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Review Article

Laccase mediator system as a potential technology for decolorization of textile dyes — Overview and perspectives

Bharti Rana[1,a] & J N Chakraborty[2]

[1]Department of Textile and Fibre Engineering, Indian Institute of Technology Delhi, New Delhi 110 016, India

[2] Department of Textile Technology, Dr. B.R. Ambedkar National Institute of Technology, Jalandhar 144 008, India

Discharge of the exhausted dye bath at the end of dyeing imposes a huge waste-water load, necessitating the use of fresh water for further processing. Due to the limited availability of fresh water, industries are compelled to remove the dyes from drained-out liquor to protect the environment and recycle the treated water for future processing. Discharged dyes create a film or layer of color on the water's surface, impeding the natural breakdown of substances and harm aquatic organisms. A significant proportion of effluent comprises synthetic dyes from azo, anthraquinone, triphenylmethane, indigo, and other groups. Several azo dyes and their cleaved by-products have been discovered to impact human health. Various established techniques are commonly employed to remove the color from dye; however, they have inherent restrictions. Due to increased demand, there is a growing need to explore cost-effective alternative treatments, such as biological integrated systems. The utilization of laccase for the decolorization of dyes presents a possible technological solution to address the drawbacks associated with current approaches. These enzymes can chemically react with and break down diverse substances. Laccases have been verified for their capacity to break down synthetic dyes in wastewater, resulting in the degradation of less hazardous products than the original dyes. This study examines the advantages and disadvantages of different technologies used to remove color from dyes. It also discusses the degradation mechanisms of several synthetic dyes catalyzed by laccase and the impact of factors, such as temperature, *p*H, dye concentration, etc. Additionally, the most recent studies on decolorization utilizing laccase are reviewed.

Keywords: Dye, Decolorization, Effluent treatment, Laccase, Synthetic dye

1 Introduction

Phenolic substances, such as tannins, lignins, and organic colorants, are responsible for the presence of color[1]. Textile effluent is mainly composed of dyes and dye intermediates. There are several types of dyes, including reactive, direct, sulfur, vat, acid, basic, mordant, and dispersed dyes[2]. The presence of color in wastewater can pose several risks, including chronic effects on aquatic organisms as well as on other living beings, environmental concerns, etc. Textile effluents can be handled using two methods: decoloration, which involves the use of physical and chemical techniques to remove dyes and biodegradation[3]. Biological techniques are both economically and environmentally favorable. The products resulting from enzymatic reactions are less dangerous than the synthetic dyes themselves[4-6].

Laccases, commonly referred to as multicopper oxidases, can facilitate the conversion of oxygen into water while simultaneously oxidizing a substrate. Laccases are widely present in plants and fungi[7,8]. Deska and Konczak[9] identified laccase as a green catalytic agent because it can be immobilized on a carrier and recovered from the post-process mixture. It allows for its reuse in various sectors[9]. Researchers conducted experimental tests to examine the function of the laccase mediator system as a potentially effective technology for removing dyes[10,11]. The efficacy of laccase in decolorizing dyes can be influenced by variations in *p*H, temperature and concentration levels.

This paper reviews the potential application of a laccase mediator system in the decolorization of various dyes. It analyses the different types of waste produced during various phases of textile chemical processing and investigates potential remedies for eradicating pollutants. This study also discusses the numerous types of reactions that laccase can catalyze and the mechanism of decolorization for different dyes.

2 Waste from Scouring, Bleaching, Dyeing and its Effects

Textile wet processing encompasses many procedures, such as singeing, desizing, bleaching, mercerizing, dyeing, printing, and finishing. The textile sector poses around 17-20% of the

[a]Corresponding author.
E-mail: bhartirana2048@gmail.com

total pollution in industrial wastewater. Direct consequences of the pollutants emitted by these sectors include acid rain, ozone layer depletion and global warming. It is imperative to decrease the pollutant emissions from the textile industry since they pose a significant threat to the environment, human lives, and other species on Earth. It induces harmful diseases and health problems in individuals and has a negative impact on aquatic life that comes into contact with these contaminants. The toxic substances, such as chlorine, sulphides, chromium, and others also cause the aforementioned issues. Prior to its emission, the treated effluent must be free of any contaminated chemicals.

Typically, the waste released by the textile sector contains various chemicals that originate from various processes within textile manufacturing. Pretreatment processes, such as desizing, produce pollutants like starch and its derivatives, cellulosic derivatives (e.g. glucose, CMC, methylcellulose), polyacrylates, polyvinylalcohol(PVA), lipids, and waxes. The desizing process employs several chemicals, such as sodium persulphate or sodium bromite, as oxidizing agents and hydrochloric acid for acid desizing, which contribute to effluent pollution and possess corrosive properties.

Scouring of textiles can also produce potential pollutants, including sodium hydroxide (NaOH), waxes, greases and sodium carbonate (Na_2CO_3). Various bleaches, such as sodium hypochlorite [Na (OCl)], hydrogen peroxide (H_2O_2), and chlorine dioxide (ClO_2), are employed to eliminate colored impurities from fabrics, but these bleaches are harmful to the environment.

The process of textile mercerization negatively impacts the environment due to the use of highly concentrated NaOH. Most effluents contain salt concentrations ranging from 2000 to 3000 parts per million (ppm). These pretreatment processes alone can lead to 35-50 % of the total biological oxygen demand (BOD) of wastewater.

The dyeing and printing procedures involve the usage of several polluted chemicals, including alkali salts, ammonia, heavy metals, mordants and pigments. Approximately 40% of dyes include carcinogenic substances, such as chromium. In addition to dyeing, several solvents, such as toluene, xylene, and methanol[12], are employed in textile printing. Starch, tallow, and various finishing compounds in the effluent are also responsible for the contamination. Coloration significantly impacts the water, rendering it unsuitable for some industrial applications when it is drained from water supplies. The textile wet processing industries are mostly linked to chemical dangers. Dyes pose risks depending on the extent of their exposure. Reactive dyes typically pose a greater risk for respiratory problems. Prior to waste management, it is crucial to systematically identify the different forms of waste and their characteristics. Based on this information, various methods can be employed to treat wastewater and eliminate pollutants from the discharged liquid.

