DECOLORIZATION/DETOXIFICATION/DEGRADATION OF TEXTILE DYES AND DYE WASTEWATERS USING *Cyathus bulleri* LACCASE/LACCASE-MEDIATORS

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by

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Submitted

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Dedicated to
Nirankari Babaji, Rajmata ji, Pujya mata ji
and my parents

CERTIFICATE

This is to certify that the thesis entitled **"Decolorization/detoxification/degradation of textile dyes and dye wastewaters using *Cyathus bulleri* laccase/ laccase-mediators"** being submitted by **Ms. Meenu Chhabra** to the Indian Institute of Technology Delhi for the award of the degree of **'Doctor of Philosophy'**, is a record of the bonafide research work carried out by her, which has been prepared under our supervision in conformity with the rules and regulations of the "Indian Institute of Technology Delhi". The research results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

Prof. Saroj Mishra **Prof. T. R. Sreekrishnan**

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Meenu Chhabra

ABSTRACT

Decolorization of different reactive and acidic dyes, used in wool industry, was performed using laccase from *Cyathus bulleri* in the presence of common natural and synthetic mediators. 2, 2'–Azino-bis(3- ethylbenzthiazoline-6-sulphonic acid) or ABTS was the most effective, among a large number of synthetic mediators tested, and resulted in 80-95% decolorization of the studied dyes. Natural mediators like vanillin were less effective and mediated less (0-45 %) color removal. Laccase-ABTS treatment also led to ~80% color removal of the simulated effluent. No co-relation was found between laccase activity on different mediators and their ability to decolorize. Optimum pH for mediator oxidation coincided with the optimum pH for decolorization using that mediator. The treated and untreated dye samples were also evaluated for toxicity in model microbial systems. Simulated effluent was not toxic to *Pseudomonas putida* but showed cytotoxicity, as indicated by the thinning of the background lawns of *Salmonella typhimurium* in Ames test. The laccase-mediator system removed cytotoxicity and mutagenicity associated with the dye mixture.

Effect of laccase (and mediator assisted laccase) action on two triarylmethane dyes, namely, Basic Green 4 and Acid Violet 17 and an azo dye Acid Red 27 was studied and various products of dye degradation and their toxicity were evaluated. It was found that rates of decolorization were accelerated by 600-800 folds for Basic Green 4 and by nearly 2000-fold for Acid Violet 17 in the presence of the mediator. Acid Red 27 was completely resistant to laccase treatment. However laccase-1-HOBT system led to nearly 90% and laccase-violuric acid system led to nearly 80% decolorization of this dye. Liquid chromatography-electron spray ionization-tandem mass spectrometric analysis was performed on the untreated dyes, dyes treated with laccase alone and dyes treated with

different laccase-mediator combinations to elucidate the pathway of degradation. Decolorization of Basic Green 4 by laccase alone was mainly due to N- demethylation reactions. In the presence of the mediator, apart from N-demethylation, a number of other reactions were initiated from the carbinol form of the dye. Benzaldehyde and N, N dimethyl aniline were identified as the major end products of degradation. Similarly, laccase alone carried out some N-deethylation steps on Acid Violet 17, which were insufficient to exhibit any significant decolorization. Mediator assisted reactions initiated with the formation and oxidation of the carbinol form of the dye. A number of subsequent oxidation reactions then resulted in formyl benzene sulphonic acid, carboxy benzene sulphonic acid and benzene sulphonic acid as the major end products. Laccase-mediator action on Acid Red 27 showed the dye breakdown into small molecular weight fragment initially but it followed the formation of unidentified complex polymeric end products. Toxicity analysis indicated that Basic Green 4 was toxic and treatment with laccase-mediators resulted in 80-100% detoxification. Acid Violet 17 was less toxic and this could be reduced by laccase-mediator treatment. Acid Red 27 was not toxic but was found to be mutagenic. ***The laccase-mediator assisted dye decolorization pathways for these dyes are proposed for the first time in this study.***

Laccase immobilization by entrapment was performed in alginate beads cross linked with calcium, copper or zinc and poly vinyl alcohol (PVA) beads crosslinked with boric acid or nitrate. Zinc alginate and copper alginate gave 80 % and 95 % decolorization respectively. Zinc alginate and PVA-nitrate retained stable 65 and 70 % laccase activity after 5 months of storage at 4°C. However, no increased resistance to chloride, EDTA and sodium azide was noted with IC_{50} values nearly identical to free laccase. It was found that efficient decolorization of Acid Violet 17 could be achieved for

5 cycles for Zinc alginate and PVA nitrate, whereas around 20 cycles of decolorization were observed for Basic Green 4 by PVA-nitrate. PVA-nitrate beads were mechanically and chemically more stable than the alginate beads. Continuous decolorization of the simulated effluent was performed in a packed bed bioreactor using immobilized laccase in calcium alginate, zinc alginate and PVA-nitrate beads. Laccase leaching was substantially reduced in PVA-nitrate beads and almost 95% decolorization could be maintained for more than 108 h.

Continuous decolorization using free enzyme was performed in an enzyme membrane reactor (EMR). Effective decolorization (>95 %) was achieved for a period of more than 25 days and nearly 45 % laccase activity could be recovered after 25 days. ABTS recovery from the treated effluent was performed by ammonium sulphate precipitation. The reactor was operated at different hydraulic retention times (HRT) and effect on decolorization achieved, mediator recovery and membrane fouling was studied. With an increase in HRT from 4-16 h, the decolorization varied from 82-95 % and the membrane fouling increased from nil to 42.36 % and ABTS recovery decreased from 72 to 30 %. ***The continuous decolorization in EMR while conserving both enzyme and mediator has been successfully demonstrated for the first time.***

The effectiveness of combination treatments for the decolorization of the real industrial effluent from a textile mill was also assessed. Optimized laccase-ABTS system led to nearly 60 % decolorization whereas coagulation of the dye using alum led to 90 % color removal, but with formation of dye sludge. Thus, neither of the processes alone was found to be very effective for color removal. The combination of both treatments led to >80 % color removal. Laccase-ABTS treatment followed by alum coagulation did not

prove to be very effective for continuous decolorization purpose. The sequence involving alum coagulation first followed by laccase-ABTS treatment in an EMR was found to be better for continuous decolorization of the effluent in terms of retention of laccase activity (55% activity retained after 15 days), ABTS recovery (60%) from the permeate, membrane fouling (Nil after 15 days) and long term operation (> 15 days) of the EMR.

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