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Review

Recent Topics of Laccase Focused on Chemical Reactions and Applications

Takashiro Akitsu*, Daisuke Nakane

Department of Chemistry, Faculty of Science, Tokyo University of Science, 1-3 Kagurzaka, Shinjuku-ku, Tokyo 162-8601, Japan
E-mail: akitsu2@rs.tus.ac.jp

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Abstract: Green chemistry elements should deal with enzymatic chemical reactions that are efficient or have a low environmental impact. Laccase is a readily available multicopper oxidase that has been extensively studied for a long time. In reviewing recent trends in laccase research, the authors selected themes that have not yet been evaluated, targeting open-access journals. From a chemical viewpoint, decomposition reactions (such as oxidation of substrates, toxic substances, pigments, and biomass materials), organic synthesis reactions, composite catalytic activities of oxygen reduction reactions, biosensors, batteries, biological fields, fermentation, culture, gene association, and antifungal activity.

***Keywords*:** laccase, reaction, substrate, selectivity

1. Introduction

An important element of green chemistry is enzymatic chemical reactions that are efficient or have a low environmental impact. Laccase is a readily and commonly available multicopper oxidase [1] (composed of mononuclear type 1 copper sites for the oxidation of substrates, trinuclear type 2, and type 3 copper sites for oxygen reduction) that has been extensively studied in the fields of degradation reactions (oxidation of substrates and harmful substances), organic synthesis reactions, oxygen reduction reactions, genetics, and biology [2–6]. Laccase is a member of the multicopper oxidase family composed of a single subunit, including copper ions, which receives electrons from substrates (typical of lignin) and moves to the trinuclear copper site on which oxygen is reduced to water (Figure 1). Laccase is used for decomposing organic dyes or lignin, organic reactions, oxygen sensing, and biofuel cells.

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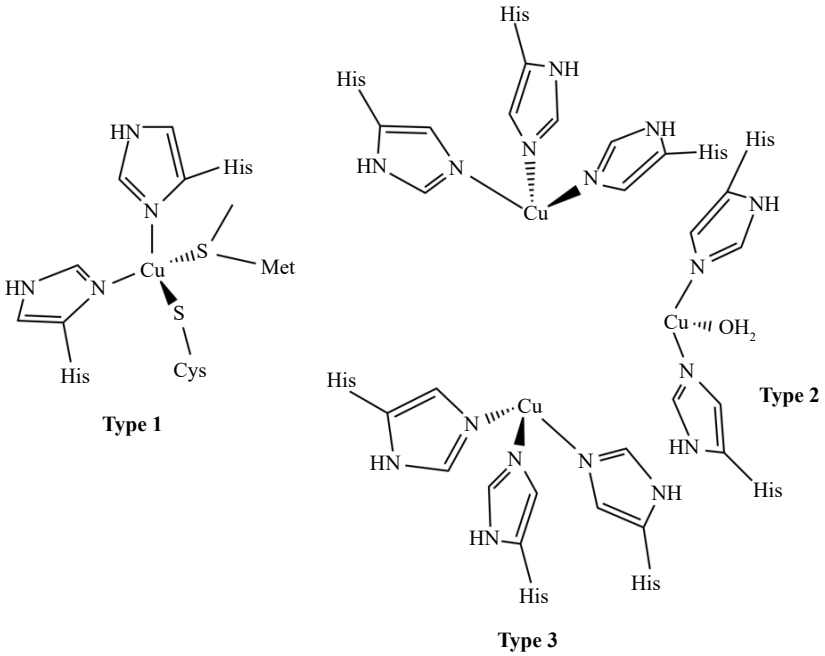

Figure 1. Type 1 and type 2 / type 3 (some (bridging) ligands are omitted) copper site structures of laccase.

Owing to various viewpoints and targets, we have focused on open-access journals and selected topics that have not yet been evaluated to conduct a new review of recent trends in laccase research. In addition to the introduction of research examples, comments focusing on substrate selectivity and selectivity for related adducts are made for characteristic ones. Molecular recognition and molecular orientation of the docking compounds were important in our "challenging" studies of metal-complex mediators for cathodes of biofuel cells, including, laccases, which catalyze oxygen reduction [7]. Alternatively, it was based on chiral oligopeptide ligation with gold electrodes and a mediator design [8]. Thus, these studies were characterized in terms of the anisotropy and symmetry of nanocomposites.

