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Laccase-based biosensors for detection of phenolic compounds

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ABSTRACT

Monitoring of phenolic compounds in the food industry and for environmental and medical applications has become more relevant in recent years. Conventional methods for detection and quantification of these compounds, such as spectrophotometry and chromatography, are time consuming and expensive. However, laccase biosensors represent a fast method for on-line and *in situ* monitoring of these compounds. We discuss the main transduction principles. We divide the electrochemical principle into amperometric, voltammetric, potentiometric and conductometric sensors. We divide optical transducers into fluorescence and absorption. The amperometric transducer method is the most widely studied and used for laccase biosensors. Optical biosensors present higher sensitivity than the other biosensors. Laccase production is dominated by a few fungus genera: *Trametes, Aspergillus,* and *Ganoderma*. We present an overview of laccase biosensors used for the determination of phenolic compounds in industrial applications.

Contents

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1. Introduction

Monitoring of phenolic compounds in the food industry and for environmental and bio-medical analyses, by using portable, cost-effective devices, has become an area of growing interest over the past decade. Phenolic compounds are widespread in nature. They can be found in fruits and vegetables, and they are responsible for the organoleptic properties of some food products, such as wine and olive oil [1,2]. Their antioxidant properties help to prevent cancer and cardiovascular diseases [3]. Phenols are also breakdown products from natural organic compounds, such as humic substances, lignins and tannins. However, some phenols are ubiquitous pollutants that come to natural waters from the effluents of chemical industrial activities, such as coal refineries, pharmaceuticals, production of resins, paints, textiles, petrochemicals, and pulp, including the manufacturing of phenol [4,5].

Consequently, aquatic organisms, including fish, are subjected to these pollutants [5], and, due to their toxicity, some phenolic compounds are subject to regulation as water pollutants. In this context, both the European Commission (EC) and the US Environmental Protection Agency (US EPA) have created lists and classifications to prioritize hazardous substances for their monitoring in drinking or natural waters, and, among them, special attention has been devoted to phenolic compounds {e.g., polycyclic aromatic hydrocarbons (PAHs) and organophosphates [6–8]}. Of particular concern are emerging pollutants, mainly those occurring in phenolic compounds, with endocrine-disrupting activity, and those represented by chlorophenols and their derivatives [9,10].

Endocrine-disrupting chemicals are substances that mimic the effects of hormones [11], producing adverse effects on reproduction in wildlife and humans; some phenolic compounds, such as bisphenol A, nonylphenol and their alkylphenolic derivatives, triclosan, genistein and others, widely used in industrial and domestic applications, produce estrogenic activity [10,12]. Chlorophenols too are harmful substances, widely used as bleaching agents in the textile and paper industries, and as pesticides in agriculture [13]. Among chlorophenols, 2,4-diclorophenol and 2,4,6-trichlorophenol are chemicals produced in large quantities, and are listed as priority environmental pollutants by the US EPA [7].

Although spectrophotometric and chromatographic techniques are the most common methods for the determination of phenolic compounds, capable of identifying and quantifying them with great accuracy, a wide variety of pollutants exist at trace levels [14], and the most recent research in monitoring techniques is mainly focused on bioanalytical tools, such as biosensors, which offer advantages over classical analytical techniques in terms of selectivity, sensitivity, short assay times, and reduced cost of analysis [14].

The use of enzymatic biosensors has increased over time, due to their specific and peculiar properties. Enzymes are versatile, efficient and specific catalysts acting in all chemical reactions that occur under mild conditions [15]. Some biosensor research has been carried out on the detection of phenolic compounds based on enzymes, such as tyrosinase [16,17] and horseradish peroxidase [18]. Nevertheless both these enzymes present some disadvantages:

- tyrosinase suffers from low stability and significant inhibition by reaction products;
- while horseradish peroxidase needs the presence of hydrogen peroxide to complete its catalytic function [19].

However, laccase appears a strong candidate as a biosensor, providing some specific advantages over other enzymes, such as its ability to catalyze electron-transfer reactions without additional cofactors, oxidize phenols and o,m,p-benzenediol compounds in the presence of molecular oxygen, and good stability [20].

The current review presents an overview of laccase biosensors used for the determination of phenolic compounds in important areas, such as clinical, bio-medical, environmental contamination, industrial and pharmaceutical analysis.

2. Laccases

The enzyme laccase (polyphenoloxidase; EC 1.10.3.2) is a member of the blue multi-copper-oxidase family. These enzymes have been studied for a long time, due to their ability to oxidize a variety of organic substrates, and to reduce molecular oxygen to water [21]. These enzymes have been detected in a variety of organisms, such as bacteria, fungi, plants, and insects, mainly as extracellular enzymes, although intracellular laccases have also been detected [22]. In plants, laccases are found in the xylem, where they presumably oxidize monolignols in the early stages of lignification, and also participate in the radical-based mechanisms of lignin polymer formation, whereas fungal laccases play a variety of roles, such as morphogenesis, pathogenesis, and lignin degradation [23]. Their unique characteristics have been widely studied and extended to several uses in agricultural, industrial, medical and environmental applications [23,24].

2.1. Occurrence

Laccases (Lac) are widely distributed in:

- plants, such as Rhus vernicifera and Rhus succedanea [25];
- wood-rotting fungi, such as Trametes versicolor, Trametes hirsuta, Trametes ochracea, Trametes villosa, Trametes gallica, Ganoderma brownie, Ganoderma curtisii, Ganoderma lobatum, Ganoderma lucidum, Cerrena maxima, Coriolopsis polyzona, Lentinus tigrinus and Pleurotus eryngii [24,26], Polyporus versicolor A, B, Pleurotus spp, Pholiata spp, Podospora anserina, Neurospora crassa, Aspergillus nidulans white-rot fungi and Pyricularia oryzae [27]; and,
- saprophytic ascomycetes, such as *Myceliophthora thermophila* and *Chaetomium thermophile*, which are involved in the humification of composts [24].

2.2. Laccase characteristics and catalytic mechanism

In general, a fungal laccase has a molecular mass of 60–80 kDa and an isoelectric point of 4–7, depending on glycosylation. Laccase is made up of a cluster of four copper atoms (type I copper; type II copper and two type III copper atoms) that form the active site of the enzyme [28]. These copper atoms are classified into three groups, depending on the characteristics obtained by UV/visible and electron paramagnetic resonance (EPR) spectroscopy:

- type I copper (T1, ligated by at least one Cys and two His) is responsible for the intense blue color of the enzyme, has a strong electronic absorption at ~600 nm and is EPR detectable;
- type II copper (T2, ligated by two His) shows no absorption in the visible spectrum (colorless) but reveals detectable EPR properties; and,
- type III copper consists of a pair of anti-ferromagnetically coupled copper atoms (T3, each ligated by three His) spectroscopically characterized by a weak adsorption at 330 nm (oxidized form) and by the absence of an EPR signal [26,29].

Upon strong anion binding (e.g., azide and fluoride), perturbation on the EPR spectra of laccase is observed, causing disruption of the anti-ferromagnetic coupling of T3 and in the T2 EPR signal intensity [30,31]. The T2 and T3 copper atoms form a trinuclear

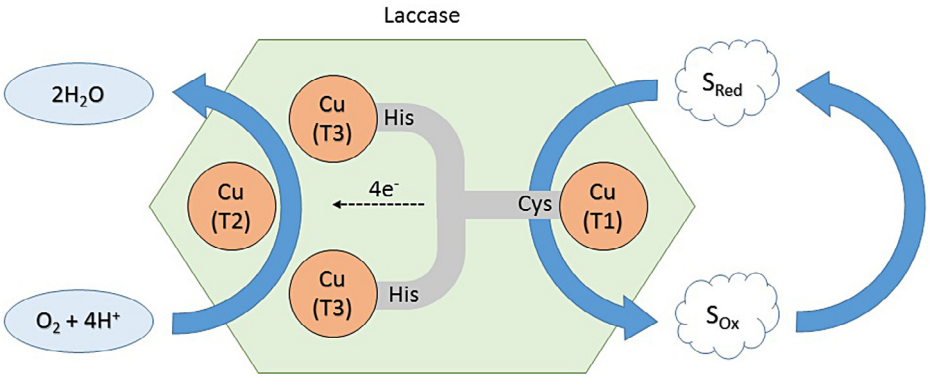

Fig. 1. A simplified reaction mechanism of laccase oxidation of suitable substrate.

cluster where reduction of molecular oxygen and release of water takes place [26,29].

Laccases have activity toward ortho- and para-diphenol groups, including mono-, di-, and poly-phenols, aminophenols, methoxyphenols, aromatic amines and ascorbate, with the concomitant four-electron reduction of oxygen to water [26,32]. The catalytic mechanism of the laccase starts with the donation of an electron to the substrate by the T1 copper site, followed by an internal electron transfer from the reduced T1 to the T2 and T3 copper sites. The T3 copper functions as a two-electron acceptor in the aerobic oxidation process, in which the presence of the T2 copper is necessary. The reduction of oxygen to water takes place at the T2 and T3 cluster and passes through a peroxide intermediate [29,33] (see Fig. 1).

Laccases are unable to oxidize directly non-phenolic substrates or large molecules with a high redox potential [34]. In this context, laccase-mediator compounds act as intermediate substrates for laccase, producing a high redox potential intermediate able to oxidize indirectly non-phenolic substrates, increasing the range of laccase-oxidizable compounds [24,26].

Being an oxidoreductase, the laccase has Cu sites with defined redox potentials (E^0). For the T1 and T3 Cu, some laccases have a "low" E^0 of ≈0.4–0.5 V (*versus* the normal hydrogen electrode), while others have a "high" E^0 of ≈0.7–0.8 V. However, the E^0 of the T2 Cu appears to be ≈0.4 V for both low and high E^0 laccase groups [35]. In general, a bell-shaped pH-activity profile (optimal pH) at ≈5–7 is observed for phenols, anilines or other substrates whose oxidation by laccase is accompanied by H^+. Because of the oxidative H^+ release, the E^0 of these substrates decreases as pH increases [35]. The subsequent increase of the ΔE^0 with laccase enhances enzymatic oxidation, contributing to the ascending part of the pH profile. However, at higher pH, the laccase inhibition by OH^- becomes more pronounced, contributing to the eventual descent of the pH profile.

2.3. Application

The ability of laccases to oxidize a broad range of phenolic compounds employed in several industrial sectors has increased their biotechnological potential. The most common uses of laccase are in:

- the textile, pulp and paper industries for bleaching and breaking down lignin [24];
- wastewater treatment for dye decolorization and xenobiotics degradation [36,37];
- the food industry [24]; and,
- the development of biofuel cells [38,39].

Laccases have also been used in the design of biosensors for the detection of phenolic compounds in food [1,40,41], and for environmental [2,42] and medical applications [43,44].

3. Immobilization of laccases in biosensors

To become viable industrial catalysts, laccases need to be subject to treatments in order to make them robust, recyclable, or heterogeneous. One of the most studied treatments is immobilization, defined as attachment of an enzyme to an insoluble support [45]. It is achieved by chemical linkage or physical adsorption/entrapment in carriers [46]. The benefits of an efficient protocol of immobilization are very important, namely prolonged use of the sensor and anticipated extended storage and working stability [47].

