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Review Article

Microorganism-Based Treatment of Azo Dyes

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Abstract

Azo Dyes are the largest class of aromatic dyes having lots of commercial interest. These dyes are mostly used in textile industries. Dyes used in textile industries such as CI disperse green, CI disperse blue, anthraquinone disperse dyes are very difficult to remove by traditional conventional methods since they are stable to light and oxidizing agents like (hydrogen peroxide and potassium dichromate) and are resistant to aerobic digestion. These dyes are carcinogenic both for animal and human beings. Biological treatment either by bacteria, fungi or consortia of both have been reported to reduce the toxicity of the dye to the permissible limit of discharge to the environment. This present review demonstrated the importance of microorganisms to reduce these azo dyes and protect the environment from the devastating effect of these dyes.

Key words: Azo dye, textile industries, microorganisms, carcinogenic

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INTRODUCTION

Environmental pollution is one of the major and most urgent problems of the modern world. Industries such as paper and pulp mills, dyestuff, distilleries, textile industries and tanneries are producing highly coloured wastewaters, polluting the environment discharging effluents from the dyeing process, with both strong persistent colour and a high Biological Oxygen Demand (BOD), both of which are aesthetically and environmentally unacceptable. In general, the final textile waste effluent can be broadly categorized into three types, high, medium and low strength on the basis of their COD (Chemical Oxygen Demand) content (Table 1) (Wang *et al.*, 2007).

The textile industry plays a major role in the economy of Asian and other countries. In India, it accounts for the largest consumption of dyestuffs at 80% (Mathur *et al.*, 2005a), taking in every type of dye and pigment produced, this amounts to close to 80,000 t. India is the second largest exporter of dyestuffs, after China. Worldwide, 10^6 t of synthetic dyes are produced annually, of which $1-1.5 \times 10^5$ t are released into the environment in wastewaters (Zollinger, 1991). This release is because not all dye binds to the fabric during the dyeing processes; depending on the class of the dye, the losses in wastewaters can vary from 2% for basic dyes to as high as 50% for reactive dyes, leading to severe contamination of surface and ground waters in the vicinity of dyeing industries. It is estimated that globally 280000 t of textile dyes are discharged in textile industrial effluent every year (Jin *et al.*, 2007). Originally dyes agent are two types, natural colouring agent and organic dyestuffs. Natural colouring agents are mainly of inorganic origin (clays, earths, minerals, metal salts and even semi-precious stones, such as malachite) or organic dyestuffs traditionally divided into 2 groups, one of animal and the other of plant origin (Ackacha *et al.*, 2003). Undoubtedly, plants are the most important sources of dyes, but few other organism like lichens, insects and shellfish were also reported to be good sources of natural dyes. Organic dyes present a broad spectrum of compounds with different physical and chemical properties (O'Neill *et al.*, 1999).

Among them, anthraquinone red colorants (e.g., cochineal, lac dye or madder root) are of special interest.

Madder root has a long tradition as a dyestuff because of its bright red colour. The red pants of Napoleon's army and the red coats of the English soldiers in the 18/19th century were dyed with madder. Moreover, not every shade is directly available from a natural source. Synthetic dyes quickly replaced the traditional natural dyes. They cost less, offered a vast range of new colors and imparted improved properties to the dyed materials (Young and Yu, 1997).

Dyes: In 1856, William Henry Perkin accidentally discovered the world's first commercially successful synthetic dye. By the end of the 19th century, 10 000 new synthetic dyes had been developed and manufactured. Nowadays, India, the former USSR, Eastern Europe, China, South Korea and Taiwan consume 600 kt of dyes per annum. Since 1995, China has been the leading producer of dyestuffs, exceeding 200 kt year⁻¹ (Wesenberg *et al.*, 2003). A large variety of dyestuffs is available, which can be natural or synthetic substances, but synthetic dyes are commonly used for textile fibers, whereas natural dyes tend to be reserved for the food industry.

Azo dyes: Azo dyes have diversity in structure but their most important structural feature is presence of azo linkage i.e., N=N-. This linkage may be present more than one time and thus mono azo dyes have one azo linkage while two in diazo and three in triazo, respectively. These azo groups are connected on both sides with aromatics like benzene and naphthalene moiety. Sometimes aromatic heterocyclic units are also present being connected with azo groups (Zollinger, 1991). Different shades of the same dye having various intensities of color are due to these aromatic side groups (McMullan *et al.*, 2001). Azo dyes containing sulfonate groups as substituent are called as sulphonated azo dyes. Azo groups in conjugation with aromatic substituents or enolizable groups make a complex structure which lead to huge expression of variation of colors in dyes (Rajaguru *et al.*, 2002).

