Dye Degradation by Fungi

Vinay Kumar, Garima Singh, and S. K. Dwivedi

Abstract Dye pollution is rising drastically due to massive use in different types of industrial activities. Synthetic dye pollution is the major issue of the environment at present due to their recalcitrant, toxic, carcinogenic and mutagenic behavior. Dye pollution affects aquatic life by impairing the sunlight permeability and can damage the aquatic ecosystem terribly. Therefore, treatment of dye-containing wastewater is required. Bioremediation seems as safe and ecofriendly approach for wastewater treatment. Fungi have massive dye decolorization potential and can be used for treatment. Studies have reported many fungal species for decolorization of dye so, for better understanding of their application in wastewater treatment process, the involved mechanism in dye decolorization should be known. This review focused on application process of fungi in dye decolorization, role of enzyme, protein, genes and surface functional group in degradation and biosorption process. The toxicity of fungal degraded dye end products is also reviewed in this chapter which is an important aspect in fungal application for dye contaminated wastewater treatment.

Keywords Dye · Fungi · Enzyme · Fungal dye degradation · Degradation mechanism · Toxicity

1 Introduction

Dyes have a great impact on aquatic life which are generated from different types of industries such textile, paper and pulp, color and printing points, leather, paints, food and cosmetic. On the basis of chemical structure of chromophore, there are almost 25 types of dye classes and over one thousand dyes are utilized in textile production for a variety of color fabrics [2, 120]. Disposal of these effluents from industries containing dye into the natural water bodies causes artistic damages and can influence the life form by declining the light permeability that affects the photosynthetic activity and availability of oxygen in the water bodies. Some of the dyes are also persistent and

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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_5

highly toxic to terrestrial as well as aquatic fauna of the environment [61]. Toxic, mutagenic and carcinogenic features of the many dyes are widely reported in the studies. It has been also found in the studies that exposure of some of the dye such azo dyes, malachite green, congo red, etc., increase the chances of chromosomal fractures, fertility loss, and can affect the respiratory enzymes in living organisms [2, 33, 113].

Due to high dissolution of dyes into the water, the physicochemical methods are less effective for their removal from the wastewater [123] and many of the downsides such as generation of huge quantity of toxic sludge, use of huge amount of chemical and energy and requirement of skilled manpower are also coupled with these techniques ([69, 61, 64–73, 101]). Microbes (fungi and bacteria) are used in microbial bioremediation processes as an eco-friendly and sustainable way for management of dye polluted wastewater. Many of the bacterial and fungal species have been reported with the potential to degrade complex dye molecules in simpler non/less toxic compounds or break down into carbon dioxide, water and others. They are also serving as decomposer in ecosystem functioning as an essential biotic component of the ecosystem that's why their utilization in treatment of dye polluted wastewater is more eco-friendly than known physicochemical techniques.

Fungi being an active agent of ecosystem as saprophytes produces many of the enzymes for instance laccase, lignin peroxidase, manganese peroxidase, etc., which can potentially catalyze various types of dye molecules (congo red, malachite green, methylene blue, etc.). Jasinska et al. [61] investigated the malachite degradation mechanism and potential of fungus *Myrothecium roridum* which produces laccase for dye degradation. In another report, *Aspergillus terreus* GS28 degraded Congo red and was found to extracellularly secrete laccase and manganese peroxidase [109]. Many of the review articles are available that deal with microbial bioremediation of dye contaminated water and wastewater. There are several types of mechanisms that have been explained in the literature for decolorization/degradation of synthetic dyes by fungi. Therefore, in this review fungi application in the meadow of bioremediation and fungal dye degradation/decolorization mechanisms are discussed. The toxicity of fungal degraded various dye end products is also narrated.

2 Dye Pollution Sources and Impact

This era is dealing with the use of fashionable clothes, paper, cosmetic, etc., that increases the heavy load on industrial activities for manufacturing of these products. The production of varieties of colors in high quantity via natural processes is unable to fill the demands of the present which necessitate the alternative methods to produce varieties of color in huge amount that can fill the need of the present. In the chemical synthesis of dye, varieties of color can be multiplied easily in huge amounts in very short duration of time. The chemically synthesized dyes have high brightness and binding capacity with substrate and are utilized in multiple industries for coloring

purposes. Most of the dyes are exploited in the textile, carpet, paper and printing industries for production of varieties of colored fibers and papers.

Due to huge application in industrial processes, dyes are produced in high amount with the generated effluents and disposed-off into the fresh water bodies like rivers, lakes and ponds without proper treatment. Synthetic dyes are exploited in diverse industries like textile, carpet, printing, paper and pulp, cosmetic, paint, laundry and leather are generated on large scale and due to their toxicity, they are related to environmental degradation. Among the dyes, Azo dye is the class of dye which is extensively being used. There are over 3,000 dyes belong to the class of azo dye [123] and it is expected that roughly 280,000 tons of synthetic dye are released from textile industries annually around the world [83, 144]. The chemical structures of some of these synthetic dyes are presented in Fig. 1. Based on their origin, dyes are categorized in two types: *Natural dyes* and *Synthetic dyes*. Natural dyes have no significant

