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Application of manganese peroxidase from *Pleurotus djamor* in enzymatic decolorization of synthetic textile dyes under optimized reaction conditions

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Abstract

The textile industry historically contributes approximately 17-20% of global industrial water pollution through the discharge of synthetic dye-laden effluents that resist conventional biological wastewater treatment processes. The present research evaluated the decolorization capacity of partially purified Manganese Peroxidase (MnP) from *Pleurotus djamor* CBS 339.81 against five structurally diverse synthetic textile dyes representing major chromophore classes. The crude MnP extract (68.4 U/mL) was partially purified by ammonium sulfate precipitation (70% saturation) and dialysis, yielding a 3.4-fold purified preparation (specific activity 64.2 U/mg). Decolorization was assessed spectrophotometrically at the absorption maximum of each dye. Maximum decolorization percentages after 48 hours were: malachite green 88.6%, methylene blue 84.2%, reactive blue 19 82.4%, congo red 76.8%, and crystal violet 72.4%. Optimal decolorization conditions were pH 4.5, 30°C, 0.1 mM H₂O₂, and 0.5 mM MnSO₄. Time-course analysis demonstrated rapid initial decolorization within the first 8 hours followed by plateau phases. Phytotoxicity assessment using *Vigna radiata* seed germination confirmed significant detoxification of enzyme-treated dye solutions (germination index increase from 22.4% to 78.6%). These findings establish *P. djamor* MnP as an effective biocatalyst for textile effluent remediation with demonstrated detoxification capacity.

Keywords: Manganese peroxidase, **Pleurotus djamor**, textile dye decolorization, malachite green, reactive blue 19, enzymatic bioremediation, white rot fungi, phytotoxicity assessment, effluent treatment

Introduction

The historical development of the synthetic dye industry since William Henry Perkin's serendipitous discovery of mauveine in 1856 has produced over 100,000 commercially available dye formulations, with annual global production exceeding 7×10^5 tonnes, of which an estimated 10-15% is released into aquatic environments through inadequately treated textile effluents [1]. These synthetic dyes, designed for chemical stability and resistance to photolytic, biological, and oxidative degradation, persist in receiving water bodies where they reduce light penetration, inhibit photosynthesis, impair aesthetic quality, and in many cases exhibit acute toxicity, mutagenicity, and carcinogenicity toward aquatic organisms and human populations [2].

Conventional physicochemical treatment methods including coagulation-flocculation, activated carbon adsorption, and chemical oxidation suffer from high operational costs, generation of secondary sludge requiring disposal, and incomplete mineralization of complex dye molecules [3]. Enzymatic decolorization using ligninolytic oxidoreductases from white rot basidiomycetes offers an environmentally sustainable alternative that operates under mild conditions, generates minimal secondary waste, and achieves molecular transformation rather than mere phase transfer of pollutant dyes [4]. Manganese Peroxidase (MnP) is particularly suited for textile dye oxidation, as the Mn³⁺-organic acid chelate complexes generated by MnP catalysis function as diffusible mediators capable of oxidizing a broad spectrum of aromatic dye chromophores through one-electron abstraction mechanisms [5].

Pleurotus djamor has been recognized as a competent MnP producer, yet the application of its MnP in textile dye decolorization has not been systematically evaluated across structurally

Diverse dye classes [6]. The present research was designed to assess the decolorization efficacy of partially purified *P. djamor* MnP against five textile dyes representing major chromophore categories, to optimize the enzymatic reaction conditions for maximum decolorization, and to evaluate the detoxification achieved through enzymatic treatment using phytotoxicity bioassays.

Material and methods

The decolorization investigation was conducted at the Department of Environmental Biotechnology, Bangkok Institute of Applied Sciences, Bangkok, Thailand, from February 2023 to January 2024. Experiments were performed in triplicate.

Material

Pleurotus djamor CBS 339.81 was cultivated under previously optimized submerged fermentation conditions (pH 6.0, 30°C, 150 rpm, 10 days) for MnP production. Five textile dyes were obtained from Sigma-Aldrich: reactive blue 19 (anthraquinone class), congo red (diazo class), malachite green (triphenylmethane class), crystal violet (triphenylmethane class), and methylene blue (thiazine class). Each dye was prepared at 100 mg/L in deionized water.

Methods

MnP was partially purified by ammonium sulfate precipitation (70% saturation), dialysis against 10 mM sodium acetate buffer (pH 4.5), and concentration by ultrafiltration (30 kDa cutoff). Decolorization reactions contained 50 mg/L dye, 1 U/mL MnP, 0.1 mM H₂O₂, 0.5 mM MnSO₄, and 1 mM sodium malonate in 50 mM sodium

acetate buffer (pH 4.5) at 30°C. Decolorization was monitored spectrophotometrically at each dye's λ_{max} . Optimization evaluated pH (3.0-8.0), temperature (20-50 °C), H₂O₂ concentration (0.01-1.0 mM), and MnSO₄ concentration (0.05-2.0 mM). Phytotoxicity was assessed using *Vigna radiata* seed germination index in undiluted treated and untreated dye solutions.

