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Advances in biotechnology and chemistry hold promise for greatly expanding the scope of products derived from lignin.

# Lignin Valorization: Improving Lignin Processing in the Biorefinery

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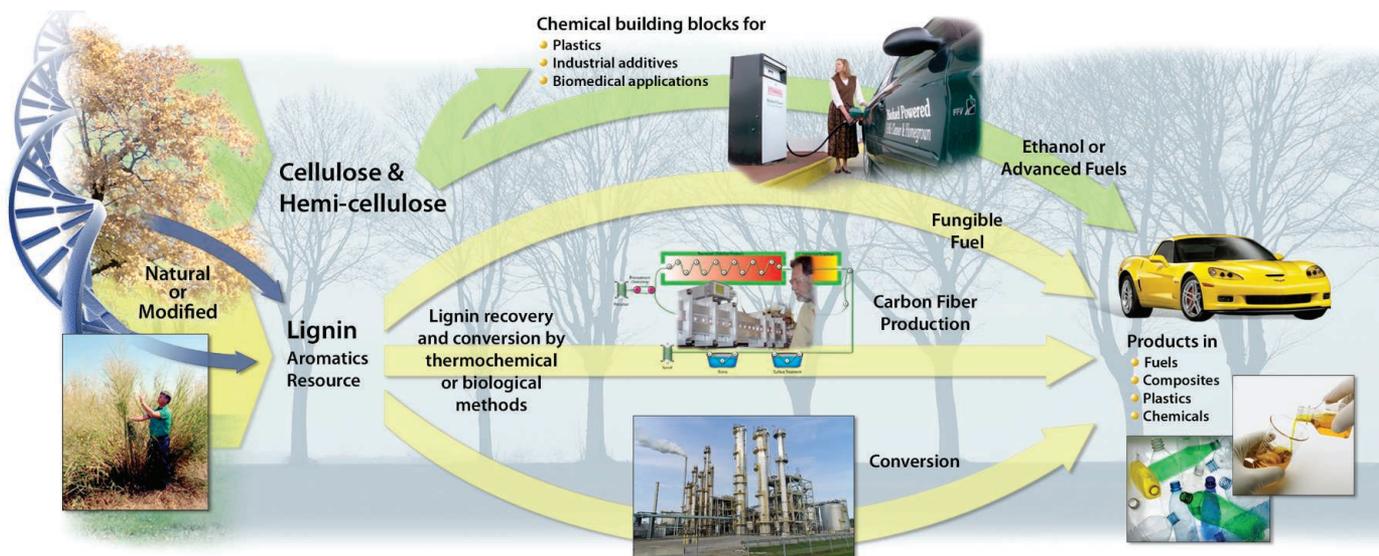


**Background:** Lignin, nature's dominant aromatic polymer, is found in most terrestrial plants in the approximate range of 15 to 40% dry weight and provides structural integrity. Traditionally, most large-scale industrial processes that use plant polysaccharides have burned lignin to generate the power needed to productively transform biomass. The advent of biorefineries that convert cellulosic biomass into liquid transportation fuels will generate substantially more lignin than necessary to power the operation, and therefore efforts are underway to transform it to value-added products.

**Advances:** Bioengineering to modify lignin structure and/or incorporate atypical components has shown promise toward facilitating recovery and chemical transformation of lignin under biorefinery conditions. The flexibility in lignin monomer composition has proven useful for enhancing extraction efficiency. Both the mining of genetic variants in native populations of bioenergy crops and direct genetic manipulation of biosynthesis pathways have produced lignin feedstocks with unique properties for coproduct development. Advances in analytical chemistry and computational modeling detail the structure of the modified lignin and direct bioengineering strategies for targeted properties. Refinement

of biomass pretreatment technologies has further facilitated lignin recovery and enables catalytic modifications for desired chemical and physical properties.

**Outlook:** Potential high-value products from isolated lignin include low-cost carbon fiber, engineering plastics and thermoplastic elastomers, polymeric foams and membranes, and a variety of fuels and chemicals all currently sourced from petroleum. These lignin coproducts must be low cost and perform as well as petroleum-derived counterparts. Each product stream has its own distinct challenges. Development of renewable lignin-based polymers requires improved processing technologies coupled to tailored bioenergy crops incorporating lignin with the desired chemical and physical properties. For fuels and chemicals, multiple strategies have emerged for lignin depolymerization and upgrading, including thermochemical treatments and homogeneous and heterogeneous catalysis. The multifunctional nature of lignin has historically yielded multiple product streams, which require extensive separation and purification procedures, but engineering plant feedstocks for greater structural homogeneity and tailored functionality reduces this challenge.



**Production of biofuels from cellulosic biomass requires separation of large quantities of the aromatic polymer lignin.** In planta genetic engineering, enhanced extraction methods, and a deeper understanding of the structure of lignin are yielding promising opportunities for efficient conversion of this renewable resource to carbon fibers, polymers, commodity chemicals, and fuels. [Credit: Oak Ridge National Laboratory, U.S. Department of Energy]

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# Lignin Valorization: Improving Lignin Processing in the Biorefinery

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Research and development activities directed toward commercial production of cellulosic ethanol have created the opportunity to dramatically increase the transformation of lignin to value-added products. Here, we highlight recent advances in this lignin valorization effort. Discovery of genetic variants in native populations of bioenergy crops and direct manipulation of biosynthesis pathways have produced lignin feedstocks with favorable properties for recovery and downstream conversion. Advances in analytical chemistry and computational modeling detail the structure of the modified lignin and direct bioengineering strategies for future targeted properties. Refinement of biomass pretreatment technologies has further facilitated lignin recovery, and this coupled with genetic engineering will enable new uses for this biopolymer, including low-cost carbon fibers, engineered plastics and thermoplastic elastomers, polymeric foams, fungible fuels, and commodity chemicals.

