

Detection of Phenolic Compounds by Laccase Biosensors: A Review

Twinkle Dahiya¹, Minakshi Sharma², JS Rana^{1*} and Chandra S Pundir^{3*}

¹Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, India

²Department of Zoology, Maharishi Dayanand University, Rohtak-124001, India

³Department of Biochemistry, Maharishi Dayanand University, Rohtak-124001, India

ABSTRACT

Laccase [EC 1.10.3.2] is the most extensively studied copper-containing oxido-reductase enzyme that can be isolated from bacteria, fungi and plants. Laccase catalyses the oxidation reaction i.e. electron-transfer from phenolic compounds in the presence of molecular oxygen without requiring a cofactor. It had high potential for oxidation of phenols and benzenediols. Several traditional methods were followed for detection of phenolic compounds like spectrophotometry and chromatography but these are in-efficient, expensive and required trained personal to operate. These limitations were overcome by laccase-based biosensors, which are simple, sensitive, highly specific, rapid and cost-effective. This review article describes various types of immobilization of laccase onto transducer in laccase biosensors, their mechanism of action, selectivity, sensitivity and applications in different fields.

*Corresponding author

Chandra S Pundir, Department of Biochemistry, Maharishi Dayanand University, Rohtak-124001, India. ORCID ID: 0000-0008-9262-4210.

JS Rana, Department of Biotechnology, Deenbandhu Chhotu Ram University of Science and Technology, Murthal-131039, India. ORCID ID: 0000-0002-0093-0537.

Received: May 30, 2025; **Accepted:** June 03, 2025; **Published:** June 13, 2025

Keywords: Biosensors, Laccase, Laccase Biosensors, Oxidation of Phenols, Detection of Phenols, Use of Laccase

Introduction

Laccase [EC 1.10.3.2] was described firstly in plant exudates *Rhus vernicifera* in 1883 and a part of multinuclear-copper-oxidase family [1]. Laccases are generally monomeric, dimeric and tetrameric glycoprotein having 50 to 130 kDa molecular weight. These are usually glycosylated proteins and found as monomer or homodimer with generally fewer saccharide units including mannose, arabinose, fucose and hexoamines, which constitute 10 – 45% of enzyme mass [2]. Various useful properties of laccase are attributed to glycosylation like- copper retention, thermal stability and proteolytic susceptibility activity [3]. Since laccase catalyses the oxidation of a broad spectrum of compounds (phenols and non-phenolic aromatic hydrocarbons), it has been a great choice of research for long time. Phenolic compounds are spread all over the world. These can be easily found in fruits and vegetables, and are responsible for the organoleptic properties of some food products, such as wine and olive oil. Its broader range of substrate specificity allows it to degrade several xenobiotics like benzenethiols and anilines [4]. The enzyme is ubiquitous in nature and detected in different bacteria, fungi, some higher plants and in many insects [5-7]. Laccase from each species exhibit particular sequence and catalytic characteristics [8]. The enzyme belongs to oxido-reductases class and acts as a promising contender in biosensor field. Laccase catalyses electron-transfer reaction in presence of aerobic oxygen without requiring any cofactor. It has high potential for oxidation of phenols and benzenediols. A number of traditional techniques were used for detection of phenolic compounds like spectrophotometry and chromatography but these are in-efficient, expensive and required a

highly-trained staff. The laccase-based biosensors are cost-effective, transient and provide a platform for rapid detection with better sensitivity and higher stability [9]. Laccase based biosensors are third generation biosensors that involves direct exchange of electron (e-) between material of electrodes and active site of enzyme without the use of any mediator. Its biosensing potential has been attributed to its better catalytic activity, making it a third-generation biosensor. The phenolic compounds in the forms of tannin (in tea processing), catecholamines (body hormone), hydroxyquinone (sewage pollutant), methomyl (pesticides) can be detected and monitored by potential use of laccase biosensors. Thus, detection of phenolic compounds in the food, environmental and bio-medical industries using portable, cost-effective devices, has become an area of growing interest over the past decade. The monitoring of phenols is essential to check quality of wine, tea processing, hormonal level diagnosis, sewage treatment and pesticides toxicity. Thus, laccase biosensors play important role in food industry, health-sector, agricultural sector and environmental quality control. However, for successful widespread practical use of this biosensor, several improvements and optimizations need to be undertaken, including large quantity of production of laccase followed by improvised immobilization techniques that reduce enzyme leakage and maintain integrity of catalytic site of the enzyme. The physical and chemical properties of biological system (like enzymes, antibodies, DNA, whole cells) are converted into electrical or other detectable signals in biosensors. The amplitude of these signals is directly proportional to analyte concentration in sample [10]. On the basis of signal transduction, biosensors are categorized into many categories such as, electrochemical, optical and thermal sensors. A biosensor consists mainly two elements as shown in figure 1.

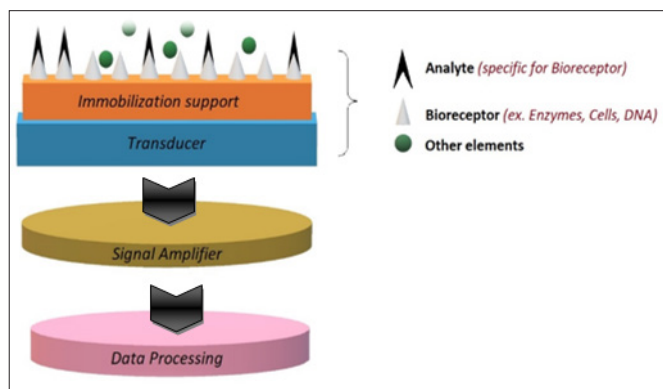


Figure 1: Transduction Mechanism in a Biosensor

- **The Bioreceptor:** (Biochemical-Recognition system) It interacts with the analyte. The interaction leads to a change in biochemical property of bioreceptor that is translated into physical/chemical output.
- **Transducer:** It transforms output signals into electrical signals, which can be either current or voltage [11]. The recognition system must have intimate integration or association with transducer.

Laccase

Laccase falls within broader description of polyphenol oxidase that linked with three specific activities:(1)Catechol oxidase/o-diphenol (oxygen oxidoreductase) [EC.1.10.3.1] activity,(2) Laccase/ p-diphenol (oxygen oxidoreductase) [EC1.10.3.2] activity, and (3) Cresolase/ monophenol monooxygenase [EC 1.18.14.1] activity. Due to its o- and p-diphenol activity, laccase show high affinity towards molecules of second groups, which belong to multi- copper oxidase family(MCO), also known by urushiol oxidases or p-diphenol oxidases [11,12]. **Polyphenols, methoxy substituted phenols, aromatic or aliphatic amines** are some substrates, which are oxidized by laccase [13]. However the substrate range vary from organism to organism.

Laccases also catalyse monoelectronic oxidation of substrates with respect to corresponding reactive radical in presence of molecular oxygen and form water as byproduct [14]. Unlike other oxidases there is no hydrogen peroxide involved in the process.

Immobilization of Laccase

Immobilization of enzyme is its attachment with an insoluble support either physically or chemically or both, in order to provide its reuse [15,16]. To construct an enzyme sensor/biosensor, the enzyme is immobilized onto transducer via adsorption or entrapment or encapsulation after crosslinking or chemical binding or sometime any of two [17].

Immobilization of enzyme ensures long term use of sensor with working stability and extended storage [18]. However, the support medium must be stable and inert and also resistance towards mechanical forces.

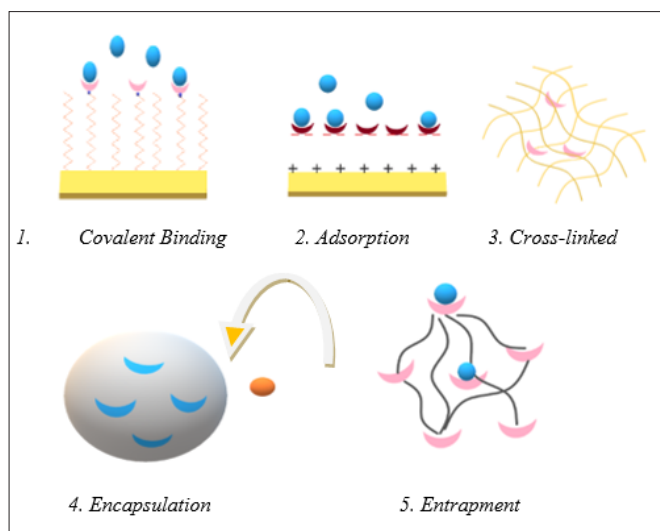


Figure 2: Immobilization Techniques for Laccase Biosensor

The selection of technique of enzyme immobilization is dependent on nature of biological recognition element and its application. The most commonly used techniques include covalent binding and, encapsulation after cross linking (Figure 2) [19].

