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Dedicated to Professor Zeno Simon
on the occasion of his 80th anniversary

COUPLED ELECTROCHEMICAL AND ENZYMATIC DEGRADATION OF TWO ANTHRAQUINONE DYES

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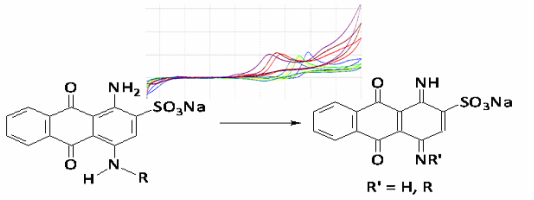
Dyes stand for one of the long-lived pollutants from textile wastewaters. For the de-pollution of these wastewaters the dye degradation is compulsory. Thus, the conditions for the degradation of two blue anthraquinone dyes, frequently used in dyeing of textile materials, have been studied. These dyes have similar chemical structure with an anthraquinone moiety as chromophore, but have different tinctorial properties, one being a reactive dye, namely C.I. Reactive Blue 19 (*RB 19*, CAS 2580-17-1) and the other an acid dye C.I. Acid Blue 62 (*AB 62*, CAS 4368-56-3). Taking into account the progress in using electrochemical methods for dye degradation, such a procedure was investigated in connection with these dyes. The influence of pH value on these compounds electro-oxidation was analyzed. For better results the combination of enzymatic and electrochemical degradation of the two antraquinone derivatives was considered. The kinetic parameters of the combined electrochemical and enzymatic degradation, such as apparent Michaelis-Menten constant (K_m^{app}) and I_{max}, were determined by means of amperometric measurements, using a glassy carbon electrode (GCE) as working electrode.



INTRODUCTION*

Since the discovery of the first synthetic dye, Mauveine, by Perkin in 1856, a great number of dyes have been synthesized, founding applications in many fields[1] such as textile, leather, food, but also in high technology fields such as electronics and printing industries.

Dyes are also one of the main water pollutants. During the textile dyeing process, the yield of reactive dyes attachment to textile material is estimated to be around 70%,[2] the resulting dye containing wastewaters being a concerning problem for the environment. Despite their low toxicity based on LD_{50},[3] these compounds have to be eliminated from the wastewaters because, by damaging the water transparency, they obstruct the photosynthetic process, and finally perturb the ecosystems equilibrium.

Among the synthetic dyes, an important place was acquired by anthraquinone dyes. Their importance is due to their stability under acidic and basic conditions, high light fastness and brilliance, as well as, the possibility to be used in other fields (food, cosmetics, etc).[4] They continue to be the second important dye class,[5] after the azo dyes.

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Regulations concerning effluents became more and more stringent, fact that imposes the application of efficient and economical processes for wastewaters treatments. Many procedures are used for dye removal from wastewaters, such as adsorption on natural or synthetic materials,[6,7] advanced oxidation by chemical reagents[8] or in photocatalytic conditions,[9] as well as biodegradation by bacteria, fungi, plants[10-12] or by free or immobilized enzymes isolated from different organisms, especially laccases.[13-16]

Electrochemical oxidation has been also used for dye degradation. [17-19] Moreover, combinations of different processes have been proposed as a solution for dye elimination from wastewaters.[20] Electrochemical oxidation seems to be a good solution for dye degradation, by itself or in combination with other procedures.

MATERIALS AND METHODS

Materials

Enzyme

The enzyme used was Laccase Roglyr Lite 1540, a commercial solid product, supplied by Hungarian Industry Products KFT and produced by Rotta Manheim. The commercial enzyme has a content of 25.7±1.2 mg protein/g commercial product.[21] The enzyme specific activity, previously determined using 2,2′-azinobis-(3-etylbenzo-thiazoline-6-sulphonate) (ABTS) as substrate, is 1301.2 μmoles g^{-1} min^{-1} at pH 3.8 and 1017.1 at pH 4.7. [21]

Dyes

The Reactive Blue 19 (**1**) [2-(3-(4-amino-9,10-dihydro-3-sulpho-9,10-dioxoanthracen-4-yl) aminobenzenesulphonyl) ethyl disodium sulfate; CAS 2580-17-1] (*RB 19*) and Acid Blue 62 (**2**) (1-amino-4-cyclohexylamino-9,10-dihydro-9,10-dioxoanthracene-2-sulfonic acid, sodium salt; CAS 4368-56-3) (*AB 62*) were kindly supplied by Bezema AG, Switzerland. All the other chemicals were purchased from Sigma, Steinheim-Germany, and were of analytical grade.

