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MINI-REVIEW

Conversion of lignin into value-added materials and chemicals via laccase-assisted copolymerization

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Abstract With today's environmental concerns and the diminishing supply of the world's petroleum-based chemicals and materials, much focus has been directed toward alternative sources. Woody biomass presents a promising option due to its sheer abundance, renewability, and biodegradability. Lignin, a highly irregular polyphenolic compound, is one of the major chemical constituents of woody biomass and is the second most abundant biopolymer on Earth, surpassed only by cellulose. The pulp and paper and cellulosic ethanol industries produce lignin on the scale of millions of tons each year as a by-product. Traditionally, lignin has been viewed as a waste material and burned as an inefficient fuel. However, in recent decades, research has focused on more economical ways to convert lignin into value-added commodities, such as biofuels, biomaterials, and biochemicals, thus developing and strengthening the concept of fully integrated biorefineries. Owing to the phenolic structure of lignin, it is possible to enzymatically graft molecules onto its surface using laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) to create exciting novel biomaterials. These environmentally friendly enzymes use oxygen as their only co-substrate and produce water as their sole by-product, and have thus found great industrial application. This mini-review highlights recent advances in the field of laccase-facilitated functionalization of lignin as well as promising future directions for lignin-based polymers.

Keywords Biomaterials · Copolymerization · Functionalization · Laccase · Lignin · Sustainability

Introduction

In the early 1900s, it was common practice to produce fuels, materials, and chemicals for industrial purposes from terrestrial plants and trees. However, midway through the century, this reliance upon biomass as a source for the production of these necessary commodities had shifted well in favor of petroleum resources (van Wyk 2001). And it is not hard to understand why: petroleum was cheap, abundant, and, contrary to biomass, inexpensively and effortlessly processed into marketable products. Therefore, it seemed logical, and for many companies was a sound business strategy. Fast-forward ahead to the twenty-first century, where petroleum supplies are dwindling, and the public is becoming increasingly aware of the impact fossil fuels have on contemporary society's carbon footprint and the associated negative environmental consequences. Thus, for life on Earth as we know it to be sustainable, the paradigm needs to be shifted once again, reverting back to renewable, carbon-neutral biomass as the primary source for fuels, chemicals, and materials.

Introducing the biorefinery—the biomass analog of the long-standing oil refinery. In an idealized model, the biorefinery will separate biomass into its basic chemical constituents and transform each individual component into a

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marketable product, maximizing yield and complete use of this renewable resource (Ragauskas et al. 2006). Woody biomass is composed of three main constituents—cellulose, hemicellulose, and lignin (Fig. 1)—and minor amounts of extractives and minerals. While the relative proportion of each varies from source to source, biomass generally contains 35–50 % cellulose, 25–30 % hemicellulose, and 15–30 % lignin (Ragauskas et al. 2006). The vast majority of the polysaccharides present in woody biomass are used to produce paper products and, more recently, enzymatically hydrolyzed and subsequently fermented to produce bioethanol, while a smaller portion is used for the production of fine chemicals (Ragauskas et al. 2006). Utilization of lignin, on the other hand, has not been as prosperous, thus it is largely regarded as a waste material.

Lignin is a highly irregular, amorphous polymer of oxidatively coupled 4-hydroxyphenylpropanoid units (Fig. 1c) and is the second most abundant terrestrial biopolymer on Earth, surpassed only by cellulose. The three main monolignols which comprise lignin are *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S), all of which are derived from phenylalanine (Fig. 2) (Vanholme et al. 2010). In woody biomass, lignin provides mechanical support, a means for water conduction throughout the tree or plant, and resistance to microbial degradation. The global pulp and paper industry produces lignin on the order of 50 million tons a year as a by-product of the pulping process (Fig. 3), with only 2 % of it being used for value-added products, such as dispersants and binders, while the remainder is burned as an inefficient fuel (Lora and Glasser 2002; Gosselink et al. 2004; Kai et al. 2016). With the advent of industrial cellulosic ethanol plants, approximately another 60 million tons/year of lignin is forecasted to be produced within the next decade in the USA alone (Langholtz et al. 2014). Thus, it is imperative that robust methods for lignin valorization are developed for the fully integrated biorefinery to be implemented (Ragauskas et al. 2014).

