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Biosensors for phenolic compounds: The catechol as a substrate model

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Abstract

The behaviour of three different laccase-based graphite biosensors was studied in view of their use in agricultural or industrial waters polluted by phenolic compounds. Catechol was used as a substrate model. Laccase from *Trametes versicolor* was immobilized on one biosensor (type A electrode) by adsorption while, on the other two biosensor types, laccase was covalently bound through the carboxylic groups created on the graphite by means of treatment with an electric potential difference (type B electrode) or with nitric acid (type C electrode). In the latter two cases, hexamethylenediamine and glutaraldehyde were used as the spacer and the coupling agent, respectively. The extension of linear response range and the sensitivity and time stability of each biosensor type were investigated. The type C biosensor gave the best results and its electrochemical properties proved comparable to those reported by other authors.
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1. Introduction

Phenolic compounds have been recognised as toxic substances and endocrine disruptors [1–4]. This definition has been used by the scientific community to classify certain chemicals of natural or synthetic origin which are capable of interfering with the endocrine system, modulating it or mimicking natural hormones [5]. The result of this interaction for humans and wildlife is the induction of serious pathologies such as developmental abnormalities and carcinogenesis [6,7]. For these reasons, the determination of phenolic compounds in environmental matrices, including tap and surface water, has become a matter of great concern and scientific interest. Determinations are usually carried out in centralised laboratories using liquid chromatography (HPLC), gas chromatography–mass spectrometry (GC–MS), or capillary electrophoresis (CE). Recent research activity has focused on the design and construction of biosensors which are capable of improving the efficiency of site monitoring and can be used for the necessary remediation activities.

Tyrosinase or laccase-based enzyme electrodes [8–17] were designed for the selective determination of phenolic compounds in environmental matrices. Their functioning is based on the reductive amperometric detection of the produced quinone species [8,18,19]. The reaction can be schematized as

$$\text{Phenol} \overset{\text{Enzyme},O_2}{\longrightarrow} \text{catechol} \overset{\text{Enzyme},O_2}{\longrightarrow} o\text{-quinone} + H_2O \quad (1)$$

according to which the enzymatic *o*-hydroxylation of phenolic compounds to catechols is followed by dehydrogenation to *o*-quinones [8,16,20].

In this paper, we will discuss the functioning of three different laccase-based enzyme electrodes, obtained by immobilizing the laccase from *Trametes versicolor* on graphite electrodes via absorption or via covalent bond. Our attention was focused on the dehydrogenation process, which is the bottleneck of the electric detection system. For this reason, we used catechol as a substrate.

Sensitivities, calibration curves and stability of the three biosensors will also be compared to similar results obtained by other authors.

Our results confirm the effectiveness of laccase-based biosensors in the determination of phenolic compounds in polluted

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waters which derive from agricultural activity (including the partial degradation of phenoxy herbicides), or from the petrol chemicals and textile industries.

2. Experimental

2.1. Materials

Laccase (EC 1.10.3.2; 26.8 U mg^{-1}) from *T. versicolor* was used as a catalyst. Laccases are cuproproteins which belong to the group of blue oxidase enzymes [21,22]. Laccase is a polyphenol oxidase that catalyzes the reaction of several inorganic substances such as phenol, with concomitant reduction of oxygen to water. The reduction of oxygen to water is accompanied by the oxidation of the phenolic substrate. Laccases have four neighbouring copper atoms which are distributed among different binding sites and classified into three types: copper types 1, 2 and 3. Copper type 1 is involved in electron capture and transfer, copper type 2 activates molecular oxygen, while copper type 3 is responsible for oxygen uptake. Substrate oxidation using laccase is a one-electron reaction which generates a free radical [23].