2.1 Categorization of Waste Produced by Textile Industry

Textile waste can be broadly classified into four main categories: hard-to-treat waste, toxic waste, high-volume waste, and dispersible waste. Each category requires distinct strategies for pollution prevention and remediation. The primary origins of this garbage are non-biodegradable organic or inorganic compounds that are difficult to handle. The composition includes colorants from dyeing procedures, phosphates from preparatory processes and dyeing phenols, surfactants, toxic organic compounds, pesticides, and phosphates. Hazardous or toxic wastes are a subset of difficult-to-process wastes. The composition comprises metallic elements, chlorinated solvents, and non-biodegradable or volatile organic substances. Industries often face challenges when dealing with large quantities of garbage. It refers to the inclusion of water used in both the preparatory and dyeing stages of continuous processing. Batch dye waste contains significant quantities of salts, acids and alkalis. Dispersible wastes encompass waste streams generated during preparation, dyeing, printing and finishing procedures. The substances encompass print paste squeeze, foam generated during coating operations, solvents emitted during dry cleaning operations, and so on[13].

2.2 Wastewater Treatments

Wastewater can be subjected to a range of physical and chemical processes[14], which can be categorized as preliminary, primary, secondary, and tertiary treatment.

2.2.1 Preliminary Treatment

It refers to physical treatments that involve eliminating many insoluble particles, i.e. rags, sticks, grit, and grease. These particles scan cause apparatus deterioration and problems related to effective functioning, as shown in Table 1.

2.2.2 Primary Treatment

It refers to removing organic matter and suspended solids from wastewater. This is achieved through

Table 1 — Outline of pre-treatment possibilities of wastewater

Treatment	Functions
Screening	To eliminate non-soluble particles from wastewater
Removal of sand and stones	To segregate larger particles with a specific gravity of around 2.65
Removal of grease	Machine grease, extraction of oils and immiscible substances
*p*H neutralization	*p*H maintenance is primarily determined
Equalization	To achieve uniformity in *p*H of effluent, it is essential to maintain low levels of both average effluent load and the effluent characteristics

Table 2 — Primary treatment possibilities of wastewater

Treatment	Functions
Coagulation	Chemical treatment eliminates total suspended solids (TSS) from wastewater, and colloids destabilisation
Flocculation	To eliminate suspended particles from water by introducing flocculant that causes aggregation and forms larger clusters called flocs
Sedimentation	Particles with greater mass descend as a result of gravitational force
Clarifiers	Removing solid particles or suspended solids from a liquid to clarify or thicken
Floatation	Eliminating TSS in form of scum

Table 3 — Secondary treatment possibilities of wastewater

Treatment	Functions
Aerobic biological treatment	To cleave the -N=N- bonds in dyes and other compounds, one can employ dissolved oxygen, microorganisms, and nutrients to support microbial activity
Anaerobic biological treatment	The process involves anaerobic microbes converting complex organic compounds into methane and carbon dioxide

Table 4 — Tertiary treatment possibilities of wastewater

Treatment	Functions
High-rate filtration	To filter residual dyes and suspended solids
Advanced oxidation	Complex oxidation procedures, such as ozonation and oxidation employing Fenton's reagents
Ultrafiltration	For bivalent salts and dissolved solids that have a weak ability to conduct electricity
Nanofiltration	For monovalent salts and dissolved solids with strong electrolytic force
Reverse osmosis	To eliminate dissolved solids, employ high-pressure pumps and membranes

physical and chemical treatments, which eliminate floating and settable materials (Table 2).

2.2.3 Secondary Treatment

This treatment utilizes biological and chemical methods to eliminate biodegradable organic materials and suspended particles, as indicated in Table 3.

2.2.4 Tertiary Treatment

It refers to a series of physical, biological and chemical processes used to eliminate any remaining suspended or dissolved solids, as outlined in Table 4.

3 Comparison of various Wastewater Treatment Technologies

Different techniques have been practised to reduce factors, such as color, BOD, COD, organic substances and suspended solids. Each technique has certain advantages and disadvantages. For example, electrochemical systems are highly efficient in pollutant removal (up to 90%), but not cost-effective because of uncontrolled radical reactions, high energy requirements, and the restrained life span of the electrode[15]. Figure 1

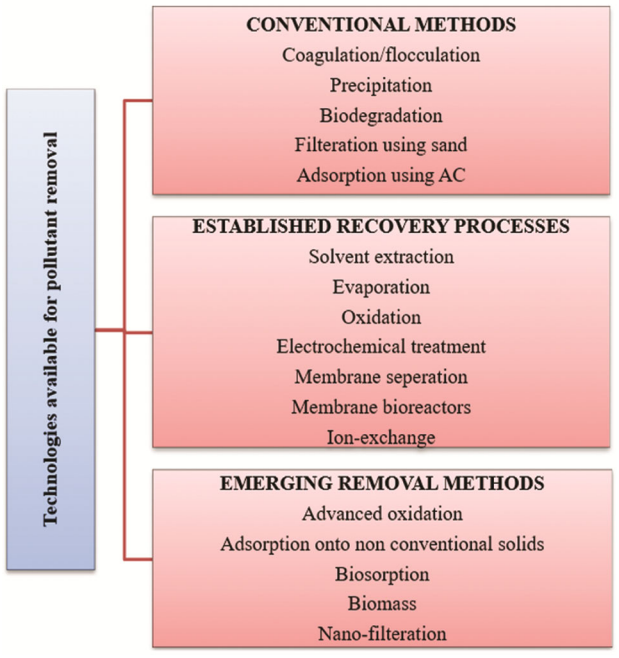

Fig. 1 — Classification of existing technologies for pollutant elimination

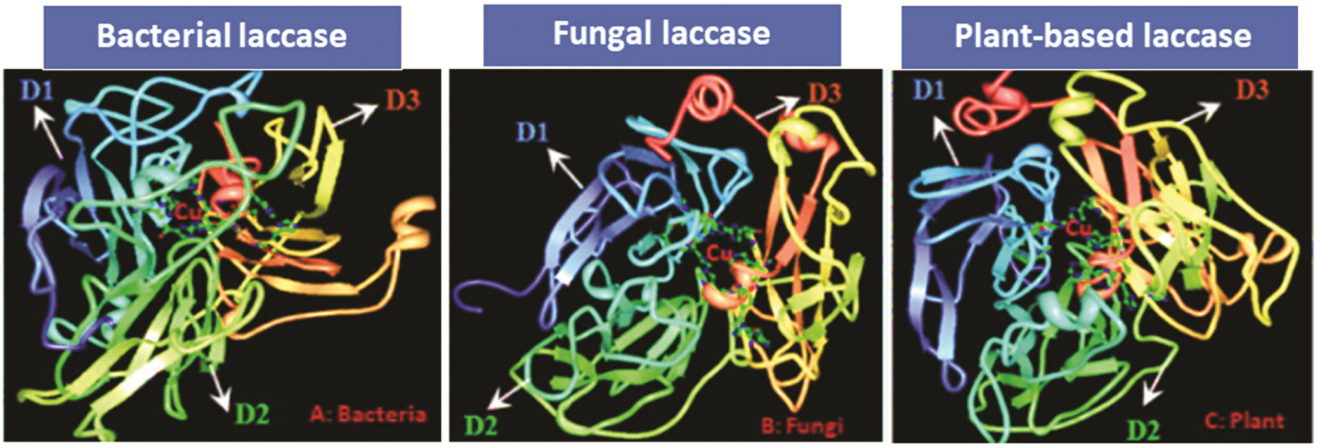

Fig. 2 — Classification of laccase based on their origin (D1— Domain 1, D2— Domain 2, D3— Domain 3 and Cu— Copper)

