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Review

Enzymatic decolorization and degradation of azo dyes – A review

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ABSTRACT

Azo dyes are aromatic compounds with one or more –N=N– groups. These dyes are the most important and largest class of synthetic dyes used in commercial applications. Several methods have already been used to treat textile effluents including physico-chemical methods such as filtration, carbon activated, coagulation and chemical flocculation. Although these methods are effective, but they are expensive and involve formation of concentrated sludge that creates a secondary disposal problem. In recent years, use of bioremediation based technologies for treating textile wastewater containing dyes has attracted much interest. The ability of microorganisms and their dye degrading enzymes to decolorize and metabolize the dyes has long been known and has proved to be the best option for bioremediation. As far as decolorization and degradation of textile dyes are concerned, azoreductases, laccases, peroxidases and many other important enzymes seem to have shown great potential to decolorize the textile dyes and these enzymes are considered as effective molecular weapon for bioremediation of azo dyes.

Contents

1. Introduction

Azo dyes make up about one-half of all dyes synthesized and are predominantly used synthetic dyes in the textile, food, paper, printing, leather and cosmetic industries (Chang and Lin, 2001). About 50,000 tons of textile dyes are discharged in the environment annually from dyeing processes globally (Lewis, 1999). They are the most visible indicator of water pollution as some dyes are visible at concentrations as low as 0.005 mg l^{-1} (O'Neill et al., 1999). It has been estimated that approximately 10–15% of the dyes used in dyeing process do not bind with the textile fibers and are found freely into the environment (Asad et al., 2007). These dyes are recalcitrant in nature. According to Jin et al. (2007) an estimated 2.8×10^5 tons of textile dyes are discharged in textile industrial effluent each year worldwide. The release of these dyes into ecosystem is harmful, not only because of its color, but also due to the fact that many azo dyes (Fig. 1) and their breakdown products (colorless amines) are toxic and/or mutagenic to living organisms (Weisburger, 2002; Xu et al., 2007). Azo dyes are believed to be electron deficient xenobiotic compounds because they possess electron withdrawing groups, generating electron deficiency in the

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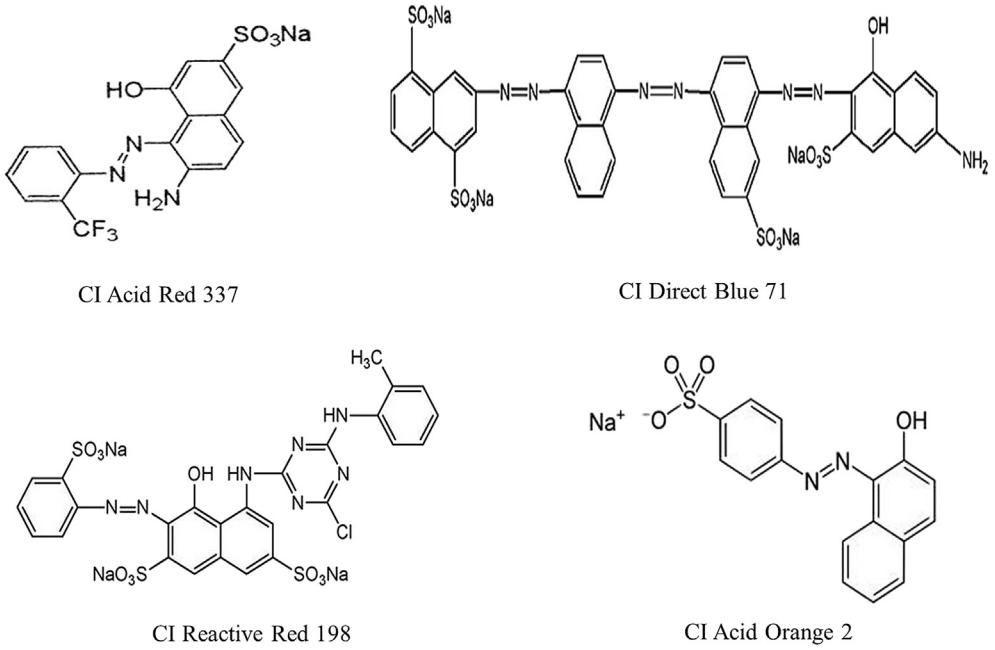

Fig. 1. Structure of some azo dyes.

molecule (dyes) making them resistant to degradation (Singh et al., 2014). About 80% of azo dyes are used in the dyeing process of textile industries. The presence of these dyes in the water ecosystem is the cause of serious environmental and health problems (Fang et al., 2004; Asad et al., 2007). Metanil yellow, an azo dye, has been proved to be hepatotoxic in albino rats (Singh et al., 1987, 1988). The metabolic disposition of metanil yellow and orange II has also been studied using rat and guinea pig as model systems (Singh, 1989, 1991a, 1991b). Metanilic acid and p-amino diphenyl amine were identified as metabolite of metanil yellow where as orange II was degraded into sulfanilic acid and 1-amino-2-nepthol. Azo dyes possess one or more azo group and are widely used in many industries because of their chemical stability and its comparatively easier synthetic processes (Raffi et al., 1997).