2. Basic Enzymatic Activity and Inhibition

The enzymatic activity of laccase may occur on the surface, the so-called hydrophobic pocket around the type 1 copper site, and the inner molecule at the type 2 and 3 sites. The selectivity should be different depending on the mechanism of the electron transfer distance or molecular recognition (including the possibility of accessing the active sites). Regarding enzymatic activity, Maniak *et al*. [9] screened a series of hydrazide compounds as laccase inhibitors from Versicolor. Hydrazides with a benzene ring and imine derivatives of aldehydes were effective inhibitors, and kinetic and molecular modeling studies were performed. The docking results for the selected compounds are also discussed.

Dettori *et al*. [10] reported tyrosinase and laccase biosensors for the IC50 of protein-ligand interactions. They prepared reversible inhibitors of tyrosinase and laccase with phenylpropanoid and hydroxylated biphenyl cores and measured their activities using spectrophotometric and electrochemical assays. In this context, research on their inhibitors could lead to the discovery of skin-lightening agents, pharmaceuticals, anti-tanning agents, and compounds for controlling harmful bacteria and insects.

3. Microfluidics

The environment of the solutions in which the laccase molecules are placed may also be anisotropically distinguishable when the solvent exhibits unidirectional flow or laminar separation. Recent developments in microfluidic systems have provided a rapid and precise method to generate monodisperse microcapsules for multiple applications. Campaña *et al*. [11] reviewed the design, fabrication, and characterization of an inexpensive microsystem for encapsulating fungal laccase from *Pycnoporus sanguineus CS43* in *Alginate microcapsules*. Simulation results overviewed the fluid behavior within the device and estimated the resulting capsule size for microcapsule production. Sheets of polymethyl-methacrylate were used to fabricate the final microsystems.

4. Biosensor

The biosensor is premised to contain a selective and distinct reaction with the chemical species to be detected. Because recent biosensors are fabricated by combining other materials, in addition to being used alone, the environment in which key enzymes can be placed are often restricted. Lulea *et al*. [12] measured laccase activity of oxygen-related species using spectroelectrochemical methods. A paper sensor impregnated with the enzyme substrate dye 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), a typical oxygen-sensitive colored indicator of radical species for laccase activity, provides semi-quantitative optical measurements. Although the paper sensor can be used alone, it allows for quantitative detection when combined with screen-printed electrodes and amperometric measurements.

Baluta *et al*. [13] investigated the electrochemical sensing detection of neurotransmitters using laccase/horseradish peroxidase-modified Pt/Au electrodes coated with poly(2,6-bis(3,4-ethylenedioxythiophene)-4-methyl-4-octyl-dithienosilole), silole derivatives, and immobilized laccase. This method has been successfully applied for the measurement of neurotransmitters in the presence of ascorbic acid, L-cysteine, and uric acid, which are interfering compounds.

Boubezari *et al*. [14] first fabricated a laccase-based biosensor by encapsulating laccase in a composite of chitosan and galactomannan. The resulting laccase-based biosensor (kept for two weeks) was an alternative to the colorimetric Folin-Ciocalteu method for estimating the antioxidant capacity of olive oil samples.

Salvo-Comino *et al*. [15] developed a biosensor platform consisting of layer-by-layer films that combine materials with different functions, which was used to obtain improved catechol biosensors. Laccase was used to form a layer-by-layer film with alternating layers of cationic linkers (chitosan) and anionic electrocatalysts, such as gold nanoparticles and sulfonated copper phthalocyanine, deposited on top. Films with different layer structures were well formed. The layer-by-layer composite efficiently improved the electron transfer pathway between laccase and the surface of the electrodes, resulting in increased intensity over the response without the layer-by-layer platform.

Baluta *et al*. [16] investigated a novel fluorescence sensing mechanism for epinephrine detection using a ceramic-based miniature biosensor involving laccase or tyrosinase immobilized on a semiconducting matrix, poly-(2,6-di([2,2'-bithiophen]-5-yl)-4-(5)-hexylthiophen-2-yl)pyridine). The detection method is based on the oxidation of the substrates. Under optimized conditions, the analysis showed good sensitivity and selectivity with a broad linear range of detection limits.

5. Biofuel Cells

Enzymatic biofuel cells are a promising green source because they can recover power from renewable and abundantly available biofuels by using enzymes as catalysts. Several studies have focused on using complex three-dimensional and expensive nanostructures as electrode supports for enzymes. Pelosi *et al*. [17] analyzed a novel flow-based biofuel cell consisting of covalently immobilized (two-dimensional material) (graphene oxide) GOx (bioanode) and laccase (biocathode) on a commercially available flat conducting polymer. Appropriate immobilization techniques based on covalent ligands have been implemented to increase the durability of enzymatic biofuel cells (Figure 2).

