Textile Dyes Degradation: A Microbial Approach for Biodegradation of Pollutants

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1 Introduction

Rapid industrialization has given rise to various unwanted elements that accumulated in the biosphere up to toxic levels to degrade the natural environment. Scientific developments are considered as key factors for progress of both developing and under developed countries, but unfortunately, most of the industries in these countries do not have proper waste treatment facilities and releasing a large quantity of effluents. A majority of xenobiotics (either untreated or partially treated) released from industries are mixed up with the natural water bodies and to the soil of the biosphere. Untreated or partially treated textile effluents are highly toxic, as they contain a large number of toxic chemicals and heavy metals. The problem of water pollution due to the discharge of industrial wastewater into natural water bodies was witnessed by western countries in 19th century and also in India after independence.

Until the discovery of synthetic alternatives, most of dyes were derived from natural sources, such as plants and shellfish. These were only present in small amounts and their extraction was often inefficient, so they were usually expensive. In 19th century, there was a need to manufacture a large quantity of cheaper dyes and pigments for textile industries. As a result synthetic dye industry became a 'high-tech' industry of Victorian times, and its acknowledged founder was an English chemist, William Henry Perkin. In 1856, Perkin, in his experiment with aniline (one of the simplest chemical components of coal tar) obtained a black precipitate and discovered purple color, which readily dyed silk and was much more stable in sunlight than any other (natural) purple dye then in use. This first

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S.N. Singh (ed.), *Microbial Degradation of Synthetic Dyes in Wastewaters*,
Environmental Science and Engineering, DOI 10.1007/978-3-319-10942-8_9

synthetic dye was patented by Perkin in August 1856, as dye aniline purple and after its success in France, it was renamed mauve (or mauveine), after the French word for the purple mallow flower (Susan 1982).

The coloring processes discharge huge quantities of dye effluents, which pollute local terrestrial habitat, rivers and others aquatic bodies. The untreated effluents and toxic chemicals, solid wastes find their way to the ground water and rivers, causing extensive damage to soil and water. In countries, such as India, China and Mauritius discharge from a large number of the textile industries go straight into the rivers. According to an annual report by Union Ministry of Environment and Forests (MoEF), about 4.4 million tons of hazardous wastes are generated by 13,011 units spread over 373 districts of the country (Ramaswamy 2003). The industrial process produces wastewater containing about one tone of dyes daily.

Dyes are natural or synthetic colored organic compounds having the property of imparting their color to the other substances, such as textile fibers. Synthetic dyes are used extensively for textile dyeing, paper printing, leather dyeing, color photography and as additives in petroleum products because of their ease and cost effectiveness in synthesis, firmness, high stability to light, temperature, detergent and microbial attack and variety in color as compared to natural dyes (Couto 2009). The dyestuff, textile, paper and leather industries, the major users of dyes, produce effluents that are usually very resistant to the biological treatment and hence, their industrial waste is a major problem to the environment. The released wastewater from textile industries is a complex mixture of many polluting substances, ranging from agro-based pesticides to heavy metals associated with dye or the dye process. Approximately, 10,000 different dyes and pigments are used in different industries and their production exceeds over 7×10^5 tonnes annually worldwide (Zollinger 1987). The textile industry generates a large volume of wastewater, which if not properly treated, can cause serious problems of environmental contamination (Kunz et al. 2002).

Many synthetic dyes are used in a number of industries, such as textiles, paper printing, color photography and the food industries (Meyer 1981) and as additive in petroleum products (Maynard 1983). The pigments used in printing ink, such as carbon black, titanium dioxide and others organic pigments, which are rendered insoluble by complexing with metal ions. Most of these pigments are prepared from azo, anthraquinone and triarylmethane dyes, and phthalocyanines. Since 50 % of dyes are used in the textile industry, azo dyes account for the largest proportion of all synthetic dyes in terms of number and amount. Approximately 70 % of all organic dyes that are currently available in the market are manufactured mainly in China, India, Korea, Taiwan and Argentina. Azo dyes, one of the largest classes of dyes used in textile industry, are released into the aquatic and terrestrial environments through the effluents emanating from textile and dyestuff industries and are normally not removed by conventional wastewater treatment system. The characteristic chemical structure (such as —C═C—, —N═N— and —C≡N—) of azo dyes makes them recalcitrant to biological break down.

