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Biosensors

BIOSENSORS BASED ON LACCASE FOR DETECTION OF COMMERCIALLY REACTIVE DYES

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The results obtained using* Trametes versicolor *laccase modified graphite electrodes for detection of eleven different commercially reactive dyes are presented for the first time herein. The increase in current upon injection of the analyzed substrate was shown to be approximated by Michaelis–Menten type dependence. The calculated kinetic constants were used to evaluate the applicability of the laccase modified graphite electrode for the detection of reactive dyes in textile effluents.

Keywords: Biosensor; Commercially reactive dye; Laccase

INTRODUCTION

The textile industry uses more than ten thousand commercial dyes that are available or incorporated in the Color Index, most of which are difficult to decolorize due to their complex aromatic molecular structure and synthetic origin (Ozer, Akkaya, and Turabik 2006).

The textile effluents are usually highly colored and, when discharged in open waters, present an obvious aesthetic problem. Coloration of textile effluents can usually be linked to the presence of water-soluble reactive dyes in the wastewater. Moreover, the dyes without an appropriate treatment can persist in the environment for extensive periods of time and are deleterious not only for the photosynthetic processes of the aquatic plants but also for all the living organisms, since the

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degradation of these can lead to carcinogenic substances (Pinheiro, Touraud, and Thomas 2004; Hao, Kim, and Chiang 2000).

Reactive dyes are widely used in the textile industry to color cellulosic fibers (Zanoni et al. 1999). Their reactive groups are able to form covalent bonds with hydroxyl groups on the fiber. A strong covalent bond would be expected, but the efficiency of the dye-fiber reaction can change from 90 to 50% (Guaratini, Fogg, and Zanoni 2001). Inefficiencies in the dyeing process result in 10–15% of all dyestuff being lost directly to wastewater. For this reason, it is absolutely necessary to find new detection and decolorization methods of the dye from the textile wastewater.

Laccase is an oxidoreductive ligninolytic enzyme used in various biotechnological and environmental applications. In comparison to other oxidoreductases, such as the peroxidades that need H_2O_2 in its catalytic process, laccase only uses oxygen for the oxidation of its reduced state (Spadaro and Renganathan 1994).

In this study, the use of a laccase modified graphite electrode for the screening of commercially available reactive dyes is presented. The obtained results were compared to the ones obtained for a model azo dye, namely, methyl orange, which had been previously studied (Zille et al. 2005).

EXPERIMENTAL PART

Chemicals

The methyl orange dye [3-(4-dimethylamino-1-phenylazo) benzene sulfonic acid sodium salt] (see Fig. 1) was synthesized by the conventional method of coupling the diazonium salt of metanilic acid with either N,N-dimethyl-p-phenylenediamine (Lisov et al. 2004). The minimum dye content was 90%. The structures of the isolated dye, as sodium salts, were confirmed by 1H NMR spectroscopy in dimethyl sulfoxide (DMSO).

All commercially reactive dyes used in this study were kindly provided by Bezema Ag, Switzerland. The general formula of a reactive dye is presented in Fig. 2. The dyes were used as received, without further purification.

Laccase (EC 1.10.3.2) from *Trametes versicolor* (20 U/mg) was kindly purchased from Sigma.

Electrode Preparation

The laccase modified electrodes were prepared using rods of solid spectroscopic graphite (SGL Carbon, Werke Ringsdorff, Bonn, Germany, type RW001, 3.05 mm diameter). The graphite rods were first polished on a wet fine-structured emery paper

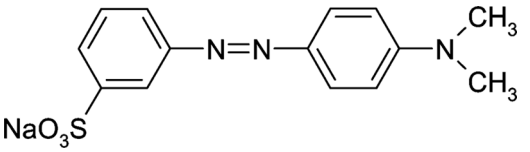

Figure 1. Structure of the 3-(4-dimethylamino-1-phenylazo) benzene sulfonic acid sodium salt.

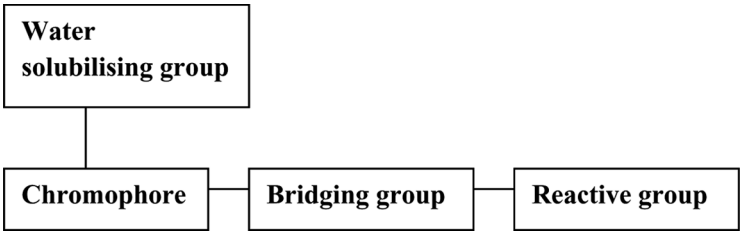

Figure 2. General structure of a reactive dye.