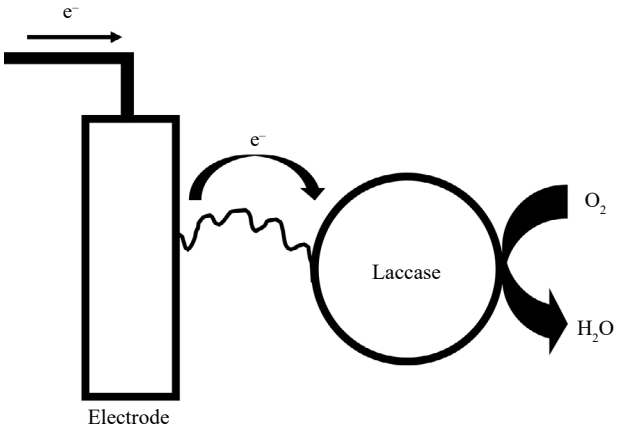

Figure 2. Tentative processes involved in laccase-immobilized bcathode of biofuel cell.

Bojang *et al*. [18] investigated the quantitative results of the electrochemistry of glucose with glucosidase, oxygen, and laccase in pH 7.0 phosphate buffer. Electrochemical analyses can be used to understand better the redox processes, charge transfer resistance, and potential mass transfer between electrode materials in phosphate buffers. The occurrence of peak separation indicates good mass transfer mechanisms and chemical reactivity within the electrode.

6. Catalysts Stabilized or Supported in Composite Materials

Enzyme immobilization and co-immobilization, used in biomass conversion, are important for catalysts that require chemical or heat stress tolerance. Thus, stabilizing or supporting enzymes (also hydrogels, see polymer decomposition and hydrogels) by composite materials may be essential treatments [19]. Enzymes are an environmentally friendly alternative for biomass processing. These enzymes, such as ligninolytic enzymes (for example, laccases and peroxidases), have excellent substrate specificity and can be reused when immobilized on magnetic nanocarriers. Magnetic nanoparticle carriers bound with laccase have the advantages of immobilizing fragile enzyme molecules and controlling separation by external operation of magnets.

Mariño *et al*. [20] reviewed the performance of biocatalysts after the formation of covalent bonds or adsorption onto magnetic nanoparticles with different functions. Functionalization strategies for these materials include silica-based surfaces prepared from sol-gel method, graphene oxide-based nanocomposites, polymer-coated surfaces, and grafted polymer brushes.

Peñaranda e*t al*. [21] investigated the design, fabrication, and testing of three magnetic microreactors based on torus geometries to increase enzyme-based conversion. The laccase bio-nanocomposite improves the dye-particle suspension and facilitates reagent interactions. The laccase enzyme was covalently immobilized on amino-terminated silanated magnetite nanoparticles (laccase magnetite) under a magnetic field, and phenol oxidation and removal were evaluated in artificial and actual wastewater.

Sotelo *et al*. tested five magnetic biofilters, including magnetic nanoparticles (142 nm), laccase (190 nm) immobilized on the nanoparticles, and permanent magnetic elements (such as neodymium magnets and metal meshes) [22]. These filters were compared by measuring the decolorization of Congo Red dye in the bioreactor, the filter half-life, and the number of magnetic nanoparticles and enzymes lost during multiple operating cycles. Filters containing laccase immobilized on magnetite, permanent magnets, and metal mesh had the highest Congo Red decolorization and longest half-life.

Metallic nanoparticles potentially have a wide range of practical applications in various industries and basic science fields. Biosensors usually function as catalysts or nanoenzymes and mediators of electron transfer. Stasyuk et al. [23] described the development of amperometric biosensors based on purified oxidases, synthetic nanoparticles of CuCe, and nanoporous gold, which were electrodeposited onto graphite electrodes. Synthetic nanoparticles of CuCe functioned an electroactive mediator and were used in laccase-based amperometric biosensors.

Dencheva *et al*. [24] reported a new method of enzyme immobilization based on passive immobilization of highly porous particles of neat magnetically responsive polyamide 4 (PA4). Laccase from Versicolor was immobilized by adsorption onto prefabricated PA4 microparticles. The activity of each conjugate was determined using complementary spectra and enzymatic activity measurements. They also reported that magnetic PA6 microparticles with immobilized laccase exhibit high performance in catechol reactions [25].