Several physico-chemical methods such as adsorption, chemical treatment and ion pair extractions have been used for azo dye decolorization, but these methods are expensive and produce large amounts of sludge after treatment. Extensively used coagulation or flocculation techniques create or generate large amounts of sludge, which requires safe disposal. Adsorption and, to a certain extent, membrane filtration techniques lead to secondary waste streams which need further treatment. There are many reports on the use of physico-chemical methods for color removal from dye containing effluents (Churchley, 1994; Vandevivere et al., 1998; Swaminathan et al., 2003; Behnajady et al., 2004; Wang et al., 2004; Golab et al., 2005; Lopez-Grimau and Gutierrez, 2006) but in present scenario biological treatment methods are most suitable and widely used due to their cost effectiveness, ability to produce less sludge, and eco-friendly nature (Song et al., 2003; Chen, 2006). Different taxonomic groups of microorganisms such as bacteria, fungi, yeast, and algae are capable of degrading azo dyes under anaerobic and aerobic conditions (Talarposhti et al., 2001). Most azo dyes are not cleaved in the presence of oxygen in conventional sewage treatment plants, but are reduced by various microorganisms under anaerobic conditions to their corresponding aromatic amines, which are further degraded in presence of oxygen (Stolz, 2001; Dos Santos et al., 2007). Azo bonds (−N=N−) in these dyes are resistant to cleavage and therefore dyes ultimately increase in the environment upto very high extent. In presence of oxygen, treatment of azo dyes with bacteria usually shows low efficiencies since oxygen is a strong electron acceptor than azo dyes. It is observed that efficient color removal of azo dyes is obtained under anaerobic and static condition with a bacterial culture. Anaerobic processes are usually not specific with regard to microorganisms involved in the reduction of dyes (Stolz, 2001). Therefore, the biological degradation of these dyes by microorganisms has potential advantages in developing decolorizing bio-treatment method of wastewater (Suzuki et al., 2001; Ooi et al., 2007; Gou et al., 2009).

Biological treatment of azo dyes is based on the enzymes synthesized by microorganism. Although these enzymes reduced certain types of azo dyes, some dyes are not degraded efficiently. To establish a biological wastewater treatment system for azo dye removal, it is necessary to screen out microorganisms that express enzymes with wide substrate (dyes) specificities.

In this review, different enzymatic mechanisms by which diverse categories of microorganism produced enzymes bring about the degradation of dyestuffs have been elucidated. This review compiles different enzymatic mechanisms in the decolorization and degradation of recalcitrant azo dyes.

2. Enzymatic decolorization and degradation of azo dyes

The primary step in bacterial decolorization of azo dyes, in either anaerobic or aerobic conditions, is the reduction of the azo bond (−N=N−) chromophore group. This reduction may involve various mechanisms, such as enzymes, low molecular weight redox mediators, chemical reduction by biogenic reductants like sulfide, or a combination of these and the location of the these reactions may be either intracellular or extracellular sites (Fig. 2).

In case of enzymatic decolorization and degradation of azo dyes, two enzymes families i.e. Azoreductases and Laccases seem to have shown great potential. Laccases have great potential to decolorize an extensive range of known industrial dyes (Rodriguez et al., 1999; Reyes et al., 1999). There are certain enzymes like Manganese

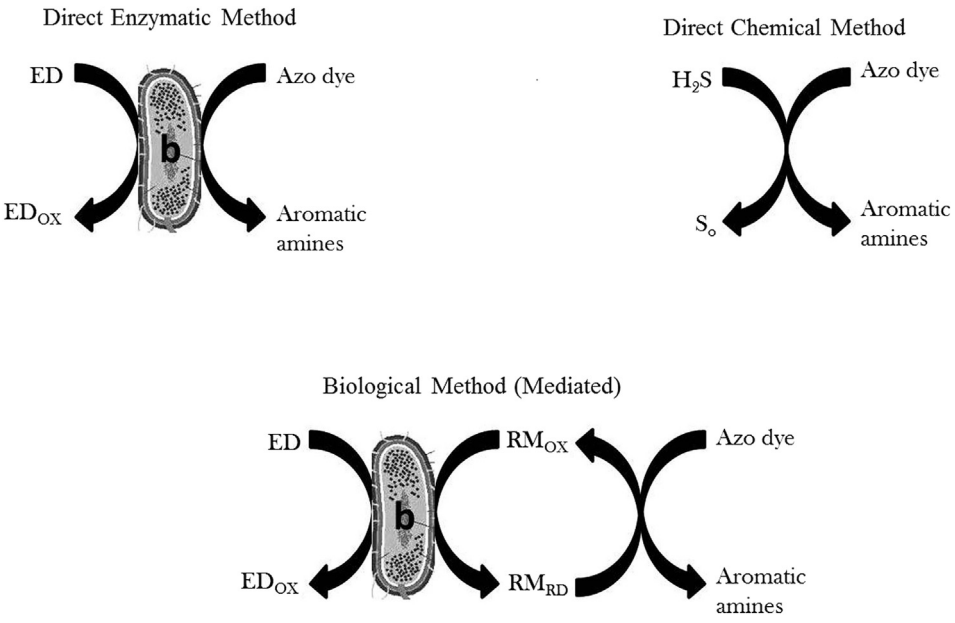

Fig. 2. Various methods of Degradation of azo dyes (RM = Redox mediator; ED = Electron donor; b = bacteria).

peroxidase (MnP), Lignin peroxidase (LiP), Polyphenol oxidase (PPO) etc. are also promising in the decolorization and degradation of azo dyes.

2.1. Decolorization and degradation of azo dyes by azoreductase

Azoreductases (EC 1.7.1.6) are major group of enzymes expressed in azo dye degrading bacteria and fungi for decolorization/degradation of these dyes. It can decolorize azo dyes into their corresponding aromatic amines (colorless products) via reductive cleavage of azo bond (Pandey et al., 2007). Azoreductases catalyze the reaction only in the presence of reducing agents like NADH, NADPH and $FADH_2$. These reducing molecules act as an electron donor and involve in the breakdown of the azo bond at intracellular or extracellular site of the bacterial cell membrane. As the azoreductase in some microorganisms can catalyze cleavage of azo groups, they have great importance in designing bio-treatment procedure of wastewater containing azo dyes (Zimmermann et al., 1982, 1984).

