Exploring the Potential Application of Genetically Engineered Laccases for the Bioremediation of Heavy Metals

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Toxic heavy metals in industrial wastewater present immediate environmental and health risks, urging scientists to explore different remediation techniques (the process of purifying pollutants from a medium). Conventional remediation methods are often costly, inefficient, and produce secondary waste in the form of sludge. In contrast, bioremediation via laccases—enzymes naturally found in fungi, plants, and bacteria—is an eco-friendly alternative that removes heavy metals through various mechanisms. However, laccases suffer from limited stability under industrial conditions, narrow optimal pH and temperature ranges, and low natural yields that prevent their implementation in industrial scales. Scientists have developed genetic engineering techniques like directed evolution, site-directed mutagenesis, and recombinant expression to combat these shortcomings. Yet, full industrial adoption is still limited by key barriers, including high large-scale production costs, lack of long-term performance data under continuous-flow industrial conditions, and incomplete analyses of economic feasibility[1]. This review discusses the structural and functional properties of laccases, their mechanism for heavy metal remediation, and recent genetic engineering advancements. Additionally, it assesses scalability for industrial use and outlines future research directions needed to translate these technologies to an industrial scale.

Introduction

Industrial operations - including mining, electroplating, and textile manufacturing - regularly discharge wastewater containing toxic heavy metals into aquatic systems. Industrial wastewater is defined as effluent discharged from manufacturing and processing facilities, with composition, pH, metal speciation, and contaminant concentrations varying by industrial sector. Mining wastewater typically contains substantial concentrations of arsenic, lead, and copper at highly acidic pH, often exceeding 400mg/L of total dissolved metals[2]. Electroplating effluents are characterized by hexavalent chromium Cr(VI), nickel and zinc, with chromium concentrations frequently reaching 200mg/L[3]. Similarly, tannery operations produce acidic, chromium-laden effluents (600mg/L total Cr from a tannery in Sialkot) from leather tanning processes[4]. This review examines laccase-based remediation developed primarily for electroplating, tannery and textile wastewater as they are three of the most extensively studied sources of hexavalent chromium pollution. However, the findings are potentially generalizable to other heavy metal contaminated industrial effluents with compatible physiochemical characteristics, such as matching pH profiles, temperature, and the absence of laccase-inhibiting co-contaminants. Studies from regions with concentrated industrial activity shows that heavy metal concentrations in wastewater often exceed permissible limits set by the World Health Organization (WHO) and other regulatory bodies. A recent example is the 2025 Sino-Metals Leach Zambia dam disaster, where approximately 50 million liters of heavy metal-laden acidic effluent were released into the Kafue River basin. This incident resulted in massive fish deaths, ecosystem damage, and heavy metal poisoning on local communities relying on the river[5]. On a smaller scale, just one gas company (LOUISVILLE GAS & ELECTRIC CO,) has released significant heavy metal pollutants into sources providing drinking water for surrounding communities, even though heavy metals can cause allergy and carcinogenicity at high concentrations[6].

Unlike organic pollutants that degrade over time, heavy metals persist indefinitely, accumulating in sediments and undergoing biomagnification through aquatic food chains – a process where toxins increase in concentration as they move up trophic levels. Due to their inherent harmful nature, researchers have developed different methods to address heavy metal contamination in wastewater[7]. Conventional physiochemical methods for wastewater include chemical filtration, ion exchange, membrane filtration and electrocoagulation. While they can achieve high removal efficiencies (89 – 99% for lead, copper nickel and cadmium), these methods face many operational challenges. Chemical precipitation generates substantial metal hydroxide sludge requiring specialized disposal. Ion exchange suffers from high regeneration costs and fouling issues[8].

