

Review Article

Microbial degradation of Azo Dyes: A review

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ABSTRACT

Keywords

Azo dyes;
enzymatic
reduction;
toxicity;
Microbial
degradation;
enzymes.

A dye is used to impart color to materials of which it becomes an integral part of human life. Azodyes account for the major produced synthetic dyestuffs because they are extensively used in the textile, leather, pharmaceutical and cosmetics industries, pose a threat for all life forms. The physico-chemical method of industrial effluent treatment does not remove the dyes effectively. Microbial degradation and decolorization of azodyes has gained more attention recently because of eco-friendly and inexpensive nature. Microbes could decolorise the dyes by both aerobic and anaerobic metabolism. Further, the efficacy of microbial decolorizing enzymes on biotransformation of toxic azodyes has been discussed. This review provides a general idea of microbial decolorization and degradation of azodyes with various physicochemical parameters and highlights the application of these processes for the treatment of azodye-containing wastewaters.

Introduction

Water is not a commercial product but, Over the last few decades, increasing globalization, urbanization, and industrialization have causes different environmental pollution. Among various industries, the textile dyeing industries discharge large volume of waste water after dyeing process (Zolinger *et al.*, 1987). It is estimated that around 10 -15% of the dyes are lost in the effluent during the dyeing processes (Baban *et al.*, 2003). The excessive discharge of the effluents from

the textile industries contains toxic chemicals such as azo dyes, and reactive dyes which adversely affect the natural resources, soil fertility, and aquatic organisms and disturb the integrity of the ecosystem (Mester and Tien 2000; Puvaneswari *et al.*, 2006) by alters the pH, increases the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), and greatly affect water quality. Discharge of effluents without adequate removal of these dyes will remain in the

environment and cause serious issues (Olukanni *et al.*, 2006). So far, various methods were adapted for the reduction of azo dyes to achieve decolorization which include physiochemical methods (Droste, 2004) and biological methods.

Dyes

Dyes are chemicals which bind to material and imparts color to that material. The color of a dye is due to the presence of chromophore group. They are widely used to color the substrate like textile fiber, paper, leather, hair, fur, plastic material, wax, a cosmetic base and food stuff. (Masitah Binti Hasan., 2008). Based on Chemical structure of chromophore there are 20 -30 different groups of dyes. Azo (Monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are the most important groups (Safwat Mohammad., 2005). The majority of industrial important azo dyes belong to the following classes: Acid dyes, Basic dyes, Direct dyes, Disperse dyes, Mordant dyes, Reactive dyes and Solvent dyes. The Acid, Basic, Direct and Reactive azo dyes are ionic dyes (Anliker *et.al.*, 1981).

Dyes contain atleast one nitrogen-nitrogen (N=N) double bond, however many different structures exist, For example, in the azo dyes, monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds respectively. The azo groups are generally connected to benzene and naphthalene rings. These side groups are necessary for imparting the color of the dye, with many different shades and intensities being possible (Zollinger, 1991). These dyes have different absorption spectrum (Table-1) and associated with electronic transition

between molecular orbital.

of synthetic dyes are produced annually worldwide. During dying process, a substantial amount of azo dye is lost in waste water (Ollgaard *et al.*, 1998). Zollinger (1987) reported that about 10-15% of dyes were lost in effluent during dyeing process.

Azo Dyes

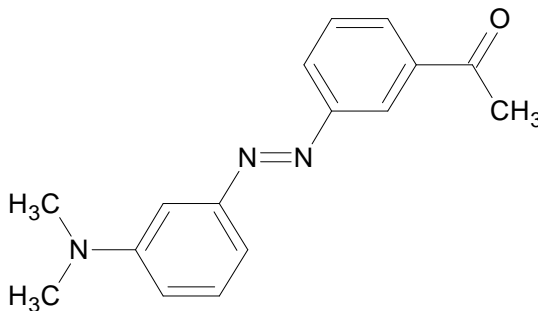
Azo dyes are the largest class of synthetic aromatic dyes composed with one or more (-N=N-) groups and sulfonic (-SO³⁻) groups with lots of commercial interest (Vandevivere *et al.*, 1998; Barragan *et al.*, 2007). Azo dyes are water-soluble synthetic organic compounds. Generally, azo dyes contain one, two or three azo linkages, linking phenyl, naphthyl rings that are usually substituted with some functional groups including triazine amine, chloro, hydroxyl, methyl, nitro, and sulphonate (Bell *et al.*, 2000). There are more than 3000 azo dyes which include Astrazon Red GTLN, Maxilon Blue GRL, and Sandolan Yellow are widely used by the textile, leather, cosmetics, food coloring and paper production industries (Lorimer *et al.*, 2001; Elbanna *et.al.*, 2010). About 80% of azo dyes are used in the dyeing process of textile industries. It had been estimated that approximately 10% of the dyes used in dyeing process do not bind to the fiber and are released into the environment (Asad *et al.*, 2007). They possess toxicity like lethal effect, genotoxicity, mutagenicity, and carcinogenicity to plants and animals (Puvaneswari *et.al.*,2006).

