Bacterial Enzymes and Their Role in Decolorization of Azo Dyes

Amar A. Telke, Avinash A. Kadam and Sanjay P. Govindwar

1 Introduction

Azo dyes are one of the largest groups of textile dyes, extensively used for dyeing purposes. A significant proportion of these dyes were entering into the surrounding environment in the form of wastewater. Discharge of such wastewater into natural water resources has created an aesthetic problem to aquatic life and human being. Several physico-chemical methods have been employed for azo dye decolorization, but they have facing several problems, such as generation of toxic by-products and economical unfeasibility. Several reports have been stated that bacteria, fungi and plants have ability to decolorize and detoxify azo dyes. However, ubiquitous nature of bacteria makes them as invaluable tool for the textile dye decolorization. Bacterial genera, such as *Pseudomonas*, *Bacillus*, *Rhodococcus*, were reported for rapid azo dye decolorizing biological agents (Kalyani et al. 2008; Telke et al. 2008). The azo dye decolorization by bacteria has been associated with the production of oxidoreductive enzymes, such as lignin peroxidase (Ghodake et al. 2008), laccase (Telke et al. 2009), azoreductases and other non specific reductases (Chen et al. 2005; Dhanve et al. 2008a, b). Azo dye decolorization by purified bacterial enzymes was demonstrated in several reports. Previous reviews have indicated that azo dye decolorization was associated with reductive cleavage of azo bond by

A.A. Telke (✉)
Center for System Microbiology, Department of System Biology, Denmark Technical University, Kongens Lyngby, Denmark
e-mail: telkeaa@gmail.com

A.A. Kadam
Department of Environmental Engineering, Kyungpook National University, 1370 Sankyuk-Dong, Buk-Gu, Daegu 702-701, Republic of Korea

S.P. Govindwar
Department of Biochemistry, Shivaji University, Kolhapur 416004, India

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reductase (Dos Santos et al. 2007; Pandey et al. 2007). However, this chapter has focused on the various oxidoreductive enzymes involved in decolorization and detoxification of textile azo dyes and their mechanism of azo dye decolorization.

2 Bacterial Decolorization of Textile Azo Dyes

Bacteria degrade the textile dyes under both aerobic and anaerobic conditions. The optimum pH for decolorization of textile dyes varied from acidic to basic range. The bacteria, used for decolorization of textile dyes, were isolated from textile dye contaminated soil by enrichment techniques and identified on the basis of 16s rRNA sequence and biochemical characteristics (Kalyani et al. 2008; Dawkar et al. 2008; Dhanve et al. 2008a, b; Telke et al. 2008). However, certain microorganism, which was not isolated from textile dye-contaminated soil, but showed excellent dye decolorization property.

3 Decolorization of Azo Dye Under Anaerobic Condition

At present, only mechanism of azo dye decolorization under anaerobic condition is well known. Anaerobic azo dye reduction involves the reduction of azo bond by the transfer of reducing equivalents resulting in the formation of aromatic amines (Chung and Cerniglia 1992). As removal of a large amount of azo dyes from wastewater is a major concern, research on bacterial azo dye reduction has mainly focused on the activity of (facultative) anaerobic bacteria. Various anaerobic bacteria were used in bioreactor for treatment of textile effluent containing azo dyes (Işık and Sponza 2005; Maas and Chaudhari 2005; Mezohegyi et al. 2008).

4 Decolorization of Azo Dyes under Anoxic Condition

Pure bacterial strains, such as *Pseudomonas luteola*, *Aeromonas hydrophila*, *Bacillus subtilis*, *Pseudomonas* sp. and *Proteus mirabilis* were utilized for azo dyes decolorization under anoxic condition (Chen et al. 1999, 2003; Chang et al. 2001; Yu et al. 2001). Although, most of these cultures were able to grow aerobically, but decolorization were achieved only under anaerobic conditions. Anoxic decolorization of various azo dyes by mixed aerobic and facultative anaerobic microbial consortia has been reported by many researchers (Nigam et al. 1996; Kapdan et al. 2000; Padmavathy et al. 2003; Khehra et al. 2005; Moosvi et al. 2005). Azo dye decolorization by pure as well as mixed cultures have required complex organic carbon sources, such as, yeast extract, peptone, or a combination of complex organic source and carbohydrate (Chen et al. 2003; Khehra et al. 2005). The decolorization

of azo dyes in anoxic condition was influenced by various substrates used in cell growth medium. Although, azo dye decolorization under anoxic condition was non-specific, limitation of this method was requirement of yeast extract or peptone. This makes the process economically in viable for industrial-scale application unless alternate cheaper sources are identified (Nigam et al. 1996; Chen et al. 2003; Moosvi et al. 2005).