classifies the varieties of techniques into three groups, viz conventional techniques, established recovery processes, and emerging removal methods used for wastewater pollutant removal. Table 5 presents the advantages and limitations of various methods that have been practiced[16-27]. Each technique has its limitations in terms of expenditure, efficiency, viability, consistency, sludge production, ecological impact, pre-treatment necessities, process difficulty, and the formation of hazardous by-products.

To resolve the environmental issues created by conventional processes, eco-friendly techniques such as enzymatic processing are increasingly used.

4 Enzymes

Enzymes serve as catalysts since they accelerate chemical reactions without changing their molecular structure in the process[28,29]. The six main classes of dye-decolorizing enzymes are oxidoreductases, hydrolases, transferases, ligases, isomerases, and lyases. Laccase facilitates the process of oxidation-reduction processes. The majority of them are commonly referred to as dehydrogenases[30,31]. This group of enzymes is involved in oxidation-reduction processes, in which one chemical undergoes oxidation and another undergoes reduction. These enzymes typically catalyze oxidation-reduction processes between two substrates, S and S', as shown in the following equation:

$$S\ (reduced) + S'(oxidized) \rightarrow S\ (oxidized) + S'\ (reduced) \quad \ldots(1)$$

They facilitate electron transfer processes. The enzymes in this category are the oxidoreductases that catalyze reactions involving C=O, CH-OH, CH-NH_2, CH=NH, and CH-CH groups.

5 Laccase

The extraction of laccase from the Japanese lacustrine tree *Rhus vernicifera* marks a significant achievement. Laccases are enzymes found in bacteria, plants, and fungi; each with varied functions depending on their source. These multicopper oxidoreductases have been utilized for research purposes to assess their potential since 1990s. Theyare designated as EC 1.10.3.2, indicating a type of p-diphenol: dioxygen oxidoreductase enzyme. These enzymes, categorized as phenol oxidases, facilitate substrate oxidation while simultaneously reducing oxygen in water[32–35].

Laccases are glycoproteins that exist as monomers, dimers, or tetramers, with each monomer containing four copper atoms in its catalytic site. The copper of Type 1 (Cu T1) exhibits paramagnetic properties, resulting in a blue coloration and facilitating the oxidation of the substrate. The trinuclear group, consisting of one Type 2 copper atom (Cu T2) and two Type 3 copper atoms (Cu T3), significantly reduces molecular oxygen to two water molecules[36,37]. Laccases are categorized into bacterial, fungal and plant types based on their 3-D structure, characterized by three ferredoxin-like domains (Fig. 2). Prior studies have demonstrated that laccase facilitates a sequence of reactions, rendering it well-suited for application in many industries[38,39]. Fungal laccases are more desirable than bacterial laccases due to the latter's lower redox potential.

5.1 Types of Reactions Catalyzed by Laccase

5.1.1 Type A Reactions

Type-A reactions entail the direct oxidation of simple organic molecules, e.g. mono-phenols, di-phenols, polyphenols, and their derivatives, without

Table 5 — Pros and cons of the traditional techniques employed for wastewater treatment

Process	Description	Pros	Cons
Oxidation utilising Fenton reagents	Oxidation reaction primarily utilises H_20_2-Fe(II)	▪ Efficient decolorization of soluble and insoluble dyes ▪ High throughput rates	▪ Sludge generation ▪ Efficiency depends upon type of oxidant ▪ Management of oxidants required in production and transport
Ozonation	Oxidation reaction utilising ozone gas	▪ No change in volume when converted to a gaseous state ▪ Good elimination of color and ozone ▪ High throughput rates	▪ Short half-time, i.e. 20 min ▪ No diminution of COD values or limited effect ▪ No impact on the level of saltiness
Photochemical	Oxidation reaction primarily utilizes hydrogen peroxideH_20_2 and UV light	▪ Zero sludge generation ▪ Straightforward procedure -Applicable to highly concentrated effluents or sludges ▪ Remarkably effective ▪ Completely eradicates all forms of organic matter	▪ Formation of byproducts ▪ Initial investment costs ▪ High running costs ▪ Formation of dioxins and other pollutants, i.e. metals, etc.
NaOCl	The oxidation reaction involves the use of the Cl^+ group to target and attack the amino group	▪ Efficient treatment for cyanide and sulfide removal ▪ Facilitates and expedites the breaking of azo bonds ▪ High throughput rates	▪ Release of aromatic amines and volatile compounds ▪ Sludge generation
Electrochemical destruction	Electrochemical oxidation reaction	▪ Breakdown compounds are non-hazardous	▪ High cost of electricity
Activated carbon	Adsorption for dye removal	▪ Technologically simple ▪ Highly effective ▪ Effective elimination of a diverse range of dyes ▪ Extremely effective method when combined with coagulation to decrease the number of suspended materials	▪ Regeneration difficulties ▪ Relatively high investment ▪ High cost of materials ▪ Nondestructive ▪ The performance of a system is contingent upon specific characteristics of material being used ▪ Need multiple types of adsorbents
Membrane filtration	Physical separation	▪ Elimination of every kind of dye, salt and mineral derivatives ▪ Produces high-quality treated effluent ▪ No chemicals required ▪ Minimal amount of solid waste being produced	▪ Production of very concentrated sludge ▪ Investment costs are often too high ▪ High energy requirements ▪ Membrane filtering systems can vary considerably in their design ▪ Quick accumulation of blockages in the membrane ▪ Slow rates of processing
Ion exchange	Ion exchange resin	▪ Recreation facilities, i.e. no adsorbent loss ▪ Simple equipment ▪ Compatible with other procedures, such as precipitation and filtration in a comprehensive wastewater treatment system ▪ Fast and effective ▪ Generate effluent of superior quality	▪ Not suitable for all types of dyes, e.g. disperse dyes ▪ Economic constraints ▪ Large volume requires large columns ▪ Rapid saturation and clogging of the reactors
Electrokinetic coagulation	Addition of ferrous sulfate and ferric chloride	▪ Economically feasible ▪ Increases biodegradability ▪ *p*H control is not necessary ▪ Rapid organic matter separation ▪ Highly efficient at eliminating suspended particles, dissolved metals, tannins, and dyes	▪ High sludge production ▪ Requires addition of chemicals coagulants, i.e. flocculants and salts ▪ Sludge deposition on electrodes ▪ Efficiency depends strongly on bubble sizes ▪ Requires post-treatment to remove high concentrations of iron and aluminum ions separation

