Title

Review of advances in the development of laccases for the valorization of lignin to enable the production of lignocellulosic biofuels and bioproducts

Authors

Leynaud Kieffer Curran, Laure MC
Pham, Le Thanh Mai
Sale, Kenneth L
et al.

Publication Date

2022

DOI

10.1016/j.biotechadv.2021.107809

Biotechnology Advances 54 (2022) 107809

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Review of advances in the development of laccases for the valorization of lignin to enable the production of lignocellulosic biofuels and bioproducts

Laure M.C. Leynaud Kieffer Curran [a,b,c], Le Thanh Mai Pham [b,d], Kenneth L. Sale [b,d], Blake A. Simmons [b,c,*]

[a] Swiss Federal Institute of Technology Lausanne, Lausanne, Vaud, Switzerland
[b] Joint Bioenergy Institute, Emeryville, CA, United States of America
[c] Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, CA, United States of America
[d] Sandia National Laboratories, Livermore, CA, United States of America

ARTICLE INFO

Keywords:
Biofuels
Biorefining
Lignin valorization
Lignin degrading enzymes
Enzymatic depolymerization
Laccase

ABSTRACT

Development and deployment of commercial biorefineries based on conversion of lignocellulosic biomass into biofuels and bioproducts faces many challenges that must be addressed before they are commercially viable. One of the biggest challenges faced is the efficient and scalable valorization of lignin, one of the three major components of the plant cell wall. Lignin is the most abundant aromatic biopolymer on earth, and its presence hinders the extraction of cellulose and hemicellulose that is essential to biochemical conversion of lignocellulose to fuels and chemicals. There has been a significant amount of work over the past 20 years that has sought to develop innovative processes designed to extract and recycle lignin into valuable compounds and help reduce the overall costs of the biorefinery process. Due to the complex matrix of lignin, which is essential for plant survival, the development of a reliable and efficient lignin conversion technology has been difficult to achieve. One approach that has received significant interest relies on the use of enzymes, notably laccases, a class of multi-copper green oxidative enzymes that catalyze bond breaking in lignin to produce smaller oligomers. In this review, we first assess the different innovations of lignin valorization using laccases within the context of a biorefinery process, and then assess the latest economical advances that these innovations offered. Finally, we review laccase characterization and optimization, as well as the prospects and bottlenecks of this class of enzymes within the industrial and biorefining sectors.

1. Lignin valorization

1.1. From plants to biofuels and bioproducts

Plants use photosynthesis to capture and convert solar energy and CO_2 into the stored energy deposited in plant cell walls (Grätzel, 2007). Renewable plant biomass, such as the lignocellulose located in plant cell walls, has long been envisioned as a sustainable feedstock for production of biofuels and bioproducts, and its widespread adoption is predicted to significantly reduce CO_2 emissions in the transportation sector (Lippke et al., 2012). In comparison with starch and sugar crops as the carbon source for biofuels and bioproducts, lignocellulosic biomass offers several significant advantages. Lignocellulose is a non-food biomass that accounts for 15 to 40% by weight of most terrestrial plants and is therefore an abundant source of energy. Additionally, it can add value to agro-based and forestry wastes, and it is a carbon-neutral substrate that does not compete with food crops (Hahn-Hägerdal et al., 2006; Naik et al., 2010). Lignocellulosic biomass is primarily composed of three natural polymers: cellulose, hemicellulose and lignin (Higuchi, 1997). Cellulose consists of glucose monomers which, when extracted and depolymerized, can be fermented into biofuels and other valuable bioproducts (Galbe and Zacchi, 2002). Hydrolysis of hemicellulose generates pentoses and hexoses that can also be used as a feedstock for biofuels and bioproducts fermentation (Ottenheim et al., 2014). Lignin is an energy dense, three-dimensional amorphous polymer and its presence hinders the efficient extraction of cellulose and hemicellulose,

Abbreviations: S, syringyl type unit; G, guaiacyl type unit; H, hydroxyphenol type unit; ILs, ionic liquids; LiP, Lignin peroxidase; MnP, manganese peroxide; VP, versatile peroxidase; LPMOs, polysaccharide monooxygenases; ABTS, 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid); HOBt, hydroxybenzotriazole.

* Corresponding author at: Joint Bioenergy Institute, Emeryville, CA, United States of America.
E-mail address: basimmons@lbl.gov (B.A. Simmons).

https://doi.org/10.1016/j.biotechadv.2021.107809
Received 25 May 2021; Received in revised form 23 July 2021; Accepted 24 July 2021
Available online 29 July 2021

which increases the costs associated with the conversion of lignocellulose into biofuels and bioproducts (Grotewol and Jones Prather, 2014). Valorization of lignin could unlock significant economical value for biofuels and bioproducts and is essential for building an economically viable biofuels industry (Ragauskas et al., 2014). Biological valorizing of lignin requires first depolymerizing it to fragments amenable to uptake and conversion by engineered host organisms. Consequently, the development of technologies to reduce biomass recalcitrance caused by the inherent heterogeneity of lignin has been the focus of intensive research (Xu et al., 2019). There is particular interest in engineering enhanced feedstocks with altered lignin composition, technologies that enhance the efficiency of biomass deconstruction and lignin extraction, and microbes that could convert all of the lignocellulosic intermediates generated into biofuels and other valuable bioproducts.

Effective and affordable lignin depolymerizing enzymes, such as laccases and peroxidases, are an essential step in transforming lignin into bioavailable substrates that can be converted into biofuels and bioproducts by microorganisms. In the first part of this review, we will discuss the different methods employed for lignocellulose depolymerization and the importance of lignin valorization for biofuels and bioproducts production in each. Secondly, we will address the potential of laccase enzymes for the depolymerization of lignin, its recent advances and bottlenecks towards its commercialization within biorefineries and other relevant industrial sectors.

1.2. Lignin

The first scientist to discover the presence of lignin in plants was the botanist A. P. de Candolle in 1819, which was 19 years before A. Payer discovered cellulose in 1838 (de Candolle, 1842). de Candolle named lignin after the word *lignum* (Latin for wood), due to its fibrous properties and the insolubility of lignin in water and alcohol (de Candolle and Buek, 1842). Lignin is critical to plant health and growth, providing mechanical support, impermeability, disease and pest resistance, and water and nutrient transport (Liu et al., 2018). It is the second most abundant biopolymer on earth, after cellulose, and composes 20–30% of the dry biomass (Wyman, 2003). Lignin is primarily composed of syringyl (S type unit), guaiacyl (G type unit), and hydroxyphenol (H type unit) phenylpropanoid monolignols (Fig. 1), but other monolignols can be present in smaller amount depending on the type of the plants, such as caffeyl alcohol, flavonoid tricin, hydroxybenzaldehydes, dihydrohydroxycinnamyl alcohol, and acylated monolignols (Anderson et al., 2015). The biosynthesis of monolignol starts in the cytosol from the amino acid phenylalanine (Boerjan et al., 2003). Then the monolignol is attached to a glucose molecule which confers water-solubility and can be transported through the plant cell wall (Li and Chapple, 2010). The S:G:H ratio varies between plants and genotypes, giving rise to the many different forms of lignin (Kishimoto et al., 2010). For example G units are more abundant than H units in softwood, while in hardwood, G and S units are both similarly present (Lourenço and Pereira, 2018). Another characteristic of this complex biopolymer is the variety of its linkages that occurs during lignin polymerization. The predominant linkage present in lignin is β-O-4 aryl ether linkage, present at 50% in softwood vs 60% in hardwood (Adler, 1977). Other important linkages present in lignin are β-5 (phenylcoumaran) linkage, but others are also found in small various amount, such as, β-β (resinol) linkage, 5–5′ (biphenyl) linkage, and 4-*O*-5 (diaryl ether) linkage (Fig. 1) (Lahive et al., 2018). The diversity of bonds makes lignin among the most complex biomolecules and leads to its recalcitrance (Simmons et al., 2010).

Lignin biosynthesis in plants is activated by oxidative enzymes such as laccases and peroxidases (Meents et al., 2018). Laccases catalyze the O_2-dependent radical oxidation of monolignols, allowing the polymerization of lignin. Similarly, peroxidases use H_2O_2 to oxidize monolignols. It is only after the deposition of the polysaccharides, cellulose and hemicellulose, that the coupling of the radicalized monolignols occurs, initiating lignin polymerization (Aminzadeh et al., 2017). In this process, the polysaccharides act like a framework of the plant cell wall, and lignin reinforces the structure by providing a highly engineered complex support that forms a type of composite material that enables structural support (Tobimatsu and Schuetz, 2019).

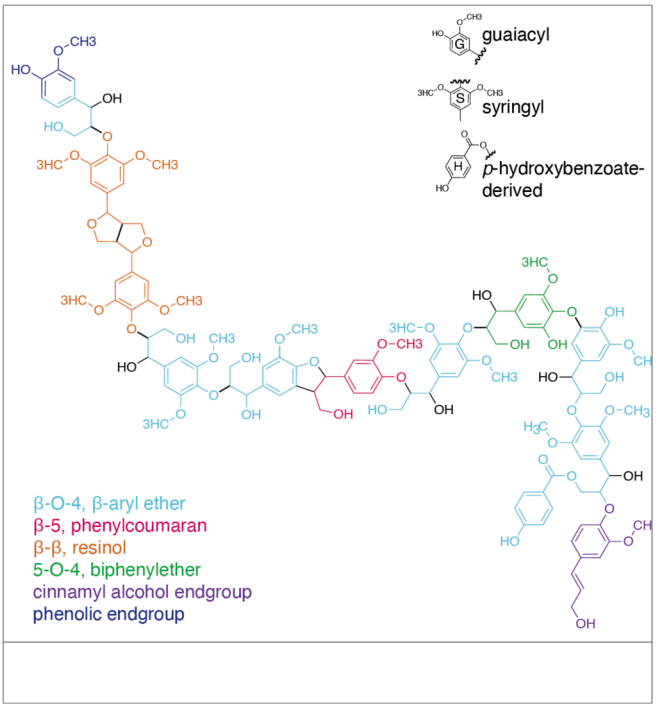

Fig. 1. Lignin structure, components and linkages from a wild type poplar, adapted from Stewart et al., 2009 (Stewart et al., 2009).

What is the path from this highly complex molecule to valuable biofuels and bioproducts? The three major steps are: (1) selection and growth of bioenergy crops, (2) deconstruction of those crops into targeted intermediates, such as sugars, monomers and oligomers of lignin, and (3) conversion of those intermediates into biofuels and bioproducts using microorganisms (Mielenz, 2001).

1.3. Feedstock

The choice of the bioenergy crop is a key element since it impacts the entire conversion downstream processes needed. Many bioenergy crops have been studied such as switchgrass (*Panicum virgatum*), sorghum (*Sorghum spp*), and poplar (*Populus spp*) (Fig. 2a) (Langholtz et al., 2016). Each plant species has different lignin structure (linkages) and content (S:G:H ratios) (Guragain et al., 2015). One of the strategies to reduce the downstream costs, is to engineer the lignin biosynthesis by the plant and so reduce the biomass recalcitrance by modifying the lignin content and composition (Verma and Dwivedi, 2014). The genetic engineering of the lignin biosynthesis pathway to reduce lignin content has direct consequences on the overall growth and development of the plant. Lignin reduction can lead to problems such as reduced plant viability, lower biomass content, and altered secondary metabolism (Welker et al., 2015). Due to the unpredictability of the results, this strategy is laborious. However, other approaches have been developed to increase the cell-wall degradability, by influencing the lignin composition rather than the content, resulting in more promising outcomes. Altering the composition of S:G:H shows that it affects the biomass digestibility characteristics. For example, a high S-lignin content can increase the enzymatic hydrolysis of the biomass, because it presents a lower polymerization degree, which is the number of monolignol units within the macromolecule lignin (Li et al., 2010). A similar approach has been used to reduce the degree of polymerization of lignin, by expressing in the lignifying tissue a bacterial hydroxycinnamoyl-CoA hydratase-lyase,

