



Bio-removal of Azo Dyes: A Review

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Abstract

Synthetic dyes are widely used in textile, paper, food, cosmetics and pharmaceutical industries with the textile industry as the largest consumer. Among all the available synthetic dyes, azo dyes are the largest group of dyes used in textile industry. Textile dyeing and finishing processes generate a large amount of dye containing wastewater which is one of the main sources of water pollution problems worldwide. Several physico-chemical methods have been applied to the treatment of textile wastewater but these methods have many limitations due to high cost, low efficiency and secondary pollution problems. As an alternative to physico-chemical methods, biological methods comprise bacteria, fungi, yeast, algae and plants and their enzymes which received increasing interest due to their cost effectiveness and eco-friendly nature. Decolorization of azo dyes by biological processes may take place either by biosorption or biodegradation. A variety of reductive and oxidative enzymes may also be involved in the degradation of dyes. This review provides an overview of decolorization and degradation of azo dyes by biological processes and establishes the fact that these microbial and plant cells are significantly effective biological weapon against the toxic azo dyes.

Keywords: azo dye; microorganism; decolorization; biosorption; enzyme; nanoparticle

Introduction

Dyes are the important industrial coloring chemical compounds. Organic chemicals that own color are known as dyes. Dyes are classified by (i) chromophore groups in their chemical structures as azo dyes, anthraquinone dyes and phthalocyanine dyes etc. and (ii) their usage or application method as disperse dyes for polyester and reactive dyes for cotton (Singh *et al.*, 2012). Azo (monoazo, diazo, triazo and polyazo), anthraquinone, triarylmethane and phthalocyanine dyes are main groups of dyes. Azo dyes absorb light in the visible region because of their chemical structure, which is characterized by one or more azo bonds (-N=N-) (Chang *et al.*, 2001). Globally, 2.8×10^5 tons of textile dyes are poured into water ecosystem every year (Jin *et al.*, 2007). Azo dyes are the most common (more than 3000 different varieties) of all textile dyes produced because of their easier biosynthesis, chemical stability and the diversity of colors available as compared to natural dyes (Chang *et al.*, 2004). About 80% of azo dyes are used in the dyeing process of textile industries. They are widely used in the textile, leather, food, paper, cosmetics and pharmaceutical industries. It has been estimated that about 10-15% of the dyes used in

dyeing process goes unbound with the textile fibers and are discharged into the environment (Asad *et al.*, 2007). Release of dye containing effluent derived from various industrial practices into water bodies and surrounding industrial areas is of major concern (Mugdha and Usha, 2012) which have several adverse effects on life including decreased aquatic photosynthesis, ability to exhaust dissolved oxygen and toxic effect on flora, fauna and humans. Presence of dyes in the textile effluent causes an unpleasant appearance by imparting the color and also their breakdown products (colorless amines) are toxic, carcinogenic and mutagenic (Xu *et al.*, 2005).

Harmful Effect of Azo Dyes

Azo dyes are present in textile effluent are a major problem due to their toxic nature. Azo dyes having a nitro group are proved to be mutagenic in nature (Chung and Cerniglia, 1992) and after breakdown they generate toxic products such as 1, 4-phenylenediamine, o-tolidine etc. (Rosenkranz and Kolpman, 1990). Also it is reported that, sulfonated azo dyes posed less or no mutagenic effect as compared to unsulfonated azo dyes (Jung *et al.*, 1992). A unique aromatic amine, 3-methoxy-4-aminoazobenzene, has been found to be potent hepatocarcinogen in rats and a

potent mutagen in bacteria (Ferraz *et al.*, 2011). A commercial textile azo dye Acid Violet 7 has ability to induce lipid peroxidation, chromosomal aberrations and inhibition of enzyme acetyl cholinesterase. The toxicity of Acid Violet 7 dye increases during the biodegradation process by *Pseudomonas putida* due to its corresponding metabolites 4-aminoacetanilide and 5-acetamido-2-amino-1-hydroxy-3,6-naphthalene disulfonic acid (Mansour *et al.*, 2010). Similarly, Methyl Red is also a mutagenic dye and its microbial degradation product N, N-dimethyl-phenylenediamine (DMPD) is a toxic and mutagenic aromatic amine (Wong and Yu, 1999) that remains unchanged in the culture (Ayed *et al.*, 2011). Tsuboy *et al.* (2007) tested azo dye Disperse Blue 291 and found that it has genotoxic, mutagenic, cytotoxic effect and also leads to the formation of micronuclei and DNA fragmentation in human hepatoma cells. Sunset Yellow, Carmoisine, Quinoline Yellow, Allura Red, Tartrazine and Ponceau 4R are azo compounds which are proved to be harmful to children when used as additives in food and drinks (Parliament, Council, The, & Union, 2008). Some dyes are also mentioned which induces bladder cancer in humans and splenic sarcomas, hepatocarcinomas and nuclear abnormality in experimental animals (Rafii *et al.*, 1997; Puvaneswari *et al.*, 2006). 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; Singh *et al.*, 1991a,b).

Due to these harmful effects of azo dyes on human health and on the environment, there is a crying need to restrict their entry in the environment. But, practically it is not feasible. So a solution to this problem is adopting such treatments methods that reduce or remove the dyes from the wastewaters.

This review compiles all the dimensions of biological methods used for biodecolorization and biodegradation of azo dyes from different sources.

Biological Methods of Decolorization and Degradation of Azo Dyes

Decolorization and degradation of azo dyes may take place by two methods either adsorption on the microbial biomass (biosorption) or biodegradation of the dyes by the living cells. Biological treatment of textile azo dyes has been proved to be the best method due to its ability to degrade almost all dye stuff and also overcome many disadvantages posed by the physico-chemical processes. Numerous studies, available with emphasis on the use of microorganisms to degrade dyes, suggest that biodegradation is an eco-friendly and cost effective method for dye containing wastewater treatment (Vitor and Corso, 2008; Pajot *et al.*, 2011).

Microorganisms (bacteria, fungi, algae and yeast), plants and their enzymes can be successfully utilized in removal of color of a wide range of azo dyes through anaerobic, aerobic and sequential anaerobic-aerobic treatment processes.

Biosorption

The uptake or accumulation of chemicals by microbial mass has been termed as biosorption (Kumar *et al.*, 1998). Biomass of bacteria, yeast, filamentous fungi and algae has been used successfully to remove toxic dyes by biosorption (Bhatnagar and Sillanpaa, 2010). This property of microorganism is due to the cell wall components such as heteropolysaccharides and lipids, which consists of different functional groups including, amino, hydroxyl, carboxyl, phosphate and other charged groups, creating strong attractive forces between the azo dye and cell wall (Srinivasan and Viraraghavan, 2010; Das and Charumathi, 2012). Won *et al.* (2005) found bacterium *Corynebacterium glutamicum* as a latent biosorbent of azo dye Reactive Red 4. Mnif *et al.* (2015) isolated bacterial strain *Bacillus weihenstephanensis* RI12 which removes Congo Red by biosorption with the use of SPB1 biosurfactant. Similar to bacteria mediated biosorption; fungi have also been used as latent sorbent for decolorization of azo dyes from industrial effluents and have achieved considerable attention. Effective biosorption depends on several conditions such as pH, temperature, ionic strength, time of contact, adsorbent, dye concentration, dye structure and type of used microorganism (Renganathan *et al.*, 2006; Vijayaraghavan and Yun, 2007; Erden *et al.*, 2011; Ambrosio *et al.*, 2012).

