Lignin: Structure and characterization

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Assignment
Learning outcomes

• Where does lignin come from?
• Principles of lignin biosynthesis
• Classification of lignins
• Importance of lignin to plants
• Distribution of lignin in plant cell walls
• Overview of reactions under thermochemical conditions
• Industrial sources & relevance
• Isolation and characterization
• Current and emerging applications
Contents

(1) Occurrence and functions in plants
(2) Biosynthesis and the diversity of molecular structures
(3) Industrial relevance
(4) Fundamental properties and reactivity
(5) Isolation and purification
(6) Derivatizations
(7) Characterization
(8) Applications
Lignin

- Main component of plant biomass (in addition to cellulose and hemicelluloses)
- Makes 25-35% of wood dry weight and 15-25% in annual plants
- Structurally, it is a polydisperse mixture of methoxylated polyphenols
- Naturally brown, hence the colour of wood
- Dissolved from wood chips in chemical pulp production: combusted for chemical recovery and heat and power production
- New uses for lignin are sought in materials and chemicals
1. Occurrence and functions in plants

- Lignin is a natural resin that fills spaces between plant cells and strengthens cell walls by covering cellulose microfibrils.

- Functions of lignin in plants:
  - Provides strength
  - Barrier against the attack of insects and microbes
  - Enables transportation of water and ions from the soil

Wheat straw xylem tissue seen in optical microscope.
Distribution of lignin in plant cell walls

- CC, ML enriched in lignin, presumably because lignification is initiated there\(^\text{[1]}\)
- Secondary walls contain the largest proportion of the total lignin in cell walls

\(^\text{[1]}\) Donaldson, Phytochem. 57 (2001) 859–873
\(^\text{[3]}\) Hale, 1969 "Structural and physical properties of pulpwood"
Imaging distribution of lignin in plant cell walls

Bright areas in pine wood arise from autofluorescence of lignin\cite{1}

Raman imaging of spruce lignin at the area of six mature cells\cite{2}

\begin{flushright}
\cite{1} Donaldson, Phytochem. 57 (2001) 859–873
\cite{2} Agarwal, Planta (2006) 224:1141–1153
\end{flushright}
Botanical classification of lignins

Additionally, lignin in softwood compression wood can be classified as GH lignin[1-2]

Gymnosperms
"Softwood"
G-lignin

Angiosperm dicotyledons
"Hardwood"
GS-lignin

Graminoids
"Grasses"
HGS-lignin

2. Biosynthesis and the diversity of molecular structures

• Growing trees and other plants do not ”just grow” – they are living organisms with subtle control over biosynthesis
• Lignin is synthesized principally from three precursor monomers which are referred to as monolignols
• Amino acid phenylalanine is the starting point:

[Diagram showing the process of phenylalanine, monolignols, and lignin polymers]
Several enzymes are needed in the monolignol synthesis

Common sites of lignin engineering (mutation)

- CAD: cinnamyl alcohol dehydrogenase
- COMT: caffeic acid O-methyltransferase
- F5H: ferulate 5-hydroxylase

Chemical structures:
- p-coumaryl alcohol
- coniferyl alcohol
- sinapyl alcohol

Zhao and Dixon, Trends Plant Sci. 2011
Lignin polymer is formed from monolignols in enzyme-initiated radical polymerization

Note: lignification occurs under chemical control i.e. it does not use template control (such as in protein synthesis)

• Result = mixtures of molecules (not one well-defined compound)

Example of radical delocalization

Coniferyl alcohol ion

Polymerization of lignin by radical coupling
Dimerization reactions of monolignols

a) Coniferyl alcohol Mg dimerization

b) Sinapyl alcohol Ms dimerization

Polymerization reactions of monolignols

Radical coupling:

- Monolignols react endwise with the growing polymer
- The resulting structure of the lignin macromolecule is “random” in the sense that several linkage types are possible at each step
- S-S coupling is an exception since this predominantly gives rise to $\beta$-O-4 units
Naming of the units in the lignin polymer

p-hydroxyphenyl (H)  Guaiacyl (G)  Syringyl (S)
Major structural units of lignin polymer

- Bolded bonds are formed in radical coupling and other in dimerization reactions

