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Review Article
Microbial Strategies for Discoloration and Detoxification of Azo Dyes from Textile Effluents

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Abstract

Wastewater from the textile industries contains a variety of pollutants, particularly dyes. In this review, different methods used for decolorization and detoxification of azo dyes have been discussed. Various physical, chemical and biological treatment methods are being employed for decolorization of azo dyes. The majority of treatment methods work either by concentrating the color in to sludge, solid supports or by complete destruction of the dye molecules. Physical and chemical methods employed for textile wastewater treatment are insufficient, not always environment friendly and usually generate unacceptable levels of secondary pollution in the form of sludge. Bioremediation is being viewed as a clean and practicable alternative means to remediate color from dye containing wastewaters. The ability of microorganisms to decolorize and metabolize dyes has been well established. The use of bioremediation based technologies for treatment of textile azo dyes may be a more viable option for sufficiently cleaning dye containing wastewaters hazardous to human health and to the environment.

Key words: Azo dyes, bioremediation, decolorization, environment, textile waste

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INTRODUCTION

Most of the water used in dyeing process is discharged as highly colored effluent from the textile industry. The colored effluents are not only aesthetically unacceptable, but also prevent the passage of sunlight through contaminated waterways. This reduces the photosynthetic activity of aquatic flora, which causes depletion of dissolved oxygen, ultimately leading to death and putrefaction of aquatic fauna. It finally contributes to anaerobic oxidation which can be sensed from putrefying odor of receiving water bodies. Azo dyes are mostly used in textile, rubber, enamel, plastic, cosmetic and many other industries due to their ease and cost effectiveness. The azo dyes are toxic, mutagenic and carcinogenic not only to aquatic animals, but also to humans who come in contact with dyes/colored effluents.

The textile dyes are highly reactive and during processing, it is difficult to treat. About 100,000 commercially available dyes are known and nearly 1 million tons of dyes are produced throughout the year, whereas out of the total usage, 10% of dyes are released in environment as dyestuff waste. Azo dyes are poorly biodegradable because of their structure. The treatment of such dye containing effluent was initially carried out by using physical and chemical treatment processes including adsorption, concentration and chemical transformation. However, the physicochemical methods employed for dye decolorization are less efficient, costly and generate secondary waste, which create disposal problem. Alternatively, the treatment approach is shifting towards the use of conventional biological methods to treat such effluents and wastewater containing dyes and toxic chemicals. Traditional biological treatment methods alone or in combination with physical and/or chemical methods have also been attempted. However, the advance biological processes have received increasing attention due to low cost, their effectivity, less secondary sludge generation and eco-friendly nature[1].

The bacterial treatment process has been reported to be much faster than fungal decolorization/degradation of dyestuffs[2]. Hence, bacterial treatment has been preferred now-a-days. Bacterial decolorization of dyes is often initiated under static/anaerobic/microaerophilic conditions by an enzymatic transformation reaction[3] in which reductive cleavage of azo bond leads to the formation of colorless aromatic amines. These toxic amines are further oxidized to simpler non-toxic forms under aerobic conditions only[4,5].

Most of the research work conducted so far is centered on the first anoxic step of treatment leading merely to decolorization of colored azo dyes. The resultant intermediate metabolites are secondary amines, which are more toxic than the original dye, thereby posing serious health hazard to the ecosystem. This latter aspect has not been studied by most of the researchers. Therefore, the interest is now focused on bacteria which can perform high rate of dye decolorization under anoxic/microaerophilic conditions and subsequent aerobic oxidation of secondary amines.