Translational biorefinery research and development has become a near-global effort in response to a variety of drivers, including energy security, rural development, and environmental concerns. The approved Summary for Policymakers in the Intergovernmental Panel on Climate Change (IPCC) Working Group I Fifth Assessment Report report states that “Human influence on the climate system is clear. This is evident from the increasing greenhouse gas concentrations in the atmosphere, positive radiative forcing, observed warming, and understanding of the climate system.” These findings add urgency to the need to develop viable, sustainable, biorefining technologies that maximize yields of renewable fuels, chemicals materials, and biopower (1). Recent results have demonstrated that biomass conversion into biofuels can deliver a sustainable and renewable energy source for liquid transportation fuels (2–4). For

the United States alone, a recent study suggested that more than 1.3 billion tons annually of biomass could be sustainably produced from agricultural and forestry sources (5). Indeed, coupling advanced biomass-conversion technologies with land-use changes could meet the nation’s need for liquid transportation fuels without affecting food, feed, and fiber production (6). Although widespread, cost-competitive biofuels production at the industrial scale must overcome multiple technical and economic challenges (7), several commercial cellulosic ethanol plants have been commissioned (8), and the first plant now generates 75 million 1 year<sup>-1</sup> of cellulosic ethanol in Italy (9).

Currently, most integrated biologically based biorefinery concepts comprise four major core sections: feedstock harvest and storage, pretreatment, enzymatic hydrolysis, and sugar fermentation to ethanol or other fuels (10). Lignin, the second most abundant terrestrial polymer on Earth after cellulose, is underutilized in these first-generation cellulosic projects, with about 60% more lignin generated than is needed to meet internal energy use by its combustion (11, 12). Therefore, new processes are needed that generate value-added products from lignin (13). Lignin is the only large-volume renewable feedstock that is composed of aromatics (14). The U.S. Energy Security and Independence Act of 2007 mandates the development of 79 billion liters of second-generation biofuels annually by 2022. Assuming a yield of 355 liters per dry ton of biomass, 223 million tons of biomass will be used annually, producing about 62 million tons of lignin (15). Without new product streams, the lignin produced would far exceed the current world market for lignin used in specialty products (16).

Although fundamental research has historically focused on converting lignin to chemicals,

materials, and fuels, very little of this effort has been translated into commercial practice. So what has changed to address this paradigm? In brief, research and commercialization efforts surrounding cellulosic ethanol have tilted the tables through several important developments: (i) bioengineering of lignin to modify and/or incorporate atypical components that reduce recalcitrance of the cell walls to bioprocessing and facilitate ease of recovery and conversion; (ii) advances in analytical chemistry and computational modeling that couple developments in genetic engineering of lignin to targeted physical and chemical properties; and (iii) biomass pretreatment technologies that facilitate lignin recovery and catalytic modifications that yield tailored chemical and/or physical properties.

## Lignin Biosynthesis

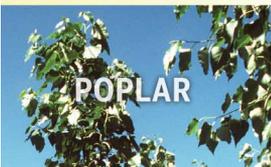
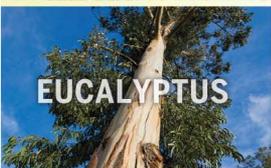
Lignin is derived from the radical polymerization of substituted phenyl propylene units. Figure 1 highlights the relative amounts of lignin and plant polysaccharides in several key agroenergy plants and woody resources.

Lignin modification in plants has been extensively investigated to reduce lignin levels or to alter its structure to facilitate pulping, improve forage digestibility, or overcome recalcitrance for bioenergy feedstocks (17, 18). The biosynthetic pathway to the three classical monolignol building blocks of lignin was thought to be understood more than 10 years ago (19), although a recent revision suggests that we may still have more to learn (20, 21). Eleven recognized enzymes are involved in the conversion of L-phenylalanine to the primary monolignols, p-coumaroyl alcohol, coniferyl alcohol, and sinapyl alcohol that generate the hydroxyphenyl (H), guaiacyl (G), and syringyl (S) lignin subunits, respectively (Fig. 2), and most of these enzymes have been targeted for down-regulation to generate plants with reduced lignin levels (22–24). Lignin levels can also be effectively reduced by targeting pathways to precursors for monolignol biosynthesis, such as C1 metabolism to supply methyl groups (25), manipulation of transcription factors (26), or introduction of engineered enzymes that generate monolignol analogs incapable of polymerization (27). In many cases, the digestibility or saccharification potential of biomass has been considerably enhanced (28), sometimes at the expense of plant growth (29–31). In some studies, reduction of lignin by ~50% or less from the wild-type levels has made pretreatment unnecessary for efficient saccharification (32).

Gymnosperm lignins lack S units and, as a result, are generally more branched than the classical G/S-rich lignin of angiosperms, which are rich in β-O-aryl ether linkages and cross-linked to cell wall polysaccharides via coupling of feruloylated xylenes to lignin or by nucleophilic addition of cell wall sugars to lignin quinone-methide intermediates yielding ether-linked lignin carbohydrate linkages (33). Ferulates and

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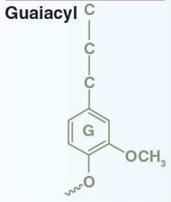
Plant resource	% Hemicellulose	% Cellulose	% Lignin*
 MISCANTHUS	24-33	45-52	9-13
 SWITCHGRASS	26-33	37-32	17-18
 CORN STOVER	31	37	18
 POPLAR	16-22	42-48	21-27
 EUCALYPTUS	24-28	39-46	29-32
 PINE	23	46	28

\*Typical aromatic polymer containing:

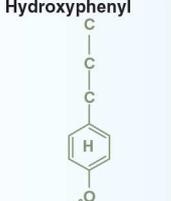
**Syringyl C**



**Guaiacyl C**



**Hydroxyphenyl**



Depending on the bioresource and isolation methodology, molecular weights for native lignin have been reported from 78,400 [in spruce (118)] to 8300 [in Miscanthus (119)] g mol<sup>-1</sup>, which are derived from C9 monolignols as described in Fig. 2.

