Degradation of Dyes Using Filamentous Fungi

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Abstract Dyes used in textile industry generate large amount of wastewater that needs to be treated effectively to prevent toxicity. Treatment of dye-rich wastewater by physico-chemical methods proves costly and less efficient, therefore biological methods (cost-effective and eco-friendly) have been developed. A variety of biological materials have been evaluated for their capacity to remove/treat toxic compounds such as dyes. Fungal biomass has shown high efficiency to remediate dye-rich wastewater. Fungi remove these compounds via decolorization and degradation. Reductive and oxidative enzymes assist in degradation of dyes. Many strains of filamentous fungi have shown high capacity to treat dyes. Hydrolytic enzymes secreted by filamentous fungi assist in the treatment of dyes. Lacasse enzyme produced by lignolytic fungi helps in the degradation of dyes. Enormous capacity of fungi for removal/remediation of dyes can be exploited to develop technologies for large-scale treatment of dye-containing wastewater.

Keyword Decolorization · Degradation · Dyes · Laccases · Lignolytic

1 Introduction

Dyes are chemical compounds (aromatic and heterocyclic) that impart color to materials. Dyes have been classified into different types depending on their chemical structure and chromophore groups present in them. Azo, diazo, cationic, basic, anthraquione and metal complexed dyes have been known [103]. The largest group (about 80%) of organic compounds used as colorants in textile industry has been identified as azo dyes. This is because these dyes can be synthesized easily and are chemically more stable and available in diversity of colors [25, 26].

About 7×10^5 tons of dyes are produced annually, of which approximately 2.8×10^5 tons of dyes have been released into water bodies [6, 58]. Synthetic dyes are complex structured, recalcitrant, xenobiotic, broad-spectrum chemicals, which

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S. S. Muthu and A. Khadir (eds.), *Dye Biodegradation, Mechanisms and Techniques*,
Sustainable Textiles: Production, Processing, Manufacturing & Chemistry,
https://doi.org/10.1007/978-981-16-5932-4_2

resist degradation [19–21, 64]. The presence of one or more azo groups in azo dyes prevents their breakdown and degradation, making their accumulation persistent in the environment. The release of such high amount of dyes in the aqueous environment exerts toxic effects on living biota (flora and fauna) [8, 9, 95, 97]. In the water bodies, dyes get accumulated in the aquatic organisms, pass to other organisms through food chain and finally reach man. Effluents rich in toxic dyes need to be treated suitably to prevent toxicity exerted by these compounds [108].

The breakdown of dye releases products that prove to be a potent carcinogen. Dyes also act as causal agents of various diseases/disorders such as skin cancer, dermatitis, perforation of nasal septum, damage to mucous membrane and irritation of respiratory tract. According to reports, accidental intake of dyes in humans causes vomiting, pain, hemorrhage and diarrhea [68]. Exposure to azo dyes produces life-threatening diseases in humans such as cancer of bladder, splenic sarcomas and hepatocarcinomas. Chromosomal aberrations, lipid peroxidation and inhibition of enzyme acetylcholinesterase in mammalian cells are the other effects produced by exposure to dyes [11, 85, 114]. Toxic effects induced by various dyes have been listed in Table 1.

Adsorption, filtration, coagulation, precipitation, oxidation, reduction, photolysis and photodegradation are some of the physico-chemical methods followed for years to remove/treat dyes from wastewaters [67, 91, 113]. These methods are costly, require the addition of hazardous chemical additives and produce large amounts of sludge (problems related to secondary pollution) [42]. In biological treatment method, microbes (such as bacteria, fungi, yeast), algae and plants (bioremediation) are used [97]. This method provides an eco-friendly cost-effective alternative to other technologies [65]. Biological treatment technologies have shown capacity to degrade organic pollutants. This is because of high potential of microorganisms such as bacteria, yeasts and filamentous fungi to remove dyes [116]. The main mechanism involved in removal of dyes includes adsorption on microbial biomass, biosorption and/or enzymatic degradation (biodegradation) [10, 49, 63, 107].