Santos *et al*. reported an amperometric biosensor of carbon paste electrodes modified with laccase enzyme, glutaraldehyde, and gold nanoparticles to measure the neurotransmitter dopamine [26]. The proposed biosensor was successfully applied to the measurement of dopamine in biological and environmental samples.

Rutin is a flavonoid glycoside that is primarily transported through the circulatory system of the human body. Monitoring the blood levels of rutin is crucial in many fields, including pharmacology and pharmacokinetics. Song et al. constructed biosensors for multi-walled carbon nanotubes (MWCNTs), cetyltrimethylammonium bromide (CTAB), hydroxyl fullerenes, and laccase nanocomposite-modified glassy carbon electrodes [27]. CTAB was used to disperse MWCNTs and improve their hydrophilicity and biocompatibility, while laccase promoted the oxidation of the catechol structure of rutin. Therefore, the sensitivity and selectivity of the modified electrode are significantly enhanced. This biosensor has practical value in medical fields, such as quality testing of rutin drug and monitoring of the clinical blood drug concentration.

Sorrentino *et al*. [28] functionalized few-layer graphene exfoliated from graphite in the presence of hydrophobin using a chimeric enzyme based on the gene fusion of laccase and hydrophobin domains. The as-produced biofunctionalized few-layer graphene was used for the biosensing of phenols (dopamine and catechol). This strategy paves the way for the functionalization of nanomaterials with the hydrophobin domains of chimeric enzymes and their use in various electrochemical applications.

Rouhani *et al*. reported the green synthesis of sulfonamides involving the immobilization of laccase from *Trametes versicolor* onto Fe3O4-graphene nanocomposites via glutaraldehyde cross-linking (laccase/Fe3O4/GO). [29] The performance of nanobiocatalysts significantly exceeded that of free laccases in terms of the stability of immobilized laccases to temperature and storage conditions. Moreover, the nanobiocatalyst exhibited better recycling performance, as evidenced by the observation that it retained 85% of its original activity after eight cycles of repeated use.

The analysis of antioxidants in various foods has become an active research area, leading to the development of several antioxidant assays. Because many antioxidants exhibit intrinsic electroactivity, the use of electrochemical methods are feasible for assessing the overall antioxidant activity of nutraceutical matrices without adding reactive species, can be an approach. Munteanu *et al*. [30] reported on the electrochemical properties of three screen-printed electrodes. The second method is based on gold nanoparticles (GNP), and the third method is based on carbon nanotube (CNT) and gold nanoparticles (CNT-GNPs). All three electrodes were modified with laccase enzyme using glutaraldehyde as a cross-linker between the amino groups of laccase and aldehyde groups of the reticulating agent. Because this enzyme is a thermostable catalyst, it significantly improves the performance of the biosensor. The electrooxidative properties of the catechins were also investigated using cyclic voltammetry.

In particular, laccases have attracted attention as efficient and promising catalysts for phenol degradation; therefore, the facile construction of functional nanomaterials with laccase-like activity is important in green and sustainable chemistry. Lei *et al*. [31] reported a facile method for synthesizing nanoenzymes with enhanced laccase-like activity through the self-assembly of copper ions with various imidazole-related compounds. They are believed to have important applications in environmental protection and pollutant detection.

Wang *et al*. [32] investigated the removal of a triphenylmethane dye by a simultaneous enzymatic photocatalytic adsorption treatment. A desirable synergistic effect for dye treatment was achieved by modifying the surface of TiO2 sol-gel-coated polyacrylonitrile/organically modified montmorillonite nanofibers prepared via electrospinning with laccase. Compared to free laccase, the immobilized laccase exhibited a higher degradation efficiency for the initial dye. Under UV illumination, the removal efficiency was further improved, and it had the potential for industrial dye degradation owing to the combined effect of immobilized enzymes and polymer supports.

Zdarta *et al*. [33] reported that a three-dimensional chitin scaffold from the sponge *Aplysina archeri* was used for the immobilization and adsorption of laccase from *Trametes versicolor*. The resulting chitin-enzyme biocatalytic system was used to remove tetracycline. The storage and thermal stability of the immobilized biomolecules were considerably improved compared with those of the free enzyme. The resulting biocatalytic system also exhibited good reusability.

