Journal of Applied Biology & Biotechnology Vol. 5 (03), pp. 072-085, May-June, 2017
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2017.50313

Polyphenol oxidase (PPO) based biosensors for detection of phenolic compounds: A Review

Ijaz Gul[1], M. Sheeraz Ahmad[1*], S. M. Saqlan Naqvi[1], Ansar Hussain[1], Rahmat Wali[2], Ammad Ahmad Farooqi[3], Ibrar Ahmed[4]

[1]Department of Biochemistry, Pir Mehar Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. [2]Department of Biology, Pir Mehar Ali Shah Arid Agriculture University, Rawalpindi, Pakistan. [3]Institute of Biomedical and genetic Engineering (IBGE), Islamabad, Pakistan. [4]Department of Biochemistry, Faculty of Biological Sciences Quaid-i-Azam University Islamabad, Pakistan.

ARTICLE INFO

Article history:
Received on: 28/06/2016
Accepted on: 03/11/2016
Available online: 19/06/2017

Key words:
Polyphenol oxidase; biosensor; phenolics; tyrosinase, laccase

ABSTRACT

The present review summarizes the literature on applications and development of polyphenol oxidase-based biosensors for detection of phenolic compounds present in industrial waste waters. Phenolic compounds including phenol and its derivatives: bisphenol A, catechol, and cresol are widely used in industrial processes. These compounds cause toxicity to living organisms and can be bioaccumulated in environment and food chain. Global production of phenolic compounds is about 50,000 tons per annum. The presence of these compounds in air, water, and food poses toxicity risks to human health and environment. Monitoring of concentration of phenolic compounds is necessary to avoid the risks posed by these compounds. Conventional methods for the detection and quantification of these compounds include laboratory-based spectrophotometric and chromatographic methods. Biosensors can be an efficient alternative to conventional methods due to their inherent specificity, simplicity and quick responsiveness. Biosensors can play an important role to improve the quality of life. Polyphenol oxidase-based biosensors can potentially be applied to detect phenolic compounds in various biological and non-biological materials.

1. INTRODUCTION

The use of portable and cost effective devices for monitoring of phenolics present in food, environmental and biological samples has become an area of growing interest. Phenolics are ubiquitous in nature. These phenolics cause browning in food items and decrease their market value. Phenolic compounds show antioxidant properties as well. They fight against cancer and heart diseases. Some neurotransmitters are phenolic in nature, for example L-DOPA [1, 2, 3]. The breakdown of lignins, tannins and humic substances produce phenolic compounds. However, some phenolic compounds are ubiquitous pollutants. These phenolics are originated from industrial effluents, products of chemical and pharmaceutical industries, paper industry, paints, textile industry and production of resins. These phenolic compounds contaminate natural waters. [4, 5].

Global production of phenolic compounds is about 50,000 tons per annum [6].These are used in various industrial processes to manufacture plastics, resins, polymers, plasticizers, detergents, pesticides, and wooden products. The industrial waste products of these phenolic compounds leach into the water reservoirs and contaminate medical, nutritional and environmental matrices [7, 8, 9]. The biologically safe concentration of phenolic compounds in water is 0.001 mg/L (1 ppm, or 10–2l μM). Beyond the safety limits, these phenolic compounds are highly toxic. Therefore, it is essential to monitor the concentration of phenolic compounds to limit them within the safe range for aquatic life [10, 11]. Phenol concentration is usually determined by spectrophotometry and chromatography, but these methods are complex and laborious [12]. Alternate methods include liquid–liquid extraction; flame ionic detection and electron capture detection methods of gas chromatography. The higher concentrations of phenols are determined by liquid-liquid extraction, gas chromatographic and mass spectrometer based methods [13]. However, these methods involve complicated sample-handling, expensive equipment and these are time consuming as well [14].

* *Corresponding Author*
M. Sheeraz Ahmad , PMAS-Arid Agiculture University rawalpindi, Pakistan. Email:dr.sheeraz @ uaar.edu.pk

Hence development of a portable, simple and sensitive system to detect the phenolic compounds is need of the age. Enzyme-based biosensors show a considerable potential as an effective alternate to the methods mentioned above. The biosensors based on polyphenol oxidase have been constructed to detect phenolics [15].

Polyphenol oxidase (PPO) shows a strong potential to use as a biosensor, providing some specific advantages over other enzymes. These advantages include the ability of polyphenol oxidase to catalyze electron-transfer reactions without need of additional cofactors, oxidation of phenolic compounds in the presence of oxygen and its good stability [1, 16]. Laccase and tyrosinase are two major groups of phenol oxidases that catalyze the oxidation of phenolic compounds. Polyphenol oxidase (PPO) is a generic word that has been used in may publications to indicate laccase and tyrosinase [17]. The development of polyphenol oxidase-based biosensors for monitoring of phenolics appears to be at a mature stage of technology. Polyphenol oxidase based biosensor systems show high selectivity, sensitivity, good stability and reduced assay times [18]. Polyphenol oxidase based biosensors developed on the principles of amperometry have been largely reported in recent publications. These biosensors are extensively reported in literature because of their high sensitivity, simplicity, quick responsiveness and easy to miniaturize [19]. The performance and stability of a biosensor largely depends upon the material used for enzyme immobilization and method of enzyme immobilization [20]. To date, different materials have been used for immobilization of PPO. Such materials include magnetic nanoparticles, sol–gels, self-gelatinizable graft copolymer of poly(vinyl alcohol) with 4-vinylpyridine, magnetic nanoparticles-coated carbon nanotubes (CNTs) nanocomposite, clays, polymers, inorganic nanomaterials, filter papers, carbon paste, chitosan, alginate and self-assembled monolayers [21]. Optical, thermal and volatametric PPO biosensors have also been developed for detection of phenolic compounds but use of amperometric biosensors is more as compared to other types of biosensors [19].
Keeping in view the importance of biosensors for detection of phenolic compounds we have reviewed the literature on polyphenol oxidase –based biosensors. Different authors have reviewed only tyrosinase based biosensors. There is not any paper which reviews all types of PPO biosensors so we have reviewed all types of polyphenol oxidase based biosensors including tyrosinase-based biosensors. In coming paragraphs we first introduced the polyphenol oxidase and then reviewed the PPO-based biosensors.

2. POLYPHENOL OXIDASE

Polyphenol oxidase (PPO) is produced in almost all living organisms, including animals, plants and microorganisms [22,23]. This enzyme has copper as a prosthetic group and utilizes molecular oxygen as a co-substrate. It catalyzes two important reactions: the first includes a cresolase activity, adding a hydroxyl group to a monophenol at ortho position to convert it into an *o*-diphenolic compound. The second consists of a catecholase activity, converting the diphenolic compound into quinone which is polymerized into red, brown or black pigments [24, 25, 26]. In living tissues, enzyme and phenolic compounds are separated from each other. During extraction process or due to cell damaging, PPO and its substrates interact with each other leading to browning, which lowers the nutritive value of food and alters the protein functions as well [27]. Based on substrate specificity and mechanism of action, polyphenol oxidases are classified into three types: tyrosinase, catechol oxidase and laccase [28].

2.1. Tyrosinase

Tyrosinase uses copper as a prosthetic group [29, 30]. It catalyzes two reactions. In the first reaction, a hydroxyl group is added at ortho position of a monophenolic compound, converting it into an *o*-diphenolic compound (monophenolase or cresolase activity).This diphenolic compound is subsequently oxidized into *o*-quinones by diphenolase or catecholase activity. Tyrosinase uses monophenols as well as diphenols as a substrate. The reaction is illustrated in Figure 1 [31].

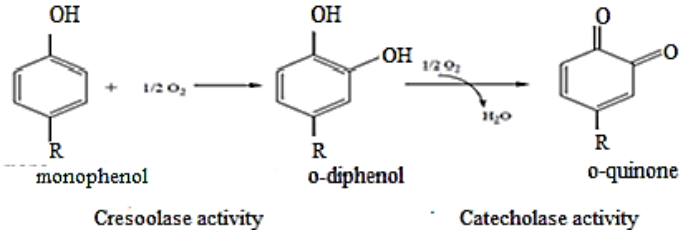

Fig. 1: Reaction mechanism of tytrosinase [31].

2.1.1 Occurrence

According to Jaenicke and Decker [32] tyrosinase present in different organisms is not common. It varies due to size of protein, sequence of amino acids and glycosylation patterns. According to Wichers *et al*. [33, 34] tyrosinase is present in clusters in higher organisms like plants, animals and fungi. There is high similarity within clusters and less similarity between groups. They have conserved active site in all groups where histidine residues make bond with copper atom.

2.1.2. Characteristics of tyrosinase

The active form of PPO has been studied and structure of polyphenol oxidase has been described in different plants and microbes [35]. The crystal structure of tyrosinase obtained from *Strptomyces* shows features different from other PPOs [35]. Tyrosinase shows hydroxylase and catecholase activities. The presence of histidine confers these activities [36].

The tyrosinase from *Aspergillus oryzyae* was expressed in *Escherichia coli* to know the importance of histidine residues not known previously [37]. Site directed mutagenesis was used to know that one Cu atom is linked to three histidine residues and second Cu atom is liganded by four histidine residues.

2.2. Catechol Oxidase

Catechol oxidase is a type3 copper protein and ubiquitous in nature. It uses diphenolic compounds as a substrate whereas, tyrosinase uses monophenolic as well as diphenoli compounds as a substrate. This oxidation reaction is oxygen dependent [38]. The oxidation reaction of catechol oxidase results in highly unstable compound called quinone. This quinone polymerizes to form a brown mesh which is assumed to protect the plant from further damage from insects and pathogens [39].

2.2.1. Characteristics of catechol oxidase

Catechol oxidases (*o*-diphenol oxidases) oxidize *o*-diphenols into the respective o-quinones in oxygen dependent reactions (Figure 2) [40, 41]. A pair of coupled cupric ions is present in active site of catechol oxidase rendering them a status as type-3 copper proteins. Type-3 copper proteins also contain tyrosinase and hemocyanins. Absence of a hydroxylase activity distinguishes catechol oxidase from tyrosinase. Due to lack of this activity; catechol oxidase cannot use monophenols as its substrate [42, 43].