The ideal support should be inert, stable and resistant to mechanical forces. It is also important to consider shape, distribution and pore size and expandability. Stability, selectivity and activity of an enzyme are obtained by combining immobilization techniques with proper selection of the support [48]. For example, the method by which an enzyme is immobilized at an electrode surface is a critical factor to establish efficient electron transference between the enzyme and the electrode surface [49]. Furthermore, covalent coupling to a solid carrier may give the enzyme increased resilience against pH or thermal inactivation, although the immobilization may lead to a significant loss in activity of the original enzyme [46].

3.1. Immobilization methods

The selection of the immobilization method depends on the nature of the biological element and its application. For biosensing applications, it is necessary to take into account the type of transducer used, the physicochemical properties of the analyte and the operating conditions in which the biosensor is to function [50]; all these considerations will allow the biological element to exhibit maximum activity and help the stability and the reusability of the device. Common methods of laccase immobilization in biosensors are covalent binding, adsorption, cross-linking, encapsulation and entrapment (see Fig. 2) [48].

Covalent binding is based on the chemical activation of groups in the support matrix so that they react with functional groups in the biomaterial, which are not essential to catalytic activity. This method presents high stability and allows the enzyme to have some resistance to the effects of temperature, pH and other conditions; however, possible changes in the structure of the active center can occur [51].

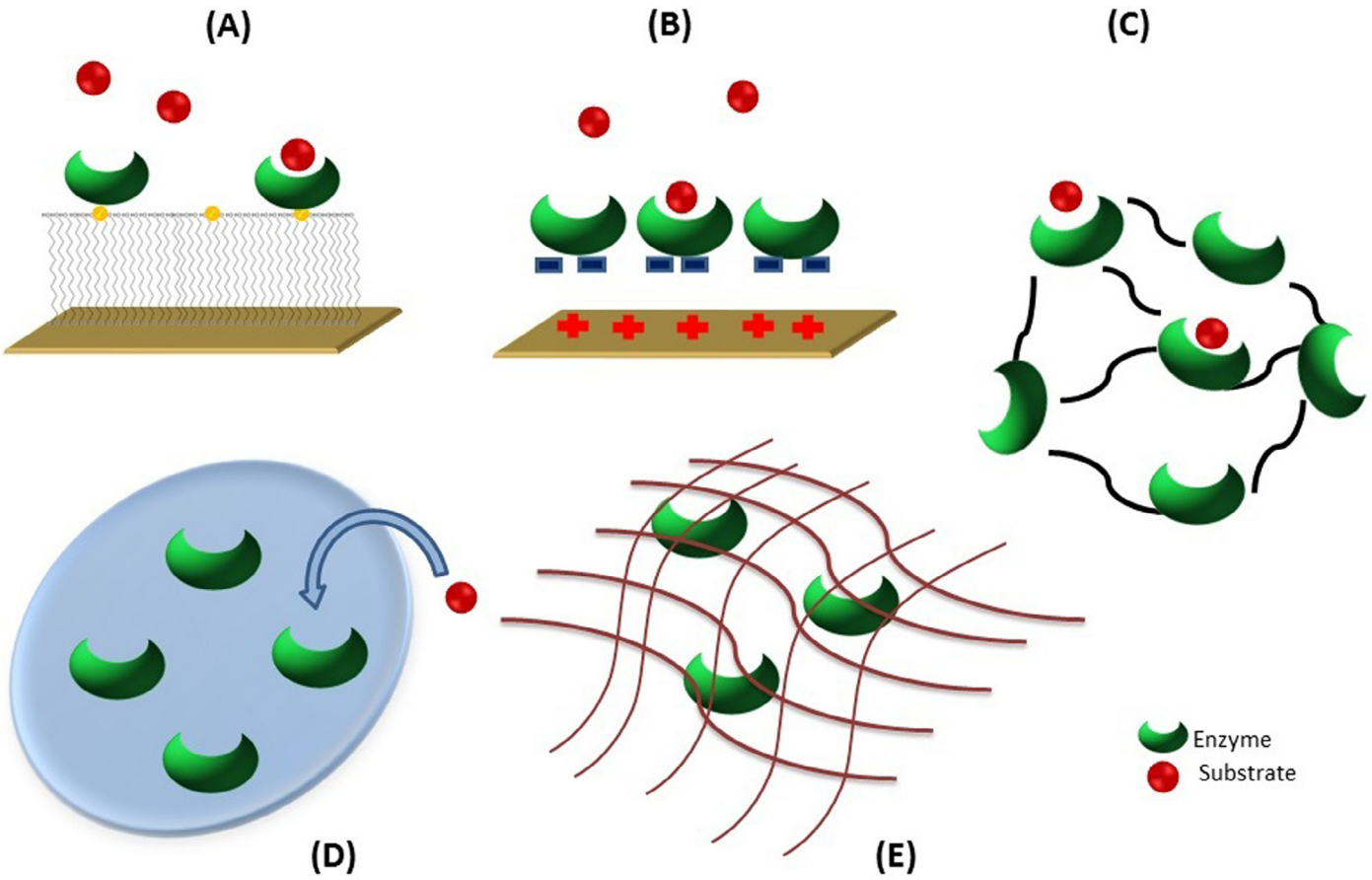

Fig. 2. Common methods of immobilization used in biosensing: (A) covalent binding; (B) adsorption; (C) cross-linking; (D) encapsulation; and, (E) entrapment.

Adsorption is a simple, low cost and fast immobilization method. The enzyme is bound to a support via ionic interactions or weak forces, such as van der Waals and hydrogen bridges. However, the biological elements immobilized through this method are mechanically unstable and can be easily desorbed under operating conditions [52].

Cross-linking bonding uses bifunctional reactives to generate intramolecular bonds between the molecules of the enzyme. These cross-linkers include dialdehydes, diiminoesters, diisocyanates and diamines activated by carbodiimide [51]. Under this type of immobilization, the complex-enzyme formed is resistant to extreme conditions of pH and temperature. However, large quantities of enzyme are needed, and factors, such as pH and ionic strength, must be controlled [53].

In encapsulation, the bioactive agent is confined to the core of micron-sized spheres made from a semi-permeable material, such as polymers [54]. Entrapment refers to a mixture of the biomaterial with a monomer solution that is then polymerized to a gel, trapping the biomaterial within the interstitial spaces of the polymeric gel. It can also be occluded within the microcavities of a synthetic fiber [55]. The enzyme suffers minimum alteration, but the method has some disadvantages, such as continuous leakage of enzyme due to variable pore-size, uneven distribution in the gel and reduced substrate accessibility to the enzyme by diffusional limitation [48].

Table 1 shows examples of immobilized laccase systems in biosensing applications.

Table 1
Methods of laccase immobilization reported by support and the immobilization characteristics of the solid biocatalyst

Ref.	Immobilization/material Support	Comments
Method of immobilization: Absorption		
[56]	Carbon nanotubes (CNTs) in surface of modified glassy-carbon electrode	- Good mechanical and electronic properties - Biocompatibility with a variety of enzymes - Ability to facilitate electron transfer - In aqueous solution, poor solubility of CNTs, which need some organic solvent to solubilize
Method of immobilization: Covalent binding of the enzyme and cross-linking with glutaraldehyde		
[57]	N-succinimidyl-3-thiopropionate (NSTP) in Gold electrodes	- With the cross-linking immobilization procedure a larger amount of enzyme was present on the electrode surface, much more sensitive than the covalent binding - Cross- linking three-dimensional network plays an important role in substrate accessibility to active centers of the enzyme
Method of immobilization: Covalent attachment of the enzyme in cellulose acetate support modified with ionic liquid		
[58]	Carboxymethyl cellulose with the ionic liquid (IL) 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide /silica or a polymer	- Immobilization of more active forms of the species with the establishment of a porous contact region between the phases within the structure - Highly efficient and robust biocatalysts

4. Transduction principles in laccase biosensors

A biosensor is an analytical device, which converts into an electric or other kind of signal, the physical or chemical properties of a biological system (e.g., enzymes, antibodies, receptors, cells, or microorganisms), which is in direct contact with the sample. The amplitude of the signal depends on the concentration of defined analytes in the solution [59].

A biosensor (Fig. 3) has two basic components:

- the bioreceptor (biochemical-recognition system), which translates information from the biochemical domain into a chemical or physical output; and,
- a transducer, which converts the output chemical signal of the recognition system to an electrical domain [60].

Enzymes were historically the first molecular recognition elements used in biosensors, and continue to be the basis of a significant amount of published research, due to the relative simplicity of modifying their catalytic properties or substrate specificity by genetic engineering. Catalytic amplification can also be achieved by modulating the enzyme activity with respect to the target analyte [61].

In this context, laccase appears a strong candidate for biosensing applications, providing some specific advantages over other enzymes:

- the ability to catalyze electron-transfer reactions without additional cofactors;
- the ability to oxidize phenols and o,m,p-benzenediols in the presence of molecular oxygen; and,
- good stability [20].

4.1. Electrochemical biosensors

Electrochemical biosensors are normally based on the production of electrons by enzymatic catalysis. The target analyte is involved in the reaction, which takes place on the active electrode surface, and the ions produced create a potential, which is subtracted from that of the reference electrode to give a measurable signal [14,62]. Depending on the property to be measured by the detector system, electrochemical biosensors may be divided into conductometric, potentiometric and voltammetric/amperometric biosensors.

The ability of laccases to catalyze the oxidation of phenolic and non-phenolic compounds, coupled with the reduction of molecular oxygen to water, makes them valuable for commercial