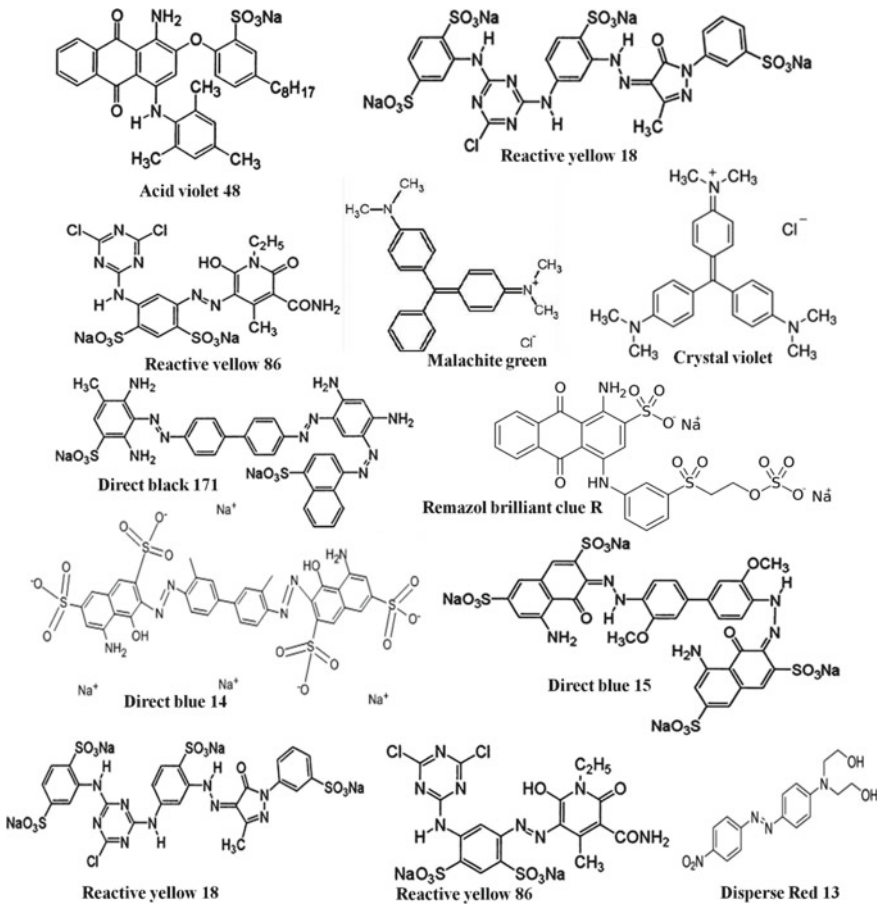

Fig. 1 Chemical structure of some synthetic dyes

impact on the environment, while synthetic dye possesses great concern. Synthetic dyes contains one or more than one chromophore such as acridine, anthraquinone, Azo (-N=N-), diazonium, oxazin, nitro, thiazin, phthalocyanine and triarylmethane which generate various color by absorbing the light in visible region (400–700 nm) ([31], [115]).

Accumulation of dyes into water bodies reduces the permeability of light and affects the aquatic ecosystem drastically by reducing the photosynthetic process. In addition, accumulated dye can also raise the chemical oxygen demand (COD) along with biological oxygen demand (BOD) of fresh water body and generate a noxious environment that turns into a degraded ecosystem [66, 105]. Synthetic dyes are extremely toxic and can influence the growth and metabolic process of the living creatures such as Reactive brilliant red can cause disturbance in the function of human serum albumin [80], disperse orange 1 can enhance the frequencies of micronuclei in lymphocytes and HepG2 cells which cause DNA damage [103], orasol navy blue 2RB can increase frame shift mutation without metabolic activation [124]. Many of the dyes have carcinogenic and mutagenic properties (Table 1). Dyes have tendency to be recalcitrant in aerobic environment which leads to their accumulation in soil and sediment at the physicochemical treatment location and also responsible for their transport to municipal water supply system.

3 Why Fungi?

Fungi normally occur in almost all the environmental conditions either normal or stressed situations such as drought, alkaline and acidic in the presence of contaminants (like heavy metal, pesticides and dye, etc.). The wastewater is accomplished with multiple types of pollutants that may hamper the growth of bioremediators but many of the fungal species exhibit tolerance toward different pollutants. Being saprophytes they use and produce numerous extracellular enzymes to degrade organic substrate and utilize the degraded substrate as energy and nutrients source for their growth and development. The extracellular release of cluster of enzymes associated with the degradation of dye is the basic criteria to select them to treat wastewater polluted with dyes. In addition, the application of growing form of fungi has self-replenishment ability which promises no need for addition of consortium from time to time and can be used continuously for a long time. From technical point of view, fungi are easily separable from the treated wastewater if used in bioreactor, easy to handle and use, can grow in low graded substrate and do not cause any type of environmental damages after their disposal. There is no requirement of high energy, chemicals and skilled manpower with the use of fungi for treatment perspective that make it more sustainable and environmental friendly than other techniques.

Table 1 Effect of dyes on living beings

Dye	Toxicity	References
Acid violet 7	Lipid peroxidation, Aberration in chromosome	Ben Mansour et al. [84]
Disperse red-1	Functional change in human lymphocytes	Chequer et al. [27]
Reactive black-5	Decline activity of urease cause ammonification in arginine rate of terrestrial ecosystem	Topac et al. [121]
Disperse blue-291	Mutagenic, cytotoxic and genotypic effects	Tsuboy et al. [122]
Malachite green	Carcinogenesis and Mutagenesis	Jasińska et al. [61]
Congo red	Carcinogen and Mutagen	Asses et al. [9]
Mordent red-73	Lead to release of the toxic chromium (IV) salt into the environment	Elmorsi et al. [39]
Disperse red-1	DNA damage caused which increase the number of micronuclei in lymphocyte of human being	Chequer et al. [27]
Disperse orange-1	Enhance the frequencies of micronuclei in lymphocytes sand HepG2 cells which cause DNA damage	Ferraz et al. [43]
Astrazon blue FGRL	Alteration of few enzymatic activity and increase in glutathione reductase	Gongord et al. [48]
Sudan I	Liver and urinary bladder carcinogen in mammals	Stiborová et al. [114]
Direct black 38	Urinary bladder cancer, Liver carcinogen	Robens et al. [103]
Disperse red 13	DNA damage in human hepatoma cells	Oliveira et al. [91]
Direct blue 6	Teratogenes is in rats during pregnancy	IARC [57]
Direct red 28	Carcinogen	Ding et al. [37]
Basic red 9	Bacterial DNA damage and hypertrophy of thyroid in Mice	IARC [57]
Reactive orange 16	Mutagenic effect	Novotný et al. [89]
Rodamine 6G	Mutagenesis	Nestmann et al. [88]
Acid blue 80	Incensement of apoptosis in epithelial cells line RTL-W1	Bae and Freeman [10]
Benzopurpurine 4B	Endocrine disrupting agents	Bazin et al. [16]
Methyl orange	Carcinogenic and mutagenic effect	Purnomo et al. [98]
Methylene blue	Increase chemical oxygen demand which lead to death of aquatic organism	Rizqi and Purnomo [102]
Orasol navy blue 2RB	Without metabolic activation increased frame shift mutation	Venturini and Tamaro [124]
Disperse orange 37	Mutagenic response	Lima et al. [34]

4 Fungi and Dye Degradation

Disposal of inadequately treated dyes containing wastewater generated from industrial activities causes high pollution load on natural water body that affect the ecosystem functioning and results into degraded ecosystem by various ways. Dyes have been reported to cause various types of abnormalities in human beings as well as environment. Due to dye's toxicity, carcinogenic and mutagenic characteristics their removal from wastewater is necessary. Many attempts have been made by the researchers for dye decolorization/removal from polluted water. Biological method is more appropriate and significant than other known methods due to their applicability and environmental friendliness. In this regards many of the fungal species have been explored to remove different types of pollutants including synthetic dyes. Fungi exhibit several types of enzymes that have the degradation potential of synthetic dyes and other organic contaminants. In addition, fungal cell is made up of lipid, protein and carbohydrates that provide attractive characteristics to its cell surface that is biosorption features. Adsorptive characteristics of fungi give a unique feature to it and increase its potential for removal and degradation of pollutants. The fungi that have been reported to decolorize different types of dyes belong to the class ascomycetes, basidiomycetes and duteromycetes especially white rot-ligninolytic fungi which have high potential to produce various enzymes including laccase, manganese peroxidase and lignin peroxidase. These enzymes play crucial function as a biocatalyst in the dye degradation process.