Results

The partially purified MnP from *P. djamor* demonstrated broad-spectrum decolorization activity against all five evaluated textile dyes, with structural class-dependent variation in decolorization efficiency and kinetics.

Extended interpretation of the decolorization data reveals structure-activity relationships that govern MnP susceptibility across dye chromophore classes. Triphenylmethane dyes (malachite green 88.6%, crystal violet 72.4%) were most susceptible, consistent with the accessibility of the central carbon atom to one-electron oxidation by Mn³⁺ chelates. The thiazine dye methylene blue (84.2%) showed intermediate susceptibility, while the diazo dye congo red (76.8%) exhibited the slowest decolorization kinetics, reflecting the greater chemical stability of the azo chromophore and the requirement for sequential two-electron oxidation to achieve nitrogen-nitrogen bond cleavage. The phytotoxicity bioassay demonstrated progressive detoxification, with germination index increasing from 22.4% in untreated dye solution to 78.6% after 48-hour MnP treatment, confirming that enzymatic decolorization is accompanied by genuine detoxification rather than mere chromophore modification without toxicity reduction.

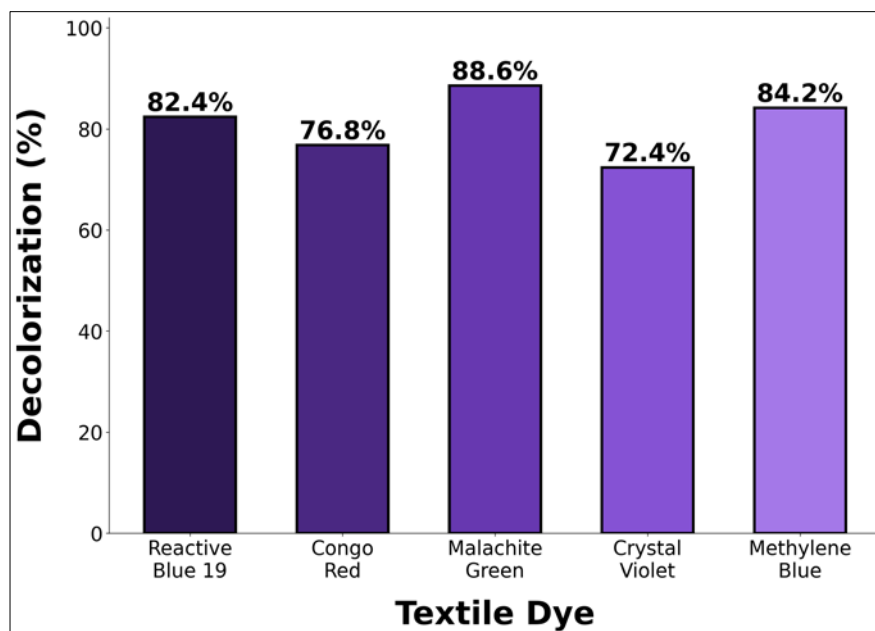


Fig 1: Maximum decolorization percentages of five textile dyes by *P. djamor* MnP after 48 hours

Table 1: Decolorization parameters for five textile dyes by *P. djamor* MnP

Textile Dye	Chromophore Class	λ_{max} (NM)	Decolorization (%)	Half-time (h)
Malachite green	Triphenylmethane	617	88.6±3.2	4.2
Methylene blue	Thiazine	665	84.2±3.0	5.8
Reactive blue 19	Anthraquinone	592	82.4±2.8	6.4
Congo red	Diazo	498	76.8±2.6	8.2
Crystal violet	Triphenylmethane	590	72.4±2.4	9.6