In the last few years, several azoreductase producing bacteria were reported by many researchers (Table 1). Catalytic proteins having azoreductase activity have been identified and characterized from a large number of bacteria, such as *Xenophilus azovorans* KF46F, *Pigmentiphaga kullae* K24, *Enterococcus faecalis, Staphylococcus aureus, Escherichia coli, Bacillus* sp. OY1-2, *and Rhodobacter sphaeroides* (Nakanishi et al., 2001; Suzuki et al., 2001; Blumel et al., 2002; Blumel and Stolz, 2003; Yan et al., 2004; Chen et al., 2004, 2005). However, involvement of intracellular azoreductase in bacterial decolorization has been doubted in recent years because many azo dyes have high polarities and complex structures, and it is difficult for dyes to diffuse through cell membranes.

The enzymatic degradation of azo dyes have been observed in various organisms, which comprise the rat liver enzyme, cytochrome P450 (Zbaida and Levine, 1990), rabbit liver aldehyde oxidase (Stoddart and Levine, 1992) and azoreductases from the intestinal microbiota, environmental fungi and bacteria (Chen, 2006) as well as halophilic and halotolerant microorganisms (Asad et al., 2007).

Additionally, a classification system for enzymes which is based on the secondary and tertiary amino acid analysis has been proposed by Abraham and John (2007). On the basis of function, another classification system is used in which azoreductases are grouped as either flavin dependant azoreductases (Nakanishi et al., 2001; Chen et al., 2004, 2005) or flavin independent azoreductases (Blumel et al., 2002; Blumel and Stolz, 2003). The flavin dependent azoreductases are further grouped into three categories; the

Table 1
Decolorization of various azo dyes by Azoreductase producing bacterial culture.

S. no.	Bacterial culture	Name of dye	Time (h)	Decolorization (%)	References
1	*Acinetobacter radioresistens*	Acid Red	48	>70	Ramya et al. (2010)
2	*Alcaligenes* sp. AA09	Reactive Red BL	24	100	Pandey and Dubey (2012)
3	*Bacillus lentus* BI377	Reactive Red 141	6	99.11	Oturkar et al. (2013)
4	*Bacillus megaterium*	Red 2G	NA	64.89	Khan (2011)
5	*Bacillus* strain SF	Reactive Black 5	6	86	Maier et al. (2004)
		Mordant Black 9	24	38	
6	*Bacillus subtilis* ORB7106	Methyl Red	48	40–98	Leelakriangsak and Borisut (2012)
7	*Brevibacterium* sp. strain VN-15	RY107	96	98	Franciscon et al. (2012)
8	*Escherichia coli* JM109 (pGEX-AZR)	Direct Blue 71	12	100	Jin et al. (2009)
9	*Enterococcus gallinarum*	Direct Black 38	20	100	Bafana et al. (2009)
10	*Mutant Bacillus* sp. ACT2	Congo Red	37–48	12–30	Gopinath et al. (2009)
11	*Pseudomonas aeruginosa*	Remazol Orange	24	94	Sarayu and Sandhya (2010)
12	*Proteus* sp.	Congo Red	48	67	Perumal et al. (2012)

enzymes using NADH only (Nakanishi et al., 2001; Chen et al., 2004), NADPH only (Chen et al., 2005) or both (Ghosh et al., 1992; Wang et al., 2007) as coenzymes which provide reducing power for reduction of azo dyes. NAD(P)H dependent reduction by azoreductase of azo compounds forms their corresponding amines, which involves the breakdown of the azo linkages (−N═N−), resulting in degradation of azo dye (Nakayama et al., 1983; Chen, 2006). Although azoreductases reduce certain types of azo dyes, there are number of dyes that do not degrade efficiently. So, for desired optimum effective biological wastewater treatment system for azo dye removal, it is advantageous to search for microorganisms that express azoreductases with large substrate specificities. Most azo dyes are substituted with sulphonate group and are with high molecular weight that's why they are unable to pass through cell membranes into intracellular environment of microorganism. Therefore, the reducing property attributed to the dye does not depend upon the intracellular uptake of the dye (Robinson et al., 2001). Bacterial degradation of azo dyes is frequently initiated by cleavage of azo bonds by azoreductases which further get converted into corresponding amines by aerobic degradation (Chen, 2006). Aranganathan et al. (2013) reported intracellular azoreductase mediated degradation products, sulfanilic acid and 1-amino-2-napthol of orange II by *Pseudomonas oleovorans* PAMD1. Gingell and Walker (1971) showed that low molecular weight redox mediator compounds can act as electron shuttles between the azo dye and NADH (nicotinamide adenine dinucleotide) dependent azo reductase that is found in outer membrane of bacteria. According to Russ et al. (2000) these mediator compounds are either metabolic products of certain substrates used by bacteria or they may be externally added. However, if the extracellular environment is aerobic, oxygen prevents the reduction of azo dye due to the preferential oxidation of reduced redox mediator by oxygen rather than by the azo dye. Kudlich et al. (1997) supported the suggestion that membrane-bound azo reductase activity, mediated by redox compounds, is different from the soluble cytoplasmic azo reductase that is responsible for reduction of non-sulphonated dyes that enters through the cell membrane. For that reason, the membrane bound and cytoplasmic azoreductases are two separate enzyme systems (Kudlich et al., 1997). Fig. 3 shows proposed mechanism for the redox mediator dependent reduction of azo dyes using entire bacterial cells, under anaerobic conditions. However, the outcome of the reduction of the azo dyes in the cell supernatant is a dominantly chemical redox reaction and the redox mediators rely on cytoplasmic reducing enzymes to supply electrons (Yoo et al., 2001). It is also possible that this chemical redox reaction works together with a direct enzymatic reaction involving an azoreductase, which may be a cytoplasmic dehydrogenase enzyme that is synthesized and secreted without accumulation in the cell interior (Bragger et al., 1997). Sahasrabudhe et al. (2014) isolated an enzyme Azoreductase from *E. faecalis* YZ66 that was able to degrade and detoxify the toxic sulfonated azo dye direct red 81under static condition.