Impact of azo dyes: Azo dyes produce clear and ambient colors. They are primarily used for colouring cotton, leather, cosmetics and food items. Azo dyes belong to a group of organic compounds. The azo 14 group of dyes binds to an aromatic ring. Through mineralization, these dyes can be splitted 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 and intake may lead to the risk of cancer and allergic reactions, an irritant for the eyes and extremely dangerous, if inhaled or consumed (Nikulina *et al.*, 1995). For example, para-phenylene diamine

Table 1: Some characteristics of typical wastewater effluent

Wastewater type	COD (mg L ⁻¹)	Conductivity (μsec cm ⁻¹)
High strength	1500	2900
Medium strength	970	2500
Low strength	460	2100

(PPD) also called 1,4-diamino benzene or 1,4-phenylene diamine, is an aromatic amine, which is a major component of azo dyes. The PPD-containing azo dyes are toxic and cause skin irritation, contact dermatitis, chemosis, lacrimation, exophthalmos 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 (Young and Yu, 1997). Some azo dyes are carcinogenic and mutagenic. 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 (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).

Reactive dyes cause asthma rhinitis and dermatitis (Nilsson *et al.*, 1993) allergic contact dermatitis (mutagenicity (Mathur *et al.*, 2005b), genotoxicity (Dogan *et al.*, 2005), carcinogenicity (De Roos *et al.*, 2005; Gonzales *et al.*, 1988) (Table 2). Dyes have a very low rate of removal ratio for BOD to COD (less than 0.1). Therefore, industrial effluents containing dyes should be processed before their discharge into the environment (Wong *et al.*, 2003).

Treatment of effluent from textile industry: The textile fishing industry has been put under immense pressure to

reduce use of harmful substances, especially mutagenic carcinogenic and allergenic effects of textile chemicals and textile dyes. There are regulations regarding the colour limits in effluents, which vary in different countries. Textile dye wastewater remediation is based not only in colour removal (decolorization), but also in the degradation and mineralization of the dye molecules (Chang *et al.*, 2001). Indeed, decolorization occurs when the molecules are removed from the solution or when the chromophore bond is broken, but the molecule in the first case and the major fragments in the second, remain intact. The absorption of light by the associated molecules shifts from the visible to the ultraviolet or infrared region of the electromagnetic spectrum. A wide range of technologies has been developed for the removal of synthetic dyes from waters and wastewaters to decrease their environmental impact. These include physical, chemical and biological methods (Table 3).

Microbial decolorization of dyes: Effluents from the textile industries contain reactive dye in a concentration range of 5-1500 mg L⁻¹. Processing of dye contaminated effluents is currently a primary environmental problem (Lata *et al.*, 2007). Conventional treatment methods such as activated sludge process, chemical coagulation carbon absorption, chemical oxidation, photo decomposition, electro-chemical treatment, reverse osmosis, hydrogen peroxide catalysis etc. Some of these techniques are even effective, although they have some shortcomings, excess amount of chemical usage with obvious disposal problem, costly plant requirements or operating

Table 2: Effect of Azo dyes on environment and human health

Name of the dye	Effects	References
Reactive brilliant red	Inhibits function of human serum albumin	Li <i>et al.</i> (2010)
Acid violet 7	Induce chromosomal aberration, lipid peroxidation, acetyl cholinesterase in mice	Ben Mansour <i>et al.</i> (2010)
Disperse red-1 and Disperse orange-1	Increase the frequency of micronuclei in human lymphocytes	Chequer <i>et al.</i> (2009)
Reactive black 5	Decrease urease activity, arginine ammonification rate in terrestrial ecosystem	Topac <i>et al.</i> (2009)
Disperse blue 291	Mutagenic, Cytotoxic, genotypic effects, formation of micronuclei, DNA fragmentation in human hepatoma cells	Tsuboy <i>et al.</i> (2007)

Table 3: Effluent treatment methods

Physical	Chemical	Biological
Sedimentation	Neutralization	Stabilisation
Filtration	Reduction	Aerated lagoons
Floatation	Oxidation	Trickling filters
Foam fractionation	Catalysis	Activated sludge
Coagulation	Ion exchange	Anaerobic digestion
Reverse osmosis	Electrolysis	Fungal treatment
Solvent extraction	-	Flocculation
Ionisation radiation	-	-
Adsorption	-	-
Incineration	-	-
Distillation	-	-
Membrane treatment	-	-

Table 4: Merits and demerits of physical treatment method (Robinson *et al.*, 2001)

