From Single to Complex Pollutants: In-silico Tools to Decipher the Decisive Role of Basidiomycetous Fungi for Dye Transformation

Khushi Negi and Krishna Sundari Sattiraju*

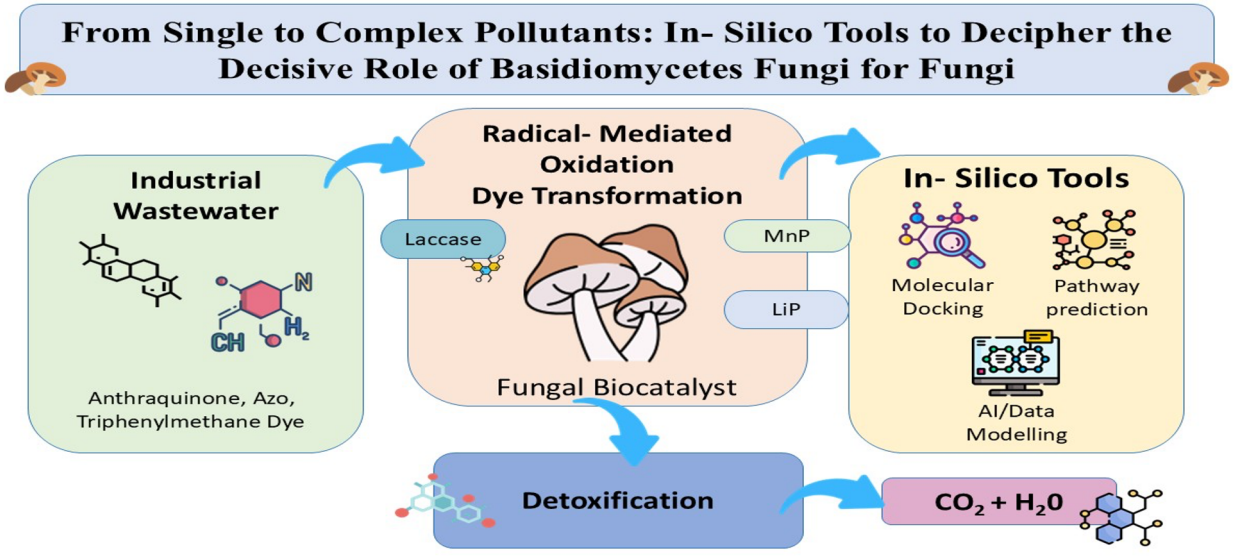
Department of Biotechnology, Jaypee Institute of Information Technology, A-10, Sector-62 201309, Naida, Uttar Pradesh

*Corresponding author: s.krishna.sundari@mail.jiit.ac.in

Abstract

The industrial discharge of synthetic dyes from textile production and related processes creates environmental contamination because these synthetic dyes exist as complex pollutant mixtures instead of single harmful substances. Basidiomycetous fungi serve as potential biocatalysts for dye transformation research because their extracellular enzymes function as ligninolytic systems which include laccases and manganese peroxidases and lignin peroxidases to activate oxidative processes that affect multiple aromatic compounds. The review studies how basidiomycetes use enzymes to transform dyes through three processes which involve radical oxidation and mediator reactions and the creation of metabolic intermediate products that exist in different dye categories. The research paper examines three analytical methods which include spectrophotometry and chromatography and spectroscopic techniques to assess how these methods can differentiate between apparent decolorization and actual chemical changes and mineralization processes. The research uses omics studies to produce system-level insights which show how extracellular oxidative processes connect with intracellular metabolic pathways. The assessment identifies methodological research limitations in current research through three main factors which include simplified experimental systems and limited metabolite identification and study variability. The research discusses three emerging computational methods which include molecular docking and biodegradation pathway prediction frameworks and machine-learning models as tools that support experimental studies by explaining how enzymes interact with substrates and how dyes transform in natural ecosystems.

Key words: Basidiomycetous fungi, Dye biodegradation, Mixed pollutants, Ligninolytic enzyme, In-silico analysis, Fungal bioremediation.



Graphical Abstract

1. Introduction

The textile and dye dependent industries have expanded rapidly which has led to increased synthetic dye waste that now contaminates waterways and creates ongoing environmental problems that affect the entire world. The chemical composition of synthetic dyes has been designed to maintain their color through industrial processing

because the dyes resist degradation from light exposure and temperature changes and chemical contact [1]. The dye-containing wastewater from textile production contains multiple chemicals because it includes dyes combined with salts and surfactants and auxiliary chemicals which elevate the chemical oxygen demand and environmental damage to water bodies that receive the wastewater [2]. The world produces hundreds of thousands of tons of dyes each year but a major portion of these dyes ends up in wastewater during dyeing processes [3]. The stable aromatic structures found in many dyes together with their xenobiotic properties enable these substances to remain in aquatic environments, which decreases underwater light transmission and hampers photosynthetic processes while creating dangers to both marine life and human beings [4], [5].

The existing research on dye remediation has not achieved its complete potential because scientists have concentrated their efforts on studying how individual dye molecules break down in artificial laboratory settings. Industrial effluents exhibit complex pollutant mixtures which comprise multiple dye types that belong to different structural categories together with heavy metals and inorganic salts and organic additives [6]. The research field of remediation studies needs to evaluate the distinctions that exist between studies which examine degraded single compounds and studies which analyze complex pollutant combinations. Dyes which exist as separate entities tend to exhibit degradation patterns that experts can forecast within basic testing environments. Human activities which operate through waste disposal produce environmental hazards because they release various pollutants into the atmosphere and water supply. The treatment process becomes more complex because different dye molecules interact to create transformation intermediates which differ from those produced in single-dye studies (Periyasamy, 2024).

The structural diversity of dyes and the chemical complexity of textile effluents therefore require treatment approaches capable of addressing multiple pollutants simultaneously rather than targeting individual compounds. The processes of coagulation and adsorption and oxidation represent common methods used in physicochemical treatment for colour removal. The processes of coagulation and adsorption and oxidation require treatment to remove their secondary pollutants and sludge residues which they create during operation (Oladoye et al., 2022).Bacterial metabolic systems display restricted capability to process various dye structures because of toxic effects and their lack of necessary enzymes to complete this task [9]. The process of color removal actually consists of decolorization and adsorption because the method does not fully break down the material which creates dangerous aromatic byproducts that remain in the treatment process.