For the over expression and characterization of azoreductase, different gene engineered strains (usually as *E. coli* strains) were constructed. It has been reported that a recombinant *E. coli* strain having the azoreductase gene (azo B) from *X. azovorans* KF46F, expressed almost 50 times higher azoreductase activity in its cell extracts than that observed in *X. azovorans* KF46F. However, the resting recombinant cell suspension showed no detectable azoreductase activity (Blumel et al., 2002http://link.springer.com/article/10.1007/s11274-006-9316-0/fulltext.html). The gene of a Flavin reductase (free) functioned as azoreductase under in vitro condition was transferred to *Sphingomonas* sp. strain BN6 and found to show 30 fold increase in azoreductase activity compared to the wild type strain. But, when the whole cells of the recombinant *Sphingomonas* sp. BN6 was used, it showed only approximately three fold increase in the decolorization rate for azo dyes amaranth and mordant yellow compared to the wild type BN6 strain (Russ et al., 2000http://link.springer.com/article/10.1007/s11274-006-9316-0/fulltext.html).

2.2. Decolorization and degradation of azo dyes by laccases

Laccases (EC1.10.3.2) are the most common members of the multicopper oxidase protein family. Laccases represent a family of copper containing polyphenol oxidases (PPO) and are normally

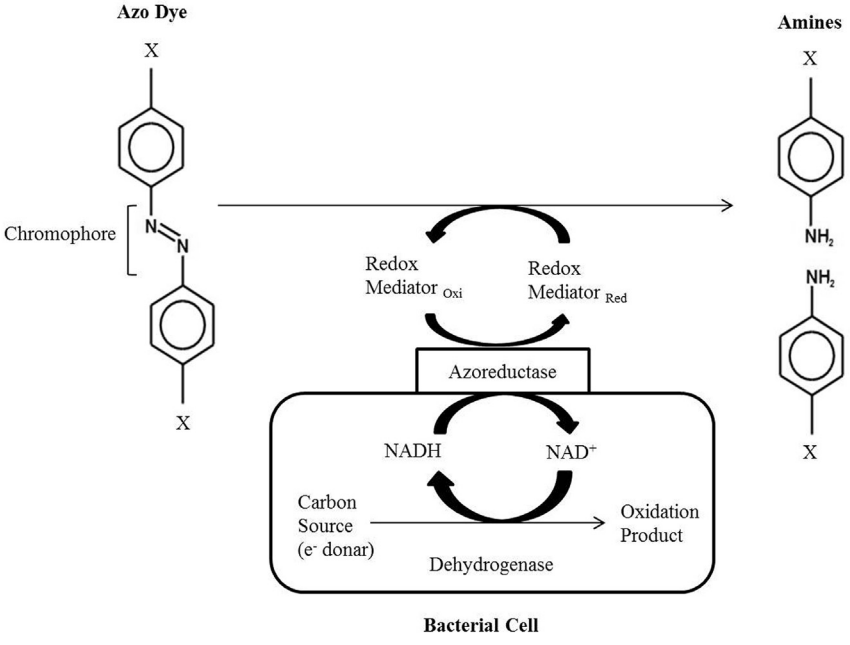

Fig. 3. Proposed mechanism for degradation of azo dyes by azoreductase (adopted from Keck et al. 1997).

called multicopper oxidases (MCO) (Birhanli and Yesilada, 2006; Arora and Sharma, 2010; Giardina et al., 2010). Laccase was first discovered in the sap of the Japanese lacquer tree *Rhus vernicifera,* and its characteristic as a metal containing oxidase was discovered by Bertrand in 1985 (Giardina et al., 2010). Husain (2006) revealed that laccase has attracted significant interest in the decolorization of colored textile wastewaters. Laccases are oxidoreductases which have great importance in different biotechnological processes mainly because of their capacity for bioremediation due to their distinct features such as non-specific oxidation capacity, no requirement for co-factors and also they do not use readily available oxygen as an electron acceptor (Telke et al., 2011; Kalyani et al., 2012). Low molecular weight compounds like 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) act as redox mediator in the real electron transfer steps of laccases (Wong and Yu, 1999). In the presence of redox mediators decolorization process of dyes could significantly be efficient (Reyes et al., 1999; Abadulla et al., 2000; Soares et al., 2001). But on the other hand, Ravikumar et al. (2013) purified the Laccase enzyme from Mushroom *Hypsizygus ulmarius* for the decolorization of methyl orange without using redox mediator. Laccases have been studied in large scale for their ability to degrade azo dyes (Chivukula and Renganathan, 1995; Kirby et al., 2000; Peralta-Zamora et al., 2003; Blanquez et al., 2004; Novotny et al., 2004). The majority of the known laccases have fungal (e.g. white-rot fungi) or plant origins, but also a small number of laccases are identified and isolated from bacteria (Gianfreda et al., 1999; Claus, 2003). Several laccase producing fungal cultures were reported for degradation of azo dyes (Table 2).

