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Dye decolorization and detoxification by laccase immobilized on porous glass beads

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ABSTRACT

The decolorization and detoxification of textile dyes by fungal laccase immobilized on porous glass beads were evaluated. Anthraquinone (Reactive blue 19 and Dispersed blue 3) and indigoid (Acid blue 74) dyes were degraded more rapidly than the azo dyes (Acid red 27 and Reactive black 5). There was no dye sorption to the enzyme bed when decolorization rates were high (>12 μM dye/U-h) but at moderate rates (8 to >0.06 μM/U-h), there was a transient color which disappeared upon prolonged exposure. With Reactive black 5, permanent adsorption occurred most likely because laccase had been totally inactivated. Although laccase treatment was more efficient at decolorizing the anthraquinone dyes, their toxicity (as determined by the Microtox assay) increased while the less efficiently decolorized solutions of azo and indigoid dyes became less toxic. These results demonstrate the potential and limitations of using immobilized laccase to enzymatically decolorize a range of different dye classes and reduce dye toxicity in a single step.

1. Introduction

When textile mill effluents are discharged to receiving waters, dyes are visible pollutants at concentrations of 0.005 mg/l (O'Neill et al., 1999). Although some dyes and/or their degradation products are toxic and potential carcinogens (Moawad et al., 2003), all reduce the amount of sunlight to photosynthetic organisms resulting in decreased oxygen levels in aquatic ecosystems. Physical color removal methods (such as activated carbon and coagulation) require subsequent disposal steps as the dyes are transferred to a different phase. Chemical methods can eliminate dyes but reagents are added and, in some cases, may generate undesirable by-products. Domestic activated sludge processes are ineffective at dye removal because the microbial consortia are typically not capable of degrading textile dyes that are adsorbed on the cell membrane (Rosa et al., 2001; Hassan et al., 2003; Wang et al., 2002).

A promising biological treatment is the use of white rot fungi (Asgher et al., 2008; Kaushik and Malik, 2009). Their extracellular enzymes including peroxidases and laccases have decolorized dyes in liquid cultures (Kirby et al., 2000; Champagne and Ramsay, 2005; Husain, 2006; Wesenberg et al., 2003). Immobilization of these enzymes is potentially more cost-effective as it would allow their re-use and may improve enzyme stability. Although purified fungal laccases have been shown to decolorize azo (Salony et al., 2006), acid (Salony et al., 2006; Ben Younes et al., 2007) and anthraquinone (Lu et al., 2007; Ben Younes et al., 2007) dyes, most studies using immobilized laccase have evaluated their ability to remove pollutants such as pesticides and phenols from synthetic wastewaters (Yinghui et al., 2002; Jolivalt et al., 2000).

Dye decolorization using laccase immobilized on imidazol-modified silica gel (Peralta-Zamora et al., 2003) or silanized alumina particles (Zille et al., 2003) occurred mainly by adsorption, and to a lesser extent, by enzymatic decolorization. More recently, Champagne and Ramsay (2007) demonstrated that laccase immobilized on controlled porosity carrier (CPC) glass beads using APTES-glutaraldehyde decolorized a single anthraquinone dye, Reactive blue 19, mainly by enzymatic degradation. However, decolorization of other dyes was not evaluated.

There are few reports on the detoxification of dyes decolorized by fungal cultures (Ramsay and Nguyen, 2002; Shin et al., 2002), and even fewer on dyes decolorized by free or immobilized laccase (Abadulla et al., 2000). Although both the culture and the purified enzymes generate decolorized dye products, fungal cultures also produce numerous metabolites and enzymes that can result in a toxic effect different from decolorization by enzymes alone. This study examines whether *Trametes versicolor* laccase immobilized on CPC-silica beads could decolorize a wider range of dye classes (specifically, Disperse blue 3 (anthraquinone dye), Acid blue 74 (an indigoid dye) and Acid red 27 and Reactive black 5 (two azo dyes)) than just Reactive blue 19 and the effect of enzymatic treatment on detoxification.

