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A Brief Introduction of Lignin

Yuhe Liao

Guangzhou Institute of Energy Conversion, Chinese Academy of Sciences, No. 2 Nengyuan Road, Tianhe, Guangzhou, 510640, China

1.1   Introduction

To reduce greenhouse gas (GHG) emissions, society should use renewable carbon resources in a sustainable way to produce chemicals, materials, and fuels next to circular use of currently available carbon feedstock. Lignocellulosic biomass has been considered as an abundant, carbon dioxide neutral, and renewable carbon resource [1]. The major compositions in lignocellulosic biomass are three oxygen-containing biopolymers: cellulose (40–60%), hemicellulose (10–40%), and lignin (15–30%), and the minor compositions include proteins, fats, pectins, inorganic matter, and others [2]. Cellulose and hemicellulose are carbohydrate-based biopolymers, whereas lignin is a complex aromatic biopolymer with a high carbon content (Figure 1.1) [3, 4]. Typically, cellulose determines the structure of cell walls in the form of microfibrils. The cross-linked lignin and hemicellulose wrap around the cellulose microfibrils (Figure 1.1). The lignin can provide additional rigidity and cause the cell walls to be hydrophobic and water impermeable. Therefore, these three main fractions are intertwined to yield the complex structure, contributing to biomass recalcitrance, which hampers the effective valorization of lignocellulosic biomass toward high-value products such as chemical and liquid fuels [5].

Different approaches have been developed to overcome the recalcitrance and valorize lignocellulosic biomass over the past decades [2, 6]. Classically, the lignocellulosic biomass is utilized to produce high-quality pulps for paper production. Emerging approaches are conversion of lignocellulose via thermal cracking (e.g. pyrolysis), biocatalysis, chemocatalysis, and integration of them toward chemicals and fuels such as bioethanol, furfurals, and levulinic acid [1, 3, 4, 7–13]. It is clear
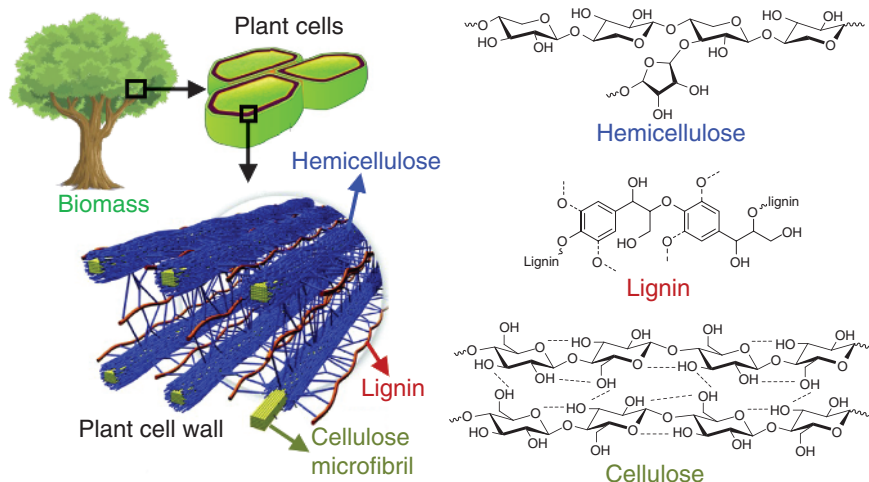
Figure 1.1 General structure of lignocellulose in plant and representative structure of cellulose, hemicellulose, and lignin. Source: From Liao et al. [2].

that these products are usually derived from cellulose and hemicellulose, whereas lignin, either left as a solid residue after conversion of cellulose and hemicellulose (such as via hydrolysis) or extracted from lignocellulose with cellulose and hemicellulose as solid residue, is considered as a waste or a low-value product for energy use. Currently, the pulping and biorefinery processes such as bioethanol production generate more than 50 million tons of lignin annually with ca. 95% used as fuel for heat and power generation due to the recalcitrance of lignin [14, 15]. The utilized 5% of lignin have several applications such as additives, surfactants, and adhesives. Although delignification (i.e. removal of lignin) of lignocellulosic biomass can facilitate the utilization of cellulose and hemicellulose to improve the economics of biorefineries, the value of biomass may not be maximized without utilization of lignin toward high-value products. Techno-economic analysis (TEA) and life-cycle assessment (LCA) have shown that valorize lignin can improve both economics and sustainability of biorefineries [16]. Hence, it is paramount to valorization of lignin with novel strategies to explore the potential of all carbon constituents of lignocellulose.

Over the past years, some progresses were achieved in the aspects of lignin characterizations to reveal the structure of lignin, isolation to obtain lignin with different properties (even native like), and valorization toward chemicals, fuels, and materials with novel approaches. Hence, this book aims to introduce the most recent advancements in these aspects, particularly the valorization methods such as oxidation, photocatalysis, electrocatalysis, and valorization of native lignin. As a preface to the following chapters, this introductory chapter will briefly introduce the structure of lignin from the point of view of monomeric units, inter-unit linkages, and biosynthesis.