During biosensor development ,it is mandatory to keep under consideration, transducer type and analyte's physiochemical properties as well as biosensor operating conditions [20]. The fore-mentioned considerations ensure the maximum activity by biological compound/tissue and the device will exhibit reusability and stability.

Covalent Binding

It involves reaction between groups in support matrix and biomaterial functional group which not involve in catalytic activity. Though it may cause structural changes in active site of enzyme but it also offers high stability and resistance to pH and temperature [21]. The material support generally used in case of laccase is N-succinimidyl-thiopropionate in Au electrode [22].

Adsorption

It involves interaction between support medium and enzyme via weak van der Waal forces, hydrogen bridges or other weak forces. This method is fast, affordable and simple. However, the immobilized elements like enzyme are not much mechanically stable thus it could be easily desorbed [23]. The material support generally used in case of laccase is Carbon Nanotubes with modified glassy carbon electrodes [24]. It causes positive change in electronic, mechanical properties and also ability to transfer electrons.

Cross Linking

It involves intramolecular bonding between molecules of enzyme via making use of bifunctional reactive. Dialdehydes, diiminoesters, diisocyanates are some of the cross linkers [21]. The resulting enzyme- complex formed is pH and temperature resistant. However, it makes use of high enzyme quantity and other factors like pH value and Ionic strength are needed to be maintained [25].

Encapsulation

In this method microspheres synthesized of semipermeable materials (polymers) is used. The immobilized biomaterial is confined to the microsphere core [26].

Entrapment

It involves mixing of biomaterial and a monomer solution to form a polymerized gel. Laccase enzyme continued to show its activity when entrapped in sol-gel silica [27]. The biomaterial is trapped within interstitial spaces of gel and sometimes it obstructed in synthetic fiber micro cavities [28]. Minimum alterations in enzymatic structures are caused. However, due to uneven pore size, the enzyme leakage continuously occurs and due to diffusional limitations, there is retardation in substrate accessibility [19].

Transducers and Associated Mechanism in Laccase Based Biosensors

Different transducers mechanisms are used by researcher for laccase biosensors, the most widely used in case of laccase enzyme are voltammetric and amperometric, which comes under electrochemical biosensors. In voltametric biosensor the current flowing through an electrode is measured. Fernandes and team developed a voltammetric laccase biosensor by using *Aspergillusoryzae* for pharmaceutical analysis. In this laccase embedded in chitosan microspheres that have been tripolyphosphate cross-linked. This laccase works optimally at a pH of 4 and acetate buffer is used to accomplish this requirement. The biosensor developed have two linear ranges for detection of rutin 5.82-13.1 μM and 0.599-3.92 μM [29]. Gupta and team developed a laccase biosensor using *Coriolushirsutus* for detection of catechol. In this on a gold electrode, laccase was bound onto amine-terminated thiol monolayers. In this acetate buffer is used to maintain the optimum pH of 5. The results show 1-400 μM concentration range and 15 $\mu\text{A}/\text{mm}$ sensitivity [30]. O.D. Leite and team constructed a voltammetric laccase biosensor by using *pleurotusostreatus* as laccase source for the detection of dopamine, adrenalin and isoprenaline. In this laccase is immobilized on carbon paste. Nujol used as working electrode, Ag/AgCl as reference and platinum auxiliary electrode. This laccase works optimally at a pH of 5.5 and citrate buffer is used to accomplish this requirement [31].

Amperometric sensors are type of electrochemical sensor in which the current generated by redox species oxidation or reduction at the electrode surface is measured, while it is kept at an adequate electrical potential. A.L Ghindilis et al. developed a amperometric laccase biosensor by using *Coriolushirsutus* for detection of catechol having important application in food industry. DEAEcellulose, DEAE-cellulose column was used as immobilization support and the linear range of 0.1-10mM was obtained [32]. Z pang and team constructed a laccase biosensor for detection of catechol in water sample. Laccase is entangled in compo site of PDA-NICNFs and the linear range obtained is 1-9100 μM . The pH of 5.5 is maintained by using 0.2 M acetate buffer solution [33,34]. D quan et al. work on the determination of p-phenylenediamine and p-chlorophenol by using laccase biosensor. This laccase works optimally at a pH of 6 and phosphate buffer is used to accomplish this requirement. Platinum electrode is used in immobilization process and the sensitivity found to be 560 nA/ μM and 195 nA/ μM respectively for p-phenylenediamine and p-chlorophenol [35].

In conductometric type of biosensor the change in electrical conductivity in sample is measured which is due to change in ionic concentration. In case of potentiometric sensors, the potential difference between two electrodes is measured. Laccase biosensors based on conductometric and potentiometric principles revealed a paucity of study, making it a future research priority.

In optical biosensor the optical phenomenon like fluorescence, chemiluminescence, absorbance etc are utilized. They provide a safe and non-electrical sensing of material. Huang and team developed the optical laccase biosensor for adrenalin determination [36].

CuTAPc-Fe₃O₄-NPs are used for immobilization of laccase and the fluorescence change due to oxidation of adrenalin is detected. pH of 7 and temperature of 25 C are taken as Measurement conditions. The biosensor developed have linear range of 0.2-0.09 μM for adrenalin [36]. J Sanz et al. took the laccase from *Trametes versicolor* and developed an optical biosensor for phenol estimation in wastewater. In this laccase is absorbed by the polyacrylamide sheet. This laccase works optimally at a pH of 6 and phosphate buffer is used to accomplish this requirement. The linear range of analyte in this is 0.109-2.5 mM [37].

L.I.B Silva et al. developed the laccase biosensor using the optical fibre transduction for the detection of catecholamines from human urine and plasma. The immobilization performed by entrapment of laccase in alginate matrix [38].

In thermometric biosensor the change in heat in the sample solution is detected. Bai & team on calorimetric based laccase biosensor. laccase is taken from *Trametes versicolor* and polyvinyl alcohol is used in immobilization of laccase. Enthalpy change in the system were used to tract enzyme reaction. Less work is occurred regarding the use of thermal transduction principle in case of laccase biosensor giving it a goal to work in future [39]. In table 1, different studied laccase biosensors with characterization and their application were.

Laccase Biosensors Selectivity and Sensitivity

Laccase biosensors are third-generation type biosensors whose functioning involves direct transport of electron in between electrodes and enzymes without presence of any mediator (43). This section is a review of selective and sensitive laccase-based biosensors made by researchers for application in environmental analysis.

Timur S.et.al.[40]. studied laccase biosensor for phenol detection in wastewater. They developed biosensor using film electrode and laccase (*Trametes versicolor* and *Aspergillus niger*) and *Agaricusbisporus* tissue. The matrix for immobilizing laccase was polyaniline. The calibration of system was done in order to detect phenolic compounds like: L-DOPA, Catechol. The maximum response time was found to be of 300 seconds. For different analytes under consideration, 0.2 to 20.0 μM detection limit was noted [40].

For detection and quantification of catechol, Gautam et al. (2003) manufactured a laccase biosensor on monolayer modified with L-cysteine, cystamine and 4-aminothiophenol on gold electrode. The detection range was 1 – 400 μM . The biosensor showed 1 month stability without any significant loss of enzymatic activity [30].

For detection of hydroquinone and syringaldazine, which are major pollutants of industrial effluents, Agnieszka et al. (2014) developed electrochemical laccase biosensors. For hydroquinone the limit of detection was 0.93 μM and sensitivity was $2.34 \pm 0.11 \mu\text{AmM}^{-1}$ [41].

For detection of pyrocatechol, Priyankiet al. (2014) developed a highly sensitive amperometric biosensors using laccase, nanocomposite matrix and carbon electrodes. The developed sensor show about 3.82 Na/nM sensitivity [42,43]. It has been confirmed by researches that copolymer plays an essential role in immobilization process.

Applications of Laccase Biosensors

Figure 3 shows the applications of Laccase biosensors in different fields.

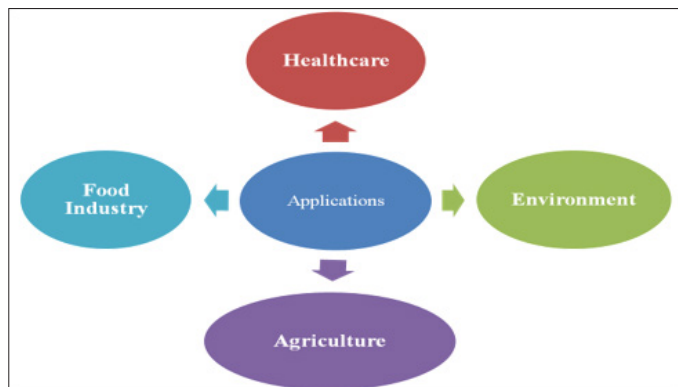


Figure 3: Applications of Laccase-Based Biosensors in Different Fields

Over the past decade, laccase-based biosensors use in food, agriculture, environment and healthcare sector shows vital area of research due to their specific properties. Phenolic compounds are detected in fruits, vegetables and also in final processed product of food industry.