Considering these shortcomings, scientists have turned to

enzymatic bioremediation, a process that uses living organisms or their enzymes to accelerate degradation reactions. Enzymes offer unique advantages for environmental applications and can exhibit extremely high efficiency for the specialized reactions they do. Some enzymes can process thousands of metal ions per catalyst molecule while operating under "regular" temperature and pressure conditions. Among the most promising are laccases (EC 1.10.3.2, p-diphenol:dioxygen oxidoreductases), due to their ability to catalyze the oxidation of a broad spectrum of substrates like phenols, amines and certain heavy metals ions[9]. Given their potential, researchers have experimented on genetically improving these enzymes. Newly developed genetically modified microorganisms (GMM's) have enhanced capabilities for heavy metal detoxification: engineered bacteria have demonstrated removal efficacies exceeding 90% for lead and cadmium under optimized conditions, demonstrating their potential for scalability in environmental applications[10]. However, challenges in enzyme stability and environmental adaptability still limit the effectiveness of laccase-based bioremediation[11]. This problem is compounded by the complex composition of industrial wastewater, which often contains pollutants that interfere with enzyme activity. Therefore, a knowledge gap currently exists in understanding how genetically engineered enzymes perform long-term under these industrial conditions. While some studies praise laccases for their broader-spectrum pollutant degradation, others mention concerns regarding their stability, economic feasibility and suboptimal remediation outcomes, hindering deployment of these biotechnologies at industrial scales[12].

This review situates laccases within the broader framework of microbial enzymatic bioremediation. It compares different mechanisms of bioremediation and explores how genetic engineering methods can improve efficiencies and performances of these enzymes for heavy metal remediation. Furthermore, this review evaluates the scalability of implementing microbial technologies on a larger, industrial scale, focusing on factors like enzyme stability, operational costs and environmental conditions. By synthesizing recent advances and addressing operational challenges, this review provides a comprehensive evaluation of laccases' suitability for heavy metal remediation in industrial wastewater.

Laccase Structure and Mechanisms of Heavy Metal Removal

Laccases are multi-copper oxidases that catalyze the oxidation of a variety of phenolic and non-phenolic compounds. Widely distributed in fungi, plants and bacteria, they are particularly prominent in bioremediation due to their high redox potential and effectiveness in degrading a wide variety of compounds. These compounds include synthetic dyes, polycyclic aromatic hydrocarbons (PAHs), and pharmaceutical residues[12]. Structurally, laccases are glycoproteins that can exist as either single units (monomeric), pairs (dimeric), or clusters of four (tetrameric). Each monomer contains four copper atoms arranged in three distinct copper centers (as shown in Figure 1).

There are two main mechanisms through which laccases transform heavy metals: direct oxidation or indirect reduction mediated by organic co-substrates. Although the direct oxidation mechanism is not as common, a well-documented exception is the conversion of Manganese (Mn(II) to Mn(III)). In a foundational study by Schlosser and Höfer, a purified laccase from Trametes versicolor directly oxidizes the soluble Mn2+ ion in the presence of Na-pyrophosphate. The oxidation occurs at the type 1 (T1) copper in the laccase active site, which is the primary electron-accepting site in the enzyme. The reaction occurs optimally at a pH of 5.0. However, this process requires chelators such as oxalate, malonate, or pyrophosphate to stabilize the Mn^{3+} product[13].

In the indirect reduction mechanism, the process also begins with the Type 1(T1) copper site. Often called the "blue copper" site due to its distinct spectroscopic properties, the T1 copper is responsible for oxidizing substrates by abstracting an electron from the organic substrate. The type 2(T2): acts as an electron transfer intermediary, shuttling electrons from the T1 site to the nuclear center (T3). Type 3 (T3) is a binuclear center that reduces molecular oxygen (O_2) to water (H_2O), completing a four-electron reduction of oxygen (equation 1). Together, the T2 and T3 copper centers comprise the trinuclear cluster (TNC). Laccases catalyze this reaction without generating harmful reactive oxygen species (e.g. superoxide radicals or hydrogen peroxide), which significantly reduces oxidative damage to non-target compounds during the bioremediation processes[14].

$$4e^- + O_2 + 4H^+ \rightarrow 2H_2O \quad (1)$$

The combination of a strong oxidative potential at the T1 site and the safe reduction of oxygen to water allows laccases to efficiently oxidize a broad range of substrates, while minimizing secondary pollution, making them ideal for various environmental applications. An example of this type of mechanism is hexavalent chromium, Cr(VI). It is a carcinogen characterized by its high bioavailability, meaning it dissolves easily (more soluble) and can be readily absorbed by living organisms. Chromium's trivalent form Cr(III) is more stable, less soluble and exhibits significantly lower toxicity[16]. Thus, it is desirable to convert Cr(VI) to Cr(III) through the following reduction reaction. (equation 2)