1.2   The Building Blocks of Lignin

Lignin, a phenolic biopolymer, is derived primarily from three kinds of 4-hydroxyphenylpropanoids (i.e. monolignols, e.g. *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol, Figure 1.2). These monolignols differ in substitution degree of methoxylation on the aromatic ring (i.e. 2 and 6 positions) and incorporate into lignin chain to produce corresponding *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units, respectively [17]. Besides, it is found that lignin is derived from numerous other building blocks, such as *p*-coumarates, ferulates, caffeyl alcohol, *p*-hydroxybenzoates, hydroxycinnamaldehdyes, tricin, and hydroxystilbenes [18, 19]. These building blocks are shown in Figure 1.2.
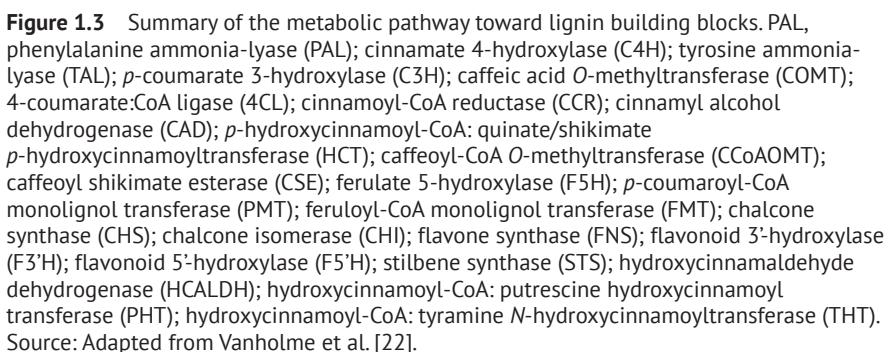
Currently, the only known route to form these building blocks is the phenylpropanoid pathway starting from phenylalanine via multiple steps in all plants with different enzymes [20, 21]. Whereas tyrosine can be an additional starting substrate for grasses. Figure 1.3 overviews the complete pathway of phenylpropanoid pathway for these building blocks. The phenylalanine was first deaminated in the presence of phenylalanine ammonia-lyase (PAL) toward cinnamic acid, which is then hydroxylated toward *p*-coumaric acid in the presence of cinnamate 4-hydroxylase (C4H). While in the case of tyrosine, *p*-coumaric acid can be produced from a shortcut pathway, direct deamination with tyrosine ammonia-lyase (TAL) or PAL [14, 23].

Then, *p*-coumaric acid is enzymatically converted toward either *p*-coumaroyl-CoA via 4-coumarate: CoA ligase (4CL) or caffeic acid through hydroxylation via *p*-coumarate 3-hydroxylase (C3H). *p*-Coumaryl alcohol is produced from reduction of *p*-coumaroyl-CoA via cinnamoyl-CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD). For caffeic acid, methylation via caffeic acid *O*-methyltransferase (COMT) forms ferulic acid, which can be transformed into feruloyl-CoA. Meanwhile, caffeic acid can be converted toward cafferoyl-CoA, which can be further methylated to feruloyl-CoA via caffeoyl-CoA *O*-methyltransferase (CCoAOMT). In addition, conversion of *p*-coumaroyl-CoA in the presence of shikimate/quinate hydroxycinnamoyl transferase (HCT) yields *p*-coumaroyl shikimate. Hydroxylation of *p*-coumaroyl shikimate via C3H can produce *p*-caffeoyl shikimate, which can be transformed toward caffeic acid and caffeoyl-CoA in the presence of caffeoyl shikimate esterase (CSE) and HCT, respectively. The shikimate intermediates are currently recognized as the favored substrates for hydroxylation [22].