Basidiomycetous fungi which include white rot fungi show potential as biological treatment systems that can convert complex organic waste materials into usable products. The fungi secrete oxidative enzymes which include laccases and manganese peroxidases and lignin peroxidases that demonstrate broad substrate range because they can oxidize numerous aromatic substances [10]. The enzyme systems developed through evolution to break down lignin, which exists as one of nature's most durable polymers, can also convert synthetic pollutants that share similar chemical structures with textile dyes. The research showed that fungal enzymes could break down and change various types of dye but the combination of fungal and bacterial systems provided better results for treating the resulting transformation products[11], [12].

However, current understanding of how fungi transform dyes through their enzymatic processes and their environmental impact and their capacity to degrade pollutants remains incomplete because researchers study specific enzymes and individual dye compounds. Research needs to develop new methods that integrate all three areas of study because scientists are starting to investigate how pollutants behave when multiple contaminants are present in their environment. The emerging computational and data-driven methods now enable researchers to study enzyme-substrate interactions while predicting how different dyes will be transformed. This review provides a summary of existing research on how Basidiomycetous fungi transform dyes while demonstrating how scientific understanding and new computer-based methods allow researchers to identify the essential function of these fungi in changing environmental pollutants from simple substances to complex mixtures.

2. Enzymatic Basis of Basidiomycete-Mediated Pollutant Transformation

2.1 Major Oxidative Enzymes Involved in Pollutant Transformation

Basidiomycete fungi are favoured with a potent extracellular oxidative enzyme system which can oxidize many structural classes of aromatic pollutant including xenobiotic monocyclic and polycyclic aromatic hydrocarbons, synthetic dyes and derivatives of petroleum-based hydrocarbons (Table 1). This has been found to be mainly apparent through the release of ligninolytic enzymes such as Lac, Mn P, and lignin peroxidase (Li P), which

exhibit a high redox potential and substrate scope capable of oxidizing complex xenobiotic compounds rich in aromatic structures that typically do not undergo other forms of biodegradation[13], [14].

Table 1. Representative studies reporting oxidative transformation of synthetic dyes by basidiomycetous fungi, highlighting enzyme systems, transformation mechanisms, and analytical evidence used to confirm degradation pathways.

Basidiomycete Species	Dye Type	Major Enzyme System	Key Transformation Mechanism	Analytical Evidence	Reference
Trametes versicolor	Azo dyes	Laccase	Radical-mediated oxidation leading to azo bond cleavage	HPLC, LC-MS metabolite analysis	[15]
Phanerochaete chrysosporium	Anthraquinone dyes	LiP, MnP	Aromatic ring oxidation and hydroxylation	GC-MS metabolite identification	[16]
Pleurotus ostreatus	Triphenylmethane dyes	Laccase	Demethylation and oxidative degradation	UV–Vis and HPLC	[13]
Trametes hirsuta	Indigo dyes	Laccase	Oxidative cleavage of indigoid structure	LC-MS and FTIR analysis	[17]
Irpex lacteus	Reactive azo dyes	MnP	Mn^{3+}-mediated oxidation of aromatic dye structures	HPLC metabolite profiling	[11]

Most microbial enzymes function intracellularly whereas the fungal ligninolytic enzymes are extracellular, so that the hyphal fungi can excavate themselves against huge, recalcitrant molecules. These enzymes catalyse radical oxidation reactions that rupture both aromatic rings and chromophoric groups in dyes. As a result, some basidiomycetes are able to degrade a variety of dyes systems such as azo, anthraquinone and triphenylmethane dyes [14], [18].

The enzyme laccase (EC 1.10.3.2), which belongs to the oxidoreductase class of enzymes, remains one of the most studied enzymes because it helps to decompose xenobiotic substances. The multicopper oxidase laccase functions through its oxidation process which removes electrons from phenolic and aromatic substrates while reducing molecular oxygen to water. The enzyme has four copper atoms which reside in sites that create catalytic centres and enable electron transfer from the substrate to oxidation. The active site of the enzyme receives electrons which result in the production of reactive radical intermediates that start the process of degrading aromatic compounds. Laccases demonstrate the ability to oxidize multiple xenobiotic substances because they can oxidize various synthetic dye classes (Legerská et al., 2016).

Laccases have important environmental value because they are used in multiple applications for dye decolorization and detoxification purposes. The research shows that higher laccase activity leads to greater dye removal success in basidiomycete fungi because this enzyme functions as a key component in their biotransformation processes [19]. The study demonstrates that both recombinant and engineered laccases exhibit better dye transformation performance through their improved stability and catalytic abilities which make them suitable for industrial wastewater treatment applications [20]. The laccase enzyme works together with manganese peroxidase to break down aromatic pollutants. The MnP enzyme oxidizes Mn^{2+} to Mn^{3+} through its reaction with hydrogen peroxide which produces oxidizing complexes that diffuse and react with phenolic substances found in complex molecules (Sosa-Martínez et al., 2020).The lignin peroxidase enzyme demonstrates high redox potential which enables it to oxidize non-phenolic aromatic structures that other enzymes cannot process [13]. The

combined action of these enzymes creates the ligninolytic enzyme system which enables basidiomycetes to break down xenobiotic compounds with different structural characteristics.

Research studies about fungal dye degradation demonstrate the effective functioning of this enzyme system because oxidative enzymes transform complex dye molecules into simple metabolites which include maleic acid and isophthalic acid, thus proving that aromatic structures undergo biodegradation [21]. The transformation reactions demonstrate how fungal enzymatic systems convert persistent synthetic compounds into less harmful intermediates which then enter central metabolic pathways. The ability of ligninolytic enzymes to act as catalysts for multiple reactions goes beyond their capacity to treat dye pollutants. The polluted sites contain aromatic pollutants which have molecular structures that resemble the chromophores used in dyes. The BTEX compounds which include benzene toluene ethylbenzene and xylene possess stable aromatic ring structures which can be broken down through oxidative processes. The scientific community links BTEX biodegradation to bacterial monooxygenases while research shows that fungal laccases and peroxidases can oxidize aromatic hydrocarbons through mechanisms that use radical-based processes [11]. The overlap between these functions shows that basidiomycete enzymes can break down both dyes and aromatic hydrocarbons which exist together in complex pollutant mixtures.