5 Decolorization of Azo Dyes Under Aerobic Condition

5.1 Decolorization of Azo Dyes Under Static Condition

The optimum pH for decolorization of azo dyesat static condition is 7.0–8.0. The pure bacterial cultures isolated from textile dye contaminated soil, such as *Pseudomonas* sp. SUK 1, *Kocuria rosea*, *Rhizobium radiobacter*, *Bacillus* sp.VUS, *Commamonas* sp. UVS,*Exiguobacterium* sp. RD3, *Proteus* sp. SUK 7; *Bacillus* sp. ADR; and *Pseudomonas* sp. SU-EBT were efficiently decolorized azo dyes at static condition than shaking condition (Kalme et al. 2007; Parshetti et al. 2007; Dawkar et al. 2008; Dhanve et al. 2008a, b; Jadhav et al. 2008; Kalyani et al. 2008; Patil et al. 2008; Telke et al. 2008, 2009). The pure bacterial cultures of *Pseudomonas* sp. SUK1 and *Rhizobium radiobacter* MTCC 8161 were able to tolerate and degrade the higher concentration (more than 1 g l^{-1}) of azo dyes (Kalyani et al. 2008; Telke et al. 2008). The recent reports showed that the combination of yeast extract with urea and agricultural wastes (bagasse powder, wheat bran, rice bran and wood shaving) were effective medium for the decolorization of azo dyes (Jadhav et al. 2008; Telke et al. 2008) at static condition.

5.2 Decolorization of Azo Dyes Under Shaking Condition

For a long time it was thought that azo dyes were recalcitrant under aerobic conditions. A bacterial strain S5, derived from *Hydrogenophaga palleronii* S1, mineralized sulfonated azo dyes by utilizing them as carbon and nitrogen source for cell growth (Blumel et al. 1998). The recent studies have shown that the pure bacterial culture (Unidentified KMK 4, strain S5, *E. coli* NO_3 and *Flavobacterium* sp. ATCC39723) and mixed bacterial culture (unidentified BF1, BF2 and *Pseudomonas putida* MTCC 1194) have ability to degrade higher concentration (1 g $^{-1}$) of azo dyes (Cao et al. 1993; Chang and Kuo 2000; Senan and Abraham 2004; Kodam et al. 2005). Aerobic bacterial cultures were reduced the azo linkage by the involvement of azo reductases and oxidases or combination of both enzymes. Azo reductases isolated from aerobic bacteria have a narrow substrate range (Kulla 1981; Zimmermann et al. 1982, 1984; Kulla et al. 1983). The involvement of peroxidase was reported in azo dye oxidation by Cao et al. (1993).

6 Mechanism of Dye Decolorization

Although for a long time, it was thought that the bacterial degradation of azo dyes in either anaerobic or aerobic conditions were associated with symmetric cleavage of the azo group (–N=N). This cleavage may be mediated through different mechanisms, such as enzymes, low molecular weight redox mediators, chemical reduction by biogenic reductants like sulfide, or a combination of both. However, the location of such type of reactions could be either intracellular or extracellular. The recent reports on azo dye decolorization have indicated involvement of peroxidase, laccase, tyrosinase, NADH-DCIP reductase, and MG reductase in decolorization of azo dyes.

6.1 Enzymatic Dye Decolorization

6.1.1 Azo Reductase

The presence of extracellular oxygen sensitive azo reductases in anaerobic bacteria, such as *Clostridium* and *Eubacterium,* were decolorized sulfonated azo dyes during their growth on solid or complex media (Rafii et al. 1990). Anaerobic azo reductases were flavoprotiens (NAD(P)H:flavin oxidoreductase), localized at intracellular or extracellular site of cell membrane, required NADH, NADPH and FADH as electron donors for reduction of azo bonds (Rafii and Cerniglia 1995; Russ et al. 2000). Co-factors like $FADH_2$, $FMNH_2$, NADH and NADPH, which provide 'H' for reduction, were located at cytoplasm (Russ et al. 2000). Lysis of cells would release co-factors at extracellular environment could be the possible reason for higher azo dye reduction rates using cell extracts or starving or lysed cells than intact or resting cells (Roxon et al. 1967; Walker 1970; Dubin and Wright 1975; Wuhrmann et al. 1980; Mechsner and Wuhrmann 1982; Russ et al. 2000).

Intracellular sulfonated azo dye reduction required the specific transport system that allows the uptake of the sulfonated azo dye into the cells (Russ et al. 2000). The role of flavin dependent azo reductase was shown by adding of flavins to resting cells of strain *Sphingomonas* strain BN6, which resulted in no significant increase in azo dye reduction. These findings suggest that in living cells with intact cell membranes, other enzyme systems and/or other redox mediators are responsible for reduction of azo dyes. In bacteria that possess electron transport systems in their membranes, as in the case of aerobic or facultative anaerobic bacteria, such as *Sphingomonas* strain BN6, the transfer of electrons from the respiratory chain to appropriate redox mediators could take place directly. If intracellular reductases are involved in the process, it is assumed that mediators, different from flavin cofactors with a higher ability to pass through the membranes, must be involved. Although