$$Cr^{6+} + 3e^- \rightarrow Cr^{3+} \quad (2)$$

One study was able to deploy purified laccase from *Gano-*

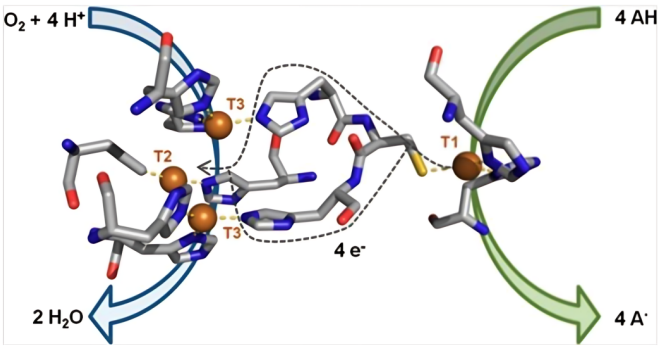

Figure 1. Laccase copper centers and electron transfer pathway. The diagram shows the electron transfer pathway from a reducing substrate to molecular oxygen. The substrate is oxidized at the Type 1 (T1) Site (Brown sphere labelled T1). Electrons are then transferred through a bridge to the trinuclear cluster (TNC), comprised of the Type 2 and 3 (T2) and (T3) respectively. At the TNC, molecular oxygen (O2) is reduced to two molecules of water (H_2O)[15].

derma multipleum to reduce >94% of Cr(VI) with 100μg/ml concentration under acidic conditions (pH 3) and a temperature of 70°C[17]. This percentage value often varies, depending on the laccases' original source and complexity of the system. Here, "complexity of the system" refers to the composition of the contaminated medium beyond a single contaminant. It encompasses factors that interfere with laccase activity, such as the presence of competing ions or fluctuating pH levels. Essentially, complex systems can reduce the overall efficiency of chromium reduction. For instance, laccase from the fungal species *Aspergillus flavus* and isolates of dark septate endophytic fungi reduced chromium by 89.1% and 99%, respectively at the concentration 50 mg/L in a simple environment with no potential interference, while *Trametes versicolor* from a white rot fungus achieved a relatively low 32.2% removal efficiency in more complex systems (Whole cell)[18–20].

Analysis of table 1 shows several patterns regarding operational parameters of fungal systems for Cr(VI) remediation. First, the environments for optimal activity are restrictive. Most systems require acidic conditions (pH of 3.0 - 5.0), which misalign with many neutral or alkaline industrial streams. Optimal temperatures values are relatively low, ranging from 25 - 30°C while some purified enzymes like that from G. multipileum are thermotolerant (70°C.) Second, performance is highly context dependent. In single-pollutant systems particularly those tested in synthetic wastewater, removal efficiencies are consistently high, ranging from 89.1% - 99.0%. However, as demonstrated by the T. versicolor in a dye-bearing co-contaminant mixture, efficiency can plummet by over 60%. Removal rates follow the same pattern, varying widely from 24 hours in single cell pollutants to 288 hours in co-contaminant mixtures. These findings on the potential and limitations of fungal bioremediation were identified through a systematic review of the literature. The methodology for this analysis is detailed in the following section.

Methods

A literature review was conducted using the electronic databases of PubMed and Google Scholar. Additional information was incorporated from government organizations, including the U.S Environmental Protection Agency. Searches were done through the keywords ("laccases" OR "laccase") AND ("heavy metals OR chromium) AND ("biodegradation" OR "remediation" OR "removal") AND ("wastewater" OR industrial effluent").

Inclusion and Exclusion Criteria

Inclusion Criteria: Original Research Articles (both in vivo and in vitro) that a) specifically investigated laccase-based remediation of heavy metals b) provided quantitative data related to the main search terms.
Exclusion Criteria: Studies were excluded if a) focused on organic pollutants without heavy metal involvement b) where full text was not accessible.

Potential Risk Bias

Several limitations and potential biases should be considered when interpreting the findings of this review. For instance, a) selection bias: studies were chosen to convey a clearer narrative regarding the comparative efficacy of laccase-based remediation. b) publication bias: journals preferentially publish studies with positive results as opposed to negative ones.

Thematic Analysis Approach

The final included studies were analyzed using a thematic synthesis approach. Key findings, mechanisms, and limitations were grouped into themes, allowing for the development of a coherent narrative that structures the review.

Results

While laccases demonstrate exceptional capabilities in heavy metal remediation, several mechanistic and operational flaws restrict their practical application[11]. For one, despite their high redox potential, laccases often cannot directly reduce certain heavy metals like Cr(VI) due to mismatches in redox potential. To overcome this issue, scientists use redox mediators such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) or 1-hydroxybenzotriazole (HBT) to shuttle electrons

Table 1 Comparison of removal efficiency of Cr(VI) by different laccase sources, including operational parameters that can influence performance.

