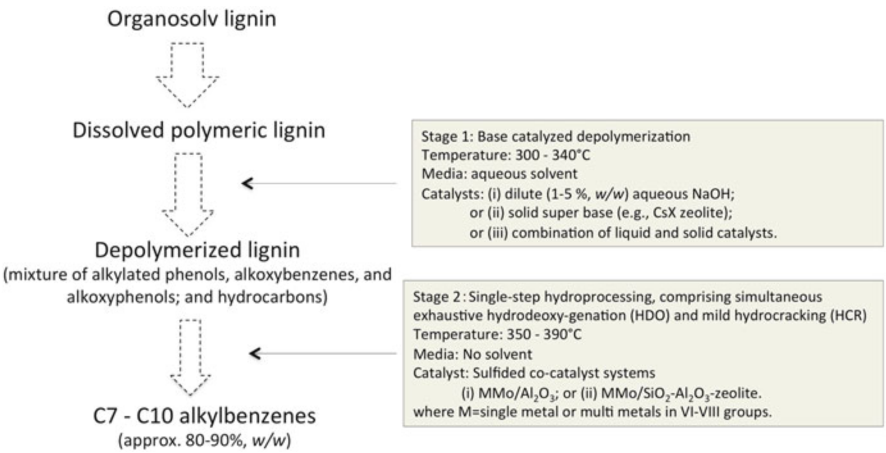


Fig. 1.9 Schematic diagram of selective conversion of lignin through two-stage pyrolysis process for alkylbenzenes as gasoline blending components (Modified from Ref. [166])

be similarly used as an alternative reaction component in synthesis of other polymer composites, such as lignosulfonic acid-doped polyamine [174], ARBOFORM [175] polyesters and polyurethanes [176, 177].

The solid portion of the residue after rapid pyrolysis of Kraft black liquor or lignin mainly contains char, fixed carbon, and inorganic carbonate [178]. Due to the large specific surface area and plenty of microspores, the lignin-char can be applied as activated carbon [138, 179, 180]. Alternatively, the carbonized lignin char is also a promising substitute supporter for preparing the sulphonated solid catalyst used in heterogeneous trans-esterification to produce biodiesel [181, 182].

On the basis of the strong mechanical effect and hydrophobic nature of lignin, the starch-based films incorporated with lignin filler has a high resistance to water with increased elongation. The improved properties have allowed composites to be developed for packaging materials [134, 183]. When used as agriculture additive, technical lignin can slow the release of fertilizers into soil [184]. Moreover, technical lignin powder can be directly blended with synthetic polymers such as polyethylene and polystyrene to improve thermal stability as well as the stabilizer stability against UV radiation [185]. Lignin acts as an antioxidant and reinforcement additive in natural or synthetic rubber [66, 186] PVC [187] and polyolefins polymer [188–191]. As a good water reducing agent, lignin can be evenly applied to the manufacture of wallboards [192, 193]. Through thermal or electrospinning of the blends of fusible lignin or lignin solutions followed by carbonization treatment, lignin based-carbon fibers can be produced for composites with the tensile and thermo stabilization being improved [194–197].

1.4 Conclusions and Future Outlook

Lignin is a complex, but important natural component in biomass. Compared to cellulose or sugars, identifying chemical constituents in lignin and lignin-derived feedstocks faces many challenges because of the nature of lignin as well as its indistinct methods of characterization. In terms of lignin chemistry and structure characterization, fundamentals behind lignin conversion through chemical, thermochemical and biological approaches, have improved as new potential applications are proposed and developed. Advanced use of lignin-based materials as specialty polymers for the paper industry, enzyme protection, biocide neutralization, precious metal recovery aids and wood preservation, have been commercialized in the market [198]. With large quantities of technical lignin originating from industry, there are great opportunities for introducing lignin-derived products into the market. There are multiple questions proposed in the field of scientific and application research on lignin that need to be addressed as listed below:

(i) In characterization of technical lignin and its derivatives, the heterogeneous properties and complexities in the structure of the polymers should be fully considered. Analytical conditions and limitations in the methods of lignin chemistry must be assessed. To confirm the results of the analyses, it is advisable to consider the characterization from multiple perspectives and to use different comparable methods in the study as much as possible.
(ii) It is notable that analysis results of lignin structures are sensitive to changes caused by derivatization, the effect of the severity of the treatment should be evaluated and strictly controlled upon application.
(iii) Although some different methods have been applied or proposed for characterization of lignin, the statistical comparison of analytical methods for the same purpose have been found to be not fully compatible, e.g., for the determination of hydroxyl groups and other functional groups [25]. Investigation of the differences in these results is necessary to reflect inadequacies in the present methods. By doing this, the method can be improved. Moreover, novel technologies capable of solving in-depth analytical problems associated with lignin can be proposed and developed for revealing more detailed structures and activities of the lignin polymers [199].
(iv) In terms of the differences in lignin according to origin and the fractionation techniques employed, dissimilar properties and reactivity of technical lignin and their derivatives offer distinct routes for subsequent end-use of lignin. Clear correlation relationships between lignin physicochemical properties and determined characters, such as lignin polymer purity, molecular weight, or concentrations of functional groups, allow good quantification of the quality of the technical lignin. Fast and reliable determination techniques that provide reliable characterization are essential for model development and for quality control of lignin [43].

(v) The de-polymerization and derivatization towards technical lignin requires multi-disciplinary research as well as much creativity. Green and viable methods that are highly efficient are in great demand. For example, valorization of lignin through conversion of ligninolytic enzymes [200] or through realizing the synergy of enzyme-microbial funneling processes with areas of substrate selection, metabolic engineering and process integration [201] are attractive.

(vi) From the technical point of view, developing or applying currently available methods for making lignin-derived products for a given market should fit within the criteria of purpose of use (product functionality) as well as technical feasibility. Marketing-scale based on scope of market demands, i.e., high volume (thousands tons or up to millions tons/year), medium volume (hundreds to thousands tons/year) or low volume (kgs to tons/year) use, is a key factor to be considered for achieving a balance between market value and product cost in facilities, raw materials, processing and marketing.

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