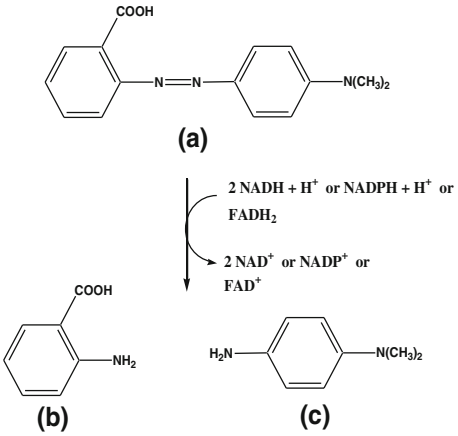

Fig. 1 Reaction catalyzed by azo reductases. **a** Methyl red; **b** 2-Amino benzoic acid; **c** *p*-dimethyl amino anilline

there are a few reports on systems which are involved in the transport into bacterial cells of other sulfonated substrates, such as *p*-toluene sulfonate, taurine and alkane sulfonates (Locher et al. 1993; Eichhorn et al. 2000). The 2-ABS degrading *Alcaligenes* sp. strain O-1 can utilize two other aromatic sulfonates, benzene and toluene sulfonate, for growth. However, cell extracts of this strain can desulfonate at least six substrates (Thurnheer et al. 1986). This suggests that the presence of highly specific transport systems for the uptake of aromatic sulfonates in these cultures. Azo reductase and flavin reductase are the potent enzymes involved in the decolorization of azo dyes, but generate the toxic amines after reduction of azo bond. The reaction catalyzed by azo reductase has been shown in (Fig. 1).

6.1.2 NADH-DCIP Reductase and Malachite Green Reductase

Significant induction of non-specific reductase, such as NADH-DCIP reductase and Malachite green reductase during decolorization of azo dyes suggests their possible participation in decolorization (Kalyani et al. 2008; Telke et al. 2008). The function of non-specific reductases is still unknown. NADH-DCIP reductase belongs to the bacterial mixed function oxidase system may takes part in the detoxification of xenobiotic compounds (Salokhe and Govindwar 1999). NADH-DCIP reductase enzyme reduced DCIP substrate using NADH as electron donor. DCIP is a blue in its oxidized form and it becomes colorless after reduction. The significant induction of non-specific reductase in the biodegradation of Malachite green was observed and termed as MG reductase (Parshetti et al. 2006). MG-reductase enzyme reduced Malachite green into leucomalachite green using NADH as electron donor. The reactions catalyzed by DCIP-reductase and MG- reductase have been reflected in Figs. 2 and 3.

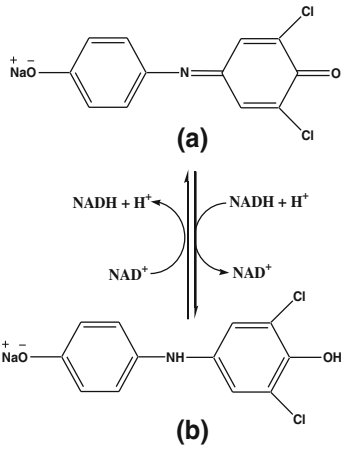

Fig. 2 Reaction catalyzed by NADH-DCIP reductase. **a** Oxidized form of DCIP; **b** Reduced form of DCIP

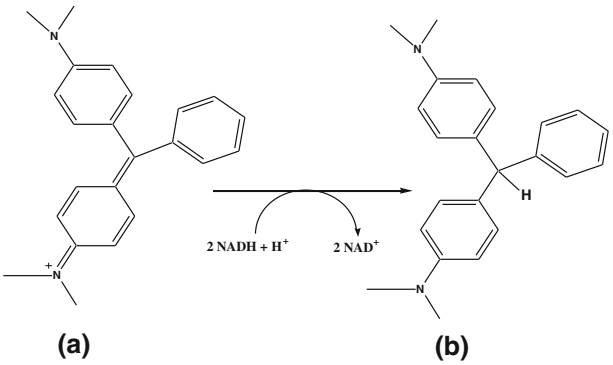

Fig. 3 Reaction catalyzed by MG reductase. **a** Malachite green; **b** Leucomalachite green

6.1.3 Lignin Peroxidase (LiP)

This enzyme belongs to the family of oxidoreductases, specifically those acting on peroxide as electron acceptor (peroxidases). The systematic name of this class enzyme was 1,2-bis(3,4-dimethoxyphenyl) propane-1,3-diol:hydrogen-peroxide oxidoreductase. LiP was *N*-glycosylated protein with molecular weight between 38 and 47 kDa. It contains heme in the active site and has shown a classical peroxidase mechanism (Tien et al. 1986). LiP catalyzed several oxidation reactions in the side chains of lignin and related compounds by one-electron abstraction to form reactive radicals (Tien and Kirk 1983; Kersten et al. 1985). The cleavage of an aromatic ring structure is also reported (Umezawa and Higuchi 1987). Several studies have found a significant induction in lignin peroxidase activity during decolorization of azo dyes by bacterial strains, suggesting their possible function in dye decolorization.