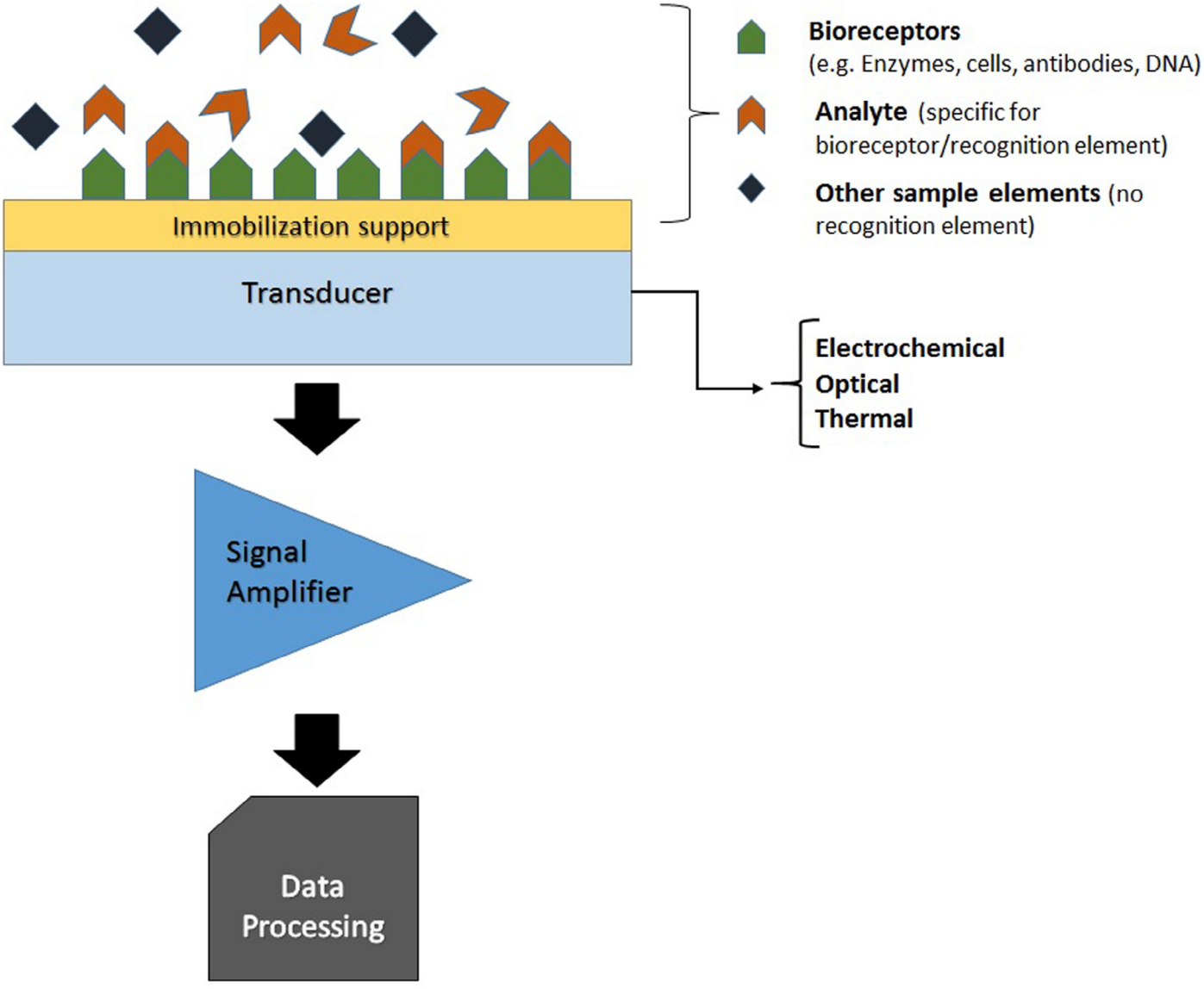

Fig. 3. Biosensor.

applications [35]. Characteristics, such as good mechanical and electronic properties, and their ability to transfer electrons make laccases good candidates to immobilize biomolecules. The surface of the electrodes allows stability and biocompatibility and promotes development and application of these techniques in the electrochemical field.

Conductometric biosensors measure changes in the conductance between a pair of metal electrodes as a consequence of the activity of a biological component; many enzyme reactions may be monitored by ion conductometric or impedimetric devices, using interdigitated microelectrodes [62].

Potentiometric measurements involve determination of the potential difference between an indicator and a reference electrode, or two reference electrodes separated by a selective membrane [62].

Voltammetric/amperometric biosensors measure the changes in the current on the working electrode due to direct oxidation of the products of a biochemical reaction [63].

4.1.1. Voltammetric sensors

Voltammetry in general is the measurement of the current that flows through an electrode as a function of the potential applied to it, and the result is a current/potential curve [64].

Fernandes et al. [65], described the construction of a biosensor based on laccase immobilized on microspheres of chitosan cross-linked with tripolyphosphate by spray drying. It is being used for rutin determination in pharmaceutical formulations, obtaining a bioelectrode that exhibits high sensitivity, good reproducibility, low detection threshold and rapid response. Santhiago et al. [66] designed a biosensor based on a carbon-paste electrode modified with laccase, produced by *Aspergillus oryzae* for the determination of l-cysteine in pharmaceutical formulations, performed in the presence of hydroquinone. Gupta et al. [67] used *Coriolus hirsutus* laccase immobilized onto amine-terminated thiol monolayers on a gold electrode to monitor catechol .

The use of biosensors based on nanocomposites is widely studied. Chen et al. [49] used zein, a natural biodegradable protein polymer, to design a new composite of laccase–gold nanoparticles (AuNPs)-cross-linked zein ultrafine fibers (CZUFs) for the determination of catechol. The results demonstrated that this biosensor possessed a high detection sensitivity, which was attributed to direct electron transfer (DET) [49].

Ionic liquids (ILs) have attracted considerable attention for electroanalysis because of their unusual physical and chemical properties, mainly resulting from their peculiar structural organization. Of particular interest are salts resulting from the combination of imidazolium cations with inorganic or organic anions that are liquid at room temperature [68]. They are widely used in laccase biosensors to prepare modified electrodes and in preparation and stabilization of nanomaterials, such as platinum NPs (PtNPs) and AuNPs dispersed in ILs [69–71].

Table 2 shows a comparison between voltammetric laccase biosensors.

4.1.2. Amperometric laccase biosensors

Amperometric sensors are a special classification of voltammetric sensors, where potential is kept constant as a function of time. The current generated by oxidation or reduction of redox species at the electrode surface, which is maintained at an appropriate electrical potential, is measured [62]. The current observed has a linear relationship with the concentration of the electroactive species. The electrode is usually constructed of platinum, gold or carbon. Adjacent to the electrode, entrapped by a membrane or directly immobilized, is the enzyme involved [14].

Recently, there was increasing focus on the use of nanomaterials, leading to the improvement of the analytical performance of enzyme electrodes. Timur et al. [78] developed a thick film sensor immobilizing laccases from different sources in a polyaniline (PANI) matrix; a conducting polymer with high conductivity, chemical durability and good environmental stability [78,79] for the determination of phenolic compounds. The measurement of oxygen consumption is related to the oxidation of the analyte.

Chawla et al. [63] described the construction of a biosensing platform for the determination of total phenolic content in fruit juices by fabricating nickel NPs (NiNPs) covered with carboxylated multiwalled-carbon nanotubes (cMWCNTs)/PANI composite electrodeposited onto a gold electrode and modified with laccase. This nano-composite-modified electrode combines the ability of CNTs and a conductive polymer to promote electron-transfer reactions with the advantages of entrapping biological material [80].

Rahman et al. [81] tested Den-AuNP nanocomposites to immobilize laccase (PDATT/ Den(AuNPs)/laccase) covalently to fabricate a third-generation catechin biosensor. The biosensor developed in this study is a promising tool for the detection of catechin in food and biological samples, combining physical and chemical properties of AuNPs and the surface reactivity of dendrimers.

Vianello et al. [82] presented a flow biosensor based on a monomolecular layer of laccase from *Rigidoporus lignosus* immobilized on a gold support. This biosensor detects phenols in the low micromolar range [i.e., below the European Community limits (0.5 mg/L)] [82].

Kulys et al. [83] employed, for the first time, recombinant fungal laccase from *Polyporus pinsitus* and thermostable recombinant laccase from *Myceliophthora thermophila* for biosensors using printed graphite electrodes suitable for continuous flow-through measurements of phenolic compounds in alarm systems.

In the food industry, to ensure the good quality of the final products, it is necessary to measure some attributes at different stages of the production process. For this reason, the development of novel analytical sensors in the food industry is needed. The determination of polyphenols in food, employing laccase biosensors, has been widely studied {e.g., in tea leaves at different stages of tea production [41] and commercial fruit juices [80]}.

There are very few papers that describe the application of laccase with the simultaneous use of other enzymes to obtain bi-enzymatic biosensors for the determination of phenolic compounds. Bauer et al. [84] developed an enzyme sensor to measure morphine based on laccase (Lac) and PQQ-dependent glucose dehydrogenase (GDH) immobilized on a Clark oxygen electrode. Laccase oxidizes morphine and the Clark electrode indicates the consumption of oxygen, which is then regenerated by glucose dehydrogenase, enhancing the assay sensitivity. Then, with the objective of discriminating between morphine and codeine in pharmaceutical drugs, a double detector was developed, using the morphine dehydrogenase (MDH)/salicylate dehydrogenase (SHL) – and the LACC/GDH sensors [84].

Tang, et al. [85] used a bi-enzyme horseradish peroxidase/laccase system for rapid, sensitive detection of *E. coli* density. Since the *E. coli* metabolism of salicylic acid (SA) forms polyphenolic compounds, the amount depending upon *E. coli* density, the biosensor was applied to detect *E. coli* density through oxidation of the polyphenols in the presence of laccase/HRP.

Table 3 shows a comparison between amperometric laccase biosensors.

4.1.3. Conductometric and potentiometric sensors

Conductometric detection involves monitoring changes in the electrical conductivity of the sample solution. As the composition of the medium changes in the course of the enzymatic reaction, whose charged products result in changes in ionic strength, the conductivity increases [120].

In the case of potentiometric sensors, the measuring principle is based on the Nernst equation through measurement of the potential between non-polarized electrodes (working electrode and

Table 2
Voltammetric laccase biosensors in terms of analytical characteristics, applications, type of immobilization and phenol analyte

Laccase sources	Laccase characteristics	Immobilization method	Measurements conditions	Electrode	Analyte	Analytical characteristics	Application	Real samples	Ref.
Aspergillus oryzae	Commercial laccase; 0.55 units/mg	Encapsulated in microspheres of chitosan cross-linked with tripolyphosphate	Acetate buffer pH 4.0; frequency 30 Hz; pulse amplitude 30 mV; and scan increment 2.0 mV	Printed graphite electrode (PGE), Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Rutin	Two linear ranges: 0.599–3.92 μm; and, 5.82–13.1 μm	Pharmaceutical analysis	Pharmaceutical formulations	[65]
Aspergillus oryzae	Commercial laccase genetically modified ; Denilite 800 U/g	Adsorption Graphite powder–Nujol–Pt.BMIPF $_6$	Phosphate buffer solution (0.1 M, pH 6.5); frequency 20 Hz; pulse amplitude 80 mV; scan 5.0 mV	Pt-BMI.PF6-laccase, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Adrenaline	Linear range 0.999–213 μm	Pharmaceutical analysis	Pharmaceutical formulations	[69]
Aspergillus oryzae	Commercial laccase genetically modified ; Denilite 800 U/g	Adsorption Graphite powder–Nujol–Pt.BMIPF $_6$	1.1 M acetate buffer solution (pH 5.0). +0.2 V	Pt-BMI.PF6-laccase, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Rosmarinic acid	Linear range 0.999–65.4 μm	Food analysis	Plant-extract samples	[68]
Aspergillus oryzae	Commercial laccase 0.29 U/ml	Cross-linked with cyanuric chloride (CC) in chitosan	0.1 M acetate buffer solution (pH 4.0); with frequency 50 Hz; pulse amplitude 100 mV and scan 5.0 mV	Lac-nanoparticles -BMI.PF $_6$ and Au/ Ag electrode	Luteolin	Linear range 0.099–5.825 μm	Food analysis	Chamomile-tea samples	[71]
Aspergillus oryzae	Commercial laccase genetically modified ; Denilite 800 U/g	Adsorption on carbon-paste electrode	Performed in the presence of hydroquinone and l-cysteine in 0.1 M phosphate buffer (pH 7.0) at an applied potential of – 0.08 V *versus* Ag/ AgCl	Carbon-paste electrode, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	L-cystein	Linear range 0.197–3.24 mm	Pharmaceutical analysis	Pharmaceutical formulations	[66]
Coriolus hirsutus	Commercial laccase; 40 U/mg	Covalently binding by glutaraldehyde on gold-thiol monolayers	1.1 M acetate buffer at pH 5	Gold electrode, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Catechol	Linear range 1–400 μm; sensitivity 15 μA/mm	Environmental analysis	Synthetic samples	[67]
Trametes versicolor	Commercial laccase	Entrapment using silica spheres as immobilization matrix	0.10 M PBS solution (pH 5.0)	Lac/Si/MWCNTs/ SPE and Ag/AgCl reference electrode	Dopamine	Linear range 1.3–85.5 μm; sensitivity 2.787×10^3 μA/mm	Clinical analysis	Synthetic samples	[72]
Aspergillus oryzae	Commercial laccase genetically modified ; Denilite 800 U/g	Adsorption on cellulose acetate /BMI·N(Tf)$_2$ support.	0.1 M acetate buffer solution (pH 5.5); frequency 90 Hz, pulse amplitude 100 mV and scan increment 4.0 mV	Lac-CA/BMI·N(Tf)2 and Ag/AgCl reference electrode	Methyldopa	Linear range 34.8–370.3 μm	Pharmaceutical analysis	Pharmaceutical formulations	[58]
Trametes versicolor	Commercial laccase	Adsorption on multi-walled carbon nanotubes-based paste electrode (MWCNTPE)	47.5 μm of 4-AMP; Britton-Robinson buffer at pH 5; scan rate of 50 mV/s	MWCNTPE, Ag/AgCl reference electrode and glassy carbon counter electrode	Pirimicarb (4-aminophenol as substrate)	Linear range 0.990–11.5 μm	Food analysis	Vegetables	[73]