Reduction of feruloyl-CoA via CCR yields coniferaldehyde, which can be transformed to coniferyl alcohol and 5-hydroxyconiferaldehyde through reduction (via CAD) and hydroxylation (via ferulate 5-hydroxylase, F5H), respectively (Figure 1.3). The main pathway to produce sinapyl alcohol is hydroxylation (via F5H) of coniferyl aldehyde followed by tandem methylation (via COMT) and reduction. Hydroxylation (via F5H) of coniferyl alcohol followed by methylation can form sinapyl alcohol as well. Oxidation of coniferylaldehyde by hydroxycinnamaldehyde dehydrogenase (HCALDH) forms ferulic acid. These synthesized monolignols are transported to the cell wall and integrated into a growing lignin chain.
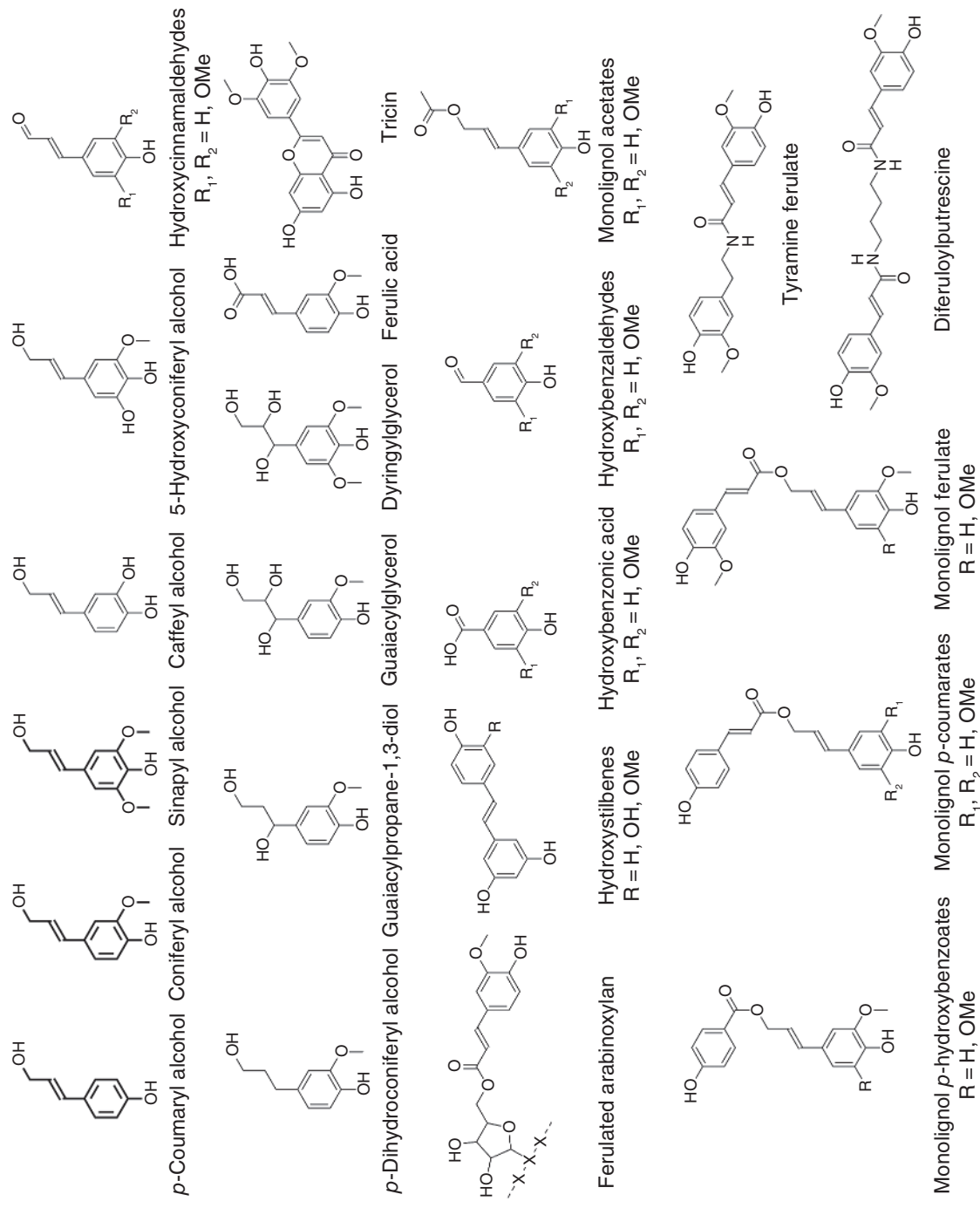
Figure 1.2 Lignin building blocks. Three main monolignols (i.e. *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol) are shown in bold.

Figure 1.3 Summary of the metabolic pathway toward lignin building blocks. PAL, phenylalanine ammonia-lyase (PAL); cinnamate 4-hydroxylase (C4H); tyrosine ammonia-lyase (TAL); *p*-coumarate 3-hydroxylase (C3H); caffeic acid *O*-methyltransferase (COMT); 4-coumarate:CoA ligase (4CL); cinnamoyl-CoA reductase (CCR); cinnamyl alcohol dehydrogenase (CAD); *p*-hydroxycinnamoyl-CoA: quinate/shikimate *p*-hydroxycinnamoyltransferase (HCT); caffeoyl-CoA *O*-methyltransferase (CCoAOMT); caffeoyl shikimate esterase (CSE); ferulate 5-hydroxylase (F5H); *p*-coumaroyl-CoA monolignol transferase (PMT); feruloyl-CoA monolignol transferase (FMT); chalcone synthase (CHS); chalcone isomerase (CHI); flavone synthase (FNS); flavonoid 3'-hydroxylase (F3'H); flavonoid 5'-hydroxylase (F5'H); stilbene synthase (STS); hydroxycinnamaldehyde dehydrogenase (HCALDH); hydroxycinnamoyl-CoA: putrescine hydroxycinnamoyl transferase (PHT); hydroxycinnamoyl-CoA: tyramine *N*-hydroxycinnamoyltransferase (THT). Source: Adapted from Vanholme et al. [22].

