University of Minho - Dept. Textile Eng.

Redox biodegradation of azo dyes

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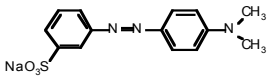
Introduction

- This study discusses the biodegradation under aerobic conditions of azo dyes with an oxidative enzyme laccase with or without mediator.

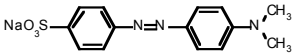
- The ability of the bio-agents to degrade azo-dyes depends on the structural characteristics of the dye, the temperature and pH of treatment, the presence of intermediates, and the difference between the redox potentials of the biocatalyst and the dye

- These approaches have been compared on the basis of the electrochemical properties of dyes and bio-agents. The question targeted by this work is whether the redox potential is a preliminary tool to predict the decolourization capacity of oxidative biocatalysts.

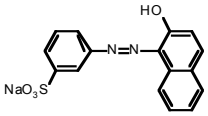
Dyes



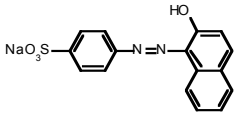
I) 3-(4-dimethylamino-phenylazo)-benzene sulfonic acid sodium salt



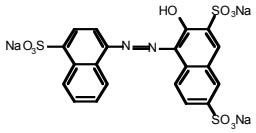
II) Acid Orange 52



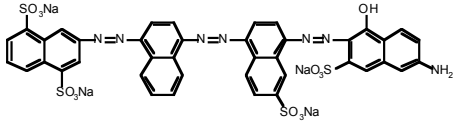
III) 3-(2-hydroxy-naphthalen-1-phenylazo)-benzene sulfonic acid sodium salt



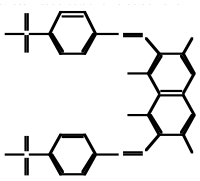
IV) Acid Orange 7



V) Acid red 27



VI) Direct blue 71



VII) Reactive Black 5

Material and methods

- **Enzyme**: Laccase (EC 1.10.3.2) from *Trametes villosa* (5.3 mg protein/mL, 600 U/mL)

- **Decolourization with laccase and mediator system:** Dye solutions (0.1 mM; 2.5 mL) buffered with 0.1 M Na-acetate buffer, pH 5, were incubated in cuvette with 20 μL of laccase and 0.5 mL of distilled water at 25ºC. The dye absorbance was measured at different times in the course of the experiment and the percentage of effluent decolourisation was calculated thereof. In the case of experiments with mediator the water volume (0.5 mL) was replaced by 0.1 mM aqueous solution of (HBT).

Electrochemical experiments

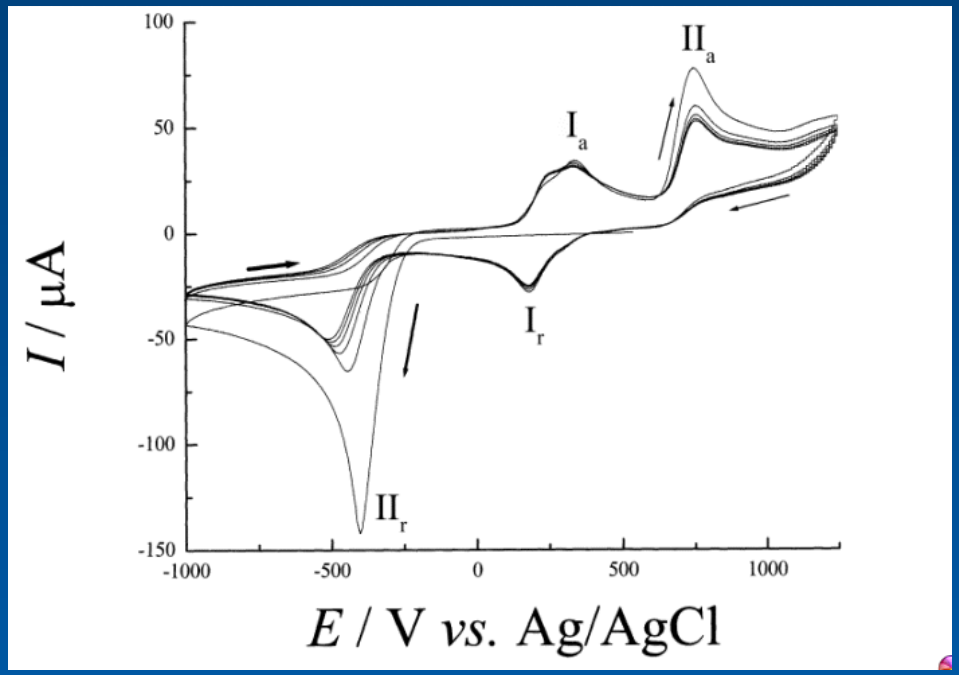
- Cyclic voltammetry of the azo dyes was performed at 100 mV/s scan rate. The experiments were performed in 0.1 M acetate buffer pH 5 at dye concentration of 0.1% w/v. Prior to analysis all solutions were purged with nitrogen for 15 min. The redox potentials recorded *vs.* Ag|AgCl reference electrode were corrected by 0.206 V to the standard hydrogen electrode (SHE).

