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Assessment of the joint effect of laccase and cellobiose dehydrogenase on the decolouration of different synthetic dyes

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ABSTRACT

In this paper the efficiency of the combined action of laccase and cellobiose dehydrogenase (CDH) to decolourise different synthetic dyes such as Remazol Brilliant Blue R (RBBR), Methyl Green (MG), Direct Violet (DV), Ponceau Xylidine (PX), Bismark Brown (BB) and Poly R-478 (PR) was assessed. It was found that the use of CDH could be a promising alternative to the utilisation of the expensive and poisonous chemical mediators such as HOBT although much research on this topic remains still to be done.

1. Introduction

Synthetic dyes are extensively used in several industries such as the textile, paper, pharmaceutical, cosmetics and food industries. Over 7×10^5 tonnes of approximately 10,000 different dyes and pigments are produced annually world-wide, of which about 50,000 tonnes are discharged into the environment [1]. The discharge of very small amounts of dyes (less than 1 mg/l for certain dyes) is aesthetically displeasing, impedes light penetration into water, retards photosynthesis, inhibits the growth of aquatic biota and interferes with gas solubility in water bodies [2]. For these reasons several countries are adopting stringent regulations for the discharge of coloured industrial effluents.

Synthetic dyes are usually treated by physical or chemical methods [3]. However, these processes are financially and often also methodologically demanding, time-consuming and mostly not very effective. Currently one of the possible alternatives for the treatment of dye-containing effluents is the use of ligninolytic fungi, which can oxidise a wide variety of organic pollutants including synthetic dyes [4–6]. This ability is due to an extracellular non-specific and non-stereoselective enzyme system consisting of lignin peroxidases (LiP, EC 1.11.1.14), manganese peroxidases (MnP, EC 1.11.1.13) and laccases (EC 1.10.3.2). The latter have been subject of recent research due to (i) laccases present a better thermostability than LiPs and MnPs and (ii) laccases only require the presence of oxygen from air but neither manganese nor hydrogen peroxide.

Laccases (*p*-diphenol:dioxygen oxidoreductases; EC 1.10.3.2) are multicopper-containing enzymes that catalyse the one-electron oxidation of phenolic substrates and aromatic amines with the simultaneous four electron reduction of molecular oxygen to water [7]. The broad substrate specificity of laccases together with the fact that they use molecular oxygen as the final electron acceptor, make these enzymes highly interesting for biotechnological applications. The range of chemical structures oxidised by laccases can be even increased by using different natural and synthetic redox mediators [8]. The basis of the laccase-mediator concept is the use of low-molecular weight compounds, which once oxidised by laccase to radicals act as redox mediators oxidising other compounds that are not substrates of laccases. Mediators having the N–OH functionality are regarded to give the best performances. Thus, Xu et al. [9] found that old newsprint could be deinked by laccase plus violuric acid (VA). Also, Rodríguez Couto and Sanromán [10] found that lac-

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case plus VA effectively decolourised the recalcitrant dyes Acid Red 97 and Acid Green 26. However, these compounds are expensive and toxic which limits their application to an industrial scale. The search for natural mediators is in progress [11]. Also, recent studies have shown that cellobiose dehydrogenase (CDH; EC 1.1.99.18), an extracellular haemo-flavo-enzyme, produced by a number of wood-degrading and phytopathogenic fungi, plays a role in the early events of lignocelluloses degradation and wood colonisation [12] by the generation of hydroxyl radicals in a Fenton type reaction in the presence of an electron donor [13]. Furthermore, hydroxy radicals should be involved in the demethylation of lignin, as they can transform non-phenolic in phenolic units and facilitate their degradation by other oxidative enzymes such as MnP or laccase [14]. In this regard, CDH has been reported to display *in vitro* a synergism with laccases in the decolouration of different classes of textile dyes [15]. In the present study, we investigated the joint effect of laccase and CDH on the decolouration of different synthetic dyes.

2. Materials and methods

2.1. Microorganism

Trametes pubescens MB89 (CBS 696.94; Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands) was obtained from the Institute of Applied Microbiology, University of Natural Resources and Applied Life Sciences (Vienna, Austria) and was maintained on malt extract agar (MEA) plates at 4 °C and subcultured every 3 months.

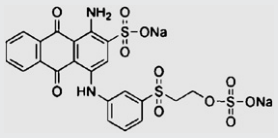
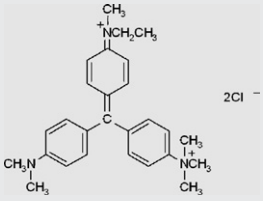
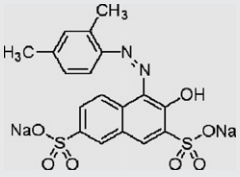
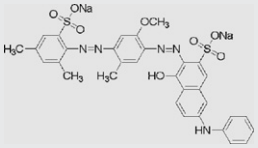
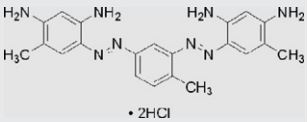
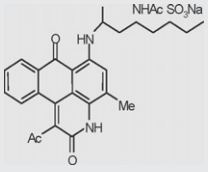
2.2. Culture conditions for laccase production

Laccase was produced as previously described [16]. Culture broth was collected at the maximum laccase activity (day 10) and clarified by centrifugation at 8000 × *g* for 15 min. After that, the supernatant was ultra-filtrated in an Amicon stirred cell apparatus (YM10 membrane). The resulting concentrated extract was used to perform the decolourising experiments.

2.3. Culture conditions for the simultaneous production of CDH and laccase

T. pubescens was cultivated as above except 10 g/l of microcrystalline cellulose powder was added as a carbon source instead of glucose to obtain the simultaneous production of CDH and laccase. The extracellular fluid was collected on day 10 when CDH activity was 49 U/l and laccase activity was 4808 U/l, centrifuged and concentrated as indicated above.

Table 1
Characteristics of the synthetic dyes used.

Dye	Class	λ_{max} (nm)	CI number	CI name	Structure
Remazol Brilliant Blue R (RBBR)	Anthraquinonic	595	61200	Reactive Blue 19	
Methyl Green (MG)	Tryarylmethane	630	42585	Basic Blue 20	
Ponceau Xylidine (PX)	Monoazo	503	16150	Acid Red 26	
Direct Violet 51 (DV)	Diazo	549	27905	Direct Violet 51	
Bismark Brown R (BB)	Diazo	468	21010	Basic Brown 4	
Poly R-478 (PR)	Polymeric	520	–	–	

CI = colour index.