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Letter

Polyoxometalates as promoters of laccase-assisted reactions

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Abstract

A synergistic effect of polyoxometalate and laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) has been observed in the aerobic oxidation of a hydrazonaphthalene type colorant. The considerable increase in the decolorization rate of Solophenyl Blue GL dye with the new catalytic system composed by laccase and heptamolybdopentovanadophosphate heteropolyanion, when compared with the treatment with enzyme or heteropolyanion alone, was tentatively explained by the redox mediation action of the heteropolyanion. © 2000 Elsevier Science B.V. All rights reserved.

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Laccases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) belonging to the class of the blue oxidases are involved in the natural biodegradation of the lignin [1]. In the catalytic cycle, these enzymes provide a four-electron transfer from substrate molecule to molecular oxygen, which plays the role of terminal electron acceptor [2]. Laccases have broad substrate specificity with respect to the electron donor. Typically, laccases catalyze the one-electron oxidation step of *ortho* and *para*-substituted mono- and polyphenolics and aromatic amines, followed by the formation of phenoxy-radical mesomeric intermediates, leading to further substrate reactions such as depolymerization/polymerization, demethoxylation and formation of quinones [3]. Recent studies [4–8] suggest that the laccase-assisted reactions strongly depend on the enzymes redox potential. Mutations of amino acid residues ‘‘near-by’’ the copper binding sites result in changes of the laccase redox potential, specific activity and optimal pH activity [5]. The practical interest in laccase-based technologies is associated to their ecologically sound perspectives. However, the efficiency of laccases for many applications (effluent treatment, delignification of lignocellulosic materials, etc.) is insufficient and they need so-called redox mediation. In the literature, several mediators from the category of phenolic [4–10] and non-phenolic types [6,9,10] have been described

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for laccase-assisted reaction thus broadening their applicability. Laccases have also showed activity towards inorganic substrates like potassium ferrocyanide [6,7]. The use of mediators in practice is hindered by their costs, regeneration problems and degradation in oxidation reactions.

Molybdovanadophosphate heteropolyanions of series $[PMo_{(12-n)}V_nO_{40}]^{(3+n)-}$ (HPA-*n*, where *n* is the number of vanadium atoms), polyoxometalates with Keggin structure, are known as highly selective catalysts in oxidative organic syntheses and in oxidative delignification [11]. HPA containing more than one vanadium atom in their composition (HPA-*n*, $n \geq 2$) are considered as multi-electron oxidants, which may be re-oxidised with dioxygen under aerobic conditions (four-electron reduction of dioxygen) [11]. The heptamolybdopentovanadophosphate heteropolyanion (HPA-5) was previously reported as an effective regenerable oxidation catalyst in the delignification of lignocellulosic materials [12] and in the oxidation of lignin phenolic model compounds [13].[1]

In this work, aiming primarily to investigate new approaches for the decolorization of effluent streams from the textile industry, laccase in the presence of HPA-5, was used as a new catalytic system in the aerobic oxidation of Solophenyl Blue GL dye (Fig. 1) The extent of the oxidative degradation of Solophenyl Blue GL dye was evaluated through the decolorization of initial aqueous blue solution. In the presence of laccase alone, 20% decolorization was reached in 60 min (Fig. 2). HPA-5 alone was able to decolorize the dye to a similar extent. When laccase and HPA-5 were used together, the HPA-5 addition did not suppress the laccase activity, as it could be expected. Surprisingly, in the same period of time, the combination of the two catalysts decolorized the dye solution to extents higher than 60% (Fig.2). This experiment was completely reproducible in 5 repetitions, with error less than 0.5%. Similar changes results were obtained with indigo and anthraquinone-type dyes structures (Indigo Carmin and Remazol Brillant Blue, respectively, Sigma, reagents). Thus, laccase and polyoxometalate clearly showed a synergistic effect in the oxidation of dyes.