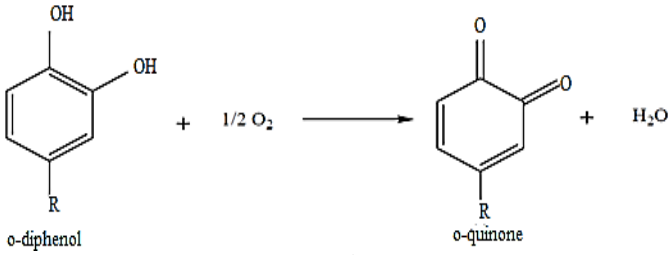

Fig. 2: Reaction mechanism of catechol oxidase [40, 41].

The secondary structure of catechol oxidase from sweet potato reported by Klabund et al [44] indicates that it consists of primarily alpha helices α2, α3, α6 and α7. The catalytic site is present in these helices and surrounded by α1 and α4 and several short β-strands. Three histidine residues coordinate each copper atom with four helices of the α-bundle (Fig. 3). His 88, His 109, and His 118 make bond with CuA. His 240, His 244 and His 274 make bond with second copper [44].

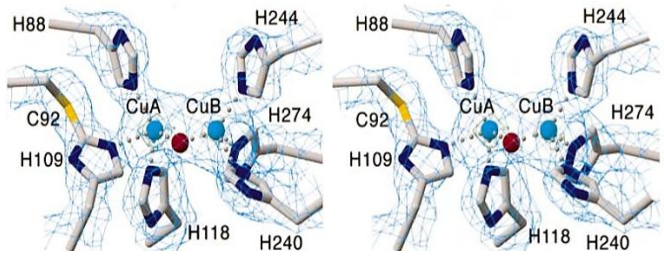

Fig. 3: Structure of active site of catechol oxidase [44].

2.3. Laccase

Laccases have multiple copper atoms and have the ability to oxidize lot of phenolics it the presence of molecular oxygen. Laccase exhibits *p*-hydroxylation activity [44].Different aspects of laccase have been studied exhaustively [45, 46, 47] like molecular genetics [48], gene expression [49, 50, 51], genetic transcription [52] and cloning [53].

2.3.1. Occurrence

Laccases are present in plants [1,54] and fungi like *Cerrenamaxima, Coriolopsispolyzona, Lentinustigrinus Trametes versicolor, T. hirsuta, T. ochracea, T. villosa, T. gallica,* [55, 56] and *Polyporus versicolor, Pholiata spp, Podospora anserina,* [57].

2.3.2. Characteristics of laccase

There are three types of laccases on the basis of the spectroscopic and functional characteristics of its copper atoms which are: copper type1, 2 and 3 [45, 58].

Electron is captured and transferred by copper type 1, molecular oxygen is activated by copper type 2 and oxygen is uptaken by copper type 3 [59]. Reaction mechanism of laccase is shown in Figure 4 [60].

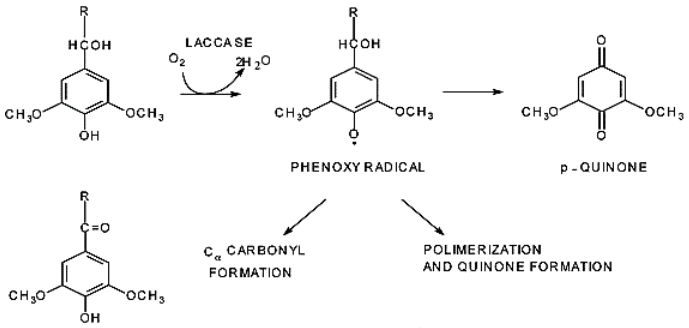

Fig. 4: Reaction mechanism of laccase [60].

3. IMMOBILIZATION OF POLYPHENOL OXIDASE IN BIOSENSORS

For efficient working of biosensors, the biological recognition element (bioreceptor i.e. biologically derived component of biosensor which detects target) and the transducer which amplifies the recognized signals should be in close contact with each other. Immobilization helps to attach the bioreceptor to the transducer and makes the sensor stable and reusable. A biorecepter may be immobilized directly on to a transducer. Alternatively, a membrane can mediate to immobilize the biorecepter that may be immobilized onto the transducer [61, 62].

The support material should be inert, stable and resistant to shearing forces. The shape, size and choice of support material is important for getting stability, selectivity and best activity of an enzyme**.** Sometimes enzyme immobilization may lead to complete loss of enzyme activity [1, 63].

3.1. Methods of enzyme immobilization

Two methods of enzyme immobilizations have been described, one is physical method in which there are weak associations between adsorbent and enzyme and second is chemical method in which there is covalent bond formation between enzyme and support [64]**.** The method of immobilization

depends upon the nature of the bio receptor, the type of transducer used, the physicochemical properties of the sample, the operating conditions of the biosensor and maximum activity of biological element in immobilized form [65]. Common methods of immobilization include adsorption, covalent binding and entrapment of bio receptor to transducer, encapsulation and cross-linking of biorecepter with transducer [66]. Adsorption is very simple, cost effective and rapid method of immobilization. The enzyme bounds to the support material by hoydrogen bonds, ionic interactions or Vander Waals forces. However adsorption does not strongly binds the biological material. It may be easily desorbed from the support material under ordinary conditions [67].

In case of covalent method of enzyme immobilization, insoluble matrix and different cross linkers are used for immobilization of the enzyme. No single method of enzyme immobilization can be specified for a specific enzyme because different enzymes and there substrates have different properties; secondly these methods have advantages as well as disadvantages [68, 69].

In encapsulation, the polymers are used for confinement of enzyme in core of micro shperes [70]. Entrapement is a process in which biomaterial is polymerized into a gel by trapping it within the interstitial spaces of the gel used for trapement. Enzyme solution is mixed with gel and then gel is allowed for polymerization [71].

4. SIGNAL TRANSDUCTION PRINCIPLES OF POLYPHENOL OXIDASE BIOSENSORS

Biosensors are electrochemical devices, comprising of a bioelement and a transducer (which may be an electrochemical, mass, optical or thermal) [72, 73]. A biosensor can be a promising technique for analysis of food and environmental monitoring. Biosensors are selective, specific and cost effective and have potential to be miniaturized and automated. These can be simple portable instruments for speedy analyses at different sites like quality control labs, food quality testing etc [74, 75]. Biosensor development has been detailed in various papers, with potential applications in clinical, environmental, agricultural and biotechnological areas. [76].

Biosensors are classified on the basis of their biological recognition elements and transducers [72]. The biological recognition element may be an enzyme, antibody, living cells, tissues etc. The sensor elements include current, conductivity, potential, intensity, electromagnetic radiations, mass and impedance [77, 78, 79]. The design of support matrix plays very important role in enzyme immobilization and signal transduction. Conducting polymers are widely used in biosensors as support matrix [80-83].

5. ELECTROCHEMICAL BIOSENSORS

In electrochemical biosensors the catalytic action of enzyme produces electrons. The analyte takes part in the reaction taking place on the active electrode and potential is created by ions. This potential is deducted from the reference electrode as a result a measurable signal is produced [84].

Various types of PPO-based biosensors have been developed, including electorchemical, optical and thermal biosensors [85]. In electrochemical biosensor devices, a bioelement is used as a biocatalyst in association with an electrical transducer [86]. A biosensor interacts with its analyte and produces certain signals in the form of current, potential difference, impedance or conductivity. Electrochemical biosensors measure change in current, voltage or conductance resulted from oxidation of analytes. Electrochemical biosensors are divided into conductimetric biosensors, potentiometric/voltametric biosensors and amperometric biosensors [87, 88].

The activity of a biological element in a biosensor changes conductance of the electrode which is directly measured. Change in conductance is correlated with the type and amount of substrate catalyzed by enzyme (conductimetric biosensors) [67].

In potentiometric biosensors change in potential difference is measured [72] in case of amperometric biosensors change in current is measured [89].

Use of catechol oxidase as a biosensor is rarely reported in literature so we reviewed only laccase and tyrosinase-based biosensors. We have subdivided these biosensors on the basis of their transduction principles.

6. LACCASE-BASED BIOSENSORS

Laccase shows a 4-electron reduction catalytic activity in which oxygen is reduced to water subsequently laccase oxidizes lot of phenolic substrate which makes laccase a potential candidate for fabrication in biosensors [90].

6.1 laccase-based amperometric biosensors

Laccase based amperometric biosensors are important class of biosensors in which current is produced as a result of enzymatic reaction and this change acts as a signal which is measured by physical techniques [1,72,90]. There is a linear relationship between observed current and amount of reactive species present. Mostly platinum, gold or carbon based electrodes are constructed [84, 90, 91].

Sarika *et al.* [92] developed three different types of amperometric biosensors for comparative studies in view of their use in waters polluted by phenolic compounds. The substrate was catechol. *Trametes versicolor* was used as a source of laccase and immobilization was done on gold electrode. Type A biosensor developed by crosslinking laccase with glutaraldehyde. In second type of biosensor (type B) laccase was immobilized by organothiol layers on gold electrode. Third biosensor (type C) was developed by covalently binding the laccase to silanized gold electrode. Among all the three electrodes type C was best with respect to specificity, sensitivity, detection limit, response time and stablitiy. pH7 was optimum for the sensor because it has shown maximum activity at this pH value.

Immobilization of laccase on silanized gold electrode of clark type DO sensor is reported by Sarika, et al. (92) (figure 5)

[90, 92]. Analysis was based on flow injection and batch types analytical systems. Biosensor was optimized for different optimal conditions. The analysis time in the flow injection mode was less than 100 seconds. The reusability of the immobilized enzyme was upto 500 detections. The enzyme activity was maximum up to 500 detections of phenolic compounds. This method was practically validated by tannin analysis in tea of different brands [92].

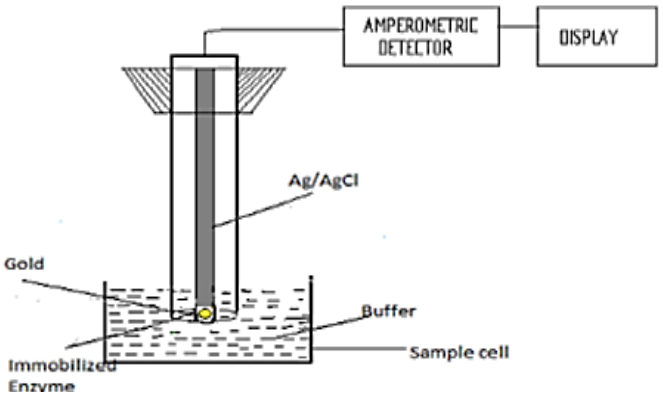

Fig. 5: Biosensor configuration [92.