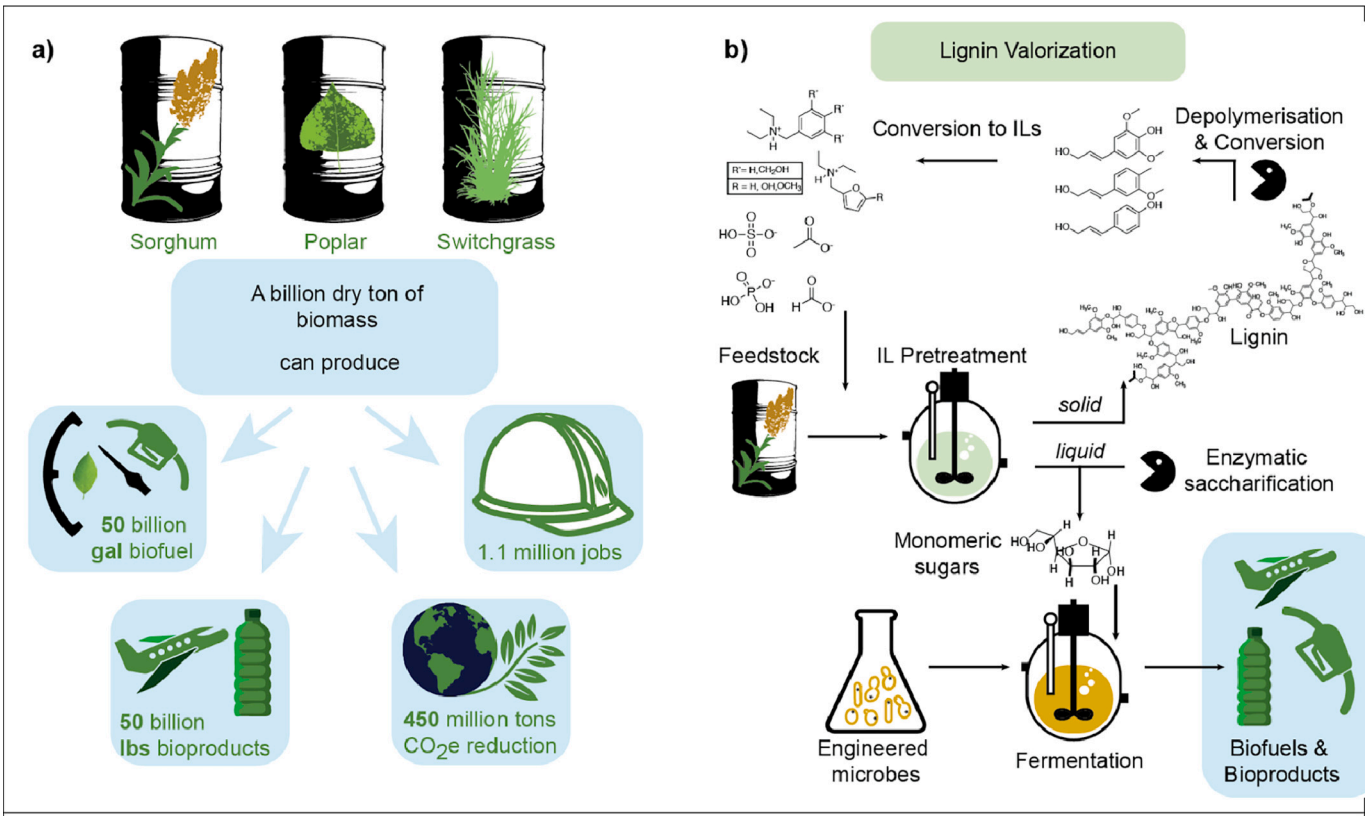

Fig. 2. a) Economical value of biofuels of second generation from sorghum, poplar and switchgrass biomass (Langholtz et al., 2016). b) A suggested biorefinery process from feedstock pretreatment to fermentation, and reconversion of lignin into ILs. Image adapted from Socha et al. 2014 (Socha et al., 2014).

reducing the biomass recalcitrance and enhancing the conversion efficiency (Eudes et al., 2012). However, the beneficial genetic transformation of lignin has been mostly applied in laboratories under ideal conditions for the plants but only few studies have been realized in the field, where conditions make plants more vulnerable to the environment (Hopkins et al., 2007).

1.4. Pretreatment

After the choice of the bioenergy crops, comes the growth of the plants, and the harvest and pretreatment of the biomass. Cellulose and hemicellulose extraction are protected by lignin. Lignin is tightly crisscrossed all around the cell wall, impairing the interaction of enzymes with cellulose and hemicellulose, which decrease the yield of biofuels and bioproducts production (Chio et al., 2019). Pretreatment is an important step that increases enzyme accessibility by solubilizing and potentially depolymerizing and extracting lignin. Some of the recent advances in biomass pretreatment for this purpose include the use of hydrothermolysis (Faga et al., 2010) ionic liquids (ILs) (Singh et al., 2009) or organic solvents (Hallac et al., 2010) and many others… (Chio et al., 2019). Hydrothermolysis is a pretreatment method used in lignocellulose, that utilizes the pressure of liquid water at high temperatures (Szczodrak et al., 1986). At high temperature, the water goes into the cell wall structure of the biomass, hydrates the cellulose, solubilizes the hemicellulose, and moderately removes lignin (He et al., 2015). Recent studies applied on pine wood shows that 35% of lignin was dissolved at 240 °C after hydrothermolysis pretreatment (Ståhl et al., 2018). Due to the complexity of lignocellulosic biomass, it is difficult to develop a single pretreatment method. Within the different methods to thermochemically extract lignin, there also exist pyrolysis and microwave methods (Chio et al., 2019). It generally requires a combination of robust and techno-economical processes (Galbe and Wallberg, 2019).

While ILs research started from a hardly noticed breakthrough by P. Walden in 1914, it is today subject to extensive research due to its numerous uses in different industrial applications (Tang et al., 2019). Walden was looking for a liquid salt at room temperature, and discovered that ethylammonium nitrate has a melting point of 12 °C (Walden, 1914). ILs boast low vapor pressures and flammability, high electrical conductivity, high thermal stability and high solvation strength. ILs can be used as a solvent to dissolve cellulose (Zhang et al., 2017), but ILs can also stabilize the hydroxyl group present in lignin, accelerating the cleavage process of the carbon-oxygen bonds, and they attack high electron deficient protonated carbon-oxygen bonds by acting as nucleophiles (Tolesa et al., 2019). The longer the pretreatment using ILs is, the better the downstream delignification efficiency is (Sun et al., 2009). After the ILs pretreatment, ILs can be isolated from the reaction, and recycled, decreasing costs (Fig. 2b) (Welton, 2018). Although the pretreatment process is one of the keys to delignification, it can be cost prohibitive and further advances are needed before a viable commercial approach can be realized (Asim et al., 2019). Moreover, certain ILs can inhibit enzyme activity either by destabilizing the enzyme structure or by other inhibitory processes if not fully removed (Elgharbawy et al., 2016). For chemical and catalytic applications for lignin treatment, there are also different methods than ILs pretreatment, such as acid, base and metallic catalysts and oxidative lignin using hydrogen peroxide for example (Chio et al., 2019).

After pretreatment, the biomass is typically separated in two output streams - a liquid and solid phase. The liquid phase, mostly constituted of cellulose and hemicellulose, can undergo direct enzymatic hydrolysis and be used to feed the downstream process of microbial conversion into biofuel and bioproducts. The solid phase, containing most of the lignin and some residual ionic liquid, goes through a different phase of saccharification, to convert and depolymerize lignin into fermentable

sugars or in ILs. ILs are isolated and recovered to be reused for the pretreatment (Fig. 2b) (Socha et al., 2014).

1.5. Enzymatic Depolymerization

The next step in lignin deconstruction is to use enzymes or microbes that can depolymerize the lignin (Fig. 2b). Enzymatic lignin depolymerization typically requires a mixture of oxidative enzymes, such laccases and peroxidases (Martínez et al., 2005), esterases and etherases (Janusz et al., 2017; Picart et al., 2015), cellobiose oxidizing enzymes, arylalcoholoxidases, and aryl alcohol dehydrogenases (Pérez et al., 2002). Several of the enzymes known to be involved in lignin depolymerization are secreted by fungi in the *Basidiomycetes* and *Ascomycetes* divisions of the fungi kingdom (Martínez et al., 2005). As an example of a *Basidiomycete*, *Phenerochaete chrysosporium*, known as white rot fungi, can grow directly on wood and has been the object of extensive research for its lignocellulosic enzyme secretion proficiency (Youn et al., 1995). In 1980, it was estimated that there are ~1700 fungal species capable of degrading lignin (Gilbertson, 1980).

Laccases, mostly secreted by wood-rotting fungi, are oxidoreductase enzymes with low redox potential that can only oxidize directly phenolic lignin units (Kääarik, 1965), and nonphenolic subunits using a mediator (Hilgers et al., 2018). In the second part of this review, we will further develop the role of laccase in other industrial applications, their mechanism of degrading lignin, and their prospects and bottlenecks towards industrial processes.

Peroxidases, a less-studied class of enzymes that includes lignin peroxidase (LiP), manganese peroxide (MnP) and versatile peroxidases (VP) were reported in 1980 in *P. chrysosporium* (Pérez et al., 2002). Peroxidases have high redox potential, particularly LiP with redox potentials measured at 1.2 V at pH 3, making this enzyme capable of oxidizing substrates that no other peroxidases would be able to (Sigoillot et al., 2012). Similar to the general peroxidase mechanism, LiP, MnP and VP contain a heme-iron in their active site that mediates catalysis. The heme-iron is first oxidized by hydrogen peroxide. Electrons are then shuttled from lignin through soluble mediators such as the phenolic veratryl alcohol, or, in the case of MnP, Manganese(II). Thus, lignin is oxidized in order to reduce hydrogen peroxide (Janusz et al., 2017). The synergy between laccases and peroxidases is still nascent and further study is needed. In nature, fungi are capable of producing more than one peroxidase and accessory oxidases at a time, such as glucose oxidase or alcohol oxidase, that are known to degrade lignin (Wang et al., 2018).

A more recently investigated hypothesized that the copper oxidoreductase enzyme, named polysaccharide monooxygenase (LPMO), catalyzes oxidative cleavage of glycosidic linkage thereby breaking down cellulose and enhancing biomass degradation (Moreau et al., 2019). LPMOs are usually used in a cocktail of enzymes, with cellulases and xylanases, to increase the saccharification of lignocellulose for the production of biofuels (Johansen, 2016).

While these lignin degrading enzymes are effective in their native ecosystems, we lack the tools and expression hosts to produce them in high quantities at low costs. For example, although *Escherichia coli* and *Saccharomyces cerevisiae* have the potential to secrete lignin degrading enzymes (Huang et al., 2014), they typically produce very low yields of soluble, active enzyme, and the high cost of downstream processes to recover and purify the enzyme makes using these organisms prohibitively expensive (Bugg et al., 2011). Moreover, enzyme depolymerization technology can be enhanced after ILs pretreatment. There is therefore a particular interest in the development of thermostable enzymes with higher tolerance for ILs (Dabirmanesh et al., 2015).

1.6. Microbe engineering

Successful lignin depolymerization yields small oligomers and monomers that can be fed into microbial biosynthesis platforms, such as microbial production of biofuels and bioproducts. Bacterial fermentation of glucose and xylose has been studied to obtain high productivity, yield and high ethanol conversion (Chandrakant and Bisaria, 1998). Today, efforts are focusing on lignin valorization, including the screening for microbes that are capable of tolerating the toxicity of some aromatic monolignol and have the catabolic potential to degrade monolignols to simpler compounds that can enter primary metabolism. An additional challenge of microbe engineering is the necessity of funneling and converting numerous different monolignols into a single product e.g., biofuel. Thus, significant effort is being invested in engineering anabolic pathways.

Rhodococcus opacus and *Pseudomonas putida* are the most studied bacteria for lignin valorization (Xu et al., 2019). Due to *R. opacus* tolerance to toxic compounds, its conversion of monolignol into lipids (a biofuel precursor), and its many different catabolic pathways, this organism has been subject to many researches. *R. opacus* has allowed the identification of aromatic catabolic pathways and substrate transporter, but also a development of a genetic toolbox to optimize catabolic pathways and transcriptional controls to create a cost-effective fermentation strain (Anthony et al., 2019). *R. opacus* accumulates up to 87% (dry *w*/w) in lipids, which can be converted into biodiesel (Alvarez et al., 1996). *P. putida* presents a high tolerance to environmental stress, and it is also capable of depolymerising high molecular weight of lignin, and then can catabolize them, making this organism a great choice for biofuels application (Salvachúa et al., 2015). *P. putida* is also well known for its capability to degrade aromatic compounds (Jiménez et al., 2002). *P. putida* remains under intensive investigation to unlock its complex metabolic pathways that allow it to degrade aromatic compounds such as found in lignin (Nikel et al., 2014). There is a clear need for more expansive studies on lignin valorization using microorganisms; better understanding of metabolic pathways for lignocellulose biomass conversion, more productive bacteria to increase the yield of biofuels and bioproducts, and finally, coupled with the upstream process, a better adaption of the microbes to the depolymerization process by making them more ILs tolerant (Fig. 2b) (Mukhopadhyay, 2015).