In addition to application of bacterial and fungal biomass in biosorption of azo dyes, algae are also considered as potent biosorbents due to their availability in both fresh and salt water (Wu and Jean, 2012). Donmez and Aksu (2002) revealed that potential of algae for biosorption is attributed to their relative high surface area and high binding affinity. Electrostatic attraction and complexation in the cell wall of algae are the physical processes that mediate algal biosorption (Satiroglu *et al.*, 2002).

Dead algal cells proved to be advantageous as biosorbents over living cells as they do not have nutrients demand, can be stored and used for long periods and can be regenerated using organic solvents or surfactants (Fu and Viraraghavan, 2001). The key advantages of biosorption process is its high selectivity, efficiency, economic, works at low concentration (Hammamni *et al.*, 2002) and have good removal performance as it is proved to be an efficient method over presently available physico-chemical technique such as ion exchange (Aksu and Donmez, 2003). An important disadvantage associated with biosorption is early saturation problem and almost no biological control over characteristic biosorbent.

Enzymatic Decolorization and Degradation

There are many reports on the use of physico-chemical methods for color removal from dye containing effluents (Golab *et al.*, 2005; Lopez-Grimau and Gutierrez, 2006). These methods include adsorption, chemical treatment, coagulation and ion pair extractions etc. but they are linked to problems such as high cost and produce large amounts of sludge after treatment which requires safe disposal.

The enzymatic approach offers alternative strategy for decolorization/degradation of azo dyes from wastewater over conventional physico-chemical treatments as it ends with less sludge production and is also cost effective. There are several enzymes involved in removal of azo dyes which proved an effective molecular weapon for decolorization of azo dyes (Singh *et al.*, 2015). Enzymes that mediate azo dye decolorization are grouped into two broad classes; Reductive and Oxidative.

Reductive enzymes

Azoreductases are the catalytic proteins, encoded by microorganisms such as bacteria (Misal *et al.*, 2011), algae (El-Sheekh *et al.*, 2009) and yeast (Vitor and Corso, 2008). Several bacterial species have been identified that have the potential of degrading azo dyes under reduced (anaerobic) conditions (Oturkar *et al.*, 2011). During the process of dye decolorization azo bond is initially cleaved by bacterial azoreductase enzyme which leads to the formation of toxic colorless aromatic amines under reduced conditions (Pandey *et al.*, 2007). Azoreductases completely rely upon reducing equivalents (e.g., NADPH, NADH and FADH) for decolorization of azo dyes (Van der Zee and Cervantes, 2009). Most of the azo dyes are substituted with sulfonate groups and have high molecular weight due to which dyes can't pass through bacterial membranes. This fact reveals that dye reducing activity is not dependent on the intracellular uptake of the dye (Pearce *et al.*, 2003). On the basis of reducing equivalent used to reduce azo linkages, enzymes have been identified as FMN-dependent reductases (Burger and Stolz, 2010), FMN-independent reductases (Burger and Stolz, 2010), NADH-dependent reductases (Misal *et al.*, 2011), NADPH-dependent reductases (Mendes *et al.*, 2011) and NADH-DCIP reductases (Phugare *et al.*, 2010). NADH-DCIP reductases are believed to be marker enzymes of bacterial and fungal mixed function oxidase systems, and mediate the detoxification of xenobiotic compounds (Bhosale *et al.*, 2006).

Oxidative enzymes

Microorganisms also encode number of oxidative enzymes for degradation of azo dyes such as polyphenol oxidases (PPO), manganese peroxidase (MnP), lignin peroxidase (LiP), laccase (Lac), tyrosinase (Tyr), N-demethylase (Oturkar *et al.*, 2011; Martorell *et al.*, 2012), dye

decolorizing peroxidases (Liers *et al.*, 2010) and cellobiose dehydrogenase (Tilli *et al.*, 2011). These oxidases have been reported in bacteria, filamentous fungi, yeast and plants. These enzymes catalyze conversion of broad range of substrates into less toxic insoluble compounds. The removal of toxic compound from waste takes place by a mechanism involving the formation of free radical followed by insoluble product (Torres *et al.*, 2003). Peroxidase is a heme containing enzyme and widely distributed in plants, microorganisms and animals (Duarte-Vazquez *et al.*, 2003). In the last few years massive research, to develop methods, relied on peroxidases from plants and fungi for the treatment of wastewater containing colored pollutants. Ferreira-Leitao *et al.* (2007) reported the role of plant Horseradish peroxidase and LiP from *Penicillium chrysosporium* in the oxidation of Methylene Blue (Basic Blue 9) and Azure B dyes. Recently Versatile Peroxidase (VP) has been purified and described as a new family of ligninolytic peroxidases, along with LiP and MnP obtained from *P. chrysosporium* (Martinez, 2002). Interestingly, these enzymes have shown the activity of both LiP and MnP and they have potential to oxidize Mn^{2+} to Mn^{3+} at around pH 5.0 while aromatic compounds at around pH 3.0, despite the presence of Mn^{2+} (Heinfling *et al.*, 1998; Ruiz-Duenas *et al.*, 2001).

Laccases are multi-copper oxidases and most studied enzyme which facilitates removal of dyes 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). Laccases catalyze decolorization of textile dyes either by direct oxidation or via indirect oxidation by using redox mediators (e.g., ABTS) to accelerate the reaction (Khlifi *et al.*, 2010).

The catalytic cycle of MnP involves the oxidation of manganous ions (Mn^{2+}) to Mn^{3+} (Hofrichter, 2002) which is further chelated with organic acids (e.g., oxalic acid). The chelated Mn^{3+} readily diffuses from the active site of the enzyme which in turn oxidizes the secondary substrates (Mester and Field, 1998).

Polyphenol oxidase is a tetramer enzyme that contains four atoms of copper per molecule, and binding sites for two aromatic compounds and oxygen involves in the removal of aromatic pollutants from various contaminated sites. PPO catalyzes o-hydroxylation of monophenols to o-diphenol. They can further catalyze the oxidation of o-diphenols to o-quinones. Waghmode *et al.* (2011a) proposed degradation pathway by *G. geotrichum* (Fig. 1a), *B. laterosporus* (Fig. 1b) and consortium GG-BL (Fig. 1c) which utilizes different microbial enzymes for the breakdown of azo dye Golden Yellow HER (GYHER).

