A Perspective of Laccase-Metal Organic Framework Biocomposites for Potential Bioremediation of Dyes

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Abstract. The persistence of synthetic dyes in aquatic systems, primarily originating from textile and industrial effluents, poses serious risks to ecosystems and human health due to their structural stability, toxicity, and resistance to conventional treatments. Enzyme-based biocatalysis has emerged as a sustainable strategy for dye degradation because of its selectivity and ability to operate under mild conditions without generating harmful by-products. However, the practical application of free enzymes is restricted by poor stability, sensitivity to operational environments, and limited recyclability. To address these challenges, metal-organic frameworks (MOFs) have been widely investigated as protective and functional supports for enzyme immobilization. Their high porosity, tunable surface chemistry, and structural versatility allow for enhanced enzyme stability, activity retention, and reusability, while simultaneously contributing to adsorption-driven pollutant removal. Notably, biocomposites such as laccase@ZIF-8 and HRP@UiO-66-NH_2 have demonstrated superior catalytic performance in degrading azo and aromatic dyes, even under harsh effluent conditions. Moreover, the adsorption of green synthesis methods which include aqueous routes, biomimetic mineralization, and mechanochemical fabrication has further improved the environmental compatibility and scalability of enzyme-MOF (E-MOF) biocomposites. These advances highlight the dual potential of E-MOFs to couple biocatalysis with adsorption within a sustainable framework. Overall, green-synthesized E-MOFs represent a promising platform for efficient dye remediation and hold strong potential for translation from laboratory studies to industrial wastewater treatment applications.

1 Introduction

The rapid expansion of the textile and allied industries has led to the extensive release of synthetic dyes into aquatic environments, posing severe ecological and health challenges. Textile dye effluents are characterized by their intense coloration, high chemical stability,

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and complex aromatic structures that render them resistant to conventional biological degradation [1,2]. These effluents often contain a wide variety of dye classes, including azo, anthraquinone, triphenylmethane, and indigoid compounds, which persist in water bodies for long periods and disrupt aquatic ecosystems by reducing light penetration and oxygen solubility [3,4]. Beyond their aesthetic and ecological impacts, many synthetic dyes and their degradation products are known to be toxic, mutagenic, and carcinogenic, thereby representing a pressing environmental concern when discharged untreated.

A number of physicochemical approaches have been developed for dye removal, such as adsorption, advanced oxidation processes (AOPs), coagulation–flocculation, and membrane filtration. Adsorption using activated carbon or biochar can effectively remove dyes but suffers from high costs for some absorbances the generation of secondary waste streams [5]. AOPs, which involve hydroxyl radical generation through Fenton's reagent, ozonation, or photocatalysis, are highly efficient but energy-intensive and produce by-products [6]. Membrane-based processes provide high removal efficiency, yet they face challenges of fouling, high operational costs, and limited selectivity for structurally diverse dyes [7]. While these technologies are effective under controlled conditions, their limitations hinder sustainable large-scale application, motivating the search for alternative eco-friendly solutions.

Biocatalysis has emerged as a promising approach for dye degradation, particularly through the use of oxidoreductases such as laccases, peroxidases, and tyrosinases. These enzymes catalyze the oxidation of phenolic and non-phenolic compounds, enabling the breakdown of complex aromatic dye structures under mild conditions without generating hazardous by-products [5]. Laccase, in particular, has attracted attention due to its broad substrate specificity and ability to utilize molecular oxygen as the final electron acceptor, yielding water as the sole by-product. However, despite their catalytic versatility, free enzymes are intrinsically unstable under harsh environmental conditions, prone to denaturation at extremes of pH and temperature, and susceptible to proteolytic degradation, thereby limiting their reusability and practical implementation [8].