Biodegradation refers to the breakdown of complex molecules to mostly smaller and simpler ones. The original complex molecules are often environmentally objectionable. The biodegradation is a biological process by which environmental

pollutants are eliminated or converted into less toxic (or even useful) substances. Natural biodegradation is often largely catalyzed by indigenous microbial or plant populations in soil or aquatic ecosystems. Biodegradation has at least three definitions (i) a minor change in an organic molecule leaving the main structure still intact, (ii) fragmentation of a complex organic molecule in such a way that the fragments could be reassembled to yield the original structure, and (iii) complete mineralization. Mineralization is the transformation of organic molecules to mineral forms, including carbon dioxide or methane, plus inorganic forms of other elements that might have been contained in the original structures. In recent years, several studies have focused on the use of microorganisms that are capable or potent to biodegrade and/or bio-accumulate toxic compounds (Aksu 2005). The bioremediation technology offers several advantages; it can be performed on site; generally has lower cost and minimum inconvenience in the process; eliminates the waste permanently; can be used in conjunction with methods of physical and chemical treatments; has minimal environmental impact and, therefore, has wide public acceptance and also encouraged by regulatory authorities (Boopathy 2000; Dias 2000).

2 Textile Effluents and Their Degradation

The wastewater treatment system is mainly based on physical and chemical procedures such as absorption, coagulation-flocculation, oxidation, filtration, and electrochemical. Although these methods are effective, but there are also some disadvantages and limitations, such as high cost, formation of hazardous by-products, operational problems and intensive energy requirements. Therefore, biological process is getting more and more attention, since it is cost-effective, environmental friendly and does not produce a large quantity of sludge (Seong et al. 1995). Microbial decolorization of dyes is currently in wide use in textile industry and also safe for environment due to non formation of toxic by-products during and after degradation. Several attempts have been made for dye degradation in the past, but yet complete dye degradation or decolorization is an important task to the researchers.

A wide variety of microorganisms (fungi, bacteria and actinomycetes) growing in biological treatment systems are the biological agents for biodegradation. Microbial degradation is usually only means for complete mineralization of organic molecules. Besides, microbes can concentrate, accumulate and absorb heavy metals inside cell or cell walls. Various microorganisms including, yeasts *Proteus* sp., *Enterococcus* sp., *Streptococcus* sp., *Bacillus subtillis* and *Streptococcus* sp. have been previously isolated to degrade azo compounds (Brown 1981). Immobilized microorganisms are also being used for water purification e.g. immobilized mycelium of *Coriolus versicolour* is being used for removing colors/pigments from Kraft mill wastes. In order to develop suitable technology to decolorize or degrade dyes discharged in the effluent and to convert them into beneficial products simultaneously, a well-planned scientifically acceptable technology is needed.

Use of biological methods for detoxification of hazardous waste is an emerging technology with great potential as an effective and inexpensive alternative to earlier methods for clean-up of polluted environments.

Among microorganisms, fungi possess some unique attributes that, in many ways, reflect their morphological and physiological diversity in different habitats. Fungal biosorption has been studied more extensively because of the availability of large amount of waste fungal biomass from fermentation industry and the amenability of the microorganisms to genetic and morphological manipulations. Paszczynski et al. (1992) have examined a new approach to increase the susceptibility of azo dyes to degradation by aerobic microorganisms, especially by *Streptomyces* spp. and *Phanerochaete chrysosporium*.

3 Fungal Dye Degradation

Dyes industries are one of the major sources of water pollution among different industrial effluents, which are directly poured into the water bodies. In India as well as in others developing countries, discharges/effluents from dye industries play a major role in deteriorating water quality. The wastes from these industries and dye process are not only responsible for water contamination, but also for many diseases as well as for the disturbance to the aquatic fauna and flora. The carcinogenic and persistence nature of azo and many other synthetic dyes, used in textile industry, have been reported extensively to be mutagenic in nature. Microbes have a great ability to degrade persistent organic compounds, which are used in dye industries. With earlier studies on microorganisms, a number of strategies have been applied to demonstrate or enhance the abilities of organisms to degrade various persistent and toxic xenobiotics. Fungi and bacteria, both are the principal degraders of organic matters, but fungi are better known for this purpose. There are about 72,000 species of fungi and the new species are being added at the rate of about 1,500 each year.

Decolorization of azo dyes by various fungi has been reported by Rafii et al. (1990). Fungal biosorption has been studied more extensively because of the availability of a large amount of waste fungal biomass from fermentation industry and the amenability of the microorganisms to genetic and morphological manipulations. About 99 % color removal was obtained by adsorption of dye to the cells of filamentous fungi. Heinfling et al. (1997) reported that *Bjerkandera adusta* and *T. versicolour* removed 95 % of HRB 8 dye within four days.