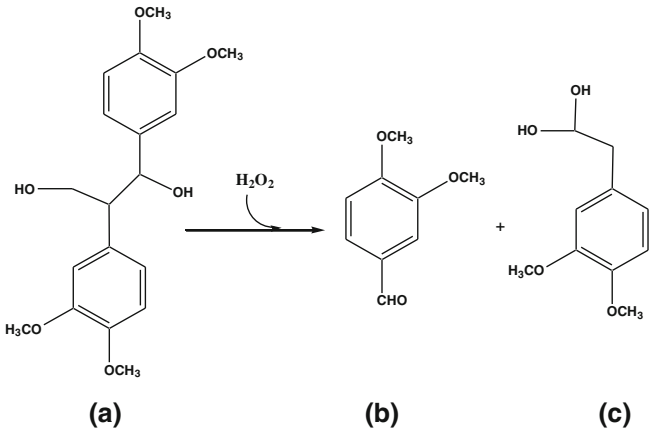

Fig. 4 Reaction catalyzed by lignin peroxidase. **a** 1, 2-bis (3, 4-dimethoxyphenyl) propane-1, 3-diol; **b** 3, 4-dimethoxybenzaldehyde; **c** 1-(3, 4-dimethoxyphenyl) ethane-1,2-diol

The role of Lip in dye decolorizationwas proved by in vitro dye decolorization assay with purified protein (Gomare et al. 2008). Reaction catalyzed by lignin peroxidase is shown in Fig. 4.

6.1.4 Laccase

Laccases (EC1.10.3.2) were mostly members of multi-copper oxidase protein family. It was catalyzes the oxidation of substituted phenolic and non-phenolic compounds in the presence of oxygen as an electron acceptor (Fig. 5). Phylogenetically, these enzymes have developed from small sized prokaryotic azurins toeukaryotic plasma proteins ceruloplasmin (Claus 2003, 2004). They contain four histidine-rich copper binding domains, which coordinate types 1–3 copper atoms that differ in their environment and spectroscopic properties (Givaudan et al. 1993). They are classified into two categories viz. blue laccase, had a blue color and characteristic absorption and EPR spectra and white or yellow-brown laccase, had no typical blue oxidase spectra and also showed atypical EPR spectra. According to Solano and Sanchez-Amat (1999), a prokaryotic melanogenic marine bacterium *Marinomonas mediterranea* containing two different polyphenol oxidases (PPO) have characteristics of both tyrosinase and laccase activity. The other PPO is a multi-potent enzyme, which is able to oxidize a wide range of substrate characteristics for both tyrosinases and laccases. The reaction catalyzed by laccase reflected in Fig. 6. The molecular weight of laccases varies from 60 to 390 kDa (Call and Mucke 1997). First prokaryotic laccase was reported in rhizospheric bacterium *Azospirillum lipoferum* (Solano et al. 1997). Laccase like activity has been found also in *Pseudomonas syringae* and *Pedomicrobium* sp. (Ridge et al. 2007). Laccase was decolorized azo dyes by non-specific free radical mechanism,

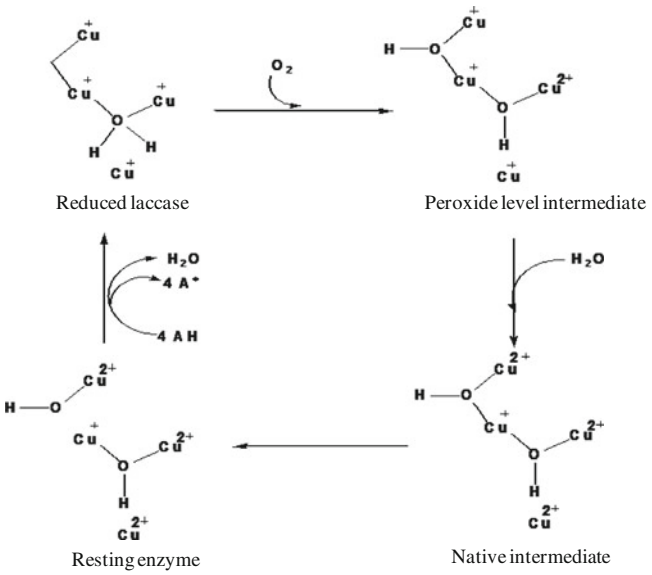

Fig. 5 Catalytic cycle of laccases

thereby avoiding the formation of toxic aromatic amines. The purified laccase from *Pseudomonas desmolyticum* NCIM 2112 and *Bacillus* sp. ADR have ability to decolorize the several textile dyes withdecolorization efficiency varying from 65 to 90 % (Kalme et al. 2009; Telke et al. 2011).