**Fig. 1. Typical biomass constituents for select plant resources.** Information for miscanthus is from (120); switchgrass, (121); corn stover, (122); poplar, (123); eucalyptus, (124); pine, (125). [Photo credits: R. Kaltschmidt, Lawrence Berkeley National Laboratory, DOE (eucalyptus); Oak Ridge National Laboratory, DOE (all other photos)]

coumarates are particularly abundant in the cell walls of grasses (34). These lignin properties contribute to recalcitrance by hindering the accessibility of cellulose microfibrils to microorganisms and enzymes but also affect coproduct value. Interruption of the monolignol pathway can alter the H/G/S ratios (35) and reduce the degree of polymerization to benefit lignin removal during pretreatment (36). Furthermore, studies on both natural and engineered lignins have demonstrated that lignin biosynthesis, and therefore structure, is more adaptable than originally believed, with variations tolerated in both the aromatic ring and side chain. For example, by up-regulating ferulate-5-hydroxylase (F5H) (Fig. 2), the entry point to S lignin biosynthesis, while simultaneously down-regulating the enzyme that O-methylates the product of F5H, *Arabidopsis* can be generated with the bulk of the lignin as 5-hydroxyguaiacyl

(5HG) units (Fig. 2, structure B) (37), and this approach would appear to be widely applicable. Such lignins have recently been shown to occur naturally in the seed coats of a limited number of species of cacti (38), and trace levels of 5HG units likely occur in lignins of perennial angiosperms. By restoring growth to plants harboring a loss-of-function mutation in the 4-coumaroyl shikimate 3'-hydroxylase gene (Fig. 2) as a result of disrupting components of the Mediator complex, it was possible to recover *Arabidopsis* plants in which the lignin was essentially comprised of only H units (21).

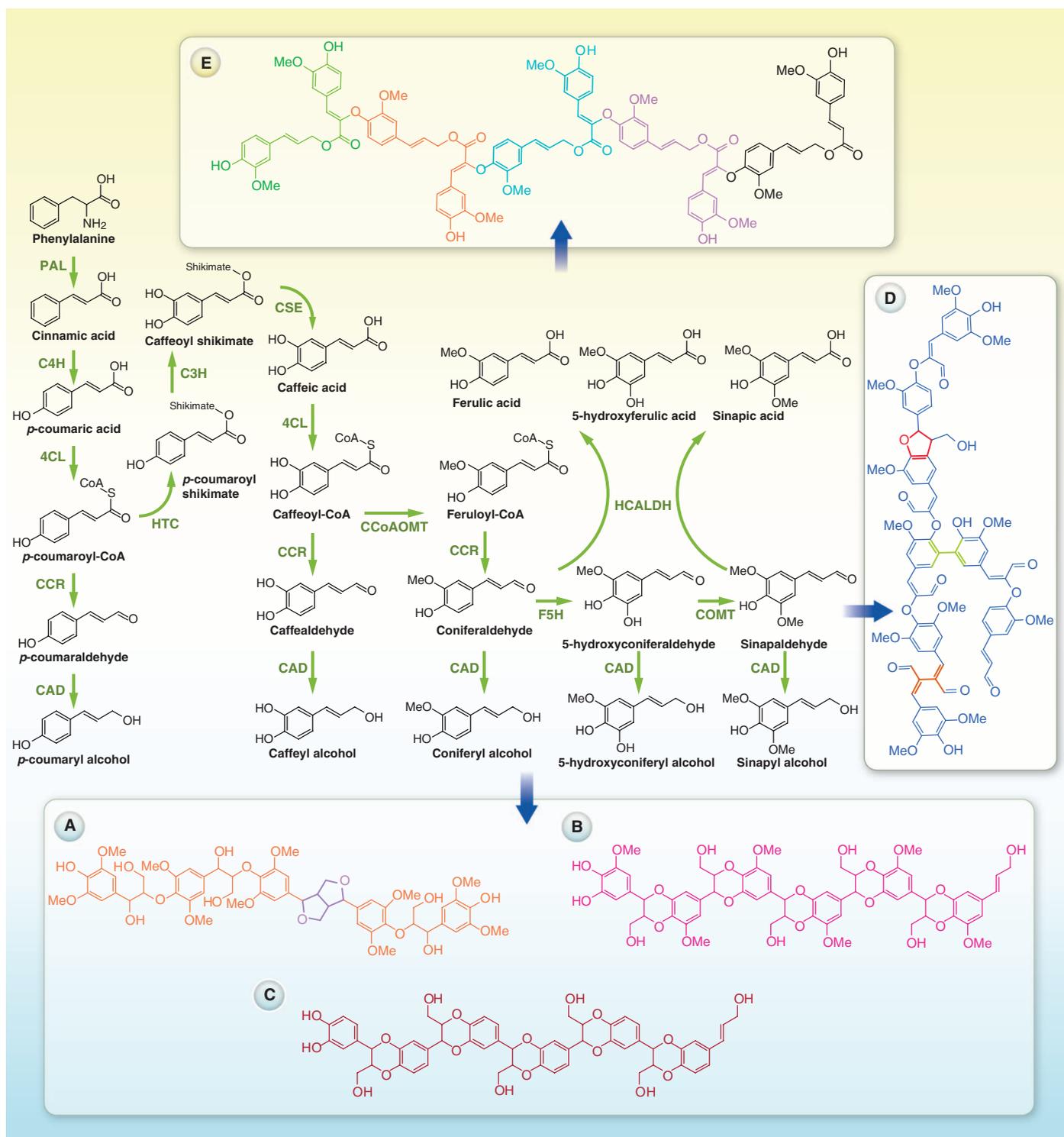
The seed coats of a larger number of unrelated and relatively exotic plant species, both monocots and dicots, contain a lignin comprising only catechyl alcohol units that are completely non-methylated and form a linear homopolymer (Fig. 2, structure C) (39, 40). In species examined

so far in which this C lignin coexists with G/S lignin, it is not attached to the classical lignin polymer (41). Down-regulation of cinnamyl alcohol dehydrogenase results in an increased aldehyde signature in lignin (42, 43), and mutants of barrel medic (*Medicago truncatula*), in which the cinnamyl alcohol dehydrogenase 1 (*CAD1*) gene has been disrupted, contain lignin that is almost exclusively composed of hydroxycinnamaldehydes rather than hydroxycinnamyl alcohol units (Fig. 2, structure D) (44), suggesting that monolignol side chains can exhibit variation without seriously compromising the lignification process. Unnatural monomers can also be introduced that will result in formation of more cleavable interunit bonds (Fig. 2, structure E) (45). Genetic engineering to incorporate unnatural lignin monomers with shortened side chains (40) reduces the degree of polymerization and facilitates biomass processing. An alternative approach was recently reported by Wilkerson *et al.* (46) whereby coniferyl ferulate feruloyl-coenzyme A (CoA) monolignol transferase was expressed in poplar. This transformation facilitated the incorporation of monolignol ferulate conjugates into lignin, which yields lignin with elevated ester linkages that are predisposed to mild alkaline depolymerization.