Physical and chemical treatment methods	Merits	Demerits
Coagulation/Flocculation	Simple, economic feasible	Large amount sludge production, handling and disposal problems, more amount chemicals required for pH adjustment
Membrane separation	All types of dyes are decolorized	High pressure, expensive, sludge generation in capable for large scale treatment
Ion exchange	Effective with no loss of redevelopment	Renewal is possible economic constraints, not effective for some dyes
Oxidation	Hasty and efficient process	High energy cost, chemical required, secondary metabolite production
Advanced oxidation process	No sludge production, little consumption of chemicals, efficiency for recalcitrant dyes	Economically impracticable
Adsorption on activated carbon	Effective	Expensive loss of absorbent
Fenton's reagent	Proficient decolorization for both soluble and insoluble dyes	Sludge formation
Photochemical Irradiation	No sludge production	Formation of secondary metabolic pollutants
Advanced oxidation process	Effective only in laboratory level	Need dissolved oxygen
Adsorption on activated carbon	No sludge production, little consumption of chemicals, efficiency for recalcitrant dyes	Economically impracticable
Fenton's reagent	Effective	Expensive loss of absorbent
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	No sludge production	Formation of secondary metabolic pollutants
	Effective only in laboratory level	Need dissolved oxygen

expenses lack of effective color reduction and particularly for sulfonated azo dyes and sensitivity to a wastewater input. Techniques by chemical oxidation using sodium hypochlorite to remove the color release a lot of aromatic amines which are carcinogenic or toxic compounds. Physical and chemical methods are effective for color removal but need of more energy and chemicals than biological processes and sometimes it causes pollution into solid or liquid side streams and it requires additional treatment or disposal (Table 4).

To alternate these techniques, microorganism can be used to completely degrade the azo dyes (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).

So, the treatment of dyes focus on some microorganisms which are to biodegrade and biosorb dye in wastewater. Number of microorganisms possesses dye decolorizing ability like bacteria (Shah, 2014), fungi, algae, actinomycetes and yeast which is reported (Table 5).

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 geastrivorus*, *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). 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; Revankar and Lele, 2007). Althoughs table 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 (Zhang and Yu, 2000; Robinson *et al.*, 2001; Mielgo *et al.*, 2001; Stolz, 2001).

Yeast degradation: Very little work has been devoted to the study of the decolourising ability of yeast, most often mentioning sorption as the main cause (Meehan *et al.*, 2000). Nevertheless, there are some reports on biodegradation by yeast strains, such as *Candida zeylanoides* (Martinez *et al.*, 1999), *Candida zeylanoides* and *Issatchenkia occidentalis* (Ramalho *et al.*, 2002, 2004, respectively). Yeast cells, like bacteria are capable of azo dye reduction to the corresponding amines. Testing adapted and unadapted cultures (Ramalho *et al.*, 2004) found that the azo dye reduction activity was due to a constitutive enzyme and that activities were dependent on intact, active cells. Moreover,

Table 5: Microbial decomposition of azo related industrial dyes

Strain	Microorganism	Dye	References
Fungi	<i>Trametes villosa</i>	Drimaren Brilliant Blue	Machado and Matheus (2006)
	<i>Pycnoporus sanguineus</i>	Remazol Brilliant Blue	Palmieri <i>et al.</i> (2005)
	<i>Pleurotus ostreatus</i>	R	Deveci <i>et al.</i> (2004)
	<i>Funalia trogii</i>	Remazol Brilliant Blue	Parshetti <i>et al.</i> (2007)
	<i>Aspergillus ochraceus</i>	R	Kuhad <i>et al.</i> (2004)
	<i>Geotrichum</i> sp.	Reactive Blue-25	Chen <i>et al.</i> (2003)
	<i>Shewanella</i> sp. NTOVI	Reactive black-5, Reactive red 158 and Reactive yellow 27	Sharma <i>et al.</i> (2009)
	<i>Phanaerochaete chrysosporium</i>	Crystal violet Orangell	
Bacteria	<i>Rhizobium radiobacter</i>	Reactive Red 141	Telke <i>et al.</i> (2008)
	<i>Citrobacter</i> sp., CK3	Reactive Red 180	Wang <i>et al.</i> (2009)
	<i>Pseudomonas</i> sp.	Reactive Red 2	Kalyani <i>et al.</i> (2009)
	<i>Enterococcus faecalis</i>	Reactive Orange II	Sahasrabudhe and Pathade (2011)
	YZ 66	Methyl Red	Keharia and Madamwar (2003)
	<i>Enterobacter agglomerans</i>	Reactive Red 195 Acid blue 113	Kalyani <i>et al.</i> (2008)
	<i>Enterobacter</i> sp.	5 Acid Orange 10 and Disperse Blue 79	Gurulakshmi <i>et al.</i> (2008)
	<i>Bacillus subtilis</i>		Kolekar <i>et al.</i> (2008)
Algae	<i>Bacillus fusiformis</i> KMK		Karacakaya <i>et al.</i> (2009)
	<i>Synechocystis</i> sp.	Reactive Red	
Actinomycetes	<i>Phormidium</i> sp.	Remazol Blue, Reactive Black B	
	<i>Streptomyces ipomoea</i>	Orange II	Molina-Guijarro <i>et al.</i> (2009)
Yeast	<i>Kluyveromyces marxianus</i> IMB3	Remazol Black B	Meehan <i>et al.</i> (2000) and Jadhav and Govindwar (2007)
	<i>Saccharomyces cerevisiae</i> MTCC463		