Overall, basidiomycetes produce extracellular oxidative enzymes which create a strong catalytic system that enables the degradation of difficult to remove pollutants. The fungi use their three enzymes which include laccase, manganese peroxidase, and lignin peroxidase to start oxidative processes that break down complex aromatic compounds. The bacteria use their extracellular enzymes to transform pollutants but researchers still need to study how these enzymes interact with cellular oxidation processes and intracellular metabolic pathways. Researchers need to study how extracellular processes interact with intracellular functions to understand how fungi break down pollutants.

2.2 Role of Extracellular and Intracellular Processes and Uncertainties in Degradation Mechanism

The extracellular oxidative enzymes serve as the main catalytic systems which basidiomycetes fungi use for pollutant degradation, yet their ability to digest complex xenobiotics requires their extracellular enzymes to oxidize materials which their cells then need to metabolize [22], [23]. The white rot fungi produce a collection of lignin-modifying enzymes which include lignin peroxidase and manganese peroxidase and laccase during their first stage of processing complex pollutants which include synthetic dyes and heavy aromatic structure dyes because this enzyme system breaks down complex aromatic structures into radical intermediates [22], [23].

Extracellular oxidation creates a critical issue because xenobiotic compounds cannot penetrate fungal cell membranes which block their entry due to their massive size and intricate molecular structure. The cell enzymes conduct non-specific oxidative reactions which produce compounds with lower molecular weight that become suitable for cellular metabolic processes [24]. The research with *Pycnoporus spp., Trametes spp., Pleurotus spp.* white rot fungi demonstrates that the presence of aromatic pollutants leads to higher ligninolytic enzyme production.

The removal of pollutants by fungal organisms requires research into two factors which are biosorption and the interactions between their cell surfaces. Fungal cell walls which are made up of chitin and glucans and proteins have demonstrated their ability to remove dye molecules through electrostatic binding and hydrophobic interactions which enable chemical adsorption and accumulation on the fungal surface (Rodríguez Couto, 2009).The process creates a system which promotes fast pollutant degradation through its ability to concentrate pollutants at the active sites of enzymes within their natural environment [26]. The existing scientific progress has failed to resolve multiple fundamental mechanisms which still present challenges to researchers. The experimental systems face challenges which make it hard to determine whether pollutants get removed through enzymatic degradation or through their combination with fungal biomass or through both pathways. The metabolic pathways which lead to metabolic products and their subsequent environmental impact through oxidation remain unknown [27], [28]. The existing uncertainties prevent researchers from obtaining complete knowledge about how fungi break down different materials.

The standard physicochemical treatment methods used in industrial waste management fail to effectively eliminate complex structural dyes from industrial waste streams which demonstrates the need for biological treatment methods that include fungal bioremediation (Robinson et al., 2001; Singh & Arora, 2011). The development of

effective basidiomycete biocatalytic systems for complex pollutant remediation requires researchers to comprehend how extracellular oxidation, biosorption, and intracellular metabolism work together.

3. Evidence Supporting Enzymatic Transformation Pathways

3.1 Laccase- and Peroxidase-Mediated Dye Transformation Mechanisms

The transformation of synthetic dyes by basidiomycete fungi is primarily driven by extracellular ligninolytic enzymes, particularly laccases and heme containing peroxidases such as manganese peroxidase (MnP) and lignin peroxidase (LiP). Multiple experimental studies demonstrate that dye removal is closely correlated with the production and activity of these enzymes, providing strong mechanistic evidence that enzymatic oxidation rather than simple adsorption is responsible for pollutant transformation. Enzyme activity assays frequently reveal that increases in laccase or peroxidase activity coincide with enhanced dye decolorization, confirming their direct catalytic involvement in oxidative degradation processes [15], [31].

Laccase (EC 1.10.3.2), a multicopper oxidase, is another enzyme isolated from many of the white rot fungi which catalyses the one electron oxidation of phenolic and aromatic substrates while the reduction of molecular oxygen to water. As a result of this action highly reactive radical intermediates are produced that decompose complicated dye molecules. Radical intermediates then undergo subsequent cleavage, polycondensation, or rearrangement before a structural modification and detoxification of dyes are achieved. Enzymes with broad specificity towards a wide range of azo, triphenylmethane and phenothiazine dyes structures explains the broad spectrum biocatalytic activity of basidiomycete fungi that are believed to possess in dye degradation (Abadulla et al., 2000; Chang et al., 2021; M. F. Khan et al., 2023).

Alongside laccases, fungal peroxidases such as MnP (EC 1.11.1.13) have also been shown to be involved in dye oxidation due to their capacity to catalyse radical mediated processes, given their high redox potential. For example, in the presence of hydrogen peroxide, Mn P oxidizes Mn 2 to Mn 3 , which is a diffusible oxidising agent able to attack phenolic substrates, facilitating oxidative degradation of dye molecules [32]. Several trials have successfully shown 80 99% decolorization levels for high Mn P activity cultures, confirming a central role of this enzyme in dye transformation systems [15].

LiP another key enzyme involved in the microbial transformation of dyes. Compared to MnP, LiP has very high redox potential (~1450 mV) which can oxidise nonphenolic aromatic compounds and initiate cleavage of CC and ether bonds on even the most complex structures of dyes [34]. The catalytic cycle of LiP starts with hydrogen peroxide mediated enzyme activation, followed by formation of reactive intermediates (the so called compound I and compound II), which then catalyses aromatic dyes through different types of electron transfer reactions in successive steps [35]. There are also experimental evidences showed LiP degraded dyes such as methylene blue rapidly after a few minutes at optimum conditions (Ferreiraleitao et al., 2007).