Methods

The pH values have been determined with a Multi 3401 WTW pH-meter.

All the electrochemical experiments were performed using a Voltalab 30 Potentiostat (Radiometer Analytical, France), controlled by a Voltamaster 4 (version 7.08) electrochemical software.

Electrode preparation

For all experiments a glassy carbon electrode (GCE) was used as working electrode. Prior to the experiments the surface of the GCE was successively polished with 5, 1, 0.3 and 0.05 μm alumina polish (Buehler Ltd, USA) and then rinsed with 8 M nitric acid and distilled water.

Electrochemical determinations

Experiments were carried out at room temperature. Magnetic stirring was used for solution homogenization. The working, counter and reference electrodes were respectively: GCE (0,07 cm^2), coiled platinum wire (23 cm) and an Ag|AgCl electrode filled with 3M sodium chloride (BAS, Bioanalytical Systems, West Lafayette – Indiana, USA). The supporting electrolyte used in the electrochemical cell was a solution of 0.1 M sodium acetate buffer pH 4.0. The adjustment of the pH was done by addition of solutions of sodium hydroxide or acetic acid, until the desired pH was attained. All the solutions were deoxygenated through bubbling nitrogen for 20 min before measurements.

The effects of pH on the E_{ox} value were investigated for solution of different pH, with dye samples of 320μmoles mL^{-1}.

For kinetic measurements, solutions of 10 mL acetate buffer at pH 4, with different content (80 to 800 μM) of compounds **1** and **2** have been prepared. The buffer solution has been obtained by mixing the necessary quantities of 0.2 M sodium acetate with 0.2 M acetic acid and distilled water. In each sample commercial enzyme (0.1 mg - 2.57 μg protein) has been added.

Cyclic voltammograms were recorded at a scan rate of 20 mV s^{-1}.

The degradation experiments have been performed using 10 mL acetat buffer solution (pH 4) of compound **2** (237 μM) and adding up 0.1 mg commercial enzyme (2.57 μg protein).

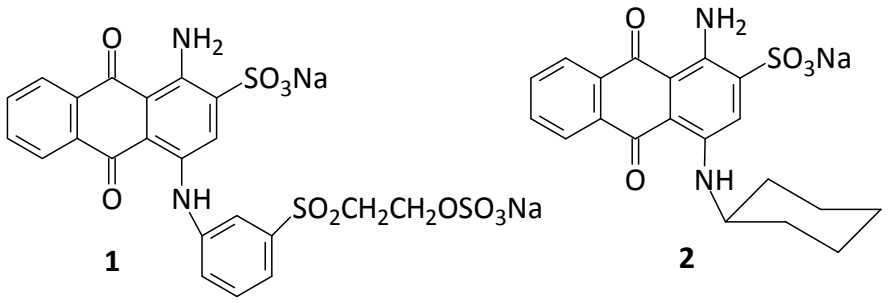
RESULTS AND DISCUSSION

The enzymatic biodegradation is an ecological solution for dye elimination from wastewaters. Laccases (benzenediol: oxygen oxidoreductase; EC 1.10.3.2) are copper oxidases, produced by fungi,

bacteria or plants. The reaction center contains 4 copper having coordinative links to the protein chain of the enzyme through amino acids like: histidine and cysteine.[22]

As previously shown, laccases have been used successfully for dye degradation, either free or immobilized.[13-16] But, some of the dyes are not so easily degraded by laccases, needing mediators as intermediates.[23] The low reactivity of the enzyme, in some cases, was explained by the low electrochemical potential of laccase compared with that of the substrate.[24] A combination of laccase treatment and electrochemical oxidation for the degradation of the two previously mentioned anthraquinone dyes may be effective.