Impact of azo dyes

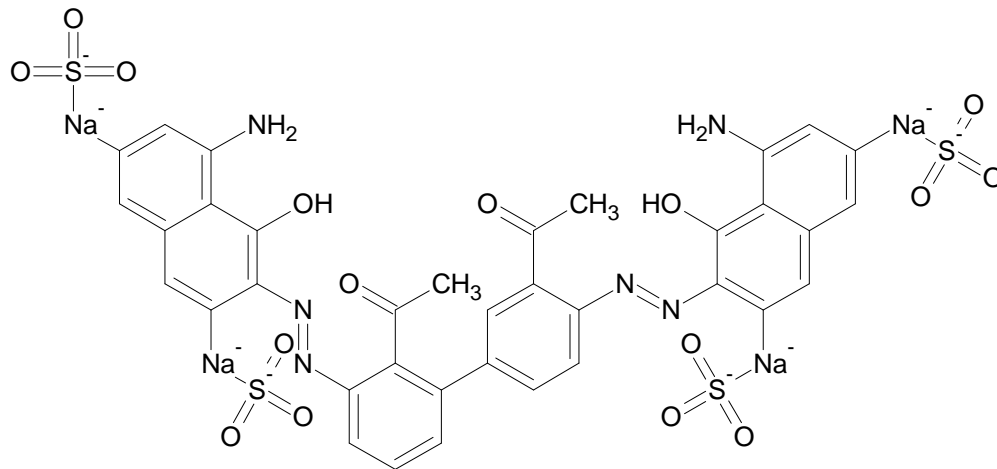
Azo dyes produce clear and strong colors. They are primarily used for colored cotton, leather, cosmetics, and food. Azo dyes

Table.1 Relationship between light absorption and color (Solomons *et.al.*, 1996)

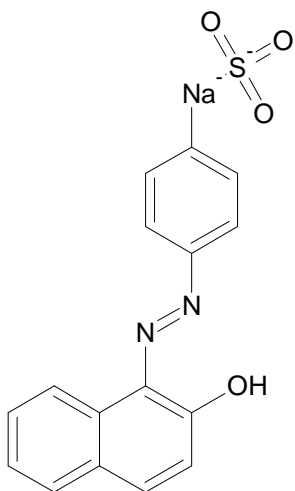
S.No	Color absorbed	Color observed	Absorbed radiation (nm)
1	Violet	Yellow-green	350-435
2	Blue	Yellow	435-480
3	Green-Blue	Orange	480-490
4	Blue-green	Red	490-500
5	Green	Purple	500-560
6	Yellow-Green	Violet	560-580
7	Yellow	Blue	580-595
8	Orange	Green-Blue	595-605
9	Red	Blue-Green	605-750