6.1.5 Polyphenol Oxidase (Tyrosinase)

Polyphenol oxidases (PPOs) were catalyze the *o*-hydroxylation of monophenols (phenol molecules in which the benzene ring contains a single hydroxyl substituent) to *o*-diphenols (phenol molecules containing two hydroxyl substituents). They can also further catalyze the oxidation of *o*-diphenols to produce *o*-quinones. The amino acid tyrosine contains a single phenolic ring that may be oxidised by the action of PPOs to form *o*-quinone (Fig. 7). Hence, PPOs may also be referred as tyrosinases. Polyphenol oxidases are enzymes that catalyze oxidation of certain phenolic substrates to quinones in the presence of molecular oxygen. Polyphenol oxidases have been reported in the bacteria viz. *Streptomyces glaucescens*, *Streptomyces antibioticus*, *Bacillus licheniformis*, *Bacillus natto* and *Bacillus sphaericus* (Whitaker 1994; Echiqo and Ritsuko 2001). The purified polyphenol oxidase was used for the oxidation of colored and phenolic substances.

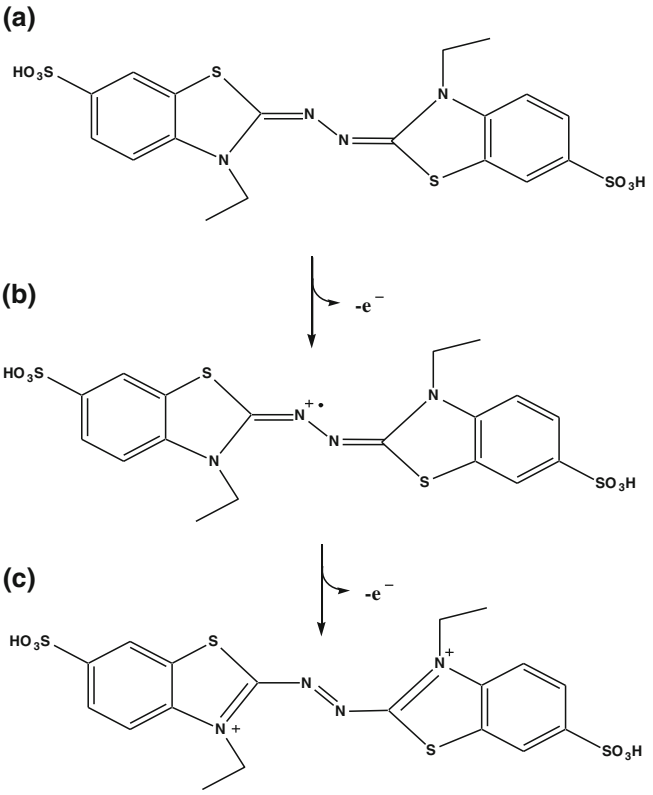

Fig. 6 Reaction catalyzed by laccases. **a** ABTS; **b** Intermediate ABTS radical; **c** Oxidized form of ABTS

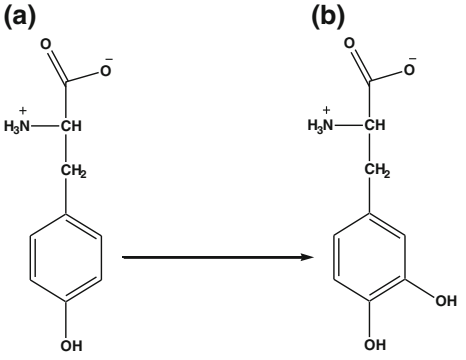

Fig. 7 Reaction catalyzed by polyphenol oxidase. **a** L-tyrosine; **b** *o*-quinone

6.1.6 Veratrol Alcohol Oxidase (VAO)

The enzyme veratrol-alcohol oxidase was catalyzes the chemical reaction as:

$$\text{Aromatic primary alcohol} + O_2 \rightleftharpoons \text{Aromatic aldehyde} + H_2O_2$$

Hence, this enzyme has two substrates i.e. aromatic primary alcohol and O_2 and their products are aromatic aldehyde and H_2O_2. This enzyme belongs to the family of oxidoreductases. VAO catalyzed the oxidation of aryl α- and α-β- unsaturated γ-alcohols to the corresponding aldehydes with concomitant reduction of O_2 to H_2O_2. The formation of H_2O_2 can be determined by the method as suggested by Tamboli et al. (2011).