(*continued on next page*)

Table 2 (*continued*)

Laccase sources	Laccase characteristics	Immobilization method	Measurements conditions	Electrode	Analyte	Analytical characteristics	Application	Real samples	Ref.
Aspergillus oryzae	Commercial laccase; Denilite II BASE	Adsorption Graphite powder–Nujol–ILs 1-butyl-3-methylimidazolium (BMI·Tf 2 N), 1-decyl-3-methylimidazolium (DMI·Tf 2 N) and 1-tetradecyl-3-methylimidazolium (TDMI·Tf 2 N)	1.1 M acetate buffer solution (pH 5.0). +0.2 V	Pt-BMI.PF6-laccase, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Rutin detection with BMI·Tf 2 N DMI·Tf 2 N TDMI·Tf 2 N	Linear ranges 4.77–46.2 μm 5.84–53.6 μm 5.84–53.6 μm	Pharmaceutical analysis	Pharmaceutical formulations	[74]
Cerrena unicolor	Purified laccase 2.23 mg/mL	Electrolytic deposition under galvanostatic conditions applying current of 1 mA	1.1 M phosphate–citrate buffer (pH 5.2); Scan rate –100 mV/s	Working platinum electrode; Pt counter electrode and saturated calomel reference electrode (SCE)	Hydroquinone	Linear range 2.0–60 μm Sensitivity 2.34 ± 0.11 μA/mm	Environmental analysis	Synthetic samples	[75]
N.r.	Activity ≥ 100 units/mg	Entrapment in Nafion matrix	0.1 m acetate buffer solution pH 5.0; scan rate 100 mV/s	Nafion/laccase-glassy carbon electrode as the working electrode, Pt wire counter electrode and Ag/AgCl reference electrode	Catechol	Linear range 0–7 μm	Environmental analysis	Real samples	[49]
Pleurotus ostreatus	Bi enzyme system Lac-Peroxidase; crude laccase extracts 15.9 U/mg	Adsorption carbon paste	0.1 M phosphate buffer solution pH 6.0 to 7.5; scan rate 40 mV/s, potential pulse 50 mV	Nujol /graphite powder laccase / peroxidase as working electrode, Ag/AgCl reference and platinum auxiliary electrodes	Dopamine adrenaline L -dopa isoprenaline	Linear ranges 6.6–390 μm 6.1–100 μm 6.7–70 μm 6.2–81 μm	Pharmaceutical analysis	Synthetic samples	[76]
Cerrena unicolor	Purified laccase; 1.62 mU/10 μL	Adsorption in graphite electrode	1.1 M citrate buffer pH 5.5; applied potential: – 50 mV	Graphite working electrode and Ag/AgCl reference electrode	Caffeic acid Prodelphinidin B3 Epicatechin gallate Catechin Epicatechin	Linear ranges 1–10 μm 1–10 μm 1–10 μm 4–40 μm 2–60 μm	Food analysis	Synthetic samples	[77]

$BMIPF_6$,1-n-butyl-3-methylimidazolium hexafluorophosphate; $BMIBF_4$, 1-n-butyl-3-methylimidazolium tetrafluoroborate; BMI·Tf_2N, 1-butyl-3-methylimidazolium; DMI·Tf_2N, 1-decyl-3-methylimidazolium; TDMI·Tf_2 N, 1-tetradecyl-3-methylimidazolium; CA, cellulose acetate; IL, Ionic liquid; MWCNT, Multiwalled carbon nanotube; N.r., not reported; SCE, Saturated calomel electrode; SPE, Screen-printed electrode.

Table 3
Amperometric laccase biosensors in terms of analytical characteristics, applications, type of immobilization and phenol analyte