Every bacterial culture exhibits specific cultural and nutritional requirements for the growth and extent of dyes decolorization. Several bacterial species capable of various dyes decolorization have been employed for the treatment of dyes under various optimized cultural (pH, temperature, anoxic/aerobic conditions, dye concentration, inoculum dose, etc.) and nutritional (carbon and nitrogen sources) conditions. Several researchers have employed various bacterial species for the decolorization and mineralization of different azo dyes [*Kocuria rosea* for malachite green[6], *Streptomyces krainskii* for reactive blue[7] 59, *Bacillus* sp. for reactive red[8] 5B, *Klebsiella* sp. for reactive yellow 107, *Comamonas acidovorans* and *Burkholderia cepacia* TM 5 for acid orange 7 and direct blue[9] 75, *Corynebacterium* sp. for reactive black 5 and reactive yellow[10] 15, *Acinetobacter calcoaceticus* NCIM2890 for amaranth[11], *Pseudomonas* sp. and *Bacillus* sp. for orange[12] 3R, *Streptococcus faecalis* and *B. subtilis* for dextile dye effluent[13], *Pseudomonas putida* for acid orange[5] 10 and *B. cereus* RMLAUI and *P. putida* SKG-1 for orange II and reactive orange 4, respectively][14-17].

Since bacterial dyes decolorization varies with varying cultural and nutritional parameters, their optimization should always be considered a prerequisite for bioremediation studies. The behavior of different bacterial strains vary for dye decolorization depending upon the dye used for the treatment process. The conditions optimized for synthetic pure dye decolorization may not be applicable to real effluent decolorization/detoxification, as the composition of textile waste water is variable and may also contain organic compounds, nutrients, salts, sulfur compounds, heavy metals and other toxicants, besides dyes. Any of these compounds may have an inhibitory effect on dye removal process. Some researchers have reviewed the treatment technologies for various textile azo dyes[18-21].

In this study, the researchers have attempted to compile the updated scattered information concerning various aspects of dyes decolorization and detoxification.

DYE DECOLORIZATION/DEGRADATION TECHNIQUES

Various physical, chemical and biological treatment techniques can be employed to remove color from dye containing wastewaters. In general, each technique has advantages and limitations. Dye removal strategies consist therefore mostly of a combination of different techniques[22] which are delineated in Fig. 1.

Microbial remediation: Alternative approaches to color removal utilizing microbial biocatalysts to reduce the dyes that are present in the effluent, offer potential advantages over the physicochemical process. Such systems are the focus of recent research groups. Over the past decades, many microorganisms are capable of degrading azo dyes, including bacteria[23-27], fungi, yeast, actinomycetes and algae[28,29].

Bacterial decolorization: The ability of bacteria to decolorize azo dyes has been investigated by a number of research groups. Several bacteria capable of dye decolorization have been reported by several researchers (Table 1). *Bacillus subtilis* was the first reported bacterial species employed for azo dye decolorization, followed by *Aeromonas hydrophila*[30] and *Bacillus cereus*[31]. Kulla[32] isolated first two *Pseudomonas* species, capable of textile dye decolorization. The extended period of adaptation in chemostat was required for the isolation of these cultures. Although, azo dyes are generally not degraded under aerobic conditions, Kulla[32] reported the ability of *Pseudomonas* strains to aerobically degrade certain azo dyes. However, the intermediates produced as a result of aerobic degradation disrupt the metabolic pathways and the dyes are not completely mineralized. Zimmermann *et al.*[23] observed that azoreductase enzyme was responsible for the initiation of orange II decolorization by the above two *Pseudomonas* isolates and that substitution of the groups near azo bond hindered the degradation process. The researchers also reported the potential of *Pseudomonas* KF46 on the azo dye orange II degradation. Thereafter, a Japanese group reported several other dye decolorizing *Pseudomonas* and *Aeromonas* species[33-35]. The azoreductase gene of efficient *Rhodococcus* species capable of decolorizing two sulphonated azo dyes (orange II and amido black) was cloned into a mutant that had lost the dye decolorization capability; the mutant regained the dye decolorization ability[36]. Hu[37] isolated *Pseudomonas luteola* strain from dyeing-wastewater-treatment sludge, which was capable of complete degradation of RBB, RP_2B and V_2RP dyes, while only decolorization of red G dye through azo bond cleavage. Nigam *et al.*[38] demonstrated that a mixture of dyes was decolorized by anaerobic bacteria in 24-30 h using free growing cells as well as by biofilms of bacteria on various support materials. Wong and Yuen[39] have reported strains of *Klebsiella pneumoniae* RS-13 and *Acetobacter liquefaciens* S-1 with the ability to decolorize azo dye methyl red and its possible suitability for the treatment of azo dye containing textile effluents. The aerobic-anaerobic decolorization and degradation of red HE7B in textile effluent was achieved employing *Pseudomonas desmolyticum*[40,41]. Zissi *et al.*[42] observed that *Bacillus subtilis* could be used to degrade a specific azo dye, p-aminobenzene. Coughlin *et al.*[43] reported that *Sphingomonas* sp., strain 1CX had an ability to decolorize 20 mg L^{-1} orange II, acid orange 8 and 10, acid red 4 and acid red dyes.