Basidiomycete fungi transform synthetic dyes through extracellular ligninolytic enzymes such as laccases, manganese peroxidases (MnP), and lignin peroxidases (LiP), which initiate radical-mediated oxidation of dye chromophores. The general pathway of fungal dye transformation, including formation of aromatic intermediates and subsequent ring cleavage leading to smaller metabolites, is illustrated in Figure 1.

Additional mechanistic evidence for dye transformation by enzyme can also be obtained through analysis of degradation intermediates, identified chromatographically and spectroscopically. These show that the degradation process involves an enzyme catalysed oxidation with chromophoric groups broken down to produce smaller aromatic components, consistent with actual chemical change rather than just bleaching [36]. Sometimes, inhibition of enzyme activity with compounds like sodium azide or EDTA, also results in the suppression of both enzyme activity and dye degradation.

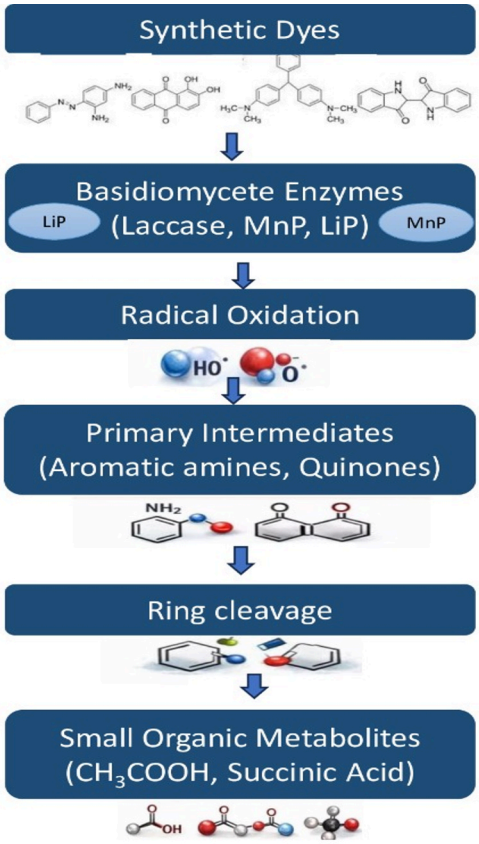


Fig1: Mechanistic pathway of fungal dye transformation[13], [26]

3.2 Mediator-Assisted Oxidation and the Influence of Dye Chemical Structure

Though ligninolytic enzymes such as laccases oxidize a broad spectrum of phenolic compounds, the majority of the textile dyes synthesized are not directly oxidized by the enzyme due to their high redox potential or steric hindrance afforded by aromatic moieties. Laccase mediator systems (LMS) have been extensively studied as an alternative method to broaden the oxidation spectrum of oxidative enzymes. Through this species, the enzyme oxidizes a mediator compound, which is capable of oxidizing molecules that do not have access to the enzymatic active centre, thus providing a means to degrade xenobiotic dyes that are structurally complex [37], [38].

A number of synthetic mediators have been studied to enhance the efficiency of enzyme catalysed oxidations. The mediators, 2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonate) (ABTS), 1hydroxybenzotriazole (HBT) and violuric acid have been found to act as redox shuttles which can transfer the oxidative equivalents from the enzyme to a higher redox potential substrates, the various dye classes of azo, anthraquinone and triphenylmethane dyes can then be oxidised by these reactive mediators through the oxidation pathways of either a radical mediated electron transfer or hydrogen atom abstraction [38], [39]. Several reports have shown that mediator amended systems have greatly increased dye decolourisation efficiencies and expanded the substrate range of laccases (Kumar et al., 2014; Moreira et al., 2014; Şaşmaz et al., 2011).

In addition to synthetic chemicals, there have been reports of natural phenolic mediators produced by lignin degradation, including syringaldehyde, acetosyringone and vanillin, to enhance laccase mediated oxidation by producing phenoxy radicals for attacking macro dye molecules [37], [43]. As would be expected, natural mediators are of particular interest due to their better environmental compatibility and relatively lower toxicity than synthetic mediators.

The effectiveness of mediator assisted oxidation depends heavily on the chemical composition of dye molecules. Numerous dyes used in industry have aromatic rings and chromophoric groups such as azo (N=N) double bonds which are particularly difficult to break down in effluent conditions [37], [43]. Oxidative enzymes Oxidative enzymes, such as laccases and peroxidases, are capable of attacking the aromatic rings through radical mediated

pathways and can be used in the degradation of persistent dyes pollutants [44]. A variation in mediator chemistry, enzyme source and dye structure may produce varying performance of these enzymes in studies.

Overall, mediator-assisted oxidation significantly expands the catalytic capabilities of fungal oxidative enzymes and represents a critical mechanism for the enzymatic transformation of structurally diverse dye contaminants.

4. Transformation Pathways of Dye Pollutants by Basidiomycete Fungi

4.1 Oxidative transformation of major dye classes

The enzyme mechanisms described in Section 3 show that basidiomycete fungi are able to oxidise many types of aromatic pollutants using their extracellular oxidative enzyme systems. The oxidising enzymes, especially laccases and ligninolytic peroxidases, catalyse the conversion of the dye molecules' chromophoric groups by radical oxidation reactions that then destabilise the conjugated aromatic structures [45], [46]. As a result, complex dye molecules undergo progressive structural modification that ultimately leads to the formation of smaller aromatic intermediates.

However, the most widely used synthetic dye class employed in the textile industries is the azo dye group. These dyes contain an azo group connecting two aromatic rings, which act as the main chromophore and provide the color of the compounds. Breakdown of the azo bond is an important step in the degradation process and often yields aromatic amines as precursors to subsequent metabolic steps [47], [48]. Other reports on microbial degradation pathways include formation of Benzene derivatives and Naphthalene structures as is evidenced by byproducts formed during azo bond cleavage as illustrated by intermediates formed in the degradation pathways of dyes [49], [50].