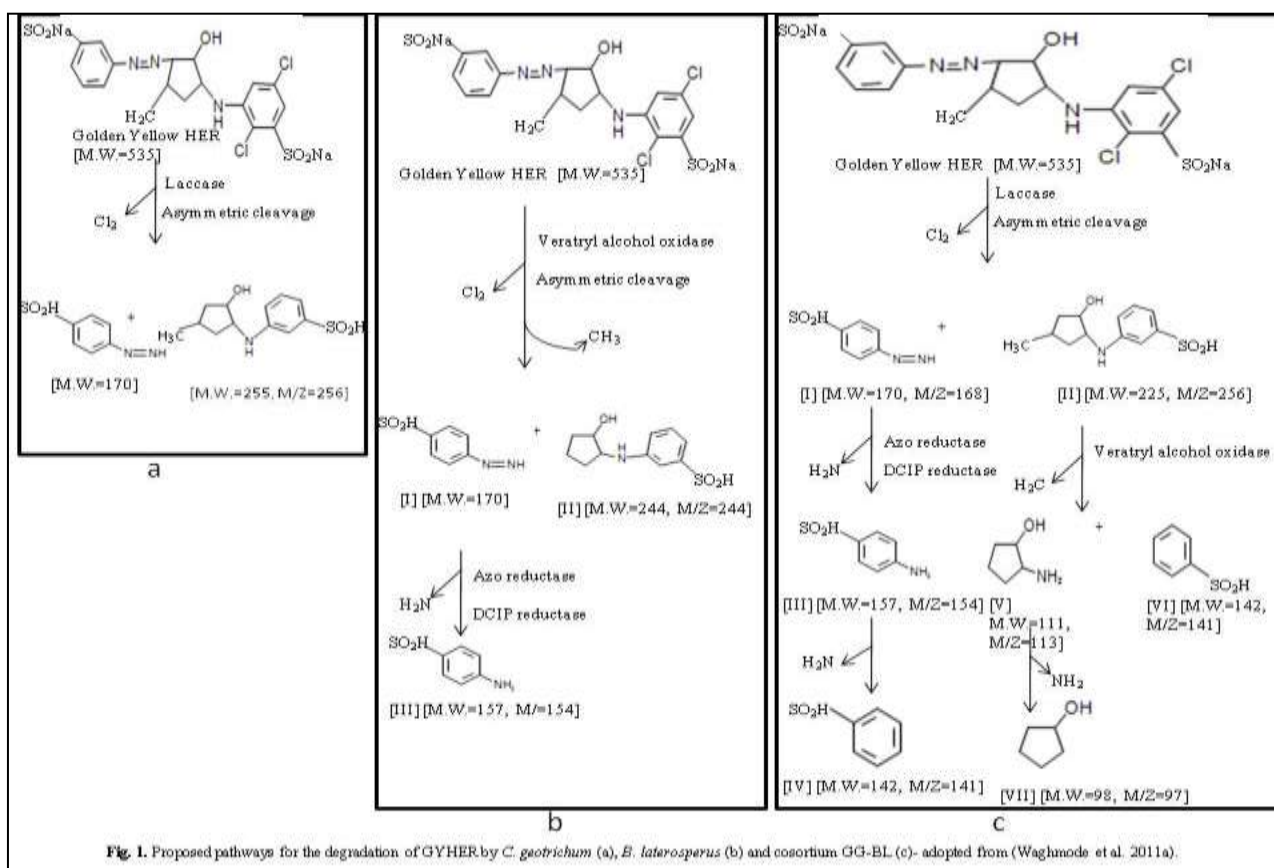


Fig. 1. Proposed pathways for the degradation of GYHER by *C. geotrichum* (a), *B. laterosporus* (b) and consortium GG-BL (c)- adopted from (Waghunode et al. 2011a).

Use of Microorganisms in Decolorization and Degradation of Azo Dye

Bacteria

Extensive work has been done to screen out the role of diverse groups of bacteria in the decolorization of azo dyes. These bacteria can be isolated from several ecological niches such as soil, water, human and animal excreta and even from contaminated food materials. Bacteria are suited fine for decolorization and complete mineralization of azo dyes as they are easy to cultivate and grow rapidly. The process of decolorization by a bacterial system may be anaerobic or aerobic or involve a combination of both (Fig. 2).

The bacterial decolorization and degradation of these dyes has been taken into account as it can ensure a higher degree of biodegradation and mineralization, is applicable to a wide range of azo dyes, low cost, eco-friendly and produce less sludge (Verma and Madamwar, 2003; Khehra et al., 2006).

Pure bacterial culture

Bacteria mediated decolorization and degradation of azo dyes involve azoreductase assisted breakdown of azo bond (-N=N-) under anaerobic condition which results in the formation of colorless hazardous aromatic amines (Van der Zee and Villaverde, 2005) which are further removed aerobically or anaerobically (Joshi et al., 2008).

Horitsu et al. (1977) first reported *Bacillus subtilis* culture capable of degrading azo dyes and after that *Aeromonas*

hydrophila (Idaka et al., 1978) and *Bacillus cereus* (Wuhrmann et al., 1980) were reported to degrade azo dye.

It is reported that azo dyes are generally resistant to bacteria mediated degradation under aerobic conditions (Ola et al., 2010) because oxygen rich environment usually hampers breakdown of the azo bond. However, some aerobic bacterial strains are reported to have ability to reduce the azo bond by oxygen insensitive or aerobic azoreductases (Lin and Leu, 2008) proving that decolorization and degradation demands oxygen rich environments (Fig. 3).

Extremophiles, an interesting bacterial community that are used in bioremediation of dye containing effluents, where normal bacterial strains could not sustain due to the high temperature and high NaCl concentration (Amoozegar et al., 2011). *Staphylococcus*, *Exiguobacterium* (Chen et al., 2011) and *Aeromonas hydrophila* (Ogugbue et al., 2011) are the halotolerant *Bacillus* strains reported for decolorization of azo dyes in the presence of high concentration of NaCl. Similarly, *Geobacillus stearothermophilus* UCP 986 is a thermophilic bacteria decolorizes Orange II at 50 °C (Evangelista-Barreto et al., 2009). Misal et al. (2011) isolated thermostable azoreductase from alkaliphilic strain *Bacillus badius* responsible for decolorization of several azo dyes, including Amaranth up to extreme temperature 85 °C. Several pure bacterial cultures are reported for removal of azo dyes (Table 1).

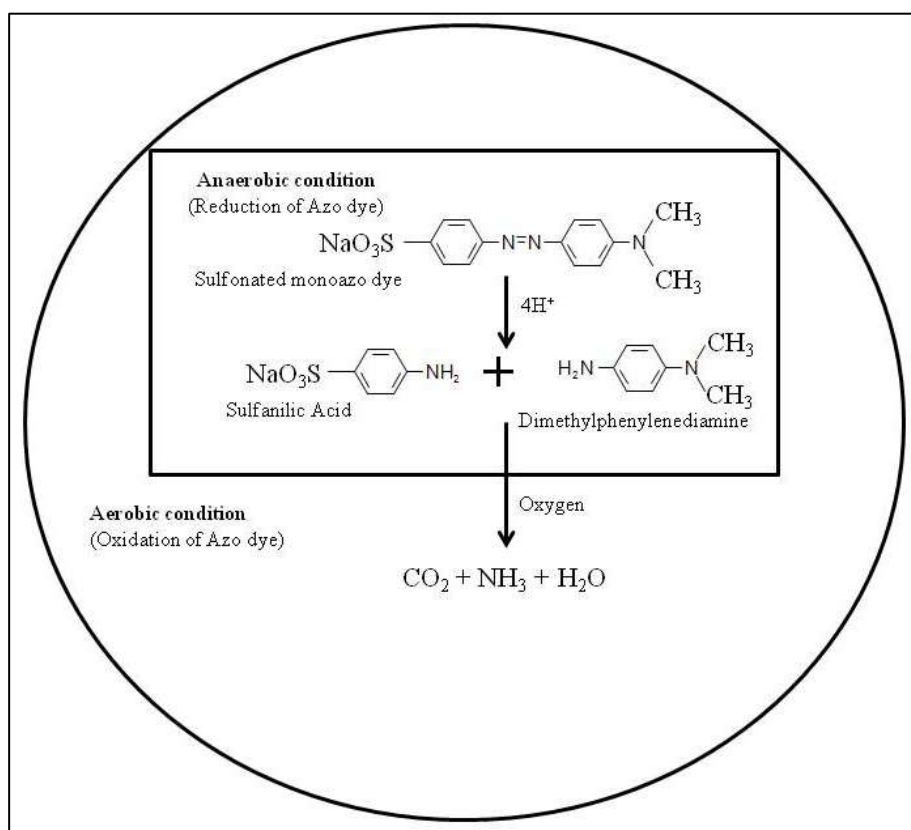


Fig. 2: Proposed mechanism for degradation of Azo dye under aerobic and anaerobic condition of bacteria