6.2 Mediated Biological Azo Dye Reduction

The high molecular weight sulfonated azo dyes are unable to pass through the cell membrane (Levine 1991). It was suggested that the reduction of these dyes could occur through the mechanism that are not dependent on transport into the cell membrane. Many reports were available on the role of redox mediators in azo bond reduction by bacteria under anaerobic conditions (Keck et al. 1997; van der Zee et al. 2001a; Dos Santos et al. 2004). Riboflavin significantly enhanced the reduction of mordant yellow 10 by anaerobic granular sludge (Field and Brady 2003). 1-amino 2-napthol, one of the constituent amines of the azo dye, AO7, increased its decolorization rate, possibly by mediating the transfer of reducing equivalents (Mendez-Paz et al. 2005). The addition of synthetic electron carriers, such as anthraquinone-2,6-disulphonate, could also greatly enhance the decolorization of many azo dyes (van der Zee et al. 2001b). Keck et al. (1997) were first example of the anaerobic cleavage of azo dyes by redox mediators formed during the aerobic degradation of a xenobiotics compound. Cell suspensions of *Sphingomonas* sp. strain BN6, grown aerobically in the presence of 2-naphthyl sulfonate (NS), shown 10–20 fold increase in decolorization rate of an azo dye under anaerobic conditions. Even the addition of culture filtrates from these cells could enhance anaerobic decolorization by cell suspensions grown in the absence of NS. Redox intermediates generated during the aerobic degradation of aromatic compounds were act as enhancer in dye decolorization reactions under anaerobic condition (Keck et al. 1997; Chang et al. 2004).

6.3 Azo Dye Decolorization by Organic and Inorganic Compounds

Azo dye decolorization can occur from purely chemical reactions with inorganic compounds, such as sulfide and ferrous ion which are formed as end products of metabolic reactions under anaerobic condition. It has been shown that H_2S generation by SRB resulted in the extracellular decolorization of azo dyes (Yoo et al. 2000; Diniz et al. 2002). Sulfate-influenced dye reduction was correlated with biogenic sulfide formation under methanogenic conditions. In the absence of sulfur compounds, dye decolorization readily occurred in the presence of granular sludge, demonstrating the importance of enzymatic mechanisms. An analysis of decolorization kinetics, in batch reactor and in laboratory scale anaerobic sludge bed reactors, indicated that the relative importance of chemical dye reduction mechanisms in high rate anaerobic bioreactors was small due to the high biomass in the reactors (van der Zee et al. 2003). The various inducers of oxidative enzymes, such as $CaCO_3$, indole, *o*-tolidine, veratrole, and vanillin, enhanced the dye decolorization (Dawkar et al. 2008). The electron donors, viz. sodium acetate, sodium formate, sodium succinate, sodium citrate, and sodium pyruvate enhanced the azo dye decolorization rate by *Bacillus* sp. ADR. Sodium acetate, sodium formate, sodium citrate, and sodium pyruvate might act as artificial electron donors for reductases involved in azo dye decolorization. 3,4-dimethoxy benzyl alcohol and *o*-tolidine increased the azo dye decolorization rate of *Bacillus* sp. ADR by stabilizing the extracellular phenol oxidase enzyme (Telke et al. 2009).

7 Decolorization of Textile Dyes by Purified Bacterial Oxidoreductase Enzymes

7.1 Azoreductase Mediated Dye Decolorization

The azoreductase enzyme was purified from *Exigubacterium* sp. RD3 which had molecular weight of 95 Kda (Dhanve et al. 2008a, b). The purified azoreductase was studied for its ability for azo dye decolorization. Azoreductase mediated azo bond cleavage in Methyl Red produced intermediates i.e., *N*, *N*-dimethyl benzene-1,4 diamine and 2-amino benzoic acid (Fig. 8) whereas, Congo red produced diamino naphthyl sulfonic acid and benzedine by azoreductase action (Fig. 9).

7.2 Laccase Mediated Dye Decolorization

Laccase was purified from *Bacillus* sp. ADR (Telke et al. 2009). Purified laccase was studied for decolorization of Methyl Red and Methyl Orange.

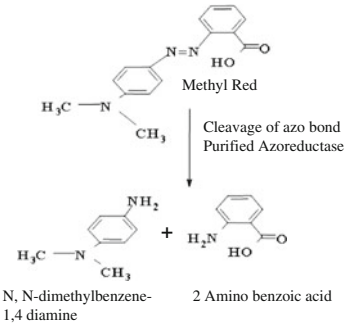

Fig. 8 Proposed degradation pathway for Methyl Red by purified azoreductase

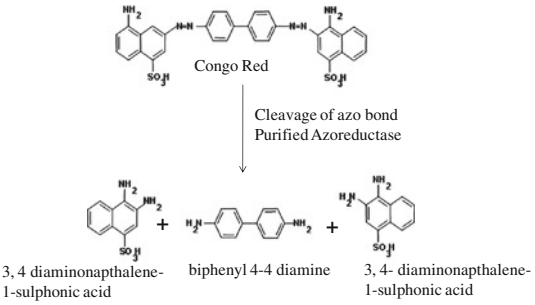

Fig. 9 Proposed degradation pathway for Congo Red by purified azoreductase

The formation of *p*-hydroperoxy dimethyl amino benzene suggests asymmetric cleavage of an azo bond in Methyl Red (Telke 2009) (Fig. 10). The formation of *p*-phenyldizine sulfonic acid and*p*-hydroxy N,N′-dimethyl amino benzene indicates asymmetric cleavage of an azo bond in Methyl Orange (Telke 2009) (Fig. 11). The biodegradation of dyes by purified laccase confirms their role in decolorization process.