Another major class of industrial colorants are the triphenylmethane dyes which includes the industrial pigments crystal violet and malachite green. Triphenylmethanes contain extensive conjugation within the aromatic moieties which is responsible for their resistivity in the environment (table 2). In fungal degradation, oxidative enzyme systems catalyse a series of demethylation and hydroxylation reactions resulting in destabilization of the central chromophore of the dyes. Laboratory biodegradation experiments have shown that crystal violet will be degraded by microbial and enzymic activity to a series of intermediate compounds. [11], [50]. Similar oxidative reactions have been observed in fungal systems capable of transforming triphenylmethane dyes into smaller aromatic compounds (Kariminiaae-Hamedaani et al., 2007).

The conjugated aromatic ring systems of the anthraquinone dyes have led to the assumption that they are more resistant to biodegradation. However, the oxidative enzymes involved in fungusmediated dye degradation may also catalyse hydroxylation and ring-modification reactions that destabilize molecules more slowly. It has been reported that fungal dye remediation has led to the production of intermediate aromatic metabolites precursors after enzymic oxidation of anthraquinone dyes [45], [49].

Table 2. Relationship between structural features of major synthetic dye classes and their susceptibility to oxidation by basidiomycete ligninolytic enzymes. The table highlights how molecular structure influences enzymatic transformation pathways and degradation mechanisms.

Dye Class	Key Structural Feature	Representative Dye Examples	Dominant Enzyme System	Major Oxidative Reaction	Typical Transformation Intermediates	Reference
Azo dyes	–N=N– azo linkage connecting aromatic rings	Congo red, Reactive Black 5	Laccase, MnP	Single-electron oxidation leading to azo bond cleavage	Aromatic amines, phenolic intermediates	[15]
Anthraquinone dyes	Quinone-based polyaromatic structure	Remazol Brilliant Blue R	LiP, MnP	Hydroxylation and quinone reduction	Hydroxyanthraquinones	[16]

Triphenylmethane dyes	Central carbon bound to three aromatic rings	Malachite green, Crystal violet	Laccase	Oxidative demethylation and radical formation	Benzophenone derivatives	[13]
Indigo dyes	Conjugated aromatic heterocyclic system	Indigo carmine	Laccase	Oxidative cleavage of indigoid structure	Isatin derivatives	[17]
Reactive textile dyes	Sulfonated aromatic rings with reactive groups	Reactive Orange dyes	MnP, Laccase	Radical-mediated oxidation and bond cleavage	Aromatic sulfonated intermediates	[11]

4.2 Formation of transformation intermediates and degradation pathways

After the first disruption of chromophoric groups, degradation of dyes involved a series of sequential biochemical reactions resulting in formation of intermediate metabolites and decrease in molecular structure. The intermediates are acknowledged as proof that the dye molecules undergo actual chemical transformation, instead of mere colored molecule adsorption or decolorization. It has been shown that aromatic intermediates such as phenolics, aromatic rings of benzene and naphthalene appear during microbial degradation of dyes. [47], [49]. Identification of these metabolites has enabled reconstruction of degradation pathways for multiple dye classes.

In the case of azo dyes, the common cleavage of azo bonds yields to aromatic amines that can be subsequently oxidized. Sequential biodegradation experiments revealed that under microaerophilic conditions, azo dyes are first reduced to aromatic amines; an aerobic phase is then required for their further mineralization into simpler aromatic compounds [48], [50]. These multi-step reactions demonstrate the complex nature of dye degradation pathways and highlight the influence of environmental conditions on the fate of degradation intermediates.

Sometimes the extracellular enzymatic oxidation of dyestuffs occurs prior to penetration of the fungal cell and the formation of intermediate breakdown products which are then transported to the inside of the cell to undergo subsequent metabolism. The coupling of an extracellular oxidation pathway to an intracellular pathway would allow the conversion of complex dye molecules into smaller metabolite units, which could then be transported into the cells for breakdown, as seen in many basidiomycete fungi (Dahiya et al., 2023; Wesenberg et al., 2003).

It is also noteworthy that the generation of intermediate metabolites are factors of measurement of the treatment efficiency. Although the broken chromophoric groups ensure the decolorization as seen to naked eye, the intermediates could be more resistant and further degraded until the complete mineralization. Hence, the monitoring of the intermediates is critical in discerned between the degradation and partial transformation.

5. Interpreting “Dye Remediation”: Decolorization, Transformation, or Degradation?

5.1 Analytical endpoints used to evaluate pollutant removal

It is important to note that visual removal of color during dye remediation studies cannot easily be equated to complete degradation of the dye molecules and thus must be carefully interpreted from an analytical perspective. Most remediation studies use UV-vis spectrophotometry during the initial visual studies in order to determine the reduction in absorption peaks that correspond to dye chromophores. As an analytical method for decolorization and subsequent dye remediation, this method provides a rapid way of monitoring dye appearance, but only identifies changes in the chromophoric regions of the molecule [51], [52]. Additional analytical approaches are to ascertain whether true biodegradation takes place.

Alternatively, chromatic and spectroscopic analysis tools such as High Performance Liquid Chromatography (HPLC), Gas Chromatography (GC) coupled with Mass Spectrometry (MS), and Fourier Transform Infra Red (FTIR) are often used to confirm the structural modification of dyes. For example, FTIR, GCMS and HPLC

analyses confirmed several benzene, biphenyl or naphthalene type intermediates, resulted from degradation of several azo dyes by *S. albidoflavus* via breakdown of the azo bonds [47]. More recently, the structural alteration of dye molecules and the consequent formation of aromatically smaller molecules could be identified using FTIR and GCMS analysis, which lends support to the degradation pathway (Bera et al., 2022; Lade et al., 2015).These reflective characterisation techniques can be used to conclusively establish dye transformation reactions over simple decolorization or physical removal.

Alongside characterization of the degradation products formed, a number of general environmental performance parameters are widely used as semiquantitative measures of extent of degradation of the pollutant. Parameters such as chemical oxygen demand (COD), BOD and total organic carbon (TOC) are widely assessed following microbial treatment of textile dyes, as they provide indicator of the extent of organic load reduction, following degradation of complex aromatic molecules to simpler metabolites (Bera et al., 2022; Lade et al., 2015).General environmental performance of the degradation process is also frequently monitored on textile wastewaters undergoing biological/enzymatic remediation [51], [53].