There are numerous reports available in public domain on degradation of synthetic dyes by diverse types of fungal species (Table 2) ([8, 93, 58, 106, 81, 104]). *Aspergillus*, *Trichderma*, *Phanerochaete* and *Pleurotus* species are widely studied in the degradation of various class of synthetic dyes including direct, disperse, azo and anthraqinone ([106, 109], [129, 44, 17, 82, 130]). The mechanisms engaged in the decolorization of dye molecules by fungi are degradation and adsorption (biosorption) [6, 9, 19, 109]. In the biological management of dyes containing water, fungi can be applied in various ways which are as follows:

4.1 Use of Growing Culture

This is a very common method for use of fungi in the degradation/decolorization of dye and mostly performed in batch study. In this method, fungi growth and dye decolorization has simultaneously happened where degradation is performed by releasing extracellular enzymes and some amount of dye is also adsorbed on the surface of fungi that enhance the decolorization performance of fungi. In an investigation, *Aspergillus terreus* GS28 was utilized for direct blue-1 decolorization in liquid culture. Almost 99.2% dye decolorization was recorded under the optimized condition at 7th day of incubation while decolorization happened via sorption and degradation. Sorption was driven by surface functional group and degradation was

Table 2 Dye degradation potential of some fungi and their degraded dye end products

Dye	Enzyme	Degraded product	Instrumental analysis	References
Mordant yellow 1	MnP, LiP and Laccase	Methyl 2-fluoro-5- nitrobenzoate, benzene and 2-methylpropionic acid	GC–MS	Liu et al. [82]
Congo red	Laccase and MnP	Naphthalene amine, biphenyl amine, biphenyl	FTIR and GC–MS	Si et al. [108]
Azo dye	Laccase	Biphenyl and naphthalene diazonium	HPLC	Krishnamoorthy et al. [67]
Procion red MX-5B	Azo-reductase	Primary and secondary amines	UV–Vis, FTIR	Almeida and Corso [6]
Congo red	MnP	Napthylamine and Benzidine	GC–MS	Wang et al. [126]
Congo red	LiP and MnP	Napthalene sulfonate and Cycloheptadienylium	LC–MS	Asses et al. [9]
Congo red	MnP	Aromatic amines	HPLC and FTIR	Chakraborty et al. [25]
Acid red 3R	MnP and LiP	4-aminonapthalene-1-sulfonic acid, 8-amino-7-hydroxynapthelene-1 and 3-disulfonic acid	GC–MS	He et al. [50]
Congo red	Laccase	Napthalene	Mass spectrometry and FTIR	Iark et al. [58]
Scarlet RR	LiP, Laccase and LiP	N-(1l3-chlorinin-2-yl)-2-{methyl[(4-oxo-3,4-dihydroquinolin-2- yl)methyl] amino}acetamide, N-(1l3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide, N-ethyl-1l3-chlorinin-2-amine and 5-({[2-(1l3-chlorinin-2-ylamino) ethyl]amino}methyl)cyclohexa-2,4-dien-1-one	FTIR, HPLC and GC–MS	Bankole et al. [13]
Malachite green	Laccase and MnP	N-demethylated primary and secondary arylamines	UV–Vis, FTIR and LC–MS	Barapatre et al. [15]

(continued)

Table 2 (continued)

Dye	Enzyme	Degraded product	Instrumental analysis	References
Brilliant green	Laccase	N-(diethylamino) phenyl) (phenyl) methylene) cyclohexadienylidine) N-methylthanaminium	UV–Vis, FTIR and LC–MS	Kumar et al. [70]
Rubine GFL	Laccase and Azo –reductase	4-[(2-methyl-4-nitrophenyl) diazenyl] phenol, 1-(2-methyl-4-nitrophenyl)-2-phenyl diazene, (2-methyl-4-nitrophenyl) diazene,	FTIR, GC–MS	Lade et al. [77]
Direct blue-1	Laccase and MnP	[4,5-Diazotricyclo [4.3.0.0 (3,7) non-4-in-2-one] and [1,2-Benzene Dicarboxylic Acid, 3-Nitro)]	GC–MS	Singh and Dwivedi [109]
Acid red 97	Laccase	3-(2-hydroxy-1-naphthylazo) benzenesulfonic acid, [(Phenol,2,6-bis(1,1-Diemethylethyl)-] and Naphthalene 1,2-dione	LC–MS	Pandi et al. [93]
Reactive blue-25	Laccase and LiP	phthalimide, di-iso-butyl phthalate	FTIR and GC–MS	Parashetti et al. [94]
Reactive red- 120		2-aminobenzenesulfonic acid and 3-methanesulfinylbut-3-en-2-one	HPLC and FTIR	Su and Lin (2013)
Disperse red 3B	LiP and MnP	Diisobotyl phthalate, 4-Hydroxy-2-butan-one, ammonia	UV–Vis, FTIR and GC–MS	Tang et al. [116]
Reactive green	Laccase, LiP and DCIP reductase	Benzoic acid, 2(-1-oxopropyl)	UV–Vis, HPLC and FTIR	Sinha and Osborne (2016)
Disperse blue 2BLN	MnP	Dibutyl phthalate and 2-(N-methyl-p-phenylenediamine) ethanol	FTIR and GC–MS	Pan et al. [92]

performed by manganese peroxidase and lignin peroxidase [109]. Similarly, brilliant green decolorization performance of three fungal species *Pleurotus forida*, *Pleurotus eryngii* and *Pleurotus sajor-caju* was reported by Naraian et al. (2018). The decolorization efficiency for brilliant green was 99, 91, 87% by *P. forida*, *P. eryngii* and *P. sajorcaju,* respectively. There are several other fungal species also have been reported for the degradation/decolorization of different type of dyes [19, 22, 25, 76, 116].