Laccase was immobilized for amperometric detection of polyphenolics. Potential of laccase catalysis was observed for different polyphenols at a particular potential. Laccase was found to respond the catechol [93, 94]. Den-AuNP nanocomposites were used by Rahman *et al.* [1,86, 141] for immobilization of laccase to detect catechin. Detection of catechin was done with that biosensor. Biosensor was responsive to other phenolic compounds as well. Vianello *et al.* [95] prepared a biosensor using mono molecular layer of laccase obtained from *Rigidoporus lignosus* by immobilization on a gold support. The biosensor was sensitive to micromolar concentration (below the European Community limits (0.5mg/L) of of phneols. Recombinant laccase was developmed by Kulys *et al* [1, 95, 96] used for the first time, recombinant fungal laccase using *Polyporus pinsitus* as a source of laccase and thermostable recombinant laccase from *Myceliophthora thermophile* for biosensors using printed graphite electrodes suitable for measuring phenolic compounds in alarm systems. A ferrocene modified graphite screen printed electrode was used to immobilize the laccase and tyrosinase to produce an amperometric bi-enzyme biosensor. Biosensor showed good results for detection of phenols in wine samples and data was compared with data obtained by Folin-ciocalteu method. The results of biosensor gave good agreement with Folin-ciocalteu method. The activity of the biosensor was inhibited due to presence of sulphur dioxide [67].

Gutierrez-sanchez [97] used a fungal species *T. hirsuta* as a source of laccase. Low density graphite electrode was used as a support material for immobilization of laccase. Real time analysis of oxygen concentration was done. Good reproducibility,sensitivity and accuracy was observed. The detection limit was below one micor molar and sensitivity was higher than 60 nA cm^{-2} M^{-1} [96, 97].

6.2. Laccase-based voltametric biosensors

Voltammetry is the measurement of the potential difference and the result is a current/potential curve [69]. Adrenaline determination was done by developing a biosensor based on laccase using *Aspergillus oryzae* as a source of laccase. 1-butyl-3-methylimidazolium cation (BMI) associated with the anions hexafuorophosphate (BMI*PF6) or bis(trifuoromethylsulfonyl)imide (BMI*Tf2N) were used for biosensor construction. BMITf2N based biosensor gave good response as compared to BMIPF6. The optimized conditions were: pulse amplitude 100 mV, frequency 10 Hz and scan increment 4.0 mV. The limit of detection of biosensor was 5.34 .x. 10^{-7}mol L^{-1}. The results obtained by laccase based biosensor were in accordance to the at the 95percent confidence level [66].

A microsphere composed of chitosan crosslinked with triphosphate by spray drying was used as a support for immobilization of laccase. The rutin determination in pharmaceutical processes can be done using this biosensor. The biosensor exhibits good sensitivity, reproducibility, low detection limit and rapid response as well [97, 98]. Laccase based electrochemical biosensor was developed by Nazari *et al* [99]. Glassy carbon electrode was developed. Laccase was immobilized on polyaniline which was electrodeposited on to glassy carbon. Electrode was characterized by voltammetry. Studies indicated that immobilization of laccase was due to covalent interaction between functional groups of glutaraldehyde and laccase. There was direct electron transfer between immobilized laccase and electrode.

Enzyme immobilization on three types of supporting materials was done to check the most effective and suitable material on the surface of a glassy-carbon electrode. The polymers used were: positively charged cetyl ethyl polyethyleneimine and negatively charged Nafion and Eastman AQ 29D polymers. The detection limits of laccase immobilized on Nafion membrane for hydroquinone and pyrocatechol were recorded as: 3.5×10^{-8} and 5.0×10^{-8} M respectively. Immobilization of laccase on Nafion and Eastman AQ 29D membranes showed more sensitivity and shortest response time. Gelatin can be used to increase the storage stability of the enzyme in immobilized form [62].

Nanocomposites based biosensors are widely studied. Chen *et al*. [99, 100] designed a biosensor using zein which is a natural biodegradable protein polymer. This polymer was used to design a new composite of laccase–gold nanoparticles (AuNPs) cross-linked zein ultrafine fibers (CZUFs) for the determination of catechol. The biosensor demonstrated high sensitivity due to direct transfer of electrons [99, 100]. Because of unique properties of ionic liquids (ILs) they are used in electroanalysis, mainly resulting from their peculiar structural organization. Salts resulting from the combination of imidazolium cations within organic or organic anions have particular importance [100]. These salts are commonly used to prepare laccase biosensors for preparation of modified electrodes. These are also used in preparation and stabilization of nanomaterials, such as platinum NPs (PtNPs) and AuNPs dispersed in ILs [101].

6.3 Laccase-based optical biosensors

In optical biosensors the spectroscopic properties of the intermediates produced are exploited in laccase biosensor [102]. Abdullah *et al* [103] developed a laccase based optical biosensor was designed using stacked films made by immobilization of MBTH and laccase. MBTH was immobilized in nafion/sol-gel silicate film and chitosan was used for immobilization of laccase. Azo-dye product was produced as a result of reaction between enzymatically produced quionones with MBTH which is a nucleophile. Detection was done by spectrophotometer. The response time of sensor was ten minutes and limit of detection was 0.33mM.

Setti *et al*. [104] reported that laccase can react with methoxy phenols in the presence of an indicator, 3-methyl-2benzothiazolinone hydrazine (MBTH) to produce azo-dy. This concept became base for Abdullah, *et al*. for the development of an optical biosensor in which MBTH was used as stacked film in Nafion/sol-gel silicate and laccase was present in chitosan for the detection of phenolics .This study also revealed that immobilization of laccase in hybrid materials makes it more specific to catechol than other analytes such as guaiacol, o-cresol and m-cresol that were also tested.

Cabaja *et al* [105] fabricated laccase in an optical biosensor. Laccase was immobilized in a ceramic based biosensing system and ammonium salt of 2,2-azibis (ABTS) was used as substrate of laccase. This biosensor a potential device for permanent monitoring phenolic compounds present in different aqueous matrices. Immobilized laccase revealed catalytic action and substrate or analyte was determined optically. For ooptical analysis change in absorbance and fluorescence are measured [106].

Mbouguen *et al* [107] deposited organoclay onto a glassy carbon electrode and laccase was immobilized on this support material. Organoclay was used to increase the sensitivity of immobilized laccase. The use of ferrocene as a mediator was compatible with bioelectrode. A three layer configuration was used to develop biosensor. Glassy carbon was coated with ferrocene first then enzyme-clay mixture was added and finally, third layer was made with enzyme free clay. The characterization was done with cyclic voltammetry and catechol was used as a substrate.

E. coli O157:H7 detector was developed by using the ability of laccase to oxidize in alkaline medium compatible to luminol. The method was based on the characterization of a luminol−H_2O_2−laccase reaction [106].

Laccase was immobilized on chitosan film; hybrid nafion/sol–gel silicate was used for immobilization of MBTH. Reaction of quinone with MBTH produces a spectrophotometrically detectable product. The biosensor demonstrated a linear response in the range of 0.5-8.0 mM concentration of catechol and limit of detection was 0.33 mM and response time of 10 minutes. The biosensor exhibited stability for two months [63]. The reaction mechanism of immobilized laccase has been shown in figure 6[(64].

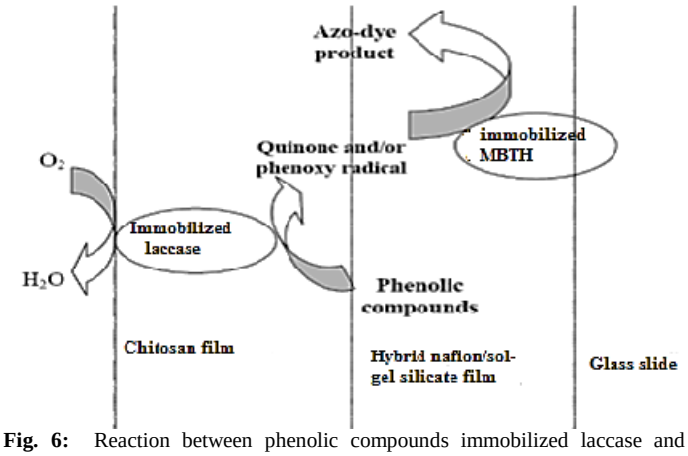

Fig. 6: Reaction between phenolic compounds immobilized laccase and immobilized MBTH [103].

A cost effective laccase-based paper biosensor has been developed for phenol detection. MBTH is used as an indicator which produces maroon-green color on reacting with quinones produced by enzymatic reaction. The optimum concentration of enzyme to be loaded on paper was 2 mg/ml while, the optimum concentration of MBTH was 24 mM. The biosensor gave good response and sensitivity for L-DOPA and catechol and its detection limit was 64 µM. The color intensity was proportional to the amount of substrate and the results were reproducible [68].

6.4. Laccase-based thermal biosensors

In calorimetric biosensos change in heat is measured.A laccase based thermal biosensor was developed by Bai *et al*. [108, 1] by using PVA as support material for enzyme immobilization.. change in heat or enthalpy was measured and correlated with concentration of phenolics present. Thermal biosensors are less common than others. Very little research has been found in this area of biosensor development and it may become a target for future research work.

7. TYROSINASE-BASED BIOSENSORS

7.1. Tyrosinase-based amperometric biosensors

Tyrosinase based various amperometric biosensors have been described in different papers. Tyrosinase was immobilized by entrapment method in a polymer, poly 3,4-ethylenedioxythiophene (PEDT), for detection of phenolics and herbicides. Glassy carbon electrode was optimized for different parameters. Amperometric measurements were done. The biosensor was sensitive for 5 to 500 nM concentrations of phenolic compounds. Herbicides inhibit tyrosinase so, their determination was based on tyrosinase inhibition. The limit of detection for the herbicides, atrazine and diuron was 1 and 0.5 mg l^{-1} respectively [109].

A tyrosinase biosensor was designed using cross linking method of immobilization. The enzyme was cross-linked at a 3-mercaptopropionic acid (MPA) self-assembled monolayer (SAM) on a gold disk electrode, to detect the phenolic compounds under

flow injection. Experimental variables such as the detection potential (−100 mV versus Ag|AgCl|KCl 3 M), flow rate (1.02 ml min^{-1}), injection volume (350 µl), and pH of the carrier solution (0.05 M phosphate buffer of pH 7.0) were optimized. The biosensor exhibited good reproducibility and no requirement for pre-treatment procedures. The life time of biosensor was five days. Under these conditions, the tyrosinase biosensor showed a good reproducibility of the FI measurements, without need of pre-treatment procedures. The biosensor was stable for five days. The biosensor was useful for real time monitoring of phenolic compounds [110].