The affordable, sustainable and scalable production of biofuels and bioproducts requires the efficient conversion of as much carbon in the plants as possible into these desired outputs. While the production and conversion of fermentable sugars has received the most significant attention to date, the efficient deconstruction and conversion of lignin is needed in order for the enterprise to be viable. This fact has been long-been recognized but has not received significant attention within the scientific community until the past decade or so. While there have been several breakthroughs over this time, there is still no proven and commercially viable approach to lignin conversion. In the second part of this review, we are focusing on the development of a particular technology, laccase enzymes, to degrade lignin. We are reviewing why laccase enzymes are central to this effort and how scientists have developed more efficient laccases at a reduced cost, and how they are still reinventing solutions to push the limit of this technology.

2. Laccase industrialization

2.1. Laccase in multiple kingdoms of life

Laccases (polyphenoloxidase; EC 1.10.3.2) are highly versatile multi-copper oxidases that can catalyze a range of bond types in lignin, leading to depolymerization of lignin into fragments amenable to biological uptake and conversion (Janusz et al., 2020). In 1983 Yoshida identified the enzyme responsible for lignin degradation, a laccase from the tree *Rhus vernicifera* (Thurston, 1994). Today, laccases are well known to be distributed within the Fungi, Plantae, and Bacteria kingdoms, and there are recent reports they also exist in insects (Futahashi et al., 2011). In Part 1 of this review, we explained the important role of laccases in plants, where, in the vascular tissue of the plants, they oxidize monolignols and cause lignification (Singh Arora and Kumar Sharma, 2010). This section will focus on the second function of laccases

- catalysis of lignin degradation. Fungal laccases are potentially more wide-spread and active in lignin degradation. It has been estimated that almost 1700 wood-rotting Basidiomycetes are present in North America (Gilbertson, 1980). Basidiomycetes play an important role in the ecosystem, as they are responsible for the recycling of carbon removed from the atmosphere by autotrophic organisms, such as plants and bacteria. Moreover, by growing on older trees, Basidiomycetes weaken them, expediting their death and providing nutrients for new trees to grow (de Mattos-Shipley et al., 2016). The Basidiomycetes contain well-studied laccase-producing fungal species, such as *Trametes species*, *Trametes versicolor*, *Cerrena unicolor*, *Agaricus bisporus* and *Phanerochaete chrysosporium* (Arregui et al., 2019; Mehra et al., 2018). Fungi are particularly interesting for their ability to secrete highly active enzymes at high concentrations. For example, LccH laccase from the white-rot Basidiomycete, *Hexagonia hirta* MSF2, reached a LccH activity of 5671.3 $U.mL^{-1}$ after phenol induction (Kandasamy et al., 2016).

Bacterial laccases are also being investigated for being produced intracellularly and extracellularly, with stable enzymes within a wide range of pH and temperature, e.g. *Bacillus subtilis* Cot A laccase reaches its maximum of activity at 75 °C, and has a half-life of 4 h at 80 °C (Chandra and Chowdhary, 2015). *Pseudomonas aeruginosa* ADN04 produced 46 $U.mL^{-1}$ after media optimization (Arunkumar et al., 2014). Other examples of bacterial laccase producers include *Bacillus spp* (very well studied and characterized), *E. coli* and *Campylobacter jejuni* (less studied since they present a lower enzymatic activity than *B. subtilis)* (Chauhan et al., 2017), *Pseudomonas putida*, *Streptomyces antibioticus*, and more (Chandra and Chowdhary, 2015).

Such a biodiversity of laccase producing species is promising in terms of prospecting for a diverse set of laccases that can be optimized for process-specific enzymes for efficient lignin degradation. To date, there are hundreds of studies in which laccases from across all kingdoms have been produced and characterized (Yang et al., 2017). However, for industrial purposes, it is difficult to optimize fermentation conditions for each species that produce laccases. Therefore, heterologous expression in tractable hosts has been the objective of much research (Wang et al., 2016). The development of genetic tools has allowed laccases to be identified, expressed and characterized in model organisms (Cullen, 1997). The most used organisms for heterologous expression that have been studied for laccase characterization are *E. coli*, *S. cerevisiae*, *Pichia pastoris*, and *Yarrowia lipolytica (*Piscitelli et al., *2010)*. Filamentous fungi, such as *Aspergillus oryzae*, *Aspergillus niger* and *Trichoderma atroviride*, are alternative host organisms to study for production and secretion of laccases, because they offer advantages such as high enzyme secretion levels, fast growth in liquid culture and availability of new genome-editing tools (Piscitelli et al., 2010; Leynaud-Kieffer et al., 2019).

2.2. Characterization of laccases

Laccases belong to the cupredoxin superfamily, among other oxidases such as manganese oxidase (Chauhan et al., 2017). Due to their broader substrate specificity, laccases have been characterized as a subgroup of enzymes, sharing a signature that distinguishes them from other multi-copper oxidase enzymes (Bourbonnais and Paice, 1990). This signature is composed of four conserved segments of length ranging from 8 to 24 amino acids. These conserved regions contain the genetic information of its active center, encoding the copper ligand coordination and maintenance of the three-dimensional folded protein (Kumar et al., 2003).

2.2.1. Structure and mechanism of laccases

Laccases have a typical molecular mass between 50 and 70 kDa, but some laccases have masses as large as 140 kDa, depending on the organism and degree to which the protein is glycosylated (Berka et al., 1997). Fungal laccases contain an N-terminal secretion signal peptide of 20–22 amino acids. Overall, the laccase protein can be glycosylated between 10 and 25%, based on increases in mass (Arregui et al., 2019). In general, the laccase structure is composed of three cupredoxin domains having β-barrel symmetry (Murphy et al., 1997).

The laccase catalytic site contains three to four copper atoms (Cu^{2+}) (Janusz et al., 2017). Fungal laccases typically have four copper atoms, with the Cu^{2+} at the surface of the structure designated as the first active site (T1), because it can interact directly with the substrate, which can be either lignin or a mediator. Three other coppers tend to form a cluster, with one comprising the second active site (T2) and the two other coppers making up the third active site (T3) (Christopher et al., 2014). Laccases function by relaying electrons between these coppers to catalyze a sequential of four electrons that substrate oxidation with concomitant reduction of molecular oxygen to two molecules of water H_2O (Fig. 3a) (Jones and Solomon, 2015).

The T1 copper is the most solvent-accessible copper, and it is the site where the substrate binds and one electron is transferred from the substrate to the laccase active center (Fig. 4). The T1 copper has an optical absorption at 600 nm and is thus named the “Blue” copper ion. It is directly coordinated to two histidines and a thiol group of a cysteine, forming a trigonal structure (Morozova et al., 2007). Depending on the organism, there may also be a methionine, phenylalanine or a leucine coordinated to the copper at T1 (Fig. 3b) (Xu et al., 1999). The maximum redox potential of laccase is determined by the capacity of its T1 active site potential, meaning that the ionization potential of a compound cannot exceed the T1 redox potential (Xu, 1997).

Following this initial electron transfer event, the electron is transferred from T1 to the buried T2/T3 cluster (12 Å) by the Cys-His pathway where the T2 and T3 sites are only separated by a distance of 4 Å (Jones and Solomon, 2015). T2 is a mononuclear copper with no detectable absorption, and experiments show that removing T2 decreases the redox capacity of laccases. The T2 copper is linked by histidines and a hydroxyl group. T3 is a pair of binuclear coppers approximately 4 Å apart and that can be detected at a wavelength of 330 nm (Solomon et al., 1996). Each copper in the T3 site is linked by three histidines (Fig. 3b).

In the laccase oxidized “resting” form, the two T3 coppers are linked together by a hydroxyl group. If reduced, then the T3 coppers separate (Fig. 3a) (Singh et al., 2011). Overall, the activity of laccases is tightly linked to the pH of the solution. Substrate oxidation by the T1 active site is improved at high pH. However, the transfer of electrons to T2/T3 is reduced at high pH due to the hydroxide anion binding to its active site (Xu, 1997). These effects tend to balance each other out and optimal activity is generally observed in the pH range between 3.0 and 5.5 for fungal laccases (Yin et al., 2019).

2.2.2. Activity on various substrates

2.2.2.1. Phenolic substrate. Phenols are oxidized to phenoxy free radicals by direct interaction with laccases, which results in either radical-coupling-based polymerization or radical rearrangement. In these reactions, the laccase generates either dead-end products (Navarra et al., 2010; Kudanga et al., 2011) or uses the phenolic substrate as a mediator in other laccase catalyzed reactions (d’Acunzo and Galli, 2003; Cañas and Camarero, 2010). Several publications have described the ability of naturally occurring phenolic compounds from lignin to act as redox mediators (Camarero et al., 2005; Camarero et al., 2007; Camarero et al., 2008). Most laccases have optimal activity towards phenolic compounds at acidic to neutral pH (Michniewicz et al., 2006; Haibo et al., 2009; Autore et al., 2009; Vasdev et al., 2005) and laccases have not been shown to catalyze lignin depolymerization under alkaline conditions (Yin et al., 2019; Hämäläinen et al., 2018; Novoa et al., 2019). Laccases present a diversity of binding modes depending on the substrate (Mehra et al., 2018), and protein engineering efforts have been carried out to understand and obtain tailor-made biocatalysts presenting the desired properties (Mehra et al., 2018; Pardo and Camarero, 2015).

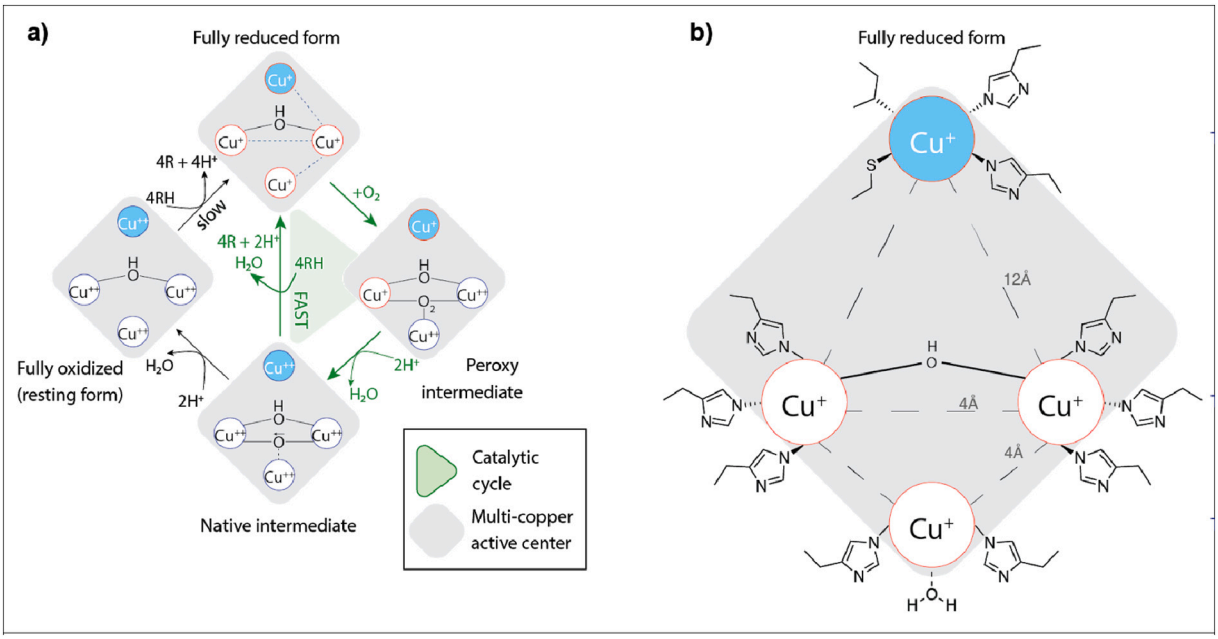

Fig. 3. a) Active multi-copper center of laccase, and mechanism from reduction to oxidation. Image adapted from Wong 2009 (Wong, 2009). b) Example of the active center structure of the white-rot fungi *Rigidoporus lignosus*, adapted from Garavaglia 2004 (Garavaglia et al., 2004).

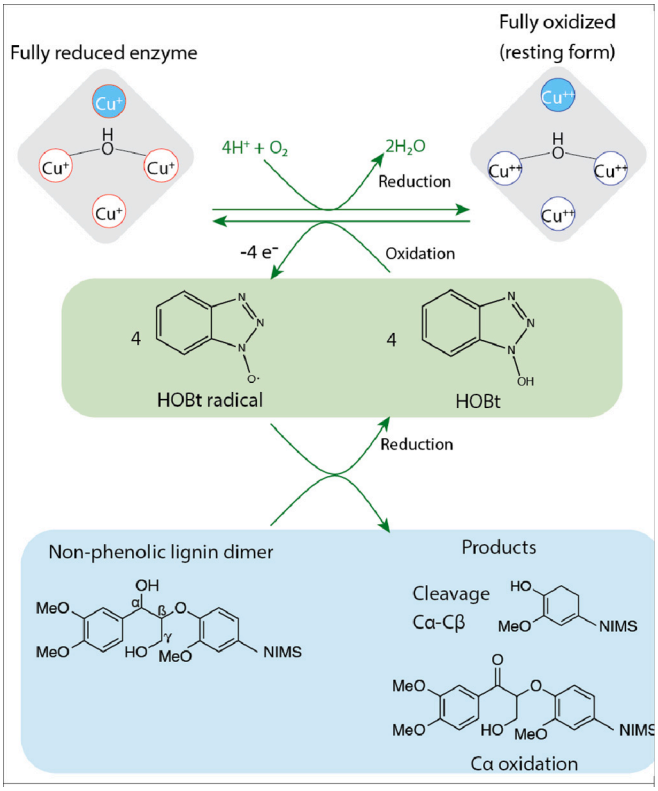

Fig. 4. Reaction mechanism of laccase oxidation using HOBt intermediaries to degrade lignin (Deng et al., 2018; Potthast et al., 2001).

