

Chapter 2

Laccases: A Blue Enzyme for Greener Alternative Technologies in the Detection and Treatment of Emerging Pollutants

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Abstract The continuous contamination of worldwide water bodies, by the presence of emerging pollutants, has raised great importance over the last decades. This group of pollutants comprises a large variety of chemicals, comprehending household and personal care products, human and veterinary drugs, as well as industrial compounds. Although, scientific data have made evident the potential threats of the emerging pollutants to public and environmental health, there is still limited information available concerning the ecotoxicity, concentration, and distribution of these compounds, which makes their ecological regulation, detection, and treatment very difficult. Thus, the search for green technologies to detect and treat potential environmental pollutants is critical for ecological and human health protection. In this context, laccases have gained scientific interest due to their broad substrate range, including recalcitrant environmental pollutants, and their ability to use only oxygen as a co-substrate. This work explores the potential of laccase enzyme as element of biosensing and bioremediation, and identifies the drawbacks that have to be overcome in order to demonstrate their feasibility and implement a large-scale process.

1 Introduction

To date, there is a critical environmental problem of contamination in water resources with persistent, bioactive, and bioaccumulative substances, which cause potential health and ecological effects (Petrovic 2003). Some of these chemicals are daily life articles, such as household and personal care products, human and veterinary drugs, which are extensively used and constantly released into the aquatic ecosystems by human activities and direct discharges from wastewater treatment plants (WWTPs) (Caliman and Gavrilescu 2009). These groups of substances are known

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as emerging pollutants and their detection and removal is crucial due to their high persistence and toxicity, even at concentrations as low as ng/L (Bolong et al. 2009; Gavrilescu et al. 2014). It is well-known that most of the emerging pollutants that pass through conventional WWTPs are not completely removed (Caliman and Gavrilescu 2009). Thus, the development of biosensors resulted as an effort in the search for analytical tools capable to detect these kinds of contaminants at low concentrations with a high specificity (Rodríguez-Mozaz et al. 2006). However, once the pollutants have been identified, a decontamination process has to be performed. In this context, bioremediation of water resources by the use of biocatalysts, such as enzymes, has been suggested in recent years. Enzymes are biomolecules that have the ability to mediate reactions; severe conditions are not required for their action and normally the by-products formed during the catalysis are benign, which implies a great opportunity for the use of enzymes in bioremediation (Senthivelan et al. 2016). Currently, several enzymes have been used in different biotechnological and industrial applications; however, laccases enzymes have received special attention due to its ability to oxidize a wide range of substrates, accompanying the reduction of oxygen to water as a by-product of reaction (Rodríguez Couto and Toca Herrera 2006). Laccases are able to oxidize, polymerize, or transform diverse recalcitrant substances into less toxic molecules; therefore, these enzymes could be suitable biocatalysts for water bioremediation (Majeau et al. 2010). However, several drawbacks have to be surpassed in order to implement the use of laccases for pollution alleviation, mainly, because of the elevated expenses that a large-scale enzyme production involves (Majeau et al. 2010).

2 Laccase

2.1 Laccase Source

As oxidoreductase enzymes, laccases possess the ability to oxidize diverse phenolic compounds with the concomitant reduction of oxygen (Yaropolov et al. 1994; Morozova et al. 2007a; Madhavi and Lele 2009). These biocatalysts have been widely found as extracellular and intracellular enzymes in several organisms, encompassing microorganisms (*Bacillus*), plants (genus *Rhus*) (Omura 1961), fungi (such as genera *Trametes*, *Cerrena*, *Ganoderma*, *Pycnoporus*, and *Coriolopsis*) (Chaubey and Malhotra 2002; Morozova et al. 2007b; Madhavi and Lele 2009), and insects (*M. sexta*) (Bailey et al. 2004). In insect species, laccases are responsible for the sclerotization process; whereas in *Bacillus* species, they play a role related to pigmentation, pathogenesis, and the assembly of UV-resistant spores (Kramer et al. 2001; Claus 2003). In plants, laccases are found in the xylem and, together with peroxidases, are involved in lignification (Omura 1961); moreover, fungal laccases play a role in delignification (Singh Arora and Kumar Sharma 2010) and humification processes (Morozova et al. 2007b).

