

**PhD Thesis Title : Treatment of complex dyes and effluents using engineered Laccase 1 of *Cyathus bulleri***

**Name : Manju**

**Entry Number: 2015BEZ8354**

## **ABSTRACT**

Textile industry accounts for nearly 14% of the total manufacturing output in India and is highly water intensive using 50-100 liters/kg textiles in wet processing. According to a recent World Bank report, 17-20% percent of global water contamination is contributed solely by finishing and coloring procedures. Fungal laccases have high biotechnological potential for bioremediation processes owing to their ability to degrade phenolics, aminophenols, aryldiamines and polyamines. In the present study, the efficiency of engineered Laccase1 isoform (LCC1) of *Cyathus bulleri* is reported in decolorizing and detoxification of Indigo Carmine (IC), effluent containing this dye as well as real effluent from a textile mill. The gene (*LCC1*) encoding wild type (WT) LCC1, was modified through site-directed mutagenesis at predefined positions generating three catalytically superior High-Activity-Variants (HAVs), viz., [LCC1-35; (Gly<sub>463</sub>Arg), LCC1-61; (Ser<sub>318</sub>Thr), LCC1-62; (Ile<sub>490</sub>Met)]. The extracellularly produced laccases in *Pichia pastoris* were partially purified and exhibited 2-9 X times higher decolorization of real effluents than the WTLCC1 even in the absence of a mediator. More than 90% decolorization (~1.6 times higher than that of the WTLCC1) was shown of IC in 18 h by the three HAVs without the addition of any mediator. To increase the rate of decolorization, several natural and synthetic mediators were screened which dramatically increased the rates and reduced the treatment time to 20 min. Maximum increase in the rate (23 X relative to the WTLCC1) was obtained with the HAV LCC1-62 with the natural mediator 2,6-Dimethoxy Phenol (DMP) making it an attractive option for mediator-assisted laccase treatment. Examination of the mediator-laccase variant combination on decolorization of IC at molecular level, through *in-silico* studies, provided evidence that His<sub>132</sub>,

Ser<sub>134</sub>, Leu<sub>475</sub>, and Glu<sub>476</sub> were associated with the hot-spot regions in the active pocket of LCC1. Apart from identifying the binding residues, the binding efficiency, strength of the hydrogen bonds, conformation of the ligand, and size of the active size pocket were identified as important factors contributing to the higher catalytic efficiency of the HAVs. Identified laccase variant, LCC1-62, was very effective in treating real effluent in the membrane reactor in batch as well as continuous mode giving 94% decolorization without any mediator. Our findings strongly suggest the feasibility of application of LCC1-62 at larger scale for textile effluent treatment.