

Determination of the mechanism and optimization of the conditions of the process of removing colored aromatic compounds by selected Basidiomycota

ABSTRACT

Synthetic dyes constitute a major environmental and public health threat due to their extensive industrial use, chemical recalcitrance, and toxicological effects on aquatic ecosystems and human health. Approximately 100,000 tons of dyes are released annually from dye-intensive industries, and in many low- and middle-income countries up to 80% of dye-containing wastewater is discharged untreated. Conventional physicochemical treatment methods are often costly, energy-intensive, and generate secondary pollution. This doctoral research investigates white-rot fungi as a sustainable biological alternative for the remediation of synthetic dye-contaminated wastewater, with particular emphasis on mechanistic understanding, process optimization and toxicity reduction.

Decolorization of dyes representing three major structural classes-azo (Evans Blue(EB), Congo Red(CR)), triphenylmethane (Brilliant Green(BG), Crystal Violet(CV)), and anthraquinone (Remazol Brilliant Blue R (RBBR))-was evaluated using *Trametes versicolor* (CB8) and *Pleurotus ostreatus* (BWPH) strains. Dye removal occurred through biodegradation and biosorption mechanisms. Optimization of operational parameters (pH, temperature, agitation, dye concentration, carbon and nitrogen sources, immobilization carrier, and biomass formulation) revealed that sponge-immobilized *T. versicolor* grown in regular medium (CB8/S2) achieved the highest decolorization efficiencies (~96%) for RBBR, Evans Blue, and Crystal Violet, followed by *T. versicolor* under shaking conditions (~90%). *P. ostreatus* showed moderate efficiencies (82–90%) under comparable conditions. ANOVA analysis confirmed that fungal species, carbon source, and nitrogen source significantly influenced decolorization ($p < 0.05$), with nitrogen source exhibiting a highly significant effect ($F = 226.64$, $p < 0.001$). Ecotoxicological assays using *Daphnia magna* and *Spirodela polyrhiza* demonstrated significant toxicity reduction in treated effluents (from Persoone class IV to III for RBBR dye via both fungal strains). Hence, post treated samples are environmentally safer than pure dye. CB8/S2 produced the highest laccase activity (20 U/L) after 96 h incubation with RBBR. Spearman correlation analysis showed strong but statistically non-significant correlations between laccase activity and RBBR decolorization ($\rho = 0.771$, $p = 0.072$) and between MnP activity and decolorization ($\rho = 0.771$, $p = 0.072$), while LiP activity showed negligible correlation ($\rho = 0.086$, $p = 0.872$), indicating that extracellular enzyme activity alone does not fully explain dye removal efficiency.

Despite extensive research on fungal dye decolorization, the molecular mechanisms underlying dye-specific metabolic adaptation in white rot fungi remain poorly understood, particularly at the systems biology level. In this study, integrated transcriptomic and proteomic analyses were employed to elucidate the global cellular response of *T. versicolor* during the biodegradation of structurally distinct dyes, RBBR and EB. Transcriptomic analysis revealed extensive differential gene expression response, with 1,106 and 1,830 genes differentially expressed ($|\log_2| \geq 2$, $p < 0.05$) in *T. versicolor* during RBBR and Evans Blue degradation, respectively.

Upregulated genes included cytochrome P450 monooxygenases, NAD(P)H-dependent oxidoreductases, keto reductases, and aldehyde dehydrogenases, along with transport-related genes. Concurrent downregulation of genes involved in cell cycle progression, cytoskeletal organization, and DNA replication indicated growth suppression under dye stress. Proteomic analysis corroborated transcriptomic trends, revealing dye-specific metabolic reprogramming, conserved stress responses involving P450s and ABC transporters, and enhanced peroxidase dependence during RBBR degradation. Collectively, this multi-omics approach provides novel mechanistic insights into fungal dye biodegradation, demonstrating that dye removal by white rot fungi involves complex intracellular regulatory networks that have not been previously characterized at this depth.

Biosorption studies demonstrated that sponge-immobilized live biosorbents (CB8/S2-BS) exhibited superior performance for triphenylmethane dyes. CB8/S2-BS achieved maximum sorption capacities of 379.4 mg/g for BG and 48.9 mg/g for CV at 400 mg/L, removing up to 90.3% and 43.9% of dyes within 6 h, which is 3-5 times higher than self-immobilized biosorbents. FT-IR analysis confirmed hydrogen bonding and electrostatic interactions as dominant binding mechanisms, and biosorbents showed effective reusability without additional treatment. Bioreactor-scale studies confirmed operational feasibility, reduced costs, and high treatment efficiency under optimized conditions.

This research delivers integrated mechanistic insights into fungal dye remediation, effectively bridging laboratory-scale findings with industrial applicability. Importantly, this work establishes a scalable framework for incorporating white-rot fungi into wastewater treatment infrastructure while advancing understanding of intracellular detoxification and transporter-mediated processes that extend beyond traditional extracellular enzyme-centric models. The results may support the development of tailored, decentralized fungal bioreactor systems optimized for specific dye classes and contribute directly to the achievement of UN Sustainable Development Goals (SDGs 6, 12, and 14).

Keywords: mycoremediation; white-rot fungi; synthetic dyes; biodegradation; biosorption; transcriptomics; proteomics; ecotoxicology; wastewater treatment; *Trametes versicolor*; *Pleurotus ostreatus*