2.2 Biocatalytic Mechanism and Applications

In an enzyme, the active site is the spot where the recognition, binding, and oxidation of the substrate take place. The active site of laccases comprises four copper nucleuses, each of them are grouped into three different classes of atoms according to both their distribution in the enzyme (T1, T2, and T3 sites) and their spectroscopic nature (Piontek et al. 2002).

The T1 and T2 sites only possess one copper atom; the two remaining atoms are found in the T3 site. Each type of copper has unique characteristics: type 1 is responsible for the emblematic bluish color of the enzyme, is a hydrophobic cavity where the substrate is oxidized during the catalytic mechanism of the laccase (Durán et al. 2002; Madhavi and Lele 2009); type 2 is colorless since no absorption in the visible region is observed; meanwhile, type 3 exhibits a weak peak at 330 nm (Ba et al. 2013). The reduced form of T1 (resulted from substrate oxidation) donates an electron, which is sent to the T2/T3 trinuclear cluster (formed from T2 and T3 copper atoms), which is where water formation occurs (reduction of oxygen) (Durán et al. 2002; Madhavi and Lele 2009) (Fig. 2.1).

The isoelectric point of laccases is around 4.0, showing their optimal performance at acidic conditions, which has been related to the growth conditions where the microorganisms produced these enzymes (Madhavi and Lele 2009). However, it has been recently reported that laccases maintain high stability (above 60% of its activity) at alkaline conditions (up to pH 8) (Ramírez-Cavazos et al. 2014b). On the other hand, the thermostability, high redox potential, and the ability to oxidize a wide range of substrate (including recalcitrant pollutants) are some other properties that make laccase of particular interest to researchers (Giroud and Minteer 2013; Ramírez-Cavazos et al. 2014b). Nowadays, the use of this enzyme is common in some industries (mostly paper, food, and textile industries) (Morozova et al. 2007b). However, the current trend is the use of laccase as biocatalysts in the bioremediation of polluted waters by emerging pollutants (Almansa et al. 2004; Junghanns et al. 2005); in the generation of energy by bioelectrocatalysis in enzymatic fuel cells (Meredith and Minteer 2012; Giroud and Minteer 2013; Holmberg et al. 2015); and in the development of biosensors using this enzyme as bioreceptor, for food

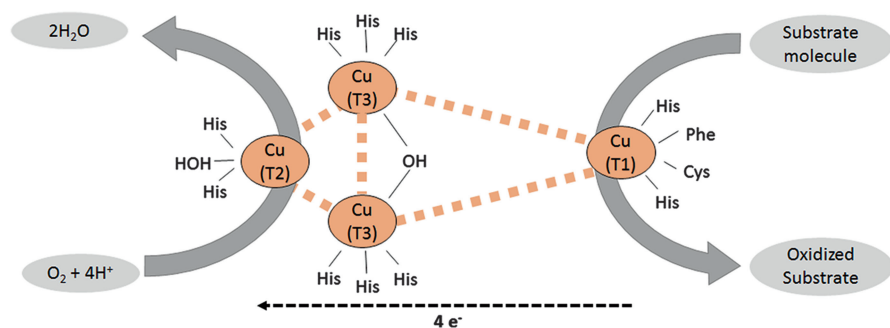


Fig. 2.1 Model of the laccase active site and catalytic cycle

(Ghindilis and Yaropolov 1992; Gamella et al. 2006; Di Fusco et al. 2010), environmental (Torrecilla et al. 2007; Tang et al. 2008), and medical applications (Quan and Shin 2004; Ferreira et al. 2009).

2.3 Laccase Production in Agro-Industrial Residues

Despite the high potential of laccase for biotechnological and industrial purposes, it is important to highlight that a large amount of enzyme is needed for a large-scale process. According to Osma et al. (2011), the culture medium represents the highest cost to the total expenses of laccase production; meanwhile, infrastructure costs are the lowest. Thus, in order to overcome this issue, several studies have been focused on (1) the development of stable genetic modifications of microorganism in order to achieve the gene expression for laccase; (2) optimization and reduction of the costs of culture media using agricultural wastes as cheap growth substrates (also helping to alleviate the environmental pollution); (3) search for new strains of microorganism capable to produce laccase (Bhattacharya et al. 2011; Yang et al. 2012; Theerachat et al. 2012; Nicolini et al. 2013; Ramírez-Cavazos et al. 2014a).