To overcome these limitations, enzyme immobilization has been extensively explored to improve stability, activity retention, and recyclability. Among the various immobilization matrices, metal–organic frameworks (MOFs) have recently emerged as attractive candidates due to their exceptionally high surface area, tunable porosity, and diverse chemical functionalities [9,10]. MOFs not only provide a protective microenvironment for enzymes but also offer synergistic adsorption–catalysis benefits in dye degradation. Furthermore, recent advances in green synthesis routes, including aqueous-based and biomimetic mineralization strategies, have improved the environmental sustainability of MOF fabrication, aligning with the global push toward circular economy practices [11].

Furthermore, recent advances in green synthesis routes have substantially improved the sustainability profile of MOFs, making them more compatible with biotechnological and environmental applications. Conventional MOF synthesis often relies on toxic organic solvents such as dimethylformamide (DMF) or dimethylacetamide (DMAc), high temperatures, and long reaction times, all of which raise environmental, economic, and safety concerns [11]. In contrast, greener methods emphasize the use of water as a benign solvent, low-temperature conditions, and scalable processes that minimize energy input and chemical waste. For example, aqueous-based synthesis of zeolitic imidazolate frameworks (ZIF-8, ZIF-67) enables rapid crystallization at room temperature, while avoiding hazardous solvent residues that could compromise enzyme activity [12–14].

One of the most notable developments is biomimetic mineralization, a strategy in which biomolecules such as enzymes, proteins, or nucleic acids act as nucleation centers for MOF growth. This process allows the direct encapsulation of delicate oxidoreductases, including laccase and peroxidase, within a protective crystalline matrix under mild aqueous conditions

[15]. The biomimetic approach not only enhances enzyme stability but also ensures high encapsulation efficiency and activity retention, offering a practical route for producing enzyme–MOF biocomposites at scale. In addition, mechanochemical synthesis, which uses ball milling or grinding with minimal solvent, has emerged as another green strategy that reduces reaction time and energy demand, while producing MOFs with sufficient crystallinity for catalytic and immobilization applications [16].

Green synthetic methods also facilitate the integration of renewable or bio-derived linkers and benign metal salts, further reducing environmental impact. For example, the substitution of petroleum-derived imidazoles with bio-based organic linkers such as caffeic acid, gallic acid, or amino acids has been reported to yield functional MOFs with improved biocompatibility and catalytic properties [17]. Such approaches not only align with the circular economy principles by valorizing renewable feedstocks but also reduce toxicity risks associated with residual MOF components in treated water streams.

The environmental compatibility of these green synthesis methods is particularly critical when MOFs are used for wastewater treatment, since toxic by-products from the support material would undermine their sustainability benefits. By adopting aqueous, biomimetic, and solvent-free routes, researchers are now able to fabricate E-MOF biocomposites that are safer, more reproducible, and industrially scalable. These advancements significantly enhance the feasibility of deploying oxidoreductase-MOF biocomposites for dye remediation, bridging laboratory studies with real-world applications in textile effluent management and clean water production.

In this context, this mini-review aims to critically examine the potential of oxidoreductase-MOF biocomposites for dye bioremediation. By highlighting current fabrication strategies, mechanistic synergies between adsorption and enzymatic catalysis, and key challenges in stability and scalability, this review seeks to identify research directions toward the sustainable deployment of enzyme–MOF systems in wastewater treatment.

2 Mechanisms of Laccase in Dye Degradation

Synthetic dyes (Fig.1) are widely employed in industries such as textiles, leather, cosmetics, paper, and food, with global annual production estimated at over 700,000 tonnes. During textile dyeing and finishing, approximately 10–20% of dyes are discharged into wastewater, leading to persistent contamination of aquatic systems [18]. The most prevalent classes of dyes in industrial effluents include azo dyes (–N=N– linkages), anthraquinone dyes, triphenylmethane dyes, and indigoid dyes [19]. Wastewater from textile facilities often contains dyes at concentrations ranging from several milligrams per litre to over 200 mg L^{-1}, which significantly reduces light penetration, interferes with photosynthetic activity, and alters aquatic ecosystem balance [3].

