

Bioelectrochemical Monitoring of a Reactive Dye Using Laccase Biosensor

F.D. Munteanu, B.I. Pancan

Department of Chemical and Biological Sciences, "Aurel Vlaicu" University of Arad, Elena Dragoi St. 2, 310330 Arad, Romania
Phone: +40257219242, Fax: +40257219331, E-Mail: florentinam@uav.ro, http://www.uav.ro

Abstract: In this paper is presented the first attempt to use a biosensor for detection of reactive dyes. The results obtained using a laccase biosensor for detection of a reactive dye, bezaktiv blue V-2R, are presented and based on the calculated sensitivity in terms of $I_{\max}/K_m^{\text{app}}$ ratio it can be concluded that it is possible to use a laccase biosensor for detection of reactive dyes that are used in textile industry.

Keywords: textile dye, laccase, electrochemistry, amperometric biosensor

1. Introduction

The textile effluents, usually highly colored, when discharged in open waters present an obvious aesthetic problem. 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 degradation of these can lead to carcinogenic substances [1, 2]. The European community has not been indifferent to this problem and in September 2003 the European directive 2002/61/EC came into force. This directive forbids the use of some products, derivatives of a restricted number of dyes. However, these restriction measures are not enough to solve the problems due to the huge amount of dyes discharged in the environment every year.

Many studies on the biological degradation of dyes are focused on the identification and characterization of the enzymes that can degrade them [3-6]. One of these enzymes, laccase, was chosen for studying in detail the processes of oxidative degradation of dyes, keeping in mind the effluent recycling processes.

Laccase is an oxidoreductive ligninolytic enzyme used in various biotechnological and environmental applications. In the last years its capacity to degrade synthetic dyes has been extensively studied [7]. In comparison to other oxidoreductases, as for example the peroxidases that need H_2O_2 in its catalytic process, laccase only uses oxygen for the oxidation of its reduced state [8].

In this work are presented the results obtained using a laccase modified biosensor for determination of bezaktiv blau V-2R.

2. Materials and methods

The salts were purchased from Sigma, St. Louis, MO. All chemicals were of high purity and used as received.

Laccase (EC 1.10.3.2) from *Trametes villosa* (5.3 mg protein/mL, 600 U/mL) was kindly provided by Novo Nordisk, Denmark.

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 wet fine-structured emery paper (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 μ l 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 0.1 M 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.

All the electrochemical experiments were performed using a Voltalab 30 Potentiostat (Radiometer Analytical, France), controlled by the Voltmaster 4 (version 5.6) electrochemical software. The working, counter and reference electrodes were respectively: glassy carbon electrode or the modified graphite electrode (0.07 cm²), coiled platinum wire (23 cm) and an Ag|AgCl electrode filled with a saturated solution of KCl (BAS, Bioanalytical Systems, West Lafayette, IN, USA). The supporting electrolyte used in the electrochemical cell was a solution of 0.1 M sodium-acetate buffer pH 5.0. 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 laccase modified graphite electrode.

3. Results and discussion

Laccase shows broad specificity in the process of oxidizing many compounds (mainly of 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. In the presence of soluble electron donors, laccase can be reduced in a mediated electron transfer (MET) mechanism (see Figure 1). In this mechanism the electron donor (substrate) penetrates the active site of the enzyme where it is oxidized in a single electron oxidation step often producing an electrochemically active compound (possibly a radical) that in turn can be re-reduced at the electrode surface in a mediated electron transfer (MET) step.

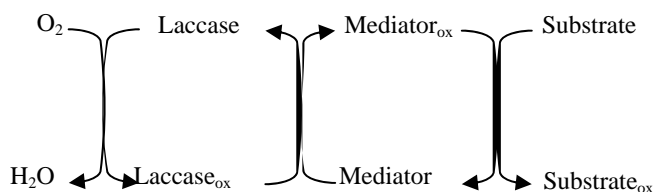


Figure 1. Role of the mediator in the enzymatic activity.

Initial experiment (applied potential -50mV versus Ag|AgCl) showed that these electrodes have low noise/background current while upon the injection of the solution of the dyes they generate a reduction current. Such a response is relatively well understood and it is usually ascribed to electrochemical reduction of laccase oxidation products [9].

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 2). 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 [10]. 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.

$$I = \frac{I_{\text{max}} [S]}{[S] + K_m^{\text{app}}} \quad (1)$$

where S is the substrate concentration, I_{max} the maximum current and K_m^{app} the apparent Michaelis–Menten constant.

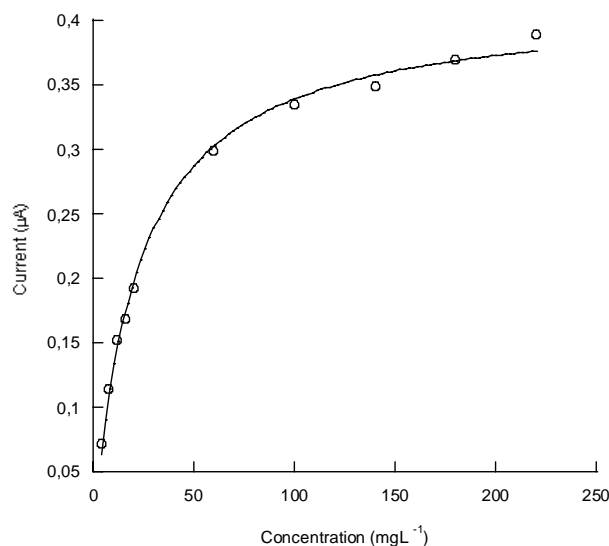


Figure 2. Calibration graph for bezaktiv blau V-2R obtained with a laccase modified graphite electrode in 0.1 M citrate buffer pH 5.0, at -50mV vs. Ag|AgCl electrode

The calculated value of K_m^{app} (calculated from the equation (1)) and the catalytic efficiency are presented in Table 1.