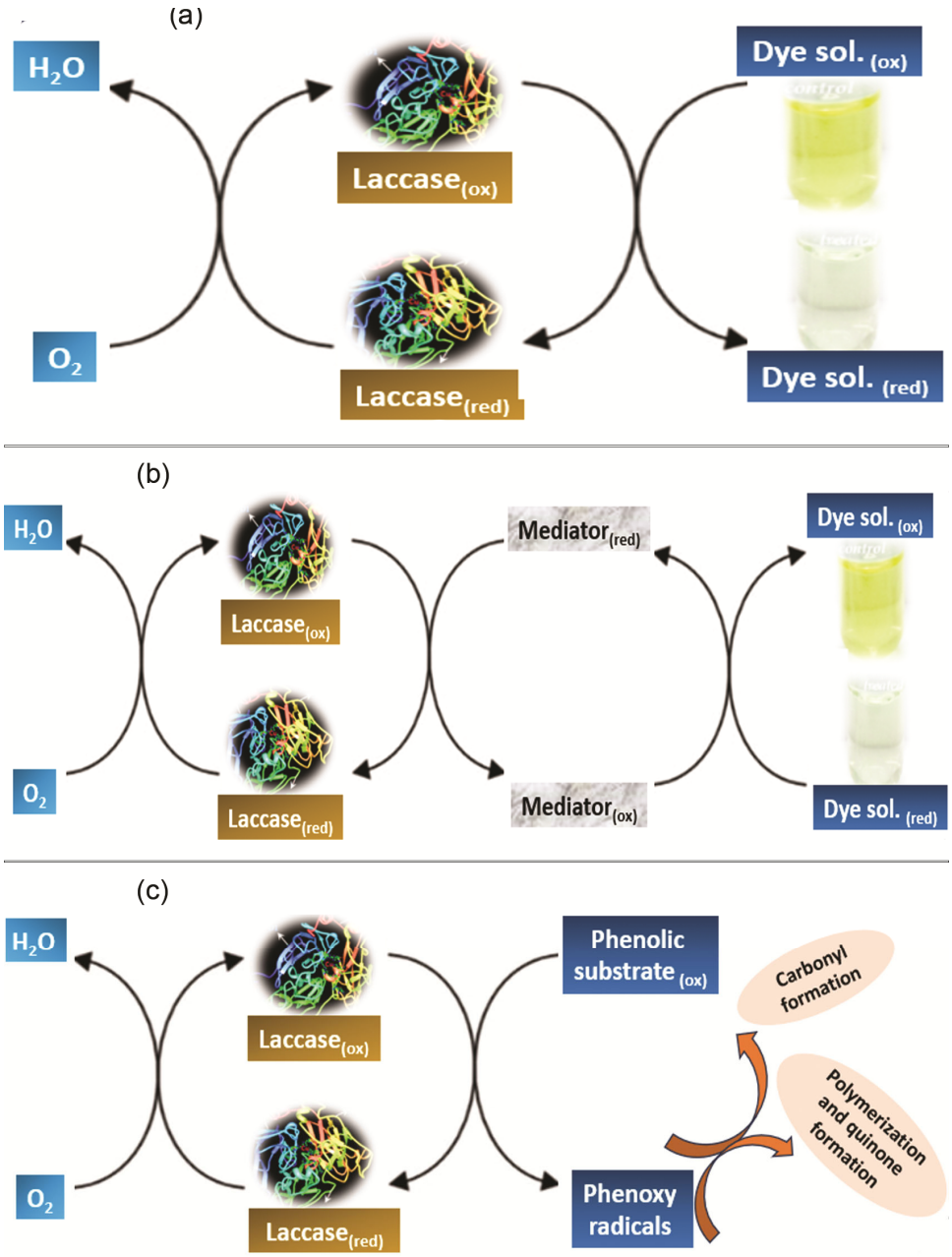

Fig. 3 — Reactions catalyzed by laccase (a) Type-A, (b) Type-B and (c) Type-C

the involvement of any mediator [Fig. 3 (a)]. These derivatives may include functional groups, such as carboxyl, amine, methoxy or sulphonic. Quinones are typically produced when phenols are oxidized by laccases[40]. Fungal laccases participate in the process of breaking down lignin. These substances have a high oxidation-reduction potential[38, 41–43], which enables them to undergo direct oxidation.

5.1.2 Type B Reactions

Type B reactions occur when phenolic and non-phenolic substrates are oxidised indirectly with the assistance of a mediator. In this category, molecules with high redox potential, such as non-phenolic chemicals, aromatic amines, or lignin complexes, experience oxidation when exposed to low molecular weight mediators, as illustrated in Fig. 3 (b). Initially, the enzyme undergoes oxidation of the mediator. Subsequently, the oxidized mediator interacts with the matching substrate, such as dye or color[44-52]. A wide range of synthetic and natural mediators are commonly utilized.

5.1.3 Type C Reactions

These are the reactions in which reactive radicals are coupled together. During these reactions, the direct oxidation of phenolic substrates leads to the formation of unstable intermediates or radicals [Fig. 3 (c)]. Polak and Jarosz[43] proposed that novel phenolic structures, such as dimers, oligomers and polymers, had the potential to be created. Enzymatic oxidation or reduction can convert these radicals into quinones[34].