7. Conversion of Compounds -Organic synthesis

New sustainable processes involving oxidase catalysis are considered alternatives to classical organic chemistry. With the growing demand for effective and new compound conversions, intelligent strategies are required to obtain a wide range of potential candidates. Laccase-catalyzed reactions have been successfully applied to synthesizing new organic compounds. In many cases, the converted organic compounds are substrates for laccase to oxidase by removing electrons. Therefore, in some cases, docking substrates around type 1 sites of the laccase surface (for a suitable situation of electron transfer) may be important for this purpose (or antibacterial activity). Mikolasch *et al*. [34] employed laccases of three different origins to generate new aminoglycoside antibiotics. Kanamycin, tobramycin, and gentamicin bind the laccase substrate 2,5-dihydroxy-N-(2-hydroxyethyl)-benzamide. The product was isolated, characterized, and investigated in vitro for antibacterial activity against various strains of Staphylococcus, including multidrug-resistant strains.

Latos-Brozio *et al*. [35] reported polymeric forms of plant flavonoids obtained by laccase enzymatic reactions. The antioxidant and antibacterial properties of flavonoids depend on their structure and polymer morphology. Naringenin is a flavonoid derived from citrus fruit, and this polyphenol is mainly found in grapefruit, oranges, and lemons. Polymerization was conducted using two enzymatic methods: laccase and horseradish peroxidase.

The unique physicochemical and bioactive properties of novel bioproducts can be obtained using fungal laccases as catalysts. Polak *et al*. studied the structure and properties of three new phenazine dyes, which were obtained from Cerrena unicolor in the presence of laccase [36]. The phenazine core structure of the product resulted from the trimolecular transformation of aminomethoxybenzoic acid and aminonaphthalenesulfonic acid isomers. The growth of Staphylococcus aureus was inhibited by 10-((2-carboxy-6-methoxyphenyl)amino)-11-methoxybenzo[α]phenazine-8-carboxylic acid, which is a compound from synthetic pigments. is ready. The new dyes showed excellent bioactivity, staining properties, antibacterial activity, and antioxidant activity.

8. Mediator-Involving Organic Reactions

In addition to the electron transfer between the electrode and laccase in biofuel cells mentioned in the Introduction, mediator-involving organic reactions may be crucial, in which the steric selectivity of substrates is relatively less important. Techniques to reduce or eliminate the toxicity of deoxynivalenol can be useful in many processes, such as maintaining the animal feed value of the byproducts of ethanol production. Shanakhat et al. used a combination of fungal laccase paired with 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) as a chemical mediator [37]. The alcohol (–OH) groups at the C3 and C15 positions of deoxynivalenol were oxidized to ketones, and this mediator was covalently attached to the C4 position (Figure 3).

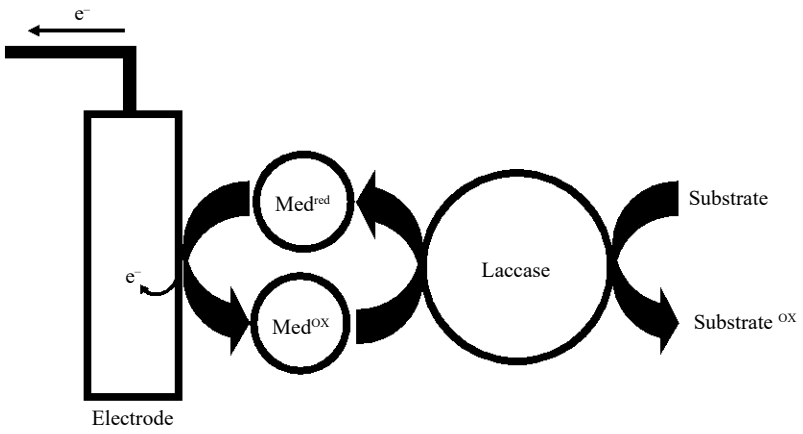

Figure 3. Tentative processes involved in mediator-supported substrate oxidizing system.

Laccase and mediator systems exhibit high scalability and productivity. The specific oxidation of the 12α-OH group of hydroxysteroids is required to synthesize keno- and ursodeoxycholic acids. Tonin *et al*. [38] compared the performances of laccase and mediator systems with those of classical and other regeneration systems in previous publications.

Overproduction of eumelanin results in a panel of unaesthetic hyperpigmented skin disorders such as melasma and blemishes. The treatment of these disorders occasionally requires the use of tyrosinase inhibitors. Tyrosinase inhibitors act as skin-lightening agents by inhibiting the preparation of eumelanin with adverse side effects. The researchers of this study reported efficient degradation of eumelanin from *Sepia officinalis*, offering an alternative procedure to traditional skin-lightening agents. Redox-active mediators exhibit synergistic effects over their single-mediator counterparts, highlighting the beneficial role of the cocktail system. The most effective laccase and cocktail system was sequentially applied to a two-component prototype of a topical whitening cream, exhibiting high degradative effects on eumelanin, as reported by Gigli *et al*. [39].

