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Green routes towards industrial textile dyeing: A laccase based approach

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ABSTRACT

Laccase-catalyzed synthesis of dye molecules represents a green choice to reduce the environmental footprint of conventional synthetic processes. Textile industry will benefit from this green technology since the synthesized dyes can be exploited to colour different fabrics.

This work describes the application of the *Pleurotus ostreatus* POXA1b laccase in polymeric dye synthesis using resorcinol and 2,5-diaminobenzenesulfonic acid (2,5-DABSA) as substrates. The potential of the resorcinol/2,5-DABSA coupling route was transferred to a chemical industry, Setaş Colour Center, by introducing a greener synthesis step within the process routinely used for textile dyeing. Dye synthesis was performed at different precursor ratios (1:1 and 1:10 2,5-DABSA: resorcinol) and their dyeing properties were compared on different fibres. The two mixtures of synthesized dyes proved to be effective on nylon and wool, with 1:10 ratio displaying the best performances in terms of dyeing efficiency and colour strength. Good and comparable end quality and "performances during use" were observed for nylon and wool coloured with both synthesized dyes.

1. Introduction

Implementation of biotechnology in the chemical synthesis aims at reducing the environmental footprint of conventional synthetic processes. Green catalytic alternatives are particularly needed in the production of dyes considering their huge global production and the harsh operative conditions required for their synthesis. For this reason, in the last decades, bio-based alternatives with the least environmental impact have been widely researched for dye synthesis looking for safe oxidant, such as oxygen and green catalyst, such as enzymes [1–4]. In this context, enzymatic processes exploiting laccases represent one of the most promising tools. Laccases (EC 1.10.3.2) are well-known multicopper phenol oxidases able to oxidize a wide spectrum of both phenolic and non-phenolic substrates to radical forms [5,6]. The oxidation of substrates by laccases takes place under mild conditions, using oxygen as co-substrate and producing water as by-product [7–10]. In particular, fungal laccases display a wider range of oxidizable substrates in comparison with bacterial ones, due to the high redox potential of their T1 copper. In addition, if these enzymes are secreted in the extracellular medium, their recovery becomes more easy and cost-effective. As a fact, taking advantage of their ability to catalyse homo- or hetero-molecular coupling of reactive intermediates, fungal laccases have been applied to the synthesis of polymeric dyes for textile and hair dyeing [11–14].

In this work a high redox potential (HRP) fungal laccase has been exploited in a biosynthetic pathway to synthesize polymeric phenolic/amino dyes based on 2,5-diaminobenzenesulfonic acid (2,5-DABSA) and resorcinol. The former has been already successfully used for the *in situ* colouration of both cotton and wool fibres, mainly coupled to catechol as dye modifier [11,12,14,15]. Oxidative coupling is the main mechanism behind this laccase catalyzed reaction, leading to C—N hetero-coupling between the two substrates on a backbone of catechol polymer resulted from a C—C homo-coupling [12,14,16]. On the other hand, resorcinol has been barely exploited in fibre dyeing. This molecule, unlike catechol, can be synthesized by a selective reaction route limiting the production of harmful by-products [17–20].

Dye synthesis was performed at alkaline pH to promote both C—N cross-coupling [14] and resorcinol polymerization. Furthermore, high pH and temperature (pH 8 and 60 °C) were applied to increase substrates solubility in order to potentially obtain the

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highest yield of coloured products for the industrial dye bath. In this frame, the use of an enzyme able to cope with high pH and temperature is mandatory.

The *P. ostreatus* laccase POXA1b displays a well-known activity and stability at the required conditions [21], addressing the reaction constraints.

The potential of the resorcinol/2,5-DABSA coupling route was transferred to a chemical industry, Setaş Colour Center, and enzymatically synthesized dyes were straight applied in their existing textile dyeing procedure. A comparison between enzymatic and chemical dyes was performed in terms of their dyeing effectiveness and fastness properties.

2. Materials and methods

2.1. Materials

Recombinant POXA1b laccase from *P. ostreatus* [22], expressed in the yeast *Pichia pastoris* under the control of *AOX1 promoter* was produced and kindly provided by Biopolis S.L. (Spain) in the frame of INDOX European Project.

All reagents were purchased from Sigma-Aldrich Corp. (St. Louis, MO).

2.2. Laccase activity

Laccase activity was determined using 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) as substrate following the reaction at 420 nm ($\varepsilon_{420} = 36 \times 10^3\ M^{-1}\ cm^{-1}$), as previously reported [23].

Determination of laccase activity towards 2,5-DABSA (1–25 mM) and resorcinol (10–50 mM) was performed by estimating the increase in absorbance at 460 and 490 nm after an incubation of 5 and 10 min at 60 °C, respectively. Assay mixture contained 1 U mL^{-1} of POXA1b laccase (estimated using ABTS as substrate) in 50 mM Tris HCl, pH 8.

