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Total phenol analysis of weakly supported water using a laccase-based microband biosensor

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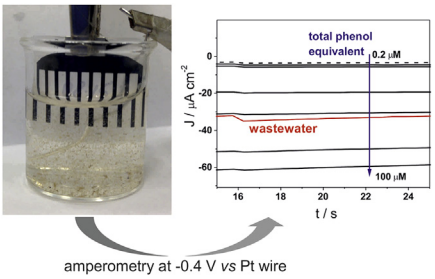
HIGHLIGHTS

- A novel screen printed microband array biosensor for *in situ* total phenol estimation in weakly supported media was developed.
- Numerical simulations using the finite element method with new periodic boundary conditions were performed.
- The use of the biosensor for tap water quality monitoring was demonstrated.
- The biosensor showed correlation with a standard method for total phenol analysis of wastewater samples.

GRAPHICAL ABSTRACT



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ABSTRACT

The monitoring of phenolic compounds in wastewaters in a simple manner is of great importance for environmental control. Here, a novel screen printed laccase-based microband array for *in situ*, total phenol estimation in wastewaters and for water quality monitoring without additional sample pretreatment is presented. Numerical simulations using the finite element method were utilized for the characterization of micro-scale graphite electrodes. Anodization followed by covalent modification was used for the electrode functionalization with laccase. The functionalization efficiency and the electrochemical performance in direct and catechol-mediated oxygen reduction were studied at the microband laccase electrodes and compared with macro-scale electrode structures. The reduction of the dimensions of the enzyme biosensor, when used under optimized conditions, led to a significant improvement in its analytical characteristics. The elaborated microsensor showed fast responses towards catechol additions to tap water – a weakly supported medium – characterized by a linear range from 0.2 to 10 μM, a sensitivity of 1.35 ± 0.4 A M^{-1} cm^{-2} and a dynamic range up to 43 μM. This enhanced laccase-based

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microsensor was used for water quality monitoring and its performance for total phenol analysis of wastewater samples from different stages of the cleaning process was compared to a standard method.

1. Introduction

Improving water resource management and increasing access to safe drinking water and basic sanitation are world-wide issues with the potential to improve the quality of life of billions of individuals [1]. The European Union Water Framework Directive, currently being implemented by all the Member states, addresses the management and protection of raw water supplies. This requires monitoring of both raw water and drinking water.

The monitoring of phenolic compounds in raw waters and wastewaters is of great importance for environmental control due to their wide use in industry and natural occurrence in various compounds [2]. The major anthropogenic sources of phenols are industrial waste from the pulp and paper, mineral and petroleum industries [3]. Many phenols are hazardous for humans, animals, plants and microorganisms and therefore the detection of low concentrations of phenols in water is an important issue [4]. Due to the high toxicity of phenols, there are many specific regulations regarding the phenol concentrations permitted in drinking and wastewaters. The European Council directives have set a limit of 0.5 μg L^{-1} for total phenol concentration in drinking water [5] and 0.1 mg L^{-1} for phenols in wastewater [4].

Various methods have been used for the determination of phenols in aqueous samples including traditional spectrophotometric methods [6] and high-performance liquid chromatography [7,8]. However, for rapid, specific and simple detection of phenolic compounds, biosensors present a more promising approach. A number of biosensors have been developed for phenol detection based on enzymes such as tyrosinase, peroxidase and laccase [9]. Laccase is used in the work presented here and is an enzyme containing copper at its active site, which catalyzes the reduction of molecular oxygen to water without the intermediate formation of hydrogen peroxide, thus simplifying the construction of a laccase-based biosensor [10]. Laccase has a broader substrate specificity than tyrosinase and peroxidase [11] and catalyzes the oxidation of various phenolic compounds, such as o-, p- and some m-diphenols, aminophenols, polyphenols, as well as phenol [12]. Laccase is therefore a candidate for total phenol measurements. A variety of biosensors for water sample analysis using laccase as a bio-recognition element have been reported recently [11,13–17]. (Table 1).

A general drawback of previously reported biosensors for phenol detection is their insufficient limits of quantification for phenols in water samples and the need for addition of electrolyte in the samples to enable the analysis. One way to improve the quantification limit and to overcome the need of additional electrolyte is by using microelectrodes, which exhibit a better signal/noise ratio than conventional-sized electrodes because they minimize the active surface area and the concomitant noise associated with the double layer capacitance, while enhancing mass transfer via multidimensional diffusion along directions where the electrode dimensions are small relative to the diffusion layer thickness [18]. However, immobilization of macromolecules such as enzymes on microelectrodes while retaining their catalytic activity is still a key challenge in the development of microelectrode-based biosensors [19]. There are only a couple of works in the literature on immobilization of laccase on microelectrodes. Kubota et *al*. [20] have investigated laccase immobilization on carbon-fiber microelectrodes (CFMEs, 10–20 fibers, 5 mm in length, 8 μm in diameter) via different immobilization techniques. The highest biosensor response was obtained using carbodiimide/glutaraldehyde for immobilization. The biosensor had a linear response to catechol between 1 and 90 μM with a sensitivity of 16 nA μM^{-1}. In another work [21], laccase was immobilized on CFMEs (7 μm in diameter and ca. 1.5 mm in length) by cross-linking of enzyme, bovine serum albumin and glutaraldehyde onto the single wall nanotube-modified CFMEs for the construction of a biofuel cell. However, the CFMEs utilized in both studies did not exhibit microelectrode behavior consistent with convergent mass transport, and thus are not suitable for analysis in electrolyte-free media. To the best of our knowledge, there is no previously reported work on a biosensor for phenol detection in weakly supported media without additional sample pretreatment, such as addition of electrolyte.

The microband design of microelectrodes [22] is a cost-effective and easily-fabricated compromise combining convergent mass transport, due to the microscale width as the critical dimension [23], and high output currents due to the macroscopic length. Among the various techniques available for microband electrode fabrication [24], screen printing stands out as an inexpensive approach [25]. The application of cross cutting [26–28] to deliver micro-scale thickness overcomes the problem of the insufficient resolution of screen printing in the lateral dimensions (approx. 50 μm).

In this study, we report on the development of a novel laccase-based microscale biosensor operating under a convergent diffusion regime. Screen printing followed by cross cutting was utilized for the fabrication of graphite microbands as a platform for further covalent immobilization of laccase. Numerical simulations utilizing the finite element method with a modified diffusion domain and new periodic boundary conditions developed for electrode arrays were used for modeling the voltammetric response of the developed microband electrodes. The diffusion domain approach is widely used for the simulation of microelectrode arrays [29–32]. Anodization followed by covalent modification via *N*-ethyl-*N*'-(3-dimethylaminopropyl)carbodiimide/N-hydroxysulfosuccinimide (EDC/NHS) chemistry was used for the electrode modification with laccase. Direct and mediated laccase bioelectrocatalytic oxidation of phenols was studied on macro- and microscale graphite electrodes. Significant enhancement of the analytical performance was achieved by the establishment of convergent diffusion at the microscale sensor which allowed, for the first time, the use of the developed amperometric biosensor for the direct analysis of weakly supported water samples without sample pretreatment. Finally, the developed microsensor was utilized to monitor phenolic compounds in wastewaters from different stages of the cleaning process.

2. Experimental

2.1. Reagents

All inorganic salts, N-hydroxysulfosuccinimide (NHS) sodium salt, 1-(3-dimethylaminopropyl)-3 ethylcarbodiimide (EDC), 1,1'-