2. Methods

2.1. Chemicals

CPC-silica beads pre-silanized with 3-aminopropyltriethoxysilane (APTES), *Trametes versicolor* laccase, Acid red 27, Reactive blue

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19, Reactive black 5 and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (diammonium salt) were purchased from Sigma–Aldrich (Oakville, ON, Canada). Dispersed blue 3, Acid blue 74 and glutaraldehyde were purchased from Acros (Belgium).

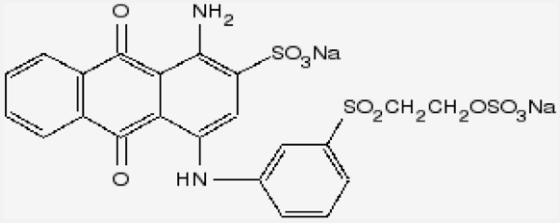
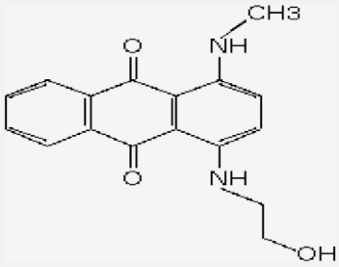
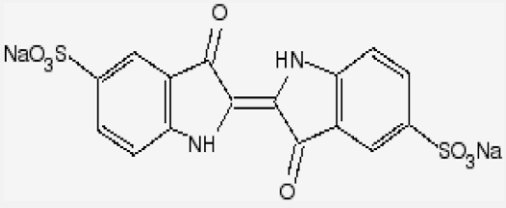
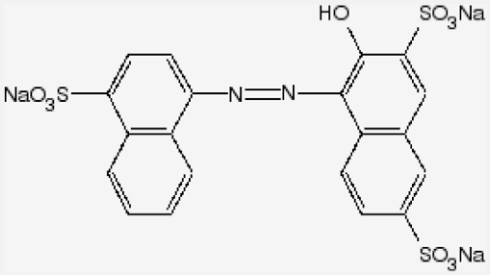
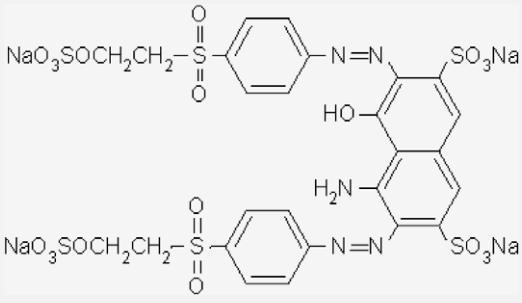
2.2. Immobilization of laccase on controlled porosity carrier (CPC) silica beads

Laccase was immobilized on pre-silanized silica beads according to Champagne and Ramsay (2007). Four grams of pre-silanized CPC-silica beads (355–600 μm in diameter, an average surface area of 42.1 m^2/g and a pore size of 37.5 nm) were immersed in degassed 2.5% glutaraldehyde in 0.1 M KH_2PO_4 at pH 5.0 for 2 h and thereafter placed in a laccase solution (~3 U/ml in 0.1 M KH_2PO_4 pH 5.0) for 36 h at 4 °C. The beads were then washed three times with distilled water and twice with phosphate buffer.

2.3. Enzyme assay

Laccase activity was measured spectrophotometrically by the generation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) radicals ($ABTS^{\bullet-}$) at 420 nm from the oxidation of ABTS (Wolfenden and Wilson, 1982) at 23 ± 1 °C using a Spectramax 250 plate reader with the SOFTmax® PRO software package (Molecular De-

Table 1
Dye structure and their maximum wavelength of absorption.

Dye	Molecular structure	MW	λ_{max} (nm)
Anthraquinone			
Reactive blue 19		626.5	592
Dispersed blue 3		296.3	719
Indigo			
Acid blue 74		466.3	609
Azo			
Acid red 27		604.5	523
Reactive black 5		991.8	597