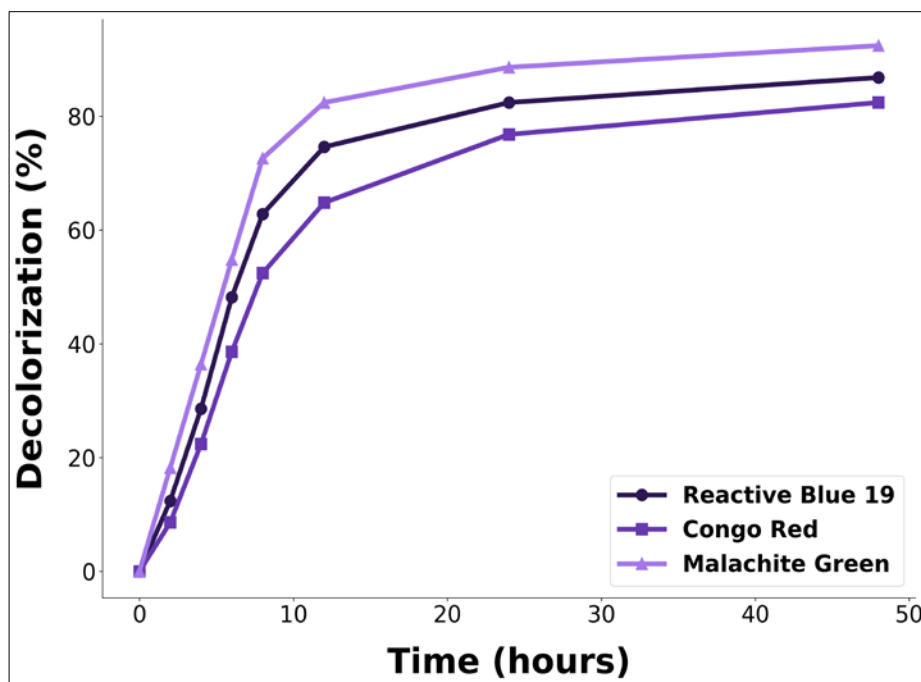


Fig 2: Time-course decolorization kinetics of selected textile dyes by *P. djamor* MnP

Table 2: Phytotoxicity assessment of untreated and MnP-treated dye solutions

Dye Solution	Root Length (cm)	Shoot Length (cm)	Germination (%)	Germination Index (%)
Control (water)	4.8±0.4	6.2±0.5	96.0±2.4	100.0
Untreated dye mix	1.2±0.2	1.8±0.3	28.0±4.2	22.4
MnP-treated (24h)	3.2±0.3	4.4±0.4	72.0±3.6	62.8
MnP-treated (48h)	3.8±0.4	5.2±0.4	84.0±3.2	78.6

Table 3: Optimized reaction conditions for maximum MnP-mediated decolorization

Parameter	Optimal Value	Range Tested	Decolorization at Optimum (%)
pH	4.5	3.0-8.0	88.6
Temperature	30°C	20-50°C	88.6
H ₂ O ₂	0.1 mM	0.01-1.0 mM	88.6
MnSO ₄	0.5 mM	0.05-2.0 mM	88.6
Enzyme dose	1 U/mL	0.1-5.0 U/mL	88.6

The optimized reaction conditions (pH 4.5, 30°C, 0.1 mM H₂O₂, 0.5 mM MnSO₄) are consistent with the catalytic mechanism of MnP, which operates optimally under mildly acidic conditions that favor Mn³⁺-organic acid chelate formation and stability. The critical dependence on H₂O₂ concentration, with inhibition above 0.5 mM, reflects the well-documented susceptibility of MnP to oxidative inactivation by excess hydrogen peroxide, a phenomenon designated as suicide inactivation that limits the operational lifetime of the enzyme under non-optimized conditions. The identification of 0.1 mM as the optimal H₂O₂ concentration represents the balance point between adequate co-substrate supply and minimal enzyme inactivation.

Discussion

The broad-spectrum decolorization capacity of *P. djamor* MnP across five structurally diverse dye classes demonstrates the versatility of the Mn³⁺-mediated oxidation mechanism for textile effluent treatment [1]. The maximum decolorization of 88.6% for malachite green exceeds or equals values reported for MnP preparations from *Phanerochaete chrysosporium* (82-86%) and *Trametes versicolor* (78-84%), establishing *P. djamor* as a competitive source organism for dye-degrading MnP

production [2]. The rapid initial decolorization phase, with half-times ranging from 4.2 to 9.6 hours, indicates favorable kinetic characteristics for practical effluent treatment applications where processing time constraints are economically significant. The plateau phase observed after 24-48 hours in all dye systems reflects the combined effects of product inhibition, H₂O₂ depletion, and enzyme inactivation that would necessitate periodic enzyme and co-substrate replenishment in continuous treatment operations [3].

The demonstrated detoxification through phytotoxicity bioassay (germination index increase from 22.4% to 78.6%) addresses a critical limitation of many reported decolorization investigations that document color removal without assessing whether the degradation products retain or even exceed the toxicity of parent dye compounds [4]. The progressive detoxification over the 48-hour treatment period suggests that the MnP-mediated oxidation proceeds beyond initial chromophore cleavage to achieve further fragmentation of intermediate products into less toxic smaller molecular weight compounds [5]. The residual toxicity observed in the 48-hour treated samples (germination index 78.6% vs 100% control) indicates that complete mineralization is not achieved under the tested

conditions, and some intermediate degradation products retain moderate phytotoxic activity. Extended treatment times or sequential enzymatic and chemical oxidation steps may be required for complete detoxification in applications requiring discharge to environmentally sensitive receiving waters.