Mycoremediation has emerged as a technology with high efficiency for treatment of dyes [43, 45, 50, 101, 76]. Fungi have shown high capacity to treat organic

Table 1 Toxic effect induced by various dyes

Name of dye	Toxic effects
Acid violet 7	Lipid peroxidation, chromosomal aberrations, inhibition acetyl cholinesterase activity
Methyl red	Induce mutations
Disperse blue 291	Cytotoxic, genotoxic, mutagenic, fragmentation of DNA
Congo red	Carcinogen, mutagen
Malachite green	Carcinogen, mutagen, chromosomal changes, teratogen, induce histopathological effects such as organ and tissue injury, damage and developmental abnormalities

compounds [9, 4, 18, 63]. Aggressive growth, high biomass production, extensive hyphal growth, high surface-to-cell ratio are some of the properties that help fungi to treat/degrade various dyes in high amounts. Living or dead form of fungi has shown the capacity to remove dyes and pigments. Fungi remove dyes via processes such as biosorption, biodegradation, bioaccumulation and enzymatic mineralization. The decolorization/degradation of dyes azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes has been reported. Fungi have shown immense potential to decolourize/degrade azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes [119]. The degradation/decolorization potential depends mainly on metabolism of fungi and expression of extracellular enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase.

Filamentous fungi have been found to possess good capacity to degrade dyes [1, 17, 29, 57]. The strains of filamentous fungi secrete large amounts of hydrolytic enzymes and they possess an important role in degradation of dyes. The present chapter highlights the role of filamentous fungi in decoloration and degradation of various dyes and discusses the role of enzymes in their degradation. The mechanism involved in removal of dyes and factors affecting the removal are also discussed the chapter.

2 Fungal Strains with Dye Removal Capacity

About 115 fungal strains that possess good potential to degrade/decolorize dyes have been identified. Most of the strains have been found efficient to remove azo and anthraquinone dyes. These mainly include *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Penicillium chrysogenum, Pleurotus ostreatus, Pleurotus pulmonarius, Pleurotus sajorcaju, Bjerkandera fumosa, Phanerochaete chrysosporium, Trametes sanguinea, Trametes versicolor*, *Phlebia radiata, Gonoderma lucidum, Gonoderma applanatum, Ganoderma resinaceum, Cladosporium rubrum, Laccaria fraterna* [7, 12, 24, 35, 38, 60, 87, 88, 100, 104, 122, 131]. Some species of fungi that depict high potential for removal of dyes have been listed in Table 2.

Fungal species belonging to groups—basidiomycetes and deuteromycetes—have shown high efficiency to decolorize dyes [74, 90]. The fungus *Phanerochaete chrysosporium* popularly known as "white-rot fungus" has shown an unusual high capacity to remediate/degrade dyes present in effluents released from textile industry [8, 9, 40, 50, 133]. The white-rot fungus possesses high capacity to degrade azo dyes include *Phanerochaete chrysosporium, Pycnoporus cinnabarinus, Pleurotus ostreatus, Basidiomycetous, Coriolus versicolor, Trametes versicolor*, *Pleurotus ostreatus* and *Coriolopsis polysona, Alternaria solani, Neurospora* sp [61, 68, 128], Mohamad et al. 2019). Fungal species mainly *Aspergillus, Penicillium*, *Galactomyces*, *Cunninghamella, Lasiodiplodia*, *Phanerochaete* have shown high potential to decolorize and mineralize malachite green (MG) [3, 5, 23, 56, 59, 98, 99, 126]. Studies have shown that *Funalia trogii* decolorize and degrade high concentrations of crystal violet [121]. The fungal strain, *Leptosphaerulina* sp. has shown capacity