Conventional methods like spectrophotometry and chromatography, used for phenol detection, are expensive and time taking, thus use of biosensor which are cost effective and take less time has been studied. To monitor phenol in raw food or its product such as tea and wine, different laccase-based biosensor was widely studied [44].

In 1977, Macholan and Schanel first reported the use of polyphenol oxidase in biosensor. They constructed a biosensor by potato or champignon polyphenol oxidase and a protein insolubilized by glutaraldehyde on polyamide surface. Then this active enzyme membrane was stretched over a Clark oxygen cell. The obtained biosensor determined phenolic content according to oxygen consumption [45]. Since then, different laccase-based biosensors were developed and a few emerging applications in different areas are discussed below:

Laccase Biosensors in Food Industry

Polyphenols are plant secondary metabolites that give wine its colour, flavour, hardness, and astringency. According to previous research, the polyphenolic concentration of red wine ranges from 2 to 5 grams per liter, whereas white wine contains 100 milligrams per liter. Polyphenolic content can be utilized to determine wine quality since polyphenol compounds have a good influence on human health due to their antioxidation action [46,47].

In year 2021, Bounegru AV and colleagues, developed a novel electrochemical biosensor from *Trametes versicolor*. It is based on carbon nanofibers-cobalt phthalocyanine-laccase and have a detection limit of 0.4-6.4 μM . It is helpful for detection of p-coumaric acid in phytoproducts.

In 2006, Gamella and colleagues determine total polyphenol content in wines using laccase biosensor. Laccase enzyme was immobilised with glutaraldehyde on a glassy carbon electrode for biosensor construction. Then diluted sample was directly added to electrochemical cell for monitoring polyphenolic compound index in wine. By this they determine detection limit for Gallic acid 2.6×10^{-3} mg/l at -200mV, while caffeic acid had 7.2×10^{-4} mg/l in wine samples. The results of phenol content estimation by biosensor and Folin-Ciocalteu method show good correlation [48]. Vasileseu

et al. work on laccase biosensor. They do modification in enzyme immobilisation by MoS₂ and GQDs (graphene quantum dots) that show change in conductivity of electrodes. Nanocomposite also provide compatible matrix for enzyme immobilisation. The modified biosensor determines caffeic acid had 0.32 μM detection limit and 17.9 nA/ μM sensitivity. This modified laccase biosensor can be used for red wine total phenol content estimation [49].

In tea processing industry, for product quality control the total reduced tannin/ catechol was estimated at different stages of processing. In 1992, Ghindlish and colleagues developed biosensor that determine catechol concentration in tea. Similarly, in 2014, S. Sadeghi and team developed biosensor to determine catechol in tea leaf sample. They used *Paraconiothyrium variable* as Laccase enzyme source with single step electrodeposition on carbon paste electrode surface. The purified laccase was entrapped in different bio composite matrix without cross-linking reagents use. The biosensor showed. shows catechol had 0.4 μM detection limit, 0.5- 80 μM liner concentration range and 1.092 μM apparent Michaelis Menten constant. In recent study laccase biosensor was developed for azo-dye tartrazine determination which is used for food colour. The biosensor was developed using laccase immobilised microspores and gold nanoparticle deposited screen-printed electrode. Laccase enzyme catalyzed the tartrazine oxidation and the current change was measured with the help of differential pulse voltammetry at 1.1V. Biosensor detected the tartrazine in linear concentration of 0.2- 14 μM and its detection limit was 0.04 μM [51].

Laccase Biosensors in Healthcare Sector

Biomarkers reflect the health status of individual. Several studies focused on the estimation of different biomarker molecules in urine, serum, plasma and pharmaceutical samples by Chemiluminescence, HPLC, fluorimetry and electrochemistry have been reported. Nowadays use of Enzyme based biosensors is one of most intensively investigated area.

Catecholamine includes epinephrine; noradrenaline and dopamine are main biomarker molecules which influences human health. Oldair D. Leite and team studied a reliable laccase (from *Pleurotus ostreatus*) carbon paste biosensor that used in pharmaceutical formulation for catecholamine determination. The adrenaline and dopamine oxidation reaction are catalysed by laccase enzyme and the current produced during this electrochemical reduction was related to catecholamines concentration in sample. The results show 6.0×10^{-5} – 7.0×10^{-4} mol/L and 7.0×10^{-5} – 4.0×10^{-4} mol/L concentration range, 7.9×10^{-6} mol/L and 9.8×10^{-6} mol/L detection limits for adrenaline and dopamine respectively [31].

Similarly, Oksana Josypčuk and his team studied four different biosensors in flow system in which minireactors were filled with either SBA-15 (mesoporous SiO₂) or MCM-41 (mesoporous SiO₂) silica powder and modified by either laccase or tyrosinase enzyme. These biosensors were used to determine L-Dihydroxyphenylalanine, dopamine, adrenaline and noradrenalin. Out of four Laccase - MCM41 reactor showed most satisfactory results. It estimates 100% relative current response for dopamine and 32.1%, 26.2% and 0.71% current response for L-Dihydroxyphenylalanine, noradrenaline and adrenaline respectively. It showed 0.004-0.2 mmol/L liner range for dopamine and statistical results proved biosensor's good accuracy and sensitivity and several months lifetime of biosensor. All these factors make this biosensor fit to use for dopamine and noradrenaline estimation in medical samples [52].

In recent study, a simple, selective and sensitive technique was demonstrated for development of laccase-based biosensor

for norepinephrine level determination. They use 9-nonyl-2,7-di(selenophen-2-yl)-9H-carbazole (semiconductor polymer) and platinum electrode that modified with laccase (extracted from *Cerrena unicolor*). The laccase enzyme was immobilized on thin polymeric film coated platinum electrode and the norepinephrine determination was based on catalytic oxidation of norepinephrine to norepinephrine quinone. The system showed relatively excellent linear range of 0.1-200x 10⁻⁶ M and detection limit of 240nM [53].

Cysteine, thiol- containing molecule, have essential role in cell metabolism and function (protein synthesis, detoxification). the disorder in metabolism results in an autosomal recessive disease cystinosis and change in Cysteine level also implicated different pathological conditions such as Alzheimer and Parkinson. Several studies for estimation of Cysteine in different pharmaceutical samples by Chemiluminescence, HPLC, fluorimetry and electrochemistry have been reported.

Nowadays use of Enzyme based biosensors is one of most intensively investigated area. In 2007, M. Santhiago and colleagues studied biosensor for L-cysteine estimation in pharmaceutical formulation. They use graphite electrode modified with laccase (from *Aspergillus oryzae*) and characterized biosensor with respect to different phenolic compounds, inhibitory molecules, pH dependence, linear range, Michaelis- Menten constant and sensitivity. The results showed 89.7 – 103.4% recovery of Cysteine and less than 1.0% relative standard deviation and 9 month lifetime of biosensor [54].

In 2021, Zhang M and team constructed a smartphone-assisted portable biosensor using laccase-mineral hybrid microflowers for colorimetric determination of epinephrine. The biosensor showed a linear range of 1-400 μ M and can be widely used in detecting the levels of this hormone in person's body.

Laccase Biosensors in Agriculture Sector

In agriculture sector, toxic pesticides use increased exponentially for high productivity but pesticide compounds adversely impact on human health and environment. The enzyme-based biosensors provide cost effective and fast analytical device for pesticide toxicity detection. Zapp et al. developed laccase-based biosensor for methomyl (Carbamate pesticides) detection. For biosensor construction Laccase isolated from *Aspergillus Oryzae* was immobilized on platinum nanoparticles matrix in liquid ionic phase. The resulted device determines methomyl concentration in tomato and carrot and the resulted values show similarity to value detected using HPLC [55]. In 2013, Oliveira and team construct biosensor for carbamate quantification by laccase (*Trametes versicolor*) immobilization on graphene doped carbon paste electrode functionalized by electrodeposited Prussian blue films. The biosensor showed 5.2x10⁻⁹ mol/L to 1 x 10⁻⁷ mol/L detection limit and suitable sensitivity, selectivity for quantification of different Carbamate such as carbofuran, carbaryl, formetanate, pirimicarb and ziram which are used mostly into tomato and potato crops [56].

In 2021, Zhong X and team constructed a biosensor by laccase (*Trametes versicolor*) immobilized in pores of mesoporous carbon sphere. The biosensor shows suitable sensitivity for electrochemical detection of tetracycline compound.