The studied dyes have the following chemical structures:



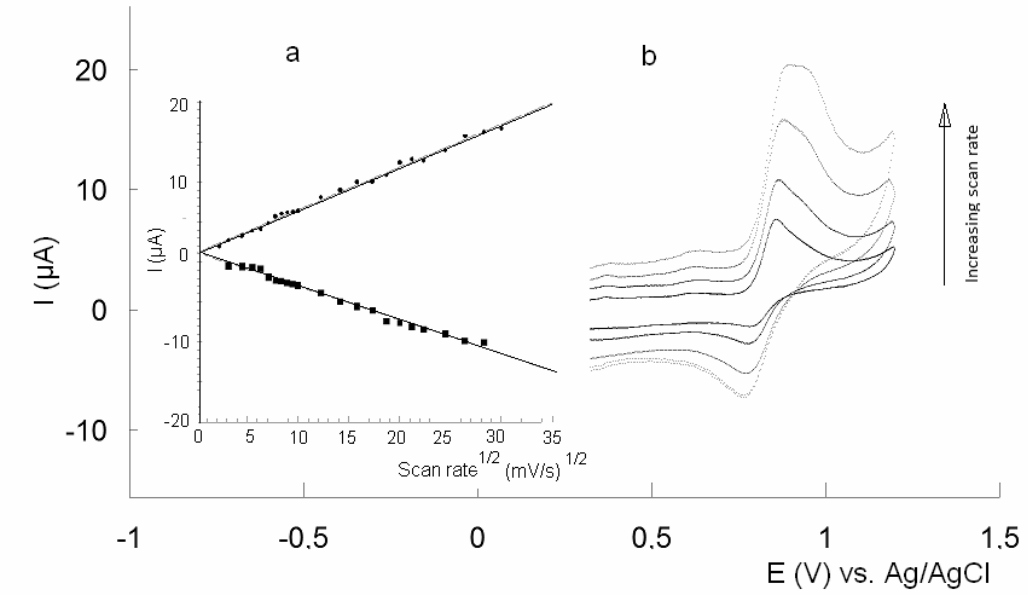


Fig. 1 – a) Dependence of the peak currents on square root of the scan rate.
b) Cyclic voltammograms of compound **1** at different scan rates.

The cyclic voltammogram performance gives information on the electrochemical behavior of these compounds. Thus, the effect of scan rate variation on the cyclic voltammograms of the studied compounds was followed in acetate buffer pH 4, over the range 5-900 mV/s.

The anodic and the cathodic current peaks increase linearly with the square root of the scan rate (see Fig. 1a). Moreover, they are moving towards more positive and negative values, respectively, fact that may be attributed to the accumulation of the oxidation or reduction products to the electrode surface.

A plot of the logarithm of the peak currents versus the logarithm of the scan rate (data not shown) gave a straight line with a slope close to the theoretical value of 0.5 indicating diffusion controlled processes.

The variation of the oxidation potential (*Eox*) with the *pH* was monitored by performing cyclic voltammograms for both compounds **1** (*RB 19*) and **2** (*AB 62*). The experimental cyclic

voltammograms for compound **1** are illustrated in Fig. 2a. All the curves display a higher intensity values for the oxidation processes compared with the reverse reduction reactions. A decrease in size of the reverse peak occurs when much of the oxidized species electrochemically produced are destroyed by subsequet chemical step.

The influence of the pH on the *Eox* value is disclosed in Fig. 2b.

The number of electron transferred may be calculated through the relation (1).[25]

$$Eox - Ered = 2.218 \frac{RT}{nF} \qquad (1)$$

Where *Eox* and *Ered* are the experimental values of potentials at which the oxidation and reduction processes occur, *R* is the universal gas constant, *T* is the absolute temperature in Kelvin degrees, and *F* is Faraday's constant. By replacing the values for R, T, and F, the relation (1) turns into (2):

$$Eox - Ered = \frac{57}{n} \text{ mV} \qquad (2)$$

From the experimental data, shown in Fig. 2a, *n* seems to be approximately 2 *Eox* - *Ered* being around 110 mV, matching to two electrons donation by the dye during the oxidation step. The amino are the electron donating groups being the preferred targets for the oxidants, in such amino anthraquinone derivatives.[26] In acid pH conditions the amino groups may be protonated the oxidation needing a higher potential. An alkaline pH makes the amino groups more reactive due to the occurence of free electrons to the unprotonated nitrogen atoms.