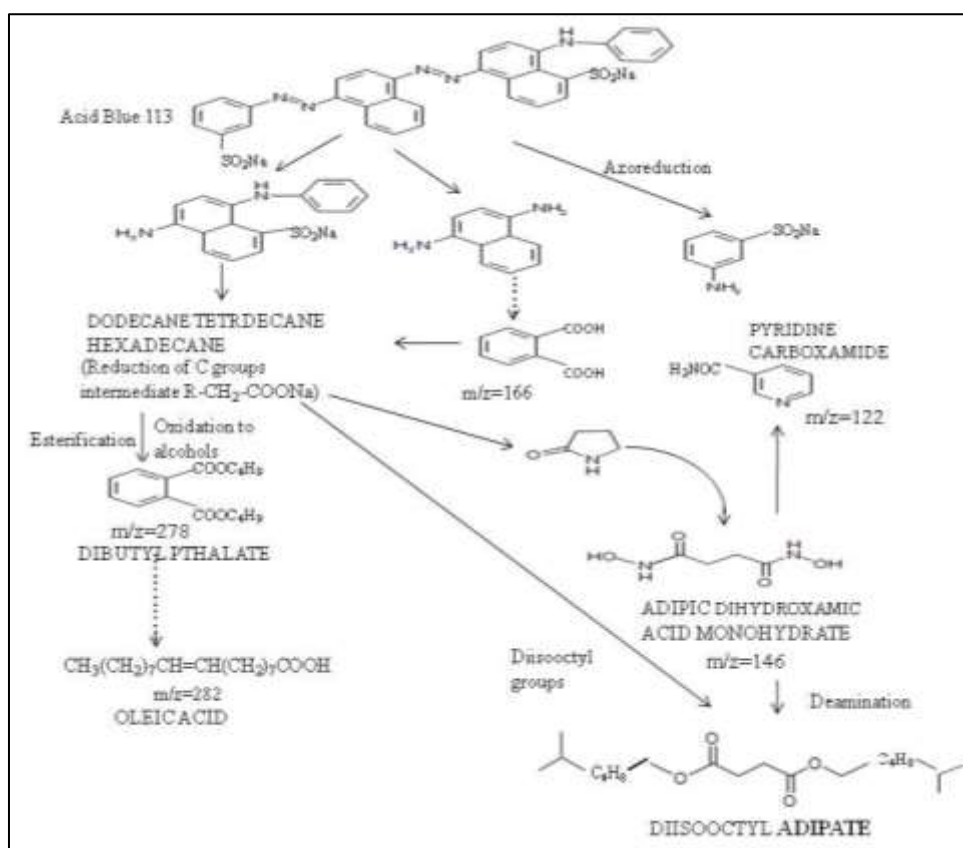


Fig. 3: Degradation pathway of Acid Blue 113 by *S. lentus* in shade flask (adapted from Sekar *et al.*, 2012)

Table 1: Decolorization of various azo dyes by pure bacterial culture.

S. N.	Bacterial culture	Name of Dye	Conditions pH, Temp(°C), Agitation (rpm), Time(h)	Decolorization (%) and Reaction mechanism	References
1.	<i>Acinetobacter baumannii</i> YNWH 226	Congo Red	7.0, 37, 180, -	99.10, Reductive and Oxidative	Ning <i>et al.</i> , 2014
2.	<i>Acinetobacter</i> sp. SRL8	Disperse Orange S-RL	7.0, 30, -, -	90.20, Microaerophilic	Zhiqiang <i>et al.</i> , 2015
3.	<i>Alcaligenes</i> sp. AA04	Reactive Red 198	7.0, 25, -, 24	90, Azoreductase	Pandey <i>et al.</i> , 2015
4.	<i>Alishewanella</i> sp. KMK6	Reactive Blue 59	7.0, 37, Static, 6	95, Azoreductase, NADH-DCIP reductase	Kolekar and Kodam, 2012
5.	<i>Bacillus cereus</i>	Cibacron Black PSG, Cibacron Red P4B	7.0, 35, -, 5 days	67, 81, Aerobic	Ola <i>et al.</i> , 2010
6.	<i>Bacillus halodurans</i> MTCC 865	Acid Black 24	9.0, 37, static, 6	90, Aerobic biodegradation	Prasad and Rao, 2014
7.	<i>Bacillus pumilus</i> HKG212	Remazol Navy Blue	8.0, 30, -, 30	>95, Anaerobic degradation	Das <i>et al.</i> , 2015
8.	<i>Bacillus</i> sp.	Congo Red	7.0, 40, -, 24	85, Aerobic	Sawhney and Kumar, 2011
9.	<i>Bacillus subtilis</i>	Reactive Red M8B	8-9, 35, -, 5 days	97, -	Arulazhagan, 2016
10.	<i>Brevibacillus laterosporus</i> MTCC 2298	Golden Yellow HER	7.0, 30, static, 48	87, Oxidative and Reductive	Gomare <i>et al.</i> , 2009
11.	<i>Comamonas</i> sp. UVS	Direct Red 5B	6.5, 40, static, 13	100, Oxidative	Jadhav <i>et al.</i> , 2008
12.	<i>Enterococcus faecalis</i> YZ66	Direct Red 81	7.0, 40, static, 1.5	100, Oxidative and Reductive	Sahasrabudhe <i>et al.</i> , 2014
13.	<i>Geobacillus stearothermophilus</i> (UCP 986)	Orange II	5.0-6.0, 50, -, 24	96-98, Aeration	Evangelista-Barreto <i>et al.</i> , 2009
14.	<i>Micrococcus glutamicus</i> NCIM- 2168	Reactive Green 19 A	6.8, 37, static, 42	100, Oxidative and Reductive	Saratale <i>et al.</i> , 2009a
15.	<i>Micrococcus luteus</i> strain SSN2	Direct Orange 16	8.0, 37, static, 6	96, -	Singh <i>et al.</i> , 2015
16.	Mutant <i>Bacillus</i> sp. ACT2	Congo Red	7.0, 37, static, 37-48	12-30, Reductive	Gopinath <i>et al.</i> , 2009
17.	<i>Pseudomonas aeruginosa</i>	Remazol Red	7.0, 40, static, 20 min	97, Veratryl alcohol oxidase, NADH-DCIP reductase	Jadhav <i>et al.</i> , 2011
18.	<i>Pseudomonas entomophila</i> BS1	Reactive Black 5	5-9, 37, static, 120	93, Azoreductase	Khan and Malik, 2016
19.	<i>Pseudomonas</i> sp.	Reactive Black 5	7.0, 35, -, 24	83, -	Mohamed, 2016
20.	<i>Rhodopseudomonas palustris</i> 51ATA	Reactive Red 195	-, 25-30, -, -	100, Anaerobic	Celik <i>et al.</i> , 2012
21.	<i>Shewanella</i> sp.	Acid Orange 7	-, -, -, 6	98, Aerobic	Wang <i>et al.</i> , 2012
22.	<i>Sphingomonas paucimobilis</i>	Methyl Red	9.0, 30, -, 10	98, Aerobic	Ayed <i>et al.</i> , 2011
23.	<i>Staphylococcus aureus</i>	Orange II, Sudan III	-, 37, Static, 48	76, -97, Azoreductase	Pan <i>et al.</i> , 2011
24.	<i>Staphylococcus hominis</i> RMLRT03	Acid Orange	7.0, 35, static, 60	94, -	Singh <i>et al.</i> , 2014