This flexibility in lignin monomer composition is potentially useful for reducing recalcitrance, enhancing extraction, and developing lignin as a high-value coproduct. For example, unlike the G lignin found in gymnosperms with complex interunit linkages, predominantly S lignin molecules engineered by overexpression of F5H (Fig. 2, structure A) have fewer interunit linkage types, facilitating separation of lignin from biomass and significantly reducing recalcitrance (47).

To date, linear C lignin has only been reported in seed coats. Simultaneous mutations in the two monolignol O-methyltransferases that should theoretically accumulate the precursor for formation of catechyl alcohol units do not lead to substantial accumulation of C lignin in stems of transgenic plants; rather, the plants are dwarfed, fail to develop properly, and contain mainly H lignin (48). The presence of naturally high levels of C lignin in *Jatropha curcas* seed coats (39), a high volume by-product of biodiesel production from the seed oil of this species, suggests a near-term resource for exploitation of this polymer.

Further development of modified lignins as feedstocks for industrial products will require a high level of production of such lignins as coproducts in lignocellulosic bioenergy crops. Considerable variation in lignin content exists in natural populations (49), but most attempts to increase lignin levels in plants by simply over-expressing one or more enzymes in the monolignol pathway have been unsuccessful. In fact, in an association study of 1100 sequenced black cottonwood (*Populus trichocarpa*) genotypes, representing the main geographic distribution of the species, none of the extreme variants in lignin



**Fig. 2. Pathways for the biosynthesis of monolignols, recently discovered nonclassical lignins, and modified lignin structures that can be obtained through genetic engineering.** The enzymatic steps (green arrows) are catalyzed by PAL, L-phenylalanine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; CCR, cinnamoyl-CoA reductase; CAD, cinnamyl alcohol dehydrogenase; HCT, hydroxycinnamoyl CoA:shikimate/quininate hydroxycinnamoyl transferase; C3'H, 4-coumaroyl shikimate 3-hydroxylase; CCoAOMT, caffeoyl-CoA 3-O-methyltransferase; COMT, caffeic acid/5-hydroxyconiferaldehyde 3-O-methyltransferase; CSE, caffeoyl shikimate esterase; F5H, ferulate/coniferaldehyde 5-hydroxylase. After transport of

monolignols to the apoplast, polymerization (blue arrows) is catalyzed by laccases (essential for initiation of polymerization *in vivo*) and peroxidases. The different lignin structures (showing only portions of the complete polymers) are A, all syringyl (S) lignin from poplar (*Populus*) overexpressing F5H; B, all 5-hydroxyguaiacyl lignin from poplar overexpressing F5H and down-regulated in COMT; C, all catechyl lignin (C lignin) from the seed coats of species within families including the Cactaceae, Cleomaceae, and Orchidaceae; D, lignin constructed from hydroxycinnamaldehydes, as found in the *M. truncatula cad-1* mutant; E, a hypothetical ester-linked linear lignin. Me, methyl group.

content, S/G ratio, or degree of polymerization contained naturally occurring mutations in the 11 enzymes of the lignin biosynthetic pathway (50). Genes controlling lignin content, molecular weight, and composition include copper transporters, vesicular trafficking genes, shikimate pathway genes, and transcription factors. Similarly, several independent mutations exist in the model species *Arabidopsis thaliana*, mostly in genes encoding transcription factors that lead to ectopic lignin deposition (51, 52). Mining natural lignin variants within existing bioenergy crops may reveal further variants in lignin composition that will favorably impact lignin as a primary biofeedstock, allowing for generation of extreme variants with unique chemical composition and physical properties that can be industrially exploited. Given the plasticity of the lignin biosynthetic pathway, unique lignins can be expected to be common rather than exceptional. Alternatively, ectopic lignin production, particularly in pith tissue that neither provides mechanical support nor enables vascular function, might facilitate the plant's accumulation of forms of lignin optimized as coproducts. However, that approach will not functionally replace classical lignins, thus allowing the tolerance of lignin for compositional diversity to be more fully exploited. Strategies have been reported for activating lignification in pith tissues in dicotyledonous plants through repression of transcription factors (53).

Two factors are critical for realizing the value of lignin in the biorefinery: ease of extraction of a lignin stream, which is generally facilitated by manipulations that reduce cross-linking to other cell wall polymers, and a structure that facilitates downstream processing, as to be discussed.

### Lignin Characterization

The ability to genetically engineer new lignin structures and control lignin deposition in plants has developed in parallel with powerful new methods for imaging lignin and analyzing its chemical structure. For example, coherent anti-Stokes Raman scattering (CARS) provides a label-free method for chemical imaging of lignin with greatly enhanced sensitivity over state-of-the-art confocal Raman scattering microscopy at a spatial resolution of about 200 nm (54). Although not as sensitive as CARS, time-of-flight secondary ion mass spectrometry (ToF-SIMS) spectral imaging can provide lignin chemical distribution maps for intact and processed biomass samples (55). Select ion monitoring in ToF-SIMS experiments makes it readily possible to visualize the distribution of S and G units in plant cross sections (56) within a single cell wall of *P. trichocarpa*. Alternatively, lignification in *A. thaliana* and *Pinus radiata* has been studied by using fluorescence-tagged monolignol analogs that penetrate plant tissue and incorporate into cell wall lignin to reveal insights into lignin deposition (57). Immunological techniques for lignin analysis have also been devel-

oped such that monoclonal antibodies can be used to detect phenylcoumaran and resinol-like structures (58) in biomass. Although still early in development, mode-synthesizing atomic force microscopy (AFM) promises to combine the analytical resolution of AFM with spectroscopy (59).