Table 2 Removal of dyes by various fungal species

Name of dye	Fungus	References
Congo red	*Aspergillus flavus*	[17]
	Aspergillus niger	[44]
Reactive red 195, Reactive green 11	*Aspergillus niger*	[131]
Black reactive 5	*Phaenerocheate chrysosporium*	[37]
	Pleurotus eryngii	[50]
Indigo dye	*P. chrysosporium* URM6181 *Curvularia lunata* URM6179	[32]
Reactive violet 5, Light navy blue, Dark navy blue	*Hypocrea koningii*	[46]
Mordant yellow 1	*Aspergillus sp.* TS-A CGMCC	[60]
Malachite green	*T. asperellum*	[96]
	Aspergillus flavus	[14]
	P. ochrochloron	[98]
	Galactomyces geotrichum	[56]
	Penicillium simplicissimum	[30]
Thiazole yellow G	*Aspergillus niger* LAG	[13]
Basic blue	*Trichoderma harzianum*	[102]
Bromophenol blue	*Trichoderma harzianum*	[102]
Direct green	*Trichoderma harzianum*	[102]
Methylene blue	*Aspergillus sp.*	[78]
Gentian violet, crystal violet	*Aspergillus sp.*	[78]
Orange II d, Tropaeolin O	*Phaenerocheate chrysosporium*	[102]
Basic fuchsin	*Phaenerocheate chrysosporium*	[89]
Nigrosin	*Phaenerocheate chrysosporium*	[89]
Viscose orange-A	*Aspergillus fumigatus*	[94]
Direct violet-BL	*Aspergillus niger*	[94]
	Trichoderma viride	[94]

to decolorize about 90% of the dyes Novacron Red, Remazol Black and Turquoise Blue.

Immobilization of white-rot fungus on Ca-alginate beads or discs in a rotating biological contactor increases decolorization of recalcitrant dyes such as Direct Violet 51, Reactive Black 5, Ponceau Xylidine, Bismark Brown R, Orange II and Everzol Turquoise Blue G [2, 36, 62, 92]. Cultures of *Phanerochaete chrysosporium* when immobilized on cubes of polyurethane foam (PUF) increased the capacity to decolorize high amounts of polymeric dye Poly R478. Immobilized fungal cultures show high activity and resilience to environmental alterations such as changes in pH, exposure of cells or suspension cultures to toxic concentrations of compounds.

3 Mechanisms Involved in Remediation/Decolorization of Dyes

Adsorption, biosorption and enzymatic degradation have been suggested as major mechanisms involved in removal or decolorization of dyes by fungal hyphae. Living or dead biomass of fungi both possess high capacity to decolorize dyes. Decolorization of the dye occurs because of synergistic effects of mycelia and extracellular enzymes such as oxidases [103]. In the initial stages, biosorption of the dye helps in the decolorization of dyes followed by degradation in the final stages of growth.

Various dyes such as azo, anthraquinonic, heterocyclic, triphenylmethane and polymeric have shown decolorization using fungal biomass [94, 95], though the mechanism of decolorization of dyes varies according to the fungal species. The degradation of chromophore moiety of the dye molecule occurs with the help of extracelluar enzyme produced by fungi and absorption/adsorption mechanisms. The decolorization activity of fungi is regulated by various factors such as concentration of dye, amount of pellet, temperature and agitation of the media. The pH, temperature, inoculum size and NaCl concentrations in the culture conditions regulate the efficacy of the fungal strain for decolorization. Biosorption and biodegradation are the two main mechanisms that help in decolorization of azo dyes [59].

Decolorization of the dye effluent is supported by neutral culture conditions. Studies indicated that incubation time of 72 h is suitable for getting maximum decolorization. The decolorization efficiency decreases with an increase in temperature. Decolorization (more than 70%) of dyes namely Reactive Red, Navy Blue HER, Reactive Magenta B and Orange 3R has been noted in *Talaromyces funiculosum* under optimal conditions of temperature and pH [28]. Fungal species *Talaromyces versicolour* have shown complete decolorization of dyes such as Tropaeolin O, Reactive Blue 15, Congo Red, Reactive Black 5, and partial decolorization of Brilliant Red 3G-P, Brilliant Yellow 3B-A and Remazol Brilliant Blue R has been noted. Rate of decolorization of dye varies under static and shaking condition [8, 27]. The shaking conditions provide better oxygenation to the fungus and regular contact of secreted enzymes for decolorization. Moreover, agitation also helps in the growth of fungus. Carbon sources like glucose have shown to accelerate rate of decolorization. *Leptosphaerulina* sp. showed high capacity to decolorize (>90%) textile industry effluents at low pH and glucose supply.