Bacterial Consortium

Consortium based treatment system achieves higher extent of biodegradation and mineralization due to the synergistic metabolic properties of the microbial community and has significant advantages over the use of pure cultures in the

degradation of azo dyes (Saratale *et al.*, 2010). Consortia can be constructed with bacteria, fungi or a combination of both (Yang *et al.*, 2009). Generally during reduction of azo bonds production of toxic, carcinogenic aromatic amines takes place but by using microbial consortium these

aromatic amines get degraded by complementary organisms, making the process more effective and efficient (Moosvi *et al.*, 2007). It is found that in a microbial consortium the individual microbial strains may attack the dye molecule at different positions or may act on metabolites produced by the co-existing strains for further decomposition (Patil *et al.*, 2008; Saratale *et al.*, 2009b). In a study, Orange II has been shown to be completely decolorized by the consortium of two bacterial species, *Enterobacter cloacae* and *Enterococcus casseliflavus* whereas individual decolorization extent is 10% and 23%, respectively in 15 min (Chan *et al.*, 2011). Another consortium PMB11, made up of three bacterial species, *Bacillus odyseeyi* SUK3, *Proteus sp.* SUK7 and *Morganella morganii* SUK5, completely decolorizes Reactive Blue 59 in 3 h, while the individual decolorization time of this dye is more than 24 h (Patil *et al.*, 2008). Recently, Lalnunhlmi and Krishnaswamy (2016) reported alkaliphilic bacterial consortium of *Bacillus flexus* strain NBN2 (SY1), *Bacillus cereus* strain

AGP-03 (SY2), *Bacillus cytotoxicus* NVH 39198 (SY3), and *Bacillus sp.* L10 (SY4) which is capable of decolorizing Direct Blue 151 and Direct Red 31 up to 97.57% and 95.25%, respectively in 5 days. Four bacterial isolates identified as *Stenotrophomonas acidaminiphila* (BN-3), *Pseudomonas putida* (BN-4), *Pseudomonas fluorescence* (BN-5) and *Bacillus cereus* (BN-7) which completely decolorized C.I. Acid Red 88 (AR-88), were used as consortium HM-4. The consortium JW-2 included three isolates *Paenibacillus polymyxa*, *Micrococcus luteus* and *Micrococcus sp.* led to complete decolorization of Reactive Violet 5R within 36h whereas individual isolates could not show decolorization even on extended incubation time (Moosvi *et al.*, 2007). Similarly, Phugare *et al.* (2011) reported that raw effluent was decolorized more effectively with a consortium (Fig. 4) containing *Providencia sp.* SDS and *Pseudomonas aeruginosa* strain BCH than the individual cultures alone. Several studies reported for biodegradation of azo dyes using bacterial consortium are given in Table 2.

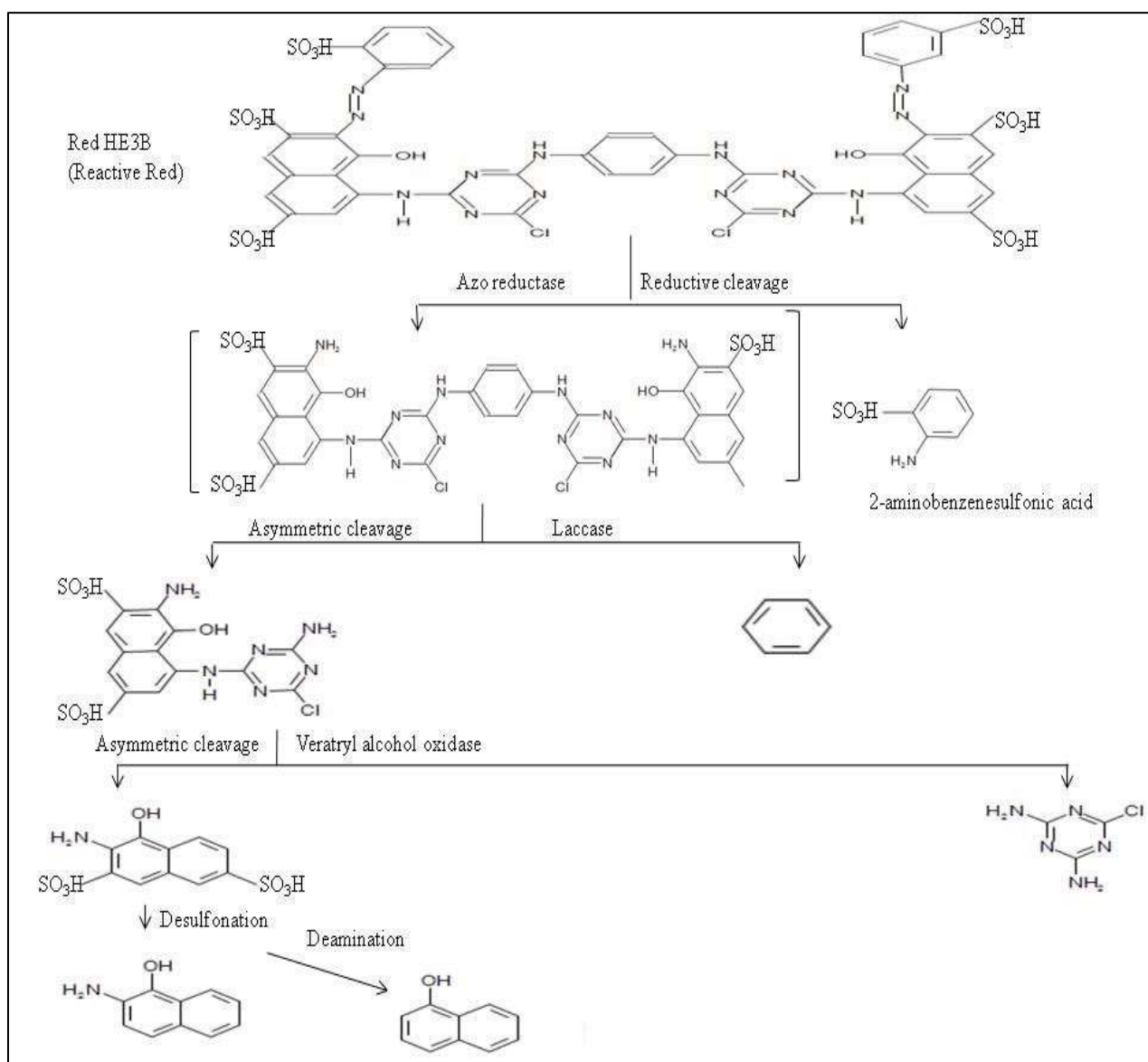


Fig 4: Proposed pathway for Red HE3B biodegradation. (Adapted from Phugare *et al.*, 2011)

Table 2 : Decolorization and degradation of azo dyes by bacterial consortium.