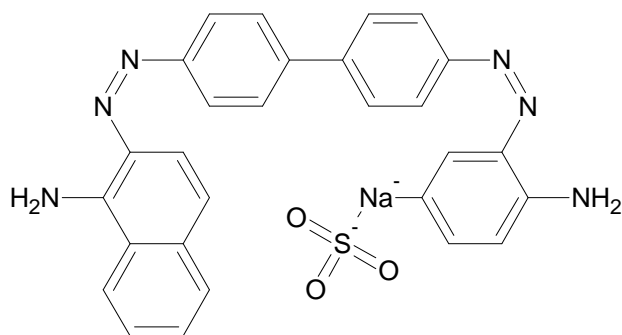
Methyl Red—monoazo dye



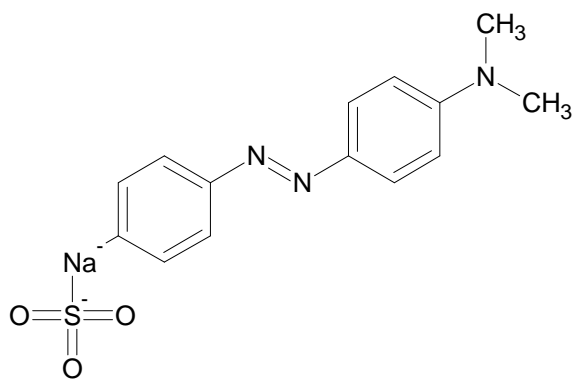
Direct Blue 15—diazo dye



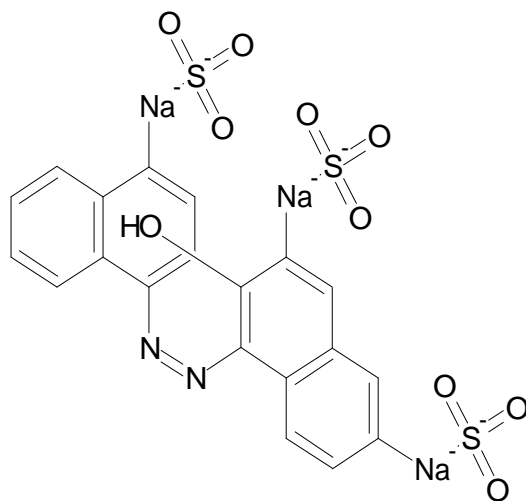
Orange II



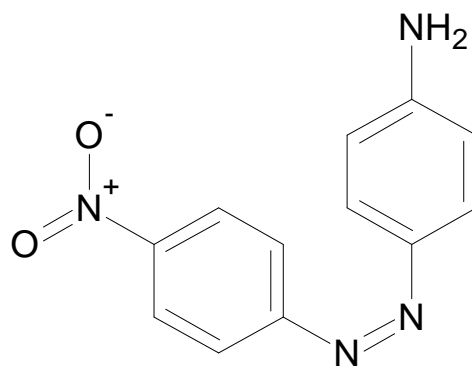
Congo Red



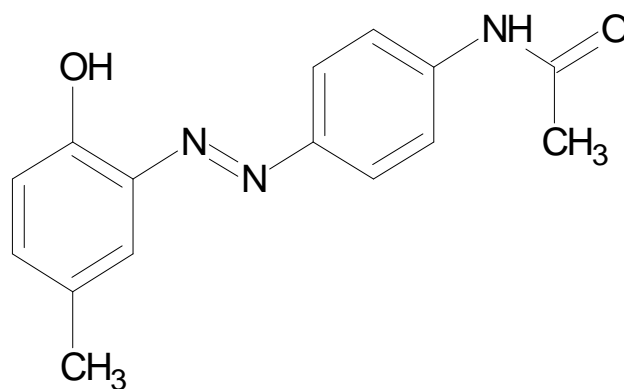
Methyl Orange



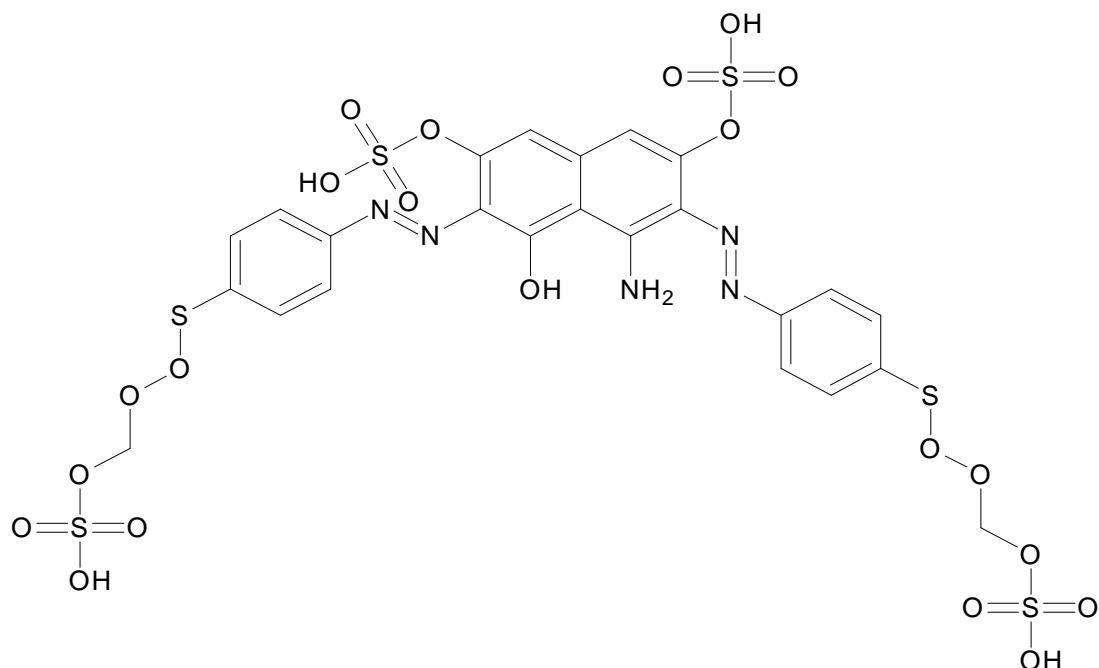
Amaranth



Disperse Orange 3



Disperse Yellow 3



Reactive Black

belong to a group of organic compounds. The azo group of dyes binds to an aromatic ring. Through mineralization, these dye can be broken down into an aromatic amine, an arylamine that is suspected to be carcinogenic. Most of the azo dyes are water soluble and readily to absorb through skin contact and inhalation leading to the risk of cancer and allergic reactions, an irritant for the eyes and highly toxic, if inhaled or consumed (Nikulina, Deveikis and pyshnov, 1995). For example, Para-phenylene diamine (PPD) also called 1,4-diamino benzene or 1,4-phenylene diamine, is an aromatic amine, which is a major component of azo dyes. PPD-containing azo dyes are toxic and causes skin irritation, contact dermatitis, chemosis, lacrimation, exophthalmose, and permanent blindness. Ingestion of PPD products leads to the rapid development of oedema on face, neck, pharynx, tongue and larynx along with respiratory distress.

In some cases, it may cause rhabdomyolysis, acute tubular necrosis supervene, vomiting gastritis, hypertension, and vertigo (Macphec *et al.*, 1975; Young *et al.*, 1977; Houk *et al.*, 1991; Sunga *et al.*, 2005). Some azo dyes are linked to human cancer, splenic sarcomas, hepatocarcinomas, and nuclear anomalies in experimental animals and chromosomal aberrations in mammalian cells (Puvaneswari *et al.*, 2006). Benzidine (BZ)-based azo dyes have been recognized as a human urinary bladder carcinogen and tumorigenic in laboratory animals (Haiey *et al.*, 1975). Toxic compounds of azo dyes readily mix with water bodies and enter into aquatic organisms (Fang *et al.*, 2004; Asad *et al.*, 2007) through food chain and ultimately reach man and cause physiological disorders such as hypertension, sporadic fever, renal damage and cramps.

Table.2 Effect of Azo dyes

S.No	Name of the dye	Effects	Reference
1	Reactive brilliant red	Inhibits function of human serum albumin	Li <i>et al.</i> , 2010