One strategy that has proved to be highly successful assisting in the conversion of lignin into marketable products is enzymatic modification (Hüttermann et al. 2001; Kalia et al. 2014). Using enzymes to modify the structure of lignin provides environmental and economic advantages over chemical or thermal processes, such as mild reaction conditions, the use of renewable and inexpensive biocatalysts, and reduction in the use of toxic chemicals. In essence, it is a completely biotechnological approach. Laccases are a class of enzyme that has received much use in the field of lignocellulosic fiber

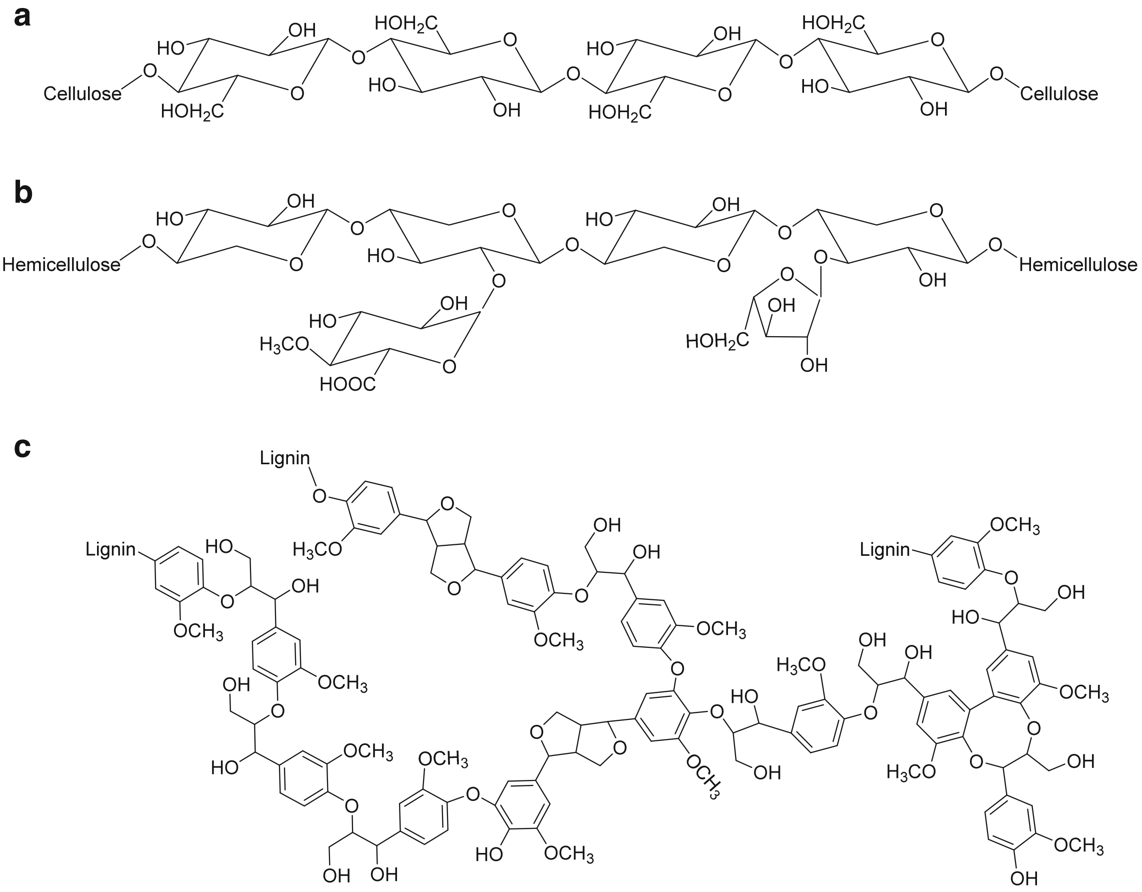


Fig. 1 The chemical structures of **a** cellulose, **b** hemicellulose, and **c** softwood lignin