For functional diversity, a few engineered laccases with increased activity towards phenolic compounds such as 2,6-dimethoxyphenol, guaiacol at alkaline conditions (pH $\geq$ 8.0) have been reported (Gunne and Urlacher, 2012). At alkaline pH, oxidation of phenolic compounds (either a phenolic mediator or a phenolic lignin dimer) to a phenoxy radical is favored by the presence of the phenolate form (d'Acunzo and Galli, 2003). At high pH, laccase activity can be inhibited by the abundance of hydroxide anion due to competitive binding to T2/T3 coppers (Xu, 1997). Towards enhanced activity at alkaline pH, more understanding of the electron transfer mechanism and T2/T3-catalyzed molecular oxygen reduction to water at the molecular level is needed for laccase engineering.

2.2.2.2. ABTS substrate. The use of mediators, such as 2,2′-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) (ABTS), helps to reduce the steric hindrance between the substrate and laccase. Substrates such as lignin and other larger phenolic compounds can have difficulties accessing the active site of a laccase due to their size. Mediators like ABTS, being smaller compounds, can be oxidized directly by laccase and then the oxidized mediator diffuses to the larger substrate where it is reduced by the substrate (Christopher et al., 2014). A classic assay used to detect any kinetic reaction of laccase activity utilizes ABTS as a substrate and oxidation of ABTS as the readout. ABTS acts as an electron donor during the oxidation reaction by laccase, reducing its form into a radical cation $ABTS^{+}$ that absorbs light at 734 nm and turns the reaction to a blue color, the higher the laccase activity is, the faster the blue color appears (Hernández-Monjaraz et al., 2018).

The mechanism of action starts when ABTS gives its electron to the T1 active site of laccase. Large substrates, such as ABTS, have a unique way to bind to the T1 active site, by delocalizing a density of electrons on multiple atoms. ABTS forms hydrogen bonds and π-π interactions with the one of the T1 histidines (Mehra et al., 2018). Then the electron gets transferred all across in its center active site at the T3 active site, and then to the T2 active site. When T2 receives the electrons, it sequentially reduces a molecule of dioxygen O_2 in two molecules of water H_2O (Jones and Solomon, 2015).

2.2.3. Assay in development: ligNIMS

Knowing that the β-*O*-4 linkage represents 50 to 60% of most of the linkage in lignin, a team of researchers developed a specific activity assay for lignin degrading enzymes (LDEs) and this specific linkage using nanostructure-initiator mass spectrometry (NIMS) (Deng et al., 2018). They synthesized two β-aryl ether substrates, one phenolic lignin like model compound and one non-phenolic lignin like model compound, both attached to a NIMS-tag to be able to run the experiments through the NIMS. They studied two different enzymes, laccase enzyme and MnP enzyme, and quantified β-*O*-4 bond breaking and measured the reaction kinetics for these enzymes on both substrates. To analyze the laccase mechanism, Deng et al. used two different assay approaches, one assay used the phenolic substrate without the use of a mediator and one assay used the non-phenolic substrate with the mediator 1-hydroxybenzotriazole (HOBt) (Fig. 4). This assay provides distinct advantages over colorimetric assays like ABTS oxidation by allowing researchers to directly measure the types of laccase catalyzed bond breaking reactions required to enzymatically depolymerize lignin to defined breakdown products. Developing technology to be able to characterize laccase depolymerization per enzymes helped to understand the variety of mechanisms of action that enzymes can offer. While laccases grew interest in the scientific community, it also expanded its possibilities of action in industry.

2.3. Laccase, a solution for many different industries

Laccases are promising biocatalysts for degrading lignin into fragments amenable to being upgraded to valuable biofuels and bioproducts but find many additional industrial applications. Due to their broad substrate specificity, and their being naturally a green chemistry, laccases are being used in the food (Mayolo-Deloisa et al., 2020), pulping and bleaching (Singh and Arya, 2019), pharmaceutical and biosensors industries (Zhang et al., 2018) and are being used for bioremediation (Arregui et al., 2019) and in water treatment technologies (Fig. 5) (Singh et al., 2018) in environmental industries.

Phenolic byproducts are present in a variety of food industries, such as fermentation of sugarcane molasses, brewing and olive oil (Mayolo-Deloisa et al., 2020). The presence of phenolic compounds in beverages affects their taste, color and overall quality. Laccases demonstrated high efficiency of removing the phenolic compounds responsible for these deleterious effects compared to other chemical treatments, and the use of laccases also demonstrated great enhancement of the color stability of the drink after treatment, particularly in the red wine industry where the color is very important (Minussi et al., 2007). In industrial baking, laccases have been used to increase the stability and strength of the machinery of the dough. Because laccase plays an oxidizing role in the dough, it improves the absorption and distribution of water, and so increases the overall volume and softness of the bread (Minussi et al., 2002). A CU1 laccase from *Trametes maxima* has been tested in the bread, and the physicochemical properties of the bread were enhanced, such as the solubility of the arabinoxylans, the proteins and phenols content, the hardness was reduced, and the height and color amplified (Niño-Medina et al., 2017).

Over the last century, industrialization and urbanisation led to a rise in pollution of the ecosystem and increased contamination of water. The broad activity of the laccases provides a sustainable solution to biotransform polluants molecules in water and to degrade organic compounds (Arregui et al., 2019). Pollutants that can be degraded by laccases include plastics, herbicides, fertilizers, nonylphenol and bisphenol A and chlorinated paraffin phthalates. Additionally, laccases can degrade environmental contaminants from pharmaceuticals, including pain killers, antibiotics, hormones and endocrine-disrupting chemicals (Bilal et al., 2017; Cabana et al., 2007). Laccases work in these cases by catalyzing degradation of these toxins to less harmful compounds. For example, laccase was shown to catalyze degradation of bisphenol A into Beta hydroxybutyric acid (Bilal et al., 2019), chloramphenicol into chloramphenicol aldehyde (Navada and Kulal, 2019), and others (Gasser et al., 2014). In the northwestern Mexico groundwater, researchers evaluated the biotransformation of Bisphenol A, ethynylestradiol, triclosan and nonylphenol catalyzed by a laccase cocktail and obtained 55 to 93% biodegradation (Garcia-Morales et al., 2015).

Another major industrial application of laccase is found in the pulping and paper industry. Pulping is a process where lignocellulose, and especially cellulose, is extracted from the wood. The cellulose is the main ingredient to make the pulp, which is then used to make paper. White liquor, containing sodium hydroxide and sodium sulfide in water, and steam are added to separate the cellulose from lignin. After digestion, the lignin is dissolved and concentrated through the Kraft process and separated from the pulp. The Kraft pulping process consists of lignin depolymerization, condensation and extraction. The pulp, commonly referred to as black liquor, contains concentrated lignin, which after filtration and chemical transformation is precipitated and purified (Froass et al., 1998). Every step in this process makes the pulp whiter and brighter. This process has several inefficiencies such as the generation of toxic byproducts and the use of large amounts of water, aspects

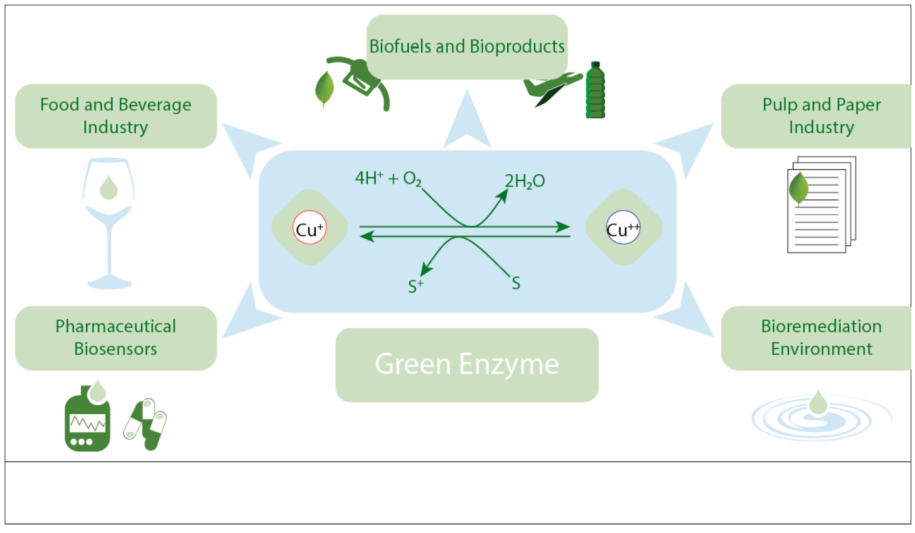

Fig. 5. Laccase enzyme, a green chemistry in the service of several industries. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

which can be improved for enhanced environmental and economic advantage (Singh and Chandra, 2019). For example, chlorophenols are used as bleaching agents and since 1999 have been known as harmful substances and listed as environmental pollutants by the United States Environmental Protection Agency (US EPA) (Rodríguez-Delgado et al., 2015). Green chemistries based on using enzymes such as laccases offer a more sustainable replacement to answer the challenges of the pulp and paper industry (Gupta and Sharma, 2017). The use of laccase in the pulp and paper industry could permit the replacement of chemicals such as chlorine dioxide, hydrogen peroxide, ozone, hydrosulfite and alkaline extraction (Virk et al., 2012), making the pulping process a biopulping, the bleaching process a biobleaching etc.… and detoxifying the paper mill effluents. Moreover, laccases demonstrated an increase in brightness after lignin digestion, and an enhancement after biobleaching of up to 12% consistency of the pulp, using laccase from *Trametes villosa* with HOBt, violuric acid (VA), and ABTS as mediators (Singh and Arya, 2019). A laccase from *Bacillus* sp. showed, after biobleaching and deinking of Old Newsprint (ONP), a brightness improvement of 13% and whiteness enhancement of 106% compared to the traditional chemical method (Gupta et al., 2015).

Biosensors are analytical devices that permit the monitoring of chemicals such as phenolic compounds by converting physical or chemical signals into an electric signal that is amplified and processed to be analyzed and directly monitored. The most commonly used biosensors today are glucose biosensors that monitor blood glucose concentration in diabetics (Yoo and Lee, 2010). To detect analytes of interest, biosensors require the use of enzymes to convert analytes of interest to detectable compounds. Laccase-based biosensors are designed to be fast, online and in situ devices for monitoring reactions with phenolic compounds. Phenol biosensors have applications in the food industry, environmental analyses, and pharmaceutical industry (Rodríguez-Delgado et al., 2015). Immobilization of laccase, meaning the capture of enzymes in a physical support, allowed the development of biosensors for phenolic compounds determination (Casero et al., 2013). Immobilization methods include covalent binding, adsorption, cross-linking, encapsulation and entrapment (Fernández-Fernández et al., 2013). The substrate molecule reacts with the immobilized laccase and is converted to a product that issues an amperometric, voltammetric, potentiometric or conductometric signal, that is amplified and analyzed by the electronics of the biosensor (Rodríguez-Delgado et al., 2015).