2	Acid Violet 7	Induce chromosomal aberration, lipid peroxidation, acetyl cholinesterase in mice	Ben Mansour <i>et al.</i> , 2010
3	Disperse Red -1 & Disperse Orange -1	Increase the frequency of micronuclei in human lymphocytes	Chequer <i>et al.</i> , 2009
4	Reactive Black 5	Decrease urease activity, arginine ammonification rate in terrestrial ecosystem	Topac <i>et al.</i> , 2009
5	Disperse Blue 291	Mutagenic, Cytotoxic, genotypic effects, formation of micronuclei, DNA fragmentation in human hepatoma cells	Tsuboy <i>et al.</i> , 2007
6	Direct Black 38	Urinary Bladder cancer in humans	Meal <i>et al.</i> , 1981 & Cerniglia <i>et al.</i> , 1986
7	Direct Blue 15	Mutagenic	Reid <i>et al.</i> , 1984

Some azo dyes are carcinogenic and mutagenic. They reduce the efficiency of seed germination and plant growth. Untreated effluents with higher concentration of dyes inhibit the elongation of shoot and roots. (Nirmalarani *et al.*, 1988).

Malachite green causes serious public health hazards and environmental problem. So far through various experimental observations it is revealed that malachite green is a multiorgan toxin; it decreases food intake, growth, fertility rates; and causes damage to liver, spleen, kidney and heart (Werth and Boiteaux, 1967, Culp *et al.*, 1999). In malachite green-fed mice, apoptosis in the transitional epithelium of the urinary bladder and thyroid follicles was observed (Culp *et al.*, 1999).

Removal of Azo dyes

Azo dyes may be toxic to aquatic

organisms and are considered as xenobiotic compounds, very resistant to natural biological degradation (Stolz, 2001; Perkowski and Ledakowicz, 2002). Many physiochemical methods like coagulation (Vandevivere *et al.*, 1998), coagulation–electrooxidation (Xiong *et al.*, 2001), adsorption (Morais *et al.*, 1999), electrolysis (D’avila-Jimenez *et al.*, 2000), photolysis (Ince, 1999) and ozonation are promising in terms of performance, while the economic aspect has become the most challenging problem. But, azo linkages are easily reduced under anaerobic conditions (Bromley-Challenor *et al.*, 2000), yielding colorless aromatic amines and are readily degraded aerobically. Therefore, a combination of anaerobic and aerobic conditions is proposed for azo compounds’ mineralization (Knackmuss, 1996).