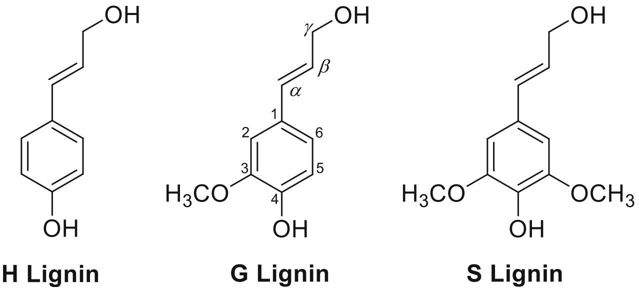


Fig. 2 Structures of monolignols: *p*-coumaryl alcohol (H), coniferyl alcohol (G), and sinapyl alcohol (S)

modification to create paper products with increased strength, hydrophobicity, and antimicrobial properties (Cannatelli and Ragauskas 2015). These multi-copper oxidases catalyze the mono-electronic oxidation of organic substrates while concomitantly reducing O_2 to $2H_2O$ (Messerschmidt 1997; Solomon et al. 2008). They are widespread throughout nature, being found in plants and fungi, where their native functions are the polymerization and degradation of lignin, respectively (Awasthi et al. 2015). Thus, this method of lignin modification mimics the very process that occurs in nature.

Laccase-assisted functionalization of lignin

Regarding the use of laccases to modify lignin to create value-added materials and chemicals, much success has been achieved in the generation of resin-free particle boards, such as medium-density fiberboard, and wood composites that have comparable strength and mechanical properties to those containing artificial resins (Nasir et al. 2015). This is particularly important given the growing concerns and knowledge on the negative health and environmental impacts of formaldehyde emissions from typical phenol-formaldehyde resins currently used as binders in particle boards (González-García et al. 2011). The binding phenomenon observed upon laccase treatment is due to laccase-generated phenoxy radicals on the lignin component of the fiber surface, which are capable of undergoing radical-radical coupling reactions with one another that lead to an increase in bonding among fibers (Felby et al. 1997; Kharazipour et al. 1997).

Following similar principles, novel lignin copolymers can be synthesized utilizing laccases to graft molecules onto the surface of lignin, via either a radical-radical coupling mechanism or a nucleophilic addition via a quinone methide intermediate (Scheme 1). In this way, novel lignin-derived biomaterials possessing biodegradable properties can be crafted. In the past, Milstein et al. (1994) were able to demonstrate the copolymerization of kraft lignin and organosolv lignin with low molecular weight compounds, such as vanillic acid, 4,4′-methylenediphenyl diisocyanate, and acrylamide, in the presence of a fungal laccase. It has also been previously established that a fungal laccase is able to graft water-soluble phenols, such as guaiacolsulfonate and 4-hydroxy phenylacetic acid, onto the surface of kraft lignin, increasing the water solubility of the lignin macromolecule (Lund and Ragauskas 2001). The remainder of this article will focus on recent developments in this trending field of research, placing much emphasis on the potential practical applications of novel lignin copolymers.

Formulation of novel lignin-based biomaterials

As mentioned previously, much research has been dedicated to the formulation of lignin-based resins so as to reduce or eliminate the use of formaldehyde-based resins. Ibrahim et al. (2013) have demonstrated the use of a fungal laccase to graft polyethylenimine (PEI), chitosan, and soy protein onto hardwood kraft lignin. The adhesive properties of the formulations were tested via tensile strength measurements using a loading machine. It was discovered that the formulation

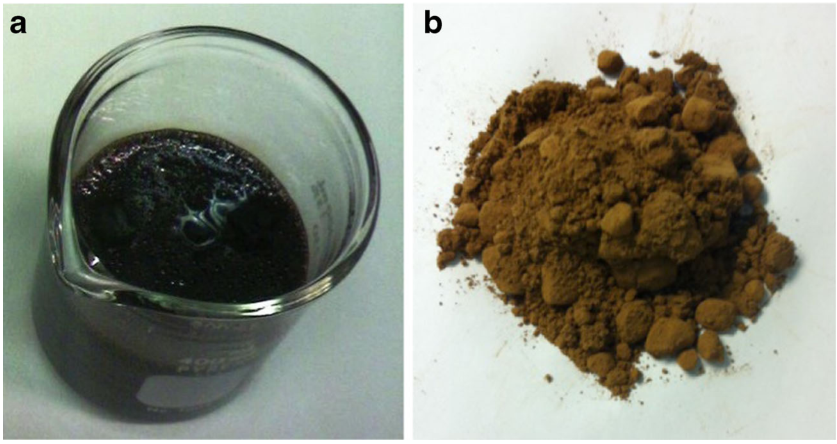
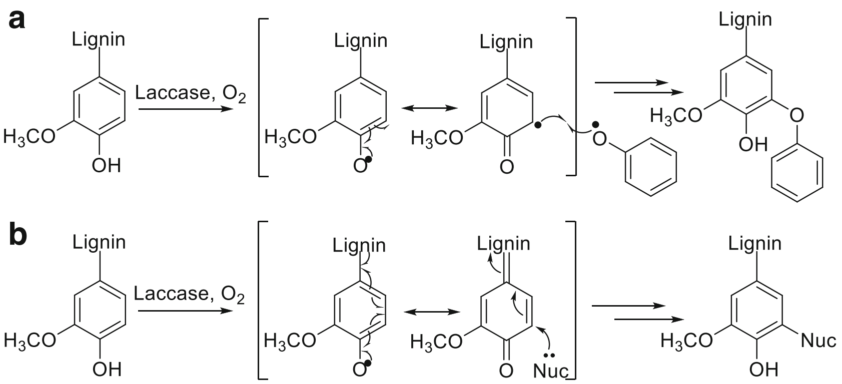