Laccase Biosensors in Environment Field

As there is an increase demand of synthetic products the large-scale production of synthetic products takes place and the phenol content in wastewater that comes out from these chemical factories has been increased to such a significant amount that it becomes a ubiquitous pollutant. Coal refineries, pharmaceutical and polymer industry also contribute largely in the addition of phenol in wastewater. This phenol causes pollution and has a harmful effect on animals, plants and humans [44]. As for estimation of phenol in the wastewater, the widely used techniques are chromatography and spectrophotometry but now a days biosensors are developed for estimation of phenol content in wastewater as this method is quick, has better sensitivity and selectivity than classical methods and detects the compound at the trace level [57]. Among all biosensor laccase-based biosensor was given prioritization as it provides broader substrate specificity and does not require additional cofactor for its reaction and also laccase provides good stability [9].

In 2022, Castrovilli MC and others, fabricated a new, low-cost, and environment-friendly laccase-based biosensor by electrospray immobilization method. This biosensor, having a range of 2-100 μ M, is able to detect catechol with high sensitivity.

Bravo I and colleagues in 2022 developed Laccase biosensor from *Trametes versicolor*. It was bioconjugated on screen printed carbon electrode for electrochemical analysis of Bisphenol.

Quintanilla-Villanueva GE and colleagues in 2021 developed a novel enzyme-based SPR strategy for detection of the antimicrobial agent chlorophene. The laccase was extracted from *Rhus vernificera* that was later immobilized covalently on gold coated surface.

Sekretaryova and his colleagues in 2016 developed a serigraphy technique-based laccase biosensor for measurement of phenol in tap water. The micro-scale graphite electrode was used. The linear range 0.2 – 10 mM and 1.35 \pm 0.4 A M⁻¹ cm⁻² sensitivity was recorded. This type of biosensor, has a lot of promises for monitoring of drinking water [58]. Zehra olcer developed the laccase biosensor source of which was *Trametes versicolor* for catechol detection. He uses gold electrode and immobilization with glutaraldehyde. This has 0.025mM - 0.8 mM linear range. The determination of catechol with laccase modified gold surface was demonstrated using cyclic voltammetry and amperometry activity tests [59].

In 2023, laccase modified on grapheme oxide disulfide (GO/TiS₂) nanocomposite amperometric biosensor was used for determination of a flavonoid antioxidant, namely (-)-epicatechin in wine and fruit juices in the presence of other phenolic compounds. The minimum detection limit (LOD) of Laccase biosensor was 0.73 μ M.

In 2025 Laccase was immobilized in a biometric environment, provided by a nanostructured Langmuir-Blodgett (LB) film of an amphiphilic material Octadecylamine (ODA) along with poly (3-hexylthiophene-2,5 diyl (B3HT) as electron mediator. This study provided valuable insight into phenol detection in food industry.

Table 1 summarises the analytic properties and applications different Laccase biosensors.

Table 1: Different Laccase Biosensors and their Analytic Properties and Applications

Source of Laccase	Immobilization Type	Analyte	Linear Range	Type	Application	Ref.
Coriolus versicolor	DEAE-cellulose covalent binding	Catechol	NA	Amperometric	Food industry	[32]
Coriolushirsutus	DEAE-cellulose Covalent binding	Catechol	0.1-10mM	Amperometric	Food industry	[32]
Cariolushirsutus	Adsorption in solid graphite electrode	Hydroquinone and catechol	Upto 2µM	Amperometric	Environment sector	[60]
Cariolushirsutus	Entrapment with gelatin and Nation	Dopamine	Upto 400nM	Amperometric	Clinical analysis	[61]
Coriolushirsutus	Entrapment in polyvinyl alcohol	Morphine	32nM-100µM	Amperometric	Pharmaceutical sector	[62]
Pleurotostreatus	Absorbtion in carbon paste	Adrenalin, Dopamine	0.06-0.7mM, 0.07-0.4mM	Voltammetric	Pharmaceutical sector	[63]
Coriolushirsutus	glutaraldehyde covalent bond with gold-thiol monolayer	Catechol	1-400µM	Voltammetric	Environment field	[64]
Polyporusinsitus	Cross-linking with BSA and glutaraldehyde	Pyrocatechol 1-naphthol o-phenylenediamine	NA	Amperometric	Environment sector	[63]
Pleurotostreatus	Adsorption carbon paste	Dopamine Adrenalin	6.6- 390 µm 6.1-100 µm	Voltammetric	Pharmaceutical sector	[64]
Trametes versicolor	Crosslinked with compound glutaraldehyde and gelatine	paracetamol with HBT, PCM Without HBT	0.5- 3µM, 2.0-15.0µM	Amperometric	Medical field	[65]
Trametes versicolor	Glutaraldehyde Cross-linked with glassy carbon electrode	Galic acid Caffeic acid	0.017µM - 4.70 µM, 0.0038-0.055 µM	Amperometric	Food sector	[48]
Rhus vernicifera	Covalent immobilization to PAMAM dendrimers	Catechins	0.1-10 µM	Amperometric	Food sector	[66]
Aspergillus oryzae	Chitosan cross linking on tripolyphosphate and microspore encapsulation	Rutin	0.599-3.92 µm And 5.823-13.1 µm	Voltammetric	Medical field	[29]
Cerrena unicolor	Entrapment in titians gel matrix	2-6 dimethoxy phenol	1.2-6.1µM	Amperometric	Environmental sector	[67]
Trametes versicolor	Entrapment on sonogel-carbon electrode (SNGC)	Caffeic, ferulic, gallic acids, etc	0.04µM -22µM	Amperometric	Food industry	[68]
Aspergillus oryzae	Adsorbtion Graphite powder -Nujol-Pt.BMIPF6	Rosmarine Acid	0.999-65.4 µm	Voltammetric	Food industry	[69]
Aspergillus oryzae	Cyanuric chlorine crosslinking with chitosan	Luteolin	0.99-5.825 µm	Voltammetric	Food industry	[70]
Trametes versicolor	Chitosan entrapment in nanocomposite MWCNTs	Caffeic, Rosmarinic, gallic and chlorogenic acid	0.73µM – 12.1 µM	Amperometric	Food sector	[71]
Trametes versicolor	Entrapment with polyazetidine prepolymer	Gallic acid	0.58µM -99.92 µM	Amperometric	Food sector	[72]
Trametes Versicolor	Entrapment in solution gel matrix of diglyceryl silane	Phenol, Gallic acid, Caffeic acid, catechin	0.339-11.067µA per mM	Amperometric	Food sector	[46]
Trametes Versicolor	Entrapment within polyvinyl alcohol photopolymer PVA-AWP	Caffeic acid, Catechol, Hydroquinone, resorcinol	0.5µM- 175µM	Amperometric	Food industry	[28]
Trametes versicolor	Cu mesoporous film carbon entrapment	Catechol	0.67-15.75µM	Amperometric	Environment sector	[73]

Pieurotusostreatus	Agrose-guar gum biopolymer matrix Entrapment	Phenols	0.5-4.5 μM	Amperometric	Environment sector	[74]
Trametes versicolor	Adsorption on gold screen-printed electrodes	Gallic and Rosmarinic acid	2 μM - 15 μM	Amperometric	Food sector	[75]
Trametes hirsute	PAP entrapment on MWC nanotubes	Gallic and Caffeic acid	0.16 μM -116.94 μM	Amperometric	Food sector	[72]
Trametes versicolor	Magnetic carbon paste silica membrane used for entrapment	Catechol	0.1-165 μM	Amperometric	Environment sector	[76]
Trametesvesicolor	Entrapment using silica sphere as immobilization matrix	Dopamine	1.3-85.5 μm	Amperometric	Clinical analysis	[77]
Ganoderma	Covalent binding by glutaraldehyde + cysteamine monolayer	Polyphenol/guaiacol	10-500 μM	Amperometric	Food sector	[78]
Ganoderma	Covalent binding on Fe ₂ O ₃ Nps/cMWCNTs/PANI/Au	TPC, Guaiacol	0.10 -10 μM , 10-500 μM	Amperometric	Food sector	[79]
Trametes versicolor	Adsorption on MWCNTs based electrodes	Pirimicarb	0.990-11.5 μM	Amperometric	Food sector	[56]
Coriolushirsutus	Cross linking using bovine serum albumin and glutaraldehyde	P-chlorophenol Guaiacol chloroguaiacol	1.0-10 μM	Amperometric	Environment sector	[80]
Trametes versicolor	Adsorption onto thionine based carbon black electrode	Bisphenol A	0.5-50 μM	Amperometric	Food sector	[81]
Trametes versicolor	Adsorption onto nanocomposition Nafion	Caffeic acid	0.2-2 μM	Amperometric	Food sector	[80]
Trametes versicolor	Glutaraldehyde use for Covalent binding on nanocomposition matrix	I. Caffeic acid II. Catechin III. Gallic acid IV. Chlorogenic acid V. Trolox	0.3-45 μM 1.7-30 μM 3.0-60 μM 1.5-30 μM 20-35 μM	Amperometric	Food sector	[82]
Trametes versicolor	Entrapment in PDA-NICNFs composite	Catechol	1-9100 μM	Amperometric	Environment sector	[34]
Trametes versicolor	Entrapment in nano matrix osmium tetroxide on PVP-MWCNT	pyrocatechol	03.98-16.71nM	Amperometric	Environmental analysis	[42]
Cerrena unicolor	Electrolytic deposition by using galvanostatic parameters	Hydroquinone	2.0-60 μM	Voltammetric	Environmental analysis	[41]
Trametes Versicolor	Nanocages of palladium-Cu alloyed	Catechol	0.005-1.15mM, 1.655-5.155mM	Amperometric	Environment Sector	[83]
Agaricusbisporus ,	Matrix of Ethylene glycol with lateral amino	Catechol	0.0025-0.05mM,	Amperometric	Environment sector	[84]
Commercialized Product	Silver nanoparticle immobilization on modified cellulose nanofibres	Catechol	4.98 μM -3.65mM	Voltammetric	Environment	[85]
Trametes versicolor	Nanorods of Polyaniline immobilized with cellulose nanofibres	Catechol	0.497 μM -2.27mM	Voltammetric	Environment	[86]
Commercialized product	carbon nanofibers loaded on nickel copper alloy nanoparticles	Hydroquinone	4x10 ⁻⁷ – 2.37x10 ⁻⁶ M	Amperometric	Environment sector	[87]