(grit size: P1200) and, then additionally, polished on paper to obtain a mirror-like surface. The electrode rods were carefully rinsed with deionized water and allowed to dry at room temperature. A 5 µl aliquot of the enzyme solution was added to each of the polished ends of the graphite rods and the electrodes were then placed at 4°C for 1 h in a glass beaker covered with sealing film, to allow the enzyme to adsorb slowly preventing rapid evaporation of the droplet of enzyme solution. The enzyme electrodes were then thoroughly rinsed with 0.1M sodium citrate buffer, pH 5.0, and if not immediately used, they were stored in the same buffer at 4°C. Weakly adsorbed laccase was desorbed before measurements, by rotating the electrode in buffer for at least 30 min.

Electrochemical Experiments

All the electrochemical experiments were performed using a Voltalab 30 Potentiostat (Radiometer Analytical, France), controlled by the Voltamaster 4 (version 5.6) electrochemical software. The working, counter, and reference electrodes were the modified graphite electrode (0.07 cm^2), coiled platinum wire (23 cm), and an Ag|AgCl electrode filled with 3M NaCl (BAS, Bioanalytical Systems, West Lafayette, IN, USA). The supporting electrolyte used in the electrochemical cell was a solution of 0.1M sodium-citrate buffer pH 5.0. All solutions were deoxygenated through bubbling nitrogen for 20 min before measurements. All experiments were performed in bulk using amperometric detection (each experiment was repeated 5 times). The applied potential was −50 mV *vs.* Ag|AgCl. The experiments were performed using a glassy carbon (laccase in solution) or a graphite electrode (laccase adsorbed onto the electrode surface).

RESULTS AND DISCUSSIONS

The blue copper phenol oxidases, also known as laccases, represent one enzyme activity implicated in lignin degradation (Spadaro and Renganathan 1994). Laccase benzenediol: oxygen oxidoreductases, EC 1.10.3.2) catalyzes the oxidation of ortho- and paradiphenols, aminophenols, aryl diamines, polyphenols, polyamines, and lignin, as well as some inorganic ions coupled to the reduction of molecular dioxygen to water.

Laccase showed broad specificity in the oxidation process of many compounds (mainly of the phenolic type) and can be exploited for the oxidation/biodegradation of a number of aquatic and terrestrial xenobiotics, industrial wastewaters, as well as for biotechnological treatment of industrial products.

Laccase catalyzes the oxidation of organic substrates such as phenolic compounds by molecular oxygen in homogeneous solutions. When laccase is adsorbed on graphite, bioelectrocatalytic reduction of oxygen occurs and is observed as a reduction current caused by direct (mediatorless) electron transfer (DET) from the electrode to the immobilized laccase and then further to molecular oxygen in solution.

The initial experiment (applied potential −50 mV *vs.* Ag|AgCl) showed that these electrodes had low noise/background current, whereas, upon the injection of the solution of the dyes, they generated a reduction current. Such a response is relatively well understood and it is usually ascribed to electrochemical reduction of laccase oxidation products (Haghighi et al. 2003).

The responses are dependent on the concentration of the dye in the solution of interest. At higher dye concentrations the current-concentration dependence gradually reached saturation (Figure 3). The apparent Michaelis–Menten constants (K_m^{app}) and maximal currents (I_{max}) have been calculated by fitting the variation of current–concentration dependencies of the analyzed compounds to the electrochemical Michaelis–Menten equation (Shu and Wilson 1976). K_m^{app} is an indicator of the affinity that an enzyme has for a given substrate and, hence, the stability of the enzyme-substrate complex.