Ramírez-Cavazos et al. (2014a) tested a strain of *Pycnoporus sanguineus*, native from northern Mexico, using a tomato juice as culture medium, resulting in laccase titer of 143,000 U/L. Fenice et al. (2003) obtained a production of 4600 U/L in olive-mill wastewater. Meanwhile, Songulashvili et al. (2007) reported the laccase production using food wastes such as mandarin peelings, wheat bran and soy bran, kiwi fruits, chicken feathers, and ethanol, obtaining a maximum laccase activity of 93,000–97,000 U/L, after the submerged fermentation of wheat bran and soy bran by *Ganoderma* spp. Several agro-wastes such as mandarin and banana peel are substrates rich in lignin carbohydrates and organic acids, which could act as inducers to stimulate laccase production (Osma et al. 2007). In this context, several studies have reported the use of solid supports such as grape seeds (Rodríguez Couto et al. 2006), banana skin (Osma et al. 2007), and groundnut (Couto and Sanromán 2006) as growth substrates for fungi. However, the use of these complex substrates has as drawback the subsequent use/purification of the laccase after fermentation; thus the development of robust protocols that allow the use of crude enzymes (no purification process) in biotechnological applications is required.

3 Laccase-Based Biosensor for Detection of Emerging Pollutants

3.1 Emerging Pollutants in Water Reservoirs

Over the past two decades, the constant presence of emerging pollutants in worldwide water supplies has gained great relevance. This type of pollutants includes a large variety of chemicals used in daily life such as household compounds, personal

care products, and drugs for human and animal uses (Daughton and Ternes 1999). Although scientific data have made evident the potential threats of the emerging pollutants to public and ecological health, there is still information that has not been assessed (Horvat et al. 2012) concerning the environmental toxicity, concentration, distribution, and transformation of these compounds in water bodies, which makes their ecological regulation, detection, and treatment very difficult (Deblonde et al. 2011). These pollutants are typically released into the environment via anthropogenic activities such as agriculture practices, industrial, and human discharges (Murray and Ormeci 2012). The emerging pollutants are commonly classified as endocrine disruptors, pharmaceutical compounds, and personal care products (Daughton and Ternes 1999).

The endocrine disruptors are compounds that mimetize the action of hormones in the organisms, causing the alteration of the endocrine system, which has been related to sexual disorders, cancer, and even chronic diseases (Caliman and Gavrilescu 2009; Rezg et al. 2013). Nowadays, the presence of chemicals that exhibit hormone alterations and are involved in the elaboration of plastics and household products has been widely reported, e.g., surfactants, flame retardants, parabens, and plasticizers (Rodriguez-Mozaz et al. 2004). Meanwhile, the chemicals present in personal care products and considered as emerging pollutants encompass disinfectants (triclosan), conservation agents, fragrances (celestolide, tonalide, galaxolide), and UV screens (octyl-dimethyl-PABA, octyl-methoxycinnamate, homosalate) (Caliman and Gavrilescu 2009). On the other hand, the pharmaceutical compounds, as emerging pollutants, encompass human and veterinary drugs that have been widely found in water supplies such as antibiotics, nonsteroidal anti-inflammatory drugs, and beta-blockers (Deblonde et al. 2011). Numerous studies have reported the presence of personal care products, pharmaceutical compounds, and endocrine disruptors in several water supplies at alarming concentrations (Meisenheimer et al. 2002; Mompelat et al. 2009; Einsiedl et al. 2010; Lapworth et al. 2012). Teijon et al. (2010) reported a monitoring survey of pharmaceutical compounds in the water sampled from a WWTP and from the aquifer that is recharged by the discharge of this WWTP, resulting in the detection of these chemicals at concentrations of $\mu\text{g/L}$.

A large number of analytical protocols have been developed for the detection of these kind of pollutants, mainly by chromatography and spectroscopy techniques, since these methods are extremely accurate and capable to detect very low concentrations; however, they require complicated pretreatment sample, trained personnel, high operating costs, and they lack on-site applicability (Teijon et al. 2010). In this context, the need for portable analytical instruments, capable to field monitoring with high selectivity, sensitivity, and short assay times, has promoted the design of new devices such as biosensors (Marco and Barceló 1996).