In order for maximizing the potential of biomass, Gitsov et al. [40] summarized a review about commodity monomers (not only alkene, diene, aromatic ones but also renewable resources, such as cellulose, lignin, plant oils, starches, and monoterpenes) and polymers for commercial products. Metal catalysts including metalloenzymes are important in synthesis strategies for ethylene, propylene, α-olefins, methylmethacrylate, 1,3-butadiene, 1,3-cyclohexadiene, isoprene, 1,3-propanediol, 1,4-butanediol, and terephthalic acid. Moreover, opportunities for other renewable-based monomers should be discussed from the view point of green chemistry.

Longe *et al*. [41] stated that mechanochemical treatments of biomass compounds were important in green chemistry. Typical example may be glucose and cellobiose from cellulose ball milling. They indicated that decrease in glycosidic linkage content and retention of N-acetyl groups in the mechanochemical treatments with boll mill using powder X-ray diffraction, in which clay played an important role in enhancing solid state reactions.

9. Decomposition or Degradation of Organic Compounds

In contrast, we focused on decomposition reactions instead of organic synthesis. Only a few studies (for example, [42, 43]) have investigated binding sites. Aflatoxins, which are widely found in feed and food products, can be harmful to human and animal health owing to their high toxicity. In this study [44], Bacillus amyloliquefaciens B10 was screened for its ability to degrade aflatoxin B1 (AFB1). Mutation of the three key metal-binding sites in B10 laccase abolished the degrading activity of AFB1, indicating that these three metal-binding sites play an important role in the catalytic degradation of AFB1. Among the many industrial catalytic proteins, fungal laccases are excellent and versatile catalytic oxidants that require only oxygen molecules, making them ideal candidates for biotechnological applications. Fungal laccases can degrade the phenolic component of lignin, allowing efficient reuse of lignocellulosic biomass to produce enzymes, bioactive substances, or clean energy and minimize the use of chemicals [45]. Lei *et al*. reported laccase-mediated phenol degradation [46] and also observed a Cu-ion-containing self-assembly exhibiting laccase-like activity.

10. Organic Dye Degradation

Owing to its industrial use as a bleaching agent, the degradation of organic pigments (related to textiles) is an important application of laccases. Organic dyes often act as electron-robbing substrates for laccase. For example, the mechanism (supported intermediate), efficiency (well-immobilized), and selectivity (for substrates usually contained in wastewater), studies by Dencheva *et al*. [17, 46], and hydrogels (see later section) are particularly important from the viewpoint of decolorization of bromophenol blue and other dyes. Although many studies (associated with wastewater or textile industries) have been published in recent years [47–49], the author believes that the number of studies that refer to substrate-protein binding patterns and selectivity are limited, except for optimal conditions of pH or temperature.

Trametes hirsuta secretes laccase isoenzymes, including constitutive and inducible forms, with promising applications in bioremediation of environmental pollutants. An inducible group B laccase from *T. hirsuta* MX2 was heterologously expressed in *Pichia pastori*. The optimal pH and temperature conditions of recombinant laccase (rLac1) for ABTS were 2.5 °C and 60 °C, respectively. Metal ions showed different effects on rLac1, with Mg^{2+}, Cu^{2+}, and K^{+} increasing enzymatic activity as their concentrations increased, whereas Zn^{2+}, Na^{+}, and Fe^{2+} inhibited enzymatic activity as their concentration

increased. Moreover, rLac1 showed more efficient decolorization ability against Remazol Brilliant Blue R than acid red 1, crystal violet, and neutral red. Docking simulation results indicated that Remazol Brilliant Blue R has a stronger binding affinity with laccase than other organic dyes by interacting with the substrate-binding cavity of the enzyme [50].

11. Polymer Decomposition and Hydrogels

Plastic pollution is a growing environmental problem because of the highly stable and durable nature of polymers, such as polyethylene, polyvinyl chloride, polyethylene terephthalate, and polyurethane. Because recycling is not a perfect solution, research has focused on alternative ways to break down plastic. Fungi offer a wide range of specialized enzymes for the degradation of persistent substances and are promising candidates in plastic degradation [51].

A series of polymers were derived from the enzymatic processing of arabinoxylan through the synergistic action of two catalytic proteins (feruloyl esterase and xylanase). Ferulic acid then serves as a substrate for laccase from Agaricus bisporus to enzymatically functionalize synthetic polymers [52].