The basidiomycete's fungus *Phanerochaete chrysosporium* has unusual degradative capabilities and termed as "white rot fungus" because of its ability to degrade lignin, a randomly linked phenyl propane-based polymeric component of wood. This fungus possesses a great potential and has become a model example for its commercial and biotechnological use in bioremediation of dyes and lignin-cellulosic materials, present in the textile effluents. Bumpus and Aust (1986) reported the capability of this fungus, to degrade a wide variety of structurally diverse organopollutants through its non-specific H_2O_2-dependent extracellular lignin-degrading

enzyme system, produced during secondary metabolism in nitrogen-limited medium. This fungus has also been shown to degrade polymeric dyes (Glenn and Gold 1983), Crystal Violet (Bumpus and Brock 1988) azo and heterocyclic dyes. Cripps et al. (1990) reported the aerobic degradation of three azo dyes (Congo Red, Orange II and Tropaeolin O) by the fungus *P. chrysosporium*. The degradation of azo, anthraquinone, heterocyclic, triphenylmethane and polymeric dyes by *P. chrysosporium* has been intensively studied (Bumpus 1989; Cripps et al. 1990; Ollikka et al. 1993). Spadaro et al. (1992) established that *P. chrysosporium* was capable of mineralizing a variety of toxic azo dyes and was dependent on the nature of ring substituents. Another white-rot fungus, *Thelephora* sp. was also used for decolorization of azo dyes, such as Orange G, Congo Red, and Amido Black 10B.

Freitage and Morell (1992) reported the results of screening of 170 strains of white-, brown-, and soft-rot decay fungi and non-decaying xylophilous fungi for phenol oxidase activity with the polymeric dye Poly-478. This study also explored a relation between dye decolorization and ligninolytic activity and the presence of phenol oxidase and peroxidases. Yesilada (1995) reported decolorization of crystal violet by *Coriolus versicolour* and *Funalia trogii*. Wilkolazka et al. (2002) have studied the potential of 115 strains of fungi to decolorize two structurally different dyes (azo dye and anthraquinonic dye) and observed that the fungi, which have a great ability to degrade the azo and anthraquinonic dyes, are mainly white-rot fungi as listed in Table 1.

Some brown-rot fungi were also described by same authors for decolorization of some anthraquinonic and azo dyes i.e. *Coprinus micaceus*, *Fomtopsis pinicola*, and *Gloeophyllum odoratum*. Similarly, seven different fungi were isolated from the dye effluent sites and identified as, *Aserpgillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichoderma viride*, *Fusarium oxysporum*, *Penicillium chrysogenum* and *Mucor* sp. which were responsible for the degradation of a wide range of textile dyes (Saranraj et al. 2010). Machado et al. (2006) showed the potential of fungi *Trametes villosa* and *Pycnoporus sanguineus* to decolorize reactive textile dyes used for cotton manufacturing in the State of Minas Gerais, Brazil and found decolorization of 28 tested dyes by the fungus *T. villosa* and decolorization of 9 dyes by the fungus *P. sanguineus*. Higher decolorization of the synthetic effluent was also observed by mixed culture of these two fungi. The biodegradation of some

Table 1 Fungal strains mainly used for biodegradation of azo and anthraquinonic dyes

Fungi	
Strains	*Abortiporus biennis*, *Bjerkandera fumosa*, *Cerrana unicolour*, *Clitocybula dusenii*, *Dichomitus albiodoffuscus*, *Diplomitiporus crustulinus*, *Flammulina velutipes*, *Gonoderma lucidum*, *G. applanatum*, *Heterobasidion annosum*, *Keuhneromyces mutabilis*, *Lentinus edodes*, *Nematoloma frowardii*, *Panus tigrinus*, *Perenniporia subacida*, *Phanerocheate chrysosporium*, *Phlebia radiate*, *Pholiota glutinosa*, *Pleurotus pullmonarius*, *Pycnoporus coccineus*, *Stropharia rugosoannulata*, *Tremetes sanguinea*, *T. versicolour*, *Agrocybe cylidracea*, *Coprinus micaceas*, *Fomitopsis pinicola*, *Geotrichum sp. Gloeophyllum odoratum*, *Pestalotia sp. Pholiota glutanosa*

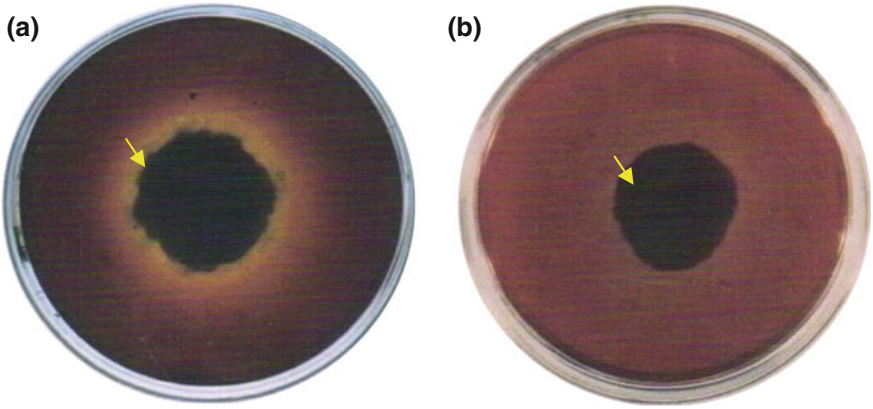

Fig. 1 Decolorization of Bromophenol blue (**a**) and Congo red (**b**) dyes by fungus *Aspergillus flavus*. *Arrows* indicating the zone of *yellow* color developed by the applied fungus during the dyes decolorization/degradation (Singh and Singh 2010b)

