Title

Bacterial diversity and composition in major fresh produce growing soils affected by physiochemical properties and geographic locations

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REVIEW ARTICLE

Detoxification of azo dyes by bacterial oxidoreductase enzymes

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Abstract

Azo dyes and their intermediate degradation products are common contaminants of soil and groundwater in developing countries where textile and leather dye products are produced. The toxicity of azo dyes is primarily associated with their molecular structure, substitution groups and reactivity. To avoid contamination of natural resources and to minimize risk to human health, this wastewater requires treatment in an environmentally safe manner. This manuscript critically reviews biological treatment systems and the role of bacterial reductive and oxidative enzymes/processes in the bioremediation of dye-polluted wastewaters. Many studies have shown that a variety of culturable bacteria have efficient enzymatic systems that can carry out complete mineralization of dye chemicals and their metabolites (aromatic compounds) over a wide range of environmental conditions. Complete mineralization of azo dyes generally involves a two-step process requiring initial anaerobic treatment for decolorization, followed by an oxidative process that results in degradation of the toxic intermediates that are formed during the first step. Molecular studies have revealed that the first reductive process can be carried out by two classes of enzymes involving flavin-dependent and flavin-free azoreductases under anaerobic or low oxygen conditions. The second step that is carried out by oxidative enzymes that primarily involves broad specificity peroxidases, laccases and tyrosinases. This review focuses, in particular, on the characterization of these enzymes with respect to their enzyme kinetics and the environmental conditions that are necessary for bioreactor systems to treat azo dyes contained in wastewater.

Keywords

Aromatic compounds, Azo dyes, bioremediation, toxicity, wastewater

History

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Introduction

Azo dyes are the largest group of synthetic dyes used in the textile and dye products industries, most of which are located in developing countries around the world. While it is well established that many of these compounds are toxic, mutagenic and genotoxic to wildlife and humans (Dafale et al., 2010; Fraga et al., 2009; Osugi et al., 2009; Tsuboy et al., 2007), every year, thousands of tons of azo dyes are released into the environment by direct discharge to waterways. Depending on the types of dyes that are used, the amounts that are discharged in wastewater can range from 2% of the original concentration for basic dyes to as high as 50% for reactive dyes (Boer et al., 2004; Tan et al., 2000). Subsequent human exposure can then occur by dermal exposure to wastewater, use of contaminated groundwater for human consumption or by less well-studied indirect routes involving plant uptake and food chain transfer. Still other effects include reduction of light penetration into surface water that has been contaminated with dye wastewater, and increases in biological oxygen demand (BOD) (Bae & Freeman 2007), leading to eutrophication of waterways. For these reasons, there is an urgent need to develop low cost, effective strategies for the treatment of dye-polluted water, as well as development of new non-toxic synthetic dyes to prevent their deleterious effects on human and aquatic life.

Although various physical, chemical and biological strategies are used for treatment of azo dyes, the process varies for individual dyes depending on their molecular structure. Many dyes are not easily degraded by either biological or physical treatment (Hsueh et al., 2009) and physico-chemical methods can produce additional waste products that makes use of these methods impractical (Sharma et al., 2013). For this reason, complete mineralization of dyes using microorganisms is an attractive option that takes advantage of the metabolic versatility of microorganisms that can target broad classes of dye chemicals (Khalid et al., 2012; Mohanty et al., 2006; Sadettin & Donmez, 2007; Saratale et al., 2011). A variety of bacteria, fungi and algae are able to degrade azo compounds via reductive and oxidative enzymes, many of which function over the range of environmental conditions relevant to wastewater treatment (Prasad & Aikat, 2014). Along with the development of inocula that can be used for wastewater treatment, azo dyes also can be removed from wastewater by direct enzymatic treatment using purified or partially purified enzymes (Rojas-Melgarejo et al., 2006). Enzymatic methods also provide

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advantages such as better standardization of the treatment process, easy handling and storage and essentially no dependence on the need to maintain cultures of active bacterial cells (Husain & Husain 2008; Husain et al., 2009).

Toxicity of azo dyes and their intermediates

Many azo dyes and their intermediates are toxic, mutagenic and carcinogenic (Shah et al., 2013) and affect higher organisms in both aquatic and terrestrial systems (Puvaneswari et al., 2006). The toxicity of azo dyes varies depending on their structural complexity, substitution groups and reactivity (Maran & Sild, 2003). Recently, several researchers have reported the toxicity of synthetic dyes in contaminated ecosystems (Dafale et al., 2010; Fraga et al., 2009; Osugi et al., 2009). According to Ferraz et al. (2010), the azo dyes Disperse Red 1 and Disperse Red 13 also are mutagenic to *Salmonella*, suggesting that they may affect the activity and composition of microbial communities comprised of bacteria that are sensitive to these toxins.

Dye structure-associated toxicity

Since azo dyes exhibit wide variability in their chemical structure, it is not possible to generalize mutagenic responses for all azo compounds. However, it is well established that differences in the toxicity and mutagenic activity of azo dyes are strongly dependent on their chemical structure (Pissurlenkar et al., 2007; Umbuzeiro et al., 2005). Specific features that contribute to mutagenicity of azo dyes are related to differences in substitution sites, and the number and position of hydroxyl and sulpho groups adjacent to the azo bond (Table 1). For example, dyes containing a hydroxyl group at the *ortho* position are more toxic than those containing a hydroxyl group at the *para* position (Tauber et al., 2005). Likewise, differences in biodegradability are associated with particular features. In studies examining this phenomenon, Pasti-Grigsby et al. (1992) found that a hydroxyl group at position 2 of the naphthol ring increased biodegradability, while Zimmermann et al. (1982) reported that sulpho groups at *ortho* and *para* positions hindered biodegradation.

Many researchers have observed that particular chemical structures affect the rates of biodegradation for different dyes. Hsueh & Chen (2007) observed a rapid biodegradation of Methyl Orange azo dye containing a sulfonic group (a strong electron-withdrawing group) at the *para* position relative to the azo bond (–N = N–). The presence of sulphonic groups on the benzene ring of some dyes confers detergent properties to the dye molecule, which can exert an inhibitory effect on microbial growth (Shah, 2014). In another study, Hsueh et al. (2009) found that the biodegradation of naphthol type azo dye with a hydroxyl group at *ortho* to azo bond was faster than that of non-naphthol type azo dye without a hydroxyl group. Likewise, azo dyes with electron-withdrawing groups (e.g. sulfo group in Reactive Red 198, Reactive Black 5 and Reactive Red 141) decolorize faster than the azo dyes with the electron-releasing groups (–NH-triazine in RB171 and RG19). This work also suggested that the number of electron withdrawing groups is very important with respect to degradation of azo dyes as they observed fast decolorization rates for those dyes that have more electron withdrawing groups such as Reactive Red 198, Reactive Black 5 and Reactive Red 141 (Hsueh et al., 2009).

The presence of particular substitution elements, especially chlorine, also contributes to the mutagenic activity of dyes. For instance, the dye Disperse Red 1 is more toxic than Disperse Red 13 due to the presence of additional chlorine atoms in its crystal lattice structure. Similarly, all of the azo dyes with nitro groups are more toxic and have higher mutagenic activity than dyes without this moiety (Brown et al., 1978; Nestmann et al., 1981). Azo dye toxicity also depends on the nature and position of the aromatic rings and the amino nitrogen atom. For example, 2-methoxy-4-aminoazobenzene is an extremely weak mutagen, whereas 3-methoxy-4- aminoazobenzene is a strong hepatocarcinogen and mutagen (Esancy et al., 1990; Garg et al., 2002). Likewise, the presence of sulphonic groups on aromatic amines decreases their mutagenicity whereas the acetoxy

Table 1. Structure-related toxicity of azo dyes.

Dye name	Major structural characteristics of dyes or their degradation products causing toxicity	References
2-Hydroxyphenyl azo-2′-naphthol azo dye	Naphthalen-2-ol and 4-aminophenol	Deb et al. (2011)
Acid Violet 7	Acetoxy ($COCH_3$) substituent on the aromatic amine	Ben Mansour et al. (2009b)
Acid Violet 7	4′-Aminoacetanilide exhibited a strong genotoxicity, which was imputed to the presence of the acetoxy ($COCH_3$) substituent on the aromatic amine	Ben Mansour et al. (2009a)
Acid Violet 7 and Acid Green 25	Presence of the acetoxy ($COCH_3$) substitute	Fabbri et al. (2010)
Acids yellow 17, Violet 7 and Orange 52	4′-Aminoacetanilid (4′-AA) imputed to the presence of the acetoxy ($COCH_3$) substituent on the aromatic amine	Ben Mansour et al. (2009c)
Direct Black 38 (DB38)	Benzidine and 4-aminobiphenyl (4-ABP)	Bafana et al. (2009)
Direct Blue 76, Direct Blue 218	Copper present in these direct dyes	Bae & Freeman (2007)
Disperse Blue 373, Disperse Orange 37, Disperse Violet 93	Tertiary amine (–N (CH_2CH_3)(CH_2CH_2OH)	Carneiro et al. (2010)
Disperse Orange 1, Disperse Red 1 and Disperse Red 13	Tertiary amine (–N (CH_2CH_3)(CH_2CH_2OH)	Osugi et al. (2009)
Orange 52	N,N′-dimethyl-p phenylenediamine and sulfanilic Acid	Ben Mansour et al. (2007)
Reactive Black 5 and Procion dyes	1-Amino-2-naphthol and vinyl sulphone	Gottlieb et al. (2003)
Reactive Red 141	2-Aminophenol (2AP) and 3- aminophenol (3AP)	Chen et al. (2009)
Remazol Black-5	Aromatic amines formed during anoxic conditions	Dafale et al. (2010)

($COCH_3$) substituent increases their mutagenic effects (Pasha et al., 2008; Roy et al., 2006).