Trametes versicolor	Flow injection SPCE	Hydroquinone	1-50 μM	Amperometric	Environment sector	[88]
Trametes versicolor	Situ-printed carbon-fibre microelectrodes	TPC	0.2-10 μM ,	Amperometric	Environment sector	[89]
Rhus vermicifera	TiO ₂ loaded Cu nanofibers	Hydroquinone	1-89.8 μM	Voltammetric	Environment sector	[90]
Cerrena unicolor	Dithienotetraphenyl polymer	Catechol	5-160 μM	Amperometric	Environment Sector	[91]
Commercialized Product	AnthraCene-functionalised MWCNTs	Arsenate	Na	Amperometric	Environment sector	[92]
Trametes versicolor	Nanofibers of bacterial Cellulose -AuNPs hybrid	Hydroquinone	30-100nM	Amperometric	Environment sector	[93]
Trametes versicolor	Polyaniline nanofiber porous matrix	Catechol	NA	Amperometric	Environment sector	[94]
Trametesvernicifera	Iron oxide nanocrystal on CPE	Catechol	8-800 μM	Amperometric	Environment Sector	[95]
Terametes versicolor	Titanium/ nafion	Catechol	0.75-150 μM	Amperometric	Environment Sector	[96]
Trametes versicolor	Zinc oxide nanoparticles mixed in Carbon xerogel-ZnO composites	Catechol	6.91-453 μM	Amperometric	Environment sector	[97]
Trametes versicolor	Polydopamine reduced Graphene oxide	Catechol	0.1-263 μM	Amperometric	Environment sector	[98]
Rhus Vernicifera	Nanofibres of Cu and Carbon with silver doped titanium oxide nanoparticles	Hydroquinone	1.20-176.50 μM	Voltammetric	Environment sector	[99]
Trametes versicolor	Sol-gel matrix	Hydroquinone, catechol, Resorcinol	1.4-0.2mM	Optical	Environment sector	[100]
Trametes versicolor	Ureasile-chalcogenide glass with silver nanoparticle	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)	NA	Amperometric	Environment sector	[101]
Trametes versicolor	Polygraphene oxide nanosheets	Catechol	0.036 -0.35 μM , 0.35-2.5 μM	Amperometric	Environment sector	[102]
Trametes versicolor	magnetic nanoparticle immobilized on Au surface	ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)	NA	Amperometric	Environment sector	[103]
Trametes versicolor	Iron oxide yolk shell nanoparticles	I. 2-6-Dimethyl oxyphenol II Guaiacol	0.025-750 μM 0.10-250 μM	Amperometric	Environment sector	[104]
Trametes versicolor	SiO ₂ Yolk shell nanospheres	Catechol	12.5-450 μM	Amperometric	Environment sector	[105]
Trametes versicolor	photocross-linked polymers	phenols	NA	Amperometric	NA	[106]
Pycnoporussanguineus	carbon paper electrode, two-dimensional molybdenum disulfide	dopamine	0.1-0.5 μM 1-5 μM	Amperometric	Pharmaceutical	[107]
Moniliafructicola		Bisphenol A		Amperometric	Environment sector	[108]
Rhus vernicifera	fabricated with SPCE	para-cresol	0.2-25ppm	Amperometric	Environment sector	[109]
NA	Immobilized over Au/MXene electrode	Catechol	0.05-0.15 μM	Amperometric	Environment sector	[110]
Rhus vernicifera	Covalent on gold coated surface	Chlorophene	NA	SPR biosensor	Environment sector	[111]

Trametes versicolor	Covalent with mineral hybrid microflowers	Epinephrine	1–400 μ M		Medical sector	[112]
NA	Covalent with APT-CDs	Dopamine	0-30 μ M	fiber-optic biosensor	Medical sector	[113]
Trametes versicolor	Embedded in pores of mesoporous carbon sphere@UiO-66-NH ₂	tetracycline	NA	electrochemical	electrochemical	[114]
Rametes versicolor	Cross linking	p-coumaric acid	0.4–6.4 μ M	voltammetric	Food and Pharmaceutical sector	[115]
Agaricus Bisporus	Covalent bond with reduced graphene oxide	Emerging pollutants	NA	Electroanalytic	Environment sector	[116]
NA	Electrospray ionization	catechol	2-100 μ M	NA	Environment sector	[117]
Trametes versicolor	Bioconjugate on screen printed carbon electrode	Bisphenol A	NA	electrochemical	Environment sector	[118]

NA= Not Available

Conclusion

According to European Community Directive, Japan Ministry of Health and Labour and Welfare and the US EPA, Laccase-based biosensors indicate significant potential for detection of phenolic compounds with high sensitivity and specificity. However, it is essential that approaches used in growing biosensors which avoid electrode surface contamination and hindrance caused by harmful chemicals present in the sample to be studied. A key challenge for future researches needs to be more focused to formulation and design of support media with least amount of enzyme loss and/or leakage after immobilization, refinement of established methods of immobilization to ensure direct enzyme adhesion, safeguarding the wholeness of catalytic site of enzyme, exploiting the use of genetic engineering for refining laccase recycling potential and stability. Hence, the future research should be focused on miniaturization of biosensors that works with minimum number of samples and reagents [119,120].

References

- Giardina P, Faraco V, Pezzella C, Piscitelli A, Vanhulle S, et al. (2010) Laccases: a never-ending story. Cellular and Molecular Life Sciences 67: 369-385.
- Leonowicz A, Wilkolazka A, Rogalski J (2004) Fungal laccases from basidiomycetes and their inducibility. Mushroom Science and Production 2: 127-139.
- Xu F, Palmer AE, Yaver DS, Berka RM, Gambetta GA, et al. (1999) Targeted Mutations in a Trametes villosa Laccase. Journal of Biological Chemistry 274: 12372-12375.
- Xu F (1996) Oxidation of Phenols, Anilines, and Benzenethiols by Fungal Laccases: Correlation between Activity and Redox Potentials as Well as Halide Inhibition. Biochemistry 35: 7608-7614.
- Hullo MF, Moszer I, Danchin A, Martin Verstraete I (2001) Cot A of Bacillus subtilis Is a Copper-Dependent Laccase. Journal of Bacteriology 183: 5426-5430.
- Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates. FEBS Letters 267: 99-102.
- Mayer AM (1986) Polyphenol oxidases in plants-recent progress. Phytochemistry 2: 11-20.
- Mayer A (2002) Laccase: new functions for an old enzyme. Phytochemistry 60: 551-565.
- Munteanu FD, Lindgren A, Emnéus J, Gorton L, Ruzgas T, et al. (1998) Bioelectrochemical Monitoring of Phenols and Aromatic Amines in Flow Injection Using Novel Plant Peroxidases. Analytical Chemistry 70: 2596-2600.
- Dzyadevych SV, Arkhypova VN, Soldatkin AP, El'skaya AV, Martelet C, et al. (2008) Amperometric enzyme biosensors: Past, present and future. IRBM 29: 171-180.
- Rogers K (2001) Viscosity and binder composition effects on tyrosinase-based carbon paste electrode for detection of phenol and catechol. Talanta 54: 1059-1065.
- Riva S (2006) Laccases: blue enzymes for green chemistry. Trends in Biotechnology 24: 219-226.
- Thurston CF (1994) The structure and function of fungal laccases. Microbiology 140: 19-26.
- Kim C, Lorenz WW, Hoopes JT, Dean JFD (2001) Oxidation of Phenolate Siderophores by the Multicopper Oxidase Encoded by the Escherichia coli yacK Gene. Journal of Bacteriology 183: 4866-4875.
- Upadhyay P, Shrivastava R, Agrawal PK (2016) Bioprospecting and biotechnological applications of fungal laccase. 3 Biotech 6: 15.
- Arica MY, Altıntaş B, Bayramoğlu G (2009) Immobilization of laccase onto spacer-arm attached non-porous poly (GMA/EGDMA) beads: Application for textile dye degradation. Bioresource Technology 100: 665-669.
- Durán N, Rosa MA, D'Annibale A, Gianfreda L (2002) Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. Enzyme and Microbial Technology 31: 907-931.
- Liu Y, Qu X, Guo H, Chen H, Liu B, Dong S (2006) Facile preparation of amperometric laccase biosensor with multifunction based on the matrix of carbon nanotubes-chitosan composite. Biosensors and Bioelectronics 21: 2195-2201.
- Fernández Fernández M, Sanromán MÁ, Moldes D (2013) Recent developments and applications of immobilized laccase. Biotechnology Advances 31: 1808-1825.
- Singh M, Verma N, Garg A, Redhu N (2008) Urea biosensors. Sensors and Actuators B: Chemical 134: 345-351.
- Arroyo M (1998) Inmovilización de enzimas. Fundamentos, métodos y aplicaciones. Ars Pharmaceutica 39: 111-127.
- Mena ML, Carralero V, González Cortés A, Yáñez Sedeño P, Pingarrón JM (2005) Laccase Biosensor Based on N-Succinimidyl-3-Thiopropionate-Functionalized Gold Electrodes. Electroanalysis 17: 2147-2155.
- Brady D, Jordaan J (2009) Advances in enzyme immobilisation. Biotechnology Letters 31: 1639-1650.
- Liu Y, Huang L, Dong S (2007) Electrochemical catalysis and