A biosensor is an instrument capable of measuring a specific target molecule in a sample, taking advantage of its affinity toward a specific bio-element of recognition (bioreceptors, e.g., immunoreagents, enzymes) (Dzyadevych et al. 2008). There are three essential elements that comprise a biosensor (Fig. 2.2). The bioreceptor interacts specifically with the analyte present in the sample, producing a biochemical

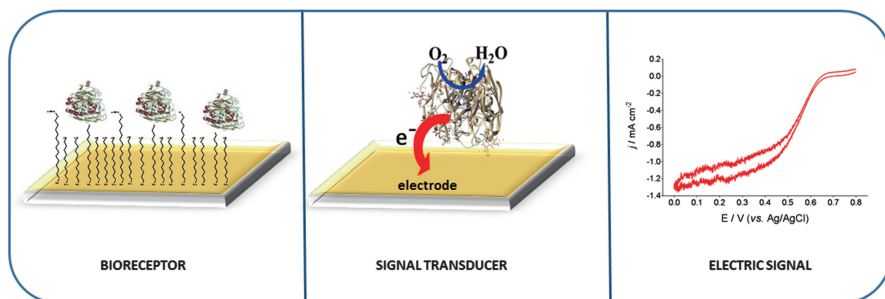


Fig. 2.2 Essential components of a biosensor: bioreceptor, electrochemical transducer, electric pathway in the production of the biosensor response and typical electrical response of the laccase-based electrode biosensors

reaction derived from this interaction, which is amplified and converted into quantifiable electrical signals (Dzyadevych et al. 2008). The transducer is in intimate contact with the bioreceptor and is responsible for the translation of the biochemical event into an electric signal (Dzyadevych et al. 2008).

The use of enzymes as bioreceptors has been widely employed in biosensing due to their stability and easier control of their recognition properties in comparison with other biomolecules such as antibodies or cells (Rogers 2006). In this context, the oxidase enzymes (e.g., tyrosinase, peroxidase, and laccase) appear as good candidates due to their ability to catalyze reactions where electron transference occurs, which can be used as the transduction principle. Laccase enzymes have some benefits over the other oxidases, which make them highly interesting for biosensing applications; their thermostability, versatility to react with a wide range of substrates, no cofactors are needed to perform the catalysis and the formation of water as by-product are some of these advantages (Munteanu et al. 1998).

3.2 Immobilization Methods

In general, an efficient protocol of immobilization is developed to facilitate the biomolecule recovery and reusability. Thus, the immobilization of the bioreceptor would prolong the life of the biosensor and assure its work stability (Liu et al. 2006). The method of immobilization to apply must provide the best conditions to assure the highest stability of the bioreceptor and this will depend on the inherent properties of the recognition element; the operational requirements of the measurement, the target molecule, and the transduction principle are also important factors to take into account in the design of a biosensor (Singh et al. 2008). The immobilization methods most commonly employed for laccase attachment are covalent coupling, adsorption, cross-linking, encapsulation, and entrapment (Fig. 2.3) (Fernández-Fernández et al. 2013).

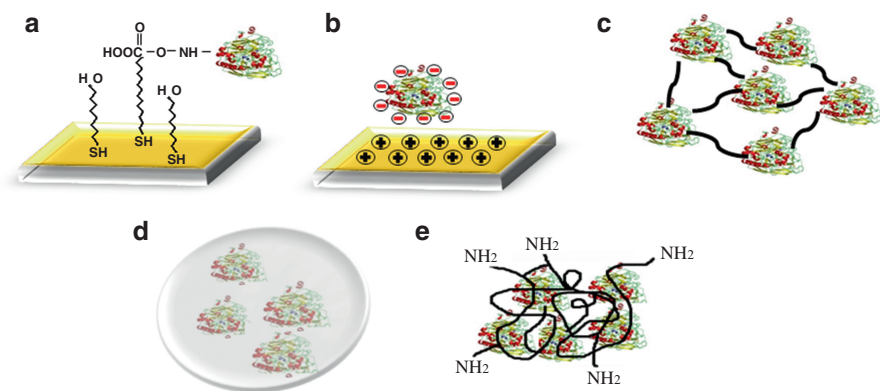


Fig. 2.3 Immobilization methods employed in biosensors. (a) Covalent coupling, (b) Adsorption, (c) Cross-linking, (d) Encapsulation, (e) Entrapment

The covalent coupling requires the chemical reaction between the functional groups in the carrier with the biomolecule (mainly, through the amino acid residues in their structure) forming covalent bonds; however, it is important to avoid the binding of the enzyme through amino acids within the active site, since that would compromise the biocatalyst activity, resulting in their inhibition (Arroyo 1998). The immobilization via adsorption takes place by weak interactions between the biomolecules and the solid support, e.g., Van der Waals interactions or ionic forces; despite the simplicity and inexpensive requirements of the protocol, this scheme presents the disadvantage of an unsteady fixation of the bioreceptors under pH changes, modification of the polarity or the ionic strength alterations, resulting in the leakage of the molecules (Brady and Jordaan 2009).