Aromatic amines/intermediate-linked toxicity

Most azo dyes are manufactured from precursor compounds that are known carcinogens, such as benzidine, naphthalene and other aromatic compounds. Aromatic amines are released after cleavage of azo bonds by microflora containing azoreductases (Prasad & Aikat, 2014). Moreover, aromatic amines are also produced when azo dyes come in contact with sweat, saliva or gastric juices of living organisms (Pielesz, 1999; Pielesz et al., 2002). Similarly, ingested azo dyes can be reduced to constitutive aromatic amines by the action of anaerobic intestinal microflora or by mammalian azoreductases that are produced in the intestinal wall or liver (Chequer et al., 2011). Aromatic amines are resistant to the traditional wastewater treatment methods, so they are more persistent in the environment than dyes (Chen et al., 2009). Within the broad class of azo dyes, those that are produced from aromatic amines, including benzidine and 4-biphenylamine, 4-aminobiphenyl, monoacetylbenzidine and acetylaminobiphenyl, pose a particularly serious threat to the environment (Cerniglia et al., 1986; Chung, 2000). Nitroanilines are commonly generated during the biodegradation of azo dyes under anaerobic conditions (Khalid et al., 2009; van der Zee & Villaverde, 2005). Toxicity of these metabolic products also affects performance and ability of the dye decolorizing bacteria that are used for wastewater treatment.

Toxicity to human, plants and aquatic life

Toxicity of azo compounds to higher organisms commonly involves transformation of the parent molecules. In mammals, transformation of azo dyes by hepatic cytochrome P450 enzymes leads to the formation of epoxides and/or reactive oxygen species that damage DNA. Ben Mansour et al. (2007) demonstrated a significant pro-oxidant effect, which suggests that the mutagenicity mechanism occurs through a free radical generation process. According to Umbuzeiro et al. (2005) and Tsuboy et al. (2007), the acetoxy group ($COCH_3$) located on the benzene ring can be metabolized by the P450 enzyme and other hepatic enzymes generating radical mutagenic intermediates. Likewise, mutagenicity of these compounds may occur through free radical generation and formation of reactive oxygen species (Ben Mansour et al., 2007). Reactivity of the azo group is critical for mutagenic activity (Osugi et al., 2009). Biochemical activation through N-hydroxylation, followed by sulfation, esterification or acetylation reactions, generates reactive intermediates that are able to bind to DNA and largely accounts for the carcinogenicity of arylamines (Pinheiro et al., 2004). Toxicity is directly related to the concentration of these compounds and high doses can be lethal, causing formation of micronuclei, DNA fragmentation and increased apoptotic index in human hepatoma cells (Chequer et al., 2009). Some dyes are reported to cause allergy, dermatitis, skin irritation, eye irritation and respiratory irritation in humans (Keharia & Madamwar, 2003). Dyes are also generally linked with the induction of bladder cancer in humans, and of splenic sarcomas, hepatocarcinomas and nuclear anomalies in experimental animals (Puvaneswari et al., 2006; Rafii et al., 1997). In the 1970s, intestinal cancer was relatively more common in highly industrialized societies, and was investigated in relation to the use of azo dyes (Chung et al., 1978; Wolff & Oehme, 1974). Recent studies have indicated that azo dyes also cause phytotoxicity (Ayed et al., 2011; Khaliq et al., 2013).

Toxicity of azo dyes and their metabolic products can be examined using various systems. For example, a micronucleus assay was used by Rajaguru et al. (1999) to study the mutagenic activity of azo dyes in mouse bone marrow, whereas Tsuboy et al. (2007) examined the toxicity of azo dyes using a heptoma cell test (HepG2). Likewise, the Ames assay and the SOS chromotest have been used to study the mutagenicity of azo dyes and their metabolites in *Salmonella* and *Escherichia coli*, respectively (Ben Mansour et al., 2007, 2009b).

Bioremediation of azo dyes by bacteria

A wide range of organisms including bacteria, fungi, algae and in some cases, plants have been reported to degrade azo compounds. However, bacteria are receiving attention worldwide due to their capabilities to degrade a variety of dyes efficiently under anaerobic or aerobic conditions (Table 2). Bacteria can remove azo compounds from the environment through a number of different mechanism(s) including biosorption, bioaccumulation, reduction, oxidation and sequential reduction–oxidation processes. Biosorption makes use of live or dead microbial biomass to remove dyes from the contaminated wastewater (Du et al., 2012; Khehra et al., 2005). In the case of bioaccumulation, the dyes are degraded after intracellular uptake by the cells (Xin et al., 2010).

Biodegradation via reduction process

A large number of bacterial species have been identified that are capable of degrading azo dyes under reduced (anaerobic) conditions (Oturkar et al., 2011; Sha et al., 2014). These bacteria initially cleave the azo bond with the help of an azoreductase enzyme. This process, called decolorization, leads to the formation of colorless aromatic amines under reduced conditions. To date, several bacterial species have been reported to decolorize azo dyes under reduced conditions (Table 2).

Several studies have demonstrated that the decolorization of azo dyes is enhanced under micro-aerophilic conditions (Joshi et al., 2008; Sandhya et al., 2005; Xu et al., 2007). An accelerated degradation of reactive azo dyes has been observed under partially reduced conditions (Khalid et al., 2012). The reduction of azo dyes under partially reduced conditions may require an alternative redox mediator to transfer electrons from NADH to the dye molecule (Chang et al., 2001; Isik & Sponza, 2003). Some azo dyes also are degraded under aerobic conditions (Sarayu & Sandhya, 2010; Ayed et al., 2011). However, the efficiency of the process may decrease in the presence of oxygen (Pearce et al., 2006), due to competition between oxygen and the dyes as electron acceptors. Although a concomitant increase in microbial biomass has been shown to occur in dye polluted water under aerobic conditions, the degradation process does not appear to

Table 2. Biodegradation of structurally different dyes by bacteria via oxidation–reduction processes.

Bacterial species	Dyes	Conditions	Comments	References
Acinetobacter calcoaceticus	Direct Brown MR	Aerobic and anaerobic/anoxic	Decolorization 91% after 48 h under static condition, 59.3% decolorization under aeration.	Ghodake et al. (2009)
Aeromonas hydrophila	Crystal Violet, Fuchsin Green, Brilliant Green, Malachite Green	Anaerobic	About 90% color of dye was removed in 10 h.	Ren et al. (2006)
Bacillus subtilis	Orange	Static	94% decolorization in 24 h.	Sha et al. (2014)
Bacillus lentus	Reactive Red 120	Static	About 97% decolorization was achieved within 12 h.	Oturkar et al. (2011)
Bacillus cereus	Reactive Red 195	Anaerobic	97% dye degraded after 72 h.	Modi et al. (2010)
Bacillus sp. VUS	Orange T4LL	Static anoxic	Complete (100%) decolorization was achieved in 24 h.	Dawkar et al. (2010)
Bacillus sp.	Methyl Red, Orange dye	Shaking	Up to 78 and 47% degradation of dyes, achieved in 5 days.	Gayathri et al. (2014)
Clostridium bifermentans SL186	Reactive Red 3B-A, Reactive Black 5, Reactive Yellow 3G-P	Anaerobic	90% of dyes decolorized in 36 h.	Joe et al. (2008)
Enterobacter spp.	Congo red	Static	Complete decolorization (200 mg L^{-1}) observed in 93 h.	Prasad & Aikat (2014)
Enterococcus casseliflavus and *Enterobacter cloacae*	Orange II	Microaerophilic conditions	95% decolorization after 15 min.	Chan et al. (2011)
Enterococcus faecalis	Acid Red 27, Reactive Red 2	Anaerobic	Decolorization was between 95–100% within 12 h.	Handayani et al. (2007)
Escherichia coli JM 109	Direct Red 71	Anaerobic	Decolorization up to 82% was achieved in 12 h.	Jin et al. (2009)
Kocuria rosea MTCC 1532	Methyl Orange	Anaerobic	Complete color removal was attained in 72 h.	Parshetti et al. (2010)
Lysinibacillus sp. AK2	Metanil Yellow	Anaerobic	Complete (100%) dye decolorization was recorded in 12 h.	Anjaneya et al. (2011)
Pseudomonas aeruginosa	Direct Orange, Disperse Brown, Reactive Green	Aerobic	94 % removal of dyes after 7 days incubation aerobic conditions.	Ahmed (2014)
Pseudomonas aeruginosa	Reactive Red 2	Static	Dye removal was 97% in 10 h.	Bheemaraddi et al. (2013)
Pseudomonas fluorescens	Reactive Orange – M2R, Reactive Blue –M58, Reactive Yellow – M4G, Reactive Black – B	Shaking	Dye decolorization 59, 90, 77 and 79%, respectively, within 16 days.	Sriram et al. (2013)
Pseudomonas oleovorans	B15	Anaerobic/anoxic	Complete removal of dye in 24 h.	Silveira et al. (2011)
Pseudomonas putida	Tectilon Yellow 2G (TY2G)	Anaerobic	Maximum decolorization was 70% after 15 h.	Srinivasan et al. (2011)
Pseudomonas sp.	Reactive Blue 13	Aerobic/anaerobic	Dye was completely decolorized in 120 h.	Lin et al. (2010)
Shewanella decolorationis S12	Fast Acid Red GR	Anaerobic/Microaerophilic	Compete decolorization under anaerobic and microaerophllic conditions in 10 and 68 h, respectively.	Xu et al. (2007)
Shewanella putrefaciens AS96	Acid Red 88, Reactive Black 5, Disperse Orange 3, Direct Red 81	Partial anaerobic	Complete decolorization in 4 h.	Khalid et al. (2008)
Sphingomonas paucimobilis	Methyl Red	Aerobic	100% dye decolorized in 10 h.	Ayed et al. (2011)

be as efficient as under reduced conditions (Isik & Sponza, 2003; Xu et al., 2007). This implies that the dye decolorization process is mainly dependent on oxygen concentration, not on microbial biomass. Similarly, the process is also very much dependent on the availability and nature of electron donors (Brige et al., 2008; Hong et al., 2008; Modi et al., 2010). Most of the anaerobic reduction processes are location specific but can take place both in cytoplasmic and membrane fractions isolated from bacterial cells (Kudlich et al., 1997). However, an essential component required for electron transport from electron donors to azo compounds is thought to be located in membrane fractions from bacterial cell extracts (Hong et al., 2007a, 2009). This implies that the coupling of oxidation of electron donor and reduction of azo dyes is a universal biochemical phenomenon for azo dyes degradation.