Toyo lacquer is a natural polymer coating with a satin-like texture and excellent properties, such as chemical resistance and durability. However, the low lightfastness makes the natural aromatic structure of urushiol unsuitable for outdoor use. Chang et al. [53] improved the lightfastness and influenced the coating and film properties of a refined Toyo lacquer. The modification of the drying process affected the curing time and film properties.

In the case of biopolymers, starches recovered from pea pods, an agricultural waste product, were enzymatically modified and used to synthesize cryogels for use as drug carriers. The enzymatic modification of starch was conducted at various molar ratios using a laccase system with (2,2,6,6-tetramethylpiperidin-1-yl) oxyl TEMPO [54].

Conversely, as a topic of polymers, hydrogels are also important for enhancing biocatalytic functions and enzyme immobilization. For example, Labus *et al*. reported immobilized biocatalysts (laccase) in natural hydrogel matrices [55]. As an effective carrier, appropriate conditions are crucial, as described in their study. Scheibel and Gitsoc presented block copolymer synthesis using laccase in immobilization media [56]. In the hydrogels, high laccase activity and thermal stability were observed. Qian *et al*. reported the immobilization of enzymes during the synthesis of poly[bis(methacrylate) phosphazene] from hexachloro-cyclotriphosphazene using methacrylate-substituted polyphosphazene [57].

12. Lignin Degradation

Laccases directly oxidize phenolic lignin compounds, whereas lignin peroxidases and versatile peroxidases act on more intractable non-phenolic lignins (Figure 4). Mediators or co-oxidants enhance the catalytic ability of these catalytic proteins and greatly expand the substrate range to those with higher redox potentials or more complex structures. Laccase and peroxidase are not strictly substrate selective [58]. Laccase-mediated grafting has also been used to modify lignin and other polymers to obtain new functional groups that can bind to small and large biomolecules. Kim *et al*. [59] described the biochemical features of mushroom lignin-degrading enzymes and their potential applications in catalytic reactions involving lignin and its derivatives, highlighting value-added chemicals and novel materials for lignin valorization. In addition, Cui *et al*. discussed the major pathways of lignin biodegradation and the use of degradation products [60]. Ligninolytic bacteria or enzymes can be used in combination with chemical pretreatments to generate value-added chemicals from lignin, thus offering a promising strategy for increasing the value of lignin. Jiao et al. [61] also reported catalytic reactions of laccase in the degradation of cotton straw lignin.

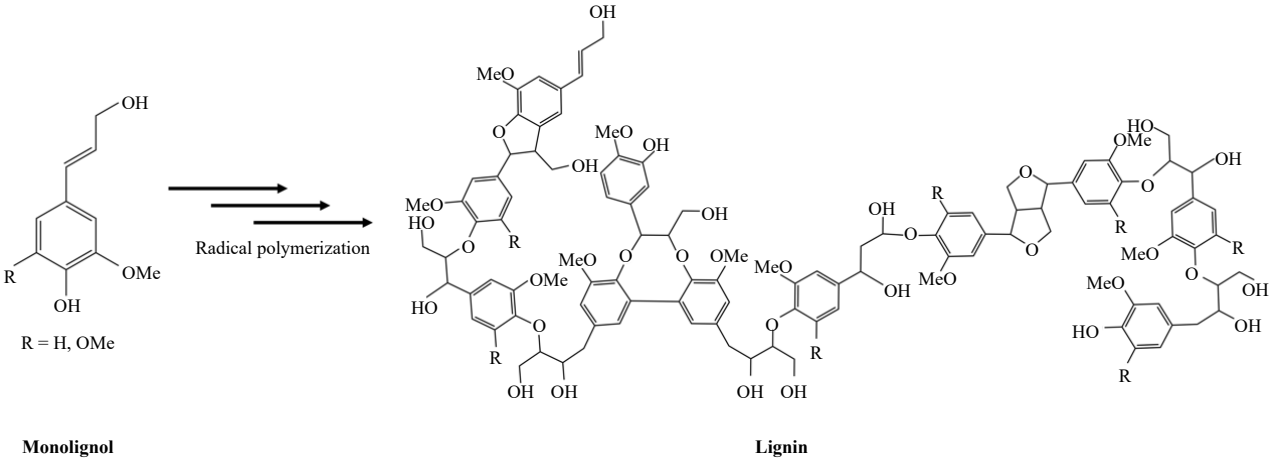

Figure 4. Typical structure of lignin.