- Redox potentials of *Trametes villosa* laccase, 1-hydroxybenzotriazole (HBT) were provided from the literature and are as follows (vs. SHE):

•LACCASE +780 mV
•HBT +1.084 mV

Cyclic voltammetry of azo dyes

- The azo dyes tested in this study presented similar cyclic voltammograms illustrated by the voltammogram of dye I in both positive and negative scans.



Cyclic voltammetry of azo dyes

- In the first positive scan of dye I an irreversible anodic peak (II_a) in the potential range of +0.9 to +1.3 V vs. SHE was observed. All dyes displayed an irreversible reduction peak in the range of -0.13 to -0.48 V vs. SHE (II_r).

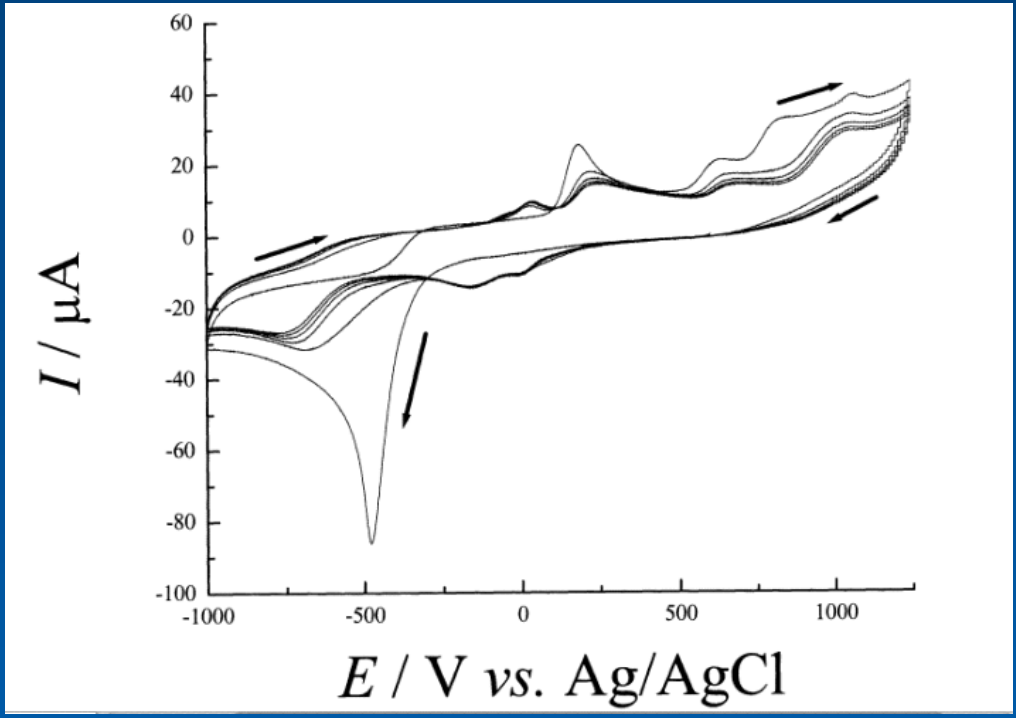
- In the following scans an apparently semi-reversible redox couple (I_a,I_r) was detected. The reductive wave I_r of the semi-reversible redox couple did not appear in the first negative scan.

- These redox couple peaks appears to be associated with the formation of unstable amine products, which were oxidised in the range of +0.15 to +0.58 V vs. SHE and reduced in the potential range of -0.1 to +0.3 V vs. SHE.