Biodegradation via oxidative processes

The colorless products consisting of aromatic compounds generated from the reductive cleavage of azo dyes are usually mineralized completely under aerobic conditions (Pandey et al., 2007). Aerobic degradation is a multiple step process and may include autoxidation, desulfonation, demethylation and deamination reactions (Oturkar et al., 2011; Singh, 2006). Gan et al. (2011) reported that different enzymes such as protocatechuate dioxygenases and aromatic ring hydroxylases are responsible for the cleavage of aromatic rings. Similarly, Dawkar et al. (2008) reported that laccases are involved in the demethylation of aromatic compounds. Hydroxylation dioxygenases have been reported to be involved in oxidative deamination reactions forming phenol like compounds (Lin et al., 2010). These oxidative enzymes are released in the extracellular matrix of the bacteria and hence aromatic amines could be degraded extracellularly, whereas aromatic amines with ortho-substituted hydroxyl groups may undergo autoxidation (Kudlich et al., 1999). In this case, aromatic amines are initially oxidized to oligomers and eventually to dark colored polymers with low solubility that can easily be removed from the water phase (Klibanov & Morris, 1981). Overall, there is a wide variation in the oxidative degradation of different aromatic amines. For example, in one study, Gan et al. (2011) depicted the mineralization of 4-aminobenzensulfonate by *Ralstonia* and *Hydrogenophaga* sp. In this mineralization process, oxygen was introduced and degradation occurred through aromatic ring hydroxylation carried out by dioxygenase enzymes following a beta-ketoadipate pathway (Parales & Resnick, 2006). This process leads to the formation of non-toxic end products including carbon dioxide, ammonium and sulfates. Singh et al. (2007) suggested another mechanism in which 4-aminobenzensulfonate was first oxidized into catechol-4-sulfonate by a strain of *Hydrogenophaga intermedia*, after which this metabolite was further utilized by an *Agrobacterium radiobacter* strain S_2 most likely through 3,4-dioxygenase I and 3,4-dioxygenase II enzymes.

Laccases also oxidize aromatic amines such as anilines and phenols, in the presence of oxygen (Bollag, 1992; Chivukula & Renganahathan, 1995; Hoff et al., 1985). In this reaction, the substrates are oxidized by an electron transfer step to produce the corresponding phenoxy radicals. These radicals are also polymerized to generate a phenolic polymer or can be oxidized by laccase to produce quinone (Bollag, 1992). Electrons received thereby are subsequently transferred to oxygen and reduced to water. Pereira et al. (2009) also proposed a pathway for the biotransformation of the azo dye Sudan Orange G (SOG) by CotA-laccase. In this study, azo dyes were oxidized without cleavage of the azo bond via free radical highly non-specific mechanism, forming phenolic type compounds. Toxic aromatic amines are not produced in this mechanism (Chen, 2006). Free radicals generated by a biotransformation process participate in coupling reactions with intact dye and/or intermediate molecules. Oligomeric or polymeric condensation products are the result of coupling reactions between the intermediates of dye laccase-oxidative process (Kandelbauer et al., 2004; Moldes et al., 2004).

Biodegradation via sequential reduction–oxidation processes

Recently, anaerobic–aerobic sequential processes have become popular for the treatment of azo dyes released by the dye-products industry. Biodegradation of azo dyes invariably starts with reductive cleavage of azo bond under anaerobic conditions, generating amine-related structures that are not completely degraded under anaerobic conditions (Farabegoli et al., 2010; Hong et al., 2007b). However, such amines are reported to be readily biotransformed under aerobic conditions (Elbanna et al., 2010; Steffan et al., 2005). Therefore, a sequential anaerobic–aerobic system could be useful for the complete mineralization of azo dyes in wastewater. Many researchers unequivocally support this premise. Supaka et al. (2004) used a mixed culture of the genera *Paenibacillus* and *Pseudomonas* for the degradation of reactive azo dyes Remazol Brilliant Orange 3R, Remazol Black B and Remazol Brilliant Violet 5R under anaerobic–aerobic conditions. The results of this study indicated that under anaerobic conditions, the azo dyes were converted to aromatic amines through reduction enzymes. After re-aeration of the synthetic dye wastewater, these amines were further degraded by the same isolates. Similarly, Khalid et al. (2009) reported degradation of Disperse Orange-3 and its byproduct 4-nitroaniline under sequential anaerobic–aerobic conditions using a 3-member bacterial consortium. Likewise, Elbanna et al. (2010) reported a complete mineralization of Reactive Lanasol Black B (RLB), Eriochrome Red B (RN) and 1,2 Metal Complexes I Yellow in anaerobic–aerobic sequential system using a consortium of *Lactobacillus casei*, *Lactobacillus paracasei* and *Lactobacillus rhamnosus*. Single strains can also perform the sequential process, but may be less efficient, leading to partial degradation of azo dyes (Hong et al., 2007a). In one such study, Hong et al. (2007a) used the bacterial strain *Shewanella decolorationis* S12, which completely decolorized the Amaranth dye under anaerobic conditions, but when it was exposed to aerobic conditions, the byproducts of amaranth (1-aminenaphthylene-4-sulfonic acid and 1-aminenaphthylene-2-hydroxy-3,6-disulfonic acid) were only partially mineralized. The 1-aminenaphthylene-2-hydroxy-3,6-disulfonic acid was completely removed while another component,

1-aminenaphthylene-4-sulfonic acid, was not affected when the cells were exposed to aerobic conditions. However, this strain could completely mineralize other dyes like Fast Acid Red GR under microaerophilic conditions (Xu et al., 2007). Although more decolorization was observed under anaerobic conditions, aromatic amines (aniline, 1,4-diaminobenzene and 1-amino-2-naphthol) generated from reductive cleavage of azo dyes under microaerophillic conditions may accumulate (Xu et al., 2007). These studies clearly demonstrate that bacterial consortia can be more effective for the complete mineralization of dyes than single strain cultures employing a sequential anaerobic–aerobic treatment system.

Bacterial enzymes responsible for the degradation of azo dyes

Recently, there has been interest in the development of direct enzymatic processes for the treatment of dye-contaminated wastewater (Champagne & Ramsay, 2010; Kalme et al., 2009; Misal et al., 2011; Wang et al., 2011). Many studies show that both reductive and oxidative enzymes play key roles in the biodegradation of azo dyes (Table 3). With new methods for enzyme immobilization and stabilization using inert materials or encapsulation, pure enzymes offer many advantages for development of a biotreatment process for cleanup of azo dye containing wastewater.

Reductive enzymes

It is well established that the first step of azo dyes degradation is reduction via azoreductase enzymes. Two broad types of azoreductases including flavin-dependent and non-flavin reductases have been reported (Blumel & Stolz, 2003; Maier et al., 2004; Suzuki et al., 2001). Flavin-dependent azoreductases usually reduce azo dyes through a Ping–Pong Bi–Bi mechanism (Nakanishi et al., 2001; Ryan et al., 2010; Wang et al., 2010). In this mechanism, two cycles of NADPH-dependent reduction of FMN to FMNH occur, converting azo dye to a hydrazine in the first step, and then the hydrazine to two constitutive amines in the second step (Correia et al., 2011; Ryan et al., 2010). Flavin dependent azoreductases are polymeric in nature, and flavin molecules are non-covalently attached with protein and link the dimeric sub units of azoreductases (Liger et al., 2004). Flavin contributes thermal stability to azoreductase enzyme (Natalello et al., 2007). In general, flavin enzymes exhibit broad substrate specificity with respect to the chemical structure and size of the dye substrate, making it particularly non-specific for any dye group (Goncalves et al., 2013).

The azoreductases are classified into two groups on the basis of their electron donor requirements. The first includes flavin containing azoreductases, which show preference for NADH, while the second performs better with NADPH as a reductant for azo dye decolorization (Chen et al., 2005; Maier et al., 2004; Punj & John, 2008). Burger & Stolz (2010) isolated a flavin free azoreductase enzyme from *Xenophilus azovorans* KF46F strain. It was one of the first flavin free azoreductase that was found to be oxygen tolerant. The mechanism by which this enzyme functions for reduction of azo dyes involves an ordered bioreactant reaction mechanism, which is different from the Ping–Pong mechanism used by flavin containing enzymes. These flavin free azoreductases also preferentially utilize NADPH as a reductant.

Some other enzymes like NADH-DCIP reductase and riboflavin reductase have been reported to function for reduction of azo dyes (Ghodake et al., 2009; Kalyani et al., 2008; Telke et al., 2009), but so far these have had limited application for the reduction of azo dyes. Most of these enzymes are shown to be ineffective *in vivo* (Blumel et al., 2002; Russ et al., 2000). Limited transport of dyes across the cell membrane and high reactivity of many of the *ortho* aminohydroxy aromatics that are formed after the reductive cleavage of the azo dyes restrict the reaction of azoreductases (Grundmann, 1979; Kudlich et al., 1999). Russ et al. (2000) suggested that the cytoplasmic anaerobic azoreductases are flavin reductases and could function for extracellular reduction of azo dyes via an electron mediator. These mediators allow transfer of redox equivalents from the cell membrane of bacteria to azo dyes. A pre-requisite for these mediators would be a greater ability to pass bacterial membranes than flavins. This suggests that the microbial strains with the ability to decolorize azo dyes require not only the presence of azoreductases but also a transport system which allows the absorption of dyes in cells. Thus, there is a need for research to investigate the optimal range of conditions in which pure enzymes might be used.

Oxidative enzymes

A variety of oxidative enzymes are also used for the degradation of dyes (Aftab et al., 2011). These include lignin peroxidase, laccase and tyrosinase. During decolorization of Orange T4LL, a significant induction in the activities of lignin peroxidase, tyrosinase and reductases (NADH-DCIP, azo and riboflavin) was observed (Dawkar et al., 2010). Dye degrading peroxidase degrades typical peroxidase substrates, but also degrades hydroxyl free anthraquinone, which is not transformed by other peroxidases (Marchis et al., 2011; Sugano et al., 2006). Likewise, a combination of lignin peroxidases and veratyl alcohol also enhances decolorization of azo and anthraquinone dyes (Joshi et al., 2010). Recently, it was reported that mono-rhamnolipid like molecules significantly increased the extracellular activities of lignin peroxidase and veratryl alcohol oxidase, the enzymes involved in dye degradation (Jadhav et al., 2011). This effect may be due to protection from inactivation of the enzyme by hydrogen peroxide or interactions that facilitate the complete oxidation–reduction cycle for the lignin peroxidase (Young & Yu, 1997). Telke et al. (2009) reported a novel enzyme, a laccase-like phenol oxidase that has the ability to react with non-phenolic substrates. Lignin peroxidase catalyzes the depolymerization of methylated lignin in dye degradation process (Saratale et al., 2009, 2011).