they noted that *I. orientalis* (a dye reducer strain) has an absolute requirement of oxygen. Compared to bacteria and filamentous fungi, yeasts have some of the advantages of absolute requirement of oxygen. Compared to bacteria and filamentous fungi, yeasts have some of the advantages of both; they not only grow rapidly like bacteria, but like filamentous fungi, they also have the ability to resist unfavorable environments (Yu and Wen, 2005). In yeast, the ferric 21 reductase system participates in the extracellular reduction of dyes (Ramalho *et al.*, 2005).

Bacterial degradation: Efforts to isolate bacterial cultures capable of degrading azo dyes started in the 1970s with reports of *Bacillus subtilis* (Horitsu *et al.*, 1977), followed by *Aeromonas hydrophila* (Idaka *et al.*, 1978) and *Bacillus cereus* (Wuhrmann *et al.*, 1980). Khadijah *et al.* (2009) isolated 1540 bacteria and screened them for the ability to degrade azo dyes; from the initial screening in microtitre plates, 220 isolates showed decolorization potential, of which 37 showed higher decolorized zones on dye-incorporated agar plates. In the final screening in liquid medium, 9 proved capable of degrading a wide spectrum of dyes. Bacteria degrade azo dyes reductively under anaerobic conditions to give colourless aromatic amines. These in turn need to be further degraded due to their possible toxic, mutagenic and/or carcinogenic character in humans and animals. Anthraquinonic dyes are less susceptible to anaerobic

reduction. Whole cell biodegradation is often carried out by a number of enzymes working sequentially; however, as with other microorganism, only a few of the expressed bacterial enzymes are directly involved in dye biotransformation. The bacterial enzymes involved in the reductive azo bond cleavage are usually azoreductases, whose actions may depend on the presence of other substances such as cofactors, co-substrates or mediators. To avoid the formation of carcinogenic amines, aerobic conditions are preferable in aromatic amine degradation (Balapure *et al.*, 2015), but it should also be noted that some of them may be auto-oxidized to polymeric structures in the presence of oxygen (Kudlich *et al.*, 1999). Undeniably, the isolation of bacteria capable of aerobic decolourisation and mineralization of dyes has attracted interest, although, especially for sulfonated azo dyes, things have proven difficult (McMullan *et al.*, 2001).

Contrary to the unspecific mechanism of azo dye bacterial reduction under anaerobic conditions, aerobic bacteria usually need to be specifically adapted to achieve a significant reductive process. This adaptation involves long-term aerobic growth in continuous culture in the presence of a very simple azo compound. Induction leads to the bacteria synthesis of azoreductases, specific for the reduction of the inducer azo compound or even others related compounds, in the presence of oxygen (Stolz, 2001). The use of bacteria is influenced by factors at the level of the cell, which in turn will influence the permeability and diffusion of dye molecules. Parameters, such

as cell density, enzymes per cell, enzymatic catalytic efficiency, substrate charge and even cell permeability, can be modeled in order to achieve the highest removal rate (Martinez *et al.*, 1999). Generally, unlike fungi, bacteria show better decolourisation for dyes-environmental impact and Remediation 141 and biodegradation activities at basic pH. In comparison to fungi, bacterial decolorization tends to be faster (Kalyani *et al.*, 2009).