Pleurotus ostreatus is a white-rot fungus that can effectively degrade lignin. Song *et al*. [62] efficiently expressed the *lac-2 gene of Pleurotus ostreatus* in the *Pichia pastoris* X33 yeast strain, which lays the foundation for studying the best pH or temperature conditions and mechanism of lignin degradation by laccase.

For additional metal ions, de Souza *et al*. [63] evaluated the optimized production of ligninolytic enzymes *via* response surface methodology using acidic cellulignin as an inducer, $MnSO_4$ (Mn^{2+}), $CuSO_4 \cdot 5H_2O$ (Cu^{2+}), veratryl(3,4-dimethoxybenzyl) alcohol, and Tween 80%. In addition, a fed-batch strategy for producing ligninolytic enzyme extracts from *P. lineage 2512* in a bubble column reactor was implemented. They optimized the experimental conditions in shake flasks to be 7.5 C/N ratio, 0.025 g/L Cu^{2+}, 1.5 mM Mn^{2+}, 3.0 mM VA, and 0.025 mM T80. Furthermore, several studies on lignin have been published [64–66].

13. Lignin and cellulose to biomass

Fossil-fuel disposal is a major environmental problem. Nevertheless, using biomass for chemical production is an alternative to energy generation *via* combustion. Lignocellulosic biomass (lignin and (hemi-)cellulose) is abundant and used for many purposes. Moreover, it can be depolymerized using fungal enzymatic machinery (laccase and peroxidase) [67]. Applying enzymes, such as laccases and xylanases, to prepare cellulose from lignocellulosic materials is an option for industries seeking to reduce the use of chlorine-containing bleaches and minimize the environmental impact of their processes. Mixed systems of hydrolases and oxidases are well-described in the context of biopulping and provide a good precedent for efficacy, despite their susceptibility to xylanase inactivation by laccase-forming oxidants [68]. Additionally, lignin polymers remaining in the pretreated materials affect biomass conversion by limiting enzymatic hydrolysis. Laccase is considered a powerful tool for delignification and detoxification of pretreated lignocellulosic material, facilitating subsequent saccharification and fermentation processes [69]. In this context, Olivieri *et al*. [70] focused on a novel process based on laccase catalysis applied to lignin depolymerization as an alternative to integrated physicochemical pretreatments. Laccase-based oxidation processes have been discussed in terms of their properties that may influence the development of bioreactor units, which utilize laccase or laccase-mediator systems for biomass delignification. However, few studies have dealt with the structural features of enzymes, cellulose, lignin [71–74] or other macromolecular biomass substrates, such as wood straw [75–78]. Therefore, for chemical reactions with organic substrates larger than this, mechanisms such as electron transfer (redox), which do not depend on molecular contacts, are speculated. Rizal *et al*. [79] reported that a combination of laccase and superheated steam effectively enhances the hydrolysis of oil palm biomass into glucose by laccase. The size reduction depends on the yield of glucose.

14. Further Perspective

For reactions with larger targets, such as environmental purification (including wastewater treatment), which may essentially be the decomposition of organic compounds or biodegradation [80–84], laccase-mediated extraction of pollutants from soil [85], and steric factors such as substrate selectivity and environmental anisotropy are not relevant, even though the best reaction conditions are examined. In contrast, biological approaches based on biochemical studies have focused on fermentation using laccases such as lignocellulosic or other biomasses with various bioreactors [86–90] and bioremediation (using mainly microorganisms and molecular enzymes, particularly laccase) [1], which may essentially include biochemical processes. Various studies have also been reported from a biological viewpoint, such as physiology and agricultural applications [91, 92], genetics aimed at improving chemical reactions (for example, removing organic dyes), or biochemical functions (up to laccase gene families and in silico identification) mentioned above [93–100]. Using a bioinformatics approach, Li *et al*. performed a genome-wide analysis of the laccase multigene family of switchgrasses with metal ions [101]. By developing along the biological information, related studies about proteome [102], culture [103], structural biology (steric factors are not relevant to the viewpoint of this review) [104], and microorganism associated with laccase [105, 106] were also reported. Laccases related to humans [107], dentistry such as dental care [108], and medical applications with a view to disease treatment [109, 110] have already been developed. Therefore, to utilize the properties of laccase in nanocomposites, the lessons learned from recent research, which can be said to be rough, should be the appropriate size of the substrate, use of molecular recognition, and composition of nanomaterial.

Author Contributions

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Conflict of interest

There is no conflict of interest for this study.

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