5.2 Laccase Mediators

5.2.1 Artificial Laccase Mediators

Bourbonnais identified ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) as the first confirmed laccase mediator for the oxidation of non-phenolic substances. The ABTS molecule undergoes oxidation, initially forming the cationic radical ABTS+, which subsequently undergoes further oxidation to form $ABTS^{2+}$. This process was studied specifically for the oxidation of non-phenolic lignin molecules[36]. The oxidation of organic dyes by laccase and ABTS mediator occurs through an electron transfer pathway, as depicted in Fig. 4[53,54]. Additional mediators utilized in conjunction with laccase for the oxidation of stubborn aromatic compounds include hydroxy benzotriazole (HBT), hydroxy phthalimide (HPI), hydroxy anthranilic acid (HAA), 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO), syringic acid, violuric acid (VIO), hydroxy acetanilide (NHA), and others[55-59]. Researchers have extensively investigated the process by which laccase oxidizes these specific mediators. These mediators generate highly reactive nitroxyl radicals that facilitate enzymatic extraction of an electron, subsequently leading to the release of a proton. The generated nitroxyl radical oxidizes the substrate through a hydrogen atom transfer mechanism (Fig. 4)[44,45,49]. Barreca *et al.*[52] and Cantarella *et al.*[55] studied the relationship between the newly created bond (NO–H) in the mediator and the detached bond (C–H) in the substrate. They observed that this equilibrium is the driving force behind this mechanism.

However, Fabbrini *et al.*[44] proposed that TEMPO, unlike other nitroxyl radicals, undergoes a unique transformation upon oxidation, forming an oxoammonium ion. This transformation occurs at a high redox potential, similar to fungal laccase, and follows a non-radical ionic process.

5.2.2 Natural Mediators of Laccase

Despite the notable effectiveness of synthetic mediators, they have various drawbacks, such as high cost and toxicity. Consequently, several investigations have been conducted on alternative mediators. The phenolic compounds serve as natural mediators for laccase, effectively addressing the limitations of synthetic mediators. Figure 5 shows examples of such mediators.

An analysis conducted by Eggert *et al.*[50,60] revealed that a secondary metabolite can function as a mediator in the oxidation of non-phenolic substrates catalyzed by laccase. However, Johannes and Majcherczyk[61] found that this metabolite does not facilitate the oxidation of polycyclic aromatic hydrocarbons by laccase[45]. In addition, Li *et al.*[62] reported that the oxidative coupling of 3-HAA results in the formation of cinnabarine acid. Despite this, cinnabarine acid is incapable of promoting the oxidation of non-phenolic substances due to the absence of a substituent group that can undergo additional oxidation by laccase. This is because there is no production of a phenoxy radical.

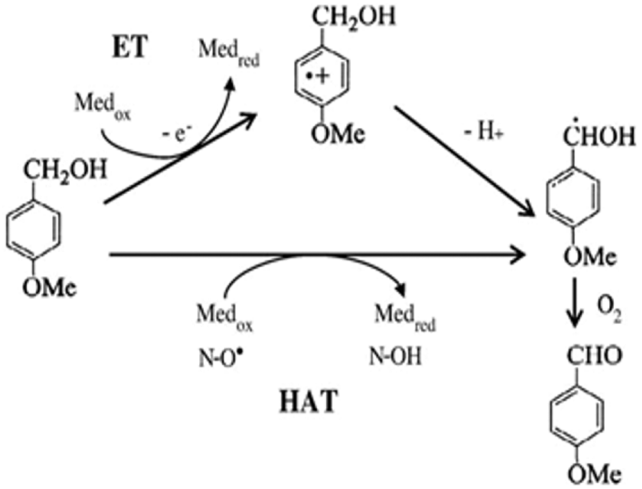

Fig. 4 — Hydrogen atom transfer mechanism

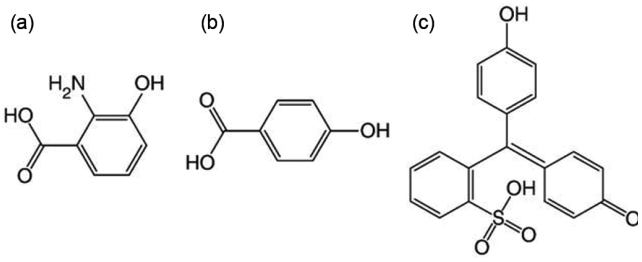

Fig. 5 — Chemical structure of natural laccase mediators (a) 3-hydroxy anthranilic acid, (b) 4-hydroxybenzoic acid and (c) phenol red

5.2.3 Natural Mediators of Laccase in Lignin Degradation

Free radicals or products originate from the biodegradation of lignin compounds or fungal strains. The lignin polymer is primarily composed of over 80% non-phenolic aromatic structures. The degradation process occurs through enzyme and radical processes. This particular reaction can be observed in the breakdown of the lignin wood polymer[42,63]. Acetosyringone, syringaldehyde, methyl vanillate, vanillin, p-coumaric acid, and acetovanillone are examples of mediators that are known to facilitate the action of laccase and aid in the removal of dye coloration. The formation of these redox mediators through the biodegradation process is also called enzymatic combustion, an oxidative process[60,64,65].

The chemical structures of these mediators are depicted in Fig. 6. These phenolic chemicals can be found in herbaceous plants or are produced through the breakdown of lignin. The type of mediator determines the various oxidation products generated from a specific substrate. These enzymes catalyze oxidation processes of substrates that cannot be directly oxidized by other enzymes due to their limited capabilities. The inclusion of these mediators during the degradation process results in increased rates of substrate transformation[50,62,66-68]. Canas *et al*[69]. and Qiu *et al.*[70] also proposed that these mediators might effectively facilitate the oxidation of certain resistant dyes by laccase.

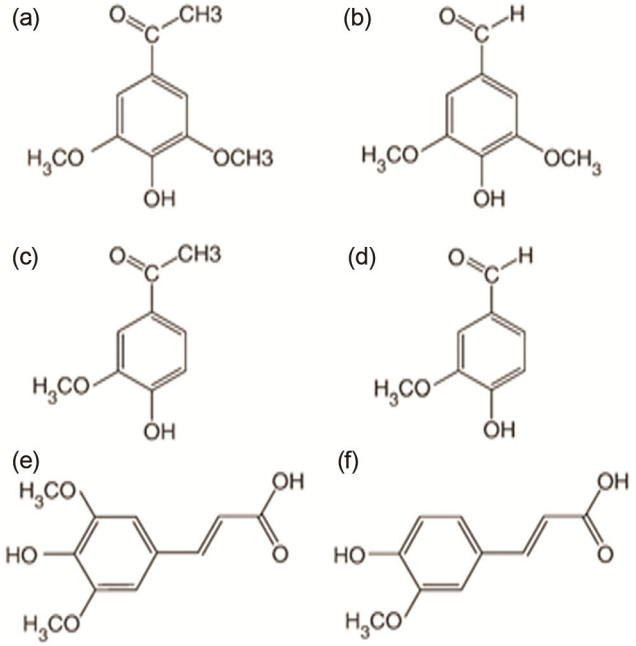

Fig. 6 — Chemical structure of compounds formed due to lignin degradation (a) syringal dehyde, (b) acetosyringone, (c) vanillin, (d) methyl vanillate, (e) acetovanillone and (f) p-coumaric acid