azo and anthraquinonic dyes by fungi *Aspergillus flavus* (Fig. 1) and *Trichoderma harzianum* was also recorded (Singh and Singh 2010a, b) which might be due to both; primary, by the adsorption and absorption of these dyes by fungal mycelia and secondary phenomenon, by producing extracellular enzymes during dye degradation (Singh and Singh 2012). However, bacteria and others microorganisms play an important role in the degradation of hazardous dyes, but among them, fungi have key role in biodegradation process.

4 Bacteria Used in Dyes Degradation

Apart from fungi, bacteria and actinomycetes also have ability to degrade the dyes and others pollutants. Various bacterial strains reduce azo dyes under both anaerobic and aerobic conditions to the corresponding amines (Meyer 1981). The bacteria *Sphingomonas xenophaga* BN6, *Agrobacterium tumefaciens*, *Ralstonia eutropha* 335, *Hydrogenophaga palleronii*, *Escherichia coli* K12 and *Flexibacter filiformis* (Gram negative), *Bacillus subtilis*, *Rhodococcus erythropolis* and *Lactobacillus plantarum*) (Gram negative) and Archea (*Halobacterium salinarum*) are reported to reduce azo dyes under anaerobic condition.

Neelambari et al. (2013) found the decolorization of azo dyes by some bacterial strains of *Pseudomonas aeruginosa*, *Alcaligenes faecalis*, *Proteus mirabilis*, *Serratia marcescens* and *Bacillus licheniformis* at static and shaking conditions. They also found that applied bacterial strains decolorized the dyes efficiently at about 3 % salt concentration and completed decolorization of dye in 48 h of incubation. The metabolites generated after the bacterial degradation were also found less toxic than original dye as confirmed by the phytotoxicity test. Jadhav et al. (2010) observed

that the *Pseudomonas aeruginosa* was able to detoxify the dye, Direct Orange 39 (1,000 ppm each day) effectively. Similarly, Roushdy and Abdel-Shakour (2011) observed zone of inhibition with control Malachite green with some microbial strains and found no growth inhibition by the degraded products. These findings clearly suggest non-toxic nature of the degradation products formed by the biological degradation.

The ability of actinomycetes to decolorize several anthraquinone and azo dyes has also been reported by several workers. The anthraquinonic dyes Remazol Brilliant Blue R, Poly B-411 and Poly R-478 were decolorized by *Streptomyces* sp. and *Thermomonospora* sp. Some species of *Streptomycetes* also decolorized azo dyes 4 (3-methoxy-4-hydroxy-phenylazo)-azobenzene-3,4′-disulfonic acid, 3-methoxy-4-hydroxy-azobenzene-4′-sulfonic acid and Orange I. Actinomycetes can catalyze hydroxylations; O, N, and S oxidations; and O- and N-dealkylation reactions against various xenobiotic compounds. *Streptomyces chromofuscus*, a member of actinomycetes group, was found capable to degrade azo dye in the presence of verytal alcohol (Paszczynski and Crowford 1991). The ability of *S. chromofuscus* to mineralize these dyes and guaiacol-substituted azo dye was of much significance, since azo dyes and some of its substitutes are resistant to aerobic degradation by most of soil bacteria. The genus *Streptomyces* has been reported to degrade benzene derivatives via classic aromatic catabolic pathways. A little research has been devoted to determining whether actinomycetes efficiently degrade condensed polycyclic aromatics, although some strains are able to metabolize naphthalene derivatives. *Streptomyces* spp. degrade some recalcitrant compounds, such as carbamates, diazinon, and bromoxynil. Actinomycetes were also found to degrade organo-chlorine compounds in spent sulfite bleach plant effluent. Further, evidences also indicate that *Streptomyces* sp. can act synergistically with other soil microorganisms to degrade recalcitrant compounds (Gunner and Zuckman 1968).