Photooxidation

The past two decades have witnessed intensive studies related to the light

induced mineralization of azo dyes (Yamashita *et al* 2000; Panduranga *et al* 2001; Subramani *et al.*, 2007). Number of literatures reported that photodegradation process of TiO₂ (Wang 2000; Neppolian *et al* 2002; Byrappa *et al* 2000; Ding *et al* 2000; Tsumura *et al* 2002), in general, occurs with the attack of organic substances by the activated oxygen species, such as hydroxyl radical and super oxide radical, generated on TiO₂ particulate surface by the reduction of dissolved oxygen in solution and/or oxidation of surface hydroxyl by TiO₂ (Poulios and Aetopoulon 1999; Carlos Gouvea *et al* 2000). Wang (2000) reported the photocatalytic degradation of reactive azo dyes in an aqueous solution and destruction of several classes of organic dyes.

Advanced Oxidation Process (AOPS)

Advanced Oxidation Processes (AOPs) are based on the generation of highly reactive species like the hydroxyl radicals (·OH) that have a strong oxidative potential. These radicals can rapidly oxidize a broad range of organic pollutants in a non-selective manner. The common AOPs includes Fenton and Fenton-like oxidation, ozonation, photochemical oxidation, electrochemical oxidation, photolysis using a H₂O₂ and O₃, Corona process, TiO₂ photolysis, radiolysis, wet oxidation and the use of electronic beams. The main principle of AOPs design is to generate and use hydroxyl free radical (HO·) as strong oxidant to destroy compound that can not be oxidized by conventional oxidant. Different approaches have been used for the treatment of industrial effluent by means of AOPs, like treatment by UV (Zhou and Smith, 2002; Stanis Law and Monika ,1999), Ozonation (Baig and Liechti, 2001, Zhou and Smith, 2002,)

combined methods like O₃/UV (Hung-Yee and Ching-Rong 1995, Perkowski and Kos 2003, Bes-Piá *et al.* 2003, Azbar *et al.* 2004), H₂O₂/UV (Georgiou *et al.*, 2002; Mariana *et al.*, 2002; Rosario *et al.*, 2002; Tanja *et al.*, 2003), Ozone/H₂O₂ (Tanja *et al.* 2003, Rein, 2001) and Ozone /UV/H₂O₂ (Arslan and Isil 2002, Azbar *et al.* 2004).

AOPs present inherent advantages, causing them to remain the most applied processes for the treatment of waste water and they are used as a cleaner, because no sludge or secondary pollution are generated and dyes are totally decomposed to low-molecular weight compounds like CO₂ and H₂O. In addition they involve a minimal capital investment and an easy and fast operation procedure, with a high efficiency in the oxidation. On the other hand they can be economically unfeasible, because of elevate energetic costs.

Biological treatment of azo dyes

Physical and chemical treatment methods such as precipitation, coagulation, adsorption, flocculation, flotation, electrochemical destruction, and mineralization and decolorization process (Gogate and Pandit, 2004) have some disadvantages such as cost, time, and release of residues. All these techniques are minimizing the toxicity level not to neutralize the toxicity (Cooper, 1993; Maier *et al.*, 2004;). To alternate these techniques, microorganism can be used to completely degrade the azo dyes (Verma and Madamwar, 2003; Moosvi *et al.*, 2005; Pandey *et al.*, 2007; Khalid *et al.*, 2008), because microorganisms reduce the azo dyes by secreting enzymes such as laccase, azo reductase, peroxidase, and hydrogenase. The reduced forms of azo dyes are further mineralized into simpler

compounds and are utilized as their energy source (Stolz, 2001). Based on the available literature, the microbial decolorization of azo dyes is more effective under combined aerobic and anaerobic conditions (Chang and Lin, 2000; Isik and Sponza, 2004; Van der Zee and Villaverde, 2005; Lodato *et al.*, 2007). A wide range of microorganisms are capable of degrading a variety of azo dyes including bacteria, actinomycetes, fungi and yeast (Gingell *et al.*, 1971; Paszczynski *et al.*, 1991; Martins *et al.*, 1999; Kirby *et al.*, 2000; Wesenberg *et al.*, 2003; Olukanni *et al.*, 2006). They have developed enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions (Anjali *et al.*, 2006).