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Trametes versicolor	Purified laccase; 400 U/mL (ABTS)	Entrapment by electrochemical deposition in polyaniline matrix	pH 4.5 (0.1 M acetate buffer); 35°C	Interdigitated sensors based on ceramic substrates with platinum working and reference electrode	Phenol catechol l -DOPA	Lineal ranges 0.40–6.0 μM 0.20–1.0 μM 2.0–20 μM	Environmental analysis	Synthetic wastewater sample	[78]
Aspergillus niger	genetically modified microorganisms; 430 U/ml (ABTS)				Phenol catechol l -DOPA	Lineal ranges 0.40–4.0 μM 0.4–15 μM 0.4–6.0 μM			
Ganoderma lucidium	Concentration of protein 40 mg mL^{-1}	Covalent binding and adsorption on NiNPs/cMWCNTs/PANI/Au	pH 5.5 (0.1 M acetate buffer) and 35°C; scan rate of 20 mV s^{-1}	Working electrode (NiNPs/cMWCNTs/PANI/AuE); reference electrode Ag/AgCl ; Pt wire as auxiliary electrode	Polyphenol/guaiacol	Linear range = 0.1–10 μM (lower range) and 10–500 μM (higher range); Sensitivity 0.694 μA μM^{-1} cm^{-2}	Food industry	Fruit juices of commercial brands	[63]
Coriolus hirsutus	Purified laccase	Covalent binding on DEAE-cellulose column	pH 4.5 (0.1 M citrate phosphate buffer); flow rate of 6 mL/min.	Oxygen electrode	Catechols	Linear range 0.1–10 mM	Food industry	Extract of tea leaves	[41]
Rhus vernicifera	Commercial enzyme	Covalent immobilization to PAMAM dendrimers	pH 6.5 PBS buffer, 30°C	PDATT/Den(AuNPs) on glassy-carbon electrode and Ag/AgCl electrode	Catechin	Linear range 0.1–10 μM	Clinical analysis/food industry	Real samples of green tea and human urine	[81]
Coriolus hirsutus	Bienzyme (GDH/LAC) system; Laccase culture ≈ 250 units/mL (catechol)	Entrapment in polyvinyl alcohol (PVA)	pH 6.5 (50 mM phosphate buffer, 10 mM glucose); flow rate of 300 μL/min	Clark oxygen electrode	Morphine	Detected 32 nM–100 μM	Pharmaceutical analysis	Real samples of morphine drug: MSI 20 (Mundipharma GmbH, Limburg/Lahn, Germany)	[84]
Trametes versicolor	Commercial laccase; 25 U/mg solid)	Entrapment in nanocomposite multiwall carbon nanotubes (MWCNTs)–chitosan (CS)	McIlvaine buffer, pH 4.50	Ag/AgCl reference electrode and gold (MWCNT)–chitosan (CS) as working electrode	Polyphenols: caffeic acid rosmarinic acid chlorogenic acid gallic acid Polyphenols: Caffeic acid rosmarinic acid chlorogenic acid gallic acid	Linear ranges 0.73–10.5 μM 0.9–12.1 μM 0.793–6.7 μM 0.79–2.1 μM Sensitivities 1.277 mA/mmol 0.846 mA/mmol 1.268 mA/mmol 3.450 mA/mmol	Food industry	*Salvia officinalis* and *Mentha piperita* extracts	[86]
Pleurotus ostreatus	Culture filtrates; 31.5 U /mL and total protein was 2.01 mg/mL	Absorption in carbon paste	Phosphate buffer solution, pH 7.0; Scan rate 30 mV s^{-1}; 1.2 units laccase/mg of carbon paste	Carbon paste-laccase as working electrode; an Ag/AgCl reference and platinum auxiliary electrode	Catecholamines: adrenaline dopamine	0.06–0.7 mM 0.07–0.4 mM	Pharmaceutical analysis	Pharmaceutical formulations	[87]
Coriolus versicolor	Bienzyme horseradish peroxidase /Laccase system; commercial	Covalent immobilization in indium tin oxide (ITO) electrode by (3-aminopropyl) triethoxysilane (APTES) monolayer	Potential applied of –0.05 V *versus* SCE in disodium hydrogenphosphate–citrate buffer solution (PCBS) of pH 6.0	Saturated calomel electrode (SCE) as reference electrode; gold wire as counter electrode and ITO electrode as working electrode	Polyphenolic compounds *of E. Coli* metabolism	1.6×10^3–1.0×10^7 cells/mL	Food industry, environmental and clinical analysis	*E. coli* solution samples	[85]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Pleurotus ostreatus	Purified laccase ; 300 U/mL	Electrostatic attachment to polyaniline (PANI)/ indium tin oxide (ITO)	Acetate buffer pH 5.5, 35°C, 4.5 μM phenol, potential 0.4 V	ITO glass plate as a working electrode, a platinum wire as counter electrode and Ag/AgCl as reference electrode	Phenol catechol	0.5–4.5 μM 0.4–15 μM	Environmental analysis	Synthetic waste water samples	[88]
Coriolus hirsutus	Purified laccase; 320–350 U/mg	Entrapment with gelatin and Nafion	Phosphate buffer pH 6	Modified glassy-carbon working electrode, platinum wire as counter electrode and Ag/AgCl as reference electrode	Dopamine	Up to 400 nM	Clinical analysis	Real sample	[89]
Coriolus hirsutus	Bienzyme (Tyrosinase/ Laccase) system; Laccase culture Filtrates; 40 U/mg	Adsorption in solid graphite electrode	0.1 M citrate buffer (pH 5.0); applied potential of –0.05; flow rate of 0.55 cm^3/min	Enzyme-modified graphite electrode , Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Catechol hydroquinone	Limit of detection 2 μM	Environmental analysis	Synthetic samples	[90]
Coriolus versicolor	Commercial enzyme	Entrapment in nanotubes–chitosan (CNTs–CS)	Phosphate buffer pH6; potential applied – 0.1 V.	CNTs–CS/GC electrode, Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Catechol	Linear range 1.2–30 μM; limit of detection 0.66 μM	Environmental analysis	Synthetic samples	[47]
Rigidoporus lignosus	(About 140 ng laccase/cm^2)	Self-assembled monolayer on a gold surface by carbodiimide chemistry	Flow rate of 100 μL/min, 0.1 M potassium phosphate buffer pH 6.5; applied potential –0.2 V	Gold surface was working electrode, SCE reference electrode and platinum wire auxiliary electrode	1,4-hydroquinone	Sensitivity of 3 nA/μM	Environmental analysis	Olive-oil wastewater.	[82]
Polyporus pinsitus	Recombinant fungal laccase	Cross-linking using bovine serum albumin and glutaraldehyde	50 mM acetate buffer pH 5.5 at 25°C.	Graphite, SCE as reference electrode and platinum wire as auxiliary electrode	Pyrocatechol 1-naphthol o-phenylenediamine	sensitivity 3.8 mA/M 1.2 mA/M 1.8 mA/M	Environmental analysis	Synthetic samples	[83]
Myceliophthora thermophila				Printed graphite electrode (PGE), Ag/AgCl reference electrode and platinum wire as auxiliary electrode	Pyrocatechol	Sensitivity 0.014 mA/M			
Trametes versicolor	Commercial enzyme	Cross-linked enzyme crystals embedded in polyvinylpropylidone (PVP) gel	0.1 M sodium-acetate buffer at pH 5.5.	Clarke-type electrode consists of a gold (Au) cathode and a reference Ag/AgCl electrode	2-amino phenol guaiacol catechol pyrogallol catechin	Linear range 0.1–0.5 mM	Food industry	Synthetic samples	[91]
N.r.	commercial laccase; 23.3 U/mg	covalently immobilized on the magnetic (Fe 3 O 4 –SiO 2) nanoparticles by glutaraldehyde	67 mM phosphate buffer solution pH5.5 ; applied potential of – 0.232 V	carbon paste electrode, saturated calomel electrode (SCE) and Pt wire electrode	Hydroquinone	0.01–137.5 μM	Environmental Analysis	Compost extracts	[92]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Trametes versicolor *Trametes hirsuta*	Commercial laccase; 30.6 U/mg 3.9 mg/mL in citrate buffer pH 5; activity: 421 U/mL	Entrapment with polyazetidine prepolymer	Flow system at a fixed potential of – 100 mV; Britton-Robinson buffer 0.1 M, pH 5	Multi-walled carbon nanotubes screen-printed electrode; Ag/AgCl as counter electrode; NHE as reference electrode	Gallic acid Gallic acid	0.587–99.92 μM 0.587–105.8 μM	Food industry	Wine samples	[93]
Cerrena unicolor	Tyrosinase/laccase bienzyme	Entrapment in titania gel matrix.	25°C; phosphate buffer pH6	Carbon electrode and saturated calomel electrode (SCE)	2,6-dimethoxyphenol 4-tertbutylcatechol 4-methylcatechol 3-chlorophenol catechol 2,6-dimethoxyphenol 4-tertbutylcatechol 4-methylcatechol 3-chlorophenol catechol	Linear ranges 1.2–6.1 μM 2.0–89.0 μM 0.21–15 μM 0.98–7.9 μM 0.20–23.0 μM Sensitivities 750.9 mA/M 684.4 mA/M 1635.5 mA/M 817.7 mA/M 5380.7 mA/M	Environmental analysis	Synthetic samples	[94]
Trametes versicolor	Tyrosinase/laccase bienzyme	Entrapment in sol–gel matrix of diglyceryl silane	Phosphate buffer 0.1 M containing KCl 0.1 M (pH 6.0); flow rate of 0.45 mL/min; V appl = +50 mV	Graphite screen-printed electrodes and Ag/AgCl reference electrode	Phenol gallic acid caffeic acid catechin	Sensitivities 11.067 μA/mM 0.339 μA/mM 1.218 μA/mM 0.435 μA/mM	Food industry	Must and wine samples	[95]
Trametes versicolor	Commercial laccase; 18,000 IU/mg	Entrapment within polyvinyl alcohol photopolymer PVA-AWP (azide-unit pendant water-soluble photopolymer)	0.1 M acetate buffer pH 4.7	Graphite screen-printed electrodes (SPE) and Ag/AgCl	Caffeic acid catechol hydroquinone resorcinol Caffeic acid catechol hydroquinone resorcinol	Linear ranges 0.5–130 μM 0.5–175 μM 1.1–130 μM 50–250 μM Sensitivities 24.91 nA/μM 18.83 nA/μM 9.44 nA/μM 0.110 nA/μM	Food industry	Tea infusions	[55]
N.r.	DeniLite (commercial product)	Covalent immobilization by glutaraldehyde	0.05 M phosphate buffer pH 5.5; applied potential 50 mV	Platinum electrode, platinum wire as counter electrode and Ag/AgCl	Catechol dopamine norepinephrine epinephrine Catechol dopamine norepinephrine epinephrine	Linear Range up to 58 μM up to 40 μM up to 55 μM up to 55 μM Sensitivity 210 nA/μM 75 nA/μM 60 nA/μM 45 nA/μM	Clinical analysis	Synthetic samples	[96],
Trametes versicolor 52J	Purified laccase 1000 U/mL	Entrapment in redox polymer [Os(bpy) 2 (PVI) 10 Cl]Cl, (Os(PVI) 10)	0.05 M acetate buffer, pH 4.5; scan rate of 5 mV/s	Os(PVI) 10-laccase electrode, Ag/AgCl reference electrode, a platinum wire auxiliary electrode and glassy carbon working electrodes	Epinephrine norepinephrine dopamine	Limits of detection 11 nM 8 nM 4 nM	Clinical analysis	Synthetic samples	[97]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Cerrena unicolor	Purified laccase 30.5 mU/10 μL	Adsorption in graphite electrode	0.1 M citrate buffer, pH 5.0	Laccase-modified graphite electrode, Ag/AgCl reference electrode	2,6-dimethoxyphenol	Linear range/ Sensitivity 0.1–2 μM/202.09 nA μM^{-1}	Food Industry, environmental and clinical analysis	Synthetic samples	[98]
					Coniferyl alcohol	0.2–6 μM/98.7 nA μM^{-1}			
					Ferulic acid	1–40 μM/69.63 nA μM^{-1}			
					Caffeic acid	1–10 μM/57.92 nA μM^{-1}			
					2-Aminophenol	1–8 μM/53.96 nA μM^{-1}			
					Dopac	1–10 μM/52.11 nA μM^{-1}			
					Hydroquinone	1–10 μM/50.99 nA μM^{-1}			
					Syringic acid	1–40 μM/44.65 nA μM^{-1}			
					ABTS	1–10 μM/ 38.63 nA μM^{-1}			
					4-aminophenol	1–10 μM/32.4 nA μM^{-1}			
					Acetosyringone	1–10 μM/29.0 nA μM^{-1}			
					Catechol	1–20 μM/24.4 nA μM^{-1}			
					Guaiacol	1–20 μM/22.38 nA μM^{-1}			
					3,4-dihydroxybenzoic	1–40 μM/14.7 nA μM^{-1}			
					Coniferyl aldehyde	1–20 μM/14.03 nA μM^{-1}			
					Dopamine	1–60 μM/11.08 nA μM^{-1}			
					4-methoxyphenol	1–100 μM/9.09 nA μM^{-1}			
					Dl-Noradrenaline	1–80 μM/6.26 nA μM^{-1}			
					3,4-Dihydroxybenz-aldehyde	10–150 μM/5.76 nA μM^{-1}			
					L-DOPA	1–40 μM/5.13 nA μM^{-1}			
					Vanillic acid	10–100 μM/3.45 nA μM^{-1}			
					Adrenaline	1–150 μM/3.16 nA μM^{-1}			
					Syringaldazine	20–100 μM/2.5 nA μM^{-1}			
					P-Cresol	10–1000 μM/0.44 nA μM^{-1}			
					Acetovanillone	10–100 μM/0.42 nA μM^{-1}			
					O-Cresol	10–1000 μM/ 0.289 nA μM^{-1}			
					Vanillin	10–400 μM/0.251 nA μM^{-1}			
					Phenol	1000–10000 μM/ 0.011 nA μM^{-1}			
					4-chlorophenol	1000–10000 μM/ 0.02 nA μM^{-1}			
					4-Hydroxybenz-aldehyde	1000–10000 μM/ 0.018 nA μM^{-1}			
Ganoderma sp. Rckk02	Purified laccase	Covalent binding by glutaradehyde + cysteamine monolayer	0.1 M acetate buffer, pH6; scan rate of 20 mV s^{-1}	Working electrode lac/AgNPs/ZnONPs/Au; reference electrode Ag/AgCl ; Pt wire as auxiliary electrode	Polyphenol/guaiacol	Linear range 10–500 μM Sensitivity 0.71 μA μM^{-1} cm^{-2}	Food industry	Wine samples	[99]
Trametes hirsute	Purified laccase 100 U/mg	Entrapment in polymers, cetyl ethyl poly-(ethyleneimine) and Nafion	Under steady-state conditions at room temperature; 0.1 M sodium citrate buffer solution, pH 5.0; T 20°C	Working electrodes: glassy-carbon/ CEPEI -laccase; laccase/Eastman electrode; laccase/ Nafion; SCE and platinum wire as reference and auxiliary electrodes	Hydroquinone	Linear range 0.1–3.0 μM	Environmental analysis	Synthetic samples	[100]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Ganoderma sp. Rckk02	Purified laccase	Covalent coupling onto Fe_3O_4 NPs/ cMWCNTs/ PANI/ Au	pH 6.0 (0.1 M sodium acetate buffer) and 35°C, when operated at 0.3 V *versus* Ag/AgCl	Lac/Fe $_3$O $_4$ NP/ cMWCNT/PANI/Au; Ag/AgCl reference electrode; platinum wire as auxiliary electrodes	Phenolic content/ guaiacol	0.1–10 μM (lower concentration range) and 10–500 μM (higher concentration range); Limit of detection 0.18 μM	Food industry	Tea infusions	[101]
T. versicolor	Purified laccase	Adsorption and covalently bound on graphite electrode	0.1 M phosphate buffer solution, pH 7.2 and T = 25°C	Graphite electrode ; Ag/AgCl reference electrode; platinum wire as auxiliary electrodes	Catechol	Linear range up to 0.1 mM Sensitivity 196 μA/mM	Environmental analysis	Synthetic samples	[102]
Trametes versicolor	Purified laccase	Adsorption on graphite electrode	0.1 M citrate buffer solution (at pH 5.0), flow rate 0.51 mL min^{-1}	Graphite electrode ; Ag/AgCl reference electrode; platinum wire as auxiliary electrodes	Coniferyl alcohol Syringic acid Ferulic acid 2-aminophenol Hydroquinone Coniferylaldehyde Dopac Guaiacol 4-aminophenol Catechol Dopamine 3,4-Dihydroxybenzoic acid 3,4-Dihydroxybenz-aldehyde L -DOPA Vanillic acid Dl -Noradrenaline P-Cresol Adrenaline DHBA Vanillin O-Cresol Phenol	0.1–2 μM 0.1–4 μM 0.1–6 μM 1–10 μM 0.5–8 μM 1–20 μM 1–10 μM 1.1–11 μM 1–10 μM 1–10 μM 1–10 μM 1–20 μM 1–40 μM 1–20 μM 10–100 μM 1–150 μM 10–100 μM 1–80 μM 1–150 μM 10–400 μM 100–4000 μM 1000–10000 μM	Food industry, environmental and clinical analysis	Synthetic samples	[103]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Trametes versicolor	Commercial laccase; 23.3 U/mg	Entrapment on Sonogel–carbon electrode (SNGC)	0.05 M acetate buffer solution of pH 5	Nafion-Lac/SNGC working electrode, Ag/AgCl and a platinum wire as reference and auxiliary electrodes	Caffeic acid	Linear range 0.04–2 μM Sensitivity 99.454 nA/μM	Food industry	Beer samples	[104]
					Ferulic acid	Linear range 0.04–2 μM Sensitivity 12.752 μM			
					Gallic acid	Linear range 0.1–22 μM Sensitivity 11.009 μM			
					(+)-Catechin	Linear range 0.04–3 μM Sensitivity 89.066 μM			
					(-)-Epicatechin	Linear range 0.04–8 μM Sensitivity 28.139 μM			
Coriolus hirsutus	Commercial laccase; 416 U/mg	Cross-linking using bovine serum albumin and glutaraldehyde	Phosphate buffer solution (pH 5.0, 10 mM)	Glassy carbon electrode, Ag/AgCl	P-chlorophenol, guaiacol, chloroguaiacol	Linear range 1.0–10.0 μM	Environmental analysis	Synthetic samples	[105]
Trametes versicolor	Commercial laccase; 22.6 U/mg	Entrapment in copper-ordered mesoporous carbon (Cu-OMC)/ chitosan (CS) film	pH 5.0 phosphate buffer solution 0.1 M	Au electrode and saturated calomel electrode (SCE)	Catechol	Linear range 0.67–15.75 μM	Environmental analysis	Synthetic samples	[106]
Pleurotus ostreatus	Purified laccase	Entrapment in agarose–guar gum composite biopolymer matrix	Acetate buffer pH 5.5, 35°C	Glassy carbon electrode; Ag/AgCl and a platinum wire as reference and auxiliary electrodes	Phenol	Linear range 0.5–4.5 μM	Environmental analysis	Synthetic samples	[107]
Trametes versicolor	Commercial laccase; 1.34 U/mg	Adsorption on the surface of the nanocomposite-Nafion	0.1 M acetate buffer pH 5.5	Carbon screen-printed-Pt-nanoparticles electrode; Ag/AgCl electrode	Caffeic acid	Linear range 0.2–2 μM	Food industry	Tea infusions	[108]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Trametes versicolor	Bienzyme system; Lac-Tyrosinase; commercial laccase; 23.3 U/mg	Entrapment on Sonogel–carbon electrode	0.05 M acetate buffer solution of pH 5	Nafion-Lac-tyr/ SNGC working electrode, Ag/AgCl and a platinum wire as reference and auxiliary electrodes	Caffeic acid Ferulic acid Gallic acid (+)-Catechin (-)-Epicatechin	Linear range 0.01–2 μM Sensitivity 167.53 nA/μM Linear range 0.03–2.5 μM Sensitivity 53.86 nA/μM Linear range 0.1–15 μM Sensitivity 14.10 nA/μM Linear range 0.01–36 μM Sensitivity 125.31 μM Linear range 0.01–9 μM Sensitivity 69.67 μM	Food industry	Beer samples	[104]
Trametes versicolor	Purified laccase; 350 U/mg	Cross-linking with gelatin and glutaraldehyde	Use of mediators: HBT (1-hydroxybenzotriazole), VLA (violuric acid) TEMPO (2,2,6,6-tetramethylpiperidine-N-oxyl radical); 0.05 M acetate buffer, pH 4.5 and 35°C	Dissolve oxygen electrode	Detection of paracetamol With HBT Without HBT	Linear range 0.5–3.0 μM 2.0–15.0 μM	Pharmaceutical analysis	Paracetamol samples	[109]
Trametes versicolor	Commercial laccase; 23.7 U/mg	Cross-linking with glutaraldehyde onto a glassy-carbon electrode	0.1 M citrate buffer pH 5.0	Glassy-carbon electrode, Ag/AgCl and a platinum wire as reference and auxiliary electrodes	Gallic acid Caffeic acid	Linear ranges 0.017–4.7 μM 0.0038–0.055 μM	Food industry	Wine samples	[1]
Ganoderma sp. Rckk02	Purified laccase	Covalent binding and adsorption on CuNPs/ cMWCNTs/ PANI/Au	pH 6 (0.1 M acetate buffer) and 35°C; scan rate of 50 mV s^{-1}	Working electrode (CuNPs/cMWCNTs/ PANI/AuE); reference electrode Ag/AgCl ; Pt wire as auxiliary electrode	Polyphenol/guaiacol	Linear range 1–500 μM Sensitivity 0.694 μA μM^{-1} cm^{-2}	Food industry	Synthetic samples	[80]
Trametes versicolor	Purified laccase; 0.5 U/mg	Entrapment into nanocomposite matrix osmium tetroxide on poly 4-vinylpyridine multiwall carbon nanotubes (MWCNT)	0.1 M sodium citrate buffer solution pH 4.7	Glassy-carbon electrode; reference electrode Ag/AgCl ; Pt wire as auxiliary electrode	Pyrocatechol	Linear range 3.98–16.71 nM Sensitivity 3.82 ± 0.31 nA/nM	Environmental analysis	Environmental samples	[110]
Cerrena unicolor	Purified laccase; 2.23 mg/ml	Electrolytic deposition under galvanostatic conditions applying current 1 mA.	Acetate buffer of pH 5.2	Working platinum electrode; Pt counter electrode and saturated calomel reference electrode (SCE)	O-aminophenol catechol phenol	N.r.	Environmental analysis	Environmental samples	[111]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
N.r.	Commercial laccase	Entrapment into matrix Nafion-carbon nanofibers (CNFs) and copper/carbon composite nanofibers (Cu/CNFs)	0.1 M acetate buffer pH 6.0	Glassy-carbon working electrode; platinum wire as the counter electrode, and Ag/AgCl as reference electrode	Catechol	Linear range 0.00995–1.13 mM Sensitivity 19.9 μA/mM Limit of detection 3.32 μM Cu/CNFs/Lac/Nafion/GCE Linear Range 0.00995–9.76 mM Sensitivity 33.1 μA/mM Limit of detection 1.18 μM	Environmental analysis	Synthetic samples	[112]
Trametes versicolor	Commercial laccase; 23.75 U /mg	Covalent binding by cross-linking with glutaraldehyde on gold electrodes	0.1 M citrate buffer of pH 5.0	Gold electrode; platinum wire as the counter electrode, and Ag/AgCl as reference electrode	Caffeic acid Catechol Dopac 4-Hydroxyphenylacetic acid 4-hydroxyphenyl-ethanol 3-Hydroxybenzoic acid 4-Hydroxyphenyl-propionic acid 4-Hydroxybenzoic acid	0.1–1 0 μM 0.1–10 μM 0.2–10 μM 10–100 μM 10–100 μM 10–100 μM 100–1000 μM 100–1000 μM	Environmental analysis	Olive-oil-mill wastewaters	[57]
N.r.	Commercial laccase; 1.34 U/mg	Adsorption onto the SiO_2–PA/GCE	0.1 M phosphate buffer solution, pH 6.0; applied potential +0.22 V.	Laccase/(h-SiO_2 –PA)/Glassy-carbon electrode; reference electrode saturated calomel electrode (SCE)	Dopamine	Linear range 0.99 – 138.40 μM Limit of detection 0.17 μM	Pharmaceutical analysis	Pharmaceutical samples and rabbit-blood serums	[113]
Trametes versicolor	Commercial laccase; 20 U/mg	Adsorption on the surface of thionine–carbon black electrode	0.05 M citrate buffer at pH 4.5; applied potential –200 mV	Screen-printed electrodes and internal Ag pseudo-reference electrode	Bisphenol A	Linear range 0.5–50 μM Sensitivity 5.0 ± 0.1 nA/μM	Food industry	Tomato-juice samples	[114]
Trametes versicolor	Commercial laccase; 23.3 U/mg	Entrapment in magnetic carbon paste -chitosan/silica membrane	67 mM phosphate buffer solution, pH 5.6	Magnetic carbon-paste electrode; reference electrode saturated calomel electrode (SCE)	Catechol	0.1 – 165 μM	Environmental analysis	Synthetic samples	[115]