- thermal stability characterization of laccase carbon nanotube-ionic liquid nanocomposite modified graphite electrode. *Biosensors and Bioelectronics* 23: 35-41.
25. Bryjak J, Kruczkiewicz P, Rekuć A, Peczyńska Czocho W (2007) Laccase immobilization on copolymer of butyl acrylate and ethylene glycol dimethacrylate. *Biochemical Engineering Journal* 35: 325-332.
 26. Rochefort D, Kouisni L, Gendron K (2008) Physical immobilization of laccase on an electrode by means of poly(ethyleneimine) microcapsules. *Journal of Electroanalytical Chemistry* 617: 53-63.
 27. Mohidem NA, Mansor AF, Zawawi WNIWM, Othman NS, Mat H (2016) Effect of synthesis conditions on physical properties, laccase catalytic activity and stability of sol gel laccase. *Journal of Sol-Gel Science and Technology* 80: 587-597.
 28. Ibarra Escutia P, Gómez JJ, Calas Blanchard C, Marty JL, Ramírez Silva MT (2010) Amperometric biosensor based on a high-resolution photopolymer deposited onto a screen-printed electrode for phenolic compounds monitoring in tea infusions. *Talanta* 81: 1636-1642.
 29. Fernandes SC, de Oliveira IRWZ, Fatibello Filho O, Spinelli A, Vieira IC (2008) Biosensor based on laccase immobilized on microspheres of chitosan crosslinked with tripolyphosphate. *Sensors and Actuators B: Chemical* 133: 202-207.
 30. Gupta G, Rajendran V, Atanassov P (2003) Laccase Biosensor on Monolayer-Modified Gold Electrode. *Electroanalysis* 15: 1577-1583.
 31. Leite OD, Fatibello Filho O, Barbosa A de M (2003) Determination of catecholamines in pharmaceutical formulations using a biosensor modified with a crude extract of fungi laccase (*Pleurotus ostreatus*). *Journal of the Brazilian Chemical Society* 14: 297-303.
 32. Ghindilis AL, Gavrilova VP, Yaropolov AI (1992) Laccase-based biosensor for determination of polyphenols: determination of catechols in tea. *Biosensors and Bioelectronics* 7: 127-131.
 33. Vodop'yanov AV, Mansfeld DA, Samokhin AV, Alekseev NV, Tsvetkov YV (2017) Production of Nano powders by the Evaporation Condensation Method Using a Focused Microwave Radiation. *Radio physics and Quantum Electronics* 59: 698-705.
 34. Li D, Luo L, Pang Z, Lei Ding, Qingqing Wang, et al. (2014) Novel Phenolic Biosensor Based on a Magnetic Polydopamine-Laccase-Nickel Nanoparticle Loaded Carbon Nanofiber Composite. *ACS Applied Materials & Interfaces* 6: 5144-5151.
 35. Quan D, You Sung K (2004) Sensing Characteristics of Tyrosinase Immobilized and Tyrosinase, Laccase Co-immobilized Platinum Electrodes. *Bulletin of the Korean Chemical Society* 25: 1195-1201.
 36. Huang J, Fang H, Liu C, Gu E, Jiang D (2008) A Novel Fiber Optic Biosensor for the Determination of Adrenaline Based on Immobilized Laccase Catalysis. *Analytical Letters* 41: 1430-1442.
 37. Sanz J, de Marcos S, Galbán J (2012) Autoindicating optical properties of laccase as the base of an optical biosensor film for phenol determination. *Analytical and Bioanalytical Chemistry* 404: 351-359.
 38. Silva LIB, Ferreira FDP, Freitas AC, Rocha Santos TAP, Duarte AC (2009) Optical fiber biosensor coupled to chromatographic separation for screening of dopamine, norepinephrine and epinephrine in human urine and plasma. *Talanta* 80: 853-857.
 39. Bai X, Gu H, Chen W, Hanchang Shi, Bei Yang, et al. (2014) Immobilized Laccase on Activated Poly (Vinyl Alcohol) Microspheres for Enzyme Thermistor Application. *Applied Biochemistry and Biotechnology* 173: 1097-1107.
 40. Timur S, Pazarlıoğlu N, Pilloton R, Telefoncu A (2004) Thick film sensors based on laccases from different sources immobilized in polyaniline matrix. *Sensors and Actuators B: Chemical* 97: 132-136.
 41. Jędrychowska A, Cabaj J, Świst A, Sołoducho J (2014) Electrochemical laccase sensor based on 3-methylthiophene/3-thiopheneacetic acid/bis(3,4-ethylenedioxythiophene)-N-nonylacridone as a new polymer support. *Journal of Electroanalytical Chemistry* 721: 64-70.
 42. Das P, Barbora L, Das M, Goswami P (2014) Highly sensitive and stable laccase based amperometric biosensor developed on nano-composite matrix for detecting pyrocatechol in environmental samples. *Sensors and Actuators B: Chemical* 192: 737-744.
 43. Sarika C, Rekha K, Narasimhamurthy B (2014) Laccase based amperometric biosensor for industrial waste waters : A comparative study on covalent immobilization methods on gold electrode. *ISOR Journals of Applied Chemistry* 7: 20-27.
 44. Rodríguez Delgado MM, Alemán Nava GS, Rodríguez Delgado JM, Graciano Dieck Assad, Sergio Omar Martínez Chapa, et al. (2015) Laccase-based biosensors for detection of phenolic compounds. *TrAC - Trends in Analytical Chemistry* 74: 21-45.
 45. Macholán L, Scháněl L (1977) Enzyme electrode with immobilized polyphenol oxidase for determination of phenolic substrates. *Collection of Czechoslovak Chemical Communications* 42: 3667-3675.
 46. Montoreali MR, Seta L Della, Vastarella W, Pilloton R (2010) A disposable Laccase Tyrosinase based biosensor for amperometric detection of phenolic compounds in must and wine. *Journal of Molecular Catalysis B: Enzymatic* 64: 189-194.
 47. Zhang Y, Lv Z, Zhou J, Fengxue Xin, Jiangfeng Ma, et al. (2018) Application of eukaryotic and prokaryotic laccases in biosensor and biofuel cells: recent advances and electrochemical aspects. *Applied Microbiology and Biotechnology* 102: 10409-10423.
 48. Gamella M, Campuzano S, Reviejo AJ, Pingarrón JM (2006) Electrochemical Estimation of the Polyphenol Index in Wines Using a Laccase Biosensor. *Journal of Agricultural and Food Chemistry* 54: 7960-7967.
 49. Vasilescu I, Eremia SAV, Kusko M, Radoi A, Vasile E, et al. (2016) Molybdenum disulphide and graphene quantum dots as electrode modifiers for laccase biosensor. *Biosensors and Bioelectronics* 75: 232-237.
 50. Sadeghi S, Fooladi E, Malekaneh M (2015) A New Amperometric Biosensor Based on Fe₃O₄/Polyaniline/Laccase/Chitosan Biocomposite-Modified Carbon Paste Electrode for Determination of Catechol in Tea Leaves. *Applied Biochemistry and Biotechnology* 175: 1603-1616.
 51. Mazlan S, Lee Y, Hanifah S (2017) A New Laccase Based Biosensor for Tartrazine. *Sensors* 17: 2859.
 52. Josypčuk O, Barek J, Josypčuk B (2018) Amperometric Determination of Catecholamines by Enzymatic Biosensors in Flow Systems. *Electroanalysis* 30: 1163-1171.
 53. Baluta S, Swist A, Cabaj J, Malecha K (2020) Point-of-care testing - Biosensor for norepinephrine determination. *International Journal of Electronics and Telecommunications* 66: 369-372.
 54. Santhiago M, Vieira IC (2007) L-Cysteine determination in pharmaceutical formulations using a biosensor based on laccase from *Aspergillus oryzae*. *Sensors and Actuators B: Chemical* 128: 279-285.
 55. Zapp E, Brondani D, Vieira IC, Carla W Scheeren, Jairton Dupont, et al. (2011) Biomonitoring of methomyl pesticide by laccase inhibition on sensor containing platinum nanoparticles