Laccases are oxidoreductases or multi-copper oxidases that are widely used to oxidize a variety of dye by-products by coupling reduction of oxygen to water with concomitant oxidation of the dye product substrates (Kurniawati & Nicell, 2007, 2009; Morozova et al., 2007). Laccases catalyze decolorization of textile dyes either by direct oxidation or via indirect oxidation using mediators to accelerate the

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Table 3. Bacterial enzymes responsible for the degradation of dyes under optimized conditions.

Bacteria	Enzyme	Co-factor/redox mediator	Optimum conditions	Dyes degraded	References
Acinetobacter calcoaceticus	Lignin peroxidase	Tryptophan	pH 1.0, 50–70 °C	Many	Ghodake et al. (2009)
Bacillus badius	Azoreductase	NADH and NADPH	pH 7.4, 60 °C	Amaranth dye	Misal et al. (2011)
Bacillus cereus	Azoreductase	NADH	pH 6–7, 40 °C	Indigo Carmine, Flame Orange, Ruby Red	Pricelius et al. (2007)
Bacillus pumilus	Laccases	2,2′-Azino-bis (3-ethylbenzthiazo-line-6-sulphonic acid), 2,6-dimethoxyphenol	pH 6.5–7.0, 70 °C	Acetosyringone, Indigocarmine	Reiss et al. (2011)
Bacillus sp. ADR	NADH–DCIP reductase	NADH	pH 7–8, 30–40 °C	Reactive Orange 16	Telke et al. (2009)
Bacillus sp. ADR	Laccase	Not required	pH 6–7, 40 °C	Many	Telke et al. (2011)
Bacillus sp. ADR	Phenol oxidase	Not required	pH 7–8, 30–40 °C	Reactive Orange 16	Telke et al. (2009)
Bacillus sp.	Azoreductase	NADH/FAD	pH 8.0, 80 °C	Reactive Black 5	Maier et al. (2004)
Bacillus subtilis	CotA-laccase	Not required	pH 8, 37 °C	Sudan Orange G	Pereira et al. (2009)
Bacillus subtilis WD23.	Laccases	Not required	pH 6.8, 60 °C	Many	Wang et al. (2011)
Bacillus velezensis	Azoreductase	NADH	No information	Direct Red 28	Bafana et al. (2008)
Comamonas	Veratryl alcohol oxidase	Not required	pH 7.0, 30–65 °C	Red HE7B and Direct Blue GLL	Jadhav et al. (2009)
Enterococcus faecalis,	Azoreductase	NADPH, FMN and NADH	pH 7.5, 25 °C	Methyl Red	Punj & John (2008)
Escherichia coli	Azoreductase	FMN cofactor	pH 7.5	Methyl Red	Ito et al. (2008)
Exiguobacterium sp. RD3	Azoreductase	NADH and NADP	pH 7, 30 °C	Reactive Yellow 84A	Dhanve et al. (2009)
Pigmentiphaga kullae K24	Azoreductase	NADPH	pH 6, 37–45 °C	Orange I	Chen et al. (2010)
Pseudomonas aeruginosa strain BCH	veratryl alcohol oxidase	Not required	pH 3.0, 55 °C	Remazol Black	Phugare et al. (2011)
Pseudomonas desmolyticum	Laccases	Not required	pH 4.0, 60 °C	Direct Blue-6, Green HE4B and Red HE7B	Kalme et al. (2009)
Shewanella oneidensis MR-1	Azoreductase	NADH	pH 6.5, 25 °C	Methyl Red	Yang et al. (2013)
Sphingobacterium sp. AT	Aryl Alcohol Oxidase	Not required	pH 3, 40 °C	Direct Red 5B	Tamboli et al. (2011)
Staphylococcus aureus	Azoreductase	NADPH	pH 6–6.6, 35–40 °C	Methyl Red	Chen et al. (2005)
Stenotrophomonasmaltophilia	Laccase	ABTS (2,2-azino-bis(3-ethyl-benzthiazoline-6-sulfonic acid)	pH 7.0, 40 °C	Reactive black-5	Galai et al. (2014)
Thermobifida fusca	DyP-type peroxidases	Heme	pH 3.5, 25 °C	Reactive Blue 19, Reactive Blue 4, Reactive Black 5	van Bloois et al. (2010)
Xenophilus azovorans	Azoreductase	NAD(P)H	pH 7.1, 25°C	Orange II	Burger & Stolz (2010)

reaction (Khlifi et al., 2010). The presence of redox mediators is required for a number of biotechnological applications, serving for oxidation of complex substrates. Normally, laccase mediators are good substrates for laccase, stable in oxidized and reduced form and have no inhibitory effect upon enzyme activity (Gonzalez et al., 2009). Relatively few bacterial laccases have been studied with respect to their ability to degrade azo dyes (Pereira et al., 2009; Singh et al., 2007). However, laccases produced by *Streptomyces* are reported to be effective for the decolorization of textile dyes (Dube et al., 2008; Lu et al., 2013; Molina-Guijarro et al., 2009). Gottlieb et al. (2003) demonstrated the usefulness of a laccase enzyme produced by *Streptomyces cyaneus* CECT 3335. Redox mediators play an important role in oxidative degradation by *Streptomyces* laccases, primarily by facilitating the movement of electrons in the system (Gonzalez et al., 2009). Dye-degrading peroxidases are reported to degrade hydroxyl-free anthraquinone dyes (Marchis et al., 2011; Sugano et al., 2006). A combination of lignin peroxidases and veratyl alcohol was found to enhance the decolorization of azo and anthraquinone dyes (Joshi et al., 2010). The role of lignin-degrading enzymes in the treatment of contaminated effluent is also established, however their utilization demands a thorough understanding of lignin degrading organisms and their enzyme systems. Although oxidative enzymes produced by fungi are also used for the degradation of azo dyes, bacterial enzymes generally have greater heat stability and broader substrate specificity (Hilden et al., 2009; Lee et al., 2003; Reiss et al., 2011). Moreover, these can be produced in a short time, operating in aqueous solvent at neutral to basic pH (Wells et al., 2006), which is a common pH of real dye-contaminated textile wastewater. Therefore, use of bacterial enzymes seems to be one of the viable options for degrading the dyes present in industrial effluents.

Structure and function of dye-degrading enzymes

Several azoreductases have been identified in bacteria but very little is known about the structural basis for substrate specificity and the nature of reaction. The subtle changes in an enzyme structure lead to substrate binding and release. These changes highlight the fine control and access to the catalytic site that are required by the Ping–Pong mechanism, and in turn, the specificity is offered by the enzyme towards different substrates. The topology surrounding the active site shows novel features of substrate recognition and binding that help to explain and differentiate the substrate specificity observed among different bacterial azoreductases (Goncalves et al., 2013). The monomer and dimer structures of the azoreductases determine their substrate specificity and thermostability (Brissos et al., 2014). Gene sequences have been determined for both dimeric azoreductases (Matsumoto et al., 2010; Nakanishi et al., 2001) and monomeric enzymes (Blumel & Stolz, 2003; Blumel et al., 2002). Dimeric azoreductases are thought to be more thermostable (Ooi et al., 2012) and these enzymes also show broad substrate specificity for azo dye reduction (Mendes et al., 2011).

Both flavin-dependent and flavin-free azoreductases have been described at the molecular level in the literature. In most cases, the genes encoding aerobic flavin-dependent azoreductases have been cloned from a variety of bacteria including *Bacillus* sp. OY1-2 (Suzuki et al., 2001), *Escherichia coli* (Nakanishi et al., 2001), *Xenophilus azovorans* KF46F (Blumel et al., 2002), *Pigmentiphaga kullae* K24 (Blumel & Stolz, 2003), *Enterococcus faecalis* (Chen et al., 2004), *Geobacillus stearothermophilus* (Mendes et al., 2011). All flavo-enzymes by definition contain a covalently or non-covalently bound flavin as a functional cofactor. Typically, they contain either flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD) having an isoalloxazine ring system, which enables them to catalyze one and two electron transfer reactions (Hefti et al., 2003). Recently, a strictly anaerobic azoreductase enzyme was also characterized from *Clostridium perfringens* by Morrison et al. (2012). The enzyme activity was highest in the presence of two cofactors, NADH and FAD. The azoreductase gene was found to be homologous to either an FMN reductase or a flavodoxin-2 conserved region.

Very few studies have reported flavin free-azo reductases. Two monomeric flavin-free azoreductases from *X. azovorans* KF46F (Blumel et al., 2002; Zimmermann et al., 1982) and *P. kullae* K24 (Blumel & Stolz, 2003) have been described. The deduced protein sequences from these cloned genes did not show significant homologies with flavin-dependent azoreductases and probably both types of reductases evolved from different origins (Chen et al., 2004).

Enzyme technology: some practical considerations

Successful treatment of dye-polluted wastewater using enzyme-based technology requires that the dye-degrading enzymes can be stably maintained in the treatment system. Thermal stability and the ability of azo dye degrading enzymes to function over a wide temperature range are thus very important for their practical application. To date, a large number of azoreductases have been shown to function over temperature ranges from 25 to 85 °C (Table 3). Sharma et al. (2013) observed maximum azoreductase activity at the normal growth temperature (37 °C) for mesophilic bacteria. Yang et al. (2013) reported an azoreductase that retained 85% of is maximal activity at 25 °C at temperatures ranging from 30 to 40 °C. Matsumoto et al. (2010) reported a thermostable azoreductase having an optimal temperature of 85 °C for degradation of Methyl Red (MR). This enzyme remained active for 1 h at 65 °C and for 1 month at 30 °C, demonstrating superior long-term stability. Another azoreductase, known as AzrA, exhibited optimal activity at temperature between 65 and 75 °C (Ooi et al., 2007), while 85 °C was the optimal temperature for AzrG that exhibited remarkable activity toward several azo compounds (Matsumoto et al., 2010). In particular, the activity toward Acid Red 88 at 85 °C was increased compared to 30 °C. Similarly, bacterial laccases have been reported to be effective for the degradation of azo dyes over a wide temperature range (40–60 °C) (Wang et al., 2009).