Bacterial degradation

The bacterial reduction of the azo dye is usually nonspecific and bacterial decolorization is normally faster. A wide range of aerobic and anaerobic bacteria such as *Bacillus subtilis*, *Pseudomonas* sp, *Escherichia coli*, *Rhodobacter* sp, *Enterococcus* sp, *Staphylococcus* sp, *Xenophilus* sp, *Corneybacterium* sp, *Clostridium* sp., *Micrococcus dermacoccus* sp, *Acinetobacter* sp, *Geobacillus*, *Lactobacillus*, *Rhizobium*, *Proteus* sp, *Morganella* sp, *Aeromonas* sp, *Alcaligenes* sp, and *Klebsiella* sp have been extensively reported as degraders of azo dyes (Stolz., 2001; Pearce *et al.*, 2003; Olukanni *et al.*, 2006; Vijaykumar *et al.*, 2007; Hsueh and Chen, 2008; Lin and Leu, 2008). Some strains of aerobic use azo dyes as sole source of carbon and nitrogen (Coughlin *et al.*, 2002); others only reduce the azo group by special oxygen-tolerant azo reductases.

A number of research groups investigated

the ability of bacteria in metabolism of azo dyes. Azo dyes are not readily metabolized under aerobic condition, and as a result of metabolic pathways it degraded into intermediate compounds but not mineralized. It can be completely degraded under coupled aerobic–anaerobic degradation (Mcmulan *et al.*, 2001). In anaerobic condition, the azo bond undergoes cleavage to generate aromatic amines and it was mineralized by non-specific enzymes through ring cleavage under aerobic condition. Therefore, coupled anaerobic treatment followed by aerobic treatment can be an efficient degradation method of azo dyes (Feigel *et al.*, 1993). Chen *et al.* (2003) have described bacterial strains which display a good growth in aerobic or agitation culture, but color removal was obtained with a high efficiency in anoxic or anaerobic culture. Mixed bacterial culture can give a better degradation rate than the individual strain.

Fungal degradation

The most widely explored fungi in regard to dye degradation are the ligninolytic fungi (Bumpus, 2004). Apart from this, *Phanerochaete chrysosporium*, *Coriolus versicolor*, *Trametes versicolor*, *Fungalia trogii*, *Penicillium gastrivorous*, *Rhizopus oryzae*, *Pleurotus ostreatus*, *Rigidoporus lignosus*, *Pycnoporus sanguineus*, *Aspergillus flavus*, and *Aspergillus niger* have been reported which are capable of degrading azo dyes (Fu and Viraraghavan, 2001; Wesenberg *et al.*, 2003).

Large literature exists regarding the potential of these fungi is to oxidize phenolic, nonphenolic, soluble and non-soluble dyes (Conneely *et al.*, 1999;

Tekere *et al.*, 2001; Kapdan and Kargi, 2002; Libra *et al.*, 2003). White-rot fungi produces lignin peroxidase, manganese-peroxidase and laccase that degrades many aromatic compounds due to their nonspecific enzyme systems (Robinson *et al.*, 2001; Wesenberg *et al.*, 2003; Toh *et al.*, 2003; Forgacs *et al.*, 2004; Harazono and Nakamura, 2005; Mohorcic *et al.*, 2006; Madhavi *et al.*, 2007).

Although stable operation of continuous fungal bioreactors for the treatment of synthetic dye solutions have been achieved, application of white-rot fungi for the removal of dyes from textile wastewaters faces many problems such as large volumes produced, the nature of synthetic dyes, and control of biomass (Palma *et al.*, 1999; Nigam *et al.*, 2000; Zhang and Yu, 2000; Robinson *et al.*, 2001; Mielgo *et al.*, 2001; Stolz, 2001). In the former type, the cells produce enzymes such as laccase, Manganese peroxidase and lignin peroxidase to mineralize the dyes (Raghukumar *et al.*, 1996, Fu and Viraraghavan, 2001). Lignin peroxidase plays a major role in the degradation of azo dyes using *P. chrysosporium* (Ollikka *et al.*, 1993).

Enzymes in azo-dye degradation

Microbial degradation of azo dyes is mediated by enzymes. The predominant enzymes are azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. Laccase and azoreductase have been shown to degenerate azo dyes (Rodriguez *et al.*, 1999; Reyes *et al.*, 1999). Dye molecules display a high structural variety, they are degraded only by few enzymes. Enzymatic processes are very promising for the decolorization of synthetic azo dyes. To understand the decolorization and degradation mechanism

of azo compounds under aerobic conditions (redox-active), exhibit relatively wide substrate specificities (Duran and Esposito 2000; Mester and Tien 2000). Wide variety of microorganisms excrete different active enzymes like laccases, phenolic oxidases, peroxidases and variety of azo-dye reductases enzymes.

Laccases

Laccase enzymes degrade the azo dye through a non-specific free radical mechanism to form phenolic compounds and thereby prevent the formation of toxic aromatic amines (Chivukula *et al.*, 1995).

The mechanism of laccase involves the removal of an H⁺ atom from the hydroxyl and amino groups of the ortho and para substituted phenolic substrates and aromatic amines.