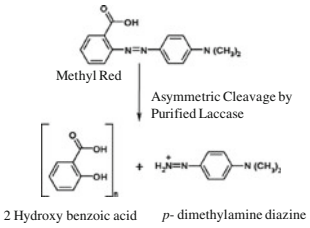

Fig. 10 Proposed degradation pathway for Methyl Red by purified laccase

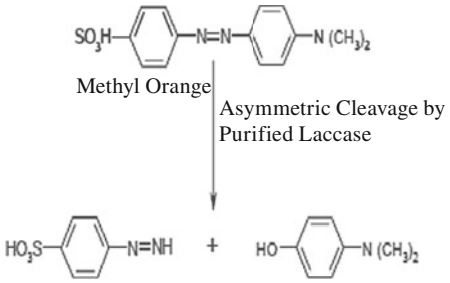

Fig. 11 Proposed degradation pathway for Methyl Orange by purified laccase

7.3 Lignin Peroxidase Mediated Dye Decolorization

The lignin peroxidase purified from *Bacillus laterosporus* was decolorizes Methyl Orange (Gomare et al. 2008). The biodegradation pathway of Methyl Orange was shown in Fig. 12.

In degradation pathway, Methyl Orange firstly forms cyclo-2, 5-ene-1-one radicals with the removal of sulfate ion, N, N di methylamine and a nitrogen molecule, where these two radicals condense to produce 1, 1′-dicyclohex-2, 5-ene-4-one moiety. Its one of the cyclohexene rings undergoes α-cleavage to form 4-substituted hexanoic acid, which on the removal of methyl radical undergoes cyclization to produce 4-cyclohexenone lactone cation. This substituted lactone cation finally forms a stable molecule of *p*-isopropanal-2,5-cyclohexa-dienone with the elimination of iminium moiety (Gomare et al. 2008).

7.4 Veratrol Alcohol Oxidase Mediated Dye Decolorization

The veratrole alcohol oxidase was purified from *Comomonas* sp. UVS and assessed fordye decolorization (Jadhav et al. 2009). Oxidative cleavage of Red HE7B (RRHE7B) yielded an unknown product. This product further undergoes desulfonation to give naphthalene-1,2,5-triol (Fig. 13). The use of purified enzyme suggested their direct involvement for dye decolorization.

8 Toxicity of Azo Dyes and Its Degradation Metabolites

The wastewater released from the textile industries after the treatment is sometime used for irrigation purpose in the agricultural fields (Paul et al. 2012). Hence, the toxicity assessment for the seed germination and plant growth of treated textile

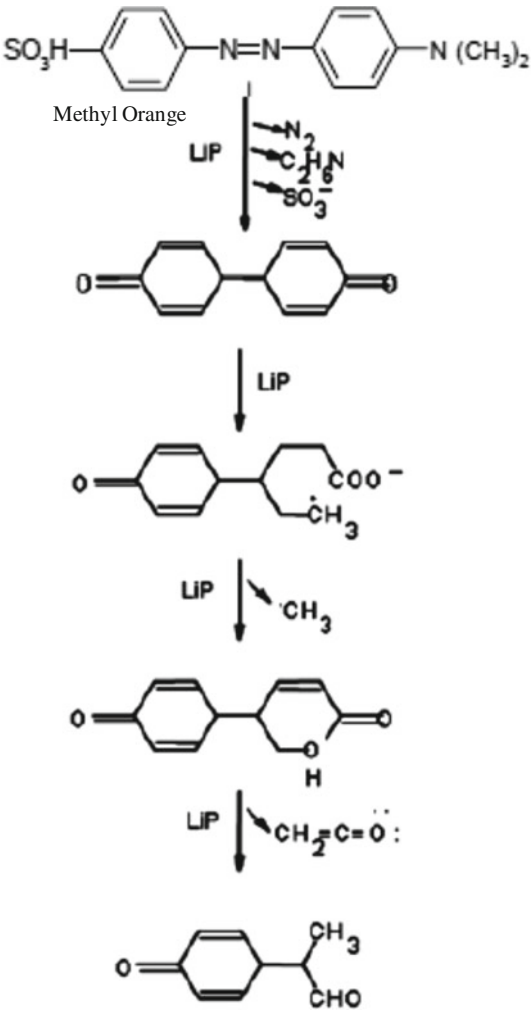

Fig. 12 Proposed degradation pathway for Methyl Orange by Lignin peroxidase

effluent is considered one of the important parameter (Paul et al. 2012). Use of plant bioassays for monitoring toxic effect of azo dyes as well as metabolites on plants was suggested by many researchers (Paul et al. 2012; Kadam et al. 2013a, b). Phytotoxicity and microbial toxicity studies revealed that the metabolites formed after azo dye decolorization were less toxic than original azo dye (Dawkar et al. 2008; Dhanve et al. 2008a, b; Jadhav et al. 2008; Kalyani et al. 2008; Telke et al. 2008). Ames test revealed that the mutagenicity of AV7 even with or without the S9 metabolizing system increased significantly after static biodegradation and totally disappeared after aerobic condition (Mansour et al. 2008).