Among other environmental factors, pH control is a key factor for practical application of dye degrading enzymes (Bibi et al., 2012; Mahmood et al., 2013). Azoreductases exhibiting dye-degradation activities have been shown to function over a wide range from as low as pH 4.0) and as high

as pH 9 (Johansson et al., 2011; Maier et al., 2004; Misal et al., 2011; Sharma et al., 2013; Yang et al., 2013). In contrast, bacterial laccases are reported to have their highest activities under neutral to alkaline conditions (Reiss et al., 2011). Metals are also widely used in dye synthesis and may influence azoreductase activity. Usually, high concentrations of metal ions such as chromium significantly inhibit enzyme activity (Aksu & Karabayir 2008; Mahmood et al., 2013). In contrast, Mg^{2+} and Mn^{2+} may enhance the activity of azoreductases (Yang et al., 2011).

During treatment processes, immobilization of dye-degrading enzymes on specific support material could be critical to determine whether introduced enzymes will function effectively for removal of azo dyes from industrial effluents. Our review of the literature suggests that there has not yet been serious work aimed at the treatment of azo dyes using immobilized azoreductases. Some researchers reported decolorization of azo dyes using immobilized or spore-bound bacterial laccases (Held et al., 2005; Hilden et al., 2009). The crude enzyme mixture containing hydrolase was self-sustaining and the respective reaction occurred without any necessary cofactors. Efficacy of these enzymes in treatment systems can possibly be enhanced by optimizing immobilization procedures. Various materials such as calcium alginate gel capsules, activated charcoal, charcoal pellets or biochar can be potential materials for immobilization of dye degrading enzymes.

Conclusions

Bacteria that produce versatile dye-degrading enzymes can be used for the bioremediation of dye-polluted effluents. The use of such bacteria for the removal of dye contaminants could be very effective due to their fast growth rate and high performance in bioreactors. Nevertheless, considerable research is still required to develop this biotechnology for the treatment of dye-contaminated wastewater on a large scale. Molecular studies should be conducted for monitoring activities of degrading bacterial community in the treatment system. Bacterial derived enzymes can also be directly used for the treatment of azo dyes. In many cases, cell-free or isolated enzymes are preferred for use over the intact organisms, especially when the effluent to be treated contains pollutants that inhibit microbial growth (Mugdha & Usha, 2012). However, incorporation of enzymes into real treatment systems requires extensive research for the optimization of system conditions. Molecular level studies of bacterial reductive and oxidative enzymes that degrade azo dyes have centered on their phylogeny and broad structural characteristics, but have not yet addressed the active sites and features affecting their specificity toward different dyes. Much research is required to develop cost effective methods for application of enzymes on a large scale. These processes are very promising for the biodegradation of synthetic azo dyes and can be a future strategy to tackle the dye contaminated wastewater problem.

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Declaration of interest

The authors have no declarations of interest to report.

References

Aftab U, Khan MR, Mehfooz M, et al. (2011). Decolourization and degradation of textile azo dyes by *Corynebacterium sp*. isolated from industrial effluent. Pak J Zool, 43, 1–8.

Ahmed SGKA. (2014). Aerobic bacterial degradation and decolorization of different azo dyes. J Biol Agric Healthcare, 4, 72–81.

Aksu Z, Karabayir G. (2008). Comparison of biosorption properties of different kinds of fungi for the removal of Gryfalan Black RL metal-complex dye. Bioresour Technol, 99, 7730–41.

Anjaneya O, Souche SY, Santoshkumar M, Karegoudar TB. (2011). Decolorization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus sp*. strain AK1 and *Lysinibacillus sp*. strain AK2. J Hazard Mater, 190, 351–8.

Ayed L, Mahdhi A, Cheref A, Bakhrouf A. (2011). Decolorization and degradation of azo dye Methyl Red by an isolated *Sphingomonas paucimobilis*: biotoxicity and metabolites characterization. Desalination, 274, 272–7.

Bae JS, Freeman HS. (2007). Aquatic toxicity evaluation of copper-complexed direct dyes to the *Daphnia magna*. Dyes Pigments, 73, 126–32.

Bafana A, Chakrabarti T, Devi SS. (2008). Azoreductase and dye detoxification activities of *Bacillus velezensis* strain AB. Appl Microbiol Biotechnol, 77, 1139–44.

Bafana A, Chakrabarti T, Muthal P, Kanade G. (2009). Detoxification of benzidine-based azo dye by *E. gallinarum*: time-course study. Ecotox Environ Saf, 72, 3960–4.

Ben Mansour H, Corroler D, Barillier D, et al. (2007). Evaluation of genotoxicity and pro-oxidant effect of the azo dyes: acids yellow 17, violet 7 and orange 52, and of their degradation products by *Pseudomonas putida* mt-2. Food Chem Toxicol, 45, 1670–7.

Ben Mansour H, Corroler D, Barillier D, et al. (2009a). Influence of the chemical structure on the biodegradability of acids yellow 17, violet 7 and orange 52 by *Pseudomonas putida*. Ann Microbiol, 59, 9–15.

Ben Mansour H, Mosrati R, Corroler D, et al. (2009b). In vitro mutagenicity of acid violet 7 and its degradation products by *Pseudomonas putida* mt-2: correlation with chemical structures. Environ Toxicol Phamacol, 27, 231–6.

Ben Mansour H, Mosrati R, Limem I, et al. (2009c). Genotoxic and antibutyrylcholinesterasic activities of acid violet 7 and its biodegradation products. Drug Chem Toxicol, 32, 230–7.

Bheemaraddi MC, Shivannavar CT, Gaddad SM. (2013). Isolation and characterization of an azo dye reactive red 2 degrading bacteria from dye contaminated soil. Int J Pharm Biol Sci, 4, 711–22.

Bibi R, Arshad M, Asghar HN. (2012). Optimization of factors for accelerated biodegradation of reactive black-5 azodye. Int J Agric Biol, 14, 353–9.

Blumel S, Knackmuss H, Stolz A. (2002). Molecular cloning and characterization of the gene coding for the aerobic azoreductase from *Xenophilus azovorans* KF46F. Appl Environ Microbiol, 68, 3948–55.

Blumel S, Stolz A. (2003). Cloning and characterization of the gene coding for the aerobic azoreductase from *Pigmentiphaga kullae* K24. Appl Microbiol Biotechnol, 62, 186–90.

Boer CG, Obici L, de Souza CG, Peralta RM. (2004). Decolorization of synthetic dyes by solid state cultures of Lentinula (Lentinus) edodes producing manganese peroxidase as the main ligninolytic enzyme. Bioresour Technol, 94, 107–12.

Bollag JM. (1992). Enzymes catalyzing oxidative coupling reactions of pollutants. Metal Ions Biol Syst, 28, 205–17.

Brige A, Motte B, Borloo J, et al. (2008). Bacterial decolorization of textile dyes is an extracellular process requiring a multicomponent electron transfer pathway. Microbial Biotechnol, 1, 40–52.

Brissos V, Goncalves N, Melo EP, Martins LO. (2014). Improving kinetic or thermodynamic stability of an azoreductase by directed evolution. PLoS One, 9, e87209.

Brown JP, Roehm GW, Brown RJ. (1978). Mutagenicity testing of certified food colours and related azo, xanthene and triphenylmethane dyes with the *Salmonella*/microsome system. Mutat Res, 56, 249–71.

Burger S, Stolz A. (2010). Characterisation of the flavin-free oxygen-tolerantbazoreductase from *Xenophilus azovorans* KF46F in