Decolorization by mixed cultures: Utilization of microorganism consortia offers considerable advantages over the use of pure cultures in the degradation of synthetic dyes (Sudha *et al.*, 2014). Using mixed cultures instead of pure cultures, higher degrees of biodegradation and mineralization can be achieved due to synergistic metabolic activities of the microbial community (Ramalho *et al.*, 2004; Khehra *et al.*, 2005; Ali, 2010). The individual strains can attack dye molecules at different positions, yielding metabolic end products that may be toxic; these can be further metabolized as nutrient sources to carbon dioxide, ammonia and water by another strain. Other species present may not be involved in bioremediation at all, but can stabilise the overall ecosystem (Kandelbauer and Guebitz, 2005). This type of mineralization is the safest way to assure that no potentially harmful and unrecognized intermediate degradation products are released into the environment. Mixed consortia usually do not require sterile conditions and have greater stability towards changes in the prevailing conditions (pH, temperature and feed composition) compared with pure cultures (Ramalho *et al.*, 2004). Therefore, the use of mixed cultures is a good strategy for bioreactors.

Aerobic/anaerobic degradation of dyes: Under aerobic conditions, bacteria produce an enzyme which helps to break down the organic compounds in wastewater. *Rhizopus oryzae*, *Cyathus bulleri*, *Coriolus versicolor*, *Funalia trogii*, *Laetiporus sulphureus*, *Streptomyces* sp., *Trametes versicolor* and other microorganisms decolorize dyes in aerobic conditions (Salony and Bisaria, 2006; Zhang *et al.*, 1999). Most of the dyes are recalcitrant for the biological degradation or nontransferable under aerobic conditions (Pagga and Brown, 1986; Rai *et al.*, 2005). Under anaerobic condition, the reactive dyes are decolorized effectively by using glucose as a carbon source (Carliell *et al.*, 1994). Conventional sewage method under anaerobic condition decolorized reactive red 141. The reactive red 141 was decomposed under anaerobic condition by the cleavage of azo bond by the microbial community resulted in the formation of 2 aminonaphthalene-1, 5 disulfonic acid (Carliell *et al.*, 1995). The addition of salts such as nitrate and sulfate decolorized reactive red 141 under

anaerobic condition. An anaerobic aerobic treatment process by mixed culture of bacterial isolated from textile dye effluent was used to decolorize the reactive azo dyes such as remazol brilliant orange 3R, remazol black B and remazol brilliant violet 5R (Supaka *et al.*, 2004; Popli and Patel, 2015). Treatment of synthetic dye waste water at the combination of anaerobic and aerobic conditions showed that the majority of colors are removed by the anaerobic process, whereas the Chemical Oxygen Demand (COD) is removed by the aerobic process (Rajaguru *et al.*, 2002; Supaka *et al.*, 2004). Mixture of bacterial isolates from domestic sewage treatment plant has been reported to be effective in decolorization of reactive azo dyes, red RB, blue M2B and yellow. The mixed cultures decolorize 95% of red RB and blue M2B. Decolorization remarkably enhance when peptone is used in the medium for growing the mixed culture (Vijaya *et al.*, 2003).

Azo based dyes are recalcitrant to degrade by conventional treatment method. The biological treatment is an effective and alternate method to decolorize and mineralize the dyes in effluent without leaving harmful by-products. In biological treatment the microorganisms biosorb/degrade the dyes with the help of some enzymes such as laccase, lignin peroxidase, manganese peroxidase etc. Both anaerobic and aerobic condition is required for complete degradation of reactive dyes (Kulla *et al.*, 1983).

Only bacteria with specialized azo dye reducing enzymes were found to degrade azo dyes under fully aerobic conditions. Due to their recalcitrance in aerobic environments, the azo dyes eventually end up in anaerobic treatment process which is much less specific (Russ *et al.*, 2000). This anaerobic reduction implies decolorization as the azo dyes are converted to usually colorless but potentially harmful, mutagens and carcinogens aromatic amines which cannot be regarded as environmentally safe end products (Chung *et al.*, 1992).

CONCLUSION

The textile, dyeing and finishing industry use wide variety of dyestuffs due to the rapid changes in the customer's demands. Discharge of effluent into open environment is serious environmental problem and the color removal of wastewater is major environmental concern. Physical and chemical methods are effective for color removal but need of more energy and chemicals processes and sometimes it causes pollution and generates high amount of sludge in environment. Hence, economical and eco-friendly techniques using bacteria can be applied for fine tuning of waste water treatment. Biotreatment offers, easy, cheaper and effective

alternative for colour removal of textile dyes. Utilization of microorganism consortia offers considerable advantages over the use of pure cultures in the degradation of synthetic textile dyes, instead of pure cultures, higher degrees of biodegradation and mineralization can be achieved due to synergistic metabolic activities of the microbial community. Consortia usually do not require sterile conditions and have greater stability towards changes in the prevailing conditions (pH, temperature and feed composition) compared with pure cultures. Therefore, the use of mixed cultures is a good strategy for bioreactors.

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