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Short communication

Decolouration of industrial azo dyes by crude laccase from *Trametes hirsuta*

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Abstract

The decolouration of several azo dyes, commonly used in the leather industry, by crude laccase obtained from *Trametes hirsuta* cultivation was assessed. Among the six dyes studied four showed a decolouration percentage higher than 50% in 4 h, whereas the other two showed more resistance to degradation. These results show the ability of laccase towards different dye structures as well as its enormous potential for the decolouration of recalcitrant azo dyes.

Keywords: Azo dyes; Decolouration; Laccase; Leather industry; *Trametes hirsuta*

1. Introduction

Azo dyes are synthetic organic compounds widely used in textile dyeing. This chemical class of dyes, which is characterised by the presence of at least one azo bond (–N=N–) bearing aromatic rings, dominates the worldwide market of dyestuffs with a share of about 70% [1]. They are designed to convey high photolytic stability and resistance towards major oxidising agents [2].

The release of azo dyes into the environment in effluent from textile dyeing plants has become a major concern in wastewater treatment, since they are highly recalcitrant to conventional wastewater treatment processes. The recalcitrance of azo dyes has been attributed to the presence of sulfonate groups and azo bonds, two features generally considered as xenobiotic [3]. In addition, some azo dyes or their metabolites may be mutagens or carcinogens [4]. Several combined anaerobic and aerobic microbial treatments have been suggested to enhance the degradation of azo dyes [5]. However, under anaerobic conditions, azo reductases usually cleave azo dyes into the corresponding amines, many of which are mutagenic and/or carcinogenic [6,7]. Furthermore, azo reductases have been shown to be very specific enzymes, thus cleaving only azo bonds of selected dyes [8]. Altogether this underlines the need for unspecific processes for the effective treatment of wastewater containing such kind of dyes.

Laccases (*p*-diphenol:dioxygen oxidoreductases; EC 1.10.3.2) are copper containing enzymes that catalyse the one-electron oxidation of phenolic substrates and aromatic amines. It has also been shown that in the presence of appropriate low-molecular-weight compounds (redox mediators), laccases are able to oxidise a wide range of other aromatic compounds [9] expanding, thus, the range of compounds that can be oxidised by these enzymes. Laccases are particularly abundant in white-rot fungi, which are the only living organisms able to degrade the whole wood components [10]. In particular, the genus *Trametes* is assumed to be one of the most efficient lignin degraders, *Trametes versicolor* the most extensive utilised representative among them. Recently, *Trametes hirsuta* has been described as a promising laccase producer [7].

The degradation of azo dyes have been extensively studied using a wide range of fungi and bacteria but work carried out using enzymes is more limited [11]. In the present study the ability of crude laccase from *T. hirsuta* cultures to decolourise several industrial azo dyes was assessed.

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Table 1
Characteristics of the azo dyes used

Dye	Conc. (g/L)	Synonym	λ_{max} (nm)	Type
DC NBS	0.133	Direct black 168	618	Mixture of both direct and acid, anionic
DB V	0.067	Acid red 119	521	Acid, anionic
SSB 4GL	0.067	Direct blue 78	601	Direct
DP 5GL	0.083		448	Acid, metal complex (Fe), anionic
DB DBN	0.05		555	Acid, anionic
SSY 4GL	0.1	Acid yellow 166	401	Acid, anionic

2. Materials and methods

2.1. Microorganism

T. hirsuta (BT 2566), obtained from Dr. G.M. Gübitz (Institute for Environmental Biotechnology, Graz University of Technology, Austria), was grown on PDA (potato dextrose agar) plates at 30 °C for about 10 days. Thereafter, the plates were maintained at 4 °C until used. The fungus was sub-cultured every 3 months.

2.2. Laccase production and crude laccase preparation

T. hirsuta was grown on crushed orange peelings in an expanded-bed bioreactor (working volume of 200 mL) under SSF conditions as described in Rodríguez Couto et al. [12]. Culture broth was harvested at the maximum laccase activity (9 days), filtered, clarified by centrifugation at 8000 × *g* for 15 min, frozen, defrosted and, then, filtered to remove the precipitated polysaccharides. The resulting clear filtrate was concentrated on an Amicon membrane with a molecular weight cut-off of 10 kDa. The experiments were performed with this concentrated clear filtrate.

2.3. Determination of laccase activity

ABTS (2,2′-azino-di-[3-ethyl-benzo-thiazolin-sulphonate]) was used as a substrate for spectrophotometric determination of laccase activity as described by Niku-Paavola et al. [13]. One activity unit was defined as the amount of enzyme that oxidised 1 μmol of ABTS per min. The activities were expressed in U L^{-1}.

2.4. Dye decolouration experiments

The dyes used were Derma Carbon NBS (DC NBS), Derma Burdeaux V (DB V), Derma Pardo 5 GL (DP 5GL) and Derma Blue DBN (DB DBN) manufactured by Clariant Ibérica SA (Spain) and Sella Solid Blue 4GL (SSB 4GL) and Sella Solid Yellow 4GL (SSY 4GL) manufactured by TFL (Germany). They are azo dyes commonly used to dye chromed leather. Table 1 describes the characteristics of these dyes. Their chemical structures have not been disclosed, since they are property of their respective companies. Stock solutions (0.5% or 0.25% (w/v) in water) were stored in the dark at room temperature.

The reaction mixture for dye decolouration consisted of an aqueous solution of dye and crude laccase (final concentration: 500 U L^{-1}) in citrate phosphate buffer (pH 5.0) in a final volume of 1.5 mL.