comparison to flavin-containing azoreductases. Appl Microbiol Biotechnol, 87, 2067–76.
Carneiro PA, Oliveira DP, Umbuzeiro GA, Zanoni MVB. (2010). Mutagenic activity removal of selected disperse dye by photoelectrocatalytic treatment. J Appl Electrochem, 40, 485–92.
Cerniglia CE, Zhuo Z, Manning BW, et al. (1986). Mutagenic activation of the benzidine-based dye direct black 38 by human intestinal microflora. Mutat Res, 175, 11–16.
Champagne PP, Ramsay JA. (2010). Dye decolorization and detoxification by laccase immobilized on porous glass beads. Bioresour Technol, 101, 2230–5.
Chan FC, Rashid NAA, Koay LL, et al. (2011). Identification and optimization of Novel NAR-1 bacterial consortium for the biodegradation of orange II. Insight Biotechnol, 1, 7–16.
Chang JS, Chou C, Lin Y, et al. (2001). Kinetic characterization of bacterial azo dye decolorization by *Pseudomonas luteola*. Water Res 35, 2841–50.
Chen H. (2006). Recent advances in azo dye degrading enzyme research. Curr Protein Pept Sci, 7, 101–11.
Chen BY, Yen C, Chen W, et al. (2009). Exploring threshold operation criteria of biostimulation for azo dye decolorization using immobilized cell systems. Bioresour Technol, 100, 5763–70.
Chen H, Hopper SL, Cerniglia CE. (2005). Biochemical and molecular characterization of an azoreductase from *Staphylococcus aureus*, a tetrameric NADPH-dependent flavoprotein. Microbiology, 151, 1433–41.
Chen H, Wang R, Cerniglia CE. (2004). Molecular cloning, overexpression, purification, and characterization of an aerobic FMN-dependent azoreductases from *Enterococcus faecalis*. Protein Expres Purif, 34, 302–10.
Chequer FMD, Angeli JPF, Ferraz ERA, et al. (2009). The azo dyes Disperse Red 1 and Disperse Orange 1 increase the micronuclei frequencies in human lymphocytes and in HepG2 cells. Mutat Res, 676, 83–6.
Chequer FMD, Dorta DJ, de Oliveira DP. (2011). Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks? In: Hauser PJ, ed. Advances in treating textile effluent. Croatia: InTech, 27–48.
Chivukula M, Renganahathan V. (1995). Phenolic azo dye oxidation by laccase from *Pyricularia oryzae*. Appl Environ Microbiol, 61, 4374–7.
Chung KT, Fulk GE, Egan M. (1978). Reduction of azo dyes by intestinal anaerobes. Appl Environ Microbiol, 35, 558–62.
Chung KT. (2000). Mutagenicity and carcinogenicity of aromatic amines metabolically produced from azo dyes. Environ Carcinog Ecotoxicol Rev, 18, 51–74.
Correia B, Chen Z, Mendes S, et al. (2011). Crystallization and preliminary X-ray diffraction analysis of the azoreductase PpAzoR from *Pseudomonas putida* MET94. Acta Cryst, 67, 121–3.
Dafale N, Agrawal L, Kapley A, et al. (2010). Selection of indicator bacteria based on screening of 16S rDNA metagenomic library from a two-stage anoxic–oxic bioreactor system degrading azo dyes. Bioresour Technol, 101, 476–84.
Dawkar VV, Jadhav UU, Jadhav SU, Govindwar SP. (2008). Biodegradation of disperse textile dye Brown 3REL by newly isolated *Bacillus* sp. VUS. J Appl Microbiol, 105, 14–24.
Dawkar VV, Jadhav UU, Tamboli DP, Govindwar SP. (2010). Efficient industrial dye decolorization by *Bacillus* sp. VUS with its enzyme system. Ecotoxicol Environ Saf, 73, 1696–703.
Deb T, Choudhury D, Guin PS, et al. (2011). A complex of Co(II) with 2-hydroxyphenyl-azo-2-naphthol (HPAN) is far less cytotoxic than the parent compound on A549-lung carcinoma and peripheral blood mononuclear cells: reasons for reduction in cytotoxicity. Chem Biol Interact, 189, 206–14.
Dhanve RS, Kalyani DC, Phugare SS, Jadhav JP. (2009). Coordinate action of exiguobacterial oxidoreductive enzymes in biodegradation of reactive yellow 84A dye. Biodegradation, 20, 245–55.
Du L-N, Wang B, Li G, et al. (2012). Biosorption of the metal complex dye Acid Black 172 by live and heated biomass of *Pseudomonas* sp. strain DY1: kinetics and sorption mechanisms. J Hazard Mater, 29, 47–54.
Dube E, Shareck F, Hurtubise Y, et al. (2008). Homologous cloning, expression, and characterisation of a laccase from *Streptomyces coelicolor* and enzymatic decolourisation of an indigo dye. Appl Microbiol Biotechnol, 79, 597–603.
Elbanna K, Hassan G, Khider M, Mandour R. (2010). Safe biodegradation of textile azo dyes by newly isolated lactic acid bacteria and detection of plasmids associated with degradation. J Bioremed Biodegrad, 1, 112. doi: 10.4172/2155-6199.1000112.
Esancy JF, Freeman HS, Claxton LD. (1990). The effect of alkoxy substituents on the mutagenicity of some aminoazobenzene dyes and their reductive-cleavage products. Mutat Res, 238, 1–22.
Fabbri D, Calza P, Prevot AB. (2010). Photoinduced transformations of Acid Violet 7 and Acid Green 25 in the presence of TiO2 suspension. J Photochem Photobiol A Chem, 213, 14–22.
Farabegoli G, Chiavola A, Rolle E, Naso M. (2010). Decolorization of Reactive Red 195 by a mixed culture in an alternating anaerobic–aerobic Sequencing Batch Reactor. Biochem Eng J, 52, 220–6.
Ferraz ERA, Umbuzeiro GA, de-Almeida G, et al. (2010). Differential toxicity of disperse Red 1 and disperse Red 13 in the Ames test, HepG2 cytotoxicity assay, and Daphnia acute toxicity test. Environ Toxicol, 26, 489–97.
Fraga LE, Anderson MA, Beatriz MLPMA, et al. (2009). Evaluation of the photoelectrocatalytic method for oxidizing chloride and simultaneous removal of microcystin toxins in surface waters. Electrochim Acta, 54, 2069–76.
Galai S, Youssoufi HK, Marzouki MN. (2014). Characterization of yellow bacterial laccase SmLac/role of redox mediators in azo dye decolorization. J Chem Technol Biotechnol, 89, 1741–50.
Gan HM, Shahir S, Ibrahim Z, Yahya A. (2011). Biodegradation of 4-aminobenzenesulfonate by *Ralstonia sp*. PBA and *Hydrogenophaga sp*. PBC isolated from textile wastewater treatment plant. Chemosphere, 82, 507–13.
Garg A, Bhat KL, Bock CW. (2002). Mutagenicity of aminoazobenzene dyes and related structures: a QSAR/QPAR investigation. Dyes Pigments, 55, 35–52.
Gayathri R, Kavi A, Meena P, et al. (2014). Biodegradation of azo dyes by using soil bacteria. Int J Eng Res Technol, 3, 738–41.
Ghodake GS, Kalme SD, Jadhav JP, Govindwar SP. (2009). Purification and partial characterization of lignin peroxidase from *Acinetobacter calcoaceticus* NCIM 2890 and its application in decolorization of textile dyes. Appl Biochem Biotechnol, 152, 6–14.
Goncalves AMD, Mendes S, de Sanctis D, et al. (2013). The crystal structure of *Pseudomonas putida* azoreductase – the active site revisited. FEBS J, 280, 6643–57.
Gonzalez MD, Vidal T, Tzanova T. (2009). Electrochemical study of phenolic compounds as enhancers in laccase-catalyzed oxidative reactions. Electroanalysis, 21, 2249–57.
Gottlieb A, Shaw C, Smith A, et al. (2003). The toxicity of textile reactive azo dyes after hydrolysis and decolourisation. J Biotechnol, 101, 49–56.
Grundmann C. (1979). Ortho-Chinone. In C. Grundmann, ed., Methoden der organischen Chemie (Houben-Weyl), part II. Chinone. 4th ed., vol. 7/3b. Stuttgart, Germany: Georg Thieme Verlag, 144.
Handayani W, Meitiniarti VI, Timotius KH. (2007). Decolorization of Acid Red 27 and Reactive Red 2 by *Enterococcus faecalis* under a batch system. World J Microbiol Biotechnol, 23, 1239–44.
Hefti MH, Vervoort J, van Berkel WJ. (2003). Deflavination and reconstitution of flavoproteins. Eur J Biochem, 270, 4227–42.
Held C, Kandelbauer A, Schroeder M, et al. (2005). Biotransformation of phenolics with laccase containing bacterial spores. Environ Chem Lett, 3, 74–7.
Hilden K, Hakala TK, Lundell T. (2009). Thermotolerant and thermostable laccases. Biotechnol Lett, 31, 1117–28.
Hoff T, Liu SY, Bollag JM. (1985). Transformation of halogen-, alkyl, and alkoxy-substituted anilines by a laccase of *Trametes versicolor*. Appl Environ Microbiol, 49, 1040–5.
Hong Y, Chen X, Guo J, et al. (2007a). Effects of electron donors and acceptors on anaerobic azo dyes reduction by *Shewanella decolorationis* S12. Appl Microbiol Biotechnol, 74, 230–8.
Hong Y, Guo J, Sun G. (2008). Characteristic and phylogenetic analysis of facultative anaerobic dissimilatory azo-reducing bacteria from anaerobic active sludge. Int Biodeter Biodegrad, 61, 313–18.
Hong Y, Guo J, Sun GP. (2009). Energy generation coupled to the azoreduction by the membranous vesicles from Shewanella decolorationis S12. J Microbiol Biotechnol, 19, 37–41.
Hong Y, Xu M, Guo J, et al. (2007b). Respiration and growth of *Shewanella decolorationis* S12 with an azo compound as sole electron acceptor. Appl Environ Microbiol, 73, 64–72.

Hsueh C, Chen B. (2007). Comparative study on reaction selectivity of azo dye decolorization by *Pseudomonas luteola*. J Hazard Mater, 141, 842–9.

Hsueh CC, Chen BY, Yen CY. (2009). Understanding effects of chemical structure on azo dye decolorization characteristics by *Aeromonas hydrophila*. J Hazard Mater, 77, 2101–8.

Husain M, Husain Q. (2008). Applications of redox mediators in the treatment of organic pollutants by using oxidoreductive enzymes: a review. Crit Rev Environ Sci Technol, 38, 1–41.

Husain Q, Husain M, Kulshrestha Y. (2009). Remediation and treatment of organo-pollutants mediated by peroxidases: a review. Crit Rev Biotechnol, 29, 94–119.

Isik M, Sponza DT. (2003). Effect of oxygen on decolorization of azo dyes by *Escherichia coli* and *Pseudomonas sp*. and fate of aromatic amines. Process Biochem, 38, 1183–92.

Ito K, Nakanishi M, Lee WC, et al. (2008). Expansion of substrate specificity and catalytic mechanism of azoreductase by X-ray crystallography and site-directed mutagenesis. J Biol Chem, 283, 13889–96.

Jadhav SB, Phugare SS, Patil PS, Jadhav JP. (2011). Biochemical degradation pathway of textile dye Remazol red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies. Int Biodeter Biodegrad, 65, 733–43.

Jadhav UU, Dawkar VV, Tamboli DP, Govindwar SP. (2009). Purification and characterization of veratryl alcohol oxidase from *Comamonas* sp. UVS and its role in decolorization of textile dyes. Biotechnol Bioprod Eng, 14, 369–76.

Jin R, Yang H, Zhang A, et al. (2009). Bioaugmentation on decolorization of C.I. Direct Blue 71 by using genetically engineered strain *Escherichia coli* JM109 (pGEX-AZR). J Hazard Mater, 163, 1123–8.

Joe M, Lim SY, Kim DH, Lee IS. (2008). Decolorization of textile dyes by *Clostridium bifermentans* SL186 isolated from contaminated soil. World J Microbiol Biotechnol, 24, 2221–6.

Johansson HE, Johansson MK, Wong AC, et al. (2011). BTI1, an azoreductase with pH-dependent substrate specificity. Appl Environ Microbiol, 77, 4223–5.

Joshi SM, Inamdar SA, Telke AA, Tamboli DP. (2010). Exploring the potential of natural bacterial consortium to degrade mixture of dyes and textile effluent. Int Biodeter Biodegrad, 64, 622–8.

Joshi T, Iyengar L, Singh K, Garg S. (2008). Isolation, identification and application of novel bacterial consortiumTJ-1 for the decolourization of structurally different azo dyes. Bioresour Technol, 99, 7115–21.

Kalme S, Jadhav S, Jadhav M, Govindwar S. (2009). Textile dye degrading laccase from *Pseudomonas desmolyticum* NCIM 2112. Enzyme Microb Technol, 44, 65–71.

Kalyani DC, Patil PS, Jadhav JP, Govindwar SP. (2008). Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas sp*. SUK1. Bioresour Technol, 99, 4635–41.