Laccases have been extensively studied for their degradation of azo dyes (Chivukula *et al.*, 1995; Kirby *et al.*, 2000; Peralta *et al.*, 2003; Blaquez *et al.*, 2004; Novotny *et al.*, 2004). These enzymes are multicopper phenol oxidases that decolorize azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines (Chivukula *et al.*, 1995; Wong and Yu, 1999).

Usually laccase oxidizes the phenolic group of the azo dye, a nucleophilic attack by water on the phenolic ring carbon bearing the azo linkages (Susana *et al.*, 2005). During this reaction, cross-coupling of the reactive species results in the formation of C-C and C-O bonds between phenolic molecules and of C-N and N-N bonds between aromatic amines (Andrea

et al., 2005). Laccase preparations obtained from *Pleurotus ostreatus*, *Schizophyllum commune*, *Sclerotium rolfsii* and *Neurospora crassa*, increased up to 25% the decolorization of individual commercial triarylmethane, anthraquinonic, and indigoid textile dyes (Abadulla *et al.*, 2000).

Lacasses is a blue copper oxidase that catalyzes the four electron reduction of molecular oxygen (O₂) to water (H₂O). These enzymes are mainly obtained from lignin degrading fungi such as *Trametes versicolor* and *T.villoa* as well as fungi like *Fusarium soloni* and *Cladospora cladosporioides*. (abedin *et.al*,2008)

Azo dye reduction by peroxidases

Azo reductase mediates the azo cleavage in the presence of reducing equivalents like FADH and NADH. Azo reduces have been identified in several anaerobic bacteria and microflora of human intestine. Azoreductase of these bacteria exhibits similar function, i.e., reduce azo dyes to aromatic amines (Rafii *et al.*, 1999)

The azoreductase gene has been identified in *Azospirillum brasilens*, *Bacillus subtilis*, *B. stearothermophilus*, *Pseudomonas aeruginosa*, and *Mycoplasma pneumoniae* (Yasuhko *et. al.*, 2001). Fungi usually degrade dyes by exo enzymes like peroxidases (Duran *et al.*, 2002) and phenol oxidases (Glenn *et al.*, 1986). The ligninolytic fungi like *P. chrysosporium* produces lignin peroxidase (LiP) (Glenn *et al.*, 1983; Tien *et al.*, 1983) and manganese peroxidase (MnP) (Wariishi *et al.*, 1988). Several reports have shown that LiP or MnP are directly involved in the degradation of various dyes (Paszczynski

et al., 1991; Pasti *et al.*, 1992; Goszczynski *et al.*, 1994;).

Manganese peroxidase was reported as the main enzyme involved in dye decolorization by *Phanerochaete chrysosporium* (Chagas and Durrant, 2001) and lignin peroxidase for *Bjerkandera adusta* (Robinson *et al.*, 2001). Some non-white-rot fungi that can successfully decolorize dyes have also been reported by Bumpus (2004).

Degradation of azo dyes by azo reductases

Azo reductases are membrane bound enzyme that catalyzes the reaction only in presence of reducing equivalents like FADH and NADH (Robinson *et al.*, 2001). So the reduction process is taken place in bacterial cells with intact cell membranes (Russ *et al.*, 2000). In Gram negative bacteria, enzyme can make direct contact with either the azo dye substrate or a redox mediator at the cell surface (Myers and Myers, 1992). In addition, some low molecular weight redox mediator compounds can act as electron shuttles between the azo dye and an NADH - dependent azo reductase that is situated in the outer membrane (Gingell and Walker, 1971). These enzymes are oxygen sensitive, so in extracellular environment, this reduction mechanism will be inhibited by oxygen. Kudlich *et al.*, (1997) reported that the membrane-bound and the cytoplasmic azo reductases are two different enzyme systems. The whole mechanism for the redox reaction was under anaerobic condition, where as the redox mediators depend on cytoplasmic reducing enzymes to supply electrons (Yoo *et al.*, 2001).