Next to synthesis of the three main monolignols, *p*-coumaroyl-CoA and feruloyl-CoA can serve as substrates to synthesize other building blocks. *p*-Coumaroyl-CoA can be transformed toward hydroxystilbenes via stilbene synthase (STS) and flavonoids (including tricin, as identified in grasses) via multistep [24–27]. For feruloyl-CoA, hydroxycinnamides (e.g. tyramine ferulate and diferuloylputrescine) and ferulated arabinoxylan can be obtained from it [28–31]. Besides, hydroxybenzaldehydes (e.g. *p*-hydroxybenzaldehyde, vanillin, and syringaldehyde) and hydroxybenzoic acids (e.g. vanillic acid and syringic acid) can be obtained from them, though the reaction pathway is not clear yet [32, 33].

Furthermore, monolignol *p*-coumarates, found in grasses, can be synthesized from *p*-coumaroyl-CoA and monolignols with the action of *p*-coumaroyl-CoA monolignol transferase (PMT), while as found in plants such as Angelica sinensis, monolignol ferulates can be obtained from feruloyl-CoA and monolignols with feruloyl-CoA monolignol transferase (FMT) [34, 35]. The three main monolignols and acetyl-CoA can be transformed in the presence of acyl transferases toward γ-O-acylated ester conjugates, acetates, which are found in a large proportion of hardwoods with low levels and in large amount in several plants such as palms, abaca, sisal, and kenaf [36, 37]. Monolignol *p*-hydroxybenzoates, widely found in poplar, aspen, palms, and willow, are the product of three main monolignols and *p*-hydroxy-benzoyl-CoA [36, 38]. It should be noted that *p*-coumarate and *p*-hydroxybenzoate are often misinterpreted as H-unit fractions, overestimating the content of H units, which is usually less than 5% and is obtained from monolignol *p*-coumaryl alcohol [14, 36, 39–41]. Caffeyl alcohol was recently found in the lignin of seed coats of some plants such as vanilla planifolia and cactaceae species [42–44]. 5-Hydroxyconiferyl alcohol was also identified in seed coats of some plants such as escobaria species (*E. dasyacantha*, *E. lloydii*, and *E. zilziana*) [45].

Another two monomers, arylpropane-1,3-diols (guaiacylpropane-1,3-diol in Figure 1.3) and hydroxyphenylglycerols, might be produced from dihydroconiferyl alcohol (in softwoods) and hydroxycinnamyl alcohols, respectively, likely via radical oxidation instead of enzymatical reaction [22, 46, 47].
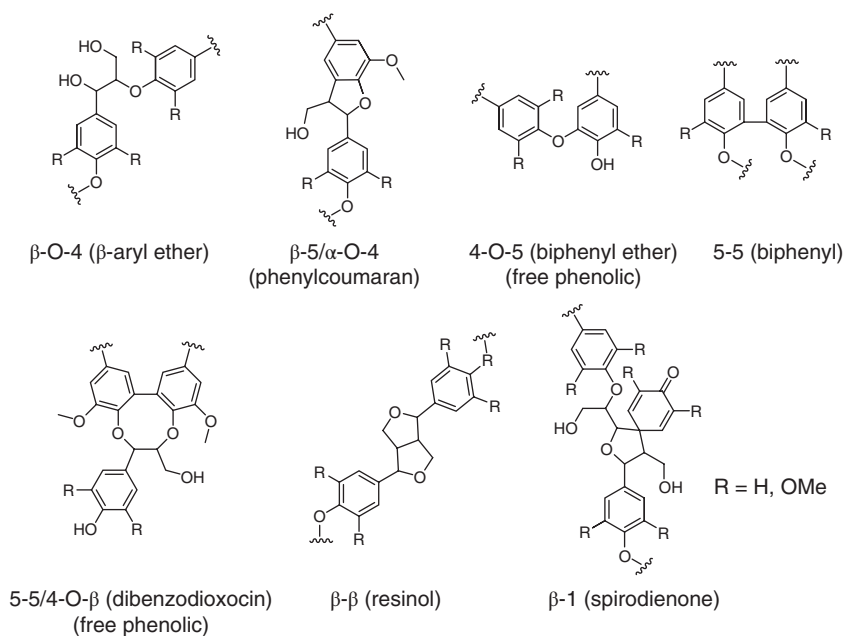
1.2.1 Interlinkages in Lignin

Lignin is obtained from polymerization of these building blocks after transportation to the cell wall. In the polymerization process, the phenols are first oxidatively converted via enzymes to form phenolic radicals, which are then subjected to coupling to form a racemic polymer. Peroxidases and laccases are involved in the oxidation step [48]. Hydrogen peroxide is the substrate of peroxidases, while laccases oxidize using oxygen. Lignification is a purely chemical process and not enzymatically determined once the radicals are formed [49].