Bacteria mediated decolorization of azo dyes under both static anoxic as well as anaerobic conditions, produces toxic aromatic amines due to enzymatic action of

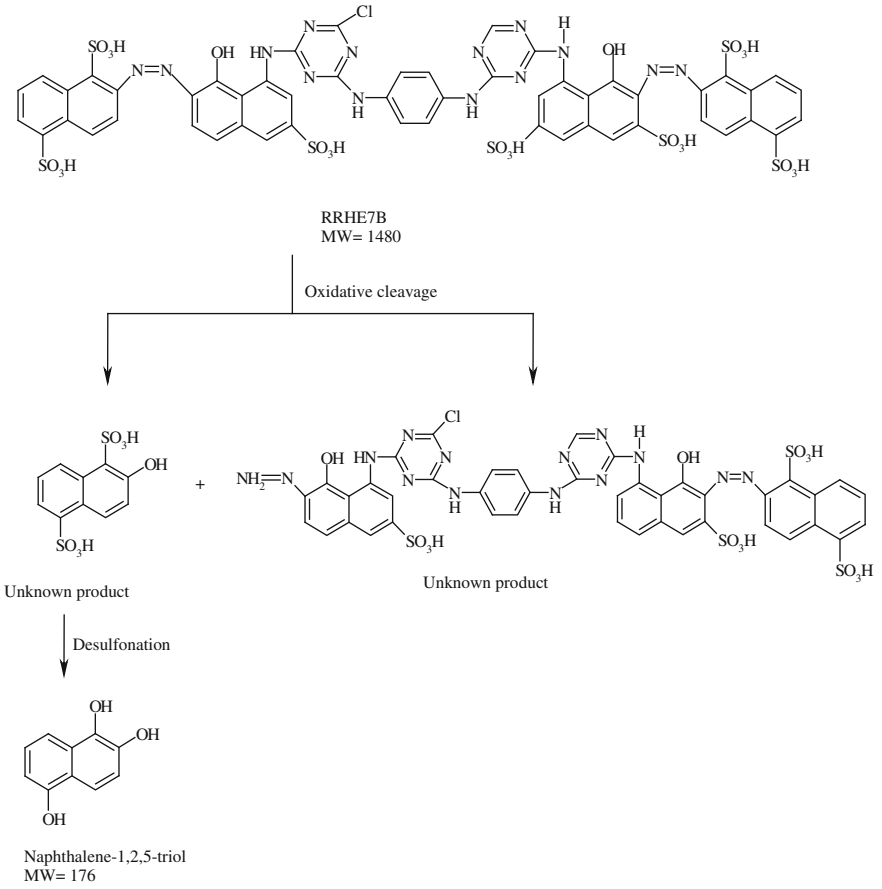

Fig. 13 Proposed degradation pathway for RRHE7B by Veratryl alcohol oxidase

azoreductase. Aromatic amines are highly toxic and carcinogenic in nature. On the other hand, decolorization of azo dyes by fungal species involves the role of oxidases which don't produce the toxic aromatic amines. Therefore, a consortium of bacteria and fungus used for decolorization of azo dyesproduces metabolite which was free from carcinogenic aromatic amines. Metabolites analyzed after decolorization of azo dye disperse Rubine GFL by consortium of *Pseudomonas* sp. SUK1 and *Aspergillus orchaceus* NCIM 1146, showed absence of the aromatic amines. However, *Pseudomonas* sp. SUK1 showed 0.14 mM concentration of aromatic amines (Lade et al. 2012). At the same time, *Aspergillus orchaceus* NCIM 1146 showed no presence of the aromatic amines. Therefore, use of enzymatic systems from fungal-bacterial co-cultures for decolorization of azo dyes enhances detoxification of azo dyes with enhanced biodegradationrate (Lade et al. 2012).

9 Conclusion

Different oxidoreductive enzymes, such as azoreductase, NADH-DCIP reductase, Riboflavin reductase, laccase, lignin peroxidase, tyrosinase and veratrol alcohol oxidase are involved in dye decolorization. The reductive enzyme system, such as azoreductase, generates toxic aromatic amines, but a combined reductive and oxidative system works better for detoxification of azo dyes. A combination of both oxidative as well as reductive enzymes removes azo dyes toxicity.

10 Futures Prospectives

Different oxidoreductive enzymes had shown their direct involvement indye decolorization. Findings of efficient enzymes, which are involved indecolorization of azo dyes, are to be explored using technologies like proteomics and genomics. Further, already knowndegradative enzymes can be cloned into single microorganism using genetic engineering technology to enhance the decolorization efficiency of synthetic dyes by microbes.

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