Treatment of textile dyes effluents by laccase mediator system

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Abstract

A screening using several laccase mediators (2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid (ABTS), 1-hydroxybenzotriazole (HBT), *N*-hydroxyacetanilide (NHA), 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO) and violuric acid (VA)) was performed on the degradation of three reactive textile dyes: Reactive Black 5 (RB5), Reactive Blue 114 (RB114) and Reactive Yellow 15 (RY15). ABTS was the most effective mediator and its efficiency depended on the type of dye and also on the pH, temperature and ABTS concentration. The optimum temperature and pH values were found at 35ºC and 5.0, respectively. ABTS has no effect on the decolourisation at low dyes concentrations, except for RB114 where at lower concentrations it promotes the best decolourisation (93%). High concentrations of ABTS inhibited the decolourisation.

Keywords: laccase, mediator, reactive textile dyes, decolourisation

1. Introduction

In textile industry, colour is applied to finished products through dyeing, resulting in the generation of different wastewaters. The final effluent contains many unfixed dyes with a high intensity of colour. Although one sees the “colored” waste in the textile effluent, the waste, in fact, may consist of hundreds of different hues, as they exhibit light absorbance in the wavelength range of 350 to 500 nm. Dyes used in the textile industry are designed to resist exposure to sweat, light, water, oxidizing agents and microbial attack (Wesenberg et al. 2002). The classes of dyes that can be used in textile industries include acid, basic, direct, disperse, mordant, reactive, sulfur, azoic, and vat dyes.

Textile industries release during the process large quantities (about 10%) of intensely coloured and toxic effluents (Young and Yu, 1997), which cause serious

environmental pollution. The rivers depend on their colour and on the clarity of water. This means that even minor releases of coloured effluents (1mg/L) may cause irregular coloration of surface waters as well as to pollute the water with toxic compounds. The dyes change the absorption and reflection of sunlight on the water creating problems to photosynthetic aquatic plants and algae. A number of biotechnological approaches have been suggested with potential interest in combating this pollution source in an eco-efficient manner (Wesenberg et al., 2003). It is known that lignolitic enzymes (e.g. Mn peroxidise, Lignin peroxidise and laccase) could be used to decolorize textile dyes (Couto and Sanromán, 2005) as well as kraft pulp (Gamelas et al. 2005; Tavares et al. 2004).

Laccases which are highly regarded to be environmentally friendly are considered to be an attractive technology for development of new methodologies of dye degradation from textile industries. Laccase is a cuproprotein belonging to a small group of enzymes denominated blue oxidases. Laccase (E.C. 1.10.3.2, *p*-benzenediol:oxygen oxidoreductase) is an oxidoreductase able to catalyze the oxidation of various aromatic compounds (particularly phenols) with the concomitant reduction of oxygen to water. In general, laccases exhibit four copper atoms, which play an important role in the enzyme catalytic mechanisms (Thurston, 1994).

The enzymatic treatment with enzymes from fungus can be simpler and more efficient than the traditional physical-chemical treatments such as adsorption or ionic exchange resins (Karam and Nicell, 1997). In recognition of these potential advantages, recent research has focussed on the development of enzyme processes for the treatment of textile wastewater. The enzymes are highly selective and can effectively treat the wastes. They are less likely to be inhibited by substances which may be toxic to living organisms and their cost could eventually be lower than that of other methods if commercially available enzymes are produced in bulk quantities. Specific mediators usually denominated by mediated systems, are being searched by investigators to extend the number of dyes decolorized. Considerable resources were exploited in the quest for a cheap, non-toxic mediator that could be used on an industrial scale (Potthast, 2001).

2. Materials and Methods

2.1. Chemicals and enzyme

Textile Dyes: reactive black 5 (Remazol Black B), reactive yellow 15 (Remazol Yellow GR) and reactive blue 114 (Levafix Brilliant Blue E-BRA) were kindly provided by DyStar (Portugal).

Enzyme: Commercial laccase (Denilite base II; 800U/g) from genetically modified *Aspergillus* was kindly provided by Novozymes.

Enzyme Mediators: 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); violuric acid (VA); *N*-hydroxyacetanilide (NHA); 1-hydroxybenzotriazole (HBT) and 2,2,6,6-tetramethylpiperidin-1-yloxy (TEMPO) were supplied by Sigma-Aldrich.

2.2. Mediator screening

The reaction mixture for mediator screening consisted of an aqueous solution of each dye (50 mg/L), laccase (0.2 U/mL), redox mediator ABTS; HBT; TEMPO; NHA and VA (0.1 mM) and 50 mM phosphate buffer (pH 5.0) in a final volume of 25 ml. The reactions were incubated at 25ºC with stirring during one day (necessary time to attain the equilibrium). A control without mediator was carried out in parallel.