- The redox peaks II_a and II_r can be associated with irreversible redox reactions leading to cleavage of the azo bonds.

Cyclic voltammetry of azo dyes

- In the voltammograms of dyes VI (tri-azo) and VII (bi-azo) the number of oxidation peaks was higher than the observed for monoazo dyes. These peaks resulted from the oxidation of the amine products generated during the disruption of more than one azo bond in these dye molecules.



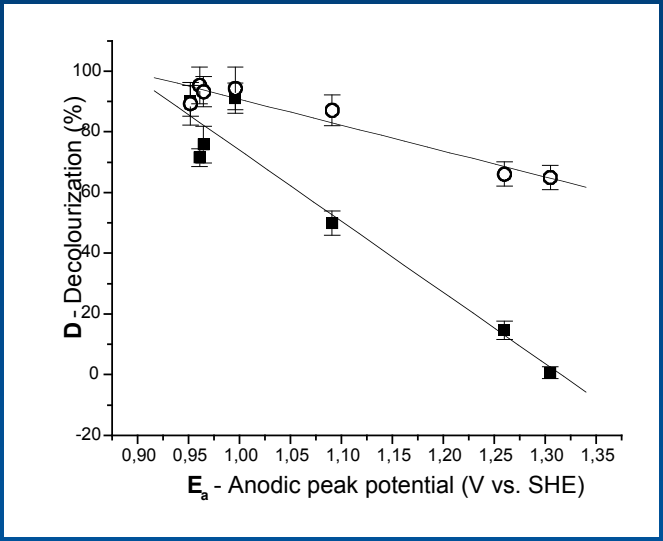
Decolourization with laccase

• For laccase–mediated oxidations, an increase in the substrate redox potential should therefore decrease the efficiency of the reaction. This hypothesis was tested by measuring the percentage of decolourization of each dye in the presence of laccase alone or laccase+HBT after 1h incubation.

Dye	% Decolourization (± standard deviation)		Oxidation peak (V)[1]
	Laccase	Laccase+HBT	
I	71 ± 3	95 ± 6	+ 0.961
II	76 ± 6	93 ± 5	+ 0.965
III	90 ± 5	89 ± 7	+ 0.952
IV	91 ± 5	94 ± 7	+ 0.996
V	15 ± 3	66 ± 4	+ 1.260
VI	50 ± 4	87 ± 5	+ 1.091
VII	0,6 ± 0.2	65 ± 4	+ 1.305

Decolourization with laccase

- A remarkably good linear correlation was found, in both systems, between the percentage decolourization of each dye and the respective anodic peak potential. The linear relationship was preserved for up to 2 h, during the initial period of decolourization.



Correlation between anodic peak potential (E_a) and decolourization % of azo dyes after 1 h with (■) Laccase and (○) Laccase/HBT mediator system. Correlation: D (■) = (308.6 ± 28.9) – (234.6 ± 26.6) E_a, r^2 = 0.97, SD = ±9.7; D (○) = (176.1 ± 10.8) – (85.4 ± 9.9) E_a, r^2 = 0.97, SD = ±3.6.

Decolourization with laccase

- The decolourization appears to depend not only on the redox potential of the enzyme but also on the reactivity of the radicals generated in the laccase-mediated reaction.

- The radical reactions can explane the dyes decolourization even so the anodic peak potentials of all the dyes are higher than the reported redox potential for laccase (+0.780 V vs. SHE).