Fig. 3 **a** Black liquor. **b** Kraft lignin powder

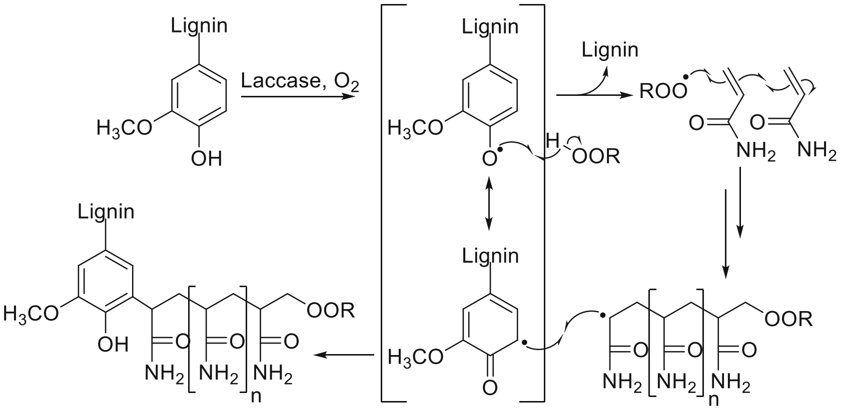


Scheme 1 Laccase-catalyzed grafting reactions. **a** Radical-radical coupling mechanism. **b** Nucleophilic addition mechanism