Kandelbauer A, Erlacher A, Cavaco-Paulo A, Gübitz GM. (2004). Laccase-catalysed decolourisation of the synthetic azo-dye diamond black PV 200 and some structurally related derivatives. Biocatal Biotransform, 22, 331–9.

Keharia H, Madamwar D. (2003). Bioremediation concepts for treatment of dye containing wastewater: a review. Indian J Exp Biol, 41, 1068–75.

Khalid A, Arshad M, Crowley DE. (2008). Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. Appl Microbiol Biotechnol, 78, 361–9.

Khalid A, Arshad M, Crowley DE. (2009). Biodegradation potential of pure and mixed bacterial cultures for removal of 4-nitroaniline from textile dye wastewater. Water Res, 43, 1110–16.

Khalid A, Kausar F, Arshad M, et al. (2012). Accelerated decolorization of reactive dyes under saline conditions by bacteria isolated from Arabian seawater sediment. Appl Microbiol Biotechnol, 96, 1599–606.

Khaliq S, Khalid A, Saba B, et al. (2013). Effect of ACC deaminase bacteria on tomato plants containing azo dye wastewater. Pak J Bot, 45, 529–34.

Khehra MS, Saini HS, Sharma DK, et al. (2005). Decolorization of various azodyes by bacterial consortium. Dyes Pigments, 67, 55–61.

Khlifi R, Belbahri L, Woodward S, et al. (2010). Decolourization and detoxification of textile industry wastewater by the laccase-mediator system. J Hazard Mater, 175, 802–8.

Klibanov AM, Morris ED. (1981). Horsheradish peroxidase for the removal of carcinogenic aromatic amines from water. Enzyme Microb Technol, 3, 119–22.

Kudlich M, Hetheridge MJ, Knackmuss H, Stolz A. (1999). Autoxidation reactions of different aromatic *ortho*-aminohydroxynaphthalenes which are formed during the anaerobic reduction of sulfonated azo dyes. Environ Sci Technol, 33, 896–901.

Kudlich M, Keck A, Klein J, Stolz A. (1997). Localization of the enzyme system involved in the anaerobic degradation of azo dyes by *Sphingomonas* sp. BN6 and effect of artificial redox mediators on azo reduction. Appl Environ Microbiol, 63, 3691–4.

Kurniawati S, Nicell JA. (2009). A comprehensive kinetic model of laccase-catalyzed oxidation of aqueous phenol. Biotechnol Prog, 25, 763–73.

Kurniawati S, Nicell JA. (2007). Variable stoichiometry during the laccase-catalyzed oxidation of aqueous phenol. Biotechnol Prog, 23, 389–97.

Lee SY, Choi JH, Xu Z. (2003). Microbial cell-surface display. Trends Biotechnol, 21, 45–52.

Liger D, Graille M, Zhou CZ, et al. (2004). Crystal structure and functional characterization of yeast YLR011wp, an enzyme with NAD(P)H-FMN and ferric iron reductase activities. J Biol Chem, 279, 34890–7.

Lin J, Zhang X, Li Z, Lei L. (2010). Biodegradation of Reactive blue 13 in a two-stage anaerobic/aerobic fluidized beds system with a *Pseudomonas sp*. isolate. Bioresour Technol, 101, 34–40.

Lu L, Zenga G, Fana C, et al. (2013). Characterization of a laccase-like multicopper oxidase from newly isolated *Streptomyces* sp. C1 in agricultural waste compost and enzymatic decolorization of azo dyes. Biochem Eng J, 72, 70–6.

Mahmood S, Khalid A, Mahmood T, et al. (2013). Potential of newly isolated bacterial strains for simultaneous removal of hexavalent chromium and reactive black-5 azo dye from tannery effluent. J Chem Technol Biotechnol, 88, 1506–13.

Maier J, Kandelbauer A, Erlacher A, et al. (2004). A new alkali thermostable azo reductase from *Bacillus s*p. Strain SF. Appl Environ Microbiol, 70, 837–44.

Maran U, Sild S. (2003). QSAR modeling of genotoxicity on non-congeneric sets of organic compounds. Artif Intell Rev, 20, 13–38.

Marchis T, Avetta P, Bianco-Prevot A, et al. (2011). Oxidative degradation of Remazol Turquoise Blue G 133 by soybean peroxidase. J Inorg Biochem, 105, 321–7.

Matsumoto K, Mukai Y, Ogata D, et al. (2010). Characterization of thermostable FMN-dependent NADH azoreductase from the moderate thermophile *Geobacillus stearothermophilus*. Appl Microbiol Biotechnol, 86, 1431–8.

Mendes S, Pereira L, Batista C, Martins LO. (2011). Molecular determinants of azo reduction activity in the strain *Pseudomonas putida* MET94. Appl Microbiol Biotechnol, 92, 393–405.

Misal SA, Lingojwar DP, Shinde RM, Gawai KR. (2011). Purification and characterization of azoreductase from alkaliphilic strain *Bacillus badius*. Process Biochem, 46, 1264–9.

Modi HA, Rajput G, Ambasana C. (2010). Decolorization of water soluble azo dyes by bacterial cultures isolated from dye house effluent. Bioresour Technol, 101, 6580–3.

Mohanty S, Dafale N, Rao NN. (2006). Microbial decolorization of Reactive Black-5 in a two-stage anaerobic–aerobic reactor using acclimatized activated textile sludge. Biodegradation, 17, 403–13.

Moldes D, Lorenzo M, Sanromán MÁ. (2004). Degradation or polymerisation of phenol red dye depending to the catalyst used. Process Biochem, 39, 1811–15.

Molina-Guijarro JM, Perez J, Munoz-Dorado J, et al. (2009). Detoxification of azo dyes by a novel pH-versatile salt-resistant laccase from *Streptomyces ipomoea*. Int Microbiol, 12, 13–21.

Morozova OV, Shumakovich GP, Gorbacheva MA, et al. (2007). ''Blue'' laccases. Biochemistry (Moscow), 72, 1136–50.

Morrison JM, Wright CM, John GH. (2012). Identification, isolation and characterization of a novel azoreductase from *Clostridium perfringens*. Anaerobe, 18, 229–34.

Mugdha A, Usha M. (2012). Enzymatic treatment of wastewater containing dyestuffs using different delivery systems. Sci Rev Chem Commun, 2, 31–40.

Nakanishi M, Yatome C, Ishida N, Kitade Y. (2001). Putative ACP Phosphodiesterase gene (*acpD*) encodes an azoreductase. J Biol Chem, 276, 46394–9.

Natalello A, Doglia SM, Carey J, Grandori R. (2007). Role of flavin mononucleotide in the thermostability and oligomerization of *Escherichia coli* stress-defense protein WrbA. Biochemistry, 46, 543–53.

Nestmann ER, Kowbel DJ, Wheat JA. (1981). Mutagenicity in *Salmonella* of dyes used by defence personnel for detection of liquid chemical warfare agents. Carcinogenesis, 2, 879–83.

Ooi T, Ogata D, Matsumoto K, et al. (2012). Flavin-binding of azoreductase: direct evidences for dual-binding property of apo-azoreductase with FMN and FAD. J Mol Catal B Enzym, 74, 204–8.

Ooi T, Shibata T, Sato R, et al. (2007). An azoreductase, aerobic NADH-dependent flavoprotein discovered from *Bacillus* sp.: functional expression and enzymatic characterization. Appl Microbiol Biotechnol, 75, 377–86.

Osugi ME, Ferraz ERA, de Oliveira DP, et al. (2009). Comparison of oxidation efficiency of disperse dyes by chemical and photoelectrocatalytic chlorination and removal of mutagenic activity. Electrochim Acta, 54, 2086–93.

Oturkar CC, Nemade HN, Mulik PM, et al. (2011). Mechanistic investigation of decolorization and degradation of Reactive Red 120 by *Bacillus lentus* BI377. Bioresour Technol, 102, 758–64.

Pandey A, Singh P, Iyengar L. (2007). Bacterial decolorization and degradation of azo dyes. Int Biodeter Biodegrad, 59, 73–84.

Parales RE, Resnick SM. (2006). Aromatic ring hydroxylating dioxygenases. In: Ramos JL, Levesque RC (Eds). Pseudomonas. Netherlands: Springer, 287–340.

Parshetti GK, Telke AA, Kalyani DC, Govindwar SP. (2010). Decolorization and detoxification of sulfonated azo dyemethyl orange by *Kocuria rosea* MTCC 1532. J Hazard Mater, 176, 503–9.

Pasha FA, Muddassar M, Chung HW, et al. (2008). Hologram and 3D-quantitative structure toxicity relationship studies of azo dyes. J Mol Model, 14, 293–302.

Pasti-Grigsby MB, Paszczynski A, Goszczynski S, et al. (1992). Influence of aromatic substitution patterns on azo dye degradability by *Streptomyces* spp. and *Phanerochaete chrysosporium*. Appl Environ Microbiol, 58, 3605–13.

Pearce CI, Christie R, Boothman C, et al. (2006). Reactive azo dye reduction by *Shewnella strain* J18 143. Biotech Bioeng, 95, 693–703.

Pereira L, Coelho AV, Viegas CA, et al. (2009). Enzymatic biotransformation of the azo dye Sudan Orange G with bacterial CotA-laccase. J Biotechnol, 139, 68–77.

Phugare SS, Waghmare SR, Jadhav JP. (2011). Purification and characterization of dye degrading of veratryl alcohol oxidase from *Pseudomonas aeruginosa* strain BCH. World J Microbiol Biotechnol, 27, 2415–23.

Pielesz A, Baranowska I, Rybak A, Włochowicz A. (2002). Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes. Ecotoxicol Environ Saf, 53, 42–7.

Pielesz A. (1999). The process of the reduction of azo dyes used in dyeing textiles on the basis of infrared spectroscopy analysis. J Mol Struct, 511–512:337–44.

Pinheiro HM, Touraud E, Thomas O. (2004). Aromatic amines from azo dye reduction: status review with emphasis on direct UV spectrophotometric detection in textile industry wastewaters. Dyes Pigments, 61, 121–39.

Pissurlenkar RRS, Shaikh MS, Coutinho EC. (2007). 3D-QSAR studies of dipeptidyl peptidase IV inhibitors using a docking based alignment. J Mol Model, 13, 1047–71.