6 Dye Decolorisation using Laccase

Chemical industries utilize a wide range of synthetic dyes, which provide a diverse range of colors. Reactive dyes are highly favored due to their numerous advantages, including superior fastness due to their strong reactivity. Dyes generally comprise three components, viz an auxochrome, a chromophore, and a reactive group. The chromophore is responsible for the coloration of dyes through the absorption of visible light. Therefore, while decolorizing dyes, this component must be carefully considered. Thus, dyes must be categorized according to the specific chromophore they contain. In industry, synthetic dyes are typically classified based on their chemical structure, which often includes chromophores such as $-NH_2$, $-(CH_3)_2N$, -OH, CH_3CO, $-OCH_3$, $-NO_2$, CH_3-, $-SO_3H$, etc.[71]. The dyes depicted in Fig.7 include azo dyes, triphenylmethane dyes, anthraquinone dyes and indigo dyes.

Jadhav *et al*[72]. demonstrated a widespread need for synthetic dyes, with over 3000 azo dyes used by various industries. However, the processing of reactive dyes has several limitations. The untreated pollutants generated are highly toxic and result in water heavily contaminated with dyes. Additionally, the water contains a high salt concentration and exhibits high BOD and COD values, which are detrimental to the environment[73,74]. The literature on laccase mediator-assisted dye degradation is summarized in Table 6.

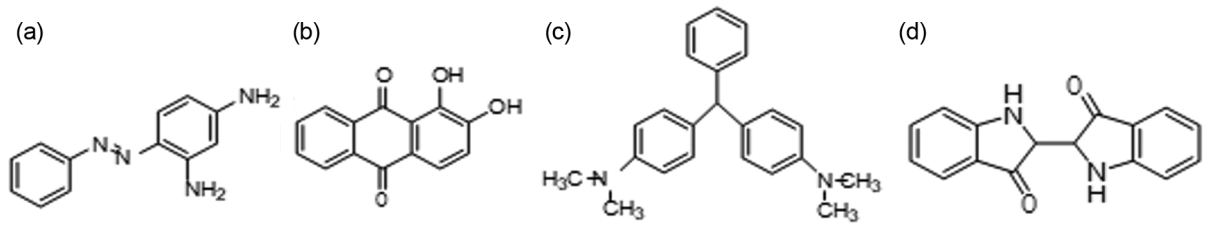

Fig. 7 — Basic structure of synthetic dyes (a) azo, (b) anthraquinone, (c) triphenylmethane and (d) indigo

Table 6 — Overview of laccase-assisted dye degradation studies

Dyes	Mediator	Optimal conditions	Decolorization efficiency	Reference
Reactive Blue 49	ABTS	-	92% (75 min)	75
Remazol Brilliant Blue, Remazol Black-5	HBT	*p*H 4.0, 60°C	92% (2 h)	76
Reactive Black 5	-	*p* H 5.0, 27°C	91% (3-4 days)	6
Reactive Levafix Blue	-	*p* H 5.5, 35°C	80% (2-3 days)	77
Acid Orange 52 and Direct Blue 71	-	*p* H 4.5, 40°C	>50% (2 h)	78
Azo dye	-	*p* H 5.0, 25°C	>70% (24 h)	79
Malachite green	ABTS	*p* H 6.0, 28°C	91.6% (172 min)	80
Reactive cold brand dye	BT, TEMPO	*p* H 5.0, 30°C	>80% (72 h)	10
Indigo carmine and malachite green	β-(10-*pH*e- nothiazyl)-propionic acid	*p* H 8.0, 50°C	>80% (1-2 h)	81
Remazol Brilliant Blue	-	*p* H 5.5, 25°C	99% (12 days)	82

6.1 Mechanisms of Dye Degradation

Azo dyes are chemical compounds with one or more azo linkages (-N=N-). Azo bonds can be linked to benzene or naphthalene using several functional groups, including -Cl, -CH_3, -NO_2, -NH_2, -OH, and –CO. Azo dyes are produced through the reaction between diazonium salts with phenols, amines, or naphthol. Numerous studies have explored the enzymatic decolorization of azo dyes. The potential reaction mechanism for the breakdown of Congo red dye is elucidated in Fig. 8. Telke *et al.*[5,84] suggested that azo dyes can undergo either symmetrical or asymmetrical breakdown during enzymatic degradation. Other studies indicate that the degradation of azo dyes by laccases is contingent upon the structure of the dye. The process begins with the asymmetric cleavage of the azo bond followed by oxidative cleavage, desulfonation, deamination, demethylation and dihydroxylation[6, 80, 85]. However, Chen *et al*[86]. and Pereira *et al*[87]. proposed that azo dyes decomposition can occur without breaking of the azo bonds. The inability of laccase to degrade azo linkages may be attributed to its redox potential. Additionally, enzymatic cleavage of azo dyes might generate perilous substances, such as amines, posing further issues. Therefore, it is imperative to prioritize a comprehensive understanding of the decolorization mechanism of reactive dyes using laccase. The laccase mediator system has also been utilized to bleach denim garments to remove indigo dye. Campos *et al.*[4] employed the laccases mediators system to facilitate the oxidation of indigo 2 indole-2,3-dione, which then undergoes further decomposition to produce anthranilic acid (2-aminobenzoic acid).

A theory concerning the indigo oxidation process entailing the progressive extraction of four electrons

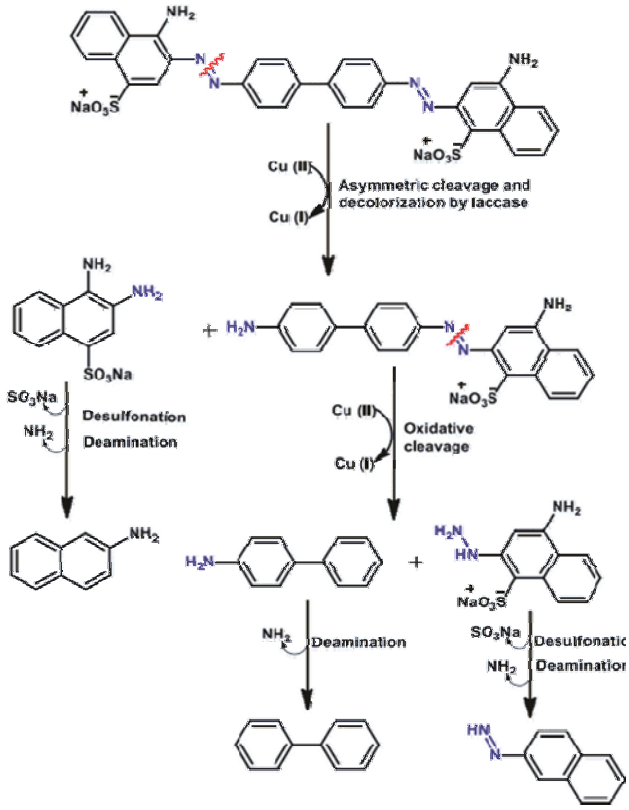

Fig. 8 — Potential reaction pathway for degradation of Congo red dye[79]