2.3. Dye synthesis

Reactions were carried out at different ratios between the two precursors in 50 mM Tris HCl, pH 8.0 at 60 °C under agitation (150 rpm) for 6 h using 1 U mL^{-1} (assayed towards ABTS) of POXA1b. Two ratios 2,5-DABSA: resorcinol were tested (1:1, 1:10), using 10, 25 or 50 mM resorcinol and the corresponding concentrations of 2,5-DABSA. When 50 mM resorcinol was used, the 1:1 ratio was not performed because of 2,5-DABSA limit of solubility in the conditions used (25 mM). Reactions were followed by hourly registering the absorbance spectra (380–800 nm), and subtracting spectra of substrates in the absence of enzyme as blank (Jasco V-530 UV/vis).

The two ratios 2,5-DABSA: resorcinol were scaled-up to 1L using 25 mM 2,5-DABSA and 25 mM resorcinol for the 1:1 reaction, and 25 mM 2,5-DABSA and 250 mM resorcinol for the 1:10. At the end of the reactions, products were analysed using a A-8453 UV–vis Spectrophotometer (A-61103)- Agilent system. Synthesized dyes were concentrated by nano-filtration equipment (handmade equipment by Setaş) using a polyamide membrane (AFC40, PCI membrane) to a final volume of 250 mL.

2.4. Multifibre test

The concentrated dyes were used for the multifibre dyeing (acetate, cotton, nylon, polyester, acrylic, wool). 5 g of each textile were dyed in 100 mL water containing 0.2 g L^{-1} Setacid VS-N, 10 g L^{-1} Setalan PM71, 20 g L^{-1} Na_2CO_3 using 8% v/v of 1:1, 1:10 ratio and Nyloset Brown N2R (reference dye). Dyeing was performed for 1 h at 102 °C in an IR Dyeing Machine (Copower Technology, LTD, Taipei Taiwan).

2.5. Fibre dyeing

Nylon fibres (5 g textile) were dyed using different concentrations (2 ÷ 8% v/v) of each concentrated dye (1:1, 1:10 ratio and Nyloset Brown N2R), in 100 mL water containing 0.2 g L^{-1} Setacid VS-N, 10 g L^{-1} Setalan PM71, 20 g L^{-1} Na_2CO_3. Dyeing was performed for 1 h at 102 °C in an IR Dyeing Machine (Copower Technology, LTD, Taipei Taiwan).

After the first dyeing procedure, the same bath was used to dye another 5 g textile piece (second bath).

Wool fiber (5 g textile) were dyed using different concentrations (2 ÷ 8% v/v) of each concentrated dye (1:1, 1:10 ratio and Nyloset Brown N2R), in 100 mL water bath containing 0.5 g L^{-1} Setalan PM 71, 3.0 g L^{-1} Ammonium Sulfate, 2 g L^{-1} Setacid VS-N, 0.2 g L^{-1} Na_2CO_3. Dyeing was performed for 1 h at 98 °C. After the first dyeing procedure, the same bath was used to dye another 5 g textile piece (second bath).

2.6. Fibre characterization

The CIELAB coordinates (L*, a*, b*) and colour strength of the samples (K/S) were evaluated using a reflectance measuring apparatus Datacolor SF600 plus. The Kubelka-Munk relationship (K/S), where K is an adsorption coefficient and S is a scattering coefficient, was applied to textiles under the assumption that light scattering is due to the fibres, while adsorption of light is due to the colorant.

The colour fastnesses of the dyed fabrics were evaluated following established test procedures: ISO 105-B02:1994-Colour fastness to artificial light: Xenon arc fading lamp test (Blue scale 1–8); ISO 105-CO6:1998-Colour fastness to domestic and commercial washing (Grey scale 1–5); ISO/DIS 105-X12:2001-Colour fastness to rubbing (Grey scale 1–5).

3. Results

3.1. Design of the process conditions

The activity of POXA1b towards 2,5-DABSA and resorcinol precursors was first assayed, and the enzyme was found able to oxidize both substrates at all the tested concentrations (Supplementary Fig. 1). In fact, a remarkable increase of absorption intensity was measured, with peaks centred at around 490 and 460 nm for resorcinol and 2,5-DABSA, respectively.

With the aim of establishing reaction conditions for dye synthesis, two different 2,5-DABSA: resorcinol ratios (1:1, 1:10) were tested setting three increasing resorcinol concentrations (10, 25, 50 mM) (Fig. 1).

As for the 1:1 ratio (Fig. 1a), at 10 mM the overall profile of the spectra increased without the presence of any peak, and a brownish colour was produced. On the other hand, at 25 mM, a peak at around 460 nm was formed, and a darker colour was observed. At 1:10 ratio (Fig. 1b) it was possible to observe the formation of a peak at around 490 nm at all the tested concentrations and higher substrate concentrations darker the colour produced. The different results obtained varying substrate ratios may reflect the different affinity/turnover of the enzyme towards the two precursors, leading to the production of different dye mixtures. In fact, at 1:1 ratio the peak at around 460 nm can be ascribable to the major conversion of 2,5-DABSA towards that of the resorcinol (Fig. 1), while when the ratio increases in favour of resorcinol (1:10 ratio), different species with an absorption peak at around 490 nm appear. Considering the operative conditions, it is not possible to rule out the existence of