Furthermore, enzymatic systems using ligninolytic enzymes, particularly laccases, have been successfully applied to the decolorization and detoxification of complex textile wastewater effluents. Such analytical monitoring of treatment has revealed significant decrease in the concentrations of residual dye and toxicity, emphasizing the need to evaluate dye pollutant removal based on a combination of spectroscopic, chromatographic and indicator variables from the environment [45]. All these analytical variables provide complementary end points that can be used to differentiate apparent colour removal from chemical transformation versus true biodegradation of a dye pollutant.

5.2 Evidence for mineralization and environmental implications of misinterpretation

While there are many reports of relatively high removal efficiencies, a problem with interpreting these findings is that decolorization does not equal mineralization. In many microbial degradation systems, the first step in azo bond cleavage is the formation of aromatic amines and various other intermediates that are often environmentally long lived or toxic. In a number of cascade biodegradation studies, azo dyes were degraded under microaerophilic conditions but had to be further degraded under aerobic conditions for complete oxidation and detoxification of the aromatic amines formed [50]. This is an example of where readers need to be careful about the difference between partial functional group transformation and complete degradation.

Evidence of actual mineralization is generally given as a significant decrease in TOC, COD and BOD, showing that the polyaromatic dye molecules have been broken down into less complex compounds such as CO_2, H_2O and inorganic metabolites. For instance, in microbial consortium-based treatment system, substantial to very high reduction in TOC and COD has been observed with degradation of azo dyes manifesting breakdown of polymeric structure of aromatic dye molecules and diminishing organic load in reaction medium [49], [54]. Similar results have also been reported in microbial degradation of textile dyes where an indication of reduction in organic load with respective dye polymeric degradation was observed to metabolites of diminished molecular weight [47], [50].

In addition to their mineralization potential, toxicity testing is also important to assess whether degradation products are still toxic to living systems. It has been found that enzymatic treatment of textile effluents employing laccase based systems greatly reduced in mutagenicity and toxicity upon dye degradation, implying that enzymatic oxidation could further transform structurally complex dye molecules into less toxic species [45]. Similar findings of reduced toxicity to aquatic animals and environmental safety upon microbial biodegradation of azo dyes and its intermediates have been reported [50].

Misinterpretation of removal efficiency will be distorted when researchers only examine spectrophotometric decolorization results. Textile dye effluents typically contain complex mixtures of dyes, auxiliaries, and transformation products, and incomplete degradation will result in the toxic intermediates remaining present in treated wastewater [51], [52]. The environmental effectiveness of biological dye remediation strategies requires complete analytical assessment which includes metabolite identification and mineralization indicators and toxicity assays.

6. Methodological Limitations and Knowledge Gaps

6.1 Experimental and analytical factors that inflate remediation claims

Although there is growing literature on fungal degradation of textile dyes, several recurring trends in the methodology which may artificially exaggerate remedial claims. Several studies evaluate the transformation of a single dye, often under controlled laboratory conditions, which do not replicate the industrial textile wastewater used in industry; wherein a multitude of dyes, salts, heavy metals, and other auxiliary chemicals are present. Textile dye effluents are chemically complex, and this can drastically impact the degradation efficiencies of microbes yet this is rarely tested in the laboratory [55][7].

A further significant limitation to many dye remediation studies is the common practice of using spectrophotometric decolorization as an indicator of removal efficiency. Despite being visually quantifiable, reduction in colour may not accurately reflect degradation of the dye to nontoxic components or degradation to uncoloured intermediates. This is particularly important in fungal remediation systems where dye removal may to result from enzymatic oxidation, bioaccumulation or by biosorption to the fungal biomass[56]. Experimental data has demonstrated that the dye removal pathway in fungal systems may be a mixture of biosorption and enzymatic degradation by lignin enzymes including laccase and manganese peroxidase[57].

Furthermore, the experimental setup also isn't representative of a typical environmental system. Numerous experiments are conducted under optimized conditions, with high fungal biomass concentrations, ideal pH values and extended incubation times, all of which would be unlikely to be present in a genuine wastewater environment. These variables have a profound effect on enzyme activity and pollution pathways[58].

6.2 Gaps in metabolite identification, toxicity assessment, and cross-study comparability

In addition to challenges associated with experimental setup, there are considerable gaps in knowledge with respect to reactions and the effects of degradation intermediates. Furthermore, despite the absence of a characterization of degradation products, some studies simply refer to "dye removal". However, degradation of azo and other aromatic dyes can produce intermediate chemicals such as aromatic amines that can be more toxic or equally toxic than the parent compounds[51].

Toxicity assessment is also limited in the fungal remediation literature. Some experiments do assess either phytotoxicity or ecotoxicity, but these are not uniformly performed (Plate, 2002), and indeed experimental work has revealed that decolorization as measured does not necessarily equate to detoxification, some degradation pathways result in intermediates that are more toxic[59]. An integrated approach to the chemical and biological assessment of the application of fungi in remediation is needed to consider toxicity and metabolite formation [23].

A further drawback concerns the lack of standardization of methodology used within studies. Use of various dyes and strains, reactor setup as well as analytical methods used in each paper prevents direct comparison of the degradation efficiencies reported in literature. Differences in nutrient, oxygen availability and dye concentration have been known to considerably alter fungal metabolic activity and enzymatic function[60]. It is therefore apparent that it remains difficult to compare results from different studies and that current understanding of fungal dye degrading capacity is fragmented; more standardized and indeed integrated experimentation is required to reliably assess the environmental significance of fungal bioremediation systems.

7. Emerging Role of In-Silico and Data-Driven Tools in Deciphering Basidiomycete-Mediated Dye Transformation

7.1 Omics Derived Insights and Challenges in Systems Level Interpretation of Biodegradation

Rapid developments in the omics arena have greatly improved our insights into the processes of fungal contaminant transformation by allowing genome wide investigations of enzyme networks and metabolic pathways. Genomics, transcriptomics, proteomics and metabolomics together have provided important information on the ways in which basidiomycete fungi orchestrate the degradation of complex aromatic pollutants including dyes and other xenobiotic substances.