2.4. Challenges in application of laccases

Laccases have been the subject of numerous studies for the last twenty years, resulting in their use in many applications with various goals (Fig. 6). As climate change raises concerns, it gives industry new challenges, such as combating pollution and applying the rule of "reduce-reuse-recycle" (Mayolo-Deloisa et al., 2020). Being an eco-friendly, swiss-knife applicant, laccases offer promising solutions due to their versatile mode of action, but not at a cheap price. First of all, one of the major drawbacks is that there is no current technology to recycle laccases in industry, and without reusing the enzyme, it impairs drastically the economic viability of the process (Gupta and Sharma, 2017). Indeed, large scale industrial applications using laccases remain arduous. The full viable commercialization of laccase would require a high production and purity level for an affordable price per ton of enzyme, and cheap intensive mediators (such as ABTS), that help to increase the laccase redox potential (Singh and Arya, 2019). The cost of production is highly increased by the downstream process, for this

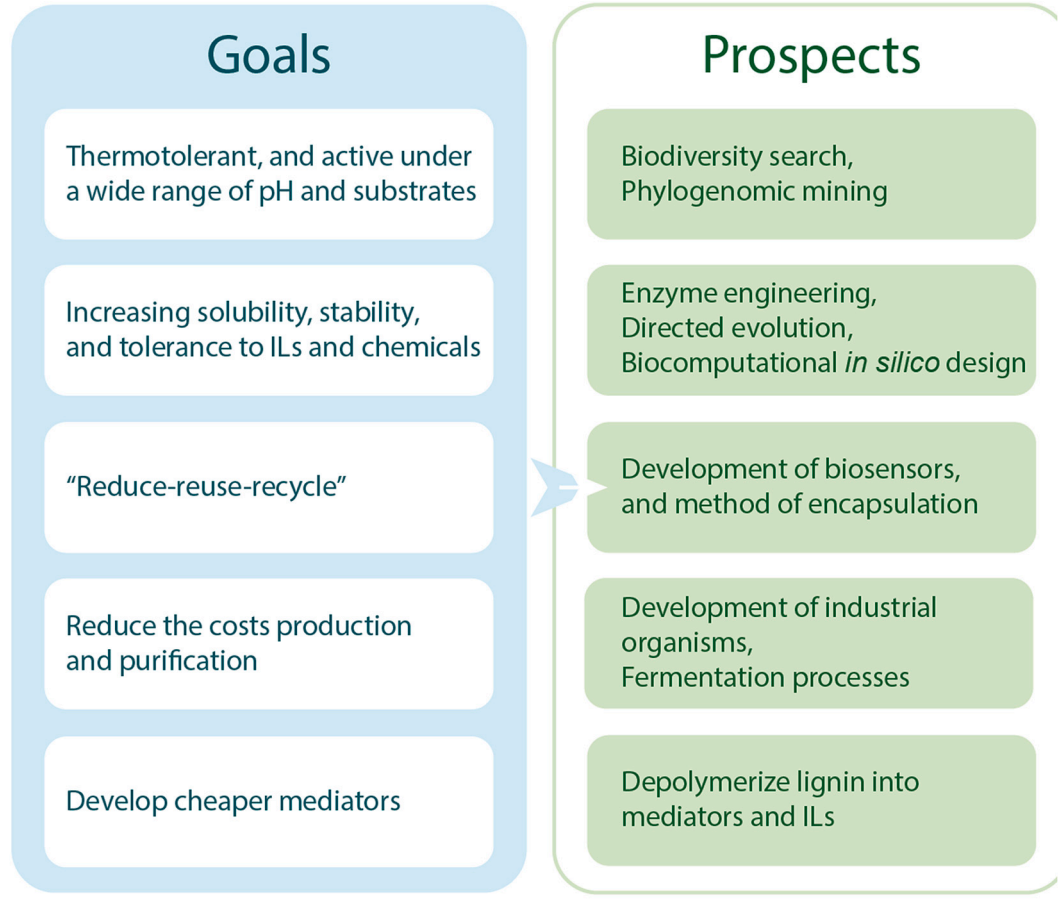

Fig. 6. Goals and prospects of laccase industrialization.

reason it is important to focus on the development of affordable purification methods (Agrawal et al., 2018). Another industrial disadvantage is the need of in vitro enzymes rather than in vivo, the activity potential is lowered after extraction of the enzymes, due to the instability and insolubility of the protein in the chosen substrate e.g. solvents (Martínez et al., 2017; Zumárraga et al., 2007). Laccase-based biosensors are facing additional challenges due to the enzyme immobilization. In fact, the costs carriers and immobilization are high due to the isolation, purification and recovery cost of the enzyme, but also because of enzyme instability after immobilization and sometimes loss of catalytic properties (Rodríguez-Delgado et al., 2015). Finally, the current available literature on laccases is heterogeneous, such as the investigation of their chemical properties and their characterization, resulting in the limit of their possible comparison and interaction (Kumar and Chandra, 2020). Today, most oxidoreductase enzymes are currently under active development for being affordable commercially by developing better purification platform processes and increasing their stability and their choice of substrate.

2.5. Development and prospect of laccase industrialization

Despite all the bottlenecks and challenges presented, research on laccases has resulted in great innovations over the last few years and researchers continue to generate new innovations for optimal exploitation of laccases (Fig. 6). A techno-economic analysis of the industrialization of lignin degrading enzymes such as laccase shows that more effort is required to lower the price of enzymes for biofuels production (Klein-Marcuschamer et al., 2012). Thanks to the development of new approaches in computational, biophysical and biochemical fields, our ability to engineer enzymes for improved stability and performance has greatly improved. This enables custom optimization of enzymes and their functionality for different applications (Mate and Alcalde, 2015; Stanzione et al., 2020). The development of bioinformatic tools is useful for metagenomic analysis and identification of new enzymes with new functionalities from non-model organisms (Dandare et al., 2019). As an example, recent promising research using directed evolution resulted in the adaptation of the Lcc9 laccase from *Coprinopsis cinerea*, expressed by *P. pastoris,* to function optimally at pH between 8.0 and 8.5 (Yin et al., 2019). The development of biosensors has led to innovative methods of enzyme immobilization, such as adsorption, entrapment, covalent-binding, encapsulation and self-immobilization, showed encouraging results to reuse laccase enzymes but the activity of the recovered enzyme after immobilization is still not always consistent (Fernández-Fernández et al., 2013). The development of novel biotechnologies has allowed researchers to discover new enzymes for lignocellulose bioconversion, by exploring the biodiversity of extreme habitats endowing the advantageous characteristics, such as this highly functional thermostable TtLMCO1 laccase from *Thermothelomyces thermophila* that has a wide spectrum of substrate specificity and has been successfully expressed in *Pichia pastoris* (Zerva et al., 2019). In order to reduce the production costs, homologous and heterologous hosts are being investigated to secrete laccases, such as *Aspergillus* sp. which is a well-known industrial host that can produce more than several $g.L^{-1}$ and could potentially reduce the purification downstream process considerably (Kumar et al., 2016). It is possible to derivatize lignin into mediators that can reduce the cost of using traditional mediators that are used today to improve the redox potential of laccases (Mate and Alcalde, 2015). Moreover, during the laccase bleaching stage, mediators undergo decomposition which limits their turnover efficiency (Christopher et al., 2014).

3. Conclusion

The efficient and affordable valorization of lignin into biofuels and bioproducts remains one of the most significant challenges facing commercial biorefineries and the bioenergy enterprise. While studied for decades, there remain significant gaps in our fundamental understanding of how lignin is broken down by biological systems. In particular, the lack of any robust and efficient biochemical methods (e.g, enzymes) capable of rapidly breaking down lignin into targeted intermediates and final products limits our ability to valorize lignin. Laccase enzymes are among the most studied class of lignin degrading enzymes and present a compelling suite of characteristics that may be key to solving these challenges. Over the last 20 years, there have been several reviews published about laccase characterization and their applications in different domains of industry. New technologies promote the potential of the enzyme and industrialization processes. However, laccases are still too expensive to be used and produced industrially, there is a need to constantly develop better tools and innovate, to make laccase an affordable industrial enzyme and to get one step closer towards affordable biofuels and bioproducts to replace fossil fuel economically and durably. Here we have presented some potential approaches based on laccases that provide a roadmap for the scientific community to work towards achieving the true potential of lignin.

Acknowledgement

This work was supported by the DOE Joint BioEnergy Institute (http://www.jbei.org) and by the U.S. Department of Energy, Office of Science, Office of Biological and Environmental Research, through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, worldwide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Adler, E., 1977. Lignin chemistry past, present and future. Wood Sci. Technol. 11, 169–218. https://doi.org/10.1007/BF00365615.

Agrawal, K., Chaturvedi, V., Verma, P., 2018. Fungal laccase discovered but yet undiscovered. Bioresour. Bioprocess. 5, 4. https://doi.org/10.1186/s40643-018-0190-z.

Alvarez, H.M., Mayer, F., Fabritius, D., Steinbüchel, A., 1996. Formation of intracytoplasmic lipid inclusions by Rhodococcus opacus strain PD630. Arch. Microbiol. 165, 377–386. https://doi.org/10.1007/s002030050341.

Aminzadeh, S., Zhang, L., Henriksson, G., 2017. A possible explanation for the structural inhomogeneity of lignin in LCC networks. Wood Sci. Technol. 1–12. https://doi.org/10.1007/s00226-017-0941-6.

Anderson, N.A., Tobimatsu, Y., Ciesielski, P.N., Ximenes, E., Ralph, J., Donohoe, B.S., Ladisch, M., Chapple, C., 2015. Manipulation of guaiacyl and syringyl monomer biosynthesis in an arabidopsis cinnamyl alcohol dehydrogenase mutant results in atypical lignin biosynthesis and modified cell wall structure. Plant Cell 27, 2195–2209. https://doi.org/10.1105/tpc.15.00373.

Anthony, W.E., Carr, R.R., DeLorenzo, D.M., Campbell, T.P., Shang, Z., Foston, M., Moon, T.S., Dantas, G., 2019. Development of Rhodococcus opacus as a chassis for lignin valorization and bioproduction of high-value compounds. Biotechnol. Biofuels. 12, 192. https://doi.org/10.1186/s13068-019-1535-3.

Arregui, L., Ayala, M., Gómez-Gil, X., Gutiérrez-Soto, G., Hernández-Luna, C.E., de Los Santos, M. Herrera, Levin, L., Rojo-Domínguez, A., Romero-Martínez, D., Saparrat, M.C.N., Trujillo-Roldán, M.A., Valdez-Cruz, N.A., 2019. Laccases: structure, function, and potential application in water bioremediation. Microb. Cell Factories 18, 200. https://doi.org/10.1186/s12934-019-1248-0.

Arunkumar, T., Alex Anand, D., Narendrakumar, G., 2014. Application of Response Surface Methodology (RSM)–CCD for the production of Laccases using submerged fermentation. Int. J. Pharm. Bio Sci. 18, 200–233.

Asim, A.M., Uroos, M., Naz, S., Sultan, M., Griffin, G., Muhammad, N., Khan, A.S., 2019. Acidic ionic liquids: promising and cost-effective solvents for processing of lignocellulosic biomass. J. Mol. Liq. 287, 110943. https://doi.org/10.1016/j.molliq.2019.110943.

Autore, F., Del Vecchio, C., Fraternali, F., Giardina, P., Sannia, G., Faraco, V., 2009. Molecular determinants of peculiar properties of a Pleurotus ostreatus laccase: analysis by site-directed mutagenesis. Enzym. Microb. Technol. 45, 507–513. https://doi.org/10.1016/j.enzmictec.2009.08.004.

Berka, R.M., Schneider, P., Golightly, E.J., Brown, S.H., Madden, M., Brown, K.M., Halkier, T., Mondorf, K., Xu, F., 1997. Characterization of the gene encoding an extracellular laccase of Myceliophthora thermophila and analysis of the recombinant