Sol-gel techniques were used for immobilization of tyrosinase for amperometric detections of phenolic compounds. Sol-gel techniques were used for the development of an amperometric biosensor for phenol determination. TA grafting copolymer was introduced into sol–gel solution and the composition of the resultant organic–inorganic composite material was optimized. The enzyme was responsive to different phenolic compounds determined at 0 mV vs. Ag/AgCI (sat. KCI). The biosensor was sensitive to catechol, phenol and *p*-cresol and sensitivity was 59.6, 23.1 and 39.4 µA/mM, respectively. 73% of enzyme original activity was maintained even after 21 days storage at 4 °C [111].

The tyrosinase was covalently immobilized to a reticulated vitreous carbon (RVC)-based flow-through electrode. The biosensor was designed for amperometric detections. The bioelectrode was tested as an amperometric detector for phenolic compounds. The biosensor was optimized for different variables like, method for immobilization, concentration of enzyme and pH of buffer. The biosensor was effective for detection of different phenolic compounds. The detection limit of the biosensor was 2 µg l^{-1} (4-chloro-3-methylphenol) to 2 mg l^{-1}. The biosensor was stable up to 20 days [112].

Amperometric tyrosinase graphite-epoxy electrode was designed for phenol determination. The enzyme was immobilized by entrapment method using a graphite-epoxy matrix. Hardener was mixed with resin. The bioelectrode was optimized for different conditions. Bioelectrode was responsive to catechol and phenol with detection limits, 1·0 µM and 0·04 µM, respectively. The enzyme showed response for consecutive 100 determinations. The enzyme activity was found stable. The stability of biosensor was dependent on storage conditions [113]. Tyrosinase biosensors were constructed using three different composite matrices for amperometric detections of phenolics. The composite electrode matrices were , graphite–Teflon; reticulated vitreous carbon (RVC)–epoxy resin; and graphite–ethylene/propylene/diene (EPD) terpolymer. The biosensor was optimized for experimental conditions. The reaction kinetics of the tyrosinase reaction was calculated for eight different phenolics. The sensitivity of biosensor for different substrates was compared which suggests that immobilization matrix affects the catalytic cycle of tyrosinase [114]. Tyrosinase was immobilized on polypyrrole film on a Pt disk electrode (figure 7). Enzyme maintained its activity in pyrole film and clearly defined reduction current proportional to the concentration of substrate was observed. Substrate concentration was observed by analyzing the changes in current. The sensitivity of biosensor was observed for different substrates and its sensitivity in decreasing order is: catechol> phenol> 2-bromophenol> 2-chlorophenol> 2-iodophenol> 2-fluorophenol. This biosensor exhibited a potential for phenol determination in different aqueous media. The biosensor exhibited high stability and sensitivity. This sensor can be used for industrial and environmental monitoring processes [115].

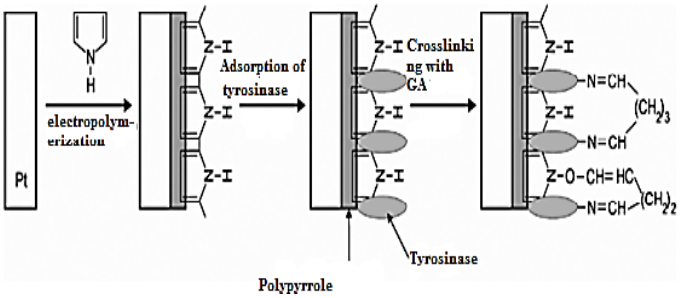

Fig. 7: Scheme of fabricating biological layer [115].

A novel amperometric tyrosinase biosensor based on Fe_3O_4 nanoparticles–chitosan nanocomposite has been reported for phenol determination. The large surface area of Fe_3O_4 nanoparticles and the porosity of chitosan led to increase the amount of enzyme and increase in bioactivity of immobilized enzyme. Biosensor characterization was done by atomic force microscopy and AC impedance spectra. Different parameters that govern the biosensor performance have been analyzed. The limit of detection shown by the biosensor lies in the range of 8.3×10^{-8} to 7.0×10^{-5} mol L^{-1}, and limit of detection is 2.5×10^{-8} mol L^{-1} [116]. A biosensor was developed by immobilizing tyrosinase in a titaniasol-gel membrane using vapour deposition method. The sensor was effective for phenol determination and exhibited high stability and good sensitivity. The response time of biosensor was below 5 seconds and sensitivity of biosensor was in descending order as: catechol then phenol and least sensitivity for p-cresol. Immobilization of tyrosinase within an Os-complex functionalized electrode position polymer can be used for the monitoring of different phenolics [117].

7.2. Tyrosinase based voltametric biosensors

A tyrosinase based biosensor was developed using boron-doped diamond (BDD) electrode modifying by tyrosinase (Ty). The immobilization was done on polyaniline (PANI) doped with polyvinyl sulfonate (PVS) composite films and detection was based on voltametric methods. Biosensor was sensitive to the substrate like L-phenylalanine and L-tyrosine. The limit of detection was $1.0x10^{-2}$ µM for both compounds. The activity was retained upto 85% after 24 hours. Sample preparation and separation procedures required for other determination methods have been eliminated by this biosensor and it can be used for phenol determination in red wine [118]. A tyrosinase biosensor

was developed by adsorbing and immobilizing the enzyme on the surface of high isoelectric point ZnO nanoparticles (nano-ZnO). This adsorption was facilitated by electrostatic interactions and glassy carbon electrode via the film forming by chitosan respectively. The biosensor does not need any other mediator for electron. Optimization studies were done.The limit of detection for biosensor was 5.0×10^{-8} mol L^{-1} [119].

Use of modified magnetic MgFe2O4 nanoparticles for the development of a tyrosinase based biosensor has been reported by Liu *et al*. [120]. First tyrosinase was covalently immobilized to core-shell ($MgFe_2O_4$–SiO_2) magnetic nanoparticles. Modification of nanoparticles was done with amino group on its surface. The resulting magnetic bio-nanoparticles were attached to carbon paste electrode (CPE) by a permanent magnet. The immobilization matrix provided good environment for tyrosinase retention. The detection range of biosensor was 1×10^{-6} to 2.5×10^{-4} M and its limit of detection was 6.0×10^{-7} M. The bionanoparticle conjugation procedure is shown in Figure 8 [120].

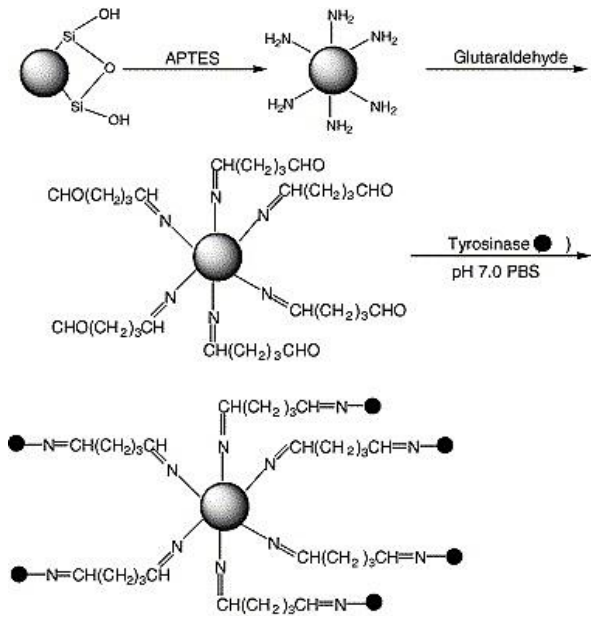

Fig. 8: Preparation of magnetic bio-nanoparticles [120].

Agarose-guar gum entrapped matrix can be used for immobilization of tyrosinase for detection of phenolic compounds. Voltametric tyrosinase biosensor was constructed for phenol determination. Tyrosinase was extracted from *Amorphophallus companulatus* and catechol was detected by reduction of quinones released by enzymatic action. The response of biosensor was linear and concentration dependent, which ranges from 6×10^{-5} to 8×10^{-4} M with a lower detection limit of 6 μM. The reusability of the biosensor was up to 20 cycles and storage time of 2 months [121].

Apetrei *et al*. [122] described the preparation of a tyrosinase-based biosensor using biomimetic Langmuir-Blodgett film where lutetium bisphthalocyanine is used as a source of electron mediation for detection of phenolic compounds voltametrically. The voltametric response consists of two steps. First step involves reduction of the quinone produced as a result of the enzymatic reaction and second step involves the electrochemical oxidation of the antioxidant. The limit of detection of the biosensor ranges from 1.98×10^{-6} -27.49×10^{-6} M. The biosensor exhibited good reproducibility and affinity for antioxidants. It shows two redox processes which makes it exceptional for phenol determination.

A novel tyrosinase biosensor was developed by Zhao *et al.* [123]. ZnO nanorod microarrays on the boron-doped nanocrystalline diamond (BDND) were used for development of this biosensor. The ZnO nanorod microarrays were firstly deposited on BDND thin film surfaces using a method known as low-temperature solution method. Then ZnO nanorods were functionalized with the mixture of 3-aminopropyltriethoxysilane (APTES) and tetraethoxysilane (TEOS) by a co-condensation technique. Finally tyrosinase was immobilized to amino-modification ZnO nanorod surfaces by the covalent binding. The biosensor was sensitive to different phenolic compounds and ZnO nanorod array shows potential for being used as a support material for other enzymes as well.

7.3. Tyrosinase based optical biosensors

A tyrosinase based optical biosensor was developed using hybrid nafion/sol-gel for the immobilization of 3-methyl-2-benzothiazolinone hydrazone (MBTH) and chitosan for immobilization of tyrosinase. MBTH stabilized the quinones produced as a result of oxidation reaction by forming of quionone-MBTH adduct. The intensity of the color increases as concentration of substrate increases. The biosensor was responsive to phenol, catechol and m-cresol when their concentrations were: 0.5-7.0, 0.5-10.0 and 1.0-13.0mg/L respectively and limit of detection was 0.18, 0.23 and 0.43mg/L, respectively. The biosensor was stable for 3 months [124].

Fiorentino *et al*. [125] developed a tyrosinase-based optical biosensor for detection of phenolic compound. The biosensor was developed by layer by layer assembly of polycation polymer poly (dimethyldiallylammonium chloride). Different features of biosensor were studied by absorption and fluorescence spectroscopy. Biosensor was used for detection of L-DOPA and exhibited good reusability and stability as well. The absorbance based measurements gave a detection limit of 23 microM and a linear response up to 350 microM. Measurements based on fluorescence gave limit of detection of 3 microM and a linear response in the range of tens of microM.