<table>
<thead>
<tr>
<th>Linkage type</th>
<th>Dimer structure</th>
<th>Approximate percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-O-4</td>
<td>Phenylpropane β-aryl ether</td>
<td>45–50</td>
</tr>
<tr>
<td>α-O-4</td>
<td>Phenylpropane α-aryl ether</td>
<td>6–8</td>
</tr>
<tr>
<td>β-5</td>
<td>Phenylcoumaran</td>
<td>9–12</td>
</tr>
<tr>
<td>5-5</td>
<td>Biphenyl and dibenzodioxocin</td>
<td>18–25</td>
</tr>
<tr>
<td>4-O-5</td>
<td>Diaryl ether</td>
<td>4–8</td>
</tr>
<tr>
<td>β-1</td>
<td>1,2-Diaryl propane</td>
<td>7–10</td>
</tr>
<tr>
<td>β-β</td>
<td>β-β-Linked structures</td>
<td>3</td>
</tr>
</tbody>
</table>

- Units linked to another phenylpropane unit from the aryl ring positions 3 and 5 (2 and 6) are referred to as "condensed linkages"
Covalent linkages between lignin and polysaccharides

- Lignin-carbohydrate complexes (LCCs) contain ether and ester bonds
- Linkages are mainly between hemicelluloses and lignin
- LCCs contribute to the cell wall integrity and difficulty of delignifying residual lignin[1]
- Lignin carbohydrate network (LCN) must be disrupted for enzymatic saccharification of plant biomass

A schematic illustration of LC-linkages in grasses[2]

Notes on structure and molecular weight of lignin

• Today, lignins are known to be more linear, less cross-linked\(^1\) and having lower Mw than previously thought
  • Lignin isolated from wood using ”mild” processing was oligomeric\(^2\)
  • Mw (or Mn) of lignin may be overestimated by size-exclusion chromatography (especially when vapor pressure osmometry gives lower Mn for the same sample)\(^3\)
• The analysis of lignin will be dealt more in-depth later in the lecture

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\(^1\) Lundqvist and Parkás, BioResources 6:2 (2011) 920-926
\(^2\) Crestini et al., Biomacromolecules 2011, 12, 3928–3935
\(^3\) Gosselink et al., Ind. Crops Prod. 19 (2004) 271–281
\(^5\) Sipponen, Doctoral thesis 2015
Presemo
3. Industrial relevance

- Lignin is removed from fibers during cellulose pulp production
  - Provides surplus energy for the pulp mills
- Kraft, soda, sulfite, organosolv processes rely on distinct reactions
- Lignin is a structural constraint and inhibitor in biochemical conversion of plant biomass into ethanol etc.
- Biorefineries produce soluble and insoluble lignin
  - A spectrum of different hydrothermal and thermochemical lignocellulose pretreatment processes are under development
4. Fundamental properties and reactivity

• Lignins are negatively charged in aqueous solutions
  • pKa of phenolic hydroxyl groups vary between 7−10,[1] depending on the neighbouring substituents; carboxylic acids have generally pKa < 4
• Solubility of lignin is important for industrial utilization and analytical work
  • Lignins are usually soluble in alkaline water and often (but not always) also in polar organic solvents and ionic liquids such as:

  - methanol (CH₃OH)
  - ethanol (CH₃CH₂OH)
  - 2-methoxyethanol (CH₂OHCH₂OCH₂CH₃)
  - formic acid (HCOOH)
  - acetic acid (CH₃COOH)
  - dimethylformamide (HCON(CH₃)₂)
  - dimethylsulfoxide (CH₃SOCH₃)
  - tetrahydrofuran (THF)
  - 1,4-dioxane
  - γ-valerolactone (GVL)
  - ethylene glycol (HOCH₂CH₂OH)
  - ethanol
  - dimethylsulfoxide (CH₃SOCH₃)
  - pyridine (C₅H₅N)
  - and many other ILs[2]

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Polydispersity of lignin

- When isolated from wood, lignin contains molecules at various molecular weights.
- Small lignin oligomers are naturally present also in plant biomass.