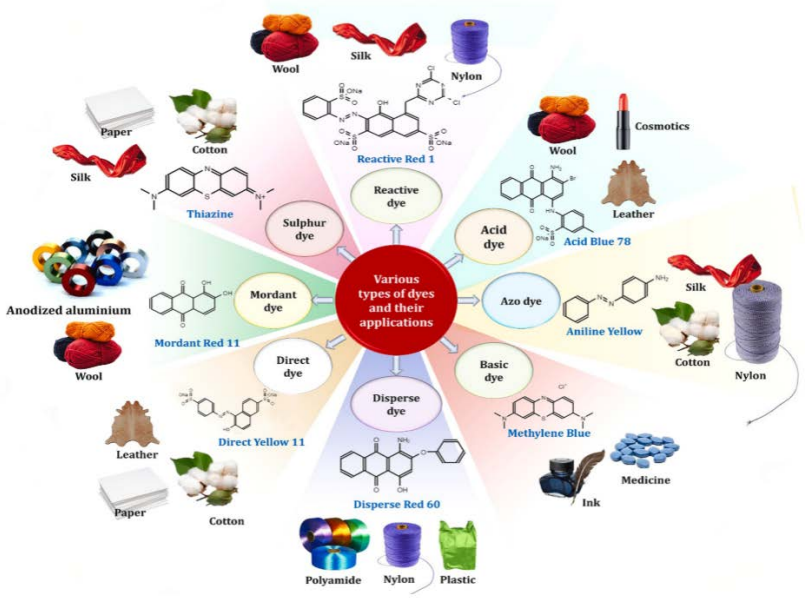

Fig. 1. Type and Chemical Structure of Common Dyes [3]

The toxicological implications of dye-contaminated water are considerable, particularly when effluents are discharged without adequate treatment. Many dyes or their degradation products are known to be mutagenic and carcinogenic, even at trace levels [20]. For instance, azo dyes can undergo reductive cleavage in the human gastrointestinal tract, generating aromatic amines such as benzidine, which are strongly associated with bladder cancer [21]. Triphenylmethane dyes, including Crystal Violet and Malachite Green, have been reported to cause organ toxicity, mutagenicity, and reproductive disorders [20]. Chronic exposure to such compounds through contaminated drinking water may result in liver and kidney dysfunctions, immune system suppression, and long-term carcinogenic effects [3].

The chemical stability of synthetic dyes derives from their highly conjugated aromatic ring systems and functional substituents such as sulfonate (–SO_3H), nitro (–NO_2), amino (–NH_2), and halogen groups, which confer high solubility and resistance to biodegradation [22,23]. Azo dyes are stabilized by azo bonds conjugated with aromatic rings, while anthraquinone dyes possess carbonyl groups that resist chemical attack. These structural features render dyes recalcitrant to conventional treatment processes. Laccase (EC 1.10.3.2), a blue multicopper oxidase, is particularly attractive for dye bioremediation due to its capacity to catalyze one-electron oxidation of phenolic and non-phenolic aromatic compounds, concomitantly reducing molecular oxygen to water [24,25]. By targeting the aromatic moieties and substituent groups of dyes, laccase is able to initiate oxidative degradation of recalcitrant structures.

The mechanism of laccase-mediated dye degradation involves oxidation of electron-rich functional groups such as phenolic hydroxyls or aromatic amines, leading to the formation of phenoxy or amine radicals [25,26]. These radicals undergo subsequent non-enzymatic reactions, including rearrangements, cleavage of azo linkages, and polymerization or breakdown of chromophoric groups, ultimately resulting in decolorization and detoxification [25,26]. In many cases, laccase requires the presence of redox mediators (e.g., 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [ABTS], 1-hydroxybenzotriazole [HBT], or natural mediators such as syringaldehyde), which extend the enzyme's substrate range by

generating diffusible radical species capable of oxidizing otherwise inaccessible dye structures [27,28]. The combined adsorption of dyes on support matrices and the catalytic oxidation by laccase offer a sustainable approach to degrading complex synthetic dyes and mitigating their environmental impact.