Generally, the monolignol is first dehydrogenated (i.e. oxidation) to form a phenol radical, which is relatively stable due to the unpaired electron delocalization [40]. Afterward, two monolignol radicals couple to yield a (dehydro)dimer, thereby forming a covalent bond. These monolignol radicals are preferred to couple at their β position, leading to the formation of β-β (resinol), β-O-4 (β-aryl ether), and β-5

(phenylcoumaran) (dehydro)dimers (Figure 1.4) [50]. These are the most common interunit linkages in lignin. These dimers are further oxidized to form radicals, which then couple with another monolignol radical to increase polymer chain. Usually, the new monolignol radical is coupled exclusively at the β position of the growing oligomer, forming β-O-4 interunit linkage. Therefore, β-O-4 interunit linkage is the most abundant linkage in lignin, and it accounts for 50–80% of total linkages in native lignin [19, 50], while isolation of lignin can decrease the amount of β-O-4 interunit linkage in isolated lignin due to degradation of lignin, and the reduced amount depends on the process conditions [14]. This type of polymerization is termed as endwise coupling, where a monolignol radical is added to the growing lignin polymer (also radical) in one reaction. As the polymerization progresses, two lignin oligomeric phenolic end units are coupled to form 5-5 (biphenyl) and 4-O-5 (diphenyl ether) interlinkages, which are not from the coupling between monomer and oligomer or between monomer and monomer [19, 50]. This is rare for S-unit rich lignin due to the presence of the two methoxy groups, while it is common in G-unit rich lignin as only one methoxy group in the monolignol. Besides, β-1 (spirodienone) linkage is formed by coupling a β-aryl ether dimeric end unit with a monolignol. The possibility of different linkages depends on the type of monolignols and the conditions in the cell wall [50].

The lignin obtained from combinatorial radical coupling is very complex and difficult to valorize and characterize. The composition (i.e. the relative abundance of units), structure, and amount of lignin differ between plant species and within the plant species [51, 52]. Besides, the lignin can vary at different growth stages of plants.

Figure 1.4 Representative interlinkages in lignin.

Generally, the lignin in a gymnosperm/softwood is composed of mostly G units, and lignin in angiosperm/dicot/hardwood contains both G and S units with trace amount of H units [19]. The lignin in (commelinid) monocot/grass contains H, G, and S units [19, 53]. The model lignin structure of these three types of plants is displayed in Figure 1.5 [39]. Additional building blocks that have been found in the past are also integrated into the chain of lignin to form new linkages. Indeed, most of the lignin has a very complex structure, but there is an exception for C-lignin, which is obtained from endwise β-O-4 coupling of caffeyl alcohol [42–44]. C-lignin is a linear homopolymer linked with benzodioxane (Figure 1.6) [43]. Therefore, selective depolymerization of C-lignin is easier than other types of lignin as they contain different C–O and C–C interlinkages. For instance, C-lignin can be nearly quantitatively converted into monomeric catechol via hydrogenolysis [54–56]. Besides, 5H-lignin, obtained from coupling of 5-hydroxyconiferyl alcohol, has the same benzodioxane units [43, 45].

Lignin has been hypothesized as a highly branched polymer for a long time, but this is now doubted. It is assumed that lignin chains connected via 5-5 (biphenyl) or 4-O-5 (diphenyl ether) interlinkages can form a Y-branch polymer. However, the yielded structures are mostly free phenolic (which is traditionally hypothesized to be branching points), as identified by 2D NMR recently, and thus the native lignin must be less branched than usually assumed [45, 57]. This was also observed for β-1 (spirodienone) linkage (i.e. free phenolic instead of etherified) [58].

1.2.2 Bioengineering of Lignin

As discussed in the previous section, the monolignols are obtained from phenylalanine and tyrosine with the action of different enzymes. Upon genetic modifications of the phenylpropanoid pathway can influence the lignin composition, structure, and amount. For instance, downregulation of the genes of PAL, C4H, and 4CL, which are the enzymes involved in the early stages of phenylpropanoid biosynthesis, can lead to decreased flux, and thus reduce the lignin content in plant [59]. Although low content of recalcitrant lignin can be formed in the plant through this strategy, detrimental agronomic effect can occur as plants need a certain amount of lignin [60].