Fungal Source	Initial Conc.	Removal Efficiency (%)	Optimal pH	Optimal Temperature (°C)	Reaction time (h)	Wastewater Type
Ganoderma multipleum	100 μg/mL	94.0	2.5 - 3.0	25 - 30	24	Synthetic / Lab
Aspergillus flavus	50 mg/mL	89.1	4.5	30	48	Electroplating
Pseudomonas aeruginosa strain ZM130	25 mg/L	76.6-98.7	Not reported	Not reported	180	Simulated Textile
Dark septate endophytic fungi (multiple)	10 – 50 mg/L	Up to 99.0	5.6	Room Temprature ∼Generally (20 - 25)	Not specified	Synthetic / Lab
Trametes Versicolor	30 mg/mL	32.2%	4.0 (may not be optimal)	Not reported	288	Dye-bearing

between the enzyme and target pollutants. Mediators can introduce several drawbacks, such as additional costs and the potential toxicity of its radicals[21]. Furthermore, laccases generally lose their activity over time. When oxidizing heavy metals, they often interact with co-substrates or mediators to produce free radicals (an atom, molecule, or ion that has at least one unpaired electron in its outer shell, making it highly reactive and unstable.) Free radicals reportedly inactivate laccases, though the reason why is relatively unexplored[10]. Additionally, since it is difficult to recover laccases from aqueous samples, they are often considered a one-time use biocatalyst[22].

Practical Limitations:

The reduction of heavy metals generally requires specific conditions (optimal pH, temperature and absence of inhibitors) to maintain activity. The same study that showed laccases reduced >94% of Cr(VI) with 100μg/ml concentration on purified laccase from *G. multipileum* noted that optimal conditions played a significant role in laccase activity, specifically requiring an acidic pH of 3 and a temperature of less than 70°C. When deviating from these optimal parameters, laccase activity decreased: at neutral to alkaline pH levels (above pH 6), enzyme activity declined by 50 – 70% compared to its maximum activity. Similarly, temperatures exceeding 70°C caused near inactivation of the enzyme[17].

Furthermore, industrial wastewater typically contains a wide variety of inorganic and organic contaminants that potentially inhibit laccase' catalytic function, exacerbating the problem of laccase efficiency in a "real-world" setting. These compounds either directly interact with the enzyme's active site or alter their conformation (essentially changing its shape), affecting how well it can work or even deactivate it entirely. Wastewater streams also frequently exhibit wide fluctuations in pH and temperature—parameters that influence laccase stability and activity. As laccases generally have narrow optimal pH and temperature windows, any deviations from optimal conditions, including those typical of many industrial settings, cause reduced degradation efficiencies[17].

Limited Yield + Production Cost:

The industrial scale production of laccase at a competitive cost is the biggest challenge behind it's application in bioremediation. A 2025 tecno-economic analysis for producing Trametes hirsuta laccase provides a clear breakdown of these costs. The cost structure shows that operational expenditures (OpEx,) from labor accounted for 51.6% of the total cost. Capital expenditures (CapEx) for equipment depreciation were also significant at 42.2%, while raw materials for the culture medium constituted only 5.0%. This production cost is 7 to 12 times cheaper than some commercial laccases, which can range from 19.27 to 31.02 CAD kU^{-1} (kilounit). However, for the vast quantities needed for large-scale wastewater remediation, this cost is still prohibitive compared to conventional methods[23].

Genetic Engineering Advancements

To improve their applicability in heavy metal remediation, scientists have attempted to remedy laccases' limitations through a variety of genetic engineering techniques, as compared in table 2.

Directed Evolution

Directed evolution is a laboratory technique that simulates natural selection to produce biological molecules with desirable traits. In the context of laccases, this approach focuses on properties related to industrial application, including pH and thermal stability, catalytic activity, and substrate specificity. The process begins with mutagenesis methods (DNA shuffling

Table 2 Summary of genetic engineering strategies for enhancing laccase properties.