enzyme expressed in Aspergillus oryzae. Appl. Environ. Microbiol. 63, 3151–3157. https://doi.org/10.1128/AEM.63.8.3151-3157.1997.
Bilal, M., Asgher, M., Parra-Saldivar, R., Hu, H., Wang, W., Zhang, X., Iqbal, H.M.N., 2017. Immobilized ligninolytic enzymes: an innovative and environmental responsive technology to tackle dye-based industrial pollutants - a review. Sci. Total Environ. 576, 646–659. https://doi.org/10.1016/j.scitotenv.2016.10.137.
Bilal, M., Iqbal, H.M.N., Barceló, D., 2019. Mitigation of bisphenol a using an array of laccase-based robust bio-catalytic cues - a review. Sci. Total Environ. 689, 160–177. https://doi.org/10.1016/j.scitotenv.2019.06.403.
Boerjan, W., Ralph, J., Baucher, M., 2003. Lignin biosynthesis. Annu. Rev. Plant Biol. 54, 519–546. https://doi.org/10.1146/annurev.arplant.54.031902.134938.
Bourbonnais, R., Paice, M.G., 1990. Oxidation of non-phenolic substrates. An expanded role for laccase in lignin biodegradation. FEBS Lett. 267, 99–102. https://doi.org/10.1016/0014-5793(90)80298-w.
Bugg, T.D.H., Ahmad, M., Hardiman, E.M., Singh, R., 2011. The emerging role for bacteria in lignin degradation and bio-product formation. Curr. Opin. Biotechnol. 22, 394–400. https://doi.org/10.1016/j.copbio.2010.10.009.
Cabana, H., Jiwan, J.-L.H., Rozenberg, R., Elisashvili, V., Penninckx, M., Agathos, S.N., Jones, J.P., 2007. Elimination of endocrine disrupting chemicals nonylphenol and bisphenol a and personal care product ingredient triclosan using enzyme preparation from the white rot fungus Coriolopsis polyzona. Chemosphere. 67, 770–778. https://doi.org/10.1016/j.chemosphere.2006.10.037.
Camarero, S., Ibarra, D., Martínez, M.J., Martínez, A.T., 2005. Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. Appl. Environ. Microbiol. 71, 1775–1784. https://doi.org/10.1128/AEM.71.4.1775-1784.2005.
Camarero, S., Ibarra, D., Martínez, Á.T., Romero, J., Gutiérrez, A., del Río, J.C., 2007. Paper pulp delignification using laccase and natural mediators. Enzym. Microb. Technol. 40, 1264–1271. https://doi.org/10.1016/j.enzmictec.2006.09.016.
Camarero, S., Cañas, A.I., Nousiainen, P., Record, E., Lomascolo, A., Martínez, M.J., Martínez, A.T., 2008. P-hydroxycinnamic acids as natural mediators for laccase oxidation of recalcitrant compounds. Environ. Sci. Technol. 42, 6703–6709. https://doi.org/10.1021/es8008979.
Cañas, A.I., Camarero, S., 2010. Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol. Adv. 28, 694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002.
Casero, E., Petit-Domínguez, M.D., Vázquez, L., Ramírez-Asperilla, I., Parra-Alfambra, A. M., Pariente, F., Lorenzo, E., 2013. Laccase biosensors based on different enzyme immobilization strategies for phenolic compounds determination. Talanta. 115, 401–408. https://doi.org/10.1016/j.talanta.2013.05.045.
Chandra, R., Chowdhary, P., 2015. Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environ Sci Process Impacts 17, 326–342. https://doi.org/10.1039/c4em00627e.
Chandrakant, P., Bisaria, V.S., 1998. Simultaneous bioconversion of cellulose and hemicellulose to ethanol. Crit. Rev. Biotechnol. 18, 295–331. https://doi.org/10.1080/0738-859891224185.
Chauhan, P.S., Goradia, B., Saxena, A., 2017. Bacterial laccase: recent update on production, properties and industrial applications. 3 Biotech. 7, 323. https://doi.org/10.1007/s13205-017-0955-7.
Chio, C., Sain, M., Qin, W., 2019. Lignin utilization: a review of lignin depolymerization from various aspects. Renew. Sust. Energ. Rev. 107, 232–249. https://doi.org/10.1016/j.rser.2019.03.008.
Christopher, L.P., Yao, B., Ji, Y., 2014. Lignin biodegradation with laccase-mediator systems. Front. Energy Res. 2 https://doi.org/10.3389/fenrg.2014.00012.
Cullen, D., 1997. Recent advances on the molecular genetics of ligninolytic fungi. J. Biotechnol. 53, 273–289. https://doi.org/10.1016/s0168-1656(97)01684-2.
Dabirmanesh, B., Khajeh, K., Ghazi, F., Ranjbar, B., Etezad, S.-M., 2015. A semi-rational approach to obtain an ionic liquid tolerant bacterial laccase through π-type interactions. Int. J. Biol. Macromol. 79, 822–829. https://doi.org/10.1016/j.ijbiomac.2015.06.002.
d'Acunzo, F., Galli, C., 2003. First evidence of catalytic mediation by phenolic compounds in the laccase-induced oxidation of lignin models. Eur. J. Biochem. 270, 3634–3640. https://doi.org/10.1046/j.1432-1033.2003.03752.x.
Dandare, S.U., Young, J.M., Kelleher, B.P., Allen, C.C.R., 2019. The distribution of novel bacterial laccases in alpine paleosols is directly related to soil stratigraphy. Sci. Total Environ. 671, 19–27. https://doi.org/10.1016/j.scitotenv.2019.03.250.
de Candolle, A.P., 1842. Regni Vegetabilis Systema Naturale, Sive Ordines, Genera et Species Plantarum Secundum Methodi Naturalis Normas Digestarum et Descriptarum/, Sumptibus Sociorum Treuttel et Würtz, Parisiis [etc.]. https://doi.org/10.5962/bhl.title.59874.
de Candolle, A.P., Buek, H.W., 1842. Genera, Species et Synonyma Candolleana: Alphabetico Ordine Disposita, seu Index Generalis et Specialis ad A.P. Decandolle, Prodromum Systematis Naturalis Regni Vegetabilis, Sumptibus Librariae Nauckianae.
de Mattos-Shipley, K.M.J., Ford, K.L., Alberti, F., Banks, A.M., Bailey, A.M., Foster, G.D., 2016. The good, the bad and the tasty: the many roles of mushrooms. Stud. Mycol. 85, 125–157. https://doi.org/10.1016/j.simyco.2016.11.002.
Deng, K., Zeng, J., Cheng, G., Gao, J., Sale, K.L., Simmons, B.A., Singh, A.K., Adams, P.D., Northen, T.R., 2018. Rapid characterization of the activities of lignin-modifying enzymes based on nanostructure-initiator mass spectrometry (NIMS). Biotechnol. Biofuels. 11, 266. https://doi.org/10.1186/s13068-018-1261-2.
Elgharbawy, A.A., Alam, M.Z., Moniruzzaman, M., Goto, M., 2016. Ionic liquid pretreatment as emerging approaches for enhanced enzymatic hydrolysis of lignocellulosic biomass. Biochem. Eng. J. 109, 252–267. https://doi.org/10.1016/j.bej.2016.01.021.
Eudes, A., George, A., Mukerjee, P., Kim, J.S., Pollet, B., Benke, P.I., Yang, F., Mitra, P., Sun, L., Cetinkol, O.P., Chabout, S., Mouille, G., Soubigou-Taconnat, L., Balzergue, S., Singh, S., Holmes, B.M., Mukhopadhyay, A., Keasling, J.D., Simmons, B.A., Lapierre, C., Loqué, D., 2012. Biosynthesis and incorporation of side-chain-truncated lignin monomers to reduce lignin polymerization and enhance saccharification. Plant Biotechnol. J. 10, 609–620. https://doi.org/10.1111/j.1467-7652.2012.00692.x.
Faga, B.A., Wilkins, M.R., Banat, I.M., 2010. Ethanol production through simultaneous saccharification and fermentation of switchgrass using *Saccharomyces cerevisiae* D5A and thermotolerant Kluyveromyces marxianus IMB strains. Bioresour. Technol. 101, 2273–2279. https://doi.org/10.1016/j.biortech.2009.11.001.
Fernández-Fernández, M., Sanromán, M.Á., Moldes, D., 2013. Recent developments and applications of immobilized laccase. Biotechnol. Adv. 31, 1808–1825. https://doi.org/10.1016/j.biotechadv.2012.02.013.
Froass, P.M., Ragauskas, A.J., Jiang, J.E., 1998. NMR studies part 3: analysis of Lignins from modern Kraft pulping technologies. Holzforschung. 52, 385–390. https://doi.org/10.1515/hfsg.1998.52.4.385.
Futahashi, R., Tanaka, K., Matsuura, Y., Tanahashi, M., Kikuchi, Y., Fukatsu, T., 2011. Laccase2 is required for cuticular pigmentation in stinkbugs. Insect Biochem. Mol. Biol. 41, 191–196. https://doi.org/10.1016/j.ibmb.2010.12.003.
Galbe, M., Wallberg, O., 2019. Pretreatment for biorefineries: a review of common methods for efficient utilisation of lignocellulosic materials. Biotechnol. Biofuels. 12, 294. https://doi.org/10.1186/s13068-019-1634-1.
Galbe, M., Zacchi, G., 2002. A review of the production of ethanol from softwood. Appl. Microbiol. Biotechnol. 59, 618–628. https://doi.org/10.1007/s00253-002-1058-9.
Garavaglia, S., Cambria, M.T., Miglio, M., Ragusa, S., Iacobazzi, V., Palmieri, F., D'Ambrosio, C., Scaloni, A., Rizzi, M., 2004. The structure of Rigidoporus lignosus Laccase containing a full complement of copper ions, reveals an asymmetrical arrangement for the T3 copper pair. J. Mol. Biol. 342, 1519–1531. https://doi.org/10.1016/j.jmb.2004.07.100.
Garcia-Morales, R., Rodríguez-Delgado, M., Gomez-Mariscal, K., Orona-Navar, C., Hernandez-Luna, C., Torres, E., Parra, R., Cárdenas-Chávez, D., Mahlknecht, J., Ornelas-Soto, N., 2015. Biotransformation of endocrine-disrupting compounds in groundwater: Bisphenol A, Nonylphenol, Ethynylestradiol and Triclosan by a Laccase cocktail from Pycnoporus sanguineus CS43. Water Air Soil Pollut. 226, 251. https://doi.org/10.1007/s11270-015-2514-3.
Gasser, C.A., Ammann, E.M., Shahgaldian, P., Corvini, P.F.-X., 2014. Laccases to take on the challenge of emerging organic contaminants in wastewater. Appl. Microbiol. Biotechnol. 98, 9931–9952. https://doi.org/10.1007/s00253-014-6177-6.
Gilbertson, R.L., 1980. Wood-rotting Fungi of North America. Mycologia. 72, 1. https://doi.org/10.2307/3759417.
Grätzel, M., 2007. Photovoltaic and photoelectrochemical conversion of solar energy. Philos. Transact. A Math. Phys. Eng. Sci. 365, 993–1005. https://doi.org/10.1098/rsta.2006.1963.
Grotewol, E., Jones Prather, K.L., 2014. Lignocellulosic Biomass for Advanced Biofuels and Bioproduct: Workshop Report. U. S. Department of Energy Office of Science, Washington DC.
Gunne, M., Urlacher, V.B., 2012. Characterization of the alkaline laccase Ssl1 from Streptomyces sviceus with unusual properties discovered by genome mining. PLoS One 7, e52360. https://doi.org/10.1371/journal.pone.0052360.
Gupta, S.K.A. Gursharan Singh Vijaya, Sharma, Prince, 2017. Enzyme technology for lignocellulosic biomass conversion and recycling to valuable paper and other products: challenges ahead. J. Mol. Biol. Biochem. Genet. 7 e52360/8pages.
Gupta, V., Garg, S., Capalash, N., Gupta, N., Sharma, P., 2015. Production of thermo-alkali-stable laccase and xylanase by co-culturing of Bacillus sp. and B. halodurans for biobleaching of kraft pulp and deinking of waste paper. Bioprocess Biosyst. Eng. 38, 947–956. https://doi.org/10.1007/s00449-014-1340-0.
Guragain, Y.N., Herrera, A.I., Vadlani, P.V., Prakash, O., 2015. Lignins of bioenergy crops: a review. Nat. Prod. Commun. 10 https://doi.org/10.1177/1934578X1501000141, 1934578X1501000.
Hahn-Hägerdal, B., Galbe, M., Gorwa-Grauslund, M.F., Lidén, G., Zacchi, G., 2006. Bio-ethanol–the fuel of tomorrow from the residues of today. Trends Biotechnol. 24, 549–556. https://doi.org/10.1016/j.tibtech.2006.10.004.
Haibo, Z., Yinglong, Z., Feng, H., Peiji, G., Jiachuan, C., 2009. Purification and characterization of a thermostable laccase with unique oxidative characteristics from Trametes hirsuta. Biotechnol. Lett. 31, 837–843. https://doi.org/10.1007/s10529-009-9945-0.
Hallac, B.B., Sannigrahi, P., Pu, Y., Ray, M., Murphy, R.J., Ragauskas, A.J., 2010. Effect of ethanol Organosolv pretreatment on enzymatic hydrolysis of *Buddleja davidii* stem biomass. Ind. Eng. Chem. Res. 49, 1467–1472. https://doi.org/10.1021/ie900683q.
Hämäläinen, V., Grönroos, T., Suonpää, A., Heikkilä, M.W., Romein, B., Ihalainen, P., Malandra, S., Birikh, K.R., 2018. Enzymatic processes to unlock the lignin value. Front. Bioeng. Biotechnol. 6, 20. https://doi.org/10.3389/fbioe.2018.00020.
He, L., Huang, H., Zhang, Z., Lei, Z., 2015. A review of hydrothermal pretreatment of Lignocellulosic biomass for enhanced biogas production. Curr. Org. Chem. 19, 437–446.
Hernández-Monjaraz, W.S., Caudillo-Pérez, C., Salazar-Sánchez, P.U., Macías-Sánchez, K. L., 2018. Influence of iron and copper on the activity of laccases in Fusarium oxysporum f. sp. lycopersici. Braz. J. Microbiol. 49 (Suppl. 1), 269–275. https://doi.org/10.1016/j.bjm.2018.06.002.
Higuchi, T., 1997. Structure and Functions of Wood, in: Biochemistry and Molecular Biology of Wood, London.
Hilgers, R., Vincken, J.-P., Gruppen, H., Kabel, M.A., 2018. Laccase/mediator systems: their reactivity toward phenolic lignin structures. ACS Sustain. Chem. Eng. 6, 2037–2046. https://doi.org/10.1021/acssuschemeng.7b03451.