5 Mechanisms and Basic Steps During Biodegradation

The aerobic and anaerobic biological wastewater treatment has been the oldest known biodegradation based process technology. Several technologies have been evolved over the past decades since the development of conventional activated sludge plant in United Kingdom in 1914. Degradation of complex compounds takes place in several stages, for example, in the case of halogenated compounds, dehalogenation often occurs early in the over all processes. Dehalogenation of many compounds containing chlorine, bromine, or fluorine occurs faster under anaerobic than aerobic conditions. The study of reductive dehalogenation, especially for its commercial application, is gaining importance. Once the anaerobic dehalogenation steps are completed, degradation of the main structure of many xenobiotics often proceeds more rapidly in the presence of O_2. Observations of natural and synthetic organic compound accumulation in natural environment, however, began to raise questions about the ability of microorganisms to degrade these varied substances

and their role in environment protection. The chemical recalcitrance resulted from the inability of microorganisms to degrade some industrially synthesized chemical compounds.

White-rot fungi are unique among eukaryotes in their ability to cleave carbon-carbon bonds in polycyclic aromatic hydrocarbons (PAHs). In fact, biological degradation of these compounds was earlier considered as an exclusively bacterial process (Gibson and Subramanian 1984). The breakdown of most organo-pollutants by ligninolytic fungi is closely linked to ligninolytic metabolism. In this process, degradation is stimulated by nutrient limitation, and it is generally believed that enzymes, whose normal function is lignin degradation, also catalyze the highly non-specific xenobiotic oxidation. The biodegradation of these types of organo-pollutants has been shown to be dependent on the lignin-degrading system of the microorganism. Initial oxidation of several organo-pollutants has been reported to be catalyzed by ligninases isolated from *P. chrysosporium* (Hammel et al. 1992). The lignin-degrading system of this fungus includes a family of lignin peroxidases or ligninases which catalyze the initial oxidative depolymerization of lignin.

Several mechanisms used by the fungi to degrade chemicals have recently been elucidated. The first step in the degradation of many chemicals by white-rot fungi often involves formation of highly reactive free radical intermediates. These free radicals are formed any time; one electron is removed or added to the ground state of a chemical. Such free radicals are very reactive and will rapidly give up or withdraw an electron from another chemical. Free radical reaction often occurs as chains in which many different radicals are generated subsequent to formation of initial radical species and free radical reactions catalyzed by the peroxidases from white-rot fungi also appear to be involved in the degradation of many pollutants. The free radical process also provides some basis for the non-specific nature of white rot fungi.

Idaka et al. (1987) described reductive fission of azo bonds by *Pseudomonas cepacia*, followed by acetylation of the resulting amino benzenes. In a continuation of the study, they also reported an oxidative pathway for degradation of amino compounds which converted these compounds to amino hydroxy compounds. These metabolites are subsequently metabolized through the Kreb's cycle after opening of ring. However, their degradation by *Streptomyces* spp. depends upon the substitution pattern of the aromatic ring. Bacterial cytochrome P-450 is believed to catalyze most of the reactions.

6 Role of Fungal Enzymes in Degradation

Production of laccase by white-rot fungus *Phanerocheate chrysosporium*, *Neurospora crassa* and some other fungi has been extensively studied for removal of pigments and phenol from liquid waste. The production of extracellular enzymes by artificially captured cells of fungi (*Trichosporon cutaneum*, *Candida tropicals* and *Phanerocheate chrysosporium*) is possible. By using gel entrapment and adherence to a matrix, encouraging results can be obtained for the wastewater treatment.

Decolorization of dye is related to the presence of extracellular peroxidases, particularly manganese peroxidases (Gold et al. 1988). However, an evidence, relating degradation of polymeric dyes to phenol-oxidizing enzymes and to lignin degradation, is largely circumstantial. Paszczynski and Crawford (1991) reported involvement of veratryl alcohol during the degradation of some azo compounds by *P. Chrysosporium* ligninase. Veratryl alcohol stimulated azo dye oxidation by ligninase, acting as a third substrate (with H_2O_2 and the azo dye) to cycle the enzyme back to its native state. These studies report aerobic biodegradation of some of the commercial textile dyes by *P. Chrysosporium*. The fungal lignin-degrading system was implicated in the decolorization process, since crude lignin peroxidase was required for the initial step of decolorization of Orange II and Tropaeolin. Paszczynski and Crowford (1991) showed that while ligninase recognized Acid yellow 9 as a substrate, Mn(II) peroxidase was responsible for decolorization of other azo dyes. The extracellular ligninolytic enzyme systems of these fungi have been directly linked to the degradation of these compounds.

The other major group of extracellular oxidative enzymes, involved in the white-rot fungal lignin degradative process, is laccases. The laccases of *T. Versicolour* catalyze the initial oxidation step in the biotransformation of anthracene and benzo [a]pyrene. This process involves either a direct laccase oxidation mechanism or an indirect mechanism involving the participation of an oxidation mediator, such as putative present in the ultrafiltrate fraction. In some experiments with *P. Chrysosporium*, manganese peroxidases were found to play a major role in the initial breakdown and decolorization of high-molecular-weight chlorolignin in bleach plant effluents and also to transform other naturally occurring polymers, such as lignite and sub-bituminous coals. This fungus demonstrated a better ability than the actinomycetes to mineralize the azo dyes (Paszczynski et al. 1992).