The favored approach is downregulation or upregulation of enzymes to yield plant lignin, which can facilitate the valorization of biomass (including lignin). For instance, the S/G ratio in angiosperms can be regulated via overexpression or underexpression of the F5H gene [59]. Overexpression of the F5H, when forced by a powerful lignin promoter, can yield linear lignin with extremely high S unit content (98.3%) [61]. The interlinkages in this type of high S lignin only involve β-O-4 and β-β, where the β-O-4 linkage accounts for 88.8% (i.e. low content of recalcitrant C–C interlinkage). Depolymerization of this type of linear lignin can produce high monomer yield, such as 78% of monomer was obtained from hydrogenolysis [61, 62]. In contrast, downregulation of F5H can obtain plant lignin with solely guaiacyl unit. Similarly, underexpression of the genes of C3H, HCT, and CSE enzymes, which are related to 3-hydroxylation steps, can produce plant lignin rich in H units [59, 63–67]. However, in the typical wild-type lignin H units are a minor
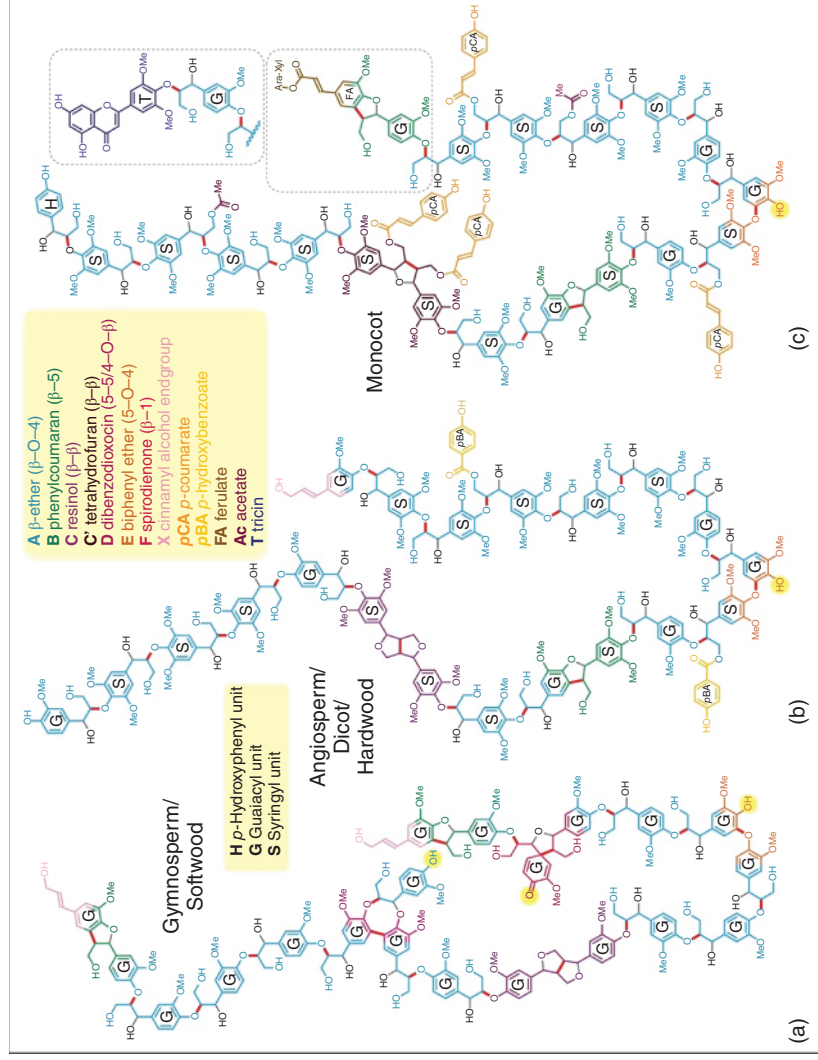
Figure 1.5 Lignin model structures containing 20 units. (a) A gymnosperm/softwood, (b) an angiosperm/dicot/hardwood, and (c) a (commelinid) monocot/grass. Source: Adapted from Ralph et al. [39].
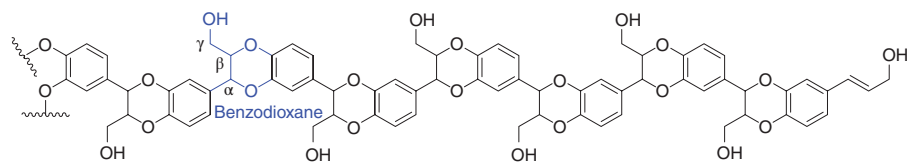
Figure 1.6 Representative chemical structural models of C-lignin. It is based on Ref. [43].

fraction. Besides, if chemically labile bonds are introduced into the lignin polymer, it will be easier to degrade lignin [68]. "Zip-lignin" is a successful example in this aspect. It introduces ester bonds, which are more chemically reactive than ether bonds, into lignin chain by acyl transferases [69]. More details about the bioengineering of lignin can be found in the decent reviews [14, 39, 59, 70].
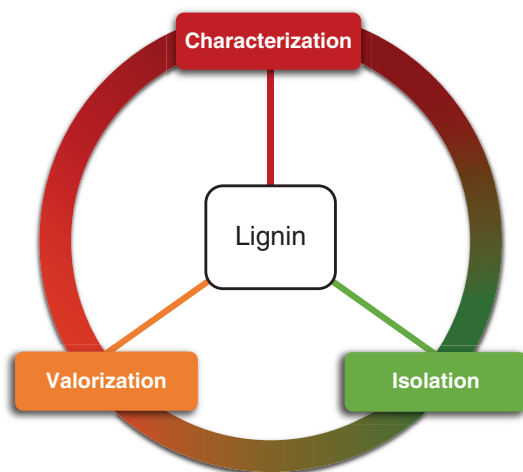
Figure 1.7 The scope of this book.