from indigo by the enzyme is illustrated in Fig. 9, which relies on the oxygen utilization rate of the laccases undergoing indigo degradation. In addition, Morsey *et al.*[88] provide a clear explanation of the malachite green dye degradation by laccase, as depicted in Fig. 10. It shows the oxidation of the dye structure, specifically the substrate, solely through the action of oxygen with laccase as a catalyst. During oxidation, the dye experiences electron depletion, with the electron being transferred between

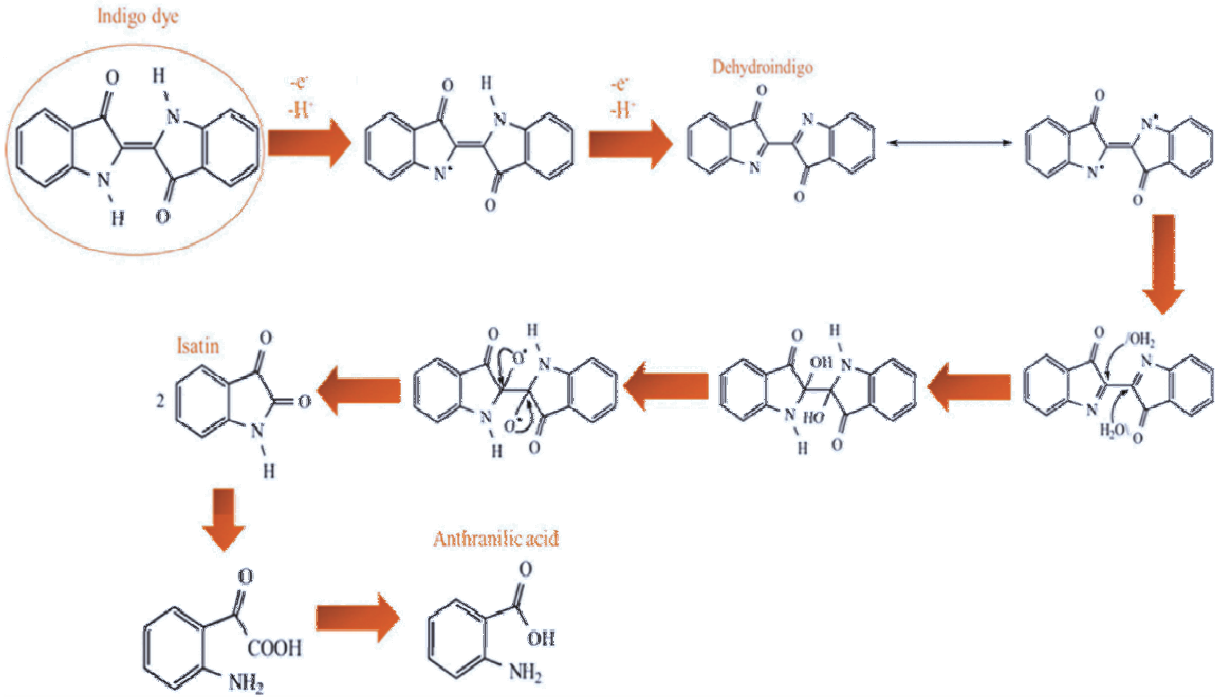

Fig. 9 — Mechanism for laccase-catalyzed degradation of indigo dye[4,83]

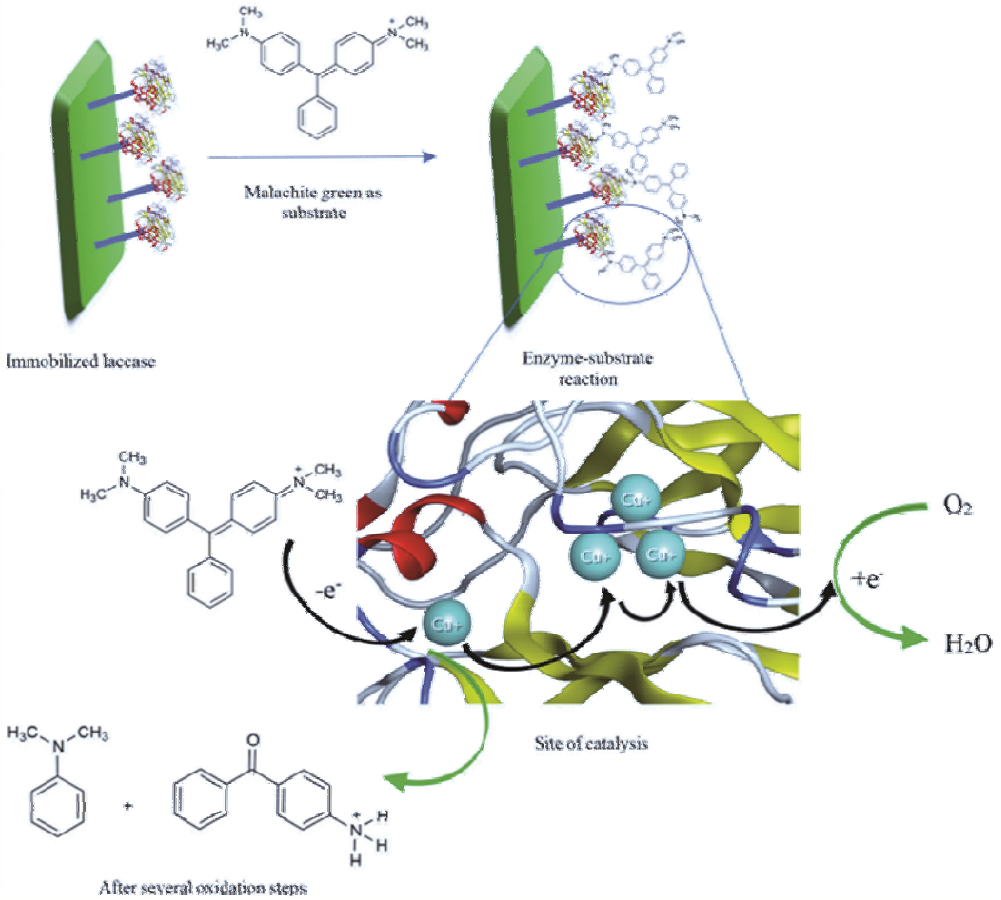

Fig. 10 — Possible malachite green dye degradation by immobilized laccase[88]

Cu^{2+} atoms inside the catalytic site. This process persists until oxygen is ultimately diminished, leading to the liberation of water.