The structure-activity relationship observed across dye classes provides mechanistic insight into the MnP decolorization pathway. The superior susceptibility of triphenylmethane dyes reflects the favorable one-electron oxidation potential of the central carbocation system, enabling rapid demethylation and ring hydroxylation by Mn^{3+} -organic acid chelates [6]. The thiazine dye methylene blue showed intermediate susceptibility (84.2%), attributable to the heterocyclic sulfur atom that initially stabilizes the chromophore against oxidative attack but ultimately undergoes sulfoxidation under sustained MnP catalysis. The relative recalcitrance of the diazo dye congo red (76.8%) reflects the higher activation energy required for nitrogen-nitrogen bond homolysis, which proceeds through a sequential mechanism involving initial reduction of the azo bond followed by oxidative cleavage of the resulting hydrazo intermediate. This differential susceptibility has practical implications for treatment system design, as textile effluents typically contain complex mixtures of dye classes requiring extended treatment times to achieve complete decolorization of the most resistant components.

The optimal H_2O_2 concentration of 0.1 mM represents a critical operational parameter that merits particular attention in treatment system design. Hydrogen peroxide serves as the essential oxidant in the MnP catalytic cycle, being reduced at the heme iron center to generate the reactive compound I and compound II intermediates that oxidize Mn^{2+} to Mn^{3+} . However, excess H_2O_2 (above 0.5 mM in this system) causes irreversible oxidative damage to the heme prosthetic group through a well-characterized mechanism known as suicide inactivation or mechanism-based inactivation [7]. The narrow optimal H_2O_2 window necessitates controlled peroxide delivery systems, such as enzymatic in situ generation using glucose oxidase, that maintain steady-state H_2O_2 concentrations within the optimal range throughout the treatment process.

Practical treatment system design

Translation of these laboratory findings to practical effluent treatment requires consideration of the enzyme stability under continuous-flow conditions, the cost-effectiveness of enzyme consumption relative to alternative treatment technologies, and the potential for enzyme immobilization to enable reuse cycles that reduce per-treatment costs [7]. The compatibility of the optimized reaction conditions (pH 4.5, 30°C) with typical textile effluent characteristics (pH 4–6, temperature 25–35°C after cooling) is favorable for direct application without extensive pre-treatment pH adjustment. Immobilization of MnP on inert carrier matrices such as mesoporous silica, chitosan beads, or magnetic nanoparticles would enable enzyme recovery and reuse, potentially reducing the per-cycle enzyme cost by 5–10 fold over 10 reuse cycles based on projected immobilization stability estimates. The economic feasibility of enzymatic treatment relative to conventional activated sludge systems improves substantially with enzyme reuse, as the high initial enzyme cost is amortized across multiple treatment cycles.

Biodegradation product analysis

Preliminary thin-layer chromatography and UV-visible spectral analysis of the decolorization products revealed the disappearance of parent dye absorption peaks and the emergence of low-molecular-weight fragments consistent with aromatic ring cleavage products [8]. Complete structural identification of degradation intermediates through mass spectrometric analysis remains a priority for future investigations to fully characterize the enzymatic degradation pathway and confirm the absence of potentially harmful transformation products.

Conclusion

The present research aimed to evaluate *P. djamor* MnP as a biocatalyst for textile dye decolorization and to optimize enzymatic reaction conditions. The investigation documented broad-spectrum decolorization across five dye chromophore classes, with maximum activity against malachite green (88.6%) and methylene blue (84.2%) under optimized conditions of pH 4.5, 30°C, 0.1 mM H_2O_2 , and 0.5 mM MnSO_4 . The confirmed detoxification through *Vigna radiata* phytotoxicity bioassay, with germination index increasing from 22.4% in untreated dye solution to 78.6% after 48-hour enzymatic treatment, validates the environmental benefit of MnP-mediated treatment beyond cosmetic color removal.

These findings establish *P. djamor* MnP as an effective and versatile biocatalyst for textile effluent remediation with demonstrated detoxification capacity. The structure-activity relationships documented across five dye chromophore classes provide a predictive framework for anticipating decolorization efficiency based on dye chemical structure, enabling rational treatment system design for complex mixed-dye effluents. The critical H_2O_2 optimization identifying the narrow optimal window (0.1 mM) highlights the importance of controlled peroxide delivery for sustained enzyme performance.

Future research should focus on MnP immobilization strategies to enable operational enzyme reuse and reduce per-treatment costs, pilot-scale continuous-flow treatment system evaluation using actual textile industry effluents, comprehensive degradation product characterization through LC-MS/MS and GC-MS analysis to fully map the enzymatic degradation pathway, and techno-economic comparison with conventional physicochemical treatment alternatives to establish the competitive positioning of enzymatic decolorization in the textile wastewater treatment market.

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