An optical biosensor was designed and reported by Silletti, et al [126] using different enzymes including tyrosinase. They monitored different chemicals and metabolites using optical features of enzymes. The detection of selected chemicals was performed through bio-mediators of algal cells (Chlamydomonas reinhardtii) and various enzymes (acetyl-cholinesterase, beta galactosidase, d-lactate dehydrogenase, tyrosinase and urease). The monitoring was performed through chlorophyll-a fluorescence for lag The analyses were performed by fluorescence that for the

algal cells was based on chlorophyll a fluorescence for C. reinhardtii cells and fluorescein 5(6)- isothiocyanate or 5(6)-carboxynaphthofluorescein for enzymes [126].

Eunji *et al.* [127] described the development of microarray-based biosensor system for phenol determination. Microarrays based on polyethylene glycol hydrogel were prepared by photopatterning of a solution having polyethylene glycol diacrylate, photoinitiator, tyrosinase, and CdSe/ZnS quantum dots (QDs). The tyrosinase was entrapped in hydrogels. The immobilized tyrosinase could carry out enzymatic oxidation of phenolic compounds which by a non-enzymatic reaction convert into quinones. The fluorescence of QDs is quenched by these quonones. As the concentration of quinones increased the fluorescence intensity of the hydrogel microarrays decreased. The biosensor was sensitive upto 1.0 µM of phenols.

A paper based bioassay has been reported by Oktem *et al* [128]. The detection of the phenolic compound is done by observing change in color produced as a result of enzymatic reaction. Tyrosinase was adsorbed on a filter paper and MBTH was over-spotted. MBTH on reaction with quinone forms adduct which is stable and allows detection of phenolic compound. The intensity of colour increases as concentration of phenolic substrate increases. Quantification of the substrate can be done by taking image of colour product and use image analysis software. The biosensor was responsive to L-DOPA [128].

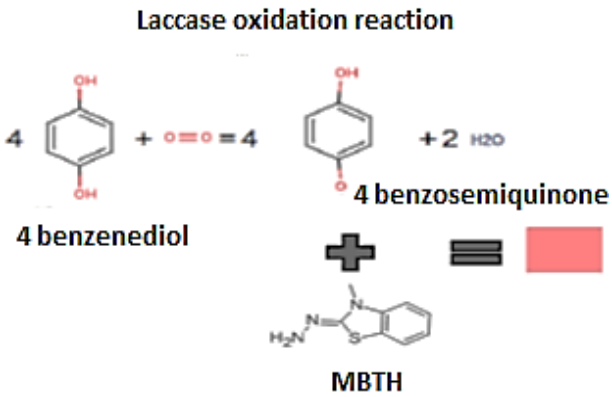

Fig. 9A: Reaction of laccase with its substrate and MBTH [128].

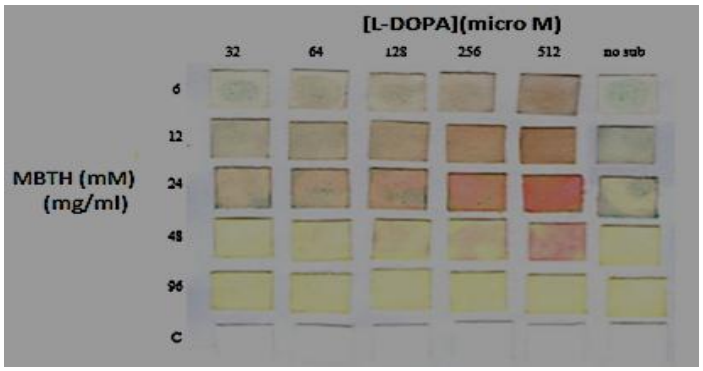

Fig. 9B: Optimization of L-DOPA and MBTH concentration (no sub represents only enzyme-MBTH mixture and C represents papers with L-DOPA only) [128].

A new type of tyrosinase-based paper biosensor was developed by Alkasir et al [129] based on colorimetric detection of phenolic compounds. The biosensor was developed by layer by layer (LbL) deposition of chitosan and alginate polyelectrolytes onto a simple filter paper and these layers physically entrapped the tyrosinase (figure 10). The change in colour was observed by naked eye and a picture can be used for more sensitive comparisons. Increase in substrate concentration increases the intensity of light [129].

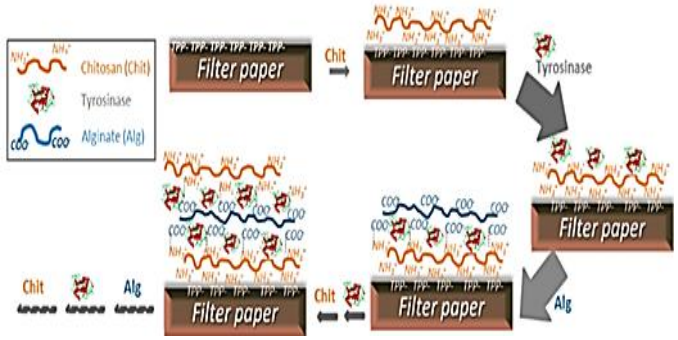

Fig. 10: A schematic representation of tyrosinase based paper biosensors showing sequential layer by layer deposition of chitosan, alginate and tyrosinase on filter paper [129].

7.4. Tyrosinase based thermal biosensors

In thermometric biosensors the heat produced or released as a result of a biological reaction is measured [130]. Thermodynamic properties of molecules were used to study the course of chemical reactions and structural dynamics of molecules [131].However the use of these properties in biosensors became base for development of thermometric devices [132]. Xie *et al.* [133] developed a biosensor in which electrochemical and calorimetric approaches were combined to detect simultaneously the electric signals as well as thermal signals. Enzyme was immobilized on a poly (pyrrole)-coated reticulated vitreous carbon (RVC) matrix. Enzyme column was constructed by platinum. Oxidation of catechol produces quinones. The heat changes are measured calorimetrically and changes in current are measured electrochemically. The cycling of catechol enhances the sensitivity of biosensor. This hybrid biosensor can be used as useful tool for comparative studies. Little research has been done to use tyrosinase as a thermal biosensor indicates an area for research.

8. COMPARISON OF DIFFERENT PPO BIOSENSORS

Table 1 gives a comparison for transduction system, substrate type, enzyme type and limit of detection for different PPO-based biosensors. We noticed that amperometry based biosensors are widely used for phenol determination. The use of thermal biosensors is very low. Polyphenol oxidase obtained from microbial sources is preferably used for development of biosensor. Electrochemical biosensors are simple, cost effective and give quick response but optical biosensors show longer stability as compared to electrochemical biosensors. Amperometric biosensors

Table 1: Comparison of PPO-based Biosensors.

Sr. No	Substrate	Enzyme	Transduction system	Detection level	Reference
1	Catechol	Tyrosinase	Amperometric	10 nM	[112]
2	Chlorophenol	Tyrosinase	Optical	$9x10^{-8}$ M/L	*[124]*
3	Phenol	Tyrosinase	Colorimetric	0.05ppm	[140]
4	Polyphenol (catechin)	Laccase	Electrochemical	$1\text{-}6x10^{-5}$M	[87]
5	Catechol	Tyrosinase	Electrochemical	$6x10^{-6}$M	[116]
6	Catechol	Laccase	Optical	0.33mM	[119]
7	*m*-cresol	Tyrosinase	Optical	1.0microM-0.56mM	[81]
8	*Catechol*	Laccase	*Optical/absorption*	*1.33M*	[125]
9	Phenol	Laccase	Optical/absorption	3.27µM	[127]
10	*Epinephrine*	Laccase	Optical	3.5pg/ml	[123]
11	*Polyphenols: Caffeic acid*	Laccase	Amperometric	*0.151µM*	[86]
12	Total phenolic content	Laccase	Amperometric	0.05µM	[80]
13	Catechin	Laccase	Amperometric	0.05±0.003µM	[141]
14	1,4hydroquinone	Laccase	Amperometric	2µM	[82]
15	Rutin	Laccase	Voltammetry	0.0623 µM	[62]
16	Adrenaline	Laccase	Voltammetry	0.293µM	[69]
17	Dopamine	Laccase	Voltammetry	0.42µM	[111]
18	Catechol	Tyrosinase	Optical	0.18mg/L	[121]
19	L-DOPA	Tyrosinase	Optical	3 µM	[120]
20	Phenols	Tyrosinase	Optical	250 µM	[128]
21	Polyphenols	Tyrosinase	Voltametric	1.98×10^{-6} M	[117]
22	Catechin	Tyrosinase	Amperometric	1.0 µM	[69]
23	Catechol	Tyrosinase	Amperometric	8.3×10^{-8}M	[73]

are better to use for detection of phenolic compounds because their detection limit is higher as compared to other biosensors.Yildiz *et al*., 2007 [117] developed a tyrosinase based amperometric biosensor. The sensor was optimized for detection of catechol and phenol. The limit of detection for catechol and phenol was 10 nM and 100 nM respectively.

Horse radish peroxidase (HrP) also comes under the category of phenol oxidases. This enzyme is used for detection and quantification of phenolic compounds and peroxides. Imabayaschi *et al*. (2001) [134] developed HrP based biosensor by immobilizing the enzyme on mercaptonic acid self-assembled monolayer (SAM) on the gold electrode. Peroxidase-based biosensors respond to phenolic compounds based on "ping-pong" mechanism which involves two substrates, hydrogen peroxide and phenolic compounds which donate the electron.

Tyrosinase based biosensors are most sensitive [135] but this class of enzyme shows low stability which leads to short life-time of tyrosinase based biosensors [136]. Laccases are used as an alternative to tyrosinase. Laccase is considered as a best candidate for determination of phenolics [137], due to its great stability [138], wide range of phenolic substrates [137] and for insusceptibility to product inhibition. Another reason to use laccase as favorite candidate for phenol determination is its sensitivity for highly toxic compounds such as chloroguaiacols and guaiacols [137]. Tyrosinase-based biosensors are less stable and exhibit product inhibition. These characteristics devalue the use of tyrosinase for determination of phenols. The drawback of HrP based biosensors is the necessity of the presence of hydrogen peroxide for completion of the biocatalytic cycle. So tyrosinase and laccase based biosensors have been proved more useful for determination of phenolic compounds present in different samples [139].