Hopkins, D.W., Webster, E.A., Boerjan, W., Pilate, G., Halpin, C., 2007. Genetically modified lignin below ground. Nat. Biotechnol. 25, 168–169. https://doi.org/10.1038/nbt0207-168.
Huang, G.L., Anderson, T.D., Clubb, R.T., 2014. Engineering microbial surfaces to degrade lignocellulosic biomass. Bioengineered. 5, 96–106. https://doi.org/10.4161/bioe.27461.
Janusz, G., Pawlik, A., Sulej, J., Swiderska-Burek, U., Jarosz-Wilkolazka, A., Paszczynski, A., 2017. Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. FEMS Microbiol. Rev. 41, 941–962. https://doi.org/10.1093/femsre/fux049.
Janusz, G., Pawlik, A., Świderska-Burek, U., Polak, J., Sulej, J., Jarosz-Wilkołazka, A., Paszczyński, A., 2020. Laccase properties, physiological functions, and evolution. Int. J. Mol. Sci. 21 https://doi.org/10.3390/ijms21030966.
Jiménez, J.I., Miñambres, B., García, J.L., Díaz, E., 2002. Genomic analysis of the aromatic catabolic pathways from Pseudomonas putida KT2440. Environ. Microbiol. 4, 824–841. https://doi.org/10.1046/j.1462-2920.2002.00370.x.
Johansen, K.S., 2016. Discovery and industrial applications of lytic polysaccharide mono-oxygenases. Biochem. Soc. Trans. 44, 143–149. https://doi.org/10.1042/BST20150204.
Jones, S.M., Solomon, E.I., 2015. Electron transfer and reaction mechanism of laccases. Cell. Mol. Life Sci. 72, 869–883. https://doi.org/10.1007/s00018-014-1826-6.
Käärik, A., 1965. The Identification of the Mycelia of Wood-Decay Fungi by their Oxidation Reactions with Phenolic Compounds, Studia Forestalia Seucia.
Kandasamy, S., Muniraj, I.K., Purushothaman, N., Sekar, A., Sharmila, D.J.S., Kumarasamy, R., Uthandi, S., 2016. High level secretion of Laccase (LccH) from a newly isolated white-rot Basidiomycete, Hexagonia hirta MSF2. Front. Microbiol. 7, 707. https://doi.org/10.3389/fmicb.2016.00707.
Kishimoto, T., Chiba, W., Saito, K., Fukushima, K., Uraki, Y., Ubukata, M., 2010. Influence of syringyl to guaiacyl ratio on the structure of natural and synthetic lignins. J. Agric. Food Chem. 58, 895–901. https://doi.org/10.1021/jf9035172.
Klein-Marcuschamer, D., Oleskowicz-Popiel, P., Simmons, B.A., Blanch, H.W., 2012. The challenge of enzyme cost in the production of lignocellulosic biofuels. Biotechnol. Bioeng. 109, 1083–1087. https://doi.org/10.1002/bit.24370.
Kudanga, T., Nyanhongo, G.S., Guebitz, G.M., Burton, S., 2011. Potential applications of laccase-mediated coupling and grafting reactions: a review. Enzym. Microb. Technol. 48, 195–208. https://doi.org/10.1016/j.enzmictec.2010.11.007.
Kumar, A., Chandra, R., 2020. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. Heliyon. 6, e03170 https://doi.org/10.1016/j.heliyon.2020.e03170.
Kumar, R., Kaur, J., Jain, S., Kumar, A., 2016. Optimization of laccase production from Aspergillus flavus by design of experiment technique: partial purification and characterization. J. Genet. Eng. Biotechnol. 14, 125–131. https://doi.org/10.1016/j.jgeb.2016.05.006.
Kumar, S.V.S., Phale, P.S., Durani, S., Wangikar, P.P., 2003. Combined sequence and structure analysis of the fungal laccase family. Biotechnol. Bioeng. 83, 386–394. https://doi.org/10.1002/bit.10681.
Lahive, C.W., Lancefield, C.S., Codina, A., Kamer, P.C.J., Westwood, N.J., 2018. Revealing the fate of the phenylcoumaran linkage during lignin oxidation reactions. Org. Biomol. Chem. 16, 1976–1982. https://doi.org/10.1039/c7ob03087h.
Langholtz, M.H., Stokes, B.J., Eaton, L.M., 2016. 2016 billion-ton report: advancing domestic resources for a thriving bioeconomy. Econom. Availab. Feedstocks. 1, 448.
Leynaud-Kieffer, L.M.C., Curran, S.C., Kim, I., Magnuson, J.K., Gladden, J.M., Baker, S.E., Simmons, B.A., 2019. A new approach to Cas9-based genome editing in Aspergillus Niger that is precise, efficient and selectable. PLoS One 14, e0210243. https://doi.org/10.1371/journal.pone.0210243.
Li, X., Chapple, C., 2010. Understanding lignification: challenges beyond monolignol biosynthesis. Plant Physiol. 154, 449–452. https://doi.org/10.1104/pp.110.162842.
Li, X., Ximenes, E., Kim, Y., Slininger, M., Meilan, R., Ladisch, M., Chapple, C., 2010. Lignin monomer composition affects Arabidopsis cell-wall degradability after liquid hot water pretreatment. Biotechnol. Biofuels. 3, 27. https://doi.org/10.1186/1754-6834-3-27.
Lippke, B., Puettmann, M.E., Johnson, L., Gustafson, R., Venditti, R., Steele, P., Katers, J. F., Katers, A., Volk, T.A., Oneil, E., Skog, K., Budsberg, E., Daystar, J., Caputo, J., 2012. Carbon emission reduction impacts from alternative biofuels. For. Prod. J. 62, 296.
Liu, Q., Luo, L., Zheng, L., 2018. Lignins: biosynthesis and biological functions in plants. Int. J. Mol. Sci. 19 https://doi.org/10.3390/ijms19020335.
Lourenço, A., Pereira, H., 2018. Compositional variability of lignin in biomass. In: Poletto, M. (Ed.), Lignin - Trends and Applications. InTech. https://doi.org/10.5772/intechopen.71208.
Martínez, A.T., Speranza, M., Ruiz-Dueñas, F.J., Ferreira, P., Camarero, S., Guillén, F., Martínez, M.J., Gutiérrez, A., del Río, J.C., 2005. Biodegradation of lignocellulosics: microbial, chemical, and enzymatic aspects of the fungal attack of lignin. Int. Microbiol. 8, 195–204.
Martínez, A.T., Ruiz-Dueñas, F.J., Camarero, S., Serrano, A., Linde, D., Lund, H., Vind, J., Tovborg, M., Herold-Majumdar, O.M., Hofrichter, M., Liers, C., Ullrich, R., Scheibner, K., Sannia, G., Piscitelli, A., Pezzella, C., Sener, M.E., Kılıç, S., van Berkel, W.J.H., Guallar, V., Alcalde, M., 2017. Oxidoreductases on their way to industrial biotransformations. Biotechnol. Adv. 35, 815–831. https://doi.org/10.1016/j.biotechadv.2017.06.003.
Mate, D.M., Alcalde, M., 2015. Laccase engineering: from rational design to directed evolution. Biotechnol. Adv. 33, 25–40. https://doi.org/10.1016/j.biotechadv.2014.12.007.
Mayolo-Deloisa, K., González-González, M., Rito-Palomares, M., 2020. Laccases in food industry: bioprocessing, potential industrial and biotechnological applications. Front. Bioeng. Biotechnol. 8, 222. https://doi.org/10.3389/fbioe.2020.00222.
Meents, M.J., Watanabe, Y., Samuels, A.L., 2018. The cell biology of secondary cell wall biosynthesis. Ann. Bot. 121, 1107–1125. https://doi.org/10.1093/aob/mcy005.
Mehra, R., Muschiol, J., Meyer, A.S., Kepp, K.P., 2018. A structural-chemical explanation of fungal laccase activity. Sci. Rep. 8, 17285. https://doi.org/10.1038/s41598-018-35633-8.
Michniewicz, A., Ullrich, R., Ledakowicz, S., Hofrichter, M., 2006. The white-rot fungus Cerrena unicolor strain 137 produces two laccase isoforms with different physico-chemical and catalytic properties. Appl. Microbiol. Biotechnol. 69, 682–688. https://doi.org/10.1007/s00253-005-0015-9.
Mielenz, J.R., 2001. Ethanol production from biomass: technology and commercialization status. Curr. Opin. Microbiol. 4, 324–329. https://doi.org/10.1016/S1369-5274(00)00211-3.
Minussi, R.C., Pastore, G.M., Durán, N., 2002. Potential applications of laccase in the food industry. Trends Food Sci. Technol. 13, 205–216. https://doi.org/10.1016/S0924-2244(02)00155-3.
Minussi, R.C., Rossi, M., Bologna, L., Rotilio, D., Pastore, G.M., Durán, N., 2007. Phenols removal in musts: strategy for wine stabilization by laccase. J. Mol. Catal. B Enzym. 45, 102–107. https://doi.org/10.1016/j.molcatb.2006.12.004.
Moreau, C., Tapin-Lingua, S., Grisel, S., Gimbert, I., Le Gall, S., Meyer, V., Petit-Conil, M., Berrin, J.-G., Cathala, B., Villares, A., 2019. Lytic polysaccharide monooxygenases (LPMOs) facilitate cellulose nanofibrils production. Biotechnol. Biofuels. 12, 156. https://doi.org/10.1186/s13068-019-1501-0.
Morozova, O.V., Shumakovich, G.P., Shleev, S.V., Yaropolov, Ya.I., 2007. Laccase-mediator systems and their applications: a review. Appl. Biochem. Microbiol. 43, 523–535. https://doi.org/10.1134/S0003683807050055.
Mukhopadhyay, A., 2015. Tolerance engineering in bacteria for the production of advanced biofuels and chemicals. Trends Microbiol. 23, 498–508. https://doi.org/10.1016/j.tim.2015.04.008.
Murphy, M.E., Lindley, P.F., Adman, E.T., 1997. Structural comparison of cupredoxin domains: domain recycling to construct proteins with novel functions. Protein Sci. 6, 761–770. https://doi.org/10.1002/pro.5560060402.
Naik, S.N., Goud, V.V., Rout, P.K., Dalai, A.K., 2010. Production of first and second generation biofuels: a comprehensive review. Renew. Sust. Energ. Rev. 14, 578–597. https://doi.org/10.1016/j.rser.2009.10.003.
Navada, K.K., Kulal, A., 2019. Enzymatic degradation of chloramphenicol by laccase from Trametes hirsuta and comparison among mediators. Int. Biodeterior. Biodegradation 138, 63–69. https://doi.org/10.1016/j.ibiod.2018.12.012.
Navarra, C., Goodwin, C., Burton, S., Danieli, B., Riva, S., 2010. Laccase-mediated oxidation of phenolic derivatives. J. Mol. Catal. B Enzym. 65, 52–57. https://doi.org/10.1016/j.molcatb.2009.12.016.
Nikel, P.I., Martínez-García, E., de Lorenzo, V., 2014. Biotechnological domestication of pseudomonads using synthetic biology. Nat. Rev. Microbiol. 12, 368–379. https://doi.org/10.1038/nrmicro3253.
Niño-Medina, G., Gutiérrez-Soto, G., Urías-Orona, V., Hernández-Luna, C.E., 2017. Effect of laccase from Trametes maxima CU1 on physicochemical quality of bread. Cogent Food Agric. 3 https://doi.org/10.1080/23311932.2017.1328762.
Novoa, C., Dhoke, G.V., Mate, D.M., Martínez, R., Haarmann, T., Schreiter, M., Eidner, J., Schwerdtfeger, R., Lorenz, P., Davari, M.D., Jakob, F., Schwaneberg, U., 2019. KnowVolution of a fungal Laccase toward alkaline pH. Chembiochem. 20, 1458–1466. https://doi.org/10.1002/cbic.201800807.
Ottenheim, C., Verdejo, C., Zimmermann, W., Wu, J.C., 2014. Hemicellulase production by Aspergillus Niger DSM 26641 in hydrothermal palm oil empty fruit bunch hydrolysate and transcriptome analysis. J. Biosci. Bioeng. 118, 696–701. https://doi.org/10.1016/j.jbiosc.2014.05.014.
Pardo, I., Camarero, S., 2015. Exploring the oxidation of lignin-derived phenols by a library of Laccase mutants. Molecules. 20, 15929–15943. https://doi.org/10.3390/molecules200915929.
Pérez, J., Muñoz-Dorado, J., de la Rubia, T., Martínez, J., 2002. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. Int. Microbiol. 5, 53–63. https://doi.org/10.1007/s10123-002-0062-3.
Picart, P., de María, P.D., Schallmey, A., 2015. From gene to biorefinery: microbial β-etherases as promising biocatalysts for lignin valorization. Front. Microbiol. 6, 916. https://doi.org/10.3389/fmicb.2015.00916.
Piscitelli, A., Pezzella, C., Giardina, P., Faraco, V., Giovanni, S., 2010. Heterologous laccase production and its role in industrial applications. Bioeng. Bugs. 1, 252–262. https://doi.org/10.4161/bbug.1.4.11438.
Potthast, A., Rosenau, T., Fischer, K., 2001. Oxidation of benzyl alcohols by the Laccase-mediator system (LMS) a comprehensive kinetic description. Holzforschung. 55, 47–56. https://doi.org/10.1515/HF.2001.008.
Ragauskas, A.J., Beckham, G.T., Biddy, M.J., Chandra, R., Chen, F., Davis, M.F., Davison, B.H., Dixon, R.A., Gilna, P., Keller, M., Langan, P., Naskar, A.K., Saddler, J. N., Tschaplinski, T.J., Tuskan, G.A., Wyman, C.E., 2014. Lignin valorization: improving lignin processing in the biorefinery. Science. 344, 1246843. https://doi.org/10.1126/science.1246843.
Rodríguez-Delgado, M.M., Alemán-Nava, G.S., Rodríguez-Delgado, J.M., Dieck-Assad, G., Martínez-Chapa, S.O., Barceló, D., Parra, R., 2015. Laccase-based biosensors for detection of phenolic compounds. TrAC Trends Anal. Chem. 74, 21–45. https://doi.org/10.1016/j.trac.2015.05.008.
Salvachúa, D., Karp, E.M., Nimlos, C.T., Vardon, D.R., Beckham, G.T., 2015. Towards lignin consolidated bioprocessing: simultaneous lignin depolymerization and product generation by bacteria. Green Chem. 17, 4951–4967. https://doi.org/10.1039/C5GC01165E.
Sigoillot, J.-C., Berrin, J.-G., Bey, M., Lesage-Meessen, L., Levasseur, A., Lomascolo, A., Record, E., Uzan-Boukhris, E., 2012. Fungal Strategies for Lignin Degradation, in: Lignins - Biosynthesis, Biodegradation and Bioengineering. Elsevier, pp. 263–308. https://doi.org/10.1016/B978-0-12-416023-1.00008-2.