Prasad SS, Aikat K. (2014). Study of bio-degradation and bio-decolourization of azo dye by *Enterobacter* sp. SXCR. Environ Technol, 35, 956–65.

Pricelius S, Held C, Murkovic M, et al. (2007). Enzymatic reduction of azo and indigoid compounds. Appl Microbiol Biotechnol, 77, 321–7.

Punj S, John GH. (2008). Purification and Identification of an FMN-dependent NAD(P)H Azoreductase from *Enterococcus faecalis*. Curr Issues Mol Biol, 11, 59–66.

Puvaneswari N, Muthukrishnan J, Gunasekaran P. (2006). Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol, 44, 618–26.

Rafii F, Hall JD, Cerniglia CE. (1997). Mutagenicity of azo dyes used in foods, drugs and cosmetics before and after reduction by *Clostridium* species from the human intestinal tract. Food Chem Toxicol, 35, 897–901.

Rajaguru P, Fairbairn LJ, Ashby J, et al. (1999). Genotoxicity studies on the azo dye Direct Red 2 using the in vivo mouse bone marrow micronucleus test. Mutat Res, 444, 175–80.

Reiss R, Ihssen J, Thony-Meyer L. (2011). *Bacillus pumilus* laccase: a heat stable enzyme with a wide substrate spectrum. BMC Biotechnol, 11, 9.

Ren S, Guo J, Zeng G, Sun G. (2006). Decolorization of triphenylmethane, azo and anthroquinone dyes by a newly isolated *Aeromonas hydrophila* strain. Appl Microbiol Technol, 72, 1316–21.

Rojas-Melgarejo F, Marin-Iniesta F, Rodriguez-Lopez JN, et al. (2006). Cinnamic carbohydrate esters show great versatility as supports for the immobilization of different enzymes. Enzyme Microb Technol, 38, 748–55.

Roy DR, Sarkar U, Chattaraj PK, et al. (2006). Analyzing toxicity through electrophilicity. Mol Divers, 10, 119–31.

Russ R, Rau J, Stolz A. (2000). The function of cytoplasmic flavin reductases in the reduction of azo dyes by bacteria. Appl Environ Microbiol, 66, 1429–34.

Ryan A, Laurieri N, Westwood I, et al. (2010). A novel mechanism for azo reduction. J Mol Biol, 400, 24–37.

Sadettin S, Donmez G. (2007). Simultaneous bioaccumulation of reactive dye and chromium (VI) by using thermophil *Phormidium sp*. Enzyme Microb Technol, 41, 175–80.

Sandhya S, Padmavathy S, Swaminathan K, et al. (2005). Microaerophilic–aerobic sequential batch reactor for treatment of azo dyes containing simulated wastewater. Process Biochem, 40, 885–90.

Saratale RG, Saratale GD, Chang JS, Govindwar SP. (2011). Bacterial decolorization and degradation of azo dyes: a review. J Taiwan Inst Chem Eng, 42, 138–57.

Saratale RG, Saratale GD, Kalyani DC, et al. (2009). Enhanced decolourization and biodegradation of textile azo dye Scarlet R by using developed microbial; consortium-GR. Bioresour Technol, 100, 2493–500.

Sarayu K, Sandhya S. (2010). Aerobic biodegradation pathway for Remazol Orange by *Pseudomonas aeruginosa*. Appl Biochem Biotechnol, 160, 1241–53.

Sha MP, Patel KA, Nair SS, Darji AM. (2014). An application of response surface methodology in microbial degradation of azo dye by *Bacillus subtillis* ETL-1979. Am J Microbiol Res, 2, 24–34.

Shah MP, Patel KA, Nair SS, Darji AM. (2013). Microbial degradation of textile dye (Remazol Black B) by *Bacillus* spp. ETL-2012. J Bioremed Biodegrad, 4, 180.

Shah MP. (2014). Bioremedial application of *Bacillus megaterium* PMS82 in microbial degradation of acid orange dye. Int J Environ Bioremed Biodegrd, 2, 93–9.

Sharma HK, Bajpai K, Shukla S, et al. (2013). Biodegradation of Reactive Red 11 azo dye using *Enterobacter* sp. and gamma proteobacterium collected from dye industry. Int J Innov Res Sci Eng Technol, 2, 1670–6.

Silveira E, Marques PP, Macedo AC, et al. (2011). Decolorization of industrial azo dye in an anoxic reactor by PUF immobilized *Pseudomonas oleovorans*. J Water Reuse Desalin, 1, 18–26.

Singh G, Capalash N, Goela R, Sharma P. (2007). A pH-stable laccase from alkali tolerant g-proteobacterium JB: purification, characterization and indigo carmine degradation. Enzyme Microb Technol, 41, 794–9.

Singh H. (2006). Fungal decolorization and degradation of dyes. In: Singh H, ed. Mycoremediation: fungal bioremediation. Hoboken, NJ: John Wiley and Sons, Inc., 420–83.

Srinivasan R, Kathiravan MN, Gopinath KP. (2011). Degradation of Tectilon Yellow 2G by hybrid technique: combination of sonolysis and biodegradation using mutant *Pseudomonas putida*. Bioresour Technol, 102, 2242–7.

Sriram MI, Kalishwaralal K, Deepak V, Gracerosepat R, Srisakthi K, Sangiliyandi G. 2011. Biofilm inhibition and antimicrobial action of lipopeptide biosurfactant produced by heavy metal tolerant strain Bacillus cereus NK1. Colloids and Surfaces B: Biointerfaces, 85, 174–181.

Steffan S, Bardi L, Marzon M. (2005). Azo dye biodegradation by microbial cultures immobilized in alginate beads. Environ Int, 31, 201–5.

Sugano Y, Matsushima Y, Shoda M. (2006). Complete decolorization of the anthraquinone dye Reactive Blue 5 by the concerted action of two peroxidases from *Thanatephorus cucumeris*. Appl Microbiol Biotechnol, 73, 862–71.

Supaka N, Juntongjin K, Damronglerd S, et al. (2004). Microbial decolorization of reactive azo dyes in a sequential anaerobic–aerobic system. Chem Eng J, 99, 169–76.

Suzuki Y, Yoda T, Ruhul A, Sugiura W. (2001). Molecular cloning and characterization of the gene coding for azoreductase from *Bacillus* sp. OY1–2 isolated from soil. J Biol Chem, 276, 9059–65.

Tamboli DP, Telke AA, Dawkar VV, et al. (2011). Purification and characterization of bacterial aryl alcohol oxidase from *Sphingobacterium* sp. ATM and its uses in textile dye decolorization. Biotechnol Bioprocess Eng, 16, 661–8.

Tan NCG, Borger A, Slenders P, et al. (2000). Degradation of azo dye Mordant Yellow 10 in a sequential anaerobic and bioaugmented aerobic bioreactor. Water Sci Technol, 42, 337–44.

Tauber MM, Guebitz GM, Rehorek A. (2005). Degradation of azo dyes by laccase and ultrasound treatment. Appl Environ Microbiol, 71, 2600–7.

Telke AA, Ghodake GS, Kalyani DC, et al. (2011). Biochemical characteristics of a textile dye degrading extracellular laccase from a *Bacillus* sp. ADR. Bioresour Technol, 102, 1752–6.

Telke AA, Kalyani DC, Dawkar VV, Govindwar SP. (2009). Influence of organic and inorganic compounds on oxidoreductive decolorization of sulfonated azo dye C.I. Reactive Orange 16. J Hazard Mater, 172, 298–309.

Tsuboy MS, Angeli JPF, Mantovani MS, et al. (2007). Genotoxic, mutagenic and cytotoxic effects of the commercial dye CI disperse blue 291 in the human hepatic cell line HepG2. Toxicol In Vitro, 21, 1650–5.

Umbuzeiro GA, Freeman HS, Warren SH, et al. (2005). The contribution of azo dyes to the mutagenic activity of the Cristais River. Chemosphere, 60, 55–64.

van Bloois E, Torres Pazmino DE, Winter RT, Fraaije MW. (2010). A robust and extracellular heme-containing peroxidase from *Thermobifida fusca* as prototype of a bacterial peroxidase superfamily. Appl Microbiol Biotechnol, 86, 1419–30.

van der Zee FP, Villaverde S. (2005). Combined anaerobic-aerobic treatment of azo dyes – a short review of bioreactor studies. Water Res, 39, 1425–40.

Wang C, Zhao M, Lu L, et al. (2011). Characterization of spore laccase from *Bacillus subtilis* WD23 and its use in dye decolorization. Afr J Biotechnol, 10, 2186–92.

Wang CJ, Laurieri N, Abuhammad A, et al. (2010). Role of tyrosine 131 in the active site of paAzoR1, an azoreductase with specificity for the inflammatory bowel disease pro-drug balsalazide. Acta Crystallogr Sect F Struct Biol Cryst Commun, 66, 2–7.

Wells A, Teria M, Eve T. (2006). Green oxidations with laccase-mediator systems. Biochem Soc Trans, 34, 304–308.

Wolff AW, Oehme FW. (1974). Carcinogenic chemicals in food as an environmental issue. J Am Vet Med Assoc, 164, 623–9.

Xin B, Chen G, Zheng W. (2010). Bioaccumulation of Cu-complex reactive dye by growing pellets of *Penicillium oxalicum* and its mechanism. Water Res, 44, 3565–72.

Xu M, Guo J, Sun G. (2007). Biodegradation of textile azo dye by *Shewanella decolorationis* S12 under microaerophilic conditions. Appl Microbiol Biotechnol, 76, 719–26.

Yang Y, Lu L, Gao F, Zhao Y. (2013). Characterization of an efficient catalytic and organic solvent-tolerant azoreductase toward methyl red from *Shewanella oneidensis* MR-1. Environ Sci Pollut Res Int, 20, 3232–9.

Yang YY, Du LN, Wang G, et al. (2011). The decolorisation capacity and mechanism of *Shewanella oneidensis* MR-1 for Methyl orange and Acid yellow 199 under microaerophilic conditions. Water Sci Technol, 63, 956–63.

Young L, Yu J. (1997). Ligninase-catalysed decolorization of synthetic dyes. Water Res, 31, 1187–93.

Zimmermann T, Kulla HG, Leisinger T. (1982). Properties of purified orange II azoreductase, the enzyme initiating azo dye degradation by *Pseudomonas* KF46. Eur J Biochem, 129, 197–203.