By using molecular oxygen as the electron acceptor, laccases catalyze the oxidation of various substituted phenolic and non-phenolic compounds (Sharma et al., 2007). Laccases have less substrate specificity and potential to degrade a wide range of xenobiotics including industrial colored wastewaters (De'Souza et al., 2006). These are copper oxidases that efficiently degrade anthraquinone dyes and dyes with phenolic moieties (Nyanhongo et al., 2002; Champagne and Ramsay, 2005). On SDS-PAGE most laccases shows electrophoretic mobilities of 60–100 kDa, glycosylation by mannose is responsible for about 10–50% of total weight of laccase. Glycosylation in laccase is responsible for copper retention thermal stability, proteolytic susceptibility, activity and secretion (Xu, 1999). The phylogenetic study revealed that these enzymes have developed from small sized prokaryotic protein azurins to eukaryotic plasma protein ceruloplasmin (Claus, 2003). They have four histidine rich copper binding domains that differ in their spectroscopic and environmental properties. These enzymes are multicopper phenol oxidases that decolorize azo dyes by using highly nonspecific free radical mechanism forming phenolic compounds, and prevent the formation of toxic aromatic amines (Chivukula and Renganathan, 1995; Wong and Yu, 1999). Laccases oxidize aromatic pollutants, such as anilines and phenols in the presence of oxygen and in this reaction; formation of phenoxy radicals takes place from their corresponding substrates, which further yield a phenolic polymer on polymerization or to produce quinine, by additional laccase oxidation (Bollag, 1992). According to Goncalves and Steiner (1996) laccases are involved in removal of a hydrogen atom from hydroxyl group of *ortho* and *para* substituted mono and polyphenolic substrates and also from aromatic amines which are further involved in depolymerization, demethylation, or quinine formations.

On the basis of fungal species and environmental conditions, it has been concluded that laccases are secreted in numerous iso forms, but it is not clearly understood that this multiplicity corresponds to an actually existing different functional roles of various isoenzymes/isozymes (Wood, 1980; Wahleithner et al., 1996; Collins and Dobson, 1997; Mansur et al., 1998). Mendes et al. (2011) found maximum decolorization and detoxification of model dye containing wastewaters when azoreductase and laccase are used simultaneously. Wong et al. (2012) cloned two laccase genes from RNA of *Lentinula edodes* L54 mycelium and showed that the efficient biodegradation of dyes and polyaromatic hydrocarbons take place by two allelic forms of *L. edodes* laccase expressed from methylotrophic yeast *Pichia pastoris.* These genes appear as allelic forms with 1573 bp long and differed only by 21 nucleotides.

Laccases oxidize the phenolic ring by one electron to generate a phenoxy radical which is oxidized once again by the enzyme to produce a carbonium ion in which the charge is localized on the phenolic ring carbon bearing the azo linkage. Nucleophilic attack by the water produces 4-sulfophenyldiazene and a benzoquinone. 4-Sulfophenyldiazene is presumably unstable in the presence of oxygen, which oxidizes it to corresponding phenyldiazene radical. The latter readily loses molecular nitrogen to produce a sulfophenyl radical, which is scavenged by O_2 to ultimately yield 4-sulfophenylhydroperoxide (SPH) (Fig. 4). SPH is unusual peroxide and is known to be formed only from peroxidase oxidation of sulfonated azo dyes, whereas organic peroxides are unstable in presence of transition metal ions.

2.3. Decolorization and degradation of azo dyes by peroxidases

It is well established that fungal systems appear to be most appropriate in the treatment of azo dyes (Ezeronye and Okerentugba, 1999). The capacity of fungi in degradation of azo dyes is attributed to the synthesis of exo enzymes such as peroxidases and phenol oxidases.

Table 2
Decolorization of various azo dyes by Laccase producing fungal culture.

S. no.	Fungal culture	Name of dye	Time (h)	Decolorization (%)	References
1	*Armillaria* sp. F022	Reactive Black 5,	96	80	Hadibarata et al. (2012)
2	*Cerrena unicolor*	Acid Red 27	24	100	Michniewicz et al. (2008)
3	*Coprinopsis cineria*	Methyl Orange	4	47.60	Tian et al. (2014)
4	*Ganoderma* sp.	Methyl Orange,	72	>90	Zhao et al. (2011)
5	*Geobacillus catenulatus* MS5	Congo Red	32	99	Verma and Shirkot (2014)
6	Immobilized *Trametes pubescens, Pleurotus ostreatus*	Remazol Brilliant Blue R, Reactive Blue 49	10 days	>95	Casieri et al. (2008)
7	*Lentinus Polychrous*	Congo Red	3	75	Suwannawong et al. (2010)
8	*Pleurotus ostreatus*	Remazol Brilliant Blue R	72	80	Palmieri et al. (2005)
9	*Pleurotus ostreatus*	Synazol Red HF6BN	24 days	96	Ilyas et al. (2012)
10	*Pycnoporus sanguineus*	Trypan Blue	24	70	Annuar et al. (2009)
11	*Thelephora* sp.	Orange G	–	19	Selvam et al. (2003)
12	*Trametes versicolor, Ganoderma lucidum, Irpex lacteus*	Black Dycem	48	90	Baccar et al. (2011)
13	*Trametes versicolor*	Reactive Black 5	30 min	42.78	Bibi and Bhatti (2012)