S. N.	Bacterial consortium	Name of Dye	Conditions pH, Temp(°C), Time(h)	Decolorization (%) and Reaction mechanism	References
1.	<i>Aeromonas caviae</i> , <i>Protues mirabilis</i> and <i>Rhodococcus globerulus</i>	Acid Orange 7 and many azo dyes	7.0, 37, 16	90, -	Joshi <i>et al.</i> , 2008
2.	Alpha, beta and gamma <i>proteobacteria</i>	Reactive Blue 59	-, -, 24	55.50, Azoreductase	Kolekar <i>et al.</i> , 2012
3.	<i>Bacillus odysseyi</i> , <i>Morganella morgani</i> , <i>Proteus</i> sp.	Reactive Blue	-, 30, 1	100, Lignin peroxidase, Laccase, Tyrosinase, NADH-DCIP reductase	Patil <i>et al.</i> , 2008
4.	<i>Bacillus vallismortis</i> , <i>Bacillus pumilus</i> , <i>Bacillus cereus</i> , <i>Bacillus subtilis</i> , <i>Bacillus megaterium</i>	Congo Red, Direct Red 7, Acid Blue 113, Direct Blue 53	-, 37, 96	96, 89.60, 81, 82.70, Aerobic degradation	Tony <i>et al.</i> , 2009
5.	<i>Enterococcus casseliflavus</i> , <i>Enterobacter cloacae</i>	Orange II	7.0, 37, 5 days	100, Aerobic degradation	Chan <i>et al.</i> , 2011
6.	<i>Galactomyces geotrichum</i> and <i>Brevibacillus Laterosporus</i>	Golden Yellow HER	9.0, 90, 24	100, Laccase, Tyrosinase, Azoreductase, Riboflavin reductase	Waghmode <i>et al.</i> , 2011a
7.	<i>P. vulgaris</i> and <i>M. glutamicus</i>	Scarlet R and mixture of 8 dyes	7.0, 37, 3	100, Reductive	Saratale <i>et al.</i> , 2009b
8.	<i>Penicillium</i> sp., <i>Exiguobacterium</i> sp.	Reactive Dark Blue K-R	7.0, 37, 24	97, Anaerobic degradation	Qu <i>et al.</i> , 2010
9.	<i>Proteus vulgaris</i> , <i>Micrococcus glutamicus</i>	Reactive Green 19	3.0, 37, 24	100, Degradation; static incubation	Saratale <i>et al.</i> , 2010
10.	<i>Providencia</i> sp., <i>Pseudomonas aeuroginosa</i>	Red HE3B, Remazol Black 5B, Red HE7B	7.0, -, -	100, Laccase, Veratryl alcohol oxidase, NADH-DCIP reductase, Azoreductase	Phugare <i>et al.</i> , 2011
11.	<i>Pseudomonas</i> sp., <i>Aspergillus ochraceus</i>	Reactive Navy Blue	-, 30, 24	80, NADH-DCIP reductase, Azoreductase, Tyrosinase	Kadam <i>et al.</i> , 2011
12.	<i>Pseudomonas</i> , <i>Arthrobacter</i> and <i>Rhizobium</i>	Acid Orange 7	-, -, -	100, Aerobic degradation	Ruiz-Arias <i>et al.</i> , 2010
13.	<i>Sphingomonas paucimobilis</i> , <i>Bacillus</i> sp., <i>Staphylococcus epidermidis</i>	Congo Red	7.0, 37, 10	100, Aerobic degradation	Ayed <i>et al.</i> , 2010

Algae (Phycoremediation)

Living and nonviable algae have been used in bioremediation of textile wastewater (Lim *et al.*, 2010). Several research groups concluded that microalgae offer a solution to global environmental problems such as the greenhouse effect and wastewater treatments (Craggs *et al.*, 1997; Parikh and Madamwar, 2005). Algae utilize three different intrinsic mechanisms for decolorization of azo dyes including use of chromophore for production of algal biomass via assimilation, production of CO₂ and H₂O during conversion of color to non-color molecule and chromophore adsorption by algal biomass. However, release of azo dyes into natural water ecosystem causes toxic effect on aquatic life but it does not significantly

reduce algal growth (Acuner and Dilek, 2004) and algae have been reported to grow in industrial effluents (Dubey *et al.*, 2011). Mechanisms of algal decolorization can involve enzymatic degradation, adsorption or both. Most studied algae that are involved in decolorization and degradation of azo dyes are blue green algae, green algae and diatoms. Several species of *Chlorella* (Acuner and Dilek, 2004) and *Oscillatoria* (Jinqi and Houtian, 1992) were found to be potent decolorizer of azo dyes and generate their corresponding aromatic amines which further metabolize these toxic aromatic amines into simpler organic compounds or CO₂ and H₂O. Similar to bacteria, algal mediated degradation of azo dyes depends upon induced form of enzyme azoreductase for breakdown

of azo bond, resulting in the production of aromatic amines (El-Sheekh *et al.*, 2009). Priya *et al.* (2011) proposed role of *Oscillatoria curviceps* derived enzymes azoreductase, laccase and polyphenol oxidase in the degradation of dye Acid Black. In contrast to bacteria and fungi, which require input of carbon and other supplements for removal of dyes, algae do not need added supplements (Omar, 2008). Patil *et al.* (2015) reported two species of green algae, *Chara* and *Scenedesmus obliquus* which successfully degraded fabric dye Congo Red and Crystal Violet by oxidative enzyme peroxidase and laccase.

Use of immobilized microalgae is another existing alternative to decolorization and degradation of dyes for example, *Chara vulgaris* (Chu *et al.*, 2009) and *S. quadricauda* (Ergene *et al.*, 2009) immobilized on alginate can scavenge higher percentage of color from textile dyes than suspended algae.

Filamentous Fungi (Mycoremediation)

Several studies are available about the potential of fungi to oxidize phenolic, nonphenolic, soluble and nonsoluble dyes (Tekere *et al.*, 2001; Libra *et al.*, 2003). The mycoremediation of dyes is a cost effective possible option to the present treatment technologies. The most widely used fungi in decolorization and degradation of dye are the lignolytic fungi of class basidiomycetes. The fungal potential of bioremediation of different complex organic pollutants is devoted to a metabolic product of a large set of intra and extracellular enzymes (Humnabadkar *et al.*, 2008). Fungi mediate a variety of complex conversion reactions such as hydroxylation of complex polyaromatic hydrocarbons, dye effluents, organic waste and steroid compounds (McMullan *et al.*, 2001). It was found that fungal mediated degradation of aromatic compound is a secondary metabolic event that arises when nutrients (C, N and S) become limiting, then these aromatic compounds are used as source of energy and nutrients essential for the propagation of the cultures (Christian *et al.*, 2005). White rot fungi produces lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase which play major role in degradation of many aromatic compounds due to its nonspecific enzyme activity (Madhavi *et al.*, 2007; Bergsten-Torralba *et al.*, 2009) and avoid production of toxic aromatic amine problem which arise during azo dye reduction. Enayatizamir *et al.* (2011) reported degradation of Azo Black Reactive 5 dye up to 92% by *P. chrysosporium* after 3 days of treatment. *P. chrysosporium* URM6181 and *Curvularia lunata* URM6179 strains decolorize effluent containing textile indigo dye up to approximately 95% after 10 days of treatment (De-Miranda *et al.*, 2013). Recently Gajera *et al.* (2015) isolated six fungal species out of which *Hypocrea koningii* was found to be most potent decolorizer of five textile azo dyes (Red HE7B, Reactive Violet 5, Red Black B, Light Navy Blue HEG, Dark Navy Blue H2GP).