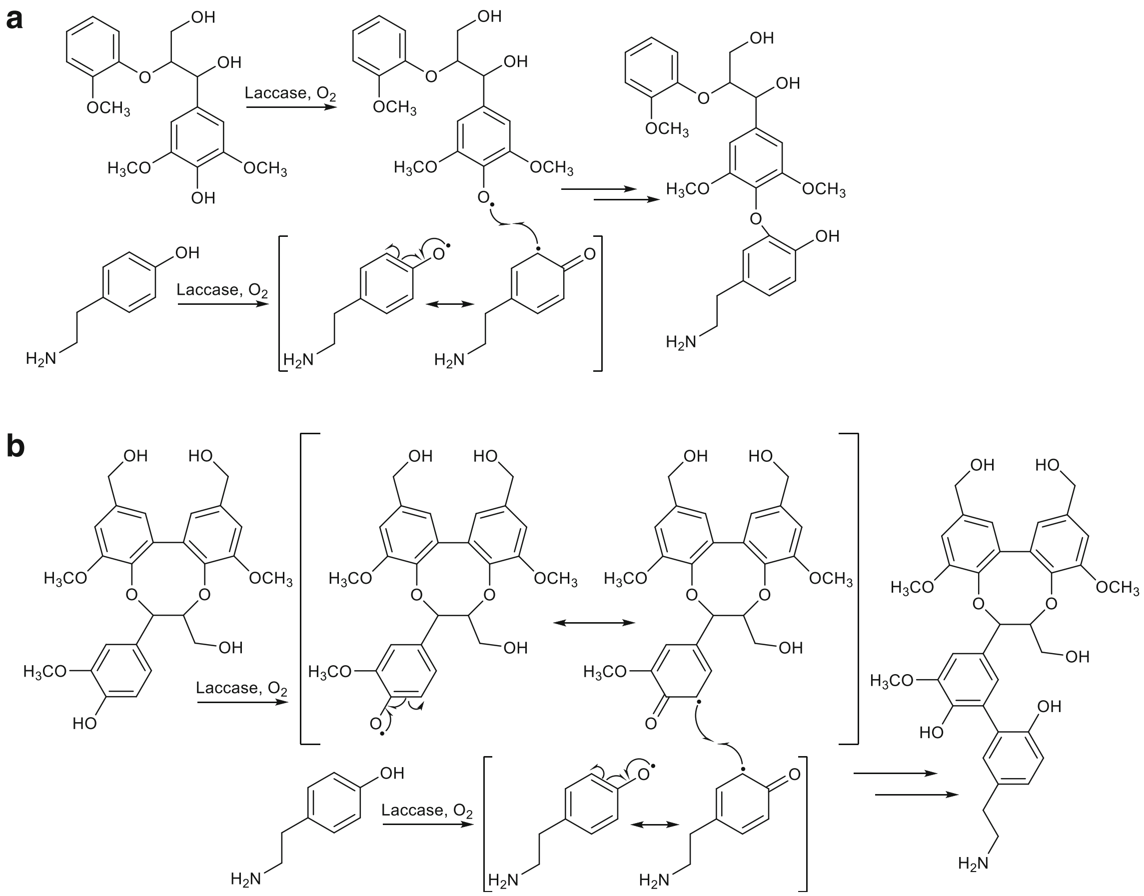
prepared by laccase-treated lignin, followed by NaBH$_4$ reduction and mixed with soy protein yielded an adhesive with greater than 50 % of the strength of commercial polyurethane adhesive and good water-resistance properties. Furthermore, the incorporation of lignin renders the adhesive with antimicrobial properties. The authors concluded that the created adhesives may find suitable use in the binding of paper and cardboard boxes. In another study, it was established that lignin may serve as a viable base material to replace synthetic latex in the formulation of an adhesive used for wool floor coverings. Aracri et al. (2014) used a fungal laccase to copolymerize a variety of technical lignins with gallic acid, tannic acid, and dopamine in an attempt to increase reactive quinonoid moieties on the surface of lignin that are capable of undergoing nucleophilic addition by amino groups present in wool to form covalent linkages between lignin and wool. Based on loop withdrawal force measurements, the lignin-based adhesives exhibited good flexibility and comparable strength performance to that of the synthetic latex adhesives.

Over the years, the research group of Mai has investigated the chemo-enzymatic grafting of acrylic compounds onto the surface of lignin. Incorporating a lignin backbone into synthetic acrylic polymers, such as polyacrylamide and polyacrylic acid, has proved to be a successful approach in creating novel engineering plastics, thickeners, fillers, and adsorbents with biodegradable properties. Initial studies demonstrated that a fungal laccase in combination with an organic peroxide, such as dioxane peroxide, was able to successfully copolymerize acrylamide with softwood organosolv lignin (Mai et al. 1999; Mai et al. 2000b). Additional studies showed that a variety of technical lignosulfonates could be copolymerized with acrylamide and acrylic acid in the presence of laccase and *t*-butylhydroperoxide, and that this system was more effective in promoting copolymerization than a Fenton-like system consisting of ferrous ion and *t*-butylhydroperoxide (Mai et al. 2000a). A follow up exploration into the mechanism of the grafting and copolymerization reactions revealed the roles of laccase and organic peroxides. It was proposed that laccase initially oxidizes lignin to generate phenoxy radicals, which then go on to oxidize peroxides to produce peroxy radicals that are capable of initiating a homopolymerization of acrylic monomers. The living polymerization of acrylic monomers is eventually quenched by radical-radical coupling reactions between the living ends of the homopolymers and lignin radicals (Scheme 2) (Mai et al. 2002).

Extensive research has been conducted over the past two decades on the laccase-catalyzed synthesis of conducting polyaniline and its applications (Karamyshev et al. 2003;



Scheme 2 Reaction mechanism for the laccase-initiated copolymerization of lignin with acrylamide (adapted from Mai et al. 1999)



Scheme 3 Reaction mechanism for the laccase-catalyzed coupling of tyramine with **a** syringylglycerol *β*-guaiacylether (adapted from Kudanga et al. 2009a) and **b** dibenzodioxocin

Shumakovich et al. 2015). Very recently, Zhang et al. (2016) synthesized a polyaniline-lignosulfonate complex via laccase catalysis. The lignosulfonate acted as a template for the synthesis of linear polyaniline. The conductive complex was able to be successfully immobilized onto the surface of cotton and therefore, may find potential use as a textile in electronic devices. Also in a recent report, the laccase-catalyzed grafting of hydrophilic compounds, such as glucosamine and the tripeptide glycil-tyrosyl-glycine, to hardwood organosolv lignin and alkali pretreated wheat straw lignin was achieved. The synthesized lignin-carbohydrate and lignin-peptide conjugates provide a framework for further functionalization and formulation of materials with distinct properties (Fițigău et al. 2015).