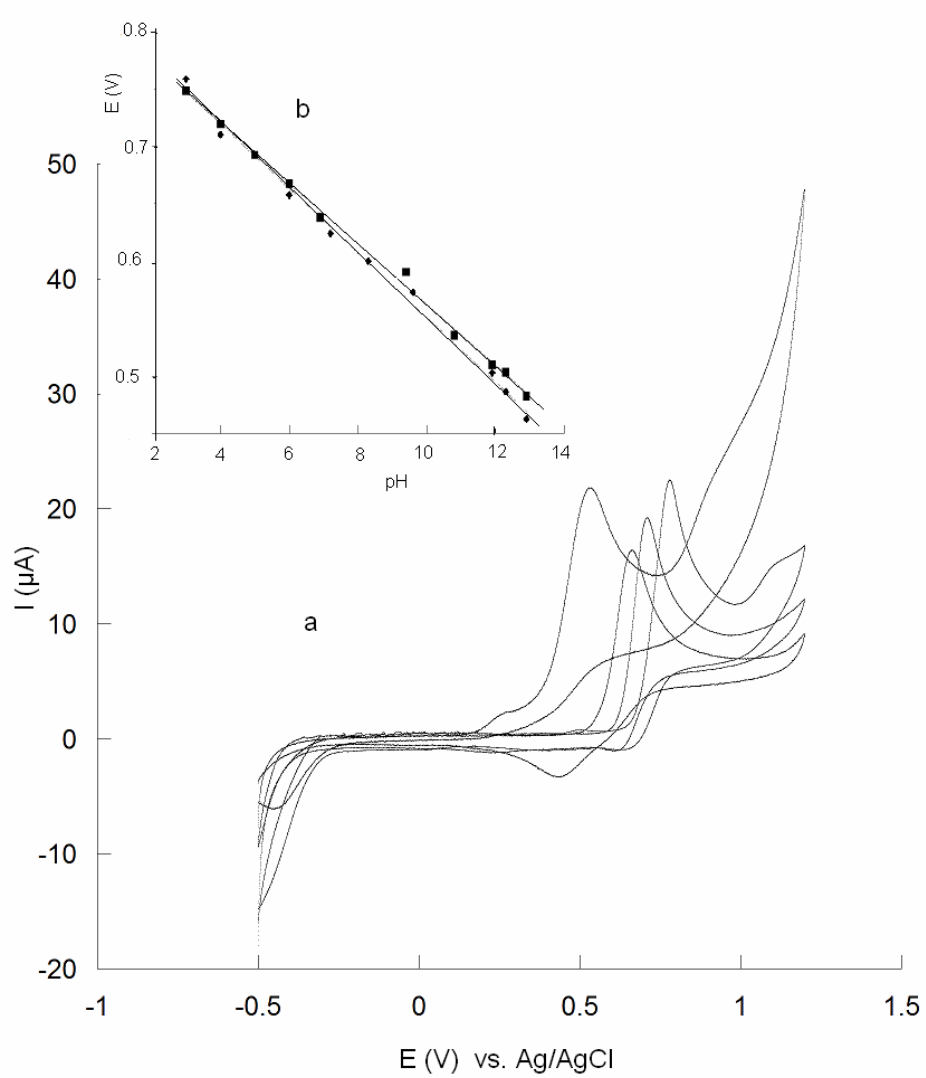


Fig. 2 – a) Cyclic voltammograms of compound **2** at different pH values (pH 12.3, 6, 4 and 3);
b) The oxidation potential (Eox) variation with pH for the two anthraquinone dyes (◆ compound **1** and ■ compound **2**).

The alkaline conditions which are proper for the anthraquinone dye oxidation are not suitable for laccases. In such conditions a strong complex Cu–OH is formed at the reaction centre, blocking the further transformation of reduced oxygen to water [22] and therefore the substrate oxidation. Thus, the experiments with laccase have been performed at pH 4, a pH value in agreement with the commercial laccase optimal activity value (see Experimental).

At first (applied potential -50 mV vs. Ag|AgCl), the GCE has low noise/background current while upon the addition of the dye solution a current is generated. The responses are dependent on the concentration of the dye in the studied solutions. By enhancing the dye concentrations the current-concentration dependency gradually reached saturation.

According the experimental data (see Fig. 3), these dyes exhibit typical Michaelis-Menten hyperbolic kinetics expressed by the following equation: [27, 28]

$$I = \frac{I_{\max}[S]}{[S] + K_M^{app}} \qquad (3)$$

Similar evolution was observed from the experimental data for the dye **1.**

The apparent Michaelis–Menten constant (K_M^{app}) and maximal current (I_{max}) have been calculated by fitting the variation of current–concentration dependency to the electrochemical Michaelis–Menten equation (3). The experimental results are presented in Table 1.

The value of 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. The value of it is higher for the compound **2** specifying a higher affinity in this case with a longer life of the complex ES (enzyme-substrate).

From the I_{max} value a higher oxidation reaction rate may be observed for compound **2** in electrochemical conditions and laccase presence.