Dye concentrations were selected in order to obtain approximately 1.5 absorbance units at the maximum wavelength in the visible spectrum (Table 1). All the reactions were incubated at room temperature, without shaking and in complete darkness. The residual dye concentration was measured spectrophotometrically from 350 to 750 nm, as shown in Fig. 1, and calculated by measuring the area under the plot according to the following expression:

$$\% = \frac{A_0 - A}{A_0} \times 100$$

where % is the decolouration percentage obtained, A_0 the initial area and A is the final area. A control test containing the same amount of a heat-denatured laccase was also performed in parallel. The assays were done twice, the experimental error being below 10%.

3. Results and discussion

The ability to decolourise different industrial azo dyes, used for dyeing chromed leather, by crude laccase from *T. hirsuta* was assessed. Decolouration was carried out directly in the spectrophotometer cuvette. Fig. 2 shows the decolouration obtained for each one of the dyes studied. The dye SSB 4GL was highly decolourised in 4 h (around 73%) followed by DC NBS (almost 67%). However, DP 5GL and especially SSY 4GL presented

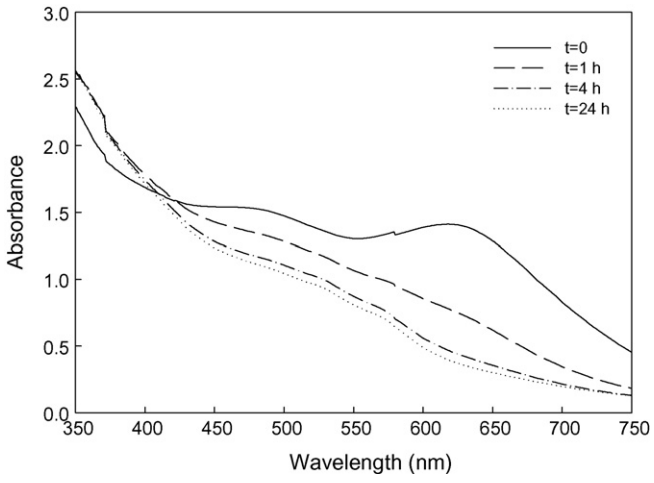

Fig. 1. Spectral scans (350–750 nm) of the decolouration of the dye DC NBS by crude laccase from *T. hirsuta*.

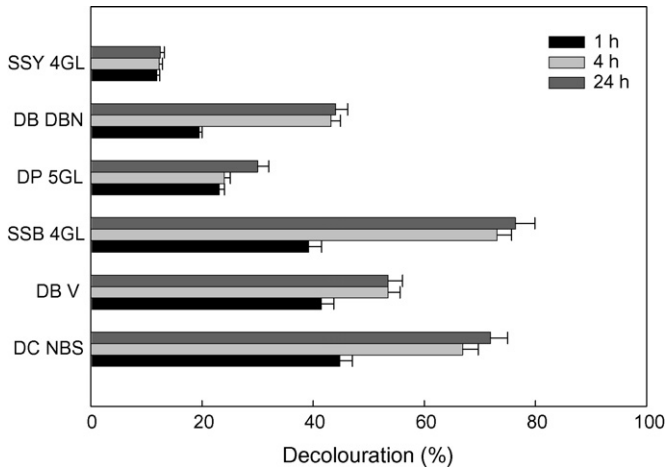

Fig. 2. Decolouration percentage of different industrial azo dyes by crude laccase from *T. hirsuta*.

more resistance to decolouration reaching a decolouration percentage of about 24% and 12% in the same incubation time, respectively. Taking into account the dye concentrations used (Table 1), the highest dye concentrations led to the lowest decolouration percentages except for the dyes DC NBS and SSB 4GL. This could be related to the purity and the molar extinction coefficients of each particular dye. Moreover, it is known that the nature and position of the dye substituents strongly affect the decolouration extent [14]. Unfortunately, we cannot know the reason for the higher decolouration of the above-mentioned dyes by laccase since their structures have not been disclosed. It is beyond the scope of the present work to provide mechanistic interpretations of the observed results as this would require analysis and identification of the reaction products.

The above results show that the individual dye structures influence the decolouration extent obtained by laccase, showing the ability of laccase towards different dye structures. From 4 to 24 h decolouration slightly increased and from there onwards (data not shown) decolouration did not improved, which could likely be due to the presence of some inhibiting sub-products generated in the dye degradation process.

As for the experiments with heat-denatured laccase, they did not show any change in their visible spectrum along incubation time. This indicates that dye decolouration was due to laccase enzyme, since it is the only enzyme present at a significant quantity in the culture filtrate.

The results obtained in the present paper are very promising since laccase from *T. hirsuta* was able to degrade several industrial azo dyes. The complex dye mixture is highly recalcitrant because they are composed of a great variety of dyes and, in addition, they contain different impurities, which make their degradation problematic.

Effluents from dyeing industries consist of a mixture of dyes together with a high salt concentration and in the case of tannery wastewater also contains high amounts of Cr^{3+}, which can highly affect enzyme stability. In a previous work [15] it was shown that inactivation of laccase from *T. hirsuta* by Cr^{3+} occurred after 7 days, so taking into account that dye decolouration occurs in few hours, the presence of Cr^{3+} does not affect laccase inactivation. This shows both the suitability and the potential of laccase enzyme from *T. hirsuta* in the treatment of colourants employed in the leather industry.

4. Conclusions

In view of the results obtained, it can be concluded that laccase from *T. hirsuta* has an enormous potential for azo dye decolouration. However, more studies in order to test the effect of the addition of redox mediators into the reaction mixture are underway in our laboratory.

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