The use of ligninolytic enzymes was investigated, but their production yields were too low for industrial applications (Bajpai 1999). Commercial enzymes, such as xylanases, are produced in the large quantities in *Trichoderma reesei* and hence used for pulp bleaching (Bajpai 1999). Application of xylanases was shown to decrease chlorine consumption, which is a environmental-friendly process on one hand and also increases the final brightness of the pulp. Other enzymes, such as laccases, have been studied for their in vivo capacity to degrade lignin (Mayer and Staples 2002). Laccases are commercially available and produced in fungal strains, such as *Aspergillus* sp. (Yever et al. 1991; Berka et al. 1997; Record et al. 2003). *Aspergillus niger* was used to produce the feruloyl esterase for pulp bleaching application (Record et al. 2003). A basidiomycetous fungus *Ganodermaa lucidum* has been found as a suitable organism for removal of Rhodamine-B and Sandolan rhodine. The decolorization of dyes, containing toxic chlorinated phenols used in Kraft bleach dyeing, has been observed by several workers. Bennett et al. (1971) reported that brownish color of the effluent in the textile industry was due to presence of chlorolignin. The ability of microfungi to degrade this component of the effluent has been studied by Cammarota and Santa-Anna (1992).

Adsorption of dyes to the microbial cell surface is the primary mechanism of decolorization (Knapp et al. 1995). Wong and Yu (1999) reported adsorption of

Acid green 27, Acid violet 7 and Indigo carmine dyes on living and dead mycelia of *Trametes versicolour*. Yong and Yu (1997) suggested the binding of dyes to the fungal hyphae, physical adsorption and enzymatic degradation by extracellular and intracellular enzymes as major mechanisms for the color removal. Besides, enzymes, such as lignin peroxidase (LiP), manganese-dependent peroxidase (MnP) and laccase, which are involved in lignin degradation, have been reported to decolorize dyes (Vyas and Molitoris 1995). Dyes with different structures are decolorized at different intrinsic enzymatic rates. Kim et al. (1996) demonstrated that presence of H_2O_2-dependent enzyme activity declourized Remozol Brilliant Blue R in the culture filtrate of *Pleurotus ostreatus* in a chemically defined medium.

Kim and Shoda (1999) have purified and characterized a novel peroxidase (Dyp) which is responsible for the dye-decolorizing activity of *Geotrichum candidum* Dec 1. Nine of the 21 types of dyes were decolorized by Dec 1, and in particular, anthroquinone dyes were effectively decolorized. Swamy and Ramsay (1999) reported that in fungus, *Trametes versicolour*, lignin peroxidase (Lip) was not detected during decolorization of the azo dye of Amaranth, while laccase and manganese peroxidase (MnP) were detected in the decolorizing cultures. A white-rot fungus, *Thelephora* sp. was isolated from the Western Ghats of South India and characterized for its lignolytic enzymes (Selvam 2000). Roushdy and Abdel-Shakour (2011) found that lignin peroxidase produced by *Cunninghamella elegans* was capable for 100 % decolorization of Malachite green dye under static condition, whereas no decolorization was observed in shaking condition. They also showed that Malachite green was degraded and decolorized under ligninolytic conditions. This indicated that ligninolytic enzymes were essential for the degradation of this dye by the fungal strain *Cunninghamella elegans*. Won et al. (2000) also stated that lignin degradation system of the fungi had been directly as well as indirectly linked to the degradation of various compound remains. Other studies reported that Malachite green was enzymatically reduced to leucomalachite green and also converted to N-demethylated and N-oxidized metabolites (Cha et al. 2001).

Traditionally, fungi have been classified as white-, brown-, or soft-rot fungi on the basis of technical decay descriptions (Nilsson 1988), regardless of their taxonomic position. Because the enzyme systems and metabolic pathways involved in the breakdown of carbohydrates and lignins are truly distinct in these fungi, rather than just modified in one or a few specific enzyme activities, decay type is of significant taxonomic importance. One important physiological characteristic of decay fungi in culture is production of extracellular phenoloxidases and peroxidases. Certain fungi produce brown diffusion zones in agar plates supplemented with 0.5 % (w/v) gallic or tannic acid, as a result of oxidation of the respective phenolic acid by extra- or intracellular phenoloxidases. Bavendamm (1928) suggested that the presence of phenoloxidases was correlated with fungi causing white-rot decay and that only these fungi were able to completely decompose lignin. Davidson et al. (1938) extended this method first time to 210 species of wood decaying fungi. Of all tested white-rot fungi, 96 % were positive on gallic acid agar, tannic acid agar, or both. Kaarik (1965) tested 173 wood-decaying species on the plates supplemented with 28 substances and found wide variations in the reactions of these strains to specific phenolic compounds.