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Table 3 (*continued*)

Laccase sources	Laccase characteristics	Immobilization type/support	Measurement conditions	Electrode	Analyte	Analytical characteristics	Application	Type of samples	Ref.
Trametes versicolor	Commercial laccase; 26 U/mg	Adsorption on gold screen-printed electrode	0.1 M citrate buffer pH 4.5; applied potential 0.200 V	Gold screen-printed; platinum wire as counter electrode, and Ag/AgCl as reference electrode	Caffeic acid Chlorogenic acid Gallic acid Rosmarinic acid	Linear range 3–15 μM Sensitivity 245.3 nA/μM Linear range 3–15 μM Sensitivity 255.0 nA/μM Linear range 2–7 μM Sensitivity 1.1 nA/μM Linear range 3–15 μM Sensitivity 173.6 nA/μM	Food analysis	Plant extracts	[116]
Trametes hirsuta *Trametes versicolor*	Commercial laccase; 421 U/mg commercial laccase; 30.6 U / mg	Entrapment in polyazetidine prepolymer (PAP) onto multi-walled carbon nanotubes	0.1 M Britton-Robinson (B-R) buffer, pH 5.5; fixed potential –100 mV *versus*	Laccase–PAP–MWCNTs working electrode ; graphite counter electrode and Ag/AgCl reference electrode	Gallic acid Caffeic acid Gallic acid Caffeic acid	Linear ranges 4.64–116.94 μM 0.16–3.33 μM Linear ranges 6.28–288.97 μM 0.16–4.38 μM	Food analysis	Wine samples	[93]
Trametes versicolor	Commercial laccase ≥ 10 U/mg	Entrapment in PDA-NiCNFs composite	pH 5.5, 0.2 M acetate buffer solution	Magnetic glassy-carbon electrode (PDA-Lac-NiCNFs/MGCE); platinum wire as counter electrode, and Ag/AgCl as reference electrode	Catechol	Linear range 1–9100 μM Sensitivity 25 μA/mM	Environmental analysis	Water samples	[117]
Tramestes versicolor	Commercial laccase; 21.8 U/mg	Covalent immobilization in nanocomposite matrix using glutaraldehyde	0.1 M citrate buffer, pH 5.0	Nanocomposites composed of NH_2 -functionalized carbon nanotubes (CNT-NH_2), gold nanoparticles (AuNPs)GC/CNT-NH 2 /AuNPs/Lac-BSA	Caffeic acid Catechin Gallic acid Chlorogenic acid Trolox	Linear range/ Sensitivity 0.3–45 μM/ 0.753 μA/μM 1.7–30 μM/ 0.142 μA/μM 3.0–60 μM/ 0.169 μA/μM 1.5–30 μM/ 0.207 μA/μM 2.0–35 μM/ 0.879 μA/μM	Food industry	Plant extracts and tea infusions	[118]
N.r.	Bienzyme system lac-tyrosinase; commercial laccase DeniLite	Covalent immobilization on platinum electrode	0.05 M phosphate buffer solution pH 6.0	Platinum disk working electrode, platinum wire counter electrode and Ag/AgCl reference electrode	P-phenylenediamine (PPD) p-chlorophenol	Sensitivity 560 nA/μM 195 nA/μM	Environmental analysis	Synthetic samples	[119]
Cerrena unicolor	Bienzyme system lac-tyrosinase; purified laccase	Entrapment in titania gel matrix	Phosphate buffer at pH 6; 25°C	Platinum wire as the counter electrode, saturated calomel electrode (SCE) as reference electrode	2,6-dimethoxyphenol 4-tertbutylcatechol 4-methylcatechol 3-chlorophenol Catechol	Linear rages 1.2–6.1 μM 2–89 μM 0.21–15 μM 0.98–7.9 μM 0.223 μM	Environmental analysis		[94]

CEPEI, Cetyl ethyl poly(ethyleneimine); cMWCNT, Carboxylated multiwalled carbon nanotube; CNT–CS/GC, Carbon nanotube-chitosan-glassy carbon; NP, Nanoparticle; N.r., Not reported; PAMAM, Polyamidoamine; PANI, Polyaniline.

reference electrode) [62]. The potentiometric sensors use an electrochemical cell with two reference electrodes to measure the potential across a membrane that selectively reacts with the charged ion of interest.