Molecular weight increases logarithmically

Baumberger et al., Holzforschung 61 (2007) 459–468
Stability of covalent bonds in lignin

- Bond dissociation enthalpies (BDE) based on density functional theory calculations:
  - $\alpha$–O–4 in GG pinoresinol, 284 kJ/mol\textsuperscript{[1]} $\beta$–O–4 (native) HG, 292 kJ/mol\textsuperscript{[2]} SS 295 kJ/mol\textsuperscript{[3]}
  - C–O are weaker than C–C bonds
  - Side-chain oxidation decreases BDE

- **Note that lignin is more reactive in thermochemical conditions because of:**
  - Formation of reactive quinone methide intermediates
  - Neighbouring group participation

\textsuperscript{[1]} Elder, Energy Fuels 2014, 28, 1175–1182
\textsuperscript{[2]} Younker et al., ChemPhysChem 2011, 12, 3556 – 3565
\textsuperscript{[3]} Kim et al., J. Phys. Chem. Lett. 2011, 2, 2846–2852
Quinone methide intermediates are important reactive forms of lignin

Reactions of lignin through the quinone methide intermediate in acidic, neutral and alkaline media.\cite{1}

\[ R' = \text{O, S, SO}_2 \text{ or internal nucleophile} \]
\[ R = \text{OAr, Ar or Alk} \]

Sulfonation of lignin

- Formation in acid sulphite pulping
- Sulfoxyl groups (pKa<2) give water-solubility within a broad pH region
- Lignosulfonates are industrial chemicals
  - Plasticizers and additives in concrete and cement production
  - Viscosity reducing additive in oil drilling mud
  - Dispersants in pesticides, dyes, carbon black
  - Vanillin production (example follows later)

Cleavage of β-O-4 bonds in pulping processes

- The most important scission reaction in lignin chemistry, why?
  - Because β-O-4 bonds are so abundant and thermochemically labile
- Basis of delignification (depolymerisation → dissolution) in cellulose pulp production
  - Increases the content of phenolic hydroxyls
  - Side reactions form new C–C bonds
**β-O-4 cleavage during kraft cooking**

Formation of active species in kraft pulping\[1\]

\[
\begin{align*}
NaOH + H_2O & \leftrightarrow Na^+ + OH^- + H_2O \quad (1) \\
Na_2S + H_2O & \leftrightarrow 2Na^+ + S^{2-} + H_2O \quad (2) \\
S^{2-} + H_2O & \leftrightarrow HS^- + OH^- \quad (3)
\end{align*}
\]

Elimination of formaldehyde

- The cooking takes place at 160-180 °C and lasts 1-2 h
- Demethylation and condensation occur also\[1\]

Formation of the reactive quinone methide intermediate

Sulfidolytic cleavage of β-aryl ether bonds

A simplified reaction scheme of kraft cooking\[2\]

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\[1\] Vuorinen, CHEMISTRY OF PULPING AND BLEACHING, https://mycourses.aalto.fi/pluginfile.php/140064/mod_resource/content/1/Supporting%20material%201.pdf
β-O-4 cleavage during soda (NaOH) cooking

Reaction occurs via neighbouring group participation

Intramolecular epoxide intermediate in the aliphatic chain

**β-O-4 cleavage in acidic conditions**

- Exact mechanism had not been fully elucidated, but the formation of β-ketones (so-called Hibbert’s ketones is well documented).

- Condensation at the α-carbon is the competitive route.

Li et al., Bioresour. Technol. 98 (2007) 3061–3068
Organosolv pulping: ethanol-water process as an example

- Ethylation of the carbocation at Cα reduces formation of condensed linkages.
- This is one reason why organosolv lignins have low Mw, Tg, and dissolve well in organic solvents.

Proposed ethylation of lignin in organosolv pulping

Formation of chemically stable "condensed" C–C linkages

• Generally, condensed linkages reduce solubility and increase molecular weight of lignin
• Occurs either in acidic or alkaline media, for example:

Acidic media:

alkaline media:

Presemo
5. Isolation and purification of lignin

Industrial lignin production

- Lignosulfonates (~1100 kt per year)
  - Borregaard LignoTech (Norway) is the main producer
- Kraft (sulfate) lignin (~100 kt per year)
  - WestRock (formerly MeadWestvaco) (US)
  - 2013: Domtar, North Carolina (US)
  - 2015: Stora Enso, Sunila (Finland)
  - 2016: West Fraser LignoForce 30 t/day demonstration plant
Isolation of lignin from spent pulping liquors