7 Mechanism of Bacterial Dye Degradation

It has been demonstrated recently that the rate of anaerobic reduction of azo dyes by *Sphingomonas xenophaga* BN6 could be significantly increased by the addition of different quinines, such as anthraquinone-2-sulfonate or 2-hydroxy-1,4-naphthoquinone. Thus, it was demonstrated that the addition of naphtha quinone and natural organic matter could significantly enhance the reduction rate of nitro aromatic compounds and hexachloroethane in the presence of bulk reductant (e.g. H_2S). Furthermore, it has also been seen that strictly anaerobic Fe (III)-reducing bacteria use the reduction of quinine moieties of humic substances (and also sulfonated anthraquinones) to transfer the reduction equivalents released during the anaerobic oxidation of organic substances. Thus, it becomes evident that in the presence of redox mediators, many heterotrophic aerobic bacteria decolorize azo dyes under anaerobic conditions. Quinones may undergo one-electron reduction processes to the corresponding hydroquinone radicals or two-electron reduction to the corresponding hydroquinones. Therefore, one-electron potentials can be used to compare the relative tendency of different quinines to take up reduction equivalents. The understanding on microbial degradation and decolorization of azo and reactive dyes is still limited and has been studied by only a few workers.

Kulla et al. (1983) employed strains of *Pseudomonas* in chemostat culture for removal of dyes. Some anaerobic bacteria and *Streptomyces* have been characterized for decolorization of chromogenic dyes. A bacterium, *Proteus mirabilis*, isolated from acclimated sludge from a dyeing wastewater treatment plant, rapidly decolorized a deep Red azo dye solution (RED RBN). Features of decolorizing process related to biodegradation and biosorption were also studied. Although *P. mirabilis* displayed good growth in shaking culture, color removal was best in anoxic static cultures and found very effective for color removal under optimum conditions (pH 6.5–7.5 and temperature 30–35 °C). The organism showed a remarkable color removal capability, even at a high concentration of azo dye. More than 95 % of azo dye was reduced within 20 h at a dye concentration of 1.0 g l^{-1}. Decolorization appears to proceed primarily by enzymatic reduction associated with a minor portion (13–17 %) of biosorption to inactivated microbial cells.

8 Main Factors Effecting Dye Degradation

Biodegradation of xenobiotics depends upon the physical, chemical and biological processes which are also governed by some environmental factors. The fungal growth and enzyme production or secretion, and consequent decolorization and degradation are influenced by numerous factors, e.g. media composition, pH value, agitation and aeration, temperature and initial dye concentration. Thus, depending on the culture characteristics, the degradation potential for dyes also varies upon the environmental conditions (Robinson et al. 2001). The structure of dyes strongly

influences their degradability by pure cultures and enzymes produced. Variations regarding dye decolorization could be possible due to the complex chemical structure of dyes, e.g. diazo dyes have more complex structure than mono-azo dyes. With recent findings, it has also been demonstrated that chemical structure of dyes affects their decolorization process (Nozaki et al. 2008). Asgher et al. (2007) evaluated the dye decolorization efficiency of mixed microbial consortia from wastewater treatment plants of different textile units and concluded that the microbial consortium is a robust process for the bioremediation of textile dye effluents. The nature of substituents on the aromatic ring has been shown to influence the enzymatic oxidation. Electron donating methyl and methoxy substituents enhanced the enzymatic degradation of azo phenols, while electron withdrawing chloro, fluoro and nitro substituents inhibited oxidation by a laccase from *Pyricularia oryzae* and MnP from *P. chrysosporium* (Chivukula and Reganathan 1995; Pasti-Grigsby et al. 1992). Some studies have demonstrated that shaking conditions do not support decolorization. The reason for no decolorization at shaking condition might be due to the competition of oxygen and dye for the reduced electron carriers under aerobic condition. Similar studies were carried out by Parshetti et al. (2006) and they reported that Malachite green was completely decolorized under static condition within 5 h by a bacterium *Kocuria rosea* MTCC 1532. The nitrogen concentration in the culture medium also influences the growth of fungi. However, decolorization of some dyes, e.g. Drimaren brilliant blue by fungi was not found dependent on the initial nitrogen concentration (Machado et al. 2006). The fungi *T. villosa* and *P. sanguineus* belong to a group of fungi whose ligninolytic systems are not regulated by nitrogen concentration, and so was the case for *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* also (Leatham and Kirk 1983; Niku-Paavola et al. 1990; Ruttimann-Johnson et al. 1993). In fact, the rates of pollutant degradation are proportional to the concentration of the chemical. The biodegradation of other commercially important classes of textile dyes, have also been addressed. Bakshi et al. (1999) found enhanced biodecoloration of synthetic commercial textile dyes by *P. chrysosporium* by improving Kirk's medium with respect to buffer, C:N ratio, Mg^{2+} and Zn^{2+}, temperature shifts, agitation, and sunflower oil addition.