The mechanism of the observed catalytic synergism of laccase and the polyoxometalate is not clear yet. However, the possibility is not excluded that HPA-5 may play the same role as the known and established organic mediators, providing the electron transfer from the substrate to the laccase. Studies on the mechanism of HPA action as promoter of laccase-assisted oxidation reactions as well as on new applications of this unusual HPA/laccase catalytic system, including delignification of lignocellulosics, are in progress. This is the first report on

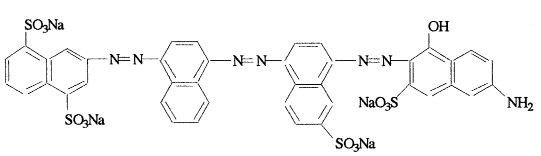

Fig. 1. Chemical structure of Solophenyl Blue GL (C.I. Direct Blue 71).

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[1] Experimental: *Preparation of the sodium salt of $[PMo_7V_5O_{40}]^{8-}$ (0.20 mol/dm^3)* (adapted from Ref. [12]): 4.55 g of V_2O_5 and 10.08 g of MoO_3 were suspended in 35 ml of water in a 100-ml flask with stirring, at room temperature. 3.71 g of anhydrous Na_2CO_3 was slowly added in portions to the stirred mixture, causing CO_2 liberation, followed by the addition of 1.38 g of NaH_2PO_4 H_2O to the obtained suspension. The mixture was refluxed for 3 h. 5 ml of 5 M H_2SO_4 were added to the resulting cold deep red solution. The final solution was stored overnight and filtered through glass filter to eliminate insoluble solids. The glass filter was washed with a small quantity of distilled water and the filtrate was added to the polyanion solution. Finally, the total volume was adjusted by evaporation to 50 ml.

Measurement of the decolorization rates: 0.75 ml enzyme solution (*Trametes villosa* laccase, Novo Nordisk, Denmark, diluted 1:20), 0.75 ml buffer (0.2 M acetate, pH 4), 50 μl HPA-5 (0.004 mol/dm^3) and 1.5 ml 0.06 g/l of dye (Solophenyl Blue GL, Ciba, Switzerland) were incubated a cuvette. Controls contained H_2O, dye and buffer; H_2O, dye, buffer and HPA-5 and finally laccase, dye and buffer. Decrease in optical density at 588 nm was monitored on a Unicam α UV-Vis spectrometer with a termostatized cuvette holder at 50°C. All chemicals were from Merck, Germany, unless otherwise stated.

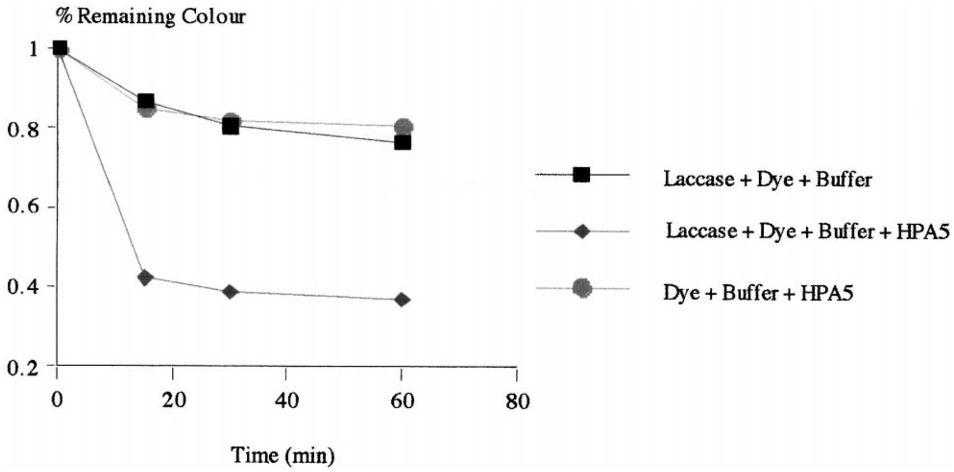

Fig. 2. Decolorization of Solophenyl Blue GL by *T. villosa* laccase, HPA-5 and their combination.

the enhancement of laccase catalytic activity by polyoxometalates.

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