Genomic analyses show that white rot fungi have large and diverse sets of genes encoding lignin degrading enzymes and auxiliary redox proteins. Integrative genomic analyses of lignin degrading fungi has identified numerous genes encoding class II peroxidases, laccases, oxidases and other redox enzymes responsible for oxidative degradation of aromatic polymers[61]. Such gene families are often found to be significantly expanded in basidiomycete genomes in line with their adaptation to, and ecological specialization for, lignocellulose decay and xenobiotic transformation[62].

Transcriptomic approaches offer additional information about the regulatory responses that appear to accompany pollutant exposure. Largescale transcriptional reprogramming occurs following fungal exposure to dye molecules and aromatics, and ultimately induces the ligninolytic enzymes, detoxification pathways, and oxidative stress responses[63]. In particular, transcriptomic studies of dye degradation have identified upregulation of genes for the production of manganese peroxidases, laccases, cytochrome P450s, and the glutathione detoxification system[64].

The above-mentioned results are further corroborated by proteomic approaches, which examine active enzymes engaged in the key steps of contaminant degradation. Proteomic analysis of white rot fungi treated with dye-based materials suggests that dye degradation by fungi involves rather an abundance of enzymes related to oxidoreduction, detoxification, and stress response pathways[65]. This suggests the fungal degradation machinery may be using complex enzyme network rather than individual reactions.

These recent developments do not address some key shortcomings in the systems level interpretation of fungal pollutant degradation. Color measurements continue to be the norm in many experiments, with a limited, or even lacking, examination of degradation products or pathways, a deficiency that is especially problematic in light of the well documented fact that decolouration dyes do not therefore lead to complete degradation or detoxification of the compound within comparison to the original. Variability in species, pollutants, and experimental conditions also ensure that many omics datasets are not directly comparable. However, the application of multiomics technologies will remain to be important in furthering our understanding of biocatalytic mechanisms utilized by fungi. The use of coupled enzymatic, metabolic, and systems level molecular datasets is likely to furnish a more detailed and accurate conceptual model explaining pollutant biotransformation by basidiomycete fungi.

7.2 In-silico analysis of enzyme–dye interactions and oxidative transformation pathways

The interpretation of complex dye molecular interactions with fungal oxidative enzymes increasingly incorporates computational structural studies as an adjunct to empirical results. Computer assisted modelling of the docking of dyes within the active pockets of fungal oxidoreductases allows the discernment of substrate orientation, catalytic accessibility and potential electron transfer routes during oxidative transformation. Docking studies on fungal laccases show aromatic dyes may complex with active residues of the type1 copper centre to give stable hydrogen bonding and hydrophobic interaction and therefore electron transfer during oxidation[66]. These structural explanations can also rationalise the reported broad substrate specificities of fungal laccases and the structural interpretation of azo and triphenylmethane dye decolourisation.

There is also a rising use of in silico techniques for predicting pathways of oxidative transformation of aromatic xenobiotics, for example textile dyes. The Biochemical Network Integrated Computational Explorer (BNICE) reaction network prediction framework works by applying a set of generalized enzymatic reaction rules to preexisting enzyme substrates, generating a model of possible degradation pathways from a given chemical structure[67]. This coordinated pathway prediction method enables the plausible formation of key oxidative intermediates in xenobiotic azo dye degradation including azo bond cleavage, aromatic hydroxylation and ring opening reactions to be identified for comparison to fungal degradation pathways. A similar modelling framework has been implemented to predict degradation of contaminants in environmental treatment systems, and predict dye transformation under different physicochemical parameters[68], [69]. Pathways combining enzyme molecular docking prediction with a metabolic scheme-based prediction framework therefore holds some promise for understanding in vivo dye transformation mechanisms.

7.3 Data-driven modelling and computational interpretation of fungal dye transformation systems

In addition to structural modelling, machine learning approaches offer a further avenue for interpreting the complex biodegradation data sets resulting from dye transformation experiments. Algorithms such as artificial neural networks, support vector regression models and ensemble learning models have been used to correlate microbial activity, parameters and degradation efficiency (Table 3) [70]. Such models can be used to analyse multivariate data sets simultaneously so that factors affecting dye degradation can be identified in wastewater systems. Similar computational models integrating artificial intelligence with process modelling have been employed to assess operational conditions affecting pollutant removal efficiency and predict the performance of complex wastewater treatment systems [71].

Table 3. *Computational and data-driven approaches used to interpret enzymatic dye transformation mechanisms, including molecular docking, biodegradation pathway prediction, and machine learning models applied to environmental pollutant degradation.*

Computational Approach	Methodology	Application in Dye Transformation Studies	Mechanistic Insight Generated	Reference
Molecular docking	Structural modelling of enzyme–substrate interactions	Analysis of laccase–dye binding orientation	Identifies catalytic residues and substrate accessibility	[66]
Biodegradation pathway prediction (BNICE)	Rule-based metabolic network generation	Prediction of dye degradation intermediates	Identification of plausible oxidative reaction pathways	[67]
Machine learning modelling	Artificial neural networks (ANN)	Prediction of biodegradation efficiency under varying environmental conditions	Identification of factors influencing degradation performance	[70]
AI-assisted wastewater modelling	Data-driven optimization of treatment systems	Prediction of dye removal in wastewater treatment processes	Process optimization and pollutant removal prediction	[71]
Integrated AI–biodegradation modelling	Hybrid computational frameworks	Evaluation of pollutant removal strategies in environmental remediation	Linking experimental degradation with predictive modelling	[65]

In recent assessments of dye remediation research, there has been evidence to suggest that a trend toward combining experimentation with computational modelling is developing, with artificial neural networks, support vector machines and combined machine learning methods used to predict pollutant removal efficiencies within various contextual environments [72]. These established, data driven techniques also aid in the interpretation of the experimental results by revealing correlations between structure, enzymology and environmental system variables. When integrated with molecular dockings studies and pathway prediction engines, these CAD tools systematize our understanding of the various scales at which dye transformation occurs from individual enzyme substrate complexes to wholes oil columns. This research paradigm may prove helpful, as the subject of environmental remediation becomes ever more complex in searching for solution strategies for mixed pollutant cargoes.