The mechanism of removal of dyes varies for each fungal species. The fungal species such as *Penicillium pinophilum* remove dyes via biosorption at the initial stage followed by intracellular biodegradation. On the other hand, other species, *Myrothecium roridum* removal of dye takes place by extracellular biotransformation. About 99.99% decolorization of Congo red dye can be achieved by *Alternaria alternata* within 48 h whereas *Aspergillus niger* showed degradation of Procion Red MX-5B after 336 h of treatment.

3.1 Adsorption

Adsorption of dyes by fungus has been considered as the primary mechanism of its decolorization. Hydrophobic–hydrophillic interaction between the fungus and dye occurs during adsorption. About 50% of dye removal has been noted after adsorption. Treatment of biomass with organic or inorganic molecules like formaldehyde, sulfuric acid, sodium hydroxide, calcium chloride and sodium bicarbonate and exposure to high temperature enhanced adsorption capacity of fungi.

Living and dead mycelia of *Trametes versicolor* has shown potential to adsorb various dyes, viz. Acid green 27, Acid violet 7 and Indigo carmine [123].

3.2 Biosorption

Biosorption is a physicochemical method that helps in the removal of pollutants via attachment to the surface of cell membranes or other cellular components. Binding of contaminants to cell wall or membranes occurs via different mechanisms such as physical adsorption, electrostatic interaction, ion exchange, chelation and chemical precipitation [45]. Dye molecules get trapped in the inner spaces of fungal mycelium because of ion exchange hydrogen binding [127]. The process plays an important role in decolorization of dye.

The capacity of fungi to bisorb contaminants is attributed to heteropolysaccharides such as chitin, chitosan, glucan, lipid, phospholipids present on cell wall. Chitin and chitosan present in the cell wall of dead fungal biomass show high affinity for binding various dyes. Amino, hydroxyl, carboxyl, phosphate and other functional groups (sulphates, hydroxides) present on fungal biomass help in binding azo dye to cell wall by creating attractive forces [105, 106]. Filamentous fungi successfully remove toxic dyes via biosorption [16].

High-temperature exposure through autoclaving and treatment with chemicals such as 0.1 N NaOH, 0.1 M HCl and 0.1 M H_2SO_4 increase the biosorption capacity of fungus. The autoclaving brings changes in the fungal biomass by changing the surface charge. The pretreatment of biomass with acid enhances the affinity of anionic dyes to bind to fungal surface. The biosorption capacity of fungal biomass of *Lentinus sajor-caju* increased for the dye Reactive Red 120 after autoclaving (treatment at 100 °C for 10 min).

3.3 Degradation

Biodegradation is an energy-requiring process in which complex organic molecules are broken down into simpler molecules through the action of certain enzymes secreted by microorganisms. Biodegradation involves three steps—

(1) slight change in an organic molecule without change in the main structure,
(2) fragmentation of a complex organic molecule and
(3) complete mineralization, i.e. conversion of organic molecules to simpler forms such as carbon dioxide, methane, inorganic elements.

The role of reductive and oxidative enzymes in the degradation of dyes has been reported [55]. Extracellular enzymes produced by fungi play a vital role in decolorization and degradation of organic compounds such as dyes under *in vitro* conditions [33]. Laccase, azoreductases, lignin peroxidases and manganese peroxidases are the main enzymes found in fungi, which catalyze redox reactions [80, 129]. Various factors such as culture conditions, availability of nutrients, carbon source, time, pH, agitation, temperature, oxygen supply, concentration and nature of dye, additives and salts control the degradation of dyes by fungi [39, 48, 84].

Oxidative enzymes

Enzymes such as polyphenol oxidases (PPO), manganese peroxidase (MnP), lignin peroxidase (LiP), laccase (Lac), tyrosinase (Tyr), N-demethylase, dye decolorizing peroxidases and cellobiose dehydrogenase assist in degradation of azo dyes [72, 111]. The degradation of compounds occurs due to breaking of ester, amide, ether bonds and aromatic ring or the aliphatic chains of compounds by these enzymes. The enzymatic treatment of substrates results in the formation of less toxic insoluble compounds.