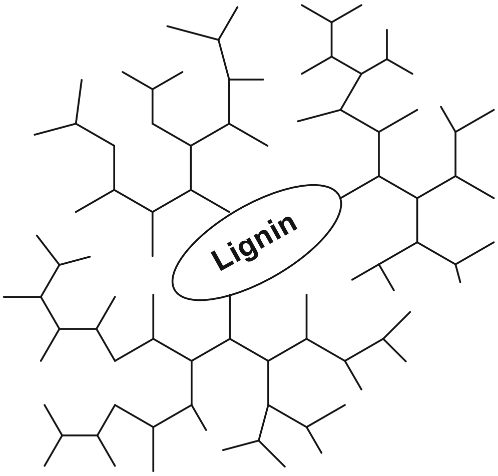


Fig. 4 Schematic of a lignin-core hyperbranched polymer

Mechanistic insights into laccase-induced lignin functionalization

So far, the recent developments in laccase-assisted functionalization and copolymerization for the generation of novel biomaterials have been discussed. Equally as important as the applications of these lignin-based copolymers are the molecular mechanisms by which they are synthesized. Kudanga et al. have studied the mechanisms of lignin functionalization by using laccases to couple small phenolic molecules to lignin model compounds. Using a bacterial laccase, the researchers were able to successfully couple the phenolic compound tyramine to the lignin model syringylglycerol *β*-guaiacylether

(Kudanga et al. 2009a). Subsequent studies with another common structural unit found in lignin, dibenzodioxocin, demonstrated that a variety of phenolic compounds could be coupled to this lignin model using laccases (Kudanga et al. 2009b; Kudanga et al. 2010). Based on the structures of the reaction products from the coupling reactions, it can be inferred that phenolic compounds form covalent linkages with lignin model compounds via radical-radical coupling reactions (Scheme 3). In the case of syringylglycerol β-guaiacylether, a model of S type lignin, coupling occurs via a phenoxy radical to form a C-O bond (Scheme 3a), whereas for dibenzodioxocin, a model for G type lignin, coupling occurs exclusively at the vacant position 5 of the aromatic ring to yield a C-C adduct (Scheme 3b). These results provide great mechanistic insight into laccase-mediated couplings of phenolic compounds onto the surface of lignin and guidance for future functionalization strategies.

Summary and outlook

As has been discussed, laccases provide an elegant, ecofriendly means for converting one of nature's most abundant biopolymers into a variety of novel biomaterials through surface modification and copolymerization with other natural and synthetic compounds. In this way, the natural system by which woody biomass is formed is mimicked, thus providing a completely biotechnological and sustainable approach to utilize lignin. Importantly, the materials that can be formulated via this approach find practical use in adhesives, engineering plastics, adsorbents, and fillers to name a few. Looking ahead, a promising research direction in the field of lignin valorization is the formation of lignin-based hyperbranched polymers, a class of polymers that exhibit unique properties, such as high functionality and high solubility (Zheng et al. 2015). In this approach, lignin serves as the core molecule to which another branching polymer is attached (Fig. 4). For example, Kai et al. (2016) were able to develop a lignin-based supramolecular hydrogel by grafting poly(ethylene glycol) methyl ether methacrylate onto the surface of kraft lignin via atom transfer radical polymerization and then mixing with α-cyclodextrin. The hydrogels possessed mechanically responsive and self-healing properties and, due to no cytotoxic effects, have potential biomedical applications. We believe that laccases may be able to assist in creating a hyperbranched macromolecular architecture using lignin as a core and building outward. Thus, it seems that laccases have, and will continue to play, a pivotal role in converting lignin into a marketable product and hence, aid in promoting and developing a more sustainable industrial society.

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Compliance with ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare that they have no conflict of interest.

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