Engineering Strategy	Laccase Variant / Source	Modification	Key Properties Enhanced	Outcome	Reference
Directed Evolution	POXA1b variant (*Pleurotus ostreatus*)	Multiple rounds of mutagenesis and screening	Thermostability, pH tolerance	Increased half life at $70^{\circ}C$; inhanced activity retention across pH 3-5	15
Site-Directed Mutagenesis	POXA1b variant (*Pleurotus ostreatus*)	Substitution of axial methionine ligand	Mediator Kinetics, Redox Potential	$\sim$60mV increase in redox potential; however stability of T1 copper center was reduced	24
Computer-Guided Mutagenesis	"GreeDo" Variant (Fungal)	Hydrophobic substitutions near T1Cu site	Redox Potential, Catalytic Efficiency	7.5-fold improvement in catalytic efficiency for high-redox mediators (e.g, $[K_4Mo(CN)_8]$) $*$ enhances laccase-mediator system not direct oxidation	25
Recombinant Expression	Laccase1 from *C.Parasitica*	Heterologous expression in i	Production Yield	Laccase activity reached 2.2 U/mL, a 6.5-fold increase over conventional media	26
Immobilization	*T. Versicolor* laccase	Enzyme immobilized on magnetic nanoparticles	Reusability, Operational Stability	Allows for enzyme recovery and reuse	10

or error-prone PCR) to introduce random mutations toward specific regions likely to influence stability. Subsequently, thousands of mutants are screened for improved thermostability, tolerance to alkaline or acidic pH ranges, or resistance to denaturing solvents. The best-performing mutants are selected for further screening rounds. Through this process, beneficial mutations are accumulated, resulting in enzymes better suited for remediation applications[27]. Researchers from the Spanish CSIC institution found that through multiple rounds of directed evolution, POXA1b variants (a gene encoding one of the laccase isoenzymes from the fungus *Pleurotus ostreatus*) showed increased half-life at 70°C and enhanced activity retention across a broader pH range (3-5)[15]. This enables laccases to be utilized in industrial wastewater environments, where heat and pH fluctuations are common.

While directed evolution relies on random mutations, another strategy for genetic engineering is site-directed mutagenesis. This involves precisely engineering laccases based on structural and functional insights. Knowing the role of each copper site is important here, as a laccases' catalytic efficiency is directly linked to its copper centers, particularly the Type 1 (T1) site responsible for substrate oxidation. The T1 copper site is the primary electron acceptor from substrates and determines the enzyme's redox potential. Experimental substitutions of the axial methionine ligand with hydrophobic residues such as leucine (Leu) or phenylalanine (Phe) have been shown to increase redox potentials by 60mV, enhancing the enzyme's oxidative capacity toward high-redox-potential substrates (Figure 2.) The results were nuanced, however, as substitutions altered coordination geometry, leading to reduced stability of the T1 copper center, showing that computational design only demonstrates laboratory scale success and that there is more research needed to be done before industrial application.

Novel approaches have also integrated computational design with targeted mutagenesis to successfully engineer high-redox-potential laccases (HRPLs') with enhancements in catalytic efficiency and operational stability. Similarly, the study introduced hydrophobic amino acid substitutions near the T1Cu site in an evolved variant, dubbed GreeDo. GreeDo displayed a near 7.5-fold improvement in catalytic efficiency toward high-redox mediators, which act as electron shuffles in laccase mediator systems. Additionally, GreeDo's stability improved. Its thermal half-life at 70°C more than doubled – from 23 minutes to 60 minutes – showing the necessary robustness for sustained industrial processes[25].

Finally, a third method of genetic engineering is called recombinant expression systems. Recombinant laccase refers

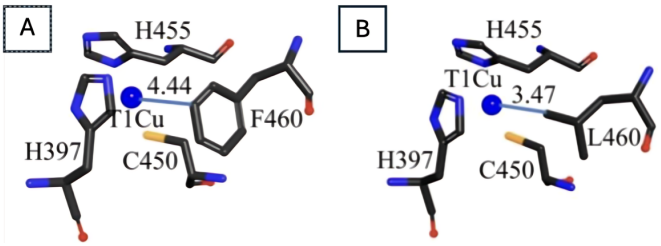

Figure 2. Engineered laccase variants with axial methionine ligand substitutions. Structural models show site-directed mutagenesis of the Thermus thermophilus SG0.5JP17-16 laccase, where the native axial methionine ligand (M460) has been replaced with (a) phenylalanine (F460) and (b) leucine (L460). The substitutions were designed to increase the enzyme's redox potential, enhancing its ability to oxidize more recalcitrant substrates. The T1 ion is depicted as a blue sphere[24].