6.2 Impact of Different Parameters on Dye Decolorization

*6.2.1 Role of p*H

The *p*H level significantly affects the decolorization of dyes using ligninolytic enzymes[89–91]. Laccase, both with and without mediators, demonstrates superior decolorization behaviour at acidic *p*H. Decolorization is reduced at neutral *p*H and minimal at alkaline *p*H, likely due to the laccasede activation at neutral *p*H, leading to denaturation. Denaturation occurs when enzymes lose their structure due to external stress or the presence of strong acids or bases. Kokol *et al*.[92] proposed that dye effluents typically have a high alkaline *p*H because of the presence of auxiliary compounds. This limits the direct use of fungal cultures, which are effective under acidic conditions only.

6.2.2 Effect of Temperature

The behavior of dye decolorization is also influenced by temperature. Reduced enzyme-substrate interactions and slower reaction rates may diminish laccase activity at lower temperatures. Dye molecule decomposition may lag, decreasing degradation efficiency. Conversely, raising the optimum temperature boosts enzyme activity and reaction kinetics, which ultimately leads to increased dye degradation rates and efficiency. Thermal energy enhances substrate molecule mobility, laccase-substrate interactions and laccase enzyme catalysis. Optimum results can be obtained at 35-40°C. Further increasing the temperature reduces dye decolorization. The probable reason for this could be enzyme inactivation at higher temperatures. This temperature-dependent behavior of dye decolorization is supported by many researchers[93–96].

6.2.3 Effect of Dye Concentration

Due to decreased dye competition for laccase enzyme active sites, the breakdown of dye molecules may be faster at lower dye concentrations. Thus, laccase enzymes degrade dye molecules efficiently. However, as dye concentration increases beyond a specific limit, various factors affect degradation. At higher dye concentrations, laccase enzyme active sites may become saturated with dye molecules, slowing degradation. As substrate-limited, degradation efficiency may plateau or decrease. Additionally, substrate inhibition or non-specific dye-laccase interactions at high dye concentrations can decrease laccase activity. This can further limit degrading efficiency. Mass transfer restrictions can also hinder dye molecule migration to laccase-active sites at high dye concentrations. This can reduce laccase-substrate interaction and degradation efficiency. Such phenomena are well-documented inthe literature[97–101].

6.2.4 Effect of Laccase Concentration

Laccase concentration affects dye removal efficiency, which is crucial for optimizing laccase mediator systems for dye decolorization. Increasing laccase concentration improves dye removal until a threshold is reached. Enzyme sites may restrict dye degradation at low laccase concentrations. This could reduce dye removal efficiency. Increasing laccase concentration within a particular range allows more enzyme molecules to oxidize dye molecules, improving dye removal effectiveness. The substrate saturation, enzyme-substrate interaction kinetics and inhibitory effects can affect the effectiveness of laccase activity. High laccase concentrations may also cause enzyme inactivation. To determine the best laccase concentration for dyeing processes and wastewater treatment, optimization entails systematic experimentation and analysis. An investigation demonstrated that as the concentration of laccase increases, the concentration of dye decreases, with all the used mediators exhibiting decolorization of the dye. Augmenting the enzyme concentration to 2.5 g/L led to a rise in decolorization efficacy. Nevertheless, increasing the quantity of the enzyme did not improve the percentage of decolorization. Other researchers utilizing crude or refined laccase also made comparable observations[76, 90, 92].

6.2.5 Effect of Mediator Concentration

Mediator concentration impacts both laccase activity and decolorization of dye. Laccase enzymes have been demonstrated to have high activity when used in conjunction with mediators, such as ABTS [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)] or HBT (1-hydroxybenzotriazole), which promote electron transfer between the enzyme and substrate. At lower mediator concentrations, the laccase's capacity to transfer electrons to dye molecules is limited, hence restricting the decolorization of dye. The activity of laccase and the decolorization of dye increase within a specific range as the concentration of mediators

increases, as a greater number of mediator molecules promote electron transport. Elevated concentrations of mediator molecules can impede the performance of laccase by either hindering the reaction with the substrate or inducing undesired reactions, resulting in reduced laccase activity and diminished effectiveness in decolorization. The concentration of the mediator is optimized to maximize the laccase activity for the decolorization of dyes. According to the literature, the highest level of decolorization was achieved when the mediator concentration was 1.5 mM[51, 62, 80, 91, 102]. Nevertheless, the concentration of the mediator does not exert a substantial impact on the behavior of dye decolorization.

7 Conclusion

The laccase mediator system effectively decolorizes various dyes with different chemical structures. Multiple technologies for dye decolorization were discussed, highlighting the difficulty in selecting only one methodology. Instead, the industry should focus on choosing the most efficient and economical method, combining selective methods to obtain optimum results. It depends upon the nature of the effluent, which is very complex as it contains various types of dyes and auxiliary chemicals. Laccase treatment, along with the use of mediators, could help achieve a good extent of decolorization, resolving the environmental issues related to textile dyeing. The combination of laccase enzymes with mediators, such as ABTS or HBT, has led to great progress in effectively eliminating different textile dyes from wastewater streams. The efficacy of laccase in decolorizing textile dyes stems from its capacity to catalyze the decomposition of intricate dye molecules into smaller, less detrimental chemicals. This approach not only helps to treat textile dye effluent but also reduces the negative impact on the environment compared to traditional methods of dye removal. Furthermore, the adaptability of laccase enzymes to operate effectively across a broad spectrum of *p*H and temperature conditions, combined with the possibility of enzyme immobilization, increases their practical usefulness in various industrial environment.

However, further research is needed to improve the operational parameters to investigate the compatibility of laccase enzymes with various textile dye formulations and to evaluate the long-term stability and reusability of the enzyme systems. Addressing cost-effectiveness and scalability is crucial for the wider implementation of this dye decolorization technique in industrial textile dyeing processes. Overall, the incorporation of laccase mediator systems offers a sustainable and environment-friendly method to reduce the adverse effects of textile dyeing on the environment. This approach supports the adoption of cleaner production methods and the conservation of water resources for future generations.

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