4.2 Use of Immobilized Fungi

In the immobilized form of fungi, mostly fungus inoculums are immobilized on the surface of any solid medium [74, 68]. This process is basically applied when fungi are used in bioreactor for decolorization of dyes. Immobilization of fungus on solid surface also enhanced the applicability of fungi and can be used in continuous treatment of dye-containing wastewater for a long time without making more effort. Sometimes it is also known as solid–liquid-phase decolorization. Andleeb et al. [7] examined the Drimarene blue K_2RL dye biodegradation potential of *Aspergillus flavus* SA2 in immobilized form in lab-scale fluidized bed reactor (FBR). The fungus was immobilized on sand with size of 0.2 mm and investigated for biodegradation. In the FBR, *Aspergillus flavus* SA2 was able to remove 71.3% of color from the simulated textile water by biodegradation and bio-decolorization mechanisms including 85.57% and 84.70% removal of BOD and COD, respectively. Recently, in an investigation, Alam et al. [5] immobilized the fungus *Trametes hirsuta* D7 in light expanded clay aggregate and utilized for decolorization of acid blue 129, reactive blue 4 and remazol brilliant blue R (RBBR). The immobilized *Trametes hirsuta* D7 showed high degradation performance for the acid blue 129, RBBR and reactive blue 4 with decolorization performance of 96%, 95% and 90%, respectively. These studies showed that fungi can give more effective results in the degradation/decolorization of dye when applied in immobilized form. Some other studies were also investigated on synthetic dye decolorization/degradation performance of various fungi as well fungal-originated enzymes such as MnP, laccase and LiP ([3, 35, 118, 125]).

4.3 Use of Fungal Extract Containing Enzymes

The ligninolytic white-rot fungi are reported to generate diverse types of enzyme having the potential for degradation/decolorization of dyes. These enzymes are LiP, MnP and laccase which have been extensively reported in the studies. In an investigation, *Phanerochaete chrysosporium* CDBB 686 extract containing MnP, LiP, laccase obtained from fermentation of corncob was used for the decolorization of congo red, poly R-478 and methyl red. Fungal extract successfully decolorized Congo red, Poly R-478 and methyl red with decolorization efficiency of 41.8%, 56.8% and 69.7%,

respectively [111]. Akpinar and Urek [4] investigated the laccase production capability of *Pleurotus eryngii* by using solid-state bioprocess utilizing Peach waste of the fruit juice industry. The obtained laccase containing extract of *P. eryngii* was investigated for methyl orange degradation and reported 43% decolorization efficiency. Many other similar reports are also available on the dye decolorization performance of fungal extract ([30, 62, 78 87]).

4.4 Use of Isolated Enzymes from Fungi

The enzymes MnP, LiP and laccase are isolated in the different studies from liquid culture of different types of fungi and were employed in decolorization/degradation of dyes and various other organic pollutants. Bouacem et al. [19] isolated two peroxidases (LiP BA45 and MnP BA30) from the fungus *Bjerkandera adusta* strain CX-9. Both enzymes LiP BA45 and MnP BA30 were monomer with molecular mass of 30.12 and 45.22 kDa and highly active at pH 3.0 and 70 °C, pH 4.0 and 50 °C, respectively. Both enzymes were analyzed for decolorization of synthetic dyes remazol brilliant violet 5R, acid blue 158, cibacet brilliant blue BG, reactive dye remazol, brilliant blue reactive, polymeric dye R, methyl green and indigo carmine significantly decolorizes, while MnP BA30 enzymes was highly effective than LiP BA45 in synthetic dye decolorization performance. Similarly, Chairin et al. [24] purified laccase from liquid culture of *Trametes polyzona* WR 710-1, a white rot fungus. Purified laccase was exploited for decolorization of synthetic dyes namely bromophenol blue, acridine orange, remazol brilliant blue R, relative black 5, methyl orange and congo red. Purified laccase showed high efficiency in the decolorization of selected dye and addition of 0.2 M 1-hydroxybenzotriazole (redox mediator) improved the efficiency of dye decolorization of fungal laccase.

5 Role of Enzymes

5.1 Laccase (E.C.1.10.3.2, p-benzenediol)

Laccase is an important enzyme that can catalyze many types of aromatic hydrocarbons such as phenolic compounds via oxidation–reduction mechanism and belong to the group oxidoreductive enzymes [26]. It can be found in bacteria, fungi as well as in plants too. Fungal laccase is broadly distributed in the species of the class Basidiomycetes, Ascomycetes, and Deuteromycetes and in white-rot fungal species, it played role in lignin degradation [58, 61, 93, 109]. Laccase molecule typically has four atoms of copper (Cu) and some time its structure also comprises three Cu atoms with a molecular mass of 50–100 kDa [51].

During dye degradation by fungi, the role of laccase is extensively reported in the studies. Laccase potentially contributed in the degradation of direct blue 1, malachite green, congo red when the fungus *Myrothecium roridum* and *Aspergillus terreus* were used [61, 109]. Abd El-Rahim et al. [1] investigated 17 fungal strains of the genus *Aspergillus* and *Lichtheimia* for the degradation of 20 dye viz. janus green B, direct blue 71, reactive orange, evans blue, fast green, crystal violet, methyl red, tartrazine, naphthol blue black, alura red AC, reactive blue 4, pararosaniline, safranin, alizarin yellow R, trypan blue, ponceau S, cibacron brilliant red 3B-A, brilliant green, direct violet 51 and direct red 80. After a critical evaluation, they found that the fungus *A. niger*, *A. terreus*, *A. oryzae*, *A. fumigatus* showed laccase activity in the degradation of direct violet while absent in the degradation of methyl red. Akpinar and Urek [4] employed Peach waste generated from fruit juice industry for fungal laccase production by *Pleurotus eryngii* and successfully used in methyl orange, reactive black, tartrazine and reactive red 2 dyes degradation. In another study, significant role of laccase was recorded in degradation of solar brilliant red 80 by *Schizophyllum commune*. These studies suggested the magnitude of laccase in the decolorization/degradation of dyes [8].