Structural analysis of lignin has become almost a subdiscipline of its own, with several textbooks written on the topic (60). One of the most promising high-throughput methods for lignin analysis is pyrolysis molecular beam mass spectrometry, which requires minimal sample amounts and routinely provides analysis of hexose and pentose sugars, lignin content, and S/G ratios (53). Structural analysis of interunit linkages of lignin is often accomplished by using advanced one- and two-dimensional (2D) nuclear magnetic resonance (NMR) (61). Likewise, detection of lignin-carbohydrate complexes increasingly relies on NMR methodologies (62). Whole-cell NMR techniques using the resolving power of 2D NMR to make structural assignments and relative signal intensity measurements have replaced the more laborious methodology that required preisolation of the lignin (63). Selective chemical fragmentation of lignin by thioacidolysis or derivatization followed by reductive cleavage and gas chromatography-mass spectrometry (GC-MS) analysis has been equally informative at identifying the main interunit linkages of lignin (64). This methodology is especially attractive for sample-size-limited lignin-related material. Regardless of the methodologies used, critically important detailed sequencing of extended interunit linkage frequencies (65, 66) and lignin carbohydrate complexes (67, 68) remains challenging.

Neutron scattering represents an emerging complementary approach to characterize lignin's structural properties. In particular, small-angle neutron scattering (SANS) provides structural information over the nm- to  $\mu\text{m}$ -length scale range, which can be interpreted by computational methods to provide an atomic-level predictive understanding of physical properties. For example, large computational atomistic simulations of models of the plant cell wall, informed by SANS, have revealed some of the fundamental physical processes involved in the phase separation of lignin from other plant matrix polymers and subsequent lignin aggregation during various types of acidic thermal treatments of biomass (69). Molecular dynamics simulations indicated that low-temperature lignin collapse is thermodynamically driven by an increase in translational entropy and solvation effects (70). The temperature dependence of the structure and dynamics of individual softwood lignin polymers was examined by using extensive molecular dynamics simulations. Lignin was found to transition from glassy, compact to mobile, extended states at temperatures above 150°C. This result is consistent with in situ SANS experiments showing lignin phase separation and aggregation to occur during the heating phase of pretreatment

(69, 71). Computational modeling offers complementary insights to advanced experimental approaches. These computational models represent a predictive tool that can help guide changes to biomass and pretreatment processes to improve the properties of extracted lignin and accelerate its separation.

### Lignin Recovery

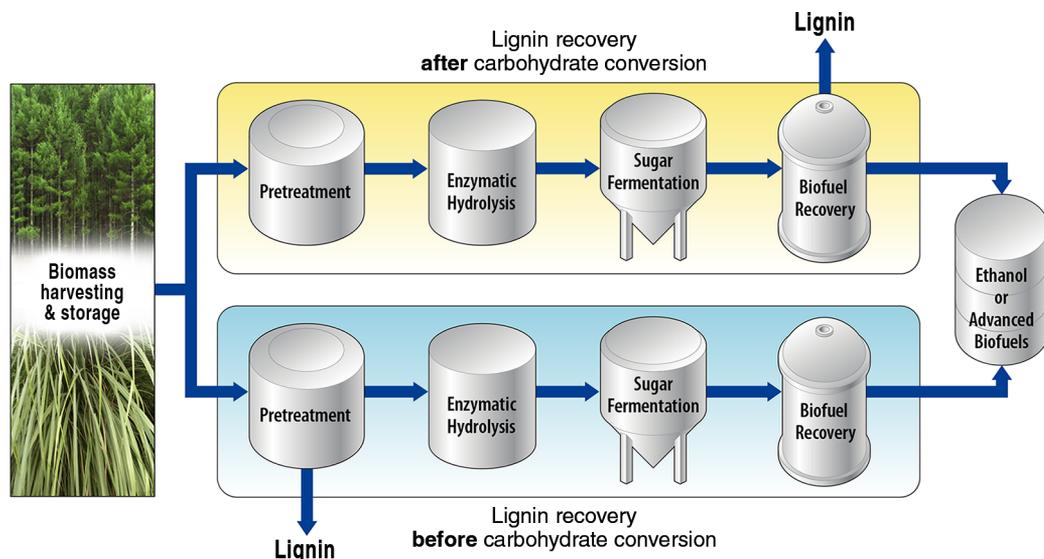
Currently, most lignocellulosic biorefineries using enzymes to deconstruct plant polysaccharides will yield lignin-rich streams by either (i) extracting the plant carbohydrates to leave most of the lignin in the solid residue (72) or (ii) applying pretreatments to fractionate biomass to extract lignin (73) before carbohydrate conversion (Fig. 3).

Some pretreatments use dilute sulfuric or other acids, or simply hot water (hydrothermal), to break down hemicellulose to sugars and to increase cellulose accessibility for enzymatic hydrolysis, after which most of the lignin is left in the solid residue (74). Others use high pH conditions by adding calcium, sodium, or potassium hydroxide to remove a large portion of the lignin and some of the hemicellulose. Ammonia (high pH) disrupts but does not necessarily remove lignin, while still making biomass more accessible to enzymes.

Pretreatment by dilute acid or hot water is known to alter the physical and chemical structure of lignin and deposit altered hemicellulose and lignin products on pretreated solids. Lignin isolated after enzymatic deconstruction currently also contains some recalcitrant polysaccharides, proteins, and mineral salts, a mixture generally viewed as limiting suitability for direct material applications. Recently, this perspective has been challenged by the use of *N,N*-dimethyl formamide to extract lignin from the solid residue of enzymatically hydrolyzed poplar (75). These separation challenges will certainly be simplified in the future as biorefinery engineering advances take hold. For example, although industrial lignocellulosic pretreatment is mostly envisioned to use plug-flow type reactors, improved benefits from flowthrough pretreatments could facilitate lignin recovery free of proteins (76, 77). Historically, this process has been challenged by excessive energy and water consumption, but some of these concerns have been addressed by using a countercurrent flowthrough design to recover less-degraded xylans and 40 to 80% of the lignin, depending on feedstock and reactor conditions (78).