Chen *et al.*[1] isolated six bacterial strains from sludge samples and mud lakes with the capabilities of textile dyes degradation. Based on the greatest extent of color removal from various dyes, *Aeromonas hydrophila* was selected, which although exhibited good growth in aerobic/agitated

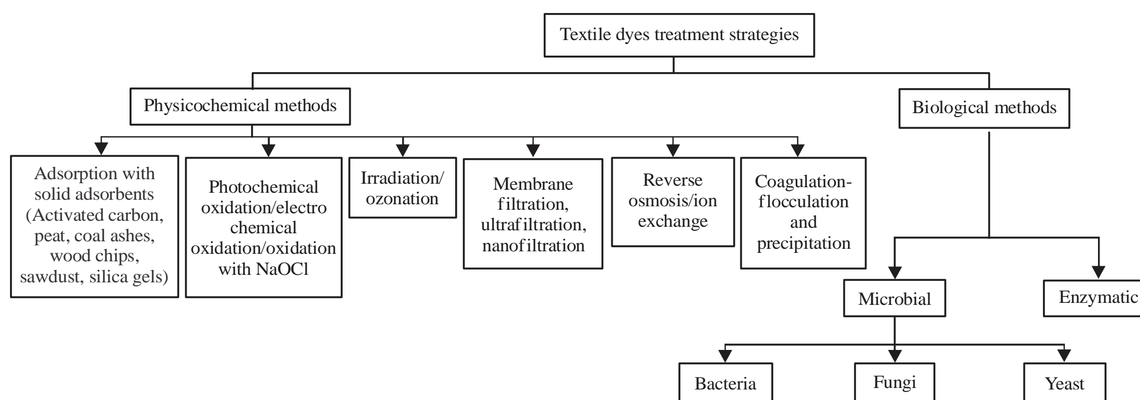

Fig. 1: Strategies for treatment of dyes from textile effluents

Table 1: Decolorization and detoxification of textile dyes by bacteria

Name of strain	Name of dye and concentration	Condition (pH, temperature (°C), agitation)	Time (h)	Decolorization (%)	Decolorization mechanism	References
Bacillus subtilis	Reactive red M8B	7.0, 37°C and 100 mg L^{-1}, shaking	120	60	-	Arulazhagan[56]
Bacillus sp. and *Pseudomonas* sp.	Provisional pink	30°C	120	9373	-	Celia and Suruthi[57]
Bacillus sp.	Navy blue and methyl red	8.0, 37°C and 0.5 g L^{-1}	24	86.068.3	-	Ezhilarasu[58]
Consortium (*Providencia rettgeri* strain HSL1 and *Pseudomonas* sp., SUK1)	Selected dyes, reactive black 5, reactive orange 16, disperse red 78 and direct red 81	37°C, 100 ppm, microaerophilic	12-30	98-99%	-	Lade *et al.*[59]
Bacillus cereus RMLAU1	Reactive orange 4	8.0, 35°C and 40 mg dye L^{-1}, 4.0% v/v inoculums, static	60	100	-	Garg *et al.*[16]
Pseudomonas putida SKG-1	Reactive orange 4	8.0, 35°C, 50 mg reactive orange L^{-1}, inoculum dose 5.0% v/v, static	72	95.2	-	Garg *et al.*[15]
Bacillus cereus RMLAU1	Acid orange 7	pH 8.0, 33°C, 100 mg dye L^{-1}, 3.0% v/v inoculums	96	68.5	-	Garg and Tripathi[17]
Bacillus cereus Hj1	Reactive black B (20 mg L^{-1})	25°C, pH 8.0, static	120	100	-	Liao *et al.*[51]
Glandularia pulchella (Sweet) Tronc. and *Pseudomonas monteilii* (plant-bacterial synergistic system)	Scarlet RR dye		48	100	-	Kabra *et al.*[52]
Alcaligenes sp., AAO9	Reactive red BL				-	Pandey and Dubey[60]
Corynebacterium sp.	Reactive black 5 (100 µg mL^{-1}), reactive yellow (100 µg mL^{-1})	7.0, 37°C	96	60 76	-	Aftab *et al.*[10]