The mechanisms of laccase in the demolition/degradation of aromatic hydrocarbon (phenolic compounds) are variously suggested by the researchers. It oxidizes phenolic group to phenoxy radical via eliminating hydrogen (H) atom from the OH (hydroxyl) group [11]. Laccase show soaring affinity toward molecular oxygen and as proposed by Burke and Cairney [21] it reacts with type-1-copper reduction by the precursor, transfers electron as of type-1-Cu to type-2-Cu as well as Type-3-Cu in a trinuclear bunch, and reduction of molecular oxygen on type-2-Cu and Type-3-Cu sites. This similar pathway was proposed for the degradation of acid red 97 by fungal laccase isolated from *Peroneutypa scoparia* where hydrogen of phenyl group is broken by laccase and converted into phenoxy radical, subsequently it reacts with OH^- and breaks the azo bond (-N=N-) of acid red 97 and forms naphthalene 1,2 diene and benzenes sulfonic acid as end products [93]. Iark et al. [58] also reported successful degradation of congo red dye by the laccase produced by fungus *Oudemansiella canarii*. In mass spectrometry and Fourier transform infrared spectroscopy (FTIR) they found four types of compound as end products of congo red but no any dye degradative clear path was suggested. Shanmugam et al. [106] utilizes *Trichoderma asperellum* laccase for degradation of malachite green (MG). They suggested that the MG degradation happened by hydroxylation of central carbon by laccase and form Michler's ketone that further catalyzed into benzaldehyde and 4-aminobenzophenone as end products via some intermediates compound formation with laccase-oxidative cleavage and mediated deamination mechanisms. So, fungal laccase may react differentially to degrade the dyes and produced various kinds of less/non-toxic end products and has crucial role in fungal dye degradation.

5.2 Manganese Peroxidase (EC 1.11.1.13)

Manganese peroxidase (MnP), a ligninolytic enzyme first noticed in *Phanerochaete chrysosporium* in 1983, Mn^{2+} was used as substrate and oxidizes into Mn^{3+} [52]. The chelators stabilized to oxidized Mn^{3+} which is turn as very active low molecular mass and diffusible oxidoreductive intermediary. It attacks nonspecifically on hydrocarbon molecules. MnP can oxide different types of organic compounds as well recalcitrant xenobiotic organic pollutants such dyes, nitroaminotaluene, phenol derivates, etc., and can also depolymerize lignin into their simpler form [128]. In fungal dye degradation, its enhanced activity has been reported in many studies. MnP involved in MG, congo red, indigo carmine and reactive red 120 degradation by fungus *Myrothecium roridum*, *Aspergillus terreus* and *Phanerochaete chrysosporium*, *P. sordida* strain YK-624, respectively ([49, 61, 104, 81]).

MnP is highly reactive agent that can catalyze dye via oxidoreductase mechanism and diminish the dye toxicity potential. In an investigation, Jasinska et al. [61] used the crude MnP produced by fungus *Phanerochaete chrysosporium* in decolorization of indigo carmine which successfully reduces indigo carmine up to 90% within 6 h. After decolorization assay, they investigated the MnP degraded end products of indigo carmine. Isatin was found as end product of indigo carmine in gas chromatography mass spectroscopic (GCMS) analysis. They suggested that Mn^{3+} first attack on indigo carmine remove $SO_2{}^{2-}$ then react with NH group of the ring to form intermediate compounds that react with H_2O and separated into isatin, end product of indigo carmine catalyzed by MnP. In another study, cDNA encoding MnP of *P. chrysosporium* was expressed in *Pichia pastoris* for MnP production. The produced MnP was utilized for degradation of MG. Where MnP mediated hydroxyl react with central carbon to mineralize MG into its N, N-dimethylaniline (N,Ndimethyl-benzenamine), 4-dimethylamino-benzophenone hydrate and methylbenzaldehyde compounds as its MnP catalyzed end products [104]. Fungal MnP have crucial role in degradation of dye.

5.3 Lignin Peroxidase (EC 1.11.1.14)

Major sources of lignin peroxidase are the white-rot basidiomycetes. Lignin peroxidase (LiP) is also called ligninase or diaryl propane peroxidase [18]. It is a globular glycoprotein extracellularly released by producers such as *Phanerochaete chrysosporium* as a secondary enzyme [42] and contains just about 343 amino acids having molecular size of 38–42 kDa [28, 60, 107]. Structurally, LiP is monomer of hemoprotein with a size of around 40 × 40 × 50 Å [95] and its folding motif have 8-alpha major and 8 minor helices and antiparallel short 3 beta-sheets [29, 40]. Its isoelectric point is in between 3.0 to 4.7 and is highly active at lower pH around 3.0 [46]. Its production can be affected by change in the ratio of source of carbon (C) and nitrogen (N) in the growth medium [54]. Its four disulfide bond upholds its

overall structure and two Ca-binding sites may provide active site for reactivity of LiP toward substrates (lignin) [97]. Many of the species of fungi reported to produce LiP but strain *P. chrysosporium* contain multiple LiP-encoding gene and produce LiP extracellularly as abundant isozyme (H10, H8, H7, H6, H2 and H1) [42, 119]. LiP can catalyze both phenolic along with non-phenolic aromatic unit of lignin either in the existence or nonexistence of mediators which is ended by Trp171 residue through long-range electron transfer that is linked with the heme group [85, 96]. LiP is noticed in many studies for its role in degradation of synthetic dyes like Azo group of dye by fungi [61, 109]. LiP producing fungal species generally belong to the class ascomycetes and basidiomycetes and white-rot ligninolytic fungi [28, 61, 107].

In an investigation, the combined activity of LiP, MnP and laccase was reported in direct blue-1 degradation by *A. terreus* GS28. The direct blue-1 was mineralized into three types of compound namely 4,5-Diazotricyclo[4.3.0.0(3,7)non-4-in-2-one], 1,2-benzene dicarboxylic acid,3-nitro and phenol,2,6-bis(1,1-Diemethylethyl) [109]. Oliveira et al. [90] investigated the LiP production capacity of *Ganoderma lucidum* (GRM117) and *Pleurotus ostreatus* (PLO9). Obtained crude LiP was immobilized on carbon nano-tubes and successfully demonstrated as a biocatalyst for decolorization of RBBR. Jadhav et al. [59] also reported the participation of LiP in biotransformation of direct blue GLL into 3-(naphthalene-1-ylazo)-naphthlene-1, 5 disulfonic acid. Disperse yellow 3 degradation was investigated in a study by *Phanerochaete chrysosporium*. LiP, horseradish peroxidase and MnP was involved in degradation of disperse yellow 3 and it was converted into 4-methyl-1,2-benzoquinone (III) or 1,2-naphthoquinone (VI) and 4-acetamidophenyldiazene that after oxidation reaction converted to acetanilide as major product [112]. The combined action of LiP, MnP and laccase was also reported in the Scarlet RR degradation by *Peyronellaea prosopidis* [13].