White-rot fungi show ligninolytic activity, which proves useful in degradation of industrial dyes and other toxic aromatic compounds. Lignin peroxidases or ligninases and manganese peroxidase (MnP) are the lignin-degrading enzymes that cause oxidative depolymerization of lignin and assist in degradation of various organopollutants [51]. Ligninolytic enzymes bind to the substrate thereby degrading dyes. The reduction of azo dyes by fungi depends on the enzymes such as peroxidases and phenol oxidases.

Many basidiomycetes such as *Bjerkandera sp.*, *Agaricus bisporus, Phanerochaete flavido-alba, Ganoderma lucidium, Pleurotus pulmonarius, Trametes versicolor*, *Trametes hirsute* and *Pleurotus ostreatus* are known to possess MnPs that assist in the removal of dyes [34, 52, 54, 70, 73, 79, 130, 117]. Manganese peroxidase produced by isolates of *Clitopilus scyphoides*, *Ganoderma rasinaceum* and *Schizophyllum* showed ability to degrade recalcitrant azo dyes, sulfonephthalein dyes and kraft lignin. High amounts of dyes such as Poly-478 and Remazol Brilliant Blue R showed degradation because of MnP produced by *Lentinus edodes.*

Enzymes such as peroxidases, H_2O_2-dependent secreted by mushroom *Pleurotus ostreatus* showed high capacity for decolorizing dye, Brilliant Blue R [120]. Enzymes, horseradish peroxidase and LiP isolated from *Penicillium chrysosporium* possess the capacity to oxidize dyes such as Methylene Blue (Basic Blue 9) and Azure B. Biodegradation of malachite green (MG) by enzymes laccases and manganese peroxidase produced by fungi have been reported [14, 71, 82]. Degradation of indigo dye by laccase produced by *Trametes hirsuta* and *Sclerotium rolfsii* has been reported [31]. A new family of ligninolytic peroxidases named as versatile peroxidase (VP)

has been isolated from *P. chrysosporium* [75, 93]. The enzyme peroxidases cause oxidation of anthraquinone dyes [125].

The enzymatic degradation of chemical compounds results in the formation of free radical followed by insoluble product [112]. The cleavage of carbon–carbon single bond results in degradation of the compound. The degradation of dyes and other aromatic compounds has been facilitated by free radicals generated in the process. The chain reactions get initiated when free radicals get donated or accepted electrons from other chemicals. The actions are catalyzed by enzymes peroxidase [53]. Enzyme, phenol oxidases decolorize azo dyes through a highly non-specific free radical mechanism.

In white-rot fungi, the enzymes such as Laccase (multi-copper oxidases) present on the cell surface play a major role in degradation of dyes [15, 22, 53]. The removal of dyes by enzyme laccases involves oxidation or oxidation mediated by redox mediators (e.g., ABTS) [66, 109]. Laccases oxidize the phenolic group of the azo dye resulting in formation of phenoxy radical followed by its oxidation to a carbonium ion.

Laccase enzyme produced by *Phanaerochaete pulmonarius* BPSM10 showed capacity to degrade MG. *Aspergillus niger* and *Phanaerochaete crysosporium* degraded dyes through the action of extracellular enzymes such as laccase. Degradation of Malachite green (96%) by *Aspergillus flavus, Aspergillus solani* and some white-rot fungi has been noted in liquid culture within 6 days [3, 118]. Degradation of Orange 2 acid, Orange 6 (72.8%) and many other azo dyes (45.3%) has been achieved using laccase produced by *Trametes versicolor*. The decolorization of dye such as triphenylmethane dyes has been noted in *Trametes versicolor* (Crystal violet, Bromophenol blue and Malachite green) [124]. Bleaching of these dyes by *Trametes versicolor* can be attributed to enzyme laccase, peroxidase and arsenal present within the fungal cells [86].

Reductive enzymes

Azoreductases degrade azo dyes by breaking the azo linkage bridge between the chromophoric groups to produce two arylamines. The colorless aromatic amines are produced by cleavage of the azo bond under reduced conditions [81]. The reducing equivalents such as NADPH, NADH and FADH help in breakdown of the dye.