- Acid precipitation from alkaline solution: recall that lignin contains acidic groups which ionize above pH 4 and pH 7–10: precipitation occurs when anionic charge is reduced so that aggregation occurs
- Complexation with metals or cationic polymers
- Anti-solvent precipitation: lignin soluble in organic solvent (ethanol) precipitates when mixed with a miscible fluid (water)
- Membrane technology using small pore sizes ~1 kDa
Commercial lignin isolation processes

• Based on the capacity of CO₂ to reduce pH and precipitate lignin at pH 10
  • Carbon dioxide precipitation of lignin from pine kraft black liquor[1]
• Lignoboost technology was further developed by Innventia and acquired by Valmet
• LignoForce technology oxidizes lignin before CO₂ precipitation[2]

Analytical lignin isolation methods

- Isolation of lignin from wood for characterization or as a model material causes always some structural alteration.
- In the Milled Wood Lignin (MWL) method, wood is ground in a ball mill and subsequently extracted with aqueous Dioxane.
- Cellulolytic enzyme lignin (CEL) and Enzymatic Mild Acidolysis Lignin (EMAL) procedures remove polysaccharides using hydrolytic enzymes before extraction of lignin with (acidic) aq. dioxane.

6. Lignin derivatizations

- In order to analyze structure of lignin or to render it more suitable to applications, chemical or enzymatic derivatizations are used.
- Some common chemical derivatizations are:
  - Acetylation
  - Methylation
  - Carboxymethylation
  - Cationization
  - Cross-linking
- Various other organic compounds such as fatty acids can be grafted to lignin.
- The enzyme laccase has been used to oxidize and cross-link lignins.
7. Characterization of lignin
Tools for characterization of lignin

- Combination of wet chemistry with spectroscopy, chromatography, and various other techniques
- Understanding the organic chemistry in use may help developing possible routes for chemical and materials production from lignin
- Characterization in liquid or solid state?
  - **Liquid state**: functional groups, molecular weight, interunit linkages
  - **Solid state**: material properties, functional groups and elemental composition, imaging of the physical structure
- A few illustrative examples follow
Solution-state analysis of lignin

- **Chemical degradations**
  - Oxidations: ozonolysis, permanganate oxidation, nitrobenzene oxidation, *cupric oxide oxidation*
  - Reductive treatments: *thioacidolysis*, DFRC
  - Chromatography is used to quantify the products
- **NMR spectroscopy**
- **Size-exclusion chromatography**
Cupric oxide oxidation

- A classical oxidative degradation method still in use today due to its simplicity
- Reaction mechanism involves radical oxidation

\[ \text{LIGNIN} \xrightarrow{\text{CuO}} \text{vanillic acid} + \text{vanillin} + \text{Acetovanillone} \]

\[ \text{CuO} \quad 4 \text{ M NaOH, 170 °C, 3 h} \]

Note that regardless of the lignin source vanillin is always the main product
Thioacidolysis

- Comparable information as from CuO oxidation, but more specific to b-O-4 cleavage and yields less degradation side-products
- Solvolytic cleavage of lignin in the presence of ethanethiol
- Malodorous (EtSH) and harmful (Et₂O-BF₃) reagents limit applicability

13C NMR

- Nuclear Magnetic Resonance (NMR) spectroscopy measures chemical shifts (changes in frequency compared to the reference frequency) when the atom is subjected to a magnetic pulse at similar frequency as its natural resonance frequency.
- 13C NMR delivers a wealth of quantitative structural information:[1]
- However, time consuming in data collection and analysis.

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HSQC NMR (2D NMR)

- Integration of $^1$H and $^{13}$C cross peaks gives semiquantitative information of the lignin interunit linkages as well as linkages to carbohydrates

\textbf{\textsuperscript{31}P NMR, a powerful method for OH groups}

- Derivatization of lignin in solution with a P-donor which is quantitatively measured against internal standard

- \textbf{TMDP = 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane}

- \textbf{IS = N-Hydroxy-5-norbornene-2,3-dicarboxylic acid imide}

Sipponen, Doctoral thesis 2015
Size-exclusion chromatography (SEC)

- Separation of molecules based on their hydrodynamic radii (size in solution)
- Organic and aqueous elution systems are in use
- Sample must dissolve fully and should not associate with the particles in the column packing material
- Molecular weight is calculated relative to known Mw standards or directly using laser scattering or viscometer

https://en.wikipedia.org/wiki/Size-exclusion_chromatography#media/File:SizeExChrom.png
Analysis of lignin as a product