1.3 Scope of this Book

Besides the inherent complexity as introduced in this chapter, the structure of lignin is modified as the result of depolymerization (i.e. cleavage of linkages) and repolymerization (i.e. formation of new linkages) in most of the biomass processing technologies. Depending on processing technologies and the severity of the process, the structure of the yielded lignin can be varied. From a chemistry and technology point of view, rational utilization of lignin for different applications is determined by three important aspects: (i) characterization of lignin to understand the structure (including linkages), (ii) isolation to obtain lignin with desired properties, and (iii) on purpose valorization of lignin based on the inherent properties (Figure 1.7). Therefore, this interdisciplinary book includes chapters focused on introducing the latest progress in these aspects, and experts from analytic chemistry, organic chemistry, chemocatalysis, biocatalysis, chemical engineering, and material chemistry will contribute to this book. The main focus is valorization of lignin toward fuels, chemicals, and materials.

References

1 Schutyser, W., Renders, A.T., Van den Bosch, S. et al. (2018). *Chemical Society Reviews* 47: 852–908.

2 Liao, Y., de Beeck, B.O., Thielemans, K. et al. (2020). *Molecular Catalysis* 487: 110883.

3 Sun, Z., Fridrich, B., De Santi, A. et al. (2018). *Chemical Reviews* 118: 614–678.

4 Li, C., Zhao, X., Wang, A. et al. (2015). *Chemical Reviews* 115: 11559–11624.

5 Himmel, M.E., Ding, S.-Y., Johnson, D.K. et al. (2007). *Science* 315: 804–807.

6 Agbor, V.B., Cicek, N., Sparling, R. et al. (2011). *Biotechnology Advances* 29: 675–685.

7 Zhang, B., Biswal, B.K., Zhang, J., and Balasubramanian, R. (2023). *Chemical Reviews*.

8 Huber, G.W., Iborra, S., and Corma, A. (2006). *Chemical Reviews* 106: 4044–4098.

9 Zhou, C.-H., Xia, X., Lin, C.-X. et al. (2011). *Chemical Society Reviews* 40: 5588–5617.

10 Alonso, D.M., Wettstein, S.G., and Dumesic, J.A. (2012). *Chemical Society Reviews* 41: 8075–8098.

11 Zhang, Z., Song, J., and Han, B. (2017). *Chemical Reviews* 117: 6834–6880.

12 Wong, S.S., Shu, R., Zhang, J. et al. (2020). *Chemical Society Reviews* 49: 5510–5560.

13 Van de Vyver, S., Geboers, J., Jacobs, P.A., and Sels, B.F. (2011). *ChemCatChem* 3: 82–94.

14 Rinaldi, R., Jastrzebski, R., Clough, M.T. et al. (2016). *Angewandte Chemie International Edition* 55: 8164–8215.

15 Zhang, C., Shen, X., Jin, Y. et al. (2023). *Chemical Reviews* 123: 4510–4601.

16 Ragauskas, A.J., Beckham, G.T., Biddy, M.J. et al. (2014). *Science* 344: 1246843.

17 Boerjan, W., Ralph, J., and Baucher, M. (2003). *Annual Review of Plant Biology* 54: 519–546.

18 Lu, F. (2019). Lignin: Biosynthesis, Functions and Economic Significance. https://novapublishers.com/shop/lignin-biosynthesis-functions-and-economic-significance/.

19 Sun, R.C. (2020). *ChemSusChem* 13: 4385–4393.

20 Barros, J., Serrani-Yarce, J.C., Chen, F. et al. (2016). *Nature Plants* 2: 1–9.

21 Jansen, F., Gillessen, B., Mueller, F. et al. (2014). *Biotechnology and Applied Biochemistry* 61: 646–654.

22 Vanholme, R., De Meester, B., Ralph, J., and Boerjan, W. (2019). *Current Opinion in Biotechnology* 56: 230–239.

23 Higuchi, T., Ito, Y., and Kawamura, I. (1967). *Phytochemistry* 6: 875–881.

24 Tropf, S., Lanz, T., Rensing, S. et al. (1994). *Journal of Molecular Evolution* 38: 610–618.

25 Eudes, A., Dutta, T., Deng, K. et al. (2017). *PLoS One* 12: e0178160.

26 Ralph, J., Kim, H., Lu, F. et al. (2023). *Recent Advances in Polyphenol Research*, vol. 8, 1–50. Wiley.

27 del Río, J.C., Rencoret, J., Gutiérrez, A. et al. (2021). *Recent Advances in Polyphenol Research*, vol. 7, 177–206. Wiley.

28 Onkokesung, N., Gaquerel, E., Kotkar, H. et al. (2012). *Plant Physiology* 158: 389–407.

29 Farmer, M.J., Czernic, P., Michael, A., and Negrel, J. (1999). *European Journal of Biochemistry* 263: 686–694.

30 del Río, J.C., Rencoret, J., Gutiérrez, A. et al. (2022). *Advances in Botanical Research* 104: 1–39.

31 Balk, M., Sofia, P., Neffe, A.T., and Tirelli, N. (2023). *International Journal of Molecular Sciences* 24: 11668.

32 Qualley, A.V., Widhalm, J.R., Adebesin, F. et al. (2012). *Proceedings of the National Academy of Sciences* 109: 16383–16388.