to laccase enzymes produced via a specific type of genetic engineering, where the laccase gene from a source organism is inserted into a different host organism, to achieve higher expression and optimized production of the enzyme compared to their native source. Host organisms commonly used for recombinant laccase expression include bacteria like *Escherichia coli* and yeast species such as *Pichia pastoris*. These hosts are favored due to their rapid growth, adaptable metabolism (they can grow and metabolize a wide range of carbon and energy sources), and production of active protein. However, achieving optimal yields and protein quality in these systems often requires fine-tuning of production conditions. The choice of host depends on the target yield, protein quality, and key production parameters such as growth medium composition, induction protocols, pH, temperature, and feeding strategies[11]. One example of successful recombinant expression of laccase is the expression of Laccase1 from *Cryphonectria parasitica* in *Saccharomyces cerevisiae*. The gene encoding for extracellular laccase from *C. parasitica* was cloned into a yeast episomal vector and transformed into *S. cerevisiae*. After successfully optimizing culture conditions, the recombinant yeast produced laccase with activity reaching 2.2 U/mL, a 6.5-fold increase compared to conventional media[26].

Industrial Application

As established before, laccases possess intrinsic limitations that constrain their direct application in industrial wastewater bioremediation. Theoretically, genetic and protein engineering approaches have been shown to effectively enhance catalytic efficiency, pH thermostability, and production yield, thereby addressing these constraints. However, a large part of industrial applications has to do with operational factors: production cost and scalability.

Production Cost

Production cost remains one of the biggest drawbacks. Although advances in genetic engineering have enabled higher yields of recombinant laccases in recombinant hosts, the cost of producing and optimizing these enzymes at an industrial scale is still substantial. Bulk enzyme production costs generally range from $10 - 320 / kg, varying significantly by process scale and purity[28]. A comprehensive laccase-based treatment considers costs across several categories: costs for cultivation, fermentation media and purification. Recombinant expression in hosts does increase yield, but necessitates the complicated optimization of conditions, adding to bulk enzyme costs[8].

Extra Costs: Immobilization + Mediators

While not genetic engineering per se, immobilization modifies how enzymes are used by confining them to a solid support. Research shows that immobilization improves reusability: immobilized laccase on activated carbon derived from pomegranate peels retained about 51% activity after 7 reuse cycles in pharmaceutical contaminant removal. The immobilized enzyme also showed better storage stability than free laccase, maintaining >90% of initial activity over a month at 25°C[22]. Essentially, immobilization is necessary for industrial scale remediation, ensuring that laccases can be reused more than once. The price for materials used for immobilization need to be taken into account. Supports such as polymeric beads, nanomaterials, and activated carbons add significant material costs, but specific percentage costs are context-dependent[29]. As mentioned before, laccase-based remediation may include mediators for enhancing substrate range. Mediator use introduces additional operational costs and raises toxicity concerns[21].

Conventional remediation costs range from about $0.30 - 0.70 per cubic meter of treated wastewater[30]. For sludge disposal alone, costs of roughly 500 euros per ton of dry mass, according to the type of treatment and disposal have been reported in European contexts, significantly higher than the original treatment cost[31]. One cost-related factor where enzymes do have an advantage over conventional methods is energy consumption. Enzyme processes run under mild temperature and pressure; hence they take up lower energy needs relative to thermal or chemical treatments. Another advantage compared to physicochemical methods is that laccase-based treatments produce significantly less secondary sludge, reducing disposal costs and environmental impact[32]. Although there is no exact estimate on how much laccase treatment would cost for the remediation of heavy metals, it is very likely that with

all the required modifications mentioned above, laccase-based wastewater treatment costs exceed those of conventional remediation methods, as summarized in Table 3.

Table 3 Table comparing costs between engineered enzymes vs. Conventional remediation methods.

Cost Category	Engineered Enzyme	Conventional Approach
Production Cost	High due to enzyme optimization	Moderate; varies depending on method
Enzyme Immobilization	Adds material cost but improves reusability	Not applicable
Mediator Usage	Adds cost and potential toxicity	Not applicable
Energy Consumption	Low; operates under mild tempratures and pressure	High; thermal and chemical inputs required
Sludge Generation	Low sludge production	High sludge production requiring costly disposal
Overall Operational Cost	Currently high but can decrease with tech improvements	Generally lower but with significant environmental costs