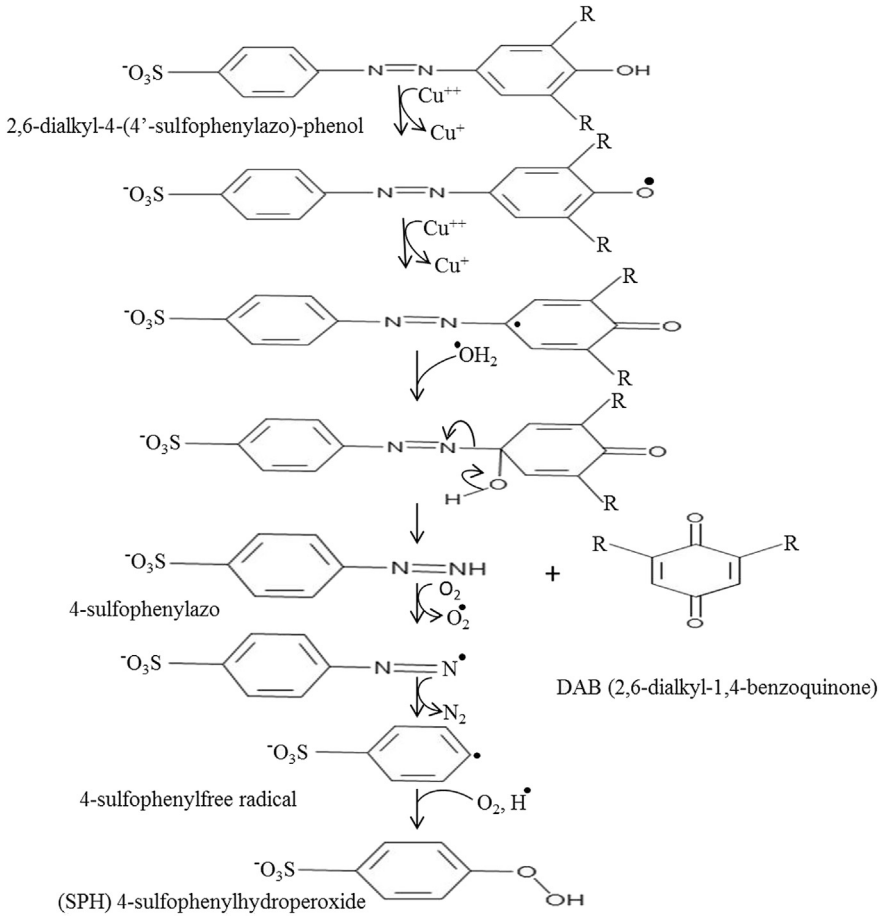

Fig. 4. Proposed mechanism for the degradation of phenolic azo dyes by *P. oryzae* laccase (Chivukula et al. 1995).

Peroxidases (EC 1.11.1.x) are hemoproteins that catalyze reactions in the presence of hydrogen peroxide (Duran et al., 2002). Heme peroxidases are a ubiquitous family of enzymes produced by almost all organisms. Koua et al. (2009) divided heme peroxidases into the following six families on the basis of organism, primary structure, and substrate. These are (a) non animal peroxidase (b) animal peroxidase (c) catalase (d) haloperoxidase (e) di-heme cytchrome-c peroxidase and (f) DyP-type peroxidase families. Peroxidases show three substrate binding sites which are denoted as heme d and c edges and an exposed tryptophan residue. By X-ray crystallographic studies substrate complex structures of d edge have been identified in many peroxidases (Gumiero et al., 2010). The decolorization or degradation of aqueous azo dyes generated by textile photography industries is a main issue in wastewater treatment. Zhang et al. (2012) studied decolorization of aqueous azo dyes such as orange G and sunset yellow in buffer solution by applying an oxidative process of H_2O_2 catalyzed by chloroperoxidase (CPO) and observed that chloroperoxidase (CPO) is a heme containing enzyme produced by *Caldariomyces fumago*.

Patil and Jadhav (2013) studied the ability of certain enzymes such as lignin peroxidase (LiP; EC 1.11.1.14), laccase, tyrosinase and DCIP reductase from roots of *Tagetes patula* for natural degradation of reactive blue 160 and they found its potential application in decolorization as well as detoxification of this textile dye. During solid state fermentation of wheat straw the white rot fungus *Pleurotus ostreatus* produced manganese peroxidase (MnP) and manganese-independent peroxidase (MIP) enzymes. Most of the sulfonephthalein (SP) dyes like bromophenol blue, thymol blue etc. were decolorized by MnP at pH 4.0. Shrivastava et al. (2005) concluded that the MnP activity prefers for methyl group at *ortho* than at the *meta* position on chromophore due to the higher Km value for *meta*-cresol purple and lower Km value for *ortho*-cresol red. Manganese peroxidase (MnP; EC 1.11.1.13) was discovered in *Phanerochaete chrysosporium* and was later shown to be produced by other white rot fungi including *P. ostreatus*. The proposed mechanism for the activity of MnP involves the oxidation of manganous ions (Mn^{2+}) to Mn^{3+}, which is then chelated with organic acids. The chelated Mn^{3+} diffuses readily from the active site of the enzyme which can further oxidize to the secondary substrates (Mester and Field, 1998). Glenn et al. (1983) first reported the decolorization of dyes by ligninolytic enzyme system of white rot fungi and measured ligninolytic activity of *Phanerochaete Chrysosporium*. McMullan et al. (2001) proposed a degradation pathway for sulfonated azo dyes by *P. Chrysosporium* (Fig. 5).

The decolorization of 12 different azo, diazo and anthraquinone dyes was carried out by using an isolated white rot fungus, strain L-25 producing manganese peroxidase. A decolorization percentage efficiency of 84.9–99.6% was obtained by culture in 14 days with an initial dye concentration of 40 mg l^{-1} (Kariminaae-Hamedaani et al., 2007).

Moreira et al. (2006) isolated and purified a novel versatile peroxidase (RBP) from *Bjerkandera* sp. strain B33/3 which has unique kinetic and spectral characteristics. This enzyme has properties of both MnP and LiP enzymes, this new class of enzymes showed high affinity for dyes and manganese. Versatile peroxidase is also able to oxidize manganese, as well as veratryl alcohol (VA)