5.4 Other Enzymes

Except Lip, MnP and Laccase many other enzymes have also been found for their active participation in degradation/decolorization of many dyes. These enzymes mostly belong to oxydoreductase enzymes group. Spadaro and Renganathan [112] reported that horseradish peroxidase accompanied the catalysis of disperse Yellow 3 into the acetanilide with MnP and LiP in *P. chrysosporium.* In *Diutina rugosa* the activity of NADH-DCIP (dichlorophenol indophenols) reductase was reported to involve in asymmetric cleavage and reduction of indigo dye escorted with laccase and LiP [14]. NADH-DCIP-mediated asymmetric cleavage, desulfonation and dehydroxylation of AR-88 were found in *Achaetomium strumarium.* In GCMS investigation, sodium naphthalene-1-sulfonate, naphthalen-2-ol, and 1,4-dihydronaphthalene were analyzed as intermediate products after action of fungal NADH-DCIP [12]. Enhanced NADH-DCIP activity was reported in *Pichia occidentalis* G1 in the degradation of acid red B [110]. In an investigation dye-decolorizing (DyP type) peroxidase enzyme was reported in *Geotrichum candidum.* DyP type enzyme vigorously associated in

trypan blue, methyl orange, eriochrome black t, and congo red dyes degradation that belong to the group of azo dye. Its molecular mass was 63 kDa and was highly active at pH 6.0 and 35 °C in degradation of methyl orange [100]. Si et al. [108] investigated the dye decolorization ability of a white rot fungus *Trametes pubescens* Cui 7571 for congo red and successful decolorization was recorded. In the congo red decolorization, tyrosinase enzyme participated in the dye degradation including laccase, MnP and LiP and four by-products of dye namely naphthalene amine, naphthalene diazonium, biphenyl and biphenyl amine were found as final dye degraded metabolites. Bilirubin oxidase (BOX) is another oxidoreductase reported in degradation of RBBR by *Myrothecium* sp. IMER1, a non-ligninolytic fungus. BOX enzyme has also the potential to degrade congo red and indigo carmine under in vitro condition [131]. Cytochrome P-450 is another factor that holds big and various number of enzymes participated in dye biodegradation. Its role in MG decolorization by *Cunninghamella elegans* ATCC 36,112 was reported by Cha et al. [23] which reduces the MG into leucomalachite green by the action of cytochrome P-450 monooxygenases.

5.5 Role of Other Bioactive Compounds

Synthetic dyes degradation by fungi is mainly driven by oxidoreductase including laccase, MnP and LiP. But several reports have suggested the role of other bioactive molecules' low-molecular weight factors in the degradation of dye. In an investigation, Jasinska et al. [61] studied the malachite green decolorization potential of *Myrothecium roridum* IM 6482. They found that laccase participated in malachite green degradation but other active factor was also involved in degradation process. To analyze active factors, heat inactivation with adding sodium azide was done to neutralize the laccase activity and obtained non-enzymatic liquid coating low-molecular masses (less than 3 kDa) were used for the malachite green (MG) degradation. Non-enzymatic liquid also showed the decolorization potential of MG with efficiency of 8 to 11%. This might be due to the Fenton-like reaction driven by peroxides, which have been proven to oxidize chemical structure through oxidation by hydroxyl radical. To confirm this hypothesis, they conducted the decolorization experiment with catalase, thiourea, superoxide dismutase (SOD) (scavengers of reactive oxygen species, ROS) and did not found significant change in MG decolorization, confirming the presence of low-molecular-mass-factor. Fenton-like reaction, a non-enzymatic mechanism for the decolorization of dye has been also proposed by several workers [47, 64, 86]. In another investigation, Hu et al. [53] purified the low-molecular weight peptide from the culture of *Phanerochaete chrysosporium* that has the phenol peroxidase activity (Pc Factor). The molecular weight of Pc factor was 600 Da with high thermostability and similar to the observation of Jasinska et al. [61].

5.6 Role of Gene

Exploration of molecular mechanism associated with dye degradation by fungi is a crucial step for better understanding of fungal application in the field of bioremediation and has great scientific interest. Gene expressions have important role in metabolic process of the fungi which establishes the growth and development in addition to tolerance toward toxic dye under contaminated environment as well as dyes degradation by regulating multiple types of enzymes activity. Various stressors (such as dyes) can induce set of the gene in the organism in turn to connect the metabolic process with dye tolerance and degradation. There are two groups of genes expression are found in stressed conditions: *regulatory gene* and *functional gene*. Regulatory group of genes encodes various types of transcriptional genes while functional group of genes encode various types of compound and enzymes that are helpful in tolerance and degradation of dye and other toxic organic and inorganic compounds [68].

In an investigation, laccase-producing *Ganoderma lucidum* molecularly analyzed and showed 15 types of laccase isoenzyme genes. Out of them, *Glac1* was highly involved in laccase production for RBBR degradation [99]. Similarly, Değerli et al. [36] found *up* and down-regulation of 10 laccase-producing genes in lichen-forming fungi that were capable to degrade RBBR dye. In mid of this, *lac8* gene was highly up-regulated and possibly associated with enhanced dye degradation. A laccase encoding *lac-T* gene was highly *up*-regulated in *Trametes hirsuta* at the time of textile effluent decolorization and during the decolorization process significant increase in laccase activity was recorded [117]. Laccase encoding gene: *lacI* in *Pichia pastoris*, *Lac1* and *Lac2* in *Trametes hirsuta* MX2 and lac48424-1 in *Trametes* sp. 48,424 overexpression was reported in the studies in the presence of dyes such as crystal violet, malachite green, methyl orange, bromophenol blue, RBBR, acid red 1 and neutral red ([41, 55, 45]). In an exploration, Lee et al. [79] conducted RBBR degradation experiment using *Phlebia brevispora.* They found manganese peroxidase was stimulated in addition to laccase enzymes in the degradation of RBBR and GeneFishing technology confirmed the differential expression of two genes that possibly involved in the enzymes' regulation. In another study, halotolerant *Pichia occidentalis* A2 showed enhanced degradation of acid red B under Static Magnetic Field (SMF) of 206.3 mT. In transcriptomic investigation, it was found that 145 genes were up-regulated and 22 genes were down-regulated under these conditions that might be encoded by the enzymes or other functional proteins [127]. Conversely, Huy et al. [56] reported that synthetic dye can directly suppress the expression level of gene *Lacc110* in *Fusarium solani* HUIB02 while induces the Lacc42 expression level.