9. FUTURE PROSPECTS OF THE STUDY

Various support materials and method of their utilization has been explored in this review. Some of the support materials are complex and time consuming for preparation. Some support materials are bad biocompatible and decrease the efficiency of immobilized enzyme. This study may help to work for development of new technology for biosensing. Cost effective and biocompatible supports may also be developed. This study is applicable at commercial level, the best example is glucose measuring biosensor. The use of biosensors in food technology is rare so biosensors can be designed for monitoring of different food processes. Research may be carried out on all building blocks of biosensor which include transducer, biomolecule, immobilization strategy and transduction principle.

10. CONCLUSION

The real-time monitoring of substances is a tendency in environmental and biotechnological studies that can be achieved by the use of sensors. The biosensors are good tools which are cost effective, simple and show good sensitivity and selectivity. An ever increasing number of papers dealing with this topic clearly show how basic knowledge gained on sensor technology and biochemistry of polyphenol oxidase can be successfully employed for biotechnological applications. Enzyme immobilization and its stability are two major problems in biosensor technology which are needed to be tackled in order to expand the use of PPO-based biosensors for industrial processes. These problems open new areas for research regarding integration of inexpensive support materials and immobilization techniques to avoid enzyme leakage during immobilization, improving its activity or using nanoparticles. PPO-based biosensors can be used for online and in

situ monitoring of phenolic compounds with high sensitivity and can be standardized for food, environmental and medical industries.

Financial support and sponsorship: The authors highly acknowledge the financial grant received from ORIC, PMAS-Arid Agriculture University Rawalpindi under university research projects.

Conflict of Interests: There are no conflicts of interest.

11. REFERENCES

1. Rodríguez-Delgado M M, Melissa M, Gibrán S. Alemán-Nava, José M, , Graciano D, Sergio O, Martínez-Chapa, Damià B, Roberto, P. Laccase-based biosensors for detection of phenolic compounds, Trends Anal. Chem. 2015; 74: 21–45.
2. Dutton J, Copeland L G, Playfer J R, Roberts N B. Measuring l-dopa in plasma and urine to monitor therapy of elderly patients with Parkinson disease treated with l-dopa and a dopa decarboxylase inhibitor. Clinic. Chem. 1993; 39 : 629–634.
3. Sukhonthara S, Kaewka, theerakulkait C, Sukhonthara S, Kaewka K, Theerakulkait C. Inhibitory effect of rice bran extracts and its phenolic compounds on polyphenol oxidase activity and browning in potato and apple puree. *Food Chem.* 2016; *190*: 922–927.
4. Kulys J, Bratkovskaja I. Antioxidants determination with laccase, Talanta. 2007; 72: 526–531.
5. Annachhatre A P, Gheewala S H. Biodegradation of chlorinated phenolic compounds. Biotechnol. Adv. 1996; 14: 35–56.
6. Baldrian P. Fungal laccases occurrence and properties, F E M S. Microbiol. Rev. 2006; 30: 215–242.
7. Riedel K, Hensel J, Rothe S, Neumann B, Scheller F. Microbial sensors for determination of aromatics and their chloro derivatives. Part II: determination of chlorinated phenols using a *Rhodo coccus* containing biosensor. Appl Microbiol Biotechnol.1993; 38: 556–559.
8. Luthria D, Sing S P, Wilson T, Vorsa N, Banuelos B S. Influence of conventional and organic agricultural practices on the phenolic content in eggplant pulp: Plant-to-plant variation. Food chem. 2010; 121: 406-411.
9. Meulenberg E P. Phenolics: occurrence and immunochemical detection in environment and food. Molecules. 2009; 14: 439-73.
10. Gardziella A, Pilato L A, Knop A. Phenolic resins: chemistry, applications, standardization, safety and ecology. *2nd ed, Springer-Vercelog, Berlin/Heidelberg*, 2010. http://doi.org/10.1016/S0924-2244(02)00155-3
11. Makarenko A A, Bezverbnaya I P, Kosheleva I A, Kuvichkina T N, Yasov P V, Reshetilov A N. Development of biosensors for phenol determination from bacteria found in petroleum fields of West Siberia. Appl Biochem Microbiol.2002; 38: 23–27.
12. Roberto Stevanato , Sabrina Fabris , and Federico Momo. New Enzymatic Method for the Determination of Total Phenolic Content in Tea and Wine. *J. Agric. Food Chem.*, 2004, 52 (20), pp 6287–6293
13. Montero L, Lonradi S, Weiss S, Popp P. Determination of phenols in lake and ground water samples by stir bar sorptive extraction-thermal desorption-gas chromatography-mass spectrometry. J Chromatogr A. 2005; 10: 71, 63-9.
14. Clesceri L S, Greenberg A E, Eaton A D. Standard methods for the examination of water and waste water. American Public Health Association, 20th edn, pp1998; 6: 73–78.
15. Rogers K R, Becker J Y, Cembrano J, Chough S H. Viscosity and binder composition effects on tyrosinase based carbon paste electrode for detection of phenol and catechol. Talanta. 2001; 54: 1059–1065.
16. Tuncagil S, Varis, Topare L. Design of a biosensor based on 1-(4- nitrophenyl)-2,5-di(2-thienyl)-1*H* pyrrole. J Mol Cat B Enzym. 2010; 64: 195-199.
17. 16. Munteanu F D, Lindgren A, Emneus J, Gorton L, Ruzgas T, Csoregi E. Bio-electrochemical monitoring of phenols and aromatic amines in flow injection using novel plant peroxidases. Anal. Chem. 1998; 70: 2596–2600.
18. Husain Q, Jan U. Detoxification of phenols and aromatic amines from polluted wastewater by using phenoloxidases. J Sci Ind Res. 2000; 59: 286–93.
19. Rubianes M, Rivas G. Amperometric biosensor for phenols and catechols based on iridium-polyphenol oxidase-modified carbon paste. *Electroanalysis*. 2000; 1159–1162
20. Gomes S A SS, Nogueira J M F, Rebelo M J F. An amperometric biosensor for polyphenolic compounds in red wine. Biosens. Bioelectron. 2004; 20: 1211–1216.
21. Singh S, Solanki P R, Pandey M K, Malhotra B D. Cholesterol biosensor based on cholesterol esterase, cholesterol oxidase and peroxidase immobilized onto conducting polyaniline films. Sens. Actuators B. 2006; 115: 534–541.
22. Tan Y, Guo X, Zhan J, Kan J. Amperometric catechol biosensor based on polyaniline–polyphenol oxidase. Biosensors and Bioelectronics. 2010; 25: 1681–1687.
23. Seo S Y K, Sharma N. Mushroom tyrosinase: recent prospects. J Agric Food Chem. 2003; 51: 2837-2853.
24. Chen I S, Charest D J, Marshall M R, Wei C I. Comparison of two treatment methods on the purification of shrimp polyphenol oxidase. I Sei Food Agric.1997; 75: 12-18.
25. Ziyan E, Pekyardimic S. Characterization of Polyphenol Oxidase from *Helianthus tuberosus*. Turk. J Chem. 2003; 27: 217-225.
26. Simsek S, Yemenicioglu A. Partial purification and kinetic characterization of mushroom polyphenol oxidase and determination of its storage stability in different lyophilized forms. Process Biochem. 2007; 42: 943-950.
27. Queiroz C, Lopes M L M, Fialho E, Valente-Mesquita V L. Polyphenol oxidase: characteristics and mechanisms of browning control. Food Rev Int. 2008; 24: 361-375.
28. Aydemir T. Partial purification and characterization of polyphenol oxidase from artichoke (Cynara scolymus L.) heads. Food Chem. 2004; 87: 59–67.
29. Espin J C, Varon R., Fenoll L G, Gilabert M A, Garcia- ruiz P A, Tudela, J, Garcia-canovas F. Kinetic characterization of the substrate specificity and mechanism of mushroom tyrosinase.. Eur. J. Biochem.2000; 267: 1270-1279.
30. Karam J, Nicell J A. Potential applications of enzymes in waste treatment J. Chem Technol Biotechnol. 1997; 69: 141.
31. Duran N, Esposito E. Potential applications of oxidative enzymes and phenoloxidase-like compounds in wastewater and soil treatment: a review. Appl Catal B Environ. 2000; *28*: 83–99.
32. Claus H, Decker H. Bacterial tyrosinases. Syst Appl Microbiol. 2006; 29: 3-14.
33. Jaenicke E, Decker H. Tyrosinases from crustaceans form hexamers. Biochem. J. 2003; 371: 515–523.
34. Wichers H J, Recourt K, Hendriks M, Ebbelaar C F M, Biancone G, Hoeberichts F A. Cloning, expression and charac-terisation of two tyrosinase cDNAs from Agaricus bisporus. Appl. Microbiol. Biotechnol. 2003; 61: 336–341.
35. Yoruk R, Maurice R M. Physicochemical properties and function of plant polyphenol oxidase: a review. J. food. Bioch. 2003; 27: 361-422.
36. Matoba Y, Kumagai T, Yamamoto A, Yoshitsu H, Sugiyama M. Crystallographic evidence that the dinuclear copper center of postharvest browning with tyrosinase inhibitors. Biol. Technol. 2006; 39: 272–277.
37. Hernandez-Romero D, Snachez-Amat A, Solano F. A tyrosinase with an abnormally high tyrosine hydroxylase/dopa oxidase ratio. Role of the seventh histidine and accessibility to the active site. FEBS J. 2006; 273: 257–270.
38. Nakamura M, Hakajima T, Ohba Y, Yamauchi S, Lee B R, Ichisima Identification of copper ligands in *Aspergillus oryzae* tyrosinase by site directed mutagenesis. Biochem. J. 2000; 350: 537–545.
39. Cary J W, Lax A R, Flurkey W H. Cloning and characterization of