Simmons, B.A., Loqué, D., Ralph, J., 2010. Advances in modifying lignin for enhanced biofuel production. Curr. Opin. Plant Biol. 13, 313–320. https://doi.org/10.1016/j.pbi.2010.03.001.

Singh, A.K., Chandra, R., 2019. Pollutants released from the pulp paper industry: aquatic toxicity and their health hazards. Aquat. Toxicol. 211, 202–216. https://doi.org/10.1016/j.aquatox.2019.04.007.

Singh Arora, D., Kumar Sharma, R., 2010. Ligninolytic fungal laccases and their biotechnological applications. Appl. Biochem. Biotechnol. 160, 1760–1788. https://doi.org/10.1007/s12010-009-8676-y.

Singh, G., Arya, S.K., 2019. Utility of laccase in pulp and paper industry: a progressive step towards the green technology. Int. J. Biol. Macromol. 134, 1070–1084. https://doi.org/10.1016/j.ijbiomac.2019.05.168.

Singh, G., Bhalla, A., Kaur, P., Capalash, N., Sharma, P., 2011. Laccase from prokaryotes: a new source for an old enzyme. Rev. Environ. Sci. Biotechnol. 10, 309–326. https://doi.org/10.1007/s11157-011-9257-4.

Singh, J., Saharan, V., Kumar, S., Gulati, P., Kapoor, R.K., 2018. Laccase grafted membranes for advanced water filtration systems: a green approach to water purification technology. Crit. Rev. Biotechnol. 38, 883–901. https://doi.org/10.1080/07388551.2017.1417234.

Singh, S., Simmons, B.A., Vogel, K.P., 2009. Visualization of biomass solubilization and cellulose regeneration during ionic liquid pretreatment of switchgrass. Biotechnol. Bioeng. 104, 68–75. https://doi.org/10.1002/bit.22386.

Socha, A.M., Parthasarathi, R., Shi, J., Pattathil, S., Whyte, D., Bergeron, M., George, A., Tran, K., Stavila, V., Venkatachalam, S., Hahn, M.G., Simmons, B.A., Singh, S., 2014. Efficient biomass pretreatment using ionic liquids derived from lignin and hemicellulose. Proc. Natl. Acad. Sci. U. S. A. 111, E3587–E3595. https://doi.org/10.1073/pnas.1405685111.

Solomon, E.I., Sundaram, U.M., Machonkin, T.E., 1996. Multicopper oxidases and oxygenases. Chem. Rev. 96, 2563–2606. https://doi.org/10.1021/cr9500460.

Ståhl, M., Nieminen, K., Sixta, H., 2018. Hydrothermolysis of pine wood. Biomass Bioenergy 109, 100–113. https://doi.org/10.1016/j.biombioe.2017.12.006.

Stanzione, I., Pezzella, C., Giardina, P., Sannia, G., Piscitelli, A., 2020. Beyond natural laccases: extension of their potential applications by protein engineering. Appl. Microbiol. Biotechnol. 104, 915–924. https://doi.org/10.1007/s00253-019-10147-z.

Stewart, J.J., Akiyama, T., Chapple, C., Ralph, J., Mansfield, S.D., 2009. The effects on lignin structure of overexpression of ferulate 5-hydroxylase in hybrid poplar. Plant Physiol. 150, 621–635. https://doi.org/10.1104/pp.109.137059.

Sun, N., Rahman, M., Qin, Y., Maxim, M.L., Rodríguez, H., Rogers, R.D., 2009. Complete dissolution and partial delignification of wood in the ionic liquid 1-ethyl-3-methylimidazolium acetate. Green Chem. 11, 646. https://doi.org/10.1039/b822702k.

Szczodrak, J., Llczuk, Z., Rogalski, J., Leonowicz, A., 1986. Intensification of oak sawdust enzymatic hydrolysis by chemical or hydrothermal pretreatment. Biotechnol. Bioeng. 28, 504–510. https://doi.org/10.1002/bit.260280406.

Tang, J., Song, H., Feng, X., Yohannes, A., Yao, S., 2019. Ionic liquid-like pharmaceutical ingredients and applications of ionic liquids in medicinal chemistry: development, status and prospects. Curr. Med. Chem. 26, 5947–5967. https://doi.org/10.2174/0929867325666180605123436.

Thurston, C.F., 1994. The structure and function of fungal laccases. Microbiology 26, 5847–5967.

Tobimatsu, Y., Schuetz, M., 2019. Lignin polymerization: how do plants manage the chemistry so well? Curr. Opin. Biotechnol. 56, 75–81. https://doi.org/10.1016/j.copbio.2018.10.001.

Tolesa, L.D., Gupta, B.S., Lee, M.-J., 2019. Degradation of lignin with aqueous ammonium-based ionic liquid solutions under milder conditions. New J. Chem. 43, 3357–3365. https://doi.org/10.1039/C8NJ05185B.

Vasdev, K., Dhawan, S., Kapoor, R.K., Kuhad, R.C., 2005. Biochemical characterization and molecular evidence of a laccase from the bird's nest fungus Cyathus bulleri. Fungal Genet. Biol. 42, 684–693. https://doi.org/10.1016/j.fgb.2005.03.013.

Verma, S.R., Dwivedi, U.N., 2014. Lignin genetic engineering for improvement of wood quality: applications in paper and textile industries, fodder and bioenergy production. S. Afr. J. Bot. 91, 107–125. https://doi.org/10.1016/j.sajb.2014.01.002.

Virk, A.P., Sharma, P., Capalash, N., 2012. Use of laccase in pulp and paper industry. Biotechnol. Prog. 28, 21–32. https://doi.org/10.1002/btpr.727.

Walden, P., 1914. Ueber die Molekulargrösse und elektrischeLeitfähigkeit einiger geschmolzenen Salze. Bull. l'Académie Impériale Des Sci. St. Pétersbourg. 8, 405–422.

Wang, B., Wang, X., Tian, Y., Li, Z., Gao, J., Yan, Y., Peng, R., Yao, Q., 2016. Heterologous expression and characterization of a laccase from*Laccaria bicolor* in*Pichia pastoris*. Biotechnol. Biotechnol. Equip. 30, 63–68. https://doi.org/10.1080/13102818.2015.1104261.

Wang, X., Yao, B., Su, X., 2018. Linking enzymatic oxidative degradation of lignin to organics detoxification. Int. J. Mol. Sci. 19 https://doi.org/10.3390/ijms19113373.

Welker, C., Balasubramanian, V., Petti, C., Rai, K., DeBolt, S., Mendu, V., 2015. Engineering plant biomass lignin content and composition for biofuels and bioproducts. Energies. 8, 7654–7676. https://doi.org/10.3390/en8087654.

Welton, T., 2018. Ionic liquids: a brief history. Biophys. Rev. 10, 691–706. https://doi.org/10.1007/s12551-018-0419-2.

Wong, D.W.S., 2009. Structure and action mechanism of ligninolytic enzymes. Appl. Biochem. Biotechnol. 157, 174–209. https://doi.org/10.1007/s12010-008-8279-z.

Wyman, C.E., 2003. Potential synergies and challenges in refining cellulosic biomass to fuels, chemicals, and power. Biotechnol. Prog. 19, 254–262. https://doi.org/10.1021/bp025654l.

Xu, F., 1997. Effects of redox potential and hydroxide inhibition on the pH activity profile of fungal laccases. J. Biol. Chem. 272, 924–928. https://doi.org/10.1074/jbc.272.2.924.

Xu, F., Palmer, A.E., Yaver, D.S., Berka, R.M., Gambetta, G.A., Brown, S.H., Solomon, E. I., 1999. Targeted mutations in a Trametes villosa laccase. Axial perturbations of the T1 copper. J. Biol. Chem. 274, 12372–12375. https://doi.org/10.1074/jbc.274.18.12372.

Xu, Z., Lei, P., Zhai, R., Wen, Z., Jin, M., 2019. Recent advances in lignin valorization with bacterial cultures: microorganisms, metabolic pathways, and bio-products. Biotechnol. Biofuels. 12, 32. https://doi.org/10.1186/s13068-019-1376-0.

Yang, J., Li, W., Ng, T.B., Deng, X., Lin, J., Ye, X., 2017. Laccases: production, expression regulation, and applications in pharmaceutical biodegradation. Front. Microbiol. 8, 832. https://doi.org/10.3389/fmicb.2017.00832.

Yin, Q., Zhou, G., Peng, C., Zhang, Y., Kües, U., Liu, J., Xiao, Y., Fang, Z., 2019. The first fungal laccase with an alkaline pH optimum obtained by directed evolution and its application in indigo dye decolorization. AMB Express 9, 151. https://doi.org/10.1186/s13568-019-0878-2.

Yoo, E.-H., Lee, S.-Y., 2010. Glucose biosensors: an overview of use in clinical practice. Sensors. 10, 4558–4576. https://doi.org/10.3390/s100504558.

Youn, H.-D., Hah, Y.C., Kang, S.-O., 1995. Role of laccase in lignin degradation by white-rot fungi. FEMS Microbiol. Lett. 132, 183–188. https://doi.org/10.1111/j.1574-6968.1995.tb07831.x.

Zerva, A., Koutroufini, E., Kostopoulou, I., Detsi, A., Topakas, E., 2019. A novel thermophilic laccase-like multicopper oxidase from Thermothelomyces thermophila and its application in the oxidative cyclization of 2′,3,4-trihydroxychalcone. New Biotechnol. 49, 10–18. https://doi.org/10.1016/j.nbt.2018.12.001.

Zhang, J., Wu, J., Yu, J., Zhang, X., He, J., Zhang, J., 2017. Application of ionic liquids for dissolving cellulose and fabricating cellulose-based materials: state of the art and future trends. Mater. Chem. Front. 1, 1273–1290. https://doi.org/10.1039/C6QM00348F.

Zhang, Y., Lv, Z., Zhou, J., Xin, F., Ma, J., Wu, H., Fang, Y., Jiang, M., Dong, W., 2018. Application of eukaryotic and prokaryotic laccases in biosensor and biofuel cells: recent advances and electrochemical aspects. Appl. Microbiol. Biotechnol. 102, 10409–10423. https://doi.org/10.1007/s00253-018-9421-7.

Zumárraga, M., Bulter, T., Shleev, S., Polaina, J., Martínez-Arias, A., Plou, F.J., Ballesteros, A., Alcalde, M., 2007. In vitro evolution of a fungal laccase in high concentrations of organic cosolvents. Chem. Biol. 14, 1052–1064. https://doi.org/10.1016/j.chembiol.2007.08.010.