Nitric acid, *N*,*N*′-dicycloexylcarbodiimide, hexamethylenediamine (HMDA) and glutaraldehyde (GA) were employed for the process of enzyme immobilization. In particular, HMDA and GA were used as the spacer and coupling agent, respectively, while the carbodiimide was used as activator of the carboxylic groups produced on the graphite electrode surface. Catechol was the substrate model for phenolic compounds.

Graphite rods (4 mm in diameter) were purchased from Agar Scientific (Agar Scientific Limited, 66a, Cambridge Road Stansted, Essex CM24 8DA, England). The platinum and the Ag/AgCl electrodes were purchased from Radiometer-Analytical (Radiometer-Analytical.SAS, Villeurbanne CEDEX, Lyon, France).

All chemicals, including the enzyme, were purchased from SIGMA (Sigma, Milan, Italy) and used without further purification.

2.2. Apparatus

The electrochemical cell (Fig. 1a) was a three electrode cell where the enzyme modified graphite electrode acted as a working electrode and the platinum electrode (type M241Pt) as a counter electrode. All measurements were carried out versus an Ag/AgCl reference electrode (type REF321), kept at −100 mV versus the working electrode. The potential difference was ensured by means of a low current potentiostat/galvanostat model 2059 from Amel (Amel, Milan, Italy) interfaced to a PC through a board (PCI-6221) purchased from National Instruments Corporation (National Instruments, Austin, TX, USA).

Electric current measurement was performed by means of a flow injection analysis (FIA) system, shown in Fig. 1b. A continuous flow of the carrier (the washing buffer solution: 0.1 M sodium acetate, pH 5.0; $T = 25\,^{\circ}C$) or of the mixture containing the catechol was injected through the electroanalytic cell under the control of an electrovalve from RS Components (RS Components s.p.a., Cinisello Balsamo, Milan, Italy). The injected volume was 200 μL and the electrical response, which constituted the output signal from the biosensor, was acquired using the Labview software package, purchased from the National Instrument Corporation (National Instruments, Austin, TX, USA). The software accounted for the values of the background current, which was continuously subtracted from the subsequent value of the measurement. The electrical current produced by the oxidation of the substrate by the immobilized enzyme according to

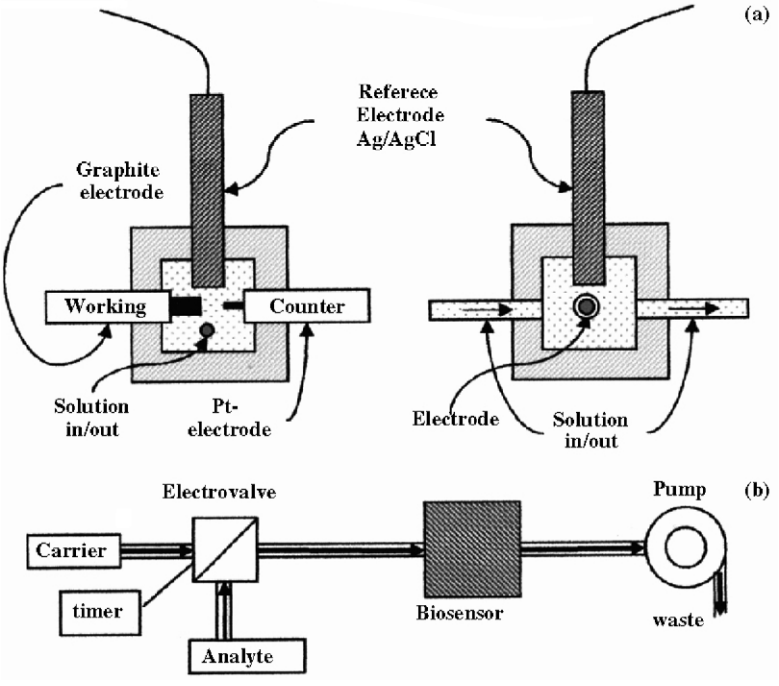

Fig. 1. (a) The electrochemical cell and (b) the FIA architecture.

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