9 Toxicity of Dyes and Their Effects on Fungi

Serious concern about textile dyes and intermediate compounds was first raised due to its toxicity and carcinogenicity that can cause damage to human health and environment (Banat et al. 1996). This is mainly due to the fact that many dyes are manufactured from known carcinogens, such as benzidine, naphthalene and other aromatic compounds (Nascimento et al. 2011). Many workers have studied the effects of dyes on microorganisms. Among them, Stearn and Stearn (1924), Dion and Lord (1944), Aiquel and Herraro (1948) have shown that basic dyes are toxic to some fungi, e.g. *Fusarium culmorum* and some Aspergilli. Further, similar studies

have also been done by many others to relate the toxicity of dyes with their structures (Mietzsch 1936; Goldacre and Phillips 1949; Fischer 1957). Michaelis and Granick (1945), Michaelis (1947, 1950) attempted to explain the toxicity by showing that basic dyes react irreversibly with nucleic acids. Similar studies have been also carried out by other workers (Neuberg and Roberts 1949; di Marco and Boretti 1950; Steiner and Beers 1958). Basu and Whitaker (1953) found that acid and basic dyes were inhibitory to the isolated cellulase of *Mlyrothecium verrucaria*. Conn (1935) disclosed the use of Crystal violet and malachite green for the preservation of cotton fish nets. Singh et al. (2007) also found an inhibition in the growth of fungus *Trichoderma harzianum* during the degradation of some hazardous dyes. In the same sequence, the toxicity of dyes and certain related compounds was also tested with six species of wood-destroying fungi, because of their economic importance to the wood-preserving industry (Weaver et al. 1959). The arylmethane basic dyes, particularly those containing three phenyl groups with two p-dialkylamino substituents, appear to be effective fungicides when tested on nutrient agar plates against six species of wood-destroying fungi. Brilliant green and Malachite green, both available commercially, are the most toxic dyes of the group, and it was concluded that alkyl substituents on the amino nitrogen atoms increase their toxicity. Malachite green is more toxic than Doebner's violet, while crystal violet is more toxic than para magenta (Weaver et al. 1959).

Most of the dyes are synthetic poly aromatic compounds and are difficult to be degraded in the environment. They are also potential to form carcinogenic breakdown products in the environment (Chung et al. 1992). Several amino-substituted azo dyes, including 4-phenylazoaniline and N-methyl- and N,N-dimethyl-4-phenylazoanilines, are mutagenic as well as carcinogenic (McCann and Ames 1975). The carcinogenicity of an azo dye may be due to the dye itself or to aryl amine derivatives generated during the reductive biotransformation of the azo linkage.

10 Future Prospectives

The work on microbial degradation/decolorization of synthetic dyes and other hazardous compounds or on the purification of wastewaters by using some ideal microbes is still not reached to development of a better alternate or superior technology. Presently, many laboratories in India and from abroad are involved in the same task and in the coming years, there would be a better option to use microbes for biodegradation. The use of microbes including fungi, bacteria, actinomycetes and algae in environmental biotechnology is still under investigation for their proper implementation. One main fact is clear that if any microbe has been shown ability to do work in vitro conditions, it doesn't mean that same work would be done in vivo conditions or vise versa. There is a need for further study on the microbes regarding the role of fungal genes to promote the degradation capacity of any microbe.

11 Conclusion

Degradation of hazardous dyes may be possible with three well known methods; physical, chemical and biological. The biological degradation or biodegradation of synthetic dyes using microbes is safe, economical and eco-friendly. A wide variety of microorganisms including, algae, bacteria, actinomycetes and fungi may be used for biodegradation process. Microbial degradation of hazardous dyes is well known and currently being adopted as a better alternative for degradation. The enzymes produced by a range of microbes, are commercially much important and are also used for the degradation of a large class of pollutants or xenobiotics. Some fungal strains, like *Phanerocheate chrysosporium*, *Aspergillus flavus*, *A. niger*, *Trichoderma harzianum* etc. are well known for the production of laccases, peroxidases and ligninases. These fungal enzymes are used for degradation of a range of organic toxic compounds including synthetic dies.

Acknowledgments The authors are thankful to the Head of the Department of Botany, University of Delhi, Delhi for providing necessary facilities during the work.

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