Although *P. chrysosporium* is the most widely studied white rot fungi in the decolorization and degradation of dyes, others fungal species have also contributed in bioremediation of azo dyes (Table 3).

Now it is a well established fact that fungal mediated removal of dyes is either by adsorption or enzymatic process. The growth, enzyme production and subsequent dye degradation by filamentous fungi are dependent and affected by many factors such as culture conditions, nutrient conditions, especially regarding nitrogen limitation, carbon source, time, pH, agitation, temperature, oxygen supply, additives and salts (Mielgo *et al.*, 2001; Zouari-Mechichi *et al.*, 2006; Evangelista-Barreto *et al.*, 2009; Parshetti *et al.*, 2010; Pilatin and Kunduhoglu, 2011; Grinhut *et al.*, 2011).

Although different fungal species are successfully employed in the decolorization and degradation of azo dyes but at the same time they encounter many problems for the removal of dyes from textile wastewaters such as large volume produces, control of biomass and the nature of synthetic dyes (Palma *et al.*, 1999; Stolz, 2001). Despite these decolorization related problems, fungal mediated decolorization is a promising alternative to replace or supplement present treatment processes.

Yeast

Similar to microalgae, the mechanisms adopted by yeast for dye decolorization are adsorption (Yu and Wen, 2005), enzymatic degradation, or a combination of both. In recent years, there has been rigorous work on dye removal by different yeast species because it shows attractive features over bacteria and filamentous fungi, as it offers lots of advantages. They not only grow rapidly like bacteria, but they also have the capacity to sustain in adverse environmental condition, such as at low pH (Yu and Wen, 2005; Martorell *et al.*, 2012). Several simple azo dyes were degraded in liquid aerated batch cultures by yeast strain *Candida zeylanoides*, the tune of color removal ranged from 44 to 90%, after 7 days of treatment (Martins *et al.*, 1999). It was observed that enzyme mediated biodegradation followed decolorization of several azo dyes which is done by some ascomycetes yeast species including *Candida tropicalis*, *Debaryomyces polymorphus* (Yang *et al.*, 2003) and *Issatchenkia occidentalis* (Ramalho *et al.*, 2004). Lucas *et al.* (2006) used *Candida oleophila* for efficient decolorization of commercial textile diazo dye Reactive Black 5. The most preferred physical condition for decolorization by yeast biomass is a low pH for example *Candida albicans* adsorb maximum Direct Violet 51 dye at pH 2.5 (Vitor and Corso, 2008) and *C. tropicalis* perform similar role at pH 4.0 for Violet 3 (Charumathi and Das, 2010). Yang *et al.* (2003) have shown that two yeasts (*D. polymorphus*, *C. tropicalis*) and a filamentous fungi (*Umbelopsis isabellina*) could completely decolorize 100 mg of Reactive Black 5 within

16-48 hours. Earlier *S. cerevisiae* MTCC463 strain was studied for decolorization of two azo dyes Malachite Green and Methyl Red (Jadhav and Govindwar, 2006; Jadhav *et al.*, 2007). Waghmode *et al.* (2011b) reported that the decolorization, COD and TOC removal of a

mixture of Golden Yellow HER, Remazol Red, Rubine GFL, Scarlet RR, Methyl Red, Brilliant Blue and Brown 3 REL is higher under aerobic than under anoxic or anaerobic conditions by using *G. geotrichum* MTCC 1360.

Table 3: Decolorization of various azo dyes by fungal culture.

S. N.	Fungal culture	Name of Dye	Conditions pH, Temp(°C), Agitation (rpm), Time(h)	Decolorization (%) and Reaction mechanism	References
1.	<i>Armillaria</i> sp. F022	Reactive Black 5	4.0, 40, -, 96	<80, Laccase	Hadibarata <i>et al.</i> , 2012
2.	<i>Aspergillus niger</i>	Congo Red	3.0, -, 60, 36	99, Degradation	Karthikeyan <i>et al.</i> , 2010
3.	<i>Coriolus versicolor</i>	Acid Orange II	-, 30, -, -	85, Degradation	Hai <i>et al.</i> , 2012
4.	<i>Cunninghamella elegans</i>	Reactive Orange II, Reactive Black 5, Reactive Red 198	5.6, 28, -, 120	93, Adsorption	Ambrosio <i>et al.</i> , 2012
5.	<i>Fusarium oxysporum</i>	Yellow GAD	-, 24, -, 144	100, Aerobic, Degradation	Porri <i>et al.</i> , 2011
6.	<i>Ganoderma</i> sp. En3	Methyl Orange, Crystal Violet, Bromophenol Blue, Malachite Green	5.5, 28, -, 72	96.7, 75, 90, 91, Laccase, Degradation	Zhuo <i>et al.</i> , 2011
7.	<i>Phanerochaete chrysosporium</i>	Direct Red 180	4.5, 30, -, 72	100, Lignin peroxidase, Aerobic, Degradation	Sen <i>et al.</i> , 2012
8.	<i>Phanerochaete chrysosporium</i>	Astrazon Red FBL	-, 37, -, 2 days	87, Degradation	Sedighi <i>et al.</i> , 2009
9.	<i>Pleurotus eryngii</i> F032	Reactive Black 5	3, 40, 120, 72	93.56, Lignin peroxidase, Manganese peroxidase, Laccase	Hadibarata <i>et al.</i> , 2013
10.	<i>Pleurotus sajor-caju</i>	Reactive Blue 220, Reactive Red 198, Reactive Yellow 15	-, -, -, 11 days	100, 100, 100, Adsorption and degradation	Munari <i>et al.</i> , 2008
11.	<i>Schizophyllum commune</i> IBL-06	Solar Brilliant Red 80	4.5, 30, shaking, 7 days	100, Laccase, Manganese peoxidase, Lignin peroxidase	Asgher <i>et al.</i> , 2013
12.	<i>Trametes</i> sp.	Orange II, Brilliant Blue R 250	-, -, -, 10 days	100, 100, Laccase, Manganese peoxidase, Degradation	Grinhut <i>et al.</i> , 2011
13.	<i>Trametes trogii</i>	Remazol Brilliant Blue R, Indigo Carmine, Indigo Carmine	4.5-7.0, 30, -, 30 min	82, 84.5, 75, Laccase, Manganese peoxidase, Degradation	Grassi <i>et al.</i> , 2011
14.	<i>Trametes versicolor</i>	Blue 49, Black 5, Reactive Brilliant Blue R, Orange 12, Orange 13	3.5-6.5, 40, -, 7 days	94, 88, 97, 83, 84, Degradation	Pilatin and Kunduhoglu, 2011

Decolorization and Degradation of Azo Dyes by Plants (Phytoremediation)