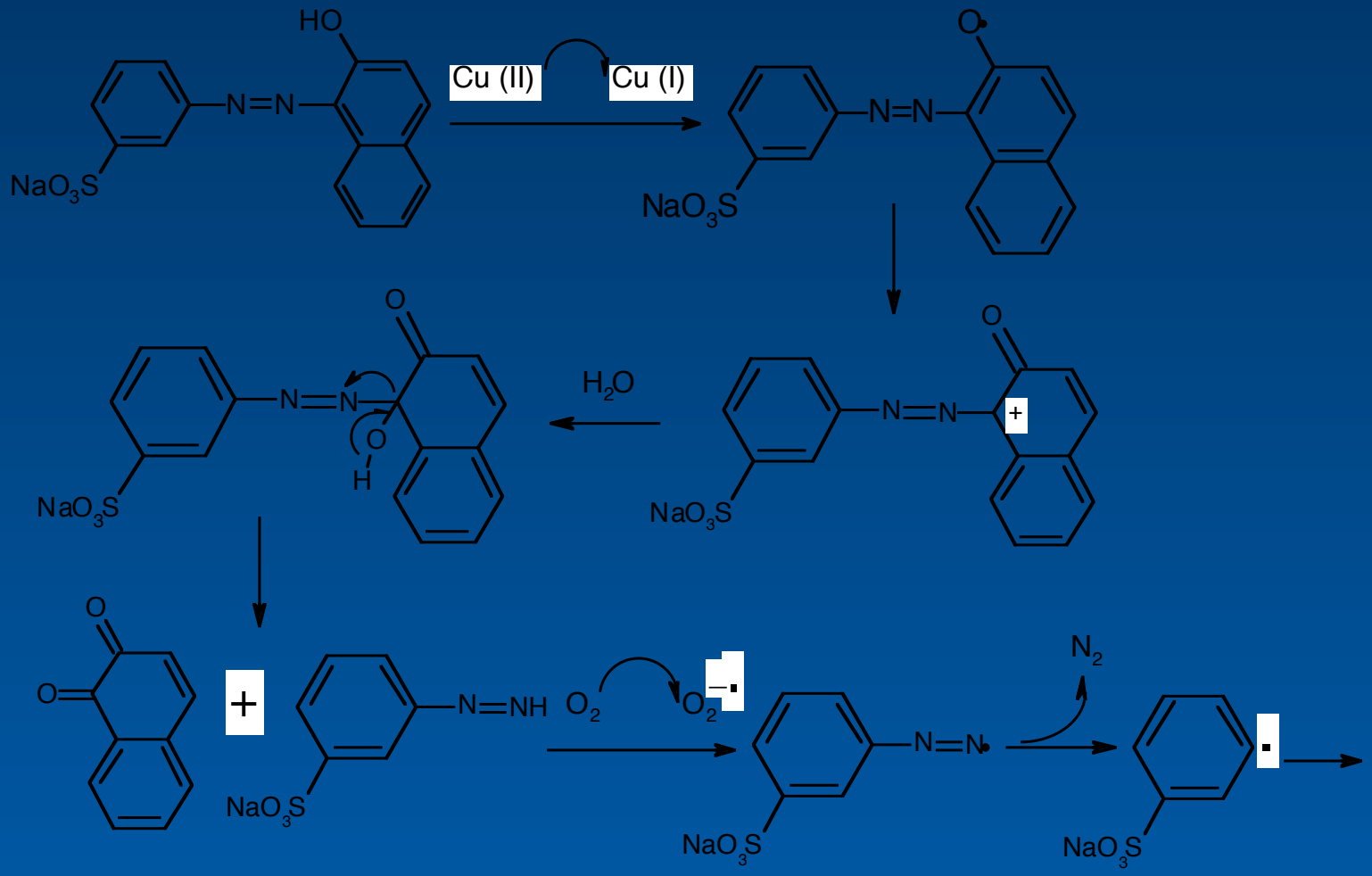
- The positive effect of HBT on the decolourization degree, this can be rationalized considering that this mediator, which is also effective through the formation of a free radical, is a stronger oxidant than laccase itself (+1.084 V vs. SHE).

Decolourization with laccase

•In the accepted mechanism for laccase-mediated oxidations the reactions do not involve direct contact between enzyme and substrate but rather the formation of free radicals, from low molecular weight compounds (redox mediators), which act as the active oxidants.

• The phenolic OH is oxidized to an unstable phenoxy radical, which in turn looses a further electron producing a carbonium ion. A nucleophylic attack by H_2O to the nitrogen-linked carbon yields a quinone and a phenyldiazene. The latter then undergoes several spontaneous reactions involving oxygen, where nitrogen is released and new free radicals are formed.

Decolourization with laccase



Conclusion

- We found a linear relationship during the initial period of decolourization of laccase and laccase/mediator system between the percentage decolourization of each dye and the respective anodic peak potential.

- It appear that Laccase mediated oxidations are not only dependent on the substrate redox potential but also on the reactivity of the free radicals that are formed during the reaction.

- The redox potential difference between the biocatalyst and the dye is a relevant indicator whether the enzyme is able to decolourize the dye.