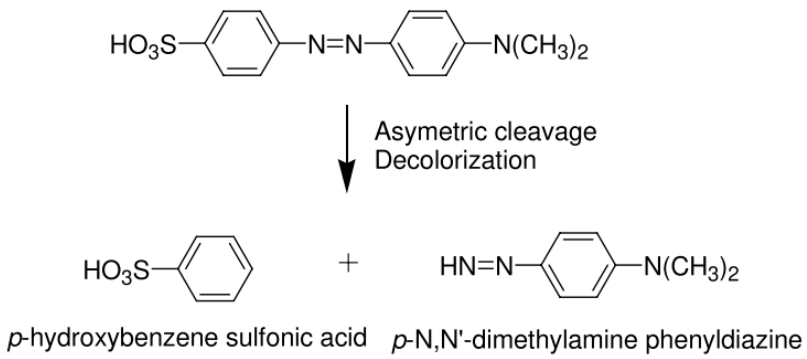

Fig. 2. Pathway of Azo Methyl Orange Dye degradation by Laccase [23]

3 Metal-Organic Frameworks as Immobilization Platform

MOFs have emerged as highly versatile platforms for enzyme immobilization due to their unique structural and chemical attributes. MOFs are crystalline porous materials constructed from the coordination of metal ions or clusters with organic linkers, giving rise to an extensive library of structures with tunable pore sizes, topologies, and surface chemistries [29,30]. Their exceptionally high surface areas, often exceeding 1000 $m^2\ g^{-1}$, coupled with ordered porosity and adjustable functional groups, provide an ideal microenvironment for hosting biomolecules. Compared to conventional immobilization supports such as silica, polymers, or activated carbon, MOFs offer superior structural regularity, uniform pore distribution, and the ability to tailor physicochemical properties through judicious choice of metal nodes and linkers [29]. These attributes enable not only efficient enzyme loading but also improved catalytic performance and stability, critical for applications in dye bioremediation.

Several classes of MOFs have been explored for enzyme immobilization in dye degradation contexts, with zeolitic imidazolate frameworks (ZIFs) being among the most widely studied. ZIF-8, composed of Zn^{2+} and 2-methylimidazole, and ZIF-67, based on Co^{2+} with the same linker, possess sodalite-type topologies with high chemical stability under neutral to basic conditions [31]. These frameworks have demonstrated excellent performance for immobilizing oxidoreductases such as laccase and horseradish peroxidase, where the microporous architecture enhances dye adsorption while simultaneously protecting enzymes from denaturation. Other notable frameworks include MIL-101 (Cr^{3+}-terephthalate), characterized by exceptionally large cages and mesoporosity, and UiO-66 (Zr^{4+}-terephthalate), which exhibits outstanding hydrothermal and chemical stability [32,33]. These systems highlight the diversity of MOFs available to accommodate enzymes of different sizes and structural sensitivities, thereby expanding their applicability in wastewater treatment.

Two principal strategies have been widely adopted for integrating enzymes into MOFs: encapsulation and surface immobilization, each with distinct advantages and limitations. Encapsulation involves the physical entrapment of enzymes within the MOF's crystalline network during in situ synthesis. The most common route is biomimetic mineralization,

wherein the enzyme itself acts as a nucleation centre, inducing the growth of MOF crystals around its surface under mild aqueous conditions [15]. This strategy offers several advantages: enzymes are shielded from denaturing agents such as extreme pH, high ionic strength, or proteases, while the rigid MOF lattice provides mechanical protection and reduces leaching during repeated catalytic cycles [34,35]. However, encapsulation is not without drawbacks. The dense crystalline packing of MOFs may lead to mass-transfer limitations, where large dye molecules (e.g., anthraquinones or triphenylmethanes) diffuse slowly through the microporous network, thereby restricting access to enzyme active sites. Strategies such as engineering mesoporous MOFs (e.g., MIL-101, mesoporous ZIFs) or introducing hierarchical porosity have been proposed to overcome these transport barriers [36].