Phytoremediation is emerging as the most promising technology for the remediation of soils and groundwater contaminated with heavy metals and organic pollutants which provide an innovative, cost effective alternative approach for biotreatment of wastewater (Patil *et al.*, 2009). Recently, some studies describe the use of plants for bioremoval of dye from wastewaters. Researchers utilized the phytoremediation potential of *Petunia*

grandiflora Juss., for remediation of a mixture of dyes and dye containing wastewater (Watharkar *et al.*, 2013). Three plant species *Brassica juncea*, *Phaseolus mungo* and *Sorghum vulgare* have been studied for its decolorization potential against azo dyes of textile effluents. These plants (*B. juncea*, *P. mungo* and *S. vulgare*) showed color removal of textile effluent up to 79, 53 and 57%, respectively (Ghodake *et al.*, 2009). Similarly, various other plants are also reported for dye decolorization for instance, *Blumea malcolmii* and *Typhonium flagelliforme*

for decolorization of Direct Red 5B (Kagalkar *et al.*, 2009) and Brilliant Blue R (Kagalkar *et al.*, 2010), respectively. Shinde *et al.* (2012) studied the potential of three fast growing plants *Parthenium hysterophorus*, *Alternanthera sessilis* and *Jatropha curcas*, for simultaneous decolorization of two textile dyes Yellow 5G and Brown R. Patil *et al.* (2009) studied decolorization potential of hairy root cultures of *Tagetes patula* L. (Marigold) for Reactive Red 198, and concluded that the inherent enzymatic system of plant is responsible for color removal. *Phragmites australis* has been applied successfully for the remediation of textile effluent containing dyes, mainly against Acid Orange 7 (Carias *et al.*, 2007). Page and Schwitzgubel (2009) pointed out that detoxification pattern of xenobiotic molecule depends upon the plant species. The use of plants for removal of toxicants is not so popular due to lack of detailed information about inherent metabolic pathways used by the plants to metabolize the toxicants (Chaudhry *et al.*, 2005).

The main advantages of dye removal by plants is that it is an autotrophic system with a large biomass which requires little nutrient cost, is easier to handle, and is generally accepted by the public due to both its aesthetic demand and environmental sustainability (Ghodake *et al.*, 2009; Kagalkar *et al.*, 2009).

Although extensive research have been carried out to establish effective and efficient phytoremediation techniques for decolorization, and degradation of azo dyes, large scale application of phytoremediation is not feasible and presently it faces a number of problems including the extent of pollutants tolerated by the plant, the bioavailable fraction of the contaminants and evapotranspiration of volatile organic pollutants as well as requirement of large areas to establish the treatment plants (Williams, 2002).

Role of Nanotechnology in Decolorization and Degradation of Azo Dyes

Biological treatment for the decolorization and degradation of azo dyes has advantages and disadvantages, which have been earlier described in this review. Bioremediation is an eco-friendly approach for the treatment of textile wastewater, but on the other hand physico-chemical characteristics of the effluents, including pH, temperature, the content of NaCl and other salts and the presence of organic compounds, can result in the deactivation of enzymes and microbial cells. Therefore, it is necessary to search for more active and versatile enzymes, microorganisms and techniques with high stability and low cost that is suitable to meet the requirements of textile industry wastewater treatment.

In the last few years various nanoparticles are widely used for the degradation of azo dyes. Nanotechnology is a very promising technique, having an important role in improvement of manufacturing technologies,

telecommunications, electronics, health and even in environmental remediation (Gross, 2001; Kim *et al.*, 2005; Moore, 2006). Recently, nanomaterials (NMs) have been used as efficient, cost effective and eco-friendly alternative to existing treatment materials, from the standpoints of both resource conservation and environmental remediation (Dimitrov, 2006; Dastjerdi and Montazer, 2010). With unique physical and chemical properties nanomaterials have a remarkable potential for toxic contaminant removal. Nanotechnology offers production and utilization of a different range of NMs, with size ranging from 1 to 100 nm and exhibit unique properties which are not found in bulk-sized materials (Stone *et al.*, 2010; Wang *et al.*, 2010). Bokare *et al.* (2008) investigated degradation of Orange G, a monoazo dye, in aqueous solutions by using Fe-Ni bimetallic nanoparticles. Transmission electron microscopy (TEM) reveals that nanoparticles have spherical particles with size of 20-40 nm. Dehghani and Mahdavi (2015) performed experiments in a batch photo-reactor on synthetic wastewater with concentrations of 0.5, 1.0, 1.5 and 2 mg l⁻¹. The study investigated the effects of factors such as irradiation time, a dose of catalyst, initial dye concentration and pH on decolorization extent of Acid Red 4092 dye by the photocatalytic process in the presence of zinc oxide nanoparticles. Iron nanoparticles were produced by using extracts of green tea leaves (GT-Fe NPs). The materials were characterized by using TEM, SEM/EDX, XPS, XRD and FT-IR techniques and were shown to contain mainly iron oxide and iron oxohydroxide. These nanoparticles were then exploited as a Fenton-like catalyst for decolorization of aqueous solutions containing Methylene Blue and Methyl Orange dyes (Shahwan *et al.*, 2011). Hairom *et al.* (2014) successfully synthesized four different types of ZnO nanoparticles via the precipitation method using oxalic acid and zinc acetate solutions for degradation of Congo Red dye. The presence of polyvinylpyrrolidone (PVP) obviously reduced the agglomerations and average particle size of the ZnO, according to the ascending order as ZnO-PVP-St < ZnO-PVP-U_s < ZnO-U_s < ZnO-St. Recently, Edison *et al.* (2016) worked on reductive cleavage of azo dyes such as Congo Red and Methyl Orange by using *Anacardium occidentale* testa derived silver nanoparticles (AgNPs) as a catalyst. The formation of highly stable AgNPs was visually confirmed by the appearance of yellow color and further verified by the existence of surface plasmon resonance (SPR) peak around 425 nm. Sharma *et al.* (2016) have studied decolorization and degradation of Methyl Red by suspended and immobilized cells of *Aeromonas jandaei* strain SCS5 under anaerobic and aerobic conditions. The complete decolorization of Methyl Red at a concentration of 100 mg l⁻¹ by *A. jandaei* strain SCS5 was recorded within 6 h for both anaerobic and aerobic suspended cultures, but the decolorization rate was faster in acidic than basic

conditions. Decolorization of azo dye MR involved the use of mediators anthraquinone-2,6-disulfonate and Fe₃O₄ nanoparticles.

Conclusions

Azo dyes account for the majority of all dyestuffs produced and employed in the textile industries. They are considered as electron-deficient xenobiotic compounds containing the aromatic functionality with one or more azo (-N=N-) groups. Textile industries are largest generator of dye containing wastewater. The release of dye containing effluent into the environment is of great concern due to its aesthetic value, toxicity, mutagenicity and carcinogenicity. Therefore, effluents from textile industries have serious environmental concern and the removal of dyes from effluent is necessary prior to their disposal. Biological processes including microorganisms such as bacteria, fungi, yeast, algae and plants overcome the limitations of physico-chemical techniques and provide an alternative approach to existing technologies for removal of azo dyes. These methods are inexpensive, environment friendly and applicable to different structural varieties of dyes. This review emphasizes the potential of biological systems and their possible mechanisms for decolorization and degradation of azo dyes. Bacteria is the most frequently applied microorganisms for the removal of dyes from textile effluents because they are easy to cultivate, adapted to survive in extreme environmental conditions and decolorize the azo dyes at a faster rate as compared to other available microorganisms. The effectiveness of microbial processes for bioremoval of azo dyes depends on the adaptability and the activity of selected microorganisms. For mineralization of azo dyes, treatment systems having mixed microbial populations or consortium are more effective due to concerted metabolic activities of the microbial community. The role of oxidative and reductive enzymes and their possible mechanisms to understand the biochemical basis of decolorization and degradation of azo dyes are also addressed in this review. Moreover, the development of innovative nanotechnologies is the future solution to the problem of colored wastewater of textile dyeing industries. Nanotechnology coupled with conventional biological processes will play a critical role in increasing environmental protection.

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