The cross-linking involves the generation of intramolecular links within the molecules of the enzyme (Arroyo 1998). Cross-linked enzyme crystals present high catalytic activities and operational stability; however, high quantities of highly purified enzyme are required; under this scheme, it is important to maintain the pH and salt concentration stable (Bryjak et al. 2007). Encapsulation refers to the confinement of the biomolecule within semipermeable spheres made of polymers (Rochefort and Kouisni 2008); meanwhile, entrapment is based on the retention of the enzyme within a polymeric grid; this method assures the integrity of the enzyme structure, however it presents diffusion problems and constant loss of the biomolecules due to differences in the size of the pore in the grid (Ibarra-Escutia et al. 2010).

3.3 Transduction Principles

3.3.1 Electrochemical Transducers in Environmental Applications

Electrochemical transducers refer to the element in a biosensor that translates the transference of electrons occurring during the reaction of an enzyme (immobilized on an electrode) with a substrate (Marco and Barceló 1996; Thévenot et al. 2001).

Laccase enzymes possess mechanical properties and electron transference abilities that make them excellent bioreceptors in electrochemical biosensors (Table 2.1).

The electrochemical transducers in biosensors are able to measure conductometry, potentiometry, and voltammetry principles (Thévenot et al. 2001). Conductometric methods monitor the development of a redox reaction through changes in the conductivity of an electrolytic solution, as a response of the charged products formed by the interaction of the bioreceptor with the analyte (Ronkainen et al. 2010). Potentiometric biosensors measure the changes of potential due to a biochemical reaction occurring between two electrodes (either, reference or indicator electrodes) (Thévenot et al. 2001). Voltammetric measurements are based on the changes of the current during a biochemical reaction on a working electrode; meanwhile, the voltage applied is varied (Chawla et al. 2012). Amperometry is a classification of voltammetry; under this scheme, the potential is maintained constant during the reaction measurement (Thévenot et al. 2001).

Chen et al. (2015) reported the immobilization of laccase in a nanocomposite of gold nanoparticles by a cross-linking method, achieving a direct electron transfer that resulted in a highly sensitive biosensor. Meanwhile, Das et al. (2014) immobilized a *Trametes versicolor* laccase in a nanocomposite matrix comprising of osmium tetroxide on poly 4-vinylpyridine, multiwalled carbon nanotubes; obtaining a current response against pyrocatechol, a genotoxic and mutagenic phenol, in the concentration range of 3.98–16.71 nM and a limit of detection of 2.82 nM with a sensitivity of 3.82 ± 0.31 nA/nM. Vianello et al. (2004) immobilized a *Rigidoporus lignosus* laccase onto a gold carrier obtaining a detection limit of 0.5 mg/L. Tang et al. (2006) reported the immobilization of an hybrid bioreceptor, consisting of horseradish peroxidase and laccase enzymes for the detection of *E. coli* density through the redox reaction of polyphenols generated by the metabolism of the *E. coli*. On the other hand, the development of new nanomaterials has improved the electrochemical analysis performed by biosensors. In this context, Mei et al. (2015) prepared a mixture of palladium and copper to cover a support of graphene oxide in order to build a platform for laccase immobilization; obtaining a detection limit of 2.0 mM for catechol. According to Rodríguez-Delgado et al. (2015), amperometric methods are highly employed in laccase-based biosensors; meanwhile, conductometric and potentiometric methods are not very often employed for this use.