Table.3 Microbial decomposition of azo related industrial dyes

Strain	Organisms	Dye	Reference
Bacteria	<i>Enterococcus faecalis</i> YZ 66	Reactive Orange II	Sahasrabudhe and pathade <i>et.al.</i> ,2011
	<i>Enterobacter agglomerans</i>	Methyl Red	Keharia <i>et.al.</i> , 2003
	<i>Enterobacter sp</i>	CI Reactive Red 195	Kalyani <i>et.al.</i> , 2007
	<i>Bacillus subtilis</i>	Acid blue 113	Gurulakshmi <i>et.al.</i> , 2008
	<i>Brevibacillus laterosporus</i> MTCC2298	Navy blue 3G	Jirasripongpun <i>et.al.</i> , 2007
	<i>Bacillus Fusiformis</i> kmk 5	Acid Orange 10 & Disperse Blue 79	Kolekar <i>et.al.</i> , 2008
Fungi	<i>Geotrichum sp</i>	Reactive black 5, Reactive red 158 & Reactive yellow 27	Kuhad <i>et.al.</i> , 2004
	<i>Shewanella sp.NTOVI</i>	Crystal violet	Chen <i>et.al.</i> , 2008
	<i>Phanaerochaete chrysosporium</i>	Orange II	Sharma <i>et.al.</i> , 2009
	<i>Aspergillus ochraceus</i> NCIM-1146	Reactive blue 25	Parshetti <i>et.al.</i> , 2007
Algae	<i>Spirogyra rhizopus</i>	Acid red 247	Ozer <i>et.al.</i> , 2006
	<i>Cosmarium sp</i>	Triphenylmethane dye & Malachite green	Daneshvar <i>et.al.</i> , 2007
Actinomycetes	<i>Streptomyces ipomoea</i>	Orange II	Molina-Guijarro <i>et.al.</i> , 2009
Yeast	<i>Kluyveromyces marxianus</i> IMB3	Ramazol black B	Meehan <i>et.al.</i> , 2000
	<i>Saccharomyces cerevisiae</i> MTCC463	Methyl red	Jadhav <i>et.al.</i> , 2007

Table.4 Enzymes mediated decolorization of some dyes

Substrates	Enzyme	Source of Enzyme	Reference
3-(4dimethyl amino-1 phenylazo) Benzene sulfonic acid	laccase	<i>Trametes villosa</i>	Zille <i>et.al.</i> , 2004
Acid Orange 6, Acid Orange 7, Methyl Orange and Methyl Red	Mixture of Bacterial Oxidoreductases	Sludge Methonogens	Kalyuzhnyi <i>et.al.</i> , 2006
Direct Yellow	Horse radish peroxidases	<i>Armoracia rusticana</i>	Maddhinni <i>et.al.</i> , 2006
Acid Blue	Laccase	<i>Cladosporium cladosporioides</i>	Vijaykumar <i>et.al.</i> , 2006
Tartrazine and Ponceau	Azoreductase	Green algae	Omar, 2008
Reactive Yellow, Reactive Black, Reactive Red and Direct Blue	Azoreductase	<i>Staphylococcus arlettae</i>	Franciscon <i>et.al.</i> , 2009.

The direct enzymatic reaction of an azo reductase, may be a dehydrogenase enzyme that is synthesized throughout the cytoplasm (Bragger *et al.*, 1997).

Enhancement of biodecolorization

Dye degradation was performed under microaerophilic conditions until no residual color was observed. The medium was subsequently aerated by stirring to promote oxidation of the aromatic amines formed by the reductive break-down of the azo bond into non-toxic metabolites. (Franciscon Elisangela *et al.*, 2009)

The of sodium pyruvate and yeast extract as carbon sources on the decolorization were also investigated, since it has been reported that the type of carbon source could affect dye decolorization and its subsequent reduction (Nigam *et al.*, 1996; Bra's *et al.*, 2001; Kim *et al.*, 2008). The amine concentrations and TOC were monitored during the biodegradation process. The degradation products were also characterized using FT-IR and UV-vis techniques, and their toxicity were measured.

A facultative *Staphylococcus arlettae* bacterium, isolated from an activated sludge process in a textile industry, was able to successfully decolorize four different azo dyes under microaerophilic conditions (decolorization percentage >97%). Using a single *Staphylococcus arlettae* strain in the same bioreactor, the sequential microaerophilic/aerobic stages were able to form aromatic amines by reductive break-down of the azo bond and to oxidize them into non-toxic metabolism (Elisangela *et al.*, 2009).

Release of textile industry effluent into water bodies creates a major threat to the

natural resources as well as human health and hygiene. Azo dyes are widely used in textile industry, and about 50% of the dye stuffs were released in effluent and discharged into nearby water bodies. It will disperse into nearby water bodies.

It will be dispersed to the ground water and leads to physiological changes in salinity, unfit for consumption, and disrupt the biodiversity by bioaccumulation and biomagnifications. The concentration of the dyes was increased at the end of food chain, which causes tumor, cancer, nervous disorder, and even lethal. To avoid all these problems, the textile effluents have to be degraded to avoid toxicity. As an emerging technique, microbial degradation is one of the best technique to detoxify the azo dyes. Now a days, most of the research is focused on the biodegradation of textile dye due to the environmental pollution.

Acknowledgement

We wish to thank our Institution of Nadar Saraswathi College for Women and also Department of Microbiology & Biochemistry for providing the necessary facilities for this study.

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