The kinetics of laccase catalyzed reactions is firstly affected by the affinity between enzyme and the substrate. An estimation of this influence can be done by amperometric measurements in terms of $I_{\text{max}}/K_m^{\text{app}}$ 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.

The results obtained using the laccase biosensor for detection of bezaktiv blau V-2R are presented in Table 1 and are compared with the results obtained in [11].

TABLE 1. Results obtained using the laccase biosensor

| | I_{max} (μA) | K_m^{app} (μM) | $I_{\text{max}}/K_m^{\text{app}}$ ($\mu\text{A} \cdot \mu\text{M}^{-1}$) |
|--------------------|------------------------------------|--------------------------------------|--|
| Bezaktiv Blau V-2R | 0.414 ± 0.001 | 22.070 ± 0.068 | 0.019 ± 0.004 |
| Methyl Orange* | 0.793 ± 0.002 | 31.497 ± 0.075 | 0.025 ± 0.003 |

* from reference [11]

As it can be observed from Table 1 the values obtained using the biosensor for determination of bezaktiv blau V-2R are similar to the values obtained with the same type of biosensor for detection of Methyl Orange.

In terms of sensitivity the results presented in here are not much different from the one obtained with a laccase biosensor for detection of catechol ($0,036 \mu\text{A} \cdot \mu\text{M}^{-1}$) in the absence of a mediator.

The reason of not using a mediator for this system is that the aim for which this kind of biosensor was tested is to be used in field. In this situations addition of another chemical to the tested effluent will further contribute to the environmental pollution.

It can be concluded that for the present study the laccase biosensor can be used for determination of reactive dyes that can be present in textile effluents.

ACKNOWLEDGMENT

This paper is part of the project CEEEX 77/2006 "Computerized filtration and separation systems activated by ultrasounds and controlled by biosensors for textile processes- FILTISOFTUS" and was supported by "Romanian Authority for Scientific Research/MATNANTECH".

REFERENCES

1. Pinheiro, H.M., Touraud, E., Thomas, O., Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters, *Dyes and Pigments*, **2004**, vol. 61, pp. 121-139.
2. Hao, O.J., Kim, H., Chiang, P.C., Decolorization of wastewater, *Crit. Rev. Env. Sci.*, **2000**, vol. 30, pp. 449-505.
3. Abadulla, E., Tzanov, T., Costa, S., Robra, K.H., Cavaco-Paulo, A., Gübitz, G.M., Decolourisation and detoxification of textile dyes with laccase from *Trametes hirsuta*, *Appl. Environ. Microbiol.*, **2000**, vol. 66, pp. 3357-3362.
4. Blümel, S., Stolz, A., Cloning and characterization of the gene coding for the aerobic azoreductase from *Pigmentiphaga kullae* K24, *Appl. Microbiol. Biotechnol.*, **2003**, vol. 62, pp. 186-190.
5. Nyanhongo, G.S., Gomes, J., Gübitz, G.M., Zvauya, R., Read, J., Steiner, W., Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*, *Water Research*, **2002**, vol. 36, pp. 1449-1456.
6. Nyanhongo, G.S., Gomes, J., Gübitz, G.M., Zvauya, R., Read, J.S., Steiner, W., Production of laccase by a newly isolated strain of *Trametes modesta*, *Bioresource Technology*, **2002**, vol. 84, pp. 259-263.
7. Mayer, A.M., Staples, R.C., Laccase: new functions for an old enzyme, *Phytochemistry*, **2002**, vol. 60, pp. 551-565.
8. Spadaro, J.T., Renganathan V., Peroxidase-Catalyzed Oxidation of Azo Dyes: Mechanism of Disperse Yellow 3 Degradation., *Arch. Biochem. Biophys.*, **1994**, vol. 312, pp. 301-307.
9. Haghghi, B., Gorton, L., Ruzgas, T., Jonsson, L.J., Characterization of graphite electrodes modified with laccase from *Trametes versicolor* and their use for bioelectrochemical monitoring of phenolic compounds in flow injection analysis, *Analytica Chimica Acta*, **2003**, vol. 487, pp. 3-14.
10. Shu, R., Wilson, G.S., Rotating ring-disk enzyme electrode for surface catalysis studies, *Anal. Chem.*, **1976**, vol. 48, pp. 1679-1686.
11. Zille, A., Munteanu, F.D., Gübitz, G.M., Cavaco-Paulo, A., Laccase Kinetics of Degradation and Coupling Reactions, *Journal of Molecular Catalysis:B*, **2005**, vol. 33, pp. 23-28.