Experiments performed with the anthraquinone dye **2** confirm the advantages of a combination of methods for degradation, namely: electrochemical and enzymatic. It shows by far a shoter time for the working solution discoloration, compared with the cases when the two procedures are applied separately.

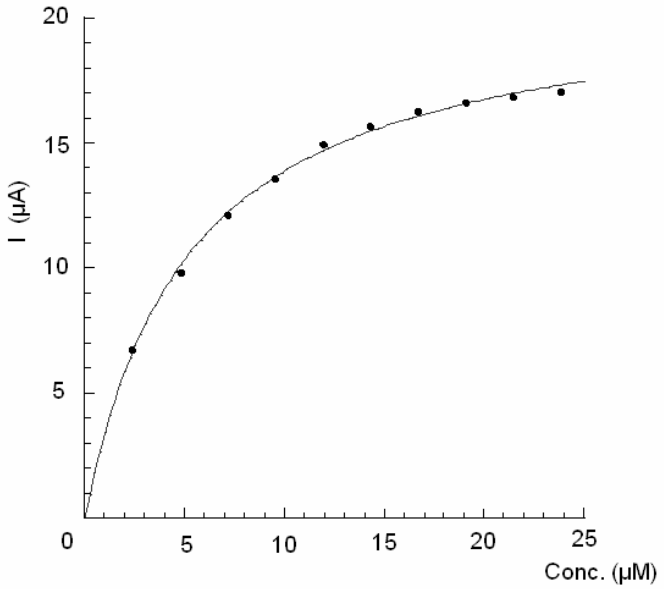


Fig. 3 – Current variation with dye **2** concentration.

Table 1

Kinetic parameters of the electrochimical oxidation catalyzed by laccase

Dye	I_{max} (μA)	K_M^{app} (μM)	I_{max}/K_m^{app} (μA/μM)
RB19 (**1**)	2.01	0.16	12.31
AB62 (**2**)	21.11	5.22	4.05

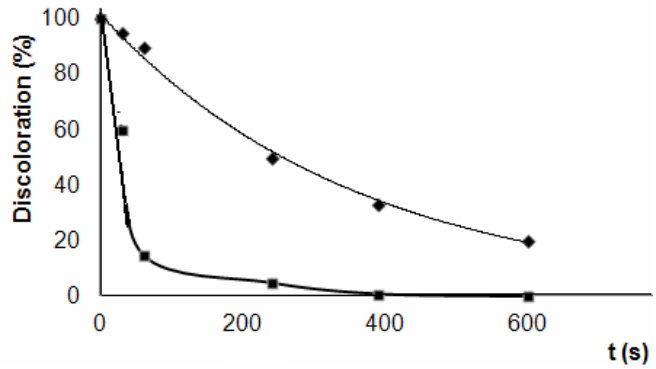


Fig. 3 – Discoloration of compound **2**: electrochem. (—◆—) and electrochem. combined with laccase treatment (—■—).

Monitoring the color disappearance (Discoloration %) for dye **2**, by measuring the decrease in time of the absorbance values for λ_{max} (585 nm), [15,29], for electrochemically oxidized solutions containing the same amount of dye, with or without laccase, the following results have been acquired.

The oxidative degradation of the acid dye **2** is almost done after 400 s in the coupled procedure (electrochemical and enzymatic treatment) while in the electrochemical case alone, after 700 s the 20% of the dye content is still preserved. The results illustrate the advantages in using the coupled procedure *versus* each of the treatments electrochemical or enzymatic.

CONCLUSIONS

The electrochemical characterization of two anthraquinone dyes, a reactive dye **1** (*RB 19*) and an acid dye **2** (*AB 62*), was performed. The choice of these dyes for our study is due to their large consumption by textile industry, as well as the difficulty to remove them from the textile effluents.

As it was shown by the experimental data, the dye **1** and **2** are easier degraded in alkaline media. These results may explain the literature data concerning their slow degradation in laccase presence, at acid pH which is optimal for the enzyme. It also urges for the search of new laccases, with optimal pH in the alkaline range for a better dye bio-oxidation.

The apparent Michaelis-Menten constant were determined for the two anthraquinone dyes using a marketable laccase. A 10 times higher value of I_{max} was evidenced for the compound **2** compared with **1**. Nevertheless, the ratio of the corresponding K_M^{app} values (32.5) compensate by far the I_{max} ratio.

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