Surface immobilization, on the other hand, refers to the attachment of enzymes onto the outer surfaces or accessible mesoporous domains of pre-formed MOFs. Immobilization can be achieved through a variety of interactions, including covalent bonding (e.g., via carbodiimide or glutaraldehyde linkers), electrostatic attraction between charged enzyme residues and functionalized MOF surfaces, or simple adsorption [37,38]. This approach generally enhances substrate accessibility, as the enzyme's active site remains directly exposed to the surrounding medium, which is particularly advantageous for the degradation of bulky dye molecules. For example, horseradish peroxidase immobilized on UiO-66-NH_2 exhibited faster catalytic degradation of azo dyes due to unobstructed access to the active heme center [39]. Nevertheless, surface-immobilized enzymes are often more vulnerable to deactivation, as they remain exposed to fluctuating environmental conditions and may detach during repeated use, especially under high ionic strength wastewater conditions.

The choice between encapsulation and surface immobilization thus requires careful consideration of the intended application. Encapsulation prioritizes enzyme stability and reusability, making it attractive for harsh industrial effluents where enzymes are prone to denaturation. Surface immobilization, by contrast, emphasizes catalytic efficiency and rapid turnover of large substrates, making it suitable for scenarios where fast decolorization is required but moderate enzyme loss can be tolerated. Increasingly, researchers are investigating hybrid strategies that combine both approaches. For example, pre-adsorbing enzymes onto MOF surfaces followed by partial encapsulation to strike a balance between stability and accessibility [40,41]. Such integrative approaches are expected to advance the practical deployment of oxidoreductase-MOF composites in real wastewater treatment plants, where effluent composition, pH, and flow dynamics vary widely.

Taken together, the unique structural advantages of MOFs, the availability of diverse frameworks, and the adaptability of immobilization strategies underscore their growing significance as platforms for oxidoreductase immobilization. When combined with eco-friendly synthesis methods, MOF-enzyme biocomposites represent a promising and sustainable solution for the bioremediation of synthetic dyes in industrial wastewater streams.

4 Green Fabrication of Enzyme-MOF Biocomposites

The integration of enzymes into MOFs has emerged as a promising strategy to develop robust and efficient biocatalytic systems for environmental and industrial applications. Traditionally, enzyme immobilization methods often rely on harsh chemical cross-linkers or organic solvents, which may compromise the structural integrity and catalytic performance of enzymes [11]. In contrast, green fabrication approaches emphasize the use of environmentally benign synthesis routes, minimal solvent consumption, and energy-efficient techniques, thereby aligning with the principles of green chemistry [42,43].

Among these approaches, mechanochemical synthesis and room-temperature self-assembly have gained attention as sustainable methods for constructing E-MOF

biocomposites. Mechanochemical synthesis, involving ball milling or grinding with little to no solvent, has been reported to significantly reduce reaction time and energy input while still producing crystalline MOFs suitable for enzyme encapsulation [16]. This not only minimizes environmental impact but also enhances the scalability of biocomposite production [44,45]. Similarly, aqueous-based synthesis provides a biocompatible medium for enzyme incorporation, ensuring that enzyme activity is preserved during the encapsulation process. Such methods eliminate the need for toxic organic solvents, which can denature proteins and generate hazardous waste [46].

Another green route for E-MOF fabrication is biomimetic mineralization, wherein the enzyme itself acts as a nucleation center for MOF growth under mild conditions [35]. This strategy allows the enzyme to be encapsulated in situ, forming protective MOF shells that improve enzyme stability against denaturation, pH fluctuations, and thermal stress [44]. Importantly, the mild aqueous conditions involved in biomimetic mineralization make this method highly compatible with delicate enzymes such as laccase, which is increasingly studied for the degradation of micropollutants in water systems [47].