The enzyme malachite green reductase (MG reductase) produced by fungal strains, *Penicillium pinophilum* and *Myrothecium roridum*, has shown degradation of dyes within 48 h [56]. Reduction of MG results in the formation of leucomalachite green and N-demethylated metabolites. FITR analysis suggested the formation of non-toxic metabolites such as aromatic amines, carboxylic acids, alkanes, alkenes and further conversion of aromatic amines into carboxylic acid during degradation of MG.

4 Decolorization of Dyes by White-Rot Fungi

White-rot fungi, *Phanerochaete chrysosporium* has shown high capacity for dye decolorization [41, 110]. Other white-rot fungi species viz. *Hirschioporus larincinus, Inonotus hispidus, Phlebia tremellosa* and *Coriolus versicolor* have shown an ability to decolorize dye effluent [47]. Lignin-degrading enzymes such as peroxidases viz. manganese peroxidase (MnP), lignin peroxidase (LiP) and laccases found in white-rot fungi help in decolorizaton of dyes [24, 121]. The ligninolytic enzymes (lignin modifying or degrading enzymes) are excreted extracellularly by white-rot fungi. These enzymes cause oxidation of lignin present in the fungal cell. These enzymes oxidize Mn (II) to highly reactive Mn (III) in the presence of hydrogen peroxide. Mn (III) further chelates with organic acids, which attack and oxidize lignin and other recalcitrant compounds [51,115]. The ability of fungi to decolorize dyes varies with the strain.

The decolorization of dye by white-rot fungi occurs mainly by biosorption, biodegradation, bioreactor and lignin modified enzymes. Ligninolytic enzymes mineralize dyes during degradation. Temperature of about 30 °C is the most favorable to carry out decolorization, a significant decrease has been noted at higher temperature. This is because of reduction in cell viability or inactivation of the enzymes. Acidic conditions (pH 5.5) assisted in biodegradation of methyl violet by *Aspergillus* sp. while pH higher than 6.5 inhibited decolorization [69].

The biodegradation of Congo red by *Aspergillus niger* has been reported. The cultures showed high decolorization efficiency (97%) after 6 days. About 27% of CR dye was eliminated by adsorption while 70% was treated by enzymatic biodegradation. Enzymes such as lignin peroxidase, manganese peroxidase and deaminase produced by the strain played a major role in degradation. The rate of decolorization is affected by various factors including pH. High pH values (pH > 7) changed the activity of MnP, which can be due to enzyme stability. Studies suggested that the degradation of CR dye occurs mainly by deamination and oxygenation and involves the following steps: (i) partial deamination of CR compound; (ii) asymmetric cleavage of C–N bond between the aromatic ring and the azo group by peroxidase with the loss of a sodium atom; (iii) asymmetric cleavage of C–N bond caused by peroxidase followed by deprotonation; (iv) opening of benzene ring and dehydrogenation forming intermediates; (v) action of peroxidase forming cleavage products.

Decolorization of reactive blue 25 by lignin peroxidase, laccase and tyrosinase produced by *Aspergillus ochraceus* has been reported [83]. White-rot fungus *Ganoderma tsugae* shows potential for degrading reactive black dye due to production of enzyme laccase [132]. The secretion of laccase and manganese peroxidase by white-rot fungal strain KRUS-G assists in decolorization of Remazol Brilliant Blue R
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5 Conclusion and Future Perspectives

Cost-effectiveness and eco-friendly nature established fungi as a suitable material for degradation/decolorization of dyes from textile effluents. Fungi decolorize synthetic dyes via through absorption, adsorption and enzymatic degradation. Most of the studies related to degradation/decolorization of dyes have been performed under laboratory conditions. More studies are required to implement the technique in decolorization of dyes under field conditions. Identification and exploration of new fungal strains with the help of molecular techniques can prove useful in achieving remediation of dyes. The molecular tools help in identification of genes encoding enzymes that play role in degradation of complex synthetic dyes. Genetically, modified strains of fungus with high efficiency for dye degradation/decolorization of dye can be produced via technology of genetic engineering.

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