Aeromonas hydrophila	Dyes mixture	pH 5.5-10.0, 20-30°C, anoxic	-	High	-	Naik and Singh[61]
Bacillus spp., VITEF 1 *Bacillus* spp., VITEF 2 *Bacillus* spp., VITEF 3	Reactive red 5B			60 57 58	-	Prasad and Rao[8]
Micrococcus glutamicus NCIM 2168	Reactive green 19 A (50 mg L^{-1})	6.8, 37, static	42	100	Oxidative and reductive	Saratale *et al.*[19]
Rhizobium radiobacter MTCC 8161	Reactive red 141 (50 mg L^{-1})	7.0, 30, static	48	90	Oxidative and reductive	Telke *et al.*[62]
Pseudomonas sp., SUK1	Reactive red 2 (5 g L^{-1})	6.2-7.5, 30, static	6	96	LiP and azoreductase	Kalyani *et al.*[48]
Comamonas sp., UVS	Direct red 5B (1100 mg L^{-1})	6.5, 40, static	13	100	LiP and laccase	Jadhav *et al.*[63]
Streptomyces krainskii SUK-5	Reactive blue-59 (reactive navy blue RX)	8.0, shaking	24		lignin peroxidase, NADH-DCIP reductase and methyl red reductase	Mane *et al.*[7]
Pseudomonas desmolyticum NCIM 2112	Red HE7B (100 mg L^{-1})	6.8-8.0, 30, static	72	95	LiP and azoreductase	Kalme *et al.*[46]
Pseudomonas sp., SUK1	Red BLI (50 mg L^{-1})	6.5 -7.0, 30, static	1	99	AND and NADH-DCIP reduction	Kalyani *et al.*[48]
Pseudomonas aeruginosa NBAR12	Reactive blue 172 (500 mg L^{-1})	7.0-8.0, 40, static	42	83	Azoreductases, flavin reductases or peroxidases	Bhatt *et al.*[64]
Four bacterial isolates consortium *Bacillus cereus* (BN-7)	Acid red 88, acid red 119 and acid red 97	7.0, 35, 100 rpm	24 24 24	78 99 94	Azoreduction	Khehra *et al.*[65]
Bacterial consortium SV5	Ranocid fast blue (100 ppm)	7.0, 37, static	24	100		Mathew and Madamwar[66]
Aeromonas hydrophila	Red RBN (3000 mg L^{-1})	5.5-10.0, 20-35	8	90	NA	Chen *et al.*[1]
Pseudomonas luteola	RP2B (Reactive red 22) (100 ppm)	NA, 28, shaking-static incubation (100 rpm, for 48 h then at static for 4 days)	120	95	Azoreduction	Hu[37]
Klebsiella pneumoniae R5-13	Methy1 red (100 mg L^{-1})	6.0-8.0, 30, 200 rpm	168	100	Reduction	Wong and Yuen[39]