3.3.2 Optical Transducers in Environmental Applications

Optical absorption and fluorescence emission measurements have been extensively used to characterize colored or chromophoric compounds. Based on this concept, the spectroscopic properties of by-products formed by laccase catalysis are employed for the detection of target molecules in optical biosensors (Zoppellaro et al. 2001). Laccase has the ability to produce a reddish dye when catalyzes the coupling oxidation of phenols in presence of 3-methyl-2 benzothiazolinonehydrazine (MBTH) (Setti et al. 1999). Based on this reaction, Abdullah et al. (2007) reported the detection of catechol at concentrations as low as 0.33 mM. The

Table 2.1 Electrochemical biosensors using laccase as bioreceptor for detection of pollutants

Laccase sources	Immobilization method	Working electrode	Target molecule	Sensing parameters	Matrix	References
<i>Coriolus hirsutus</i>	Covalent coupling	Gold	Catechol	Dynamic range 1–400 μM	Synthetic	(Gupta et al. 2003)
				Sensitivity 15 $\mu\text{A}/\text{mM}$		
<i>Cerrena unicolor</i>	Electrodeposition	Platinum	Hydroquinone	Dynamic range 2.0–60 μM	Synthetic	(Jędrychowska et al. 2014)
				Sensitivity $2.34 \pm 0.11 \mu\text{A}/\text{mM}$		
Not reported	Entrapment	Nafion/laccase glassy carbon	Catechol	Dynamic range 0–7 μM	Real samples	(Chen et al. 2015)
<i>Trametes versicolor</i>	Entrapment	Platinum	Phenol	Dynamic range 0.40–6.0 μM	Wastewater	(Timur et al. 2004)
			Catechol	0.20–1.0 μM		
			l-DOPA	2.0–20 μM		
			Phenol	Dynamic range 0.40–4.0 μM		
			Catechol	0.4–15 μM		
<i>Aspergillus niger</i>			l-DOPA	0.4–6.0 μM		
<i>Coriolus versicolor</i>	Covalent coupling	Indium tin oxide	Polyphenolic compounds of <i>E. coli</i> metabolism	1.6×10^3 to 1.0×10^7 cells/mL	<i>E. coli</i> solutions	(Tang et al. 2006)

(continued)

Table 2.1 (continued)

Laccase sources	Immobilization method	Working electrode	Target molecule	Sensing parameters	Matrix	References
<i>Pleurotus ostreatus</i>	Electrostatic attachment	Indium tin oxide	Phenol	Limit of detection 0.5–4.5 μM	Wastewater	(Kushwah et al. 2011)
			Catechol	0.4–15 μM		
<i>Coriolus hirsutus</i>	Adsorption	Graphite	Catechol hydroquinone	Limit of detection 2 μM	Synthetic	(Yaropolov et al. 1995)
<i>Coriolus versicolor</i>	Entrapment	Glassy carbon/chitosan	Catechol	Dynamic range 1.2–30 μM	Synthetic	(Liu et al. 2006)
				Limit of detection 0.66 μM		
<i>Rigidoporus lignosus</i>	Covalent coupling	Gold	1,4-Hydroquinone	Sensitivity 3 nA/ μM	Olive oil wastewater	(Vianello et al. 2004)
<i>Trametes hirsute</i>	Entrapment	Glassy-carbon/ Cetyl ethyl poly(ethyleneimine)/ Nafion	Hydroquinone	Dynamic range 0.1–3.0 μM	Synthetic	(Yaropolov et al. 2005)
<i>Trametes versicolor</i>	Adsorption and covalently coupling	Graphite	Catechol	Dynamic range up to 0.1 mM	Synthetic	(Portaccio et al. 2006)
				Sensitivity 196.0 $\mu\text{A}/\text{mM}$		
<i>Trametes versicolor</i>	Entrapment	Gold	Catechol	Dynamic range 0.67–15.75 μM	Synthetic	(Xu et al. 2010)
<i>Pleurotus ostreatus</i>	Entrapment	Glassy carbon	Phenol	Dynamic range 0.5–4.5 μM	Synthetic	(Kushwah and Bhadauria 2010)

<i>Ganoderma</i> sp. Rekk02	Covalent coupling	Gold with cooper nanoparticles- carboxylated multiwalled carbon nanotubes- polyaniline	Polyphenol/ guaiacol	Dynamic range 1–500 μM	Synthetic	(Chawla et al. 2011)
				Sensitivity 0.694 $\mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$		
<i>Trametes</i> <i>versicolor</i>	Entrapment	Glassy carbon	Pyrocatechol	Dynamic range 3.98–16.71 nM	Real samples	(Das et al. 2014)
				Sensitivity 3.82 \pm 0.31 nA/nM		
<i>Cerrena</i> <i>unicolor</i>	Electrolytic deposition	Platinum	o-Amino phenol catechol phenol	Not reported	Real samples	(Cabaj et al. 2011)
<i>Cerrena</i> <i>unicolor</i>	Entrapment	Platinum	4-tertbutylcatechol	Dynamic range 2–89 μM	Not reported	(Kochana et al. 2008)
			4-methylcatechol	0.21–15 μM		
			3-chlorophenol	0.98–7.9 μM		
			Catechol	0.2–23 μM		