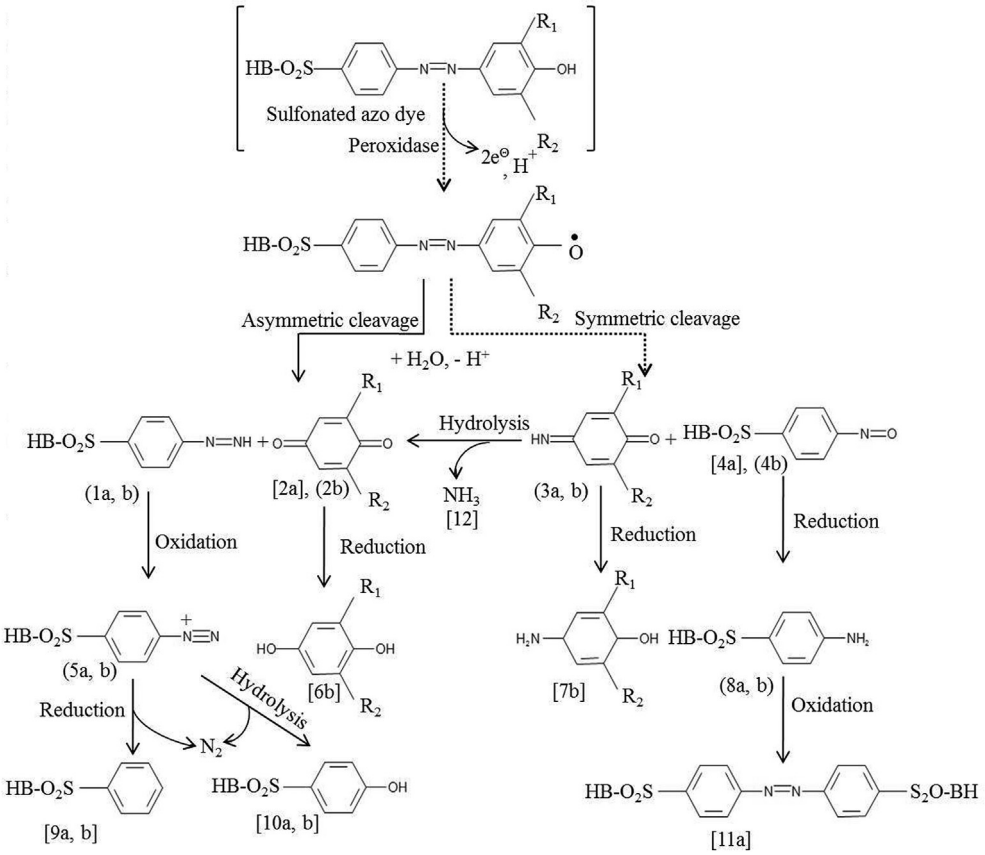

Fig. 5. Proposed pathway for peroxidase catalyzed degradation of sulfonated azo dyes (McMullan et al. 2001). In this Pathway the compounds in *parentheses* have not been found, but their existence is rationalised as necessary intermediates for the observed final products. The compounds represented by numbers in *brackets* have been found in reaction mixtures. Substitution pattern (as in I), R_1=R_2=O and B=O; substitution pattern (as in II), R_1=H, R_2=OCH, and B=NH. [2a] 2,6 dimethyl-1,4-benzoquinone, [4a] 4 nitrosobenzenesulfonic acid, [6b] 2 methoxyhydroquinone, [7b] 2-methoxy-4 aminophenol, [8a] sulfanilic acid, [8b] sulfanilamide, [9a] 4-hydroxybenznesulfonic acid, [9b] 4-hydroxybenzenesulfonamide, [10a] benzenesulfonic acid, [10b] benzenesulfonamide, [11a] azobenzene-4,4′ disulfonic acid, [12] ammonia.

and 2, 6-dimethoxyphenol (DMP) in both manganese dependent and independent reactions. The enzyme peroxidase is well known for its capacity to remove aromatic amines and phenolic compounds from aqueous solutions and also to decolorize textile effluents. This study generates an idea about the potential of the enzyme horseradish peroxidase (HRP; EC 1.11.1.7) in the decolorization of textile dyes and effluents. De'Souza et al. (2007) reported HRP decolorization efficiency to the tune of 59%, 94% and 52% on dyes remazol turquoise blue G, lanaset blue 2R and textile effluents, respectively.

Haddaji et al. (2015) studied the potential role of enzyme peroxidases found in three macrophytic plants (*Arundo donax, Typha angustifolia and Phragmites australis*) for the degradation of direct azo dyes amaranth and amido black and found that it is an effective degradation methodology.

A lignin peroxidase (LiP) enzyme derived from the fermentation of sludge from sewage treatment plant showed potential for treatment of textile effluents for decolorization of dyes. The statistical optimization approach showed that higher removal of methylene blue dye (65%) in static condition with the initial concentration of 15 mg l^{-1} and lignin peroxidase activity of 0.687 U/ml with 60 min of reaction time, while in agitated mode the MB removal was 90% with the same operation time (Alam et al., 2009). In order to develop the cost effective wastewater treatment method, the simple ammonium sulfate precipitated proteins from bitter gourd were taken for the decolorization of many dyes that are present in polluted wastewater. Partially purified preparation of bitter gourd precipitate (BGP) was obtained by adding 20–80% ammonium sulfate and this preparation possess a specific activity of 99.0 EU of peroxidase/mg protein. The experiments were designed to evaluate dye decolorization in presence of crude BGP and H_2O_2, and interestingly, it was found that BGP is a very good enzyme for dye decolorization (Akhtar et al., 2005).

2.4. Decolorization and degradation of azo dyes by polyphenol oxidase

Polyphenol oxidase or (PPO) (EC 1.14.18.1) is a tetramer enzyme that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen. The enzyme catalyzes o-hydroxylation of monophenols to o-diphenol. They can further catalyze the oxidation of o-diphenols to o-quinones. It is the rapid polymerization of o-quinones to produce black, brown or red pigments that is the cause of fruit browning. Theamino acidtyrosine contains single phenolic ring that may be oxidized by action of PPOs to form o-quinone. Hence, PPOs may also be referred to as tyrosinases. PPO is an oxidoreductive enzyme and involves in the removal of aromatic pollutants from various contaminated sites. According to Husain and Jan (2000), this enzyme can act on a broad range of substrate and have capacity for removal of organic pollutants present in very low concentration at contaminated sites. Khan and Hussain (2007) reported that ammonium sulfate protein precipitate of potato and brinjal have very high polyphenol activity, the precipitate have potential to degrade the textiles and non-textiles dyes.