- in ionic liquid phase supported in montmorillonite. *Sensors and Actuators, B: Chemical* 155: 331-339.
56. Oliveira TMBF, Fátima Barroso M, Morais S, Pedro de Lima-Neto, Adriana N Correia, et al. (2013) Biosensor based on multi-walled carbon nanotubes paste electrode modified with laccase for pirimicarb pesticide quantification. *Talanta* 106: 137-143.
 57. Marco MP, Barceló D (1996) Environmental applications of analytical biosensors. *Measurement Science and Technology* 7: 1547-1562.
 58. Sekretaryova AN, Volkov AV, Zozoulenko IV, Turner APF, Vagin MY, et al. (2016) Total phenol analysis of weakly supported water using a laccase-based microband biosensor. *Analytica Chimica Acta* 907: 45-53.
 59. Ölçer Z (2020) A Novel Biosensor Based on Laccase for The Detection of Catechol. *Celal Bayar University Journal of Science* 16: 9-14.
 60. Yaropolov AI, Kharybin AN, Emnéus J, Marko Varga G, Gorton L (1995) Flow-injection analysis of phenols at a graphite electrode modified with co-immobilised laccase and tyrosinase. *Analytica Chimica Acta* 308: 137-144.
 61. Lisdat F, Wollenberger U, Makower A, Hörtnagl H, Pfeiffer D, et al. (1997) Catecholamine detection using enzymatic amplification. *Biosensors and Bioelectronics* 12: 1199-1211.
 62. Bauer CG, Kühn A, Gajovic N, Frieder W Scheller, Alexander Makower, et al. (1999) New enzyme sensors for morphine and codeine based on morphine dehydrogenase and laccase. *Fresenius' Journal of Analytical Chemistry* 364: 179-183.
 63. Kulys J, Vidziunaite R (2003) Amperometric biosensors based on recombinant laccases for phenols determination. *Biosensors and Bioelectronics* 18: 319-325.
 64. Leite O, Lupetti K, Fatibellofilho O, Vieira I, Barbosa A (2003) Synergic effect studies of the bi-enzymatic system laccase peroxidase in a voltammetric biosensor for catecholamines. *Talanta* 59: 889-896.
 65. Oliveira TMBF, Fátima Barroso M, Morais S, Mariana Araújo, Cristina Freire, et al. (2013) Laccase-Prussian blue film-graphene doped carbon paste modified electrode for carbamate pesticides quantification. *Biosensors and Bioelectronics* 47: 292-299.
 66. Rahman MA, Noh HB (2008) Direct Electrochemistry of Laccase Immobilized on Au Nanoparticles Encapsulated-Dendrimer Bonded Conducting Polymer: Application for a Catechin Sensor. *Analytical Chemistry* 80: 8020-8027.
 67. Kochana J, Nowak P, Jarosz Wilkołazka A, Bieroń M (2008) Tyrosinase/laccase bienzyme biosensor for amperometric determination of phenolic compounds. *Microchemical Journal* 89: 171-174.
 68. ElKaoutit M, Naranjo Rodriguez I, Tamsamani KR, Hernández Artiga MP, Bellido Milla D, et al. (2008) A comparison of three amperometric phenoloxidase Sonogel Carbon based biosensors for determination of polyphenols in beers. *Food Chemistry* 110: 1019-1024.
 69. Franzoi A, Dupont J, Spinelli A, Vieira I (2009) Biosensor based on laccase and an ionic liquid for determination of rosmarinic acid in plant extracts. *Talanta* 77: 1322-1327.
 70. Franzoi AC, Vieira IC, Dupont J, Scheeren CW, de Oliveira LF (2009) Biosensor for luteolin based on silver or gold nanoparticles in ionic liquid and laccase immobilized in chitosan modified with cyanuric chloride. *The Analyst* 134: 2320.
 71. Diaconu M, Litescu SC, Radu GL (2010) Laccase MWCNT chitosan biosensor A new tool for total polyphenolic content evaluation from in vitro cultivated plants. *Sensors and Actuators B: Chemical* 145: 800-806.
 72. Tortolini C, Di Fusco M, Frascioni M, Favero G, Mazzei F (2010) Laccase polyazetidine prepolymer MWCNT integrated system: Biochemical properties and application to analytical determinations in real samples. *Microchemical Journal* 96: 301-307.
 73. Xu X, Guo M, Lu P, Wang R (2010) Development of amperometric laccase biosensor through immobilizing enzyme in copper-containing ordered mesoporous carbon (Cu-OMC)/chitosan matrix. *Materials Science and Engineering: C* 30: 722-729.
 74. Kushwah BS, Bhadauria S (2010) Development of biosensor for phenol detection using agarose-guar gum-based laccases extracted from *Pleurotus ostreatus*. *Journal of Applied Polymer Science* 115: 1358-1365.
 75. Litescu SC, Eremia SAV, Bertoli A, Pistelli L, Radu GL (2010) Laccase-Nafion Based Biosensor for the Determination of Polyphenolic Secondary Metabolites. *Analytical Letters* 43: 1089-1099.
 76. Pang Y, Zeng G, Tang L, Zhang Y, Li Z, et al. (2011) Laccase biosensor using magnetic multiwalled carbon nanotubes and chitosan/silica hybrid membrane modified magnetic carbon paste electrode. *Journal of Central South University of Technology* 18: 1849-1856.
 77. Li Y, Zhang L, Li M, Pan Z, Li D (2012) A disposable biosensor based on immobilization of laccase with silica spheres on the MWCNTs-doped screen-printed electrode. *Chemistry Central Journal* 6: 103.
 78. Chawla S, Rawal R, Kumar D, Pundir CS (2012) Amperometric determination of total phenolic content in wine by laccase immobilized onto silver nanoparticles/zinc oxide nanoparticles modified gold electrode. *Analytical Biochemistry* 430: 16-23.
 79. Rawal R, Chawla S, Devender, Pundir CS (2012) An amperometric biosensor based on laccase immobilized onto Fe₃O₄NPs/cMWCNT/PANI/Au electrode for determination of phenolic content in tea leaves extract. *Enzyme and Microbial Technology* 51: 179-185.
 80. Eremia SAV, Vasilescu I, Radoi A, Litescu SC, Radu GL (2013) Disposable biosensor based on platinum nanoparticles-reduced graphene oxide-laccase biocomposite for the determination of total polyphenolic content. *Talanta* 110: 164-170.
 81. Portaccio M, Di Tuoro D, Arduini F, Danila Moscone, Marcella Cammarota, et al. (2013) Laccase biosensor based on screen-printed electrode modified with thionine carbon black nanocomposite, for Bisphenol A detection. *Electrochimica Acta* 109: 340-347.
 82. Amatongchai M, Sroysee W, Laosing S, Chairam S (2013) Rapid screening method for assessing total phenolic content using simple flow injection system with laccase based-biosensor. *International Journal of Electrochemical Science* 8: 10526-10539.
 83. Mei LP, Feng JJ, Wu L, Zhou JY, Chen JR, et al. (2015) Novel phenol biosensor based on laccase immobilized on reduced graphene oxide supported palladium copper alloyed nanocages. *Biosensors and Bioelectronics* 74: 347-352.
 84. Akbulut H, Bozokalfa G, Asker DN (2015) Polythiophene-g-poly(ethylene glycol) with Lateral Amino Groups as a Novel Matrix for Biosensor Construction. *ACS Applied Materials & Interfaces* 7: 20612-20622.
 85. Fu J, Li D, Li G, Huang F, Wei Q (2015) Carboxymethyl cellulose assisted immobilization of silver nanoparticles onto cellulose nanofibers for the detection of catechol. *Journal of Electroanalytical Chemistry* 738: 92-99.
 86. Fu J, Pang Z, Yang J, Huang F, Cai Y, et al. (2015) Fabrication