detection was made by spectrophotometric measurements of the reddish compound formed from the coupling of MBTH films (stacked in nafion silicate) to radicals quinone and/or phenoxy produced by laccase enzymatic oxidation (Abdullah et al. 2007). Sanz et al. (2012) studied the reaction of phenol catalyzed by *Trametes versicolor* laccase, using a polyacrylamide film sensor immobilized with the enzyme, obtaining a limit of detection of 0.109 mM.

Surface plasmon resonance (SPR) phenomenon is also an optical principle employed for biosensing, since almost two decades ago. SPR measurements are performed at a fixed angle of incidence and the monitoring is done by detecting changes in the amount of the reflected light, which is correlated to changes in mass on the surface due to binding events (between the bioreceptor and the analyte) (Estevez et al. 2012). In this context, Surwase et al. (2016) reported a first attempt for the detection of phenolic compounds in water by using laccase enzyme as bioreceptor in a SPR device.

4 Laccase as Biocatalyst for Removal of Emerging Pollutants

Several studies have reported the presence of endocrine disruptors, personal care products, and pharmaceutical compounds in diverse water bodies at important concentrations (Snyder et al. 2003; Caliman and Gavrilescu 2009). Thus, the presence of these pollutants in water supplies has become an important issue in terms of treatment technologies for water cleaning, mainly because of the highly resistance of these compounds to common removal techniques. Advanced oxidation techniques based on UV/ozone exposure have obtained efficient yields of removal/inactivation (Esplugas et al. 2007); however, these processes are expensive (Lloret et al. 2012) and in some cases the by-products generated are more toxic than the parent pollutant (Sein et al. 2008). Therefore, laccases appear as strong biocatalysts to be employed in bioremediation treatments, since can react under mild conditions with a broad substrates range, encompassing recalcitrant pollutants, and generate non-harmful by-products. Fukuda et al. (2004) demonstrated the removal of the endocrine activity of the by-products from bisphenol A; catalyzed by *Trametes villosa* laccase (Fukuda et al. 2004). Likewise, Cabana et al. (2007a) reported nonestrogenic activity in the products of reaction by the catalysis of bisphenol A, triclosan, and nonylphenol by employing *Coriopsis polyzona* laccase.

The removal of emerging pollutants from water supplies using laccase could be achieved under several schemes: (1) free enzyme; (2) immobilized enzyme; and (3) cells from culture broths.

Several studies have reported the use of free laccases for emerging pollutants removal. Yang et al. (2013) reported 27% of diclofenac elimination. The total elimination of estrone, 17 β -estradiol, estriol, and 17 α -ethinylestradiol (Auriol et al. 2006, 2007, 2008), and oxybenzone (Garcia et al. 2011) was achieved by *Trametes versicolor* laccase. Almost complete removal of 2,4-dichlorophenol was observed by free laccase at acidic pH (Jia et al. 2012), temperatures around 30 and 50 °C (Zhang

et al. 2008; Gaitan et al. 2011; Qin et al. 2012; Xu et al. 2013), and using elevated concentrations of enzyme (Zhang et al. 2008). Jia et al. (2012) investigated the degradation of 2,4-dichlorophenol by a photocatalytic–enzymatic treatment, achieving 90% within 2 h with the coupled degradation process.

The use of mediators is also common during enzymatic degradations; these compounds are small-size molecules that can extend the ability of an enzyme to react toward noncommon substrates; also are stable and reusable by various cycles (Majeau et al. 2010). ABTS, 1-hydroxy-benzotriazole (HBT), nitroso-2-naphthol-2,6-disulfonic acid (NNDS), Syringaldehyde, 4-Acetylamino-TEMPO 4-hydroxy-TEMPO, Violuric acid (VIO), and p-Coumaric acid are some of the mediators most widely employed for laccase catalysis (Majeau et al. 2010). Ji et al. (2016) reported the elimination of carbamazepine using as mediators p-coumaric acid, syringaldehyde, and acetosyringone, obtaining 60% of degradation after 96 h. Meanwhile, Margot et al. (2015) assessed the potential of laccase to remove sulfamethoxazole and isoproturon with three mediators: ABTS, syringaldehyde, and acetosyringone, showing complete transformation within a few hours. Almost total biotransformation of diclofenac by laccase was also obtained using 1-hydroxybenzotriazole (Nguyen et al. 2013), syringaldehyde, and violuric acid (VA) (Lloret et al. 2010, 2013).