5.7 Role of Surface Functional Group in Dye Decolorization

Fungal cell wall is mainly made up of lipid, protein and carbohydrates that carry many types of surface functional group including –COOH, C=C, C=O, O–H, N–H and C-H [68, 75]. The surface functional groups do not participate in dye degradation but can increase the decolorization efficiency of fungi by adsorbing the dye molecules on the mycelia surface of fungi. Singh and Dwivedi [109] reported that almost 34.4% of total 98.2% decolorization of direct blue-1 dye happened via biosorption process by fungus *A. terreus* GS28 which showed that adsorption of dye on mycelia surface has crucial role in decolorization of dye by fungi. Further, in Fourier transform infrared spectroscopic investigation they found that O–H and C=C are involved in direct blue-1 adsorption via hydrogen bond and π-π interaction. Chakraborty et al. (2013 observed that morphology of *Alternaria alternata* CMERI F6 was more amorphous after decolorization of Congo red than control in scanning electron microscopic observation. Due to high amorphousness, the peak of x-ray diffraction analysis at 2 degree values of 28° was reduced which confirms the role of adsorption in congo red decolorization. In one more investigation, Asses et al. [9] examined the decolorization of congo red by fungus *Aspergillus niger* and they also found that surface adsorption of dye molecules occurred in decolorization of Congo red that was confirmed from changes FTIR spectra. Similar observations are reported in various studies in decolorization of synthetic dye by fungi [6, 62, 63, 65].

6 Fungal Genetic Engineering and Their Role in Dye Degradation

This era is dealing with heavy pollution load and the available tools and remediating agents are not sufficient to remove these pollutants. The bioremediation agents are considered as more environmental-friendly and sustainable to remove and degrade the pollutants from contaminated site. At present, several types of bioremediation agents including microorganisms and plants have been explored that can effectively degrade/remove the pollutants from the contaminated sites. These remediators cannot be used in every type of environment due to the fact that in the open environment there are multiple factors such as pH, temperature, presence of other contaminants that can affect the growth, development and pollutant removal/degradation efficiency of these bio-agents. Through the genetic engineering, functional genes of one organism that responsible for degradation and removal of pollutants can be expressed into another desired organism that contains pleasing features for its applicability in *on-site* or engineered systems. This approach can also enhance the applicability of fungi in the field of dyes degradation/decolorization. Many of the reports are available on the genetic engineering for fungi that showed enhanced dye degradation characteristic after gene manipulation ([32, 38, 129, 17, 82, 130]). In a recent study, through genetic engineering, Đurđić et al. [38] inserted LiP gene in pCTCON2 vector to find a saturation

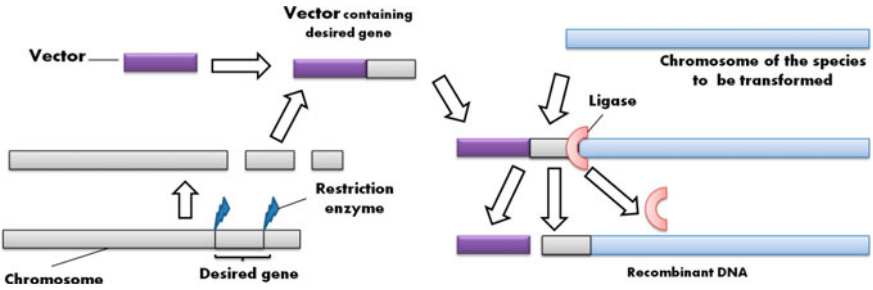

Fig. 2 The schematic diagram represents the genetic engineering process for obtaining genetically modified species for better productivity and performance of fungi

mutagenesis library. Further LiP variants genes and wild type gene were expressed in *S. cerevisiae* EBY100 and were used for degradation of structurally different azo dyes (Amido black 10B, Evans blue and Guinea green). The mutant ML3 and ML8 showed high catalytic activity toward Evans blue, ML2 and ML6 toward amido black 10B and ML3 and ML5 can catalyze Guinea green. In another investigation *Pleos-dyp1* gene of the fungus *Pleurotus ostreatus* that capable to degrade Acetyl Yellow G (AYG), RBBR and Acid Blue 129 (AB129) was successfully expressed in *Trichoderma atroviride*. Successfully genetically manipulated *T. atroviride* can degrade mono and di-azo dye, anthracenedione and anthraquinone dyes with its extracellularly produced DyP1 (dye-decolorizing peroxidase) activity. However, the recombinant *T. atroviride* was able to degrade AYG and AB129 at significant level [32]. In a study, Xu et al. [129] used cDNA library of 1092 bp full length from Manganese peroxidase producing fungus *Ganoderma lucidum* strain that designated as *GluMnP1* to construct eukaryotic expression vector pAO815::*GlMnP*. The vector pAO815::*GlMnP* was successfully transferred in *Pichia pastoris* SMD116. The recombinant *Pichia pastoris* SMD116 was capable to decolorize four dyes namely drimaren red K-4Bl, drimaren blue CL-BR, disperse navy blue HGL and drimaren yellow X8GN and phenol. Similarly, *mnp3* gene responsible for MnP production was cloned from *Cerrena unicolor* BBP6 and functionally expressed in *Pichia pastoris*. The resulting recombinant has great potential in decolorization of RBBR, methyl orange, bromophenol blue, crystal violet and brilliant blue R and two polyaromatic hydrocarbons (phenanthrene and fluorene) [130] (Fig. 2).

7 Toxicity of Fungal Degraded Products

Synthetic dyes have some level of toxicity to different types of organisms that can affect the normal physiological and metabolic process of the organism. As discussed above, fungi have huge potential for their application in bioremediation of dye contaminated wastewater and fungi can degrade the dye molecules into their smaller or constituents units. Mostly, these fungal degraded end products were less toxic

than parent dye molecules (Table 3). In a study, Assess et al. [9] conducted an experiment using *Aspergillus niger* for the degradation of congo red dye. Congo red was degraded into naphthalene sulfonate and cycloheptadienylium and in the phytotoxicity assessment *Zea maize* and *Solanum leucopersicum* showed higher germinate rate and shoot and root growth in fungal treated dye solution as compared to Congo red dye solution. In another study, *Trichoderma tomentosum* degraded Acid Red 3R into 4-aminonapthalene-1-sulfonic acid, 8-amino-7-hydroxynapthelene-1 and 3-disulfonic acid. Further, the toxicity assessment of fungal treated dye solution showed less toxicity on the germination rate and growth of *Glycine max* and *Adenanthera microsperma* as compared to pure acid red 3R solution. Conversely, some studies were also reported that fugal treated some dye solution may more toxic than their parent dye molecule. In an investigation, Almeida and Corso [6] tested the procion red MX-5B dye degradation ability of *Aspergillus terreus*. In the end-product analysis, primary and secondary amines were found as fungal degraded metabolites of procion red MX-5B. In the toxicity analysis, fungal treated dye solution showed 10-folds increase in toxicity toward shoot and root growth of *Lactuca sativa* while cause 100% death of *Artemia salina* larvae as compared to parent dye solution. Therefore, the toxicity test should be taken into consideration after treatment of dye contaminated water which can reduce the environmental risk.