The adoption of green fabrication methods for E-MOF biocomposites not only enhances the functional stability and reusability of enzymes but also ensures the sustainability of the overall process. Such approaches are particularly relevant in environmental remediation, where the dual goals of pollutant removal and eco-friendly material production must be met. Future efforts should focus on optimizing these green strategies to achieve large-scale, cost-effective fabrication, thereby accelerating the translation of E-MOF composites into practical applications for wastewater treatment, biosensing, and green catalysis.

Table 1. List of E-MOF and its Application.

E-MOF	Immobilization method	Application	Performance (%)	Reference
LAC@Cu-ZIF-90	In-situ encapsulation	Deep Green reduction	90.28	[48]
HRP@ZIF-8	In-situ encapsulation	BPA removal	98	[49]
Laccase on Fe_3O_4-NH_2@MIL-101(Cr)	Adsorption	Removal of 2,4-dichlorophenol	87	[50]
MIL-68(Al)/PVA/Lac	Chemical linking	Alizarin Green decolorization	95.86	[51]
OpdA@MIL-88A	Adsorption	Removal of organophosphorus	89.26	[52]
HRP/GOx@NZIF-8	In-situ encapsulation	BPA removal	80	[53]
CPA/PVP/ZIF-8	In-situ encapsulation	Ochratoxin A degradation	71.3	[54]

5 Future Perspectives

The green synthesis of E-MOF biocomposites represents a rapidly advancing frontier for sustainable wastewater treatment, particularly in addressing the persistent challenge of dye pollutants. While significant progress has been made in developing E-MOFs with enhanced stability, catalytic efficiency, and recyclability, several future directions must be pursued to fully realize their potential in practical remediation systems.

First, the development of E-MOFs with multifunctional architectures could accelerate dye degradation through synergistic mechanisms. For example, combining oxidative enzymes such as laccase or peroxidase with photoactive or redox-active MOFs can enhance electron transfer processes, leading to more efficient breakdown of complex dye molecules [55]. Such hybrid systems could broaden the spectrum of degradable dyes, particularly azo and anthraquinone types, which are known for their high stability and toxicity.

Second, future work should emphasize long-term stability and reusability under real wastewater conditions**,** which often involve fluctuating pH, high ionic strength, and competing organic pollutants. While green-fabricated E-MOFs have demonstrated improved tolerance compared to free enzymes, strategies such as linker modification, hierarchical porosity design, and composite integration with membranes or biochar supports may further enhance their robustness in continuous-flow treatment systems.

Finally, the translation of laboratory studies to pilot-scale and field applications remains an urgent priority [43]. Collaborative efforts between materials scientists, environmental engineers, and industry stakeholders are essential to design modular, low-energy bioreactors incorporating green-synthesized E-MOFs. Such systems could enable efficient, selective, and eco-friendly dye degradation, contributing to global efforts to achieve cleaner water and sustainable industrial practices.

In conclusion, green synthesis of E-MOFs offers a transformative pathway for dye remediation, balancing high catalytic performance with ecological responsibility. Continued innovation in synthesis strategies, structural design, and system integration will determine their successful transition from promising laboratory materials to real-world water treatment technologies.

6 Conclusion

The green synthesis of E-MOF biocomposites offers a sustainable and highly effective pathway for textile dye remediation, overcoming the limitations of conventional treatment methods and unstable free enzymes. By integrating oxidoreductases such as laccase and peroxidase within MOFs, these systems combine catalytic activity with adsorption capacity, leading to improved stability, recyclability, and efficiency under harsh wastewater conditions. Recent advances in aqueous, biomimetic, and mechanochemical fabrication further enhance their environmental compatibility and scalability, aligning with green chemistry principles. With continued innovation in structural design and system integration, green-fabricated enzyme-MOFs hold strong potential to transition from laboratory studies to real-world applications in industrial effluent management and clean water production.

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