These chemical sensors can be turned into biosensors by coating them with a biological element, such as an enzyme that catalyzes a reaction to produce the ion that the underlying electrode is designed to detect [120]. Since the reactions catalyzed by laccases proceed by the monoelectronic oxidation of a suitable substrate molecule (phenols and aromatic or aliphatic amines) to the corresponding reactive radical (a free cation) [121], the use of laccases would be suitable for the design of biosensors employing both transduction principles. However, a comprehensive search of publications about laccase biosensors using conductometric and potentiometric principles showed a lack of research in this field, making it a target area for future research.

4.2. Optical biosensors

During the enzymatic reaction of laccase enzymes, intermediates appear, with different spectroscopic properties [122]. Sanz et al. [123] followed the enzymatic reaction of laccase with phenol using these intrinsic spectroscopic properties, (molecular absorption and fluorescence) using a lac-polyacrylamide sensor film. The results observed in the study show that the lifespan of the sensor film does not depend on the storage time (stored for at least 6 months) but only on the number of measurements performed by the device [123].

The ability of laccase to oxidize methoxyphenols in the presence of 3-methyl-2 benzothiazolinonehydrazone (MBTH) to produce azo-dye compounds was studied by Setti et al. [124]. Based on this concept, Abdullah, et al. [124] developed an optical biosensor using stacked films of MBTH in hybrid Nafion/sol-gel silicate and laccase in chitosan for the detection of phenolic compounds. The same study also demonstrated that laccase immobilization in this hybrid material enabled the biosensor to be more selective to catechol, as compared with another analytes, such as guaiacol, o-cresol and m-cresol that were also tested.

Huang et al. [125] fabricated a fiber-optic biosensor for the determination of adrenaline. They immobilized laccase on CuTAPc-Fe_3O_4-NPs to catalyze the oxidation of adrenaline and to detect the consumption of oxygen with a fluorescent oxygen-sensing membrane. The adrenaline oxidation leads to a fluorescence change because the molecular oxygen acts as a dynamic quencher of fluorescence [126]. Ferreira et al. [44] combined the HPLC technique with an optic-fiber detection system using laccase as a sensing bioelement (HPLC–LacOF) for quantification of catecholamines (epinephrine, dopamine and norepinephrine) in biological fluids (i.e., plasma and urine). The system was based on changes in the refractive index of the optic-fiber-sensitive cladding (laccase + alginate matrix) caused by the linkage of the catecholamines eluted from the HPLC.

Zhang, et al. [126] developed a method based on the characterization of a luminol–H_2O_2–laccase reaction for the detection of *E. coli* O157:H7, using the ability of laccase to oxidize in a strong alkaline medium, which was compatible with the luminol system.

Table 4 summarizes laccase biosensors that employ optical principles for the detection of phenols.

4.3. Thermal sensors

Calorimetric or thermal sensors work by determining the presence or the concentration of a chemical species by measuring its enthalpy change [129].

Bai et al. [129] developed a thermometric biosensor using a poly(vinyl alcohol) (PVA) microsphere support where *Trametes versicolor* laccase was immobilized by the inverse suspension cross-linked

Table 4
Optical laccase biosensor classified in terms of analytical characteristics, applications, type of immobilization and phenol analyte

Transduction	Laccase species	Laccase characteristics	Immobilization type	Measurements conditions	Analyte	Analytical characteristics	Application	Real samples	Ref.
Optical/fluorescence	*Pycnoporus sanguineus*	10 U of laccase immobilized, 0.25 μg/mL ABTS	Covalently with glutaraldehyde on CuTAPc-Fe_3O_4	pH 7.0 and 25 °C; use of ABTS as mediator.	Adrenaline	Linear range 0.2–0.09 μM	Clinical analysis	Synthetic sample	[125]
Optical/absorption	N.r.	Commercial laccase; 0.004 U/μL	Cross-linking with glutaraldehyde in chitosan film	50 mM phosphate buffer solution pH 6.0; wavelength of 505 nm	Catechol	Linear range 0.5–8.0 mM	Environmental analysis	Synthetic sample	[127]
Optical/absorption	*Trametes versicolor*	Commercial laccase; 21.8 U/mg solid	Absorption in polyacrylamide film	Phosphate buffer solution pH 6.0; wavelength of 400 nm	Phenol	Linear range 0.109–2.5 mM Limit of quantification: 0.109 mM	Environmental analysis	Wastewater	[123]
Optical fiber	*Trametes versicolor*	Commercial laccase; 22.4 U/mg	Entrapment in alginate matrix	Column PLRP-S 100 Å 5 μm, 150 mm × 4.6 mm ID reversed-phase . Mobile phase: 5% ACN, 0.025 M sodium phosphate, 0.025 M citric acid, 0.001 M heptane and H_2SO_4 acid, pH 2.85; constant flow rate of 0.75 mL/min	Catecholamines: epinephrine dopamine norepinephrine	5–125 pg/mL	Clinical analysis	Samples of plasma and human urine	[128]

CuTAPc, Copper tetra-aminophthalocyanine; N.r., Not reported.

Table 5
Transduction methods. A comparative analysis of limits of detection, lifetimes and response times of laccase biosensors

Species	Transduction/biosensor	Analyte	Limit of detection	Useful lifetime	Response time	Ref.
Pycnoporus sanguineus	Optical/fluorescence	Adrenaline	N.r.	72 h in continuous use and 1 month stored at 4°C	30 s	[125]
N.r	Optical/absorption	Catechol	1.33 M	2 months	10 min	[127]
Trametes versicolor	Optical/absorption	Phenol	3.27 μM	15–30 measurements	N.r.	[123]
Trametes versicolor	Optical fiber	Epinephrine dopamine norepinephrine	3.5 pg/mL 2.9 pg/mL 3.3 pg/mL	2 months of continuous operation	7 min	[44]
Trametes versicolor	Amperometric	Polyphenols: Caffeic acid Rosmarinic acid chlorogenic acid gallic acid	0.151 μM 0.233 μM 0.161 μM -	15 measurements; 10% decrease	2 min	[86]
Trametes versicolor (TvL) *Aspergillus niger* (AnL)	Amperometric	Phenol Catechol l -DOPA Phenol Catechol l -DOPA	N.r. N.r.	35 cycles in 8 h; 2.5% and 6.7% decreases for TvL and AnL, respectively	300 s (200 s of stabilization and 100 s for reaction)	[78]
Ganoderma lucidium	Amperometric	Total phenolic content	0.05 μM	200 cycles over a period of 120 days (stored at 4°C)	8 s	[80]
Coriolus hirsutus	Amperometric	Catechol	N.r.	500 cycles	100 s	[41]
Rhus vernicifera	Amperometric	Catechin	0.05 ± 0.003 μM	N.r.	<10 s	[81]
Coriolus hirsutus	Bienzyme amperometric biosensor	Morphine	32 nM (with amplification)	N.r.	<1 min	[84]
Pleurotus ostreatus	Amperometric	Catecholamines: adrenaline dopamine	7.9 μM 9.8 μM	After 14 days (over 240 measurements) 25% decrease	N.r.	[87]
Pleurotus ostreatus	Amperometric	Phenol catechol	N.r.	25 days (175 measurements), decrease 30%	N.r.	[88]
Coriolus hirsutus	Amperometric	Dopamine	10 nM	14 days 21 days decrease 50%	110–160 s	[89]
Rigidoporus lignosus	Amperometric	1,4-hydroquinone	2 μM	35 days stored at 4°C; 15 days with continuous flow at 100 μL/min	N.r.	[82]
Aspergillus oryzae	Voltammetry	Rutin	0.0623 μM 0.712 μM (based on two linear ranges)	320 days (at least 930 determinations)	N.r.	[65]
N.r	Amperometric	Hydroquinone	15 nM	15 days with a negligible decrease; after 40 days, decrease 30%	60 s	[92]
Aspergillus oryzae	Voltammetry	Adrenaline	0.293 μM	90 days; 300 determinations	N.r.	[69]
Aspergillus oryzae	Voltammetry	Rosmarinic acid	0.188 μM	(300 days; 920 determinations)	N.r.	[68]
Trametes versicolor	Amperometric	Gallic acid	0.587 μM	10 days	N.r.	[40]
Trametes versicolor	Voltammetry	Dopamine	0.42 μM	30 days	N.r.	[117]

(*continued on next page*)

Table 5 (*continued*)

Species	Transduction/biosensor	Analyte	Limit of detection	Useful lifetime	Response time	Ref.
Aspergillus oryzae	Voltammetry	Methyldopa	5.5 M.	60 days; at least 350 determinations	N.r.	[58]
Trametes versicolor	Amperometric	Caffeic acid catechol hydroquinone resorcinol	0.524 μM 0.558 μM 1.071 μM 35.432 μM	>6 months	10 min	[55]
DeniLite (commercial product)	Amperometric	Catechol dopamine norepinephrine epinephrine	0.07 μM 0.2 μM 0.3 μM 0.4 μM	40–50 days	2 s	[96]
Trametes versicolor 52J	Amperometric	Epinephrine norepinephrine dopamine	11 nM 8 nM 4 nM	1 month	5 s	[97]
Trametes hirsute	Amperometric	Hydroquinone pyrocatechol	0.035 μM 0.05 μM	10 cycles	2 min	[100]
Ganoderma sp. Rckk02	Amperometric	Guaiacol	0.03 μM	150 uses over a period of 4 months; 25% decrease	3 s	[63]
T. versicolor	Amperometric	Catechol	N.r.	30 days	N.r.	[102]
Trametes versicolor	Amperometric	Catechol	0.67 μM	1 month	N.r.	[106]
Pleurotus ostreatus	Amperometric	Phenol	8 μM	2 months	N.r.	[107]
Aspergillus oryzae	Voltammetry	Rutin detection with BMI·Tf 2 N DMI·Tf $_2$ N TDMI·Tf $_2$ N	0.45 μM 0.689 μM 0.775 μM	270 days (over 850 samples)	N.r.	[74]
Trametes versicolor	Amperometric	Caffeic acid	0.09 μM	6 weeks	60 s	[108]
Cerrena unicolor	Voltammetry	Hydroquinone	0.93 μM	4 months	20 s	[75]
Ganoderma sp. Rckk02	Amperometric	Guaiacol	0.156 μM	7 months (300 measurements)	4 s	[80]
N.r	Voltammetry	Catechol	0.166 μM	1 month	N.r.	[49]
Trametes versicolor	Amperometric	Pyrocatechol	2.82 nM	3 weeks	N.r.	[110]
Cerrena unicolor	Amperometric	O-Aminophenol catechol phenol	N.r.	2 months	120 ± 20 s 140 ± 20 s 300 ± 20 s	[111]
Trametes versicolor	Amperometric	Catechol	0.0334 μM	30 days; 10% decrease	50 s	[115]
Pleurotus ostreatus	Voltammetry	Dopamine adrenaline L -dopa isoprenaline	0.027 μM 0.025 μM 0.024 μM 0.026 μM	2 months (500 determinations)	N.r.	[87]

$BMIPF_6$,1-n-butyl-3-methylimidazolium hexafluorophosphate; $BMIBF_4$, 1-n-butyl-3-methylimidazolium tetrafluoroborate; BMI·Tf_2N, 1-butyl-3-methylimidazolium; DMI·Tf_2N, 1-decyl-3-methylimidazolium; TDMI·Tf $_2$ N, 1-tetradecyl-3-methylimidazolium; N.r., Not reported.

method. The enzyme reactions were monitored by changes in the enthalpy of the reaction system.