- of polyaniline/carboxymethyl cellulose/cellulose nanofibrous mats and their biosensing application. *Applied Surface Science* 349: 35-42.
87. Li D, Lv P, Zhu J, Yao Lu, Chen Chen, et al. (2015) NiCu Alloy Nanoparticle-Loaded Carbon Nanofibers for Phenolic Biosensor Applications. *Sensors* 15: 29419-29433.
88. Upan J, Reanpang P, Chailapakul O, Jakmune J (2016) Flow injection amperometric sensor with a carbon nanotube modified screen printed electrode for determination of hydroquinone. *Talanta* 146: 766-771.
89. Sekretaryova AN, Volkov AV, Zozoulenko IV, Turner APF, Vagin MY, et al. (2016) Total phenol analysis of weakly supported water using a laccase-based microband biosensor. *Analytica Chimica Acta* 907: 45-53.
90. Yang J, Li D, Fu J, Huang F, Wei Q (2016) TiO₂-CuCNFs based laccase biosensor for enhanced electrocatalysis in hydroquinone detection. *Journal of Electroanalytical Chemistry* 766: 16-23.
91. Cabaj J, Jędrychowska A, Zajac D, Krawiec S, Sołducho J (2016) Phenolic Compounds Determination Using Laccase-based Electrode Modified with Conducting Polymer Support. *International Journal of Electrochemical Science* 11: 609-620.
92. Wang T, Milton RD, Abdellaoui S, Hickey DP, Minter SD (2016) Laccase Inhibition by Arsenite/Arsenate: Determination of Inhibition Mechanism and Preliminary Application to a Self-Powered Biosensor. *Analytical Chemistry* 88: 3243-3248.
93. Li G, Sun K, Li D, Pengfei Lv, Qingqing Wang, et al. (2016) Biosensor based on bacterial cellulose-Au nanoparticles electrode modified with laccase for hydroquinone detection. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 509: 408-414.
94. Kim JH, Hong SG, Sun HJ, Ha S, Kim J (2016) Precipitated and chemically-crosslinked laccase over polyaniline nanofiber for high performance phenol sensing. *Chemosphere* 143: 142-147.
95. Sarika C, Shivakumar MS, Shivakumara C, Krishnamurthy G, Narasimha Murthy B, et al. (2017) A novel amperometric catechol biosensor based on α -Fe₂O₃ nanocrystals-modified carbon paste electrode. *Artificial Cells, Nanomedicine, and Biotechnology* 45: 625-634.
96. Romero Arcos M, Garnica Romo M, Martínez Flores H (2016) Electrochemical Study and Characterization of an Amperometric Biosensor Based on the Immobilization of Laccase in a Nanostructure of TiO₂ Synthesized by the Sol-Gel Method. *Materials* 9: 543.
97. Li D, Zang J, Zhang J, Kelong AO, Qingqing Wang, et al. (2016) Sol-Gel Synthesis of Carbon Xerogel-ZnO Composite for Detection of Catechol. *Materials* 9: 282.
98. Li DW, Luo L, Lv PF, Qing Qing Wang, Ke Yu Lu, et al. (2016) Synthesis of Polydopamine Functionalized Reduced Graphene Oxide-Palladium Nanocomposite for Laccase Based Biosensor. *Bioinorganic Chemistry and Applications* 2016: 1-10.
99. Yang J, Li D, Pang Z, Wei Q (2016) Laccase Biosensor Based on Ag-Doped TiO₂ Nanoparticles on CuCNFs for the Determination of Hydroquinone. *Nano* 11: 1650132.
100. Lepore M, Portaccio M (2017) Optical detection of different phenolic compounds by means of a novel biosensor based on sol-gel immobilized laccase. *Biotechnology and Applied Biochemistry* 64: 782-792.
101. Kavetsky T, Smutok O, Gonchar M, Olha Demkiv, Plamen Petkov, et al. (2017) Laccase-containing ureasil-polymer composite as the sensing layer of an amperometric biosensor. *Journal of Applied Polymer Science* 134: 45278.
102. Maleki N, Kashanian S, Maleki E, Nazari M (2017) A novel enzyme based biosensor for catechol detection in water samples using artificial neural network. *Biochemical Engineering Journal* 128: 1-11.
103. Almeida I, Henriques F, Carvalho MD, Viana AS (2017) Carbon disulfide mediated self-assembly of Laccase and iron oxide nanoparticles on gold surfaces for biosensing applications. *Journal of Colloid and Interface Science* 485: 242-250.
104. Patel SKS, Anwar MZ, Kumar A, Sachin Otari, Jung Kul Lee, et al. (2018) Fe₂O₃ yolk-shell particle-based laccase biosensor for efficient detection of 2,6-dimethoxyphenol. *Biochemical Engineering Journal* 132: 1-8.
105. Zheng Y, Wang D, Li Z, Sun X, Gao T, et al. (2018) Laccase biosensor fabricated on flower shaped yolk shell SiO₂ nanospheres for catechol detection. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 538: 202-209.
106. Kavetsky T, Smutok O, Demkiv O, Sigita Kasetaitė, Jolita Ostrauskaitė, et al. (2019) Dependence of operational parameters of laccase-based biosensors on structure of photocross-linked polymers as holding matrixes. *European Polymer Journal* 115: 391-398.
107. Rubio Govea R, Hickey DP, García Morales R, Melissa Rodríguez Delgado, Mario A Domínguez Rovira, et al. (2020) MoS₂ nanostructured materials for electrode modification in the development of a laccase based amperometric biosensor for non-invasive dopamine detection. *Microchemical Journal* 155: 104792.
108. Demkiv OM, Gayda GZ, Broda D, Gonchar MV (2021) Extracellular laccase from *Monilia fruticola*: isolation, primary characterization and application. *Cell Biology International* 45: 536-548.
109. Zhao K, Veksha A, Ge L, Lisak G (2021) Near real-time analysis of para-cresol in wastewater with a laccase-carbon nanotube-based biosensor. *Chemosphere* 269: 128699.
110. Chandran M, Aswathy E, Shamna I, Vinoba M, Kottappara R, et al. (2021) Laccase immobilized on Au confined MXene based electrode for electrochemical detection of catechol. *Materials Today: Proceedings* 46: 3136-3143.
111. Quintanilla Villanueva GE, Luna Moreno D, Blanco Gámez EA, Rodríguez Delgado JM, Villarreal Chiu JF, et al. (2021) A Novel Enzyme-Based SPR Strategy for Detection of the Antimicrobial Agent Chlorophene. *Biosensors* 11: 43.
112. Zhang M, Zhang Y, Yang C, Ma C, Tang J (2021) A smartphone-assisted portable biosensor using laccase-mineral hybrid microflowers for colorimetric determination of epinephrine. *Talanta* 224: 121840.
113. Sangubotla R, Kim J (2021) Fiber-optic biosensor based on the laccase immobilization on silica-functionalized fluorescent carbon dots for the detection of dopamine and multi-color imaging applications in neuroblastoma cells. *Materials Science and Engineering: C* 122: 111916.
114. Zhong X, Wang F, Piao J, Chen Y (2021) Fabrication and application of a novel electrochemical biosensor based on a mesoporous carbon sphere@UiO-66-NH₂/Lac complex enzyme for tetracycline detection. *The Analyst* 146: 2825-2833.
115. Bounegru AV, Apetrei C (2021) Development of a Novel Electrochemical Biosensor Based on Carbon Nanofibers Cobalt Phthalocyanine-Laccase for the Detection of p-Coumaric Acid in Phytochemicals. *International Journal of Molecular Sciences* 22: 9302.
116. Bonet San Emeterio M, Felipe Montiel N, del Valle M (2021) Graphene for the Building of Electroanalytical Enzyme-Based Biosensors. Application to the Inhibitory Detection of Emerging Pollutants. *Nanomaterials* 11: 2094.
117. Castrovilli MC, Tempesta E, Cartoni A, Paolo Plescia, Paola Bolognesi, et al. (2022) Fabrication of a New, Low-Cost, and Environment-Friendly Laccase-Based Biosensor

- by Electrospray Immobilization with Unprecedented Reuse and Storage Performances. ACS Sustainable Chemistry & Engineering 10: 1888-1898.
118. Bravo I, Prata M, Torrinha Á, Delerue Matos C, Lorenzo E, et al. (2022) Laccase bioconjugate and multi-walled carbon nanotubes-based biosensor for bisphenol A analysis. Bioelectrochemistry 144: 108033.
119. Evli S, Uygun DA (2023) Laccase modified GO/TiS₂ nanocomposite based amperometric biosensor for (-)-epicatechin detection. Microchem J 188: 108501
120. Machado AC, CSalvo Comino ML, Rodriguez Mendez ML, Caseli L (2025) Enhancing biosensor capabilities through Laccase immobilization in nanostructured Langmuir-Blodgett films for phenol detection. Results in Surfaces and Interfaces 18: 100366.

Copyright: ©2025 Chandra S Pundir, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.