• Complex structure, but all-inclusive analysis is not necessary
• **Key properties in relation to the application important**
• Real application tests needed also
• Distinction of a high purity lignin from a low purity one:
  – Low amounts of contaminating carbohydrates, sulphur, inorganics
  – Good solubility and material properties
8. Applications of lignin

• 2·10^{10} tons of lignin is produced annually in Nature [1]
• Only a fraction of the total is used in products, why?
  – Lack of regular (stereochemical) structure
  – Heterogeneous repeating units (H, G, S and their linkage patterns)
  – Poor understanding of how the degree of polymerization and structure change when lignin is isolated from cell walls
• However, recent advances in lignin isolation and characterization hold promise for increased use of lignin in materials
R&D for commercialization of lignin is undergoing rapid growth

Experts say that lignin may eventually be one of the new renewable materials extracted out of the green gold of the Nordic forests. Stora Enso’s Sunne pulp mill in Finland will, in the future, extract lignin from pine wood springs.

Following the biorefinery investment announcement made in July, there is an air of excitement at the mill.

The first dedicated biorefinery investment is expected to be completed in early 2015. Mill Director Olli-Pekka Reunanen is pleased, and with good reason: installing the new machinery in the architectural milieu that dates back to 1968 is an enjoyable challenge for the mill staff. "We won’t be building new buildings; instead, the idle buildings in the mill area will be used to house the machinery required for the biorefinery," explains Reunanen.

Prior to making the 32-million-euro investment decision, Stora Enso carefully looked into areas where lignin extraction would be profitable. Sunne is Stora Enso’s first, but probably not its last, biorefinery dedicated investment.

Stora Enso has been researching lignin and its attributes for more than a decade.

"Lignin is a challenging and complex material, it’s an honour to be a pioneer in this area. At the same time, we are taking a step into new and previously unchartered markets," says Mikael Hannus of the Biomaterials’ Biorefinery and Bioenergy unit.

The initial markets are anticipated in, for example, the construction and automotive industries, where lignin offers a sustainable alternative to the phenols used in plywood and wood-paneling glues and the polyols used in foams. Other applications are also under development.

http://www.upmbiochemicals.com/lignin/Pages/Default.aspx
Industry investment in lignin production is good news to R&D in this area

**BioChoice™ Description and Applications**

BioChoice™ lignin is a bio-based alternative to the use of petroleum and other fossil fuels. A wide range of potential applications for BioChoice lignin exist, including in the energy, materials and chemical categories.

- Adhesives
- Agricultural Chemicals
- Carbon Products (e.g., carbon fiber, graphite, activated carbon, etc.)
- Coatings
- Dispersants
- Fuels and Fuel Additives
- Natural Binders
- Resins

**BioChoice Product Attributes**

- Total Lignin > 95% of Total Solids
- Low Ash
- Low Sulphur
- Single Species - Southern Pine

Current and emerging applications

- Energy (it has value!) 26.7 MJ/kg (HHV)[1]
- Vanillin, 3% from softwood (~10% of lignin)
  - Today, 20% of vanillin is produced from lignin and 80% from crude oil using the guaiacol route[2-3]
- Pyrolysis of lignin → bio-oil (15–25%), gas (20–50%), char (30–60%)[4]
- Aromatic chemicals (BTX)
- Composites and polymer blends
- Micro- and nano-scale structures (particles, capsules, sheets etc.) for novel applications
- High-performance materials such as carbon fibres are also under development

Oxidation of lignosulfonate to vanillin

Compare the reaction to the analytical cupric oxide oxidation presented above.
Carbon fibers from lignin

- Carbon fibers are high-value materials currently produced from polyacrylonitrile
- Lignin is more affordable raw material, but less linear polymer with predominant ether linkages
  - Challenges the production of carbon fibres with sufficient strength properties
  - However, this is promising results have been obtained in this research area

Kadla et al., Carbon 40 (2002) 2913–2920
Summary of keywords

condensation  aliphatic  dimerization
phenolic  oxidation / reduction
quinone  radical coupling
methide  polydisperse
intermediate  branched
methoxyl  condensed / uncondensed
monolignols  units
β-O-4  heterogeneous
aromatic  anionic
lignin  p-hydroxyphenyl
lignosulfonate  guaiacyl
kraft lignin  syringyl
condensed / uncondensed units
Assignment