Pretreatments that specifically target lignin extraction frequently have their technical origins in the chemical pulp industry. For example, alkaline pretreatments can fragment and solubilize lignin, thus providing a biomass product with highly reactive polysaccharides for biofuels production, with lignin recovery from these alkaline streams in various stages of commercial development. Alternatively, organosolv approaches treat biomass with a mixture of water and organic solvents, such as ethanol or methanol, along with

**Fig. 3. Simplified process flow diagram illustrating paths to recover lignin.** This can happen either after removal of most of the carbohydrates by hydrolysis and fermentation operations (top sequence) or by pretreatment (126, 127) before downstream carbohydrate conversion (bottom sequence). [Courtesy of Oak Ridge National Laboratory, DOE]



addition of a catalyst at 140° to 200°C. Organosolv pretreatment typically results in more than 50% lignin removal through cleavage of lignin-carbohydrate bonds and  $\beta$ -O-4 interunit linkages and subsequent solubilization in the organic solvent (79). After pretreatment, lignin is precipitated and recovered from the concentrated liquor (80). Organosolv-derived lignin is sulfur-free, rich in functionality including phenolics, exhibits a narrow polydispersity, and has limited carbohydrate contamination (81). Ionic liquids provide an alternative path for lignin removal to classical organosolv pretreatment for enhancements of subsequent enzymatic hydrolysis. For example, 1-ethyl-3-methylimidazolium acetate was used to extract lignin from promising biorefining feedstocks, such as poplar and birch, at elevated temperatures; the lignin was then recovered with the addition of an antisolvent (82). Although some changes in lignin structure were noted, most structural features were retained (83). More aggressive changes in the structure of lignin can be accomplished by using acidic ionic liquids, such as 1-*H*-3-methylimidazolium chloride, which will hydrolyze ether linkages (84). Future developments will focus on selective lignin extraction and functionalization and minimization of process costs for recovery and recycling.

Postfermentation recovery of lignin is expected to be used for low-value markets, such as process heat and electricity, because of the presence of deconstruction enzymes and fermentation components that would require additional purification for higher value uses. Lignin utilization in value-added markets that take advantage of its unique material properties can more likely afford the extra costs for prerecovery by such pretreatments as flowthrough, organosolv and ionic liquids, provided that these processes are not overly capital intensive. As discussed previously, the extraction of lignin from transgenic plants with reduced structural diversity or labile interunit linkages (i.e., esters) can further

simplify the overall extraction process in the future.

#### Lignin Valorization: Materials

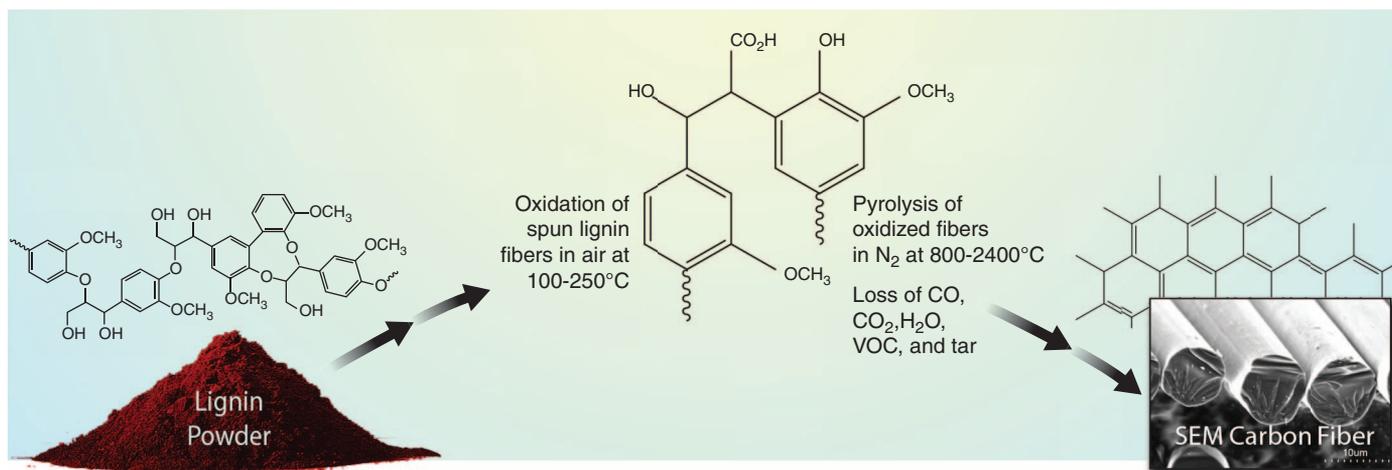
One of the greatest challenges in biorefining is to engineer lignin structures to not only reduce biomass recalcitrance but also enable lignin valorization (i.e., conversion to higher value compounds). Although low-market volume chemical additives can be derived from lignin, the amount of lignin from an industrial cellulosic ethanol plant will range from ~100,000 to 200,000 tons year<sup>-1</sup>; this scale disparity will shape lignin valorization research and development (85).

A promising lignin product platform is the global development of energy-efficient lightweight vehicles. A body-in-white design-based model has demonstrated that over 40 to 50% of the structural steel mass in a vehicle could be replaced with carbon fiber composite materials (86). However, to realize this goal, low-cost manufacturing of carbon fibers (~300 × 10<sup>6</sup> kg year<sup>-1</sup>) is needed at the commercial scale, but commercial carbon-fiber precursors derived from polyacrylonitrile (PAN) are too costly for most such applications. Lignin from cellulosic biorefining operations could become an ideal precursor for carbon fiber synthesis, as highlighted in Fig. 4, but our understanding of the fundamental chemistry involved in this process is limited, hindering advances in this process to some extent.

To obtain lignin-derived carbon fiber, isolated lignin is first processed into fibers by extruding filaments from a melt or solvent swollen gel. Then the spun fibers are thermally stabilized in air where the lignin fiber is oxidized. At this stage, the filaments become pyrolyzable without melting or fusion. During pyrolysis under nitrogen or inert atmosphere, the fibers become carbonized through the elimination of hydrocarbon volatiles, their oxidized derivatives, carbon monoxide, carbon dioxide, and moisture. Figure 4 shows a scanning electron micrograph of car-

bonized filaments with a rough cross section. The final morphology of the carbon materials depends not only on carbon precursor chemistry but also on processing methodology. So far, lignin-based carbon fibers largely derived from Kraft pulping liquors have not exhibited good mechanical properties, mainly because of fiber porosity and lack of oriented graphitic structure. Softwood and hardwood lignins, obtained from the pulping process, can be melt-processed to fibers after eliminating the high-molecular-weight fractions by solvent extraction or molecular fractionation using membranes (87, 88). Softwood lignin precursor fibers manifest more rapid oxidative cross-linking than hardwood lignin fibers, a desirable characteristic for high-yield cost-competitive carbonized derivatives. The use of lignin from switchgrass and other agricultural resources has not been explored as extensively, although recent reviews and patent claims have highlighted its potential, and future studies will undoubtedly further define this opportunity (89, 90).