$$I = \frac{I_{max}[S]}{[S] + K_M^{app}} \tag{1}$$

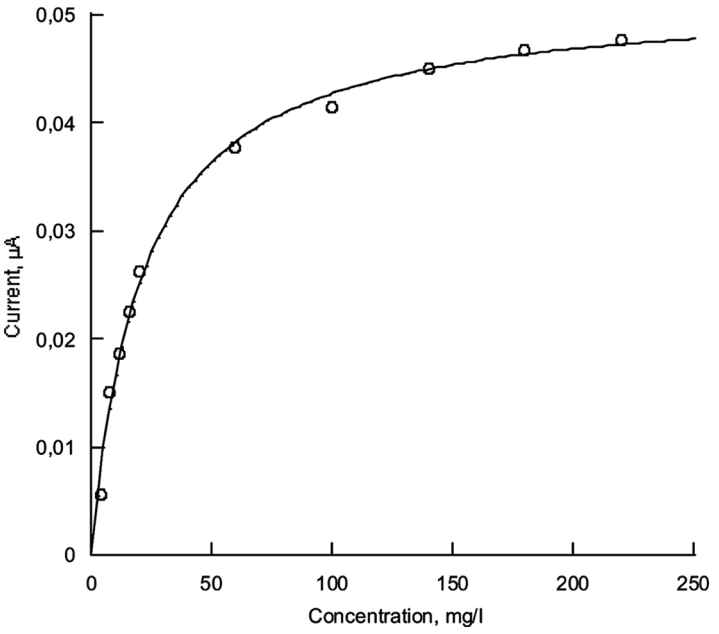

Figure 3. Calibration graph for Procion Red HE-3B obtained with a laccase modified graphite electrode in 100 mM citrate buffer pH 5.0, at −50 mV *vs.* Ag|AgCl electrode filled with 3M NaCl.

Table 1. Results obtained for the oxidation of the reactive dyes by laccase (each experiment was repeated 5 times)

Reactive dye	I_{max} (nA)	K_m^{app} (mg/l)	I_{max}/K_m^{app} nA $*$ (mg/l)$^{-1}$)
Procion Red HE-3B	$52,1 \pm 4,3$	$21,3 \pm 1,7$	$2,4 \pm 0,2$
Bezaktiv Red V-BT	$91,4 \pm 6,5$	$200,9 \pm 14,8$	$0,5 \pm 0,1$
Bezaktiv Red S-3B	$290,3 \pm 23,6$	$38,1 \pm 2,6$	$7,6 \pm 0,1$
Bezaktiv Red HE-3B	$154,2 \pm 12,3$	$25,7 \pm 1,4$	$5,9 \pm 0,1$
Bezaktiv Brilliant Orange V-3 R	$172,7 \pm 14,8$	$13,5 \pm 1,1$	$12,7 \pm 0,8$
Bezaktiv Brillant Red P-B	$34,6 \pm 3,1$	$27,2 \pm 2,3$	$1,3 \pm 0,1$
Bezaktiv Blau V-2 R	$281,8 \pm 19,3$	$98,2 \pm 8,6$	$2,9 \pm 0,2$
Bezaktiv Blau S-2 R	$123,4 \pm 10,5$	$18,3 \pm 2,4$	$6,8 \pm 0,5$
Bezaktiv Blau P-3 R	$89,2 \pm 7,2$	$30,4 \pm 3,1$	$2,9 \pm 0,2$
Bezaktiv Blau HE-RM	$71,5 \pm 6,1$	$30,9 \pm 2,5$	$2,3 \pm 0,1$
Bezaktiv Gelb HE-4 R	$202,4 \pm 17,5$	$32,5 \pm 2,7$	$6,2 \pm 0,4$
Methyl Orange – model dye	$360,5 \pm 19,8$	$29,4 \pm 3,2$	$12,2 \pm 0,7$

where S is the reactive dye concentration, I_{max} the maximum current, and K_m^{app} the apparent Michaelis–Menten constant. The kinetics of laccase catalyzed reactions is first affected by the affinity between enzyme and the substrate. An estimation of this influence can be done by amperometric measurements in terms of the I_{max}/K_m^{app} ratio. These parameters are often calculated in the design of enzymatic sensors to evaluate the sensitivity of the system proposed, which is related to the low or high affinity of the enzyme towards a specific substrate.

As it can be observed from Table 1, the highest sensitivity of the biosensor is observed for Bezaktiv Brilliant Orange V-3R, which is similar to the one obtained for the model dye used in this study [12,7 nA $*$ (mg/l)$^{-1}$, respectively, 12, 2 nA $*$ (mg/l)$^{-1}$].

The worst sensitivity is obtained for Bezaktiv Red V-BT (0,5 nA $*$ (mg/l)$^{-1}$), which might be due to steric hindrances. This dye also produced the highest value for the Michaelis-Menten constant, which proves that laccase used in these experiments has a low affinity for this dye.

CONCLUSIONS

The results obtained from these studies are the first ones obtained using a laccase based biosensor for the screening of commercially reactive dyes.

Based on the kinetic values, it can be concluded that laccase modified graphite electrodes can be used for detection of dyes from textile effluents. Moreover, these biosensors can be used for the continuous monitoring of the dye content in effluent and can be coupled with the decolorization process for a better control of the total dye content in the effluent.

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