A comprehensive search of publications on laccase biosensors using this transduction principle found very little research in this field, making it a target for future work.

5. Comparative analysis of laccase biosensors

Table 5 shows a comparative analysis of the transduction methods used in laccase biosensors regarding the limit of detection (LOD), stability, response time and lifespan of the device. In the case of electrochemical biosensors, we notice that amperometric principles are widely used in design, using mainly laccase sourced from *Trametes, Aspergillus* and *Ganoderma* genera.

The best lifespan was obtained by electrochemical devices, with stability of up to 10 months and at least 900 measurements.

The biosensor using a strain from *Ganoderma lucidium* showed a lifespan of 4 months with stability of up to 200 measurements without loss of activity.

Although electrochemical sensors have the advantages of quick response, cost efficiency and simplified operation, they also have disadvantages, such as degradation of the electrode surface by the continuous flow, leading to a lower lifespan and stability, in contrast with optical biosensors, which achieved lower LODs and longer stability.

Optical biosensors, in turn, show other disadvantages, such as requiring multi-step assays, and often large and expensive equipment, such as fluorescence, that requires labeled molecules. In this context, Ferreira et al. [44] developed an optical sensor based on *Trametes versicolor* immobilized onto an optical fiber to detect catecholamines, which maintained its stability for a period of two months of continuous operation and showed the best LOD, detecting trace levels (pg/mL).

6. Global analysis of laccase biosensors by research field

We conducted a literature review on the status and progress of research on laccase biosensor design during the period 1992–2014. The methodology was based on a bibliometric analysis, previously described elsewhere [130]. The bibliometric methodologies referred to enumeration and statistical analysis of scientific output in the form of articles, publications, citations, patents and other indicators [131].

Our literature research was based on the following: the transduction principle used the source of laccase and the immobilization technique applied. This study used the number of Web publications in the Scopus database as a reference, as has been done for other studies from different areas of knowledge such as chemical engineering, environmental sciences, separation and purification technologies [132–141]. Scopus was used as the database of choice, since it covers most of the journals included in the Thomson Reuters Web of Science [142].

Briefly, the methodology consisted of an extensive literature search (articles and conference papers) based on keywords that were matched with the article title, abstract and keywords. The keywords were the following:

- ***electrochemical principle***: laccase, biosensor, electrochemical, amperometric, voltammetric, potentiometric, conductometric, chemomechanical;
- ***optical principle***: laccase, biosensor, surface-plasmon resonance, fluorescence and absorption;
- ***thermal principle***: laccase, biosensor, thermal;
- ***organisms used as sources of laccase***: laccase, biosensor, *Trametes, Aspergillus, Ganoderma, Coriolus, Rhus Pleurotus* and *Pycnoporus*; and,
- ***immobilization technique***: laccase, biosensor, adsorption, cross-linking, covalent, carbon nanotubes, coating, and entrapment.

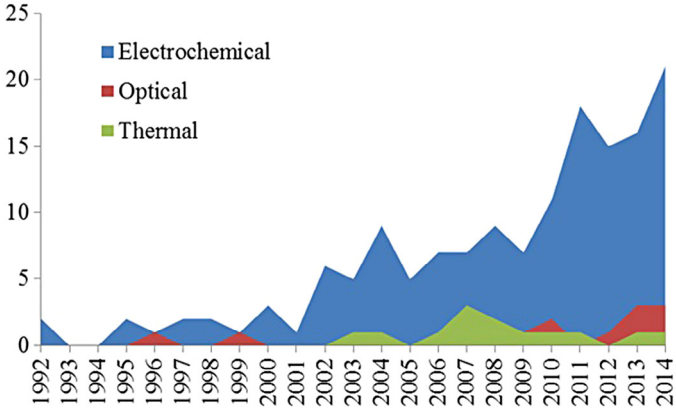

Fig. 4. Evolution of scientific research on transduction principles used for laccase-based biosensors (1992–2014).

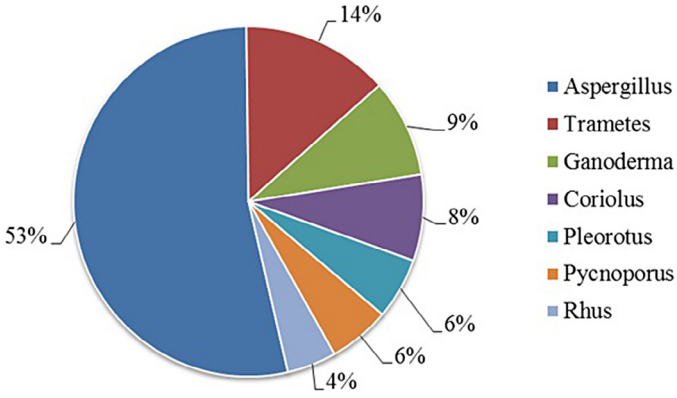

Fig. 5. Genera of organisms used as sources of laccases for biosensors worldwide (1992–2014).

We found that the electrochemical principle was the most studied method for biosensor design with laccases (Fig. 4), where 153 documents have been published. Since 2001, scientific production in this field has grown considerably. By comparison, optical and thermal biosensors using laccases received relatively little attention, with only 13 documents each. However, a deeper look into thermal research published found that this principle is only mentioned, but has not been developed.

Fig. 5 shows the main genera of organisms used as laccase source for biosensor design during the period 1992–2014 worldwide. Of the sources of laccase, the genus *Aspergillus* is the most widely used in biosensors, contributing to about 53% of laccase followed by *Trametes* (14%) *Ganoderma* (9%), *Coriolus* (8%), *Pleurotus* (6%), *Pycnoporus* (6%) and finally *Rhus* (4%). We note that more research needs to be developed for *Rhus, Pycnoporus* and *Pleurotus* as laccase-production organisms for biosensors.

Finally, Fig. 6 shows the immobilization techniques used for the design of biosensors using laccase. It can be seen that the main method is covalent immobilization (32%) and carbon nanotubes (23%), followed by adsorption (16%), cross-linking (16%), entrapment (8%) and coating (5%).

7. Barriers and solutions for laccase biosensors

Laccase biosensors show great potential for use in the food industry, environmental monitoring and biomedical analysis. Despite

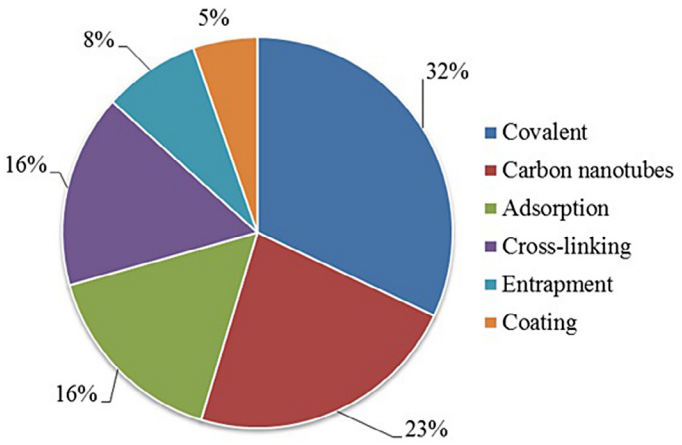

Fig. 6. Immobilization techniques used for laccase-based biosensors worldwide (1992–2014).

the advantages of laccase biosensors, some problems need to be addressed in order to generalize their use in industrial processes.

Since immobilization, one of the main steps in biosensor fabrication, might reduce enzymatic activity, other technologies could be coupled to counteract this effect. Incorporation of nanomaterials has been demonstrated to improve sensitivity and overall performance of enzymatic biosensors, especially with AuNPs, CNTs and graphene [143].

Although the electrochemical principle is the most studied in terms of laccase biosensors, there is little research regarding conductometric and potentiometric principles. The lack of research in these transducers is probably due to the instability and short life of the reactive radicals (charged products) produced by laccases, which cannot be detected before they undergo further oxidations and non-enzymatic reactions (e.g., hydration, disproportion or polymerization) [144]. However, amperometry is the transducer most used [145]. Amperometric detection is commonly used with biocatalytic sensors because of its simplicity and advantages, since the fixed potential during amperometric detection results in a negligible charging current (the current needed to apply the potential to the system), which minimizes the background signal that adversely affects the LOD [120]. Another advantage of amperometric detection is the significantly enhanced mass transport to the electrode surface. The high selectivity due to the oxidation or reduction potential used for detection is characteristic of the analyte species [59]. The high costs related to enzyme production, immobilization and biosensor fabrication might reduce its feasibility. Electrochemical sensors, based on silicon, show great potential for batch fabrication and could therefore be best suited for disposable sensors [146].

Electrochemical interference from substances, such as paracetamol, ascorbic acid or uric acid, can disturb an accurate measurement if the biosensor is operated at high applied potentials. This might be overcome by using a laccase-covered electrode and a blank electrode [147].

Enzyme instability, and consequently insufficient performance, are the main problems in microelectronic amperometric devices. Membrane fouling caused by protein adsorption leads to a decrease in sensitivity. A combination of different technologies, such as electropolymerization and photo-patternable enzyme membranes, can lead to reliable biosensor systems [147].

8. Conclusions

The use of laccase biosensors shows great potential for detection and quantification of phenolic compounds, which are regulated by different directives, such as the European Community Directive, Japan's Ministry of Health and Labor and Welfare, and the US EPA. Conventional detection methods, such as spectrophotometry, gas chromatography, liquid chromatography and capillary electrophoresis, are time consuming and expensive. The main transduction methods for laccase biosensors are electrochemical (amperometric, voltammetric, potentiometric, conductometric), optical and thermal. The most widely studied and used of these methods for laccase biosensors is the amperometric, whereas thermal and conductometric principles are the least studied and applied. Optical biosensors provide a higher sensitivity, which could be used in the food industry or in the biomedical field, where high quality is required.

In order to expand their use for industrial processes, some problems need to be tackled, which could be done by integrating inexpensive supports and immobilization techniques to avoid enzyme leakage, improving the activity recovery during the immobilization process or by incorporating nanomaterials.

Laccase biosensors represent a fast method for monitoring online and *in situ* phenolic compounds, with high sensitivity and reproducibility, and can be standardized for food, environmental and medical industries.

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