Lignin-based carbon fibers currently have poor mechanical properties compared with petroleum-derived counterparts. PAN-based fibers exhibit graphitic stacking that is so far difficult to achieve in lignin-derived carbon. The disordered carbon synthesized from natural lignins is thought to be related to its original morphology. The partially globular structure of lignin in nature forms glassy carbon during thermal pyrolysis (91). The formation of rigid oxidized segments during lignin thermal treatment is supported by a gradual increase in the glass transition temperature of the precursor with increasing thermal treatment (92). Although oxidation and cross-linking helps to increase char content in a pyrolyzed polymer (93), extensive cross-linking could be detrimental to structural carbon order (94). Therefore, new chemical modifications of lignin and/or innovative biosynthesis strategies are needed to produce linear-fiber-forming lignin, with controlled monomer ratios and chemical architectures that facilitate



**Fig. 4. Highlights of thermal chemistry involved in converting lignin to carbon fiber.** See (88, 128) for more information. [Courtesy of Oak Ridge National Laboratory, DOE]

rapid chemical transformation to infusible mass and formation of planar graphitic structure during pyrolysis.

Minimization of reactive C-O bonds in interunit lignin linkages, such as  $\beta$ -O-aryl ether, is anticipated to yield a more favorable lignin for structural carbon-fiber production (95). This type of lignin manipulation has already been accomplished in transgenic alfalfa lines down-regulated in the expression of enzymes involved in lignin biosynthesis, which exhibited higher amounts of H-lignin and consequently yielding less C-O-C reactive interunit linkages and more C-C linkages that are chemically less reactive (96). Such lignin modifications could be beneficial for high carbon yield and formation of long-range order in high-temperature carbonized filaments.

Another high-volume lignin application is plant-derived plastics and composites. The synthesis of engineering plastics and thermoplastic elastomers, polymeric foams, and membranes from lignin with comparable properties to those from petroleum products has been reported for some time (97). The most frequent lignin source for these past studies has been from chemical pulping operations that are directed primarily at lignin removal from cellulosic fibers through a series of alkaline depolymerization or lignin sulfonation reactions. Although the lignin structure from such operations may be far removed from that needed for most high-value material applications, these sources have found commercial markets, such as an additive for cement, dust suppression, and drilling fluids for oil recovery (98). A few notable exceptions have been published: for example, the intrinsic structure of select lignosulfonates has facilitated their use for expanders for lead acid batteries (99) and other select applications (100). However, process impurities, variable molecular weights, and poor processability hinder the value of most current industrial pulping lignins for composite products. Despite these concerns, oxypropylation of lignin has been shown to yield a promising

polyol for polyurethane synthesis, yielding excellent physical strength properties (101). In contrast, the application of lignin in thermoplastics has been challenging; for example, blending ~5 to 20% of hydroxyl propyl lignin with poly(methyl methacrylate) provided up to a 200-MPa increase in Young's modulus over the pure polymer but exhibited detrimental embrittlement (102). Hilburg *et al.* presented an alternative approach using a controlled polymerization of nanolignin particles with styrene or methyl methacrylate, which provided a 10-fold increase in toughness over a lignin/polymer blend equivalent (103). The latter results illustrate the potential for utilizing lignin in composites provided the structures are engineered on the molecular scale.

Future development of green lignin-based polymers pivots on new processing technologies coupled to tailor-made bioenergy crops containing lignin with desired chemical and physical properties for a host of lignin-based material applications. For example, mild isolation of C lignin (Fig. 2, structure C) could readily provide a lignin feedstock that addresses many of the current processing issues. Alternatively, lignin composed partially or exclusively of hydroxycinnamaldehydes, such as the lignin from the *Medicago cad1* mutant (Fig. 2, structure D), provides a new functional group as a natural avenue to explore formation of formaldehyde-free resins or plastics by using the intrinsic reactivity of the aldehyde for cross-linking.

#### Lignin Valorization: Fuels and Chemicals

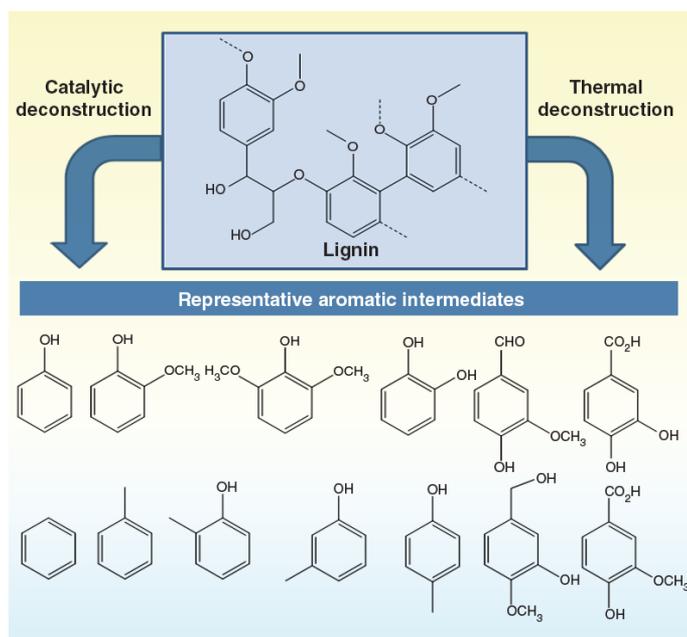
Despite the anticipated improvements in engineered lignin structures and tailored pretreatment chemistries, some lignin fractions from a biorefinery are not expected to be suitable for material applications but can still be valuable for conversion into fuels and chemicals. Lignin depolymerization is challenging given the broad distribution of bond strengths in the various C-O and C-C linkages in lignin and the tendency for

low-molecular-weight species to undergo recondensation, often to more recalcitrant species (Fig. 5). Resulting streams are subsequently difficult to upgrade, given the heterogeneity of low-molecular-weight species, which often possess diverse functional groups.