cDNAs coding for Vicia faba polyphenol oxidase. Plant Mol. Biol. 1992; 20: 245–253.
40. Dervall, B. J. Phenolase and pectic enzyme activity in the chocolate spot disease of beans. Nature. 1961; 189: 311.
41. Dinckaya E, Akyilmaz E, Akgol S, Onal S T, Zihnioglu F, Telefoncu A. A novel catechol oxidase enzyme electrode for the specific determination of catechol. Biosci Biotechnol.Biochem. 1998; 62: 2098-2100.
42. Sarkar S, Shilpa P S G, Reddy S M. Studies on oxidative enzymes (polyphenol oxidase and peroxidase) in four varieties of banana (Musa paradisiaca L.) Indian Phytopathol. 2010; 63:
43. Guell M, Siegbahn P E M. Theoretical study of the catalytic mechanism of catechol oxidase. J Biol Inorg Chem. 2007; 12: 1251-1264.
44. Klabunde T C, Eicken J C, Sacchettini, Krebs B. Crystal Structure of a Plant Catechol Oxidase Containing a Dicopper Center. Nature struct biol. 1998; 5: 1084–90.
45. Alexandre G, Zhulin I B. Laccases are widespread in bacteria. Trends Biotechnol. 2000; 18: 41-42.
46. Bajpai P. Application of enzymes in the pulp and paper industry. Biotechnol Prog. 1999; 15: 147.
47. Rodriguez E, Pickard M A, Vazquez R. Industrial dye decolorization by laccase from ligninolytic fungi. Curr Microbiol. 199; 3: 27.
48. Gianfreda L, Xu F J M. Bollag, A. Laccases a useful group of oxidoreductive enzymes. Bioremediation. 1999; 3: 1.
49. Cullen D. Recent advances on the molecular genetics of ligninolytic fungi. J Biotechnol. 1997; 53: 273.
50. Ong E, Pollock W B R, Smith, M. Cloning and sequence analysis of two laccase complementary DNSs from the ligninolytic basidiomycete *Trametes versicolor* gene.1997; 196: 113.
51. Karahanian E G, Corsini S, Lobos S, Vicuna R. Structure and expression of a laccase gene from the ligninolytic basidiomycete *Ceriporiopsis subvermispora*. Biochim Biophys Acta. 1998; 14: 43-65.
52. Jonsson L J P, Cassland A, Reimann N O, Nilvebrant. Laccase from *Trametes versicolor*: cloning expression and catalysis in M. Paice. J. Saddler (Eds.). Proceedings of the 7th International Conference on Biotechnology in the Pulp and Paper Industry, Vancouver, Canada, 1998; 7B: 175.
53. Collin P, Dowson P A D W. Regulation of laccase gene transcription in *Trametes versicolor*. Appl Environ Microbiol. 1997; 63: 34-44.
54. Hatamoto O H, Sekine E, Nakano K A. Cloning and expression of cDNA encoding the laccase from *Schizophyllum Commune*. Biosci Biotechnol Biochem. 1999; 63: 58.
55. Omura T. Studies on laccases of lacquer trees-comparison of laccases obtained *from Rhus vernicifera* and *Rhus succedanea*. J. Biochem. 1961; 50: 264– 272.
56. Morozova O V, Shumakovich G P, Shleev S V, Yaropolov Y I. Laccase-mediator systems and their applications :a review. Appl. Biochem. Microbiol. 2007; 43: 523–535.
57. Madhavi V, Lele S S. Laccase: properties and applications. Bioresources. 2009; 4: 1694–1717.
58. Chaubey A, Malhotra B D. Review mediated biosensors. Biosens.Bioelectron. 2002; 7: 441–456.
59. Tortolini C, Rea S, Carota E, Cannistraro S, Mazzei F. Influence of the immobilization procedures on the electroanalytical performances of Trametes versicolor laccase based bioelectrode . Microchemical Journal. 2012; 100: 8-13.
60. Durán N, Rosa M A, D'Annibale A, Gianfreda L. Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. Enzyme Microb. Technol. 2002; 31: 907–931.
61. Minussi R C, Pastore G M, Durán N. Potential applications of laccase in the food industry. Trends Food Sci Technol. 2002; 13: 205–216.
62. Mehrvar M B C, Scharer J, Moo-Young M, Luong. Fiber optic biosensors trends and advances. Anal Sci J. 2001; 6: 677–692.
63. D'Souza S F. Microbial biosensors. Biosens Bioelectron. 2001; 16: 337–353.
64. Sheldon R A. Enzyme immobilization: the quest for optimum performance. Adv. Synth. Catal. 2007; 349: 1289-130.
65. Arıca M, Altıntas B, Bayramoğlu G. Immobilization of laccase on tospacer-arm attached non-porous poly (GMA/EGDMA) beads: application for textile dye degradation, Bioresor. Technol. 2009; 100: 665–669.
66. Mascini M, Tombelli S. Biosensors for biomarkers in medical diagnostics. Biomarkers. 200; 13: 637-657.
67. Brady B, Jordaan J. Advances in enzyme immobilisation. Biotechnol Lett. 200; 31: 1639-1650.
68. Khan A A, Akhtar S, Husain Q.. Adsorption of polyphenol oxidase on celite 545 directly from ammonium sulphate fractionated proteins of brinjal (*Solanum melongena*). J. Sci. Ind. Res. 2005; 64: 621-626.
69. Krajewska B. Ureases. II: properties and their customizing by enzyme immobilizations: a review. J. Mol. Catal. B. Enz. 2009; 59: 22-40.
70. Bryjak J, Kruczkiewicz P, Rekuc A, Peczyn´ska-Czoch W. Laccase immobilization on co- polymer of butyl acrylate and ethylene glycol dimethacrylate. Biochem. Eng. J. 2007; 35: 325–332.
71. Rochefort D, Kouisni L, Gendron K. Physical immobilization of laccase on an electrodebymeans of poly(ethyleneimine)microcapsules. J.Electroanal.Chem. 2008; 617: 53–63.
72. Ibarra-Escutia P, Gómez J J, Calas-Blanchard C, Marty J L, Ramírez-Silva M T. Amperometric biosensor based on a high resolution photo polymer deposited onto a screen-printed electrode for phenolic compounds monitoring in tea infusions. Talanta. 2010; 81: 1636–1642.
73. Thévenot D R, Toth K, Durst R A, Wilson G S. Electrochemical biosensors: recommended definitions and classification1International Union of Pure and Applied Chemistry: Physical Chemistry Division, Commission I.7 (Biophysical Chemistry); Analytical Chemistry. Division, Commission V.5 (Electroanalytical. Biosens Bioelectron. 2001; 16: 121–131.
74. Dzyadevych S V, Arkhypova V N, Soldatkin A P, El'skaya A V, Martelet C, Jaffrezic-Renault N. Amperometric enzyme biosensors: past, present and future. I R B M.2008; 29: 171–180.
75. Luong J H T, Groom C A, Male K B. Potential role of biosensors in the food and drink industries. Biosensors and Bioelectronics. 1991; 6: 547–554.
76. Jianguo L, L Gaoxiang. Application of biosensors for diagnostic analysis and bioprocess monitoring. Sens. Act. B: Chemic. 2000; 65: 26-31.
77. Bourgeois W, Stultz J E. On-line monitoring of wastewater quality. J chem and Techno Bio Technology. 2001; 76: 33–348.
78. Mehrvar M, Abdi M. Recent Developments, Characteristics, and Potential Applications of Electrochemical Biosensors. Anal Sci. 2004; 20: 1113–1126.
79. Castillo J, Gáspár S, Leth S, Niculescu M, Mortari A, Bontidean I, Csöregi E. Biosensors for life quality. Sens Actuat B-Chem. 2004; 102: 179–194.
80. Park B W, Yoon D, Kim D S. Recent progress in bio-sensing techniques with encapsulated enzymes. Biosens Bioelectron. 2010; 26: 1–10.
81. Tsai Y C, Cheng C. Amperometric biosensors based on multiwalled carbon nanotube-Nafion-tyrosinase nanobiocomposites for the determination of phenolic compounds. Sens Actuators B-Chem. 2007; 125: 10-16.
82. Carvalho R H, Lemos F, Lemos M A N D A, Cabral J M S, Ribeiro F. Electro-oxidation of phenol on a new type of zeolite/graphite biocomposite electrode with horseradish peroxidase. J Mol Cat A Chemical. 2007; 278: 47-52.
83. Serra B, Benito B, Agui L, Reviejo A J, Pingarron J M. Graphite-teflonperoxidase composite electrochemical biosensors. A tool for the wide detection of phenolic compounds. Electroanalysis. 2001; 13: 693-700.
84. Mailley P, Cummings E A, Mailley S C, Eggins B R, Adams M E, Cosnier S. Composite carbon paste biosensor for phenolic derivatives based on in situ electrogenerated polypyrrole binder. Anal.Chem. 2003; 75: 5422-5428.