Scalability

To implement laccases in industrial settings, researchers need to establish a definitive way to produce large amounts of laccases while keeping in mind cost. Recombinant expression has made large-scale bioremediation seem feasible, yet producing these enzymes cost- effectively at large scale is still complex, which is why most applications with recombinant laccases are commercial: the first usage of recombinant laccase was recorded in 1996 for denim fishing, then for the construction of biosensors for detection of acid. Since then, laccases have been applied for bioremediation purposes, especially for the treatment of synthetic dyes, and recently, PAH's, PCB's and heavy metals[33]. However, Enzyme quantities needed for these industrial treatments are much larger. For example, the Soktas project requires treating a wastewater volume of approximately 7,000 cubic meters per day[34]. There is no definitive method to quantify the amount of laccase needed for projects like Soktas, since enzyme expression is measured in activity units per liter (U/L). An interesting yet speculative benchmark can be made, however. Researchers found that the estimated cost of treating 1L of wastewater using commercial and crude grade laccase was \$2 – 60 and \$1 - 60 respectively[35]. This implies that to treat 7000 cubic meters of wastewater, the equivalent of seven million liters, it would cost around \$7 million on the low end and \$420 million on the high end. Granted, this estimation is quite dubious and should be taken with a grain of salt, and it should only serve to illustrate the potential financial burden associated with large-scale treatment. This issue of production cost is prevalent in studies, as several authors cite the lack of cost-effective laccase production to be the primary bottleneck preventing industrial application[36].

Semi-industrial studies have validated the use of enzyme-based treatments in real wastewater streams. For example, engineered laccase from *T. Versicolor* immobilized on magnetic nanoparticles has been used to treat dye and heavy metal contaminated textile effluents, achieving over 90% removal of azo dyes and significant reduction of metals like Pb, Cr, and Cd. Similarly, recombinant laccase-expressing yeast has enabled treated electroplating wastewater to meet discharge standards while producing lower sludge volumes than chemical methods[32]. These are some of the few notable examples that utilize engineered laccases in industrial settings, bringing us to the most pressing issue: the limited data availability on long-term operational data.

Discussion

The biggest gap in laccase research is the lack of studies that evaluate the stability of laccase-based systems in continuous-flow industrial settings. A large majority of published work on laccase-based wastewater bioremediation remains at the laboratory or pilot scale. While these smaller-scale models highlight exemplary removal efficiencies under controlled conditions, there remains little published evidence documenting enzyme behavior over extended operational periods. Consequently, we do not yet know the impact of factors like the frequency and cost of re-immobilization or enzyme replenishment, or the impacts of real industrial wastewater fluctuations on laccases' lifespan. For example, most available studies report laccase performance over several hours to a few days, whereas industrial effluent treatment requires consistent function for months or more[37]. Currently, the only use cases for laccases consist of reactions under milder conditions, with applications ranging from the food industry and a select few bleaching processes to cosmetics. Application on industrial wastewater still requires further research on economic feasibility considering enzyme production, immobilization, and replenishment costs and full-scale continuous operation studies under realistic industrial conditions.

When compared quantitatively with physicochemical methods, laccase-based bioremediation offers lower energy consumption due to operation at mild temperatures and pressures and produces less secondary waste, reducing disposal costs, and environmental concerns. However, the higher enzyme production and immobilization costs render laccase treatments more expensive overall. Popular alternatives, particularly

electrocoagulation, deliver variable removal efficiencies but benefit from scalability advantages[30]. A comprehensive cost-benefit analysis is necessary to convincingly position laccase bioremediation as a competitive industrial solution. In sum, popular conventional methods, most notably electrocoagulation and filtration processes still hold an overall advantage due to their established scalability and often lower cost despite their own limitations. Further research advances must be made regarding the cost effective production of large quantities of laccase, and long term studies on laccase performance are key for their future implementation.

Conclusion

Current research on laccases for bioremediation for industrial wastewater consistently highlights limitations of native enzymes, primarily their instability in harsh wastewater conditions, reliance on mediators, and low native production yields. Genetic engineering approaches have been crucial in addressing these challenges, with techniques like directed evolution, site-directed mutagenesis and rational design demonstrating noteworthy improvements in catalytic efficiency, thermal and pH stability. However, the transition from laboratory success to industrial-scale application remains the primary bottleneck. Scientists must prioritize research on economically viable large-scale production and validate laccases in continuous-flow industrial settings. If these limitations are addressed, genetically engineered enzymes can become a cost-effective solution for heavy metal remediation in industrial wastewater.

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