In terms of immobilization, Krastanov (2000) studied the degradation of β -naphthol, observing a complete removal after a hybrid treatment with laccase from *Pyricularia oryzae* and tyrosinase. Lante et al. (2000) immobilized *P. oryzae* laccase on a polyethersulfone membrane, obtaining 18% of β -naphthol removal. Le et al. (2016) provided a novel immobilization technique for laccase on copper alginate for real wastewater treatment, showing 89.6% of triclosan removal after 8 h treatment. Nguyen et al. (2014) reported an enzymatic membrane reactor for the degradation of bisphenol A and diclofenac, obtaining >85% and >60% removal, respectively, by laccase from *Aspergillus oryzae*. Meanwhile, Chen et al. (2016) immobilized laccases on the surface of yeast cells for treatment of bisphenol A (46% removal after 6 h) and sulfamethoxazole (47% removal after 30 h of treatment). Nevertheless, there are just a few works that address the degradation of emerging pollutants in a real matrix and under real reaction conditions (pH, temperature, ionic strength); this is important to consider since some matrix components could decrease the laccase activity and therefore decrease the degradation yield. Rodríguez-Delgado et al. (2016) tested the biotransformation of the micro-pollutants: diclofenac, 5,7-diiodo-8-hydroxyquinoline, β -naphthol, and 2,4-dichlorophenol using laccase from *P. sanguineus* CS43 in groundwater samples, observing a reduced bioconversion for β -naphthol and 2,4-dichlorophenol in the real samples in comparison with the synthetic buffer matrix. Biotransformation of bisphenol A, 4-nonylphenol, 17- α -ethynylestradiol, and triclosan were tested in groundwater, as well (Garcia-Morales et al. 2015).

The presence of some ions in a reaction matrix has been reported to induce structural modifications in the active site of the enzyme (Zilly et al. 2011). For instance, halide anions have been related to the interference on the transference of electrons within the active site of the laccase enzyme (Enaud et al. 2011; Margot et al. 2013);

meanwhile, cyanide and calcium provoke the separation of the copper atoms from the enzyme (Cabana et al. 2007b). Kim and Nicell (2006) observed that bisphenol A biodegradation was adversely affected by nitrite, thiosulfate, and cyanide.

5 Future Perspectives and Conclusions

The use of laccases as bioreceptors in biosensors promotes the development of new technologies for environmental monitoring in terms of pollutants screening. However, the immobilization of the bioreceptor is a crucial step in biosensors design, thus more research has to be focused on (1) the creation of new materials for support purposes that diminish the loss of the enzymes once they are immobilized; (2) the improvement of the current immobilization methods to assure an oriented binding of the enzyme in order to safeguard the integrity of the active site, resulting in the recovery of the enzymatic activity once the immobilization was performed; and (3) the use of genetic modifications to extend the laccase stability under difficult catalysis conditions, assuring the recycling of the bioreceptor and thus the lifetime of the biosensor.

The exploitation of laccase enzymes as element of bioremediation opens up enormous possibilities in terms of future treatment technologies for water cleaning. However, unlike biosensor applications, an elevated amount of enzyme is required for bioremediation. Therefore, one of the crucial issues that avoid large-scale use of the laccase is related to the high cost of enzyme production; thus further research should be focused on diminishing the cost that represents the growth mediums for the microorganisms during the laccase production. Furthermore, future research needs to look at (1) the test of the interaction of hybrid treatments in bioremediation, e.g., photocatalytic–enzymatic, enzymatic–enzymatic, whole cell–enzymatic, etc., (2) the study of the structure and toxicity of the by-products formed by laccases catalysis under different reaction conditions, and (3) the implementation a pilot-scale process, where the laccase treatments would be performed under real reaction conditions (pH, temperature, and matrix composition), which consider possible matrix interactions over the catalysis.

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