These challenges will be substantially diminished as plant feedstocks are engineered to have predominant G, H, C, or S lignin. The latter would certainly favor thermal or chemical depolymerization, because methoxy groups at the 3 and 5 positions of the aromatic ring (structure A in Fig. 2) diminish relatively unreactive carbon-carbon (C-C) interunit linkages, yielding a lignin more reactive to thermal and catalytic fragmentation. To date, multiple strategies have emerged for lignin depolymerization and upgrading, including thermochemical treatments, homogeneous and heterogeneous catalysis, and biological depolymerization.

Transition metal catalysts have long been used for lignin hydrogenolysis and hydrodeoxygenation upgrading at high hydrogen pressures, either as single- or two-step processes (104). These approaches have received substantial academic and industrial attention for production of gasoline-range aromatics, as well as benzene, toluene, and xylene. A recent review provides a comprehensive list of the reductive depolymerization and upgrading strategies used to date (105). Most have required high temperature and pressure (>200°C and >5 MPa) in combination with catalysts developed for petroleum processing, such as Ni-Mo or Co-Mo/Al<sub>2</sub>O<sub>3</sub>, with the primary function being removal of sulfur and nitrogen heteroatoms. Despite substantial research, lignin depolymerization via hydrogenolysis remains a major technical challenge, primarily because the diverse reactivities of lignin-derived low-molecular-weight species limit yields of single products and their presence in biomass-derived streams poisons metal catalysts. Additionally, hydrodeoxygenation catalysis for the production of fuels and chemicals from

**Fig. 5. Generalized catalytic and thermal fragmentation of lignin to low-molecular-weight compounds.**



lignin-derived intermediates often leads to catalyst deactivation through high coke formation, hydrothermal instability, and catalyst sintering (106). These challenges warrant development of more robust catalysts designed for diverse substrates and tolerant of harsh environments. For example, promising new catalysts to overcome these limitations, including designs with alternative supports and bimetallic functionalities, have been investigated with model compounds (107).

Oxidative processes for lignin depolymerization have also involved catalytic side-chain oxidation and fragmentation reactions (108). Many of the targeted products from lignin oxidation, wherein the aromatic character is preserved, are aromatic acids and aldehydes with smaller market volumes. However, oxidation can also enable production of ring-opened organic acids that an effective separation method could recover as potentially valuable products. Depolymerization of lignin in nature occurs primarily via oxidative enzymes, such as laccases and peroxidases, which are secreted by white-rot fungi and some bacteria (109, 110). Application of these natural strategies has been examined, such as a mild pretreatment of whole biomass or as a means to produce low-molecular-weight aromatic compounds (111). The utilization of these approaches for lignin depolymerization and subsequent upgrading will be predicated on minimizing the degradation of sugars and reductions in the need for potentially costly enzyme cofactors.

Purely thermal routes have also been explored for lignin depolymerization. Pyrolytic approaches yield low-molecular-weight species in the vapor phase, which undergo condensation reactions upon phase change to higher-molecular-weight oligomers (112). Coupling lignin pyrolysis with hydrodeoxygenation catalysis (113) for upgrading vapor-phase monomeric species potentially offers

a route to fuels and chemicals, but the primary technical barrier is the inability to continuously feed lignin to large-scale reactors (114), an issue that may be solved with commercial-scale ligno-cellulosic biorefineries.

For chemicals production, an underlying uncertainty is the ability to meet stringent product purity specifications. Thus, rather than targeting a single commodity chemical from lignin, a more direct solution may be to produce a blended petrochemical feedstock, such as reformat, that can be integrated and upgraded within an existing petrochemical complex. The primary trade-offs for this route are efficient oxygen removal, fuel quality (because of saturation of the high-octane aromatic blend stocks), and high hydrogen consumption (115). One of the largest challenges associated with lignin valorization is whether there are economic pathways for conversion of lignin to value-added fuels and chemicals. A recent design study that focused on the biological conversion of cellulosic sugars to fuels showed that potential routes for the conversion of lignin to chemicals may lead to improvements in overall economics and sustainability for an integrated biorefinery (116). For example, the conversion of lignin to 1,4-butanediol and adipic acid improved the overall process economics and potentially reduced the greenhouse gas emissions by an order of magnitude relative to production of electricity from lignin. The well-established commercial production of vanillin from lignin provides a strong precedent for future innovative advances in this field (117).

### The Present and Future

Lignin is a major component of terrestrial ligno-cellulosic biomass. The effective utilization of lignin is critical for the accelerated development and deployment of the advanced cellulosic bio-

refinery. However, we acknowledge that, despite some markets and uses of lignin which stretch back decades, it has been long said in the pulp industry that “one can make anything from lignin except money.” This review set out to describe a set of developments over the past few years that we suggest, when taken together, represent a tipping point in the prospects for lignin as a viable, commercially relevant sustainable feedstock for a new range of materials and uses.

First, the advent of new cellulosic biorefineries will introduce an excess supply of different, nonsulfonated, native and transgenically modified lignins into the process streams. Future research will continue to establish to what extent the lignin structure in plants can be altered to yield a product that can be readily recovered via pretreatment and has the appropriate tailored structures to be valorized for materials, chemicals, and fuels. Third, although lignin sequencing remains a vision, approaches based largely on NMR, high-performance liquid chromatography–mass spectrometry (LC-MS) or GC-MS, and specific binding antibodies have greatly improved our knowledge of the structures of lignin and its products. These results need to be further integrated into improved force fields and high-performance computational modeling to provide a predictive tool of lignin’s chemical and physical properties and reactivities in multiple environments. Such insights may help redesign lignin within its cross-linked complex biological matrix to meet subsequent process and end product goals. Overall, the need to understand and manipulate lignin from its assembly within plant cell walls to its extraction and processing into value-added products aligns with our potential to obtain a deeper understanding of complex biological structures. This is especially true because the valorization of lignin cannot come at the expense of the effective utilization of other biopolymers, such as cellulose and hemicellulose.

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