85. Marco M P, Barceló D. Environmental applications of analytical biosensors. *Meas*. Sci. Technol. 1996; 7: 1547–1562.
86. Bassi A S, Tang D, Lee E, Zhu J X, Bergougnou M A. Biosensors in environmental and bioprocess monitoring and control. Food Technol Biotechnol.1996; 34: 9-22.
87. Evtyugin GA, Stoikova E E. Electrochemical biosensors based on dendrimers. J Anal Chem. 2015; 70: 517-534.
88. Somerset V. (n.d.). Environmental Biosensors *Edited by Vernon Somerset*.
89. Soldatkin O O, Shelyakina M K, Arkhypova V N, Soy E, Kirdeciler S K, Ozansoy K B, Dzyadevych S V. Nano- and microsized zeolites as a perspective material for potentiometric biosensors creation. Nano Scl Res Lett. 2015; 10: 59.
90. Meiqing G, Hefeng W, Di H, Zhijun H, Qiang L, Xiaojun W, Jing C. Amperometric catechol biosensor based on laccase immobilized on nitrogen-doped ordered mesoporous carbon (N-OMC)/PVA matrix, Science and Technology of Advanced Materials, 2014; 15: 035005, DOI: 10.1088/1468-6996/15/3/035005
91. Diaconu M, S Carmen, Litescu, G L Radu. Laccase–MWCNT–chitosan biosensor—A new tool for total polyphenolic content evaluation from in vitro cultivated plants. Sensor. Actuators. B. 2010; 145: 800–806.
92. Sarika C, Rekha K, Narasimhamurthy B. Laccase based amperometric biosensor for industrial waste waters : A comparative study on covalent immobilization methods on gold electrode. IOSR-JAC. 2014; 7: 20–27.
93. Gomes A S S, Rebelo M J F. A new la11ccase biosensor for polyphenol determination. Sensors. 2003; 3: 166–175.
94. Yaropolov A I, Shleev S V, Morozova O V, Zaitseva E A, Marko-Varga G, Emneus J, Gorton L. An amperometric biosensor based on laccase immobilized in polymer matrices for determining phenolic compounds. J Anal Chem. 2005; 60: 624–628.
95. Vianello F, Cambria A, Ragusa S, Cambria M T, Zennaro L, Rigo A. A high
96. sensitivity amperometric biosensor using a monomolecular layer of laccase as biorecognition element. Biosens. Bioelectron. 2004; 20: 315–321.
97. Gutierrez-Sanchez C, Shleev S, Lacey G, A.L.Third-generation oxygen amperometric biosensor based on *Trametes hirsuta* laccase covalently bound to graphite electrode.Chem. Pap. 2014 pp 1-4. DOI: 10.2478/s11696-014-0595-x
98. Fernandes S C, De Oliveira I R W Z, Fatibello-Filho O, Spinelli A, Vieira I C. Biosensor based on laccase immobilized on microspheres of chitosan crosslinked with tripolyphosphate. Sens. Actuators B Chem. 2008; 133: 202–207.
99. Nazari M, Kashanian S, Rafipour R. Laccase immobilization on the electrode surface to design a biosensor for the detection of phenolic compound such as catechol. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2015; 145: 130–138.
100. Santhiago M, Vieira I C. L-Cysteine determination in pharmaceutical formulations using a biosensor based on laccase from *Aspergillus oryzae*. Sens. Actuators B Chem. 2007; 128: 279–285.
101. Chen X, Li D, Luo L, Ullah N, Wei Q. Facile fabrication of gold nanoparticle on zein ultrafine fibers and their application for catechol biosensor. Appl. Surf. Sci. 2015; 328: 444–452.
102. Lepore M, M Portaccio. Optical detection of different phenolic compounds by means of a novel biosensor based on sol-gel immobilized laccase. Biotech. Appl. Biochem. 2016. doi: 10.1002/bab.1551
103. Abdullah J, Ahmad M, Heng L Y, Karuppiah N, Sidek H. An Optical Biosensor based on Immobilization of Laccase and MBTH in Stacked Films for the Detection of Catechol. Sensors, 2007; 7: 2238-2250.
104. Setti L, Giuliani S, Spinozzi G, Pifferi P G. Laccase catalyzed-oxidative of 3-methyl 2- benzothiazolinone hydrazone and methoxyphenols. Enzyme Microb. Technol. 1999; 25: 285–289.
105. Cabaja J, Agnieszka J, Karol M, Agnieszka Ś, Jadwiga S. Optical biosensor for permanent monitoring of phenol derivatives in water solutions. Chemical Engineering Transactions 2016; 47:
106. Zhang Y, Tan C, Fei R, Liu X, Zhou Y, Chen J. Sensitive chemi luminescence immunoassay for E. coli O157:H7 detection with signal dual-amplification using glucose oxidase and laccase. Anal. Chem. 2014; 86: 1115–1122.
107. Mbouguena J K, Ngamenib E, Alain W. Quaternary ammonium functionalized clay film electrodes modified with polyphenol oxidase for the sensitive detection of catechol. Biosensor. Bioelectron. 2007; 23: 269–275.
108. Bai X, Gu H, Chen W, Shi H, Yang B, Huang X. Immobilized laccase on activated poly(vinyl alcohol) microspheres for enzyme thermistor application. Appl. Biochem. Biotechnol. 2014; 173: 1097–1107.
109. Vedrine C, Fabiano, Tran-Minh C. Amperometric tyrosinase based biosensor using an electrogeneratedpolythiophene film as an entrapment support. Talanta. 2003; 59: 535-44.
110. Campuzano S, Serra B, Pedrero M, Villena F J, Pingarron J M. Amperometric flow-injection determination of phenolic compounds at self-assembled monolayer-based tyrosinase biosensors. Analytica Chimica Acta. 2003; 494: 187–197.
111. Wang B, Zhang J, Dong S. Silica sol-gel composite film as an encapsulation matrix for the construction of an amperometric tyrosinase-based biosensor. Biosens Bioelectron. 2000; 15: 397-402.
112. Pena N, Reviejo A J, Pingarron J M. Detection of phenolic compounds in flow systems based on tyrosinase-modified reticulated vitreous carbon electrodes.Talanta. 2001; 55:179-87.
113. Onnerfjord P, Emneus J, Marko-Varga G, Gorton L, Ortega F, Domınguez E. Tyrosinase graphite-epoxy based composite electrodes for detection of phenols. Biosens Bioelectron. 1995; 10: 607-619.
114. Zapp E, Brondani D, Vieira I C, Scheeren C W, Dupont J, Barbosa A M J. Biomonitoring of methomyl pesticide by laccase inhibition on sensor containing platinum nanoparticles in ionic liquid phase supported in montmorillonite. Sens. Actuators B Chem. 2011; 155: 331–339.
115. Apetrei C, Rodríguez-méndez M L, Saja J A D. Amperometric tyrosinase based biosensor using an electropolymerized phosphate-doped polypyrrole film as an immobilization support Application for detection of phenolic compounds. Electrochimica Acta. 2011; 56: 8919–8925.
116. Wang S, Tan Y, Zhao D, Liu G. Amperometric tyrosinase biosensor based on Fe3O4 nanoparticles-chitosan nanocomposite. Biosens Bioelectron. 2008; 23: 1781–7.
117. Yildiz H B, Castillo J, Guschin D A, Toppare L, Schuhmann W. Phenol biosensor based on electrochemically con-trolled tyrosinase in a redox polymer. Microchimacta. 2007; 159: 27–34.
118. Böyükbayram A E, Kiralp S, Toppare L, Yağci Y. Preparation of biosensors by immobilization of polyphenol oxidase in conducting copolymers and their use in determination of phenolic compounds in red wine. Bioelectrochemistry. 2006; 69: 164–171.
119. Li Y F, Liu Z M, Liu Y L, Yang Y H, Shen G L, Yu R Q. A mediator-free phenol biosensor based on immobilizing tyrosinase to ZnO nanoparticles. Anal Biochem. 2006; 34: 3–40.
120. Liu Z, Liu Y, Yang H, Yang Y, Shen G, Yu R. A phenol biosensor based on immobilizing tyrosinase to modified core-shell magnetic nanoparticles supported at a carbon paste electrode. Analytica Chimica Acta. 2005; 533: 3–9.

121. Tembe S, Inamder S, Haram S, Karvee M D, Souza S F. Electrochemical biosensor for catechol using agarose-guargum entrapped tyrosinase. J Biotechnol.2007; 128: 80–85.
122. Apetrei C, Alessio P, Constantino C J L, De Saja J A, Rodriguez-Mendez M L, Pavinatto F J, Oliveira O N. Biomimetic biosensor based on lipidic layers containing tyrosinase and lutetium bisphthalocyanine for the detection of antioxidants. Biosen. Bioelectron. 2011; 26: 2513–9.
123. Zhao J, Wu D, Zhi J. A novel tyrosinase biosensor based on biofunctional ZnO nanorod microarrays on the nanocrystalline diamond electrode for detection of phenolic compounds. Bioelectrochem. 2009; 75: 44–9.
124. Abdullah J, Ahmad M, Karuppiah N, Heng L Y, Sidek H. Immobilization of tyrosinase in chitosan film for an optical detection of phenol. Sensa Actuat B: Chem. 2006; 114: 604–609.
125. Fiorentino D, Gallone A, Fiocco D, Palazzo G, Mallardi A. Mushroom tyrosinase in polyelectrolyte multilayers as an optical biosensor for o-diphenols. Biosens Bioelectron. 2010; 25: 2033–7.
126. Silletti S, Rodio G, Pezzotti G, Turemis M, Dragone R, Frazzoli C, Giardi M T. An optical biosensor based on a multiarray of enzymes for monitoring a large set of chemical classes in milk. Sens Actuat B: Chem. 2015; 215: 607–617.
127. Eunji J, Son K J, Kim J, Won-Gun K. Phenol biosensor based on hydrogel microarrays entrapping tyrosinase and quantum dots. Analyst. 2010; 11: 2745-3012.
128. Oktem H A, Senyurt O, Eyidogan F I, Bayrac C, Yilmaz R. Development of Laccase based paper biosensor for the detection of phenolic compounds. J. Food Agric Environ. 2012; 10: 1030-1034.
129. Alkasir R S J, Ornatska M, Andreescu S. Colorimetric paper bioassay for the detection of phenolic compounds. Anal Chem. 2012; 84: 9729-9737.
130. Spink C, Wadso I. Calorimetry as an analytical tool in biochemistry and biology. Meth. Biochem. Anal. 1976; 23: 1–159.
131. Grime J K. Analytical Solution Calorimetry. Wiley, New York. 1985.
132. Mosbach K, Danielsson B. An enzyme thermistor. Biochim. Biophys. Acta. 1974; 364: 140–145.
133. Xie B, Tang J. Ulla W, Gillis J, Lo G, Frieder S, Danielsson B. Hybrid biosensor for simultaneous electrochemical and thermometric detection. Anal. Lett. 2006; 30:
134. Imabayashi S, Kong Y, Watanabe M. Amperometric biosensor for polyphenol peroxidase immobilized on gold electrodes. Electroanal. 2001; 13: 408-412.
135. Parellada J, Narváez A, Domínguez E, Katakis I. A new type of hydrophilic carbon paste electrodes for biosensor manufacturing: binder paste electrodes. Biosens Bioelectron. 1997; 12: 267-75.
136. Rogers K R. Recent advances in biosensor techniques for environmental monitoring .Mol. Electron. Anal. Chem. 2006; 568: 222-231.
137. Freire R S, Duran N, Kubota L T. Development of a laccasebased flow injection electrochemical biosensor for the determination of phenolic compounds and its application for monitoring remediation of Kraft E1 paper mill effluent. Anal. Chem. Acta. 2002; 463: 229- 238.
138. Domínguez A, Gómez J, Lorenzo M, Sanroman A. Enhanced production of laccase activity by trametes versicolor immobilized into alginate beads by the addition of different inducers. World J. Microb. Biotechnol. 2007; 23: 367-373.
139. Ghindilis A L, Krishnan R, Atanasov P, Wilkins E. Flow-through Amperometric immunosensor: Fast 'sandwich' scheme immunoassay. Biosens. Bioelectron. 1997; 12: 415-423.
140. Russell I M, Burton S G.The development of an immo-bilized enzyme bioprobe for the detection of phenolic pollutants in water. Anal Chim Acta.1999; 389: 161–170.
141. Rahman A, Noh H, Shim Y. Direct electrochemistry of laccase immobilized on Au nanoparticles encapsulated-dendrimer bonded conducting polymer: application for a catechin sensor. Anal. Chem. 2008; 80: 8020–8027.

How to cite this article:

Gul I, Ahmad MS, Naqvi SMS, Hussain A, Wali R, Farooqi AA, Ahmed I. Polyphenol oxidase (PPO) based biosensors for detection of phenolic compounds: A Review. J App Biol Biotech. 2017; 5 (03): 072-085.