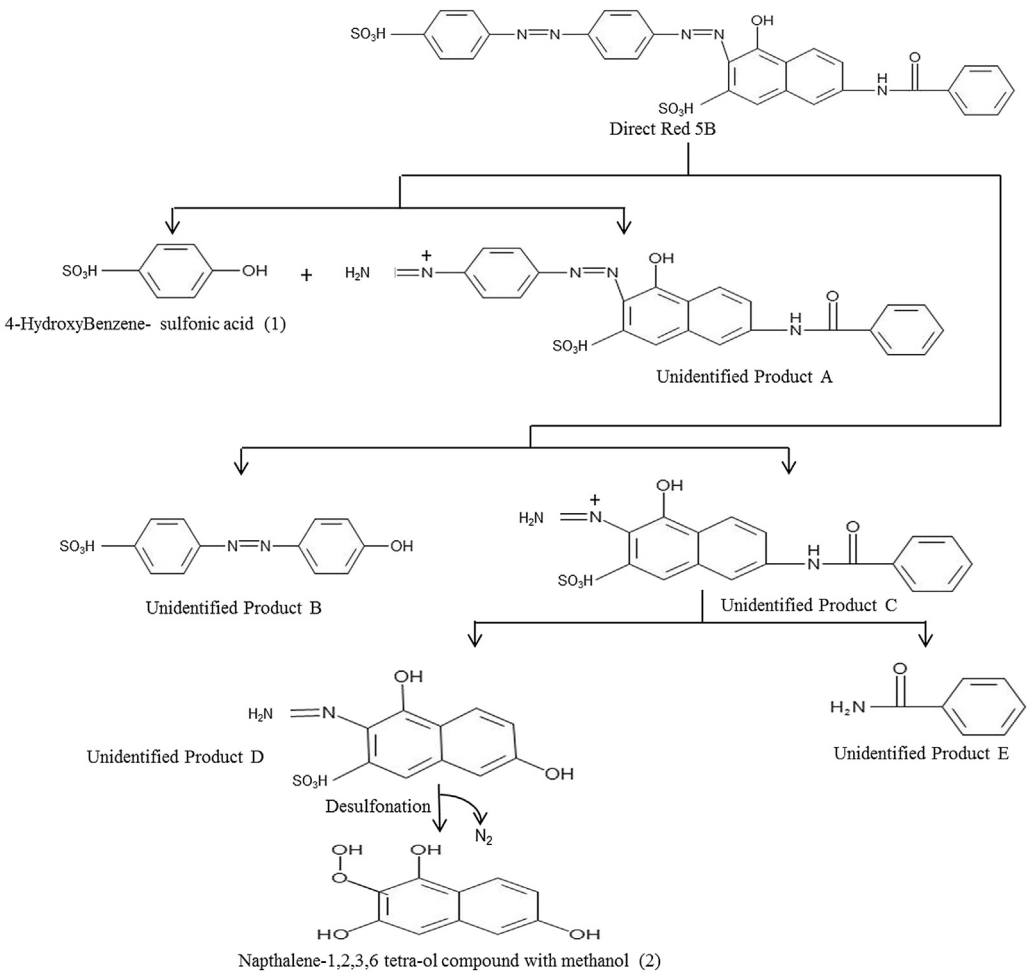

Fig. 6. Proposed Pathway for the degradation of Direct Red 5B by purified banana pulp polyphenol oxidase enzyme. In this Pathway the compound shown in Arabic number in a bracket has been found in reaction mixture. The compound showed in alphabet (A, B, C, D, E) are unidentified intermediates (Jadhav et al. 2011).

Shinde et al. (2012) studied the potential of PPO, extracted from leaves of three fast growing plants *Parthenium hysterophorus, Alternanthera sessilis* and *Jatropha curcas*, for simultaneous decolorization of two textile dyes yellow 5G and brown R. Jadhav et al. (2011) reported the degradation of textile dyes, direct Red 5B and direct Blue GLL, using purified banana pulp polyphenol oxidase. The purified enzyme could decolorize 90% direct red 5B dye within 48 h and direct blue GLL dye up to 85% within 90 h. They proposed a pathway for the degradation of direct red 5B by purified banana pulp polyphenol oxidase enzyme (Fig. 6).

3. Conclusions

Azo dyes constitute the largest and most versatile class of synthetic dyes used in a variety of industries including textile, pharmaceutical, food and cosmetics industries and represent major components in wastewater from these industrial dying processes. The presence of dyes imparts an intense color to effluents which leads to environmental as well as aesthetic problems. The selection of the best treatment option for the bioremediation of a specific type of industrial wastewater is a difficult task because of the complex composition of these effluents. Microbial and enzymatic decolorization and degradation of azo dyes have significant potential to address this problem due to their environmental friendly, inexpensive nature, and also because they do not produce large quantities of sludge. To understand the decolorization and degradation mechanism of azo dyes, detailed information is needed about the initial enzymatic breakdown of azo linkages. Enzymatic processes are very promising for the decolorization of synthetic azo dyes. In this review, initial steps in the pathways of azoreductase, laccase, peroxidase and polyphenol oxidase mediated degradation of recalcitrant synthetic azo dyes have been taken into account. Azoreductases are major group of enzymes expressed in azo dye degrading bacteria for decolorization and degradation of these dyes. Azoreductases catalyze the reaction only in presence of reducing agents like NADH, NADPH and $FADH_2$. Laccases are the most common members of the multicopper oxidase protein family. These are oxidoreductases which have great importance in bioremediation phenomenon. Peroxidases are hemoproteins that catalyze reactions in presence of hydrogen peroxide whereas polyphenol oxidases are an oxidoreductive enzyme and involves in removal of aromatic pollutants from various contaminated sites. Thus it is well established fact that these enzymes are considered as molecular weapon for bioremediation of azo dyes.

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