8 Conclusion

Fungi have enormous potential for their application in bioremediation of wastewater. This chapter discussed the application of fungi in dye decolorization process and the role of cell surface functional groups, enzyme, proteins and genes in decolorization process. Fungi in the decolorization of dye use biosorption and biodegradation mechanism. On the surface of fungi, many types of functional groups are present that are associated with biosorption of dye molecules. While, MnP, LiP and laccase extracellularly secreted by fungi that play crucial role in dye degradation. Some other enzymes including DCIP reductase, Azo-reductase and *Bilirubin oxidase* have also been reported for their role in dye degradation. Several studies have suggested that these enzymes are expressed by functional group of genes that play important role in dye degradation. Transgenic fungi have shown enhanced dye degradation potential that provide a new aspect in the field of dye decolorization by fungi and is needed more research. Genetically modified microorganisms are expected to have huge potential for future application.

Table 3 Toxicity of fungal degraded dye end products

Name of the Fungi	Dye	Degraded product	Toxicity test	Toxicity of the dye degraded products	References
Aspergillus terreus	Procion Red MX-5B	Primary and secondary amines	Phytotoxicity	Fungal degraded metabolites increased the percent inhibition of root growth (0.87 cm) around 5–50% of *Lactuca sativa* which denote a tenfold increase in toxicity as compare to pure dye. The pure dye were non-toxic for *Artemia salina* larvae while degraded product cause 100% death of larvae	Almeida and Corso [6]
Ceriporia lacerate	Congo Red	Napthylamine and benzidine		In the toxicity test, *Amaranthus mangostanus L.* and *Sesamum indicum* showed less germination rate (55.83 and 38.89%) in fungal degraded dye end products as compared to pure dye (62.50 and 60.00%)	Wang et al. [126]
Aspergillus niger	Congo Red	Napthalene sulfonate and Cycloheptadienylium	Phytotoxicity	*Zea mais and Solanum leucopersicum where degraded* showed maximum percent germination (82.30 and 81.27%), root length (3.16 and 3.20 cm) and shoot length (10.9 and 4.66 cm) in fungal degraded Congo red metabolite as compared to pure dye {germination (60.35 and 60.31%), root length (1.7 and 2.06 cm) and shoot length (6.5 and 1.8 cm)}	Assess et al. [9]

(continued)

Table 3 (continued)

Name of the Fungi	Dye	Degraded product	Toxicity test	Toxicity of the dye degraded products	References
Trichoderma tomentosum	Acid Red 3R	4-aminonapthalene-1-sulfonic acid, 8-amino-7-hydroxynapthelene-1 and 3-disulfonic acid	Phytotoxicity	After biodegradation, the toxicity test was done with two plant seed *Glycine max* and *Adenanthera microsperma*, which denote that degraded product was less toxic (percent germination-80 and 87%, root length-2.4 and 2.8 cm and shoot length-4.1 and 2.8 cm) as compared to pure dye (percent germination-73 and 83%, root length-0.9 and 1.3 cm and shoot length-3.0 and 2.6 cm)	He et al. [50]
Oudemansiella canarii	Congo Red	Napthalene	Microtox test	The microtox assay detected that degraded product was less toxic than pure dye	Iark et al. [58]
Peyronellaea prosopidis	Scarlet RR	N-(1l3-chlorinin-2-yl)-2-{methyl[(4-oxo-3,4-dihydroquinolin-2-yl)methyl] amino}acetamide, N-ethyl-1l3-chlorinin-2-amine, N-(1l3-chlorinin-2-yl)-2-{[(4-oxo-3,4-dihydronaphthalen-2-yl)methyl]amino}acetamide, and 5-({[2-(1l3-chlorinin-2-ylamino) ethyl]amino}methyl)cyclohexa-2,4-dien-1-one	Phytotoxicity	Toxicity test was analyzed with *Triticum aestivum* and *Peyronellaea prosopidis* where degraded product showed higher percent germination, root length and shoot length as compared to pure dye	Bankole et al. [13]
Aspergillus Flavus	Malachite green	N-demethylated primary and Secondary arylamines	Phytotoxicity and Microbial toxicity	On the basis of toxicity assessment, degraded product was less toxic as compare to control	Barapatre et al. [15]

(continued)

Table 3 (continued)

Name of the Fungi	Dye	Degraded product	Toxicity test	Toxicity of the dye degraded products	References
Aspergillus terreus	Direct Blue-1	[4,5-Diazotricyclo[4.3.0.0(3,7) non-4-in-2-one], [(Phenol,2,6-bis(1,1-Diemethylethyl)-] and [1,2-Benzene Dicarboxylic Acid, 3-Nitro)]	Phytotoxicity test	Toxicity test was done with *Solanum leucopersicum* and *Triticum aestivum* seed where degraded product was less toxic as compared to pure dye	Singh and Dwivedi [109]
Aspergillus niger	Reactive red-120	2-aminobenzenesulfonic acid and 3-methanesulfinylbut-3-en-2-one	Phytotoxicity test	Mung beans seed used to check the toxicity of degraded metabolites where percent germination, root length and shoot length were less toxic in comparison with Reactive red	Su and Lin (2013)
Phomopsis sp.	Remazol Brilliant Blue R	Napthylamine and Benzidine	Phytotoxicity and Microbial test	Toxicity test was analyzed with *Phaseolus mungo* seed and *Azotobacter vinelandii* (MTCC 2460), *Azospirillum brasilense* (MTCC 4034) and *Bacillus cereus* (430) microbes where results showed that degraded product was less toxic as compared to pure dye	Navada et al. [87]

Acknowledgements V. Kumar and G. Singh thankful to University Grants Commission (UGC), Government of India for providing UGC-Non NET fellowship.

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