7.4 Enzyme expression patterns and coupling of extracellular and intracellular processes

The conversion process for complex aromatic pollutants by basidiomycete fungi depends on the synchronized work between their extracellular oxidative enzymes and their internal metabolic systems. The process of pollutant transformation begins through basidiomycete fungi which use their extracellular oxidative enzymes to produce laccases and manganese peroxidases (MnP) and lignin peroxidases (LiP) and versatile peroxidases that create extremely active radical species which can damage stable aromatic compounds[73], [74]. The enzymes demonstrate ability to oxidize multiple types of aromatic pollutants because they possess broad substrate specificity which includes synthetic dyes and lignin derivatives and polycyclic aromatic compounds[33]. White-rot fungi use their complete collection of oxidative enzymes and auxiliary redox partners to perform extracellular enzyme transformations according to genome-scale studies[61]. The genomic data shows that many basidiomycete species have developed extra gene families which contain oxidoreductases and peroxidases through evolutionary processes that allow them to break down complex aromatic polymers[61].

The final stage of pollutant transformation does not typically occur through extracellular oxidation processes. The process of oxidative cleavage produces intermediate compounds which undergo further transformation via cellular metabolic systems. The research on transcriptomics shows that dye substrates activate simultaneous gene expression of oxidative metabolism genes and detoxification genes and cellular stress response genes[63]. The researchers found multiple transcriptional modules connected to laccase activity through their gene co-expression network analysis of dye-degrading fungi[63].

Intracellular enzymatic systems are involved in the detoxification and further transformation of oxidation products, via enzymes such as cytochrome P450 mono oxidases, glutathione-S-transferases and reductases, which modify and cleave aromatic intermediates formed during extracellular oxidation processes[64]. In anthraquinone-degrading cultures, activities of MnP, the laccase, glutathione metabolism enzymes and cytochrome P system increased during the exposure of pollutants, signifying the tolerance driven by oxidative and metabolic detoxification[64]

Proteomic studies also show additional indication of enzyme networks working in concert to degrade pollution in fungi. For example, shotgun proteomics analyses of white rot fungi degrading dyes show that oxidoreductases, redox cycling enzymes, and stress response proteins were enriched while fungi were degrading dye[65]

The data from these various approaches, however, do lend support to a model of basidiomycete-mediated pollutant transformation involving initial extracellular oxidation coupled to subsequent intracellular metabolism. Such a coupling of oxidase mediated oxidation with subsequent intracellular metabolism would allow fungi to effectively transform a wide range of xenobiotic substrates of varying structure, without upsetting cell redox status or cell metabolism

8. Future Perspectives and Research Directions

Further advances in basidiomycete dye transformation will likely supplement enzymatic investigations with computational pathway prediction tools. As fungal systems are capable of removing dyes with a broad range of chemical architectures in an efficient oxidative decolourisation, often only a limited number of intermediate metabolites are definitively identified, if at all, at the end of the pathway. Computational systems biology approaches to colour dye degradation provide a powerful mechanism to interpret transformation pathways by predicting plausible reaction networks and generally uncharacterised intermediate compounds. For instance, systematic prediction of all possible reaction pathways from a given xenobiotic can be undertaken by reaction rule-based approaches such as the Biochemical Network Integrated Computational Explorer (BNICE), which has been used to assess the spectrum of possible oxidative decolourisation routes[67].Applying such theory in combination with technique development to support chromatogram-based metabolite identification may greatly improve mechanistic interpretation of basidiomycete dye decolourisation.

Structural modelling strategies are predicted to be fundamentally important in the molecular level understanding of enzyme dye interactions. Investigations into the structural basis for enzyme catalysis have focused on the application of molecular docking protocols which have shown aromatic dye molecules to be interacting with the catalytic residues around the type1 copper in fungal laccases through hydrogen bonding and hydrophobic interactions. These findings have provided a structural understanding of dye oxidation processes observed experimentally[66]. Applying these modelling techniques to other lignin degrading enzyme classes and mediator assisted oxidation processes could reveal differences in substrate access and catalytic efficiency between different dye classes.

In parallel, simple machine learning, data driven modelling strategies have been employed to unpick complex dye biodegradation data streams from environmental treatment systems. Artificial neural networks, and support vector regression, for example, have been employed to assess relationships between microbial activity, operational parameters, and pollutant removal efficiencies [70][71]. Combining these predictive approaches with experimental dye biodegradation studies could provide a framework for systematic assessment of fungal treatment systems and add to the development of predictive capabilities in dye transformation within more complex environmental matrices[72].

9. Conclusion

The basidiomycetous fungi are potent oxidative biocatalysts that enable degradation of structurally complex dye pollutants by means of extracellular lignin degrading enzymes. The extensive oxidatively promiscuous range of the fungal enzyme system affords reactions that have the power to degrade the complex aromatic dye structures and facilitate oxidative transformation and detoxification of environmental contaminants. The interpretation of the mechanistic studies of dye transformation is hindered by the difficulty in accurately quantifying many intermediates and tandem oxidative reactions.

This review highlights the need for combining experimental enzymology with an appropriate *in-silico* modelling framework as a means to obtain further insight into fungal dye transformation processes. *In -silico* techniques such as molecular docking and biodegradation pathway prediction algorithms enable analysis at the enzyme substrate level and enable mechanistic hypotheses to be made regarding enzymatic oxidative routes. Additionally, databased modelling offers a complementary approach through which to investigate large data sets derived from biochemical monitoring studies by revealing correlations between conditions, enzymology and degradation efficiencies.

As environmental research progresses from a single dye pollutant paradigm to one of a mixture of contaminants, the marriage of analytical evidence to a computational interpretation becomes even more pertinent. The integration of enzymatic experimentation, sophisticated analytical techniques and insilico approaches offers a comprehensive approach to understanding the contribution of basidiomycetous fungi to dye conversion and ultimately, the design of more pragmatic fungal biocatalytic systems for the green remediation of wastewater streams.

Eventually, as both the fields of environmental biotechnology and computer modelling converge more and further, the ability to identify fungal biocatalysts to solve ever more complex pollutants in contaminated ecosystems will be enhanced.

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