2. 3. Dye decolourisation experiments

To study the effect of temperature and pH on decolourisation of the three textile dyes, the dyes, 0.1mM of ABTS as mediator and the commercial laccase were incubated in 25 mL Erlenmeyer flasks under stirring during one day. The effect of temperature was studied in phosphate buffer (50mM) pH 5. The dye solution was incubated at different temperatures. The effect of pH was studied by the incubation of dye solution in the following buffers: 50 mM of citrate/phosphate for pH 3.0 and 4.0; 50 mM of phosphate for pH 5.0 and pH 6.0. To determine the effect of ABTS concentration, different concentrations were tested.

2.4 Determination of dye degradation

Dye decolourisation was determined by monitoring the decrease in the absorbance peak at the maximum wavelength for each dye: reactive black 5 (579nm), reactive yellow 15 (416nm) and reactive blue 114 (593nm). UV-visible spectrophotometer (Thermo, model UV1) was used in all experiments. Decolourisation is reported as: % decolourisation = $(A_i - A_f)/A_i \times 100$, where A_i is the initial absorbance and A_f is the final absorbance of the dye.

3. Results and discussion

The experiments with laccase and without a mediator did not decolourise any dye along incubation time. However when a redox mediator was introduced, the catalytic mechanism of decolourisation started depending on the dye and on the mediator. The mediator screening showed that ABTS was a more effective mediator than the others for the oxidative degradation of the reactive textile dyes (table 1).

Table 1 – Mediator screening for reactive dye degradation by laccase.

Reactive Dye	Dye degradation (%)					
	No mediator	HBT	ABTS	TEMPO	NHA	VA
RB114	ND[a]	0.3	**70**	ND	1.5	5.0
RB5	ND	0.2	**42**	1.5	ND	2.5
RY15	ND	2.7	**21**	2.0	0.8	0.2

[a] ND, not detected.

The decolourization by LMS is very sensitive to temperature and pH, depending on the kind of dye. The optimum temperature and pH for decolourisation were 40ºC and pH 5.0-5.5, respectively, with a maximum decolourisation above 80%

for RB114 (Figures 1 and 2). At lower temperature (20ºC) no decolourisation was observed, except for RB5 (36%), as well as at lower pH (pH 3.0).

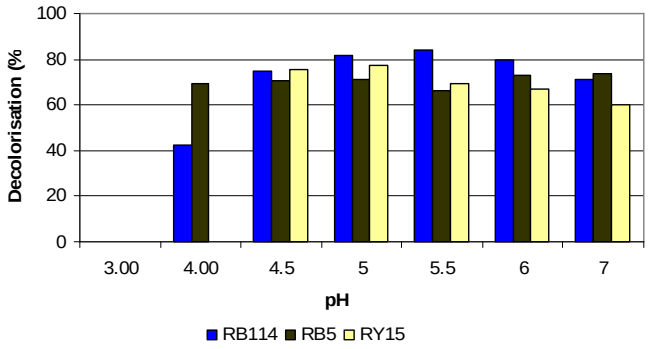

Figure 1 - Effect of pH on the decolourisation of the reactive dyes: Blue 114 (RB114), Black 5 (RB5) and Yellow 15 (RY15) by laccase mediator system.

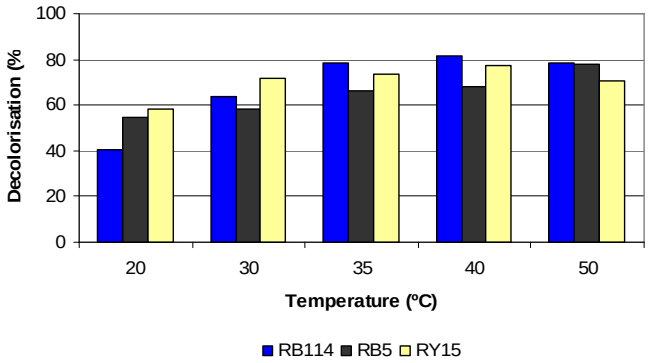

Figure 2 - Effect of temperature on the decolourisation of the reactive dyes: Blue 114 (RB114), Black 5 (RB5) and Yellow 15 (RY15)by laccase mediator system.

The decolourisation of all dyes was very dependent on ABTS concentration. When high ABTS concentrations were employed (above 0.2 mM), lower or none dye degradation was observed. The optimum ABTS concentration for RB5 and RY15 decolourisation by LMS was 0.1mM with colour reductions of 73% and 76%, respectively. The best absorbance spectrum for RB114 was obtained with ABTS 0.001mM where a very low absorbance was detected over all spectrum, with a decolourisation of 87%.

These results are very promising and reveal the high potential of LMS to reactive dye decolourisation. So, LMS can be used for treating textile dying wastewaters, particularly as a polishing process for water recycling.

4. Acknowledgments

The authors wish to thank Novozymes (Denmark) for laccase and DyStar (Portugal) for reactive dyes. R. Cristóvão thanks FCT for the Ph.D Scholarship (SFRH/BD/28529/2006) and A.P.M. Tavares thanks FCT for the Post Doctorate Scholarship (SFRH/BPD/22697/2005).

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