33 Widhalm, J.R. and Dudareva, N. (2015). *Molecular Plant* 8: 83–97.

34 Petrik, D.L., Karlen, S.D., Cass, C.L. et al. (2014). *The Plant Journal* 77: 713–726.

35 Karlen, S.D., Zhang, C., Peck, M.L. et al. (2016). *Science Advances* 2: e1600393.

36 Ralph, J. (2010). *Phytochemistry Reviews* 9: 65–83.

37 Del Río, J.C., Marques, G., Rencoret, J. et al. (2007). *Journal of Agricultural and Food Chemistry* 55: 5461–5468.

38 Lu, F., Karlen, S.D., Regner, M. et al. (2015). *Bioenergy Research* 8: 934–952.

39 Ralph, J., Lapierre, C., and Boerjan, W. (2019). *Current Opinion in Biotechnology* 56: 240–249.

40 del Río, J.C., Rencoret, J., Gutiérrez, A. et al. (2020). *ACS Sustainable Chemistry & Engineering* 8: 4997–5012.

41 Vanholme, R., Morreel, K., Darrah, C. et al. (2012). *New Phytologist* 196: 978–1000.

42 Chen, F., Tobimatsu, Y., Havkin-Frenkel, D. et al. (2012). *Proceedings of the National Academy of Sciences* 109: 1772–1777.

43 Wang, S., Shen, Q., Su, S. et al. (2022). *Trends in Chemistry*.

44 Li, Y., Meng, X., Meng, R. et al. (2023). *RSC Advances* 13: 12750–12759.

45 Chen, F., Tobimatsu, Y., Jackson, L. et al. (2013). *The Plant Journal* 73: 201–211.

46 Ralph, J., MacKay, J.J., Hatfield, R.D. et al. (1997). *Science* 277: 235–239.

47 Ralph, J., Kim, H., Peng, J., and Lu, F. (1999). *Organic Letters* 1: 323–326.

48 Barrière, Y., Ralph, J., Méchin, V. et al. (2004). *Comptes Rendus Biologies* 327: 847–860.

49 Ralph, J., Brunow, G., Harris, P.J. et al. (2009). *Recent Advances in Polyphenol Research* 1: 36–66.

50 Ralph, J., Lundquist, K., Brunow, G. et al. (2004). *Phytochemistry Reviews* 3: 29–60.

51 Lourenco, A., Rencoret, J., Chemetova, C. et al. (2016). *Frontiers in Plant Science* 7: 1612.

52 Rencoret, J., Marques, G., Gutierrez, A. et al. (2008). Structural characterization of milled wood lignins from different eucalypt species. *Holzforschung* 62: 514–526. https://www.degruyter.com/document/doi/10.1515/HF.2008.096/html

53 Buranov, A.U. and Mazza, G. (2008). *Industrial Crops and Products* 28: 237–259.

54 Li, Y., Shuai, L., Kim, H. et al. (2018). *Science Advances* 4: eaau2968.

55 Wang, S., Zhang, K., Li, H. et al. (2021). *Nature Communications* 12: 416.

56 Stone, M.L., Anderson, E.M., Meek, K.M. et al. (2018). *ACS Sustainable Chemistry & Engineering* 6: 11211–11218.

57 Li, Y., Akiyama, T., Yokoyama, T., and Matsumoto, Y. (2016). *Biomacromolecules* 17: 1921–1929.

58 Zhang, L., Gellerstedt, G., Ralph, J., and Lu, F. (2006). *Journal of Wood Chemistry and Technology* 26: 65–79.

59 Vanholme, R., Morreel, K., Ralph, J., and Boerjan, W. (2008). *Current Opinion in Plant Biology* 11: 278–285.

60 Voelker, S.L., Lachenbruch, B., Meinzer, F.C. et al. (2011). *Plant, Cell & Environment* 34: 655–668.

61 Shuai, L., Amiri, M.T., Questell-Santiago, Y.M. et al. (2016). *Science* 354: 329–333.

62 Lan, W., Amiri, M.T., Hunston, C.M., and Luterbacher, J.S. (2018). *Angewandte Chemie International Edition* 57: 1356–1360.

63 Vanholme, R., Cesarino, I., Rataj, K. et al. (2013). *Science* 341: 1103–1106.

64 Ralph, J., Akiyama, T., Coleman, H.D., and Mansfield, S.D. (2012). *Bioenergy Research* 5: 1009–1019.

65 Wagner, A., Ralph, J., Akiyama, T. et al. (2007). *Proceedings of the National Academy of Sciences* 104: 11856–11861.

66 Franke, R., Hemm, M.R., Denault, J.W. et al. (2002). *The Plant Journal* 30: 47–59.

67 Coleman, H.D., Park, J.-Y., Nair, R. et al. (2008). *Proceedings of the National Academy of Sciences* 105: 4501–4506.

68 Zhou, S., Runge, T., Karlen, S.D. et al. (2017). *ChemSusChem* 10: 3565–3573.

69 Wilkerson, C., Mansfield, S., Lu, F. et al. (2014). *Science* 344: 90–93.

70 De Meester, B., Vanholme, R., Mota, T., and Boerjan, W. (2022). *Plant Communications* 3: 100465.