culture, but color removal was the best in anoxic or anaerobic condition. The culture also displayed a growth-associated pattern of color removal. The most suitable pH and temperature for color removal were 5.5-10 and 20-35°C, respectively under anoxic conditions and >90% red BRN was decolorized within 8 days at an initial dye concentration of 3000 mg L^{-1}. Furthermore, this strain also decolorized (~90%) the media containing a mixture of dyes within 2 days, followed by an insignificant change in color for the next 10 days. Chang *et al.*[44] found that some extracellular metabolites produced during the growth of *Escherichia coli* NO3 strain enhanced its decolorization potential for azo dye reactive red 22. Tan and Field[45] reported the biodegradation of relatively simple sulphonated amino-benzene and amino-nephthalene compounds under aerobic conditions. Pourbabaee *et al.*[2] isolated *Bacillus* sp., strain PS from textile effluent, which decolorized methyl orange azo dye at 30°C aerobically under shaking (140 rpm) within 2 days of incubation. The isolate was also able to aerobically decolorize terasil black dye in the presence of glucose or starch (at 1.0%, w/v) as an exogenous carbon source, the decolorization process was still faster in the presence of 0.5% (w/v) yeast extract. Parshetti *et al.*[6] observed complete decolorization of initial 50 mg malachite green L^{-1} concentration by *Kocuria rosea* MTCC 1532 under anoxic conditions within 5 h incubation, however, no dye decolorization was evident under shaking condition. This was attributed to the competition of oxygen and dye for the reduced electron carriers under aerobic condition[46]. The strain also exhibited decolorization of azo, triphenylmethane and industrial dyes (cotton blue, methyl orange, reactive blue 25, direct blue-6, reactive yellow 81 and red HE4B). The semi-synthetic media containing molasses, urea and sucrose showed 100, 91 and 81% decolorization, respectively. The induction of malachite green reductase and 2,6-dichlorophenol indophenol (DCIP) reductase enzymes during dye decolorization was suggestive of their involvement in the color removal process. Patil *et al.*[47] reported 89, 90 and 82% decolorization of reactive blue 59 by *Bacillus odyssey*, *Morganella morganii* and *Proteus* sp., SUK 7, respectively. Kalyani *et al.*[48] reported that *Pseudomonas* sp., SUK1 decolorized (99%) red BL1 (initial 50 μg mL^{-1}) within an hour at pH range 6.5-7.0 and 30°C under anoxic condition. Grekova-Vasileva *et al.*[49] reported 100% decolorization of initial 700 and 1400 mg amaranth dye L^{-1} by *Pseudomonas* strain AZO29 isolate from wastewater treatment plant in textile factory within 24 and 72 h, respectively. The decolorization rate enhanced with increasing amaranth concentration from 0.165-0.265 mM and >90% color removal was achieved by AZO29 strain for a range of dye concentration up to 0.265 mM. Wong and Yuen[39] reported 100% methyl red (initial 100 mg L^{-1}) dye removal by *Klebsiella pneumoniae* RS-13 within 24 h. Hu[37] observed 93.2% RBB (initial 100 mg L^{-1}) dye removal by *Pseudomonas luteola* within 48 h.

Prasad and Rao[8] employed three individual bacterial isolates, viz., *Bacillus* spp., VITEF 1, 2 and 3 for reactive red 5B decolorization under aerobic conditions and reported 60, 57 and 58% color removal, respectively. Ponraj *et al.*[12] isolated four dye decolorizing isolates, viz., *Bacillus* sp., *Salmonella* sp., *Klebsiella* sp. and *Pseudomonas* sp. from the textile effluent. Out of the four isolates, *Bacillus* and *Pseudomonas* sp., exhibited maximum dye decolorization of 89% at the end of 144 h under optimum cultural and nutritional conditions; *Bacillus* sp. was found to be more efficient in dye decolorization activity. Aftab *et al.*[10] noted 60 and 76% decolorization, respectively of reactive black 5 and reactive yellow (at initial 100 μg mL^{-1}) within 4 days by *Corynebacterium* sp., isolate at optimum 37°C and pH 7.0. Tripathi and Srivastava[5] attempted to compare the decolorization potential of *P. putida* MTCC 102, *B. cereus* MTCC 3105, *Pseudomonas fluorescence* MTCC 103, *B. subtilis* NCIM 2063, *Alcaligenes* sp., NCIM 15085 and *Staphylococcus aureus* NCIM 2901 against acid orange 10 azo dye, *P. putida* was adjudged as the most potent bacterial strain. This efficient strain could decolorize acid orange 10 by 90% within 24 h at pH 7.0, 37°C, dye concentration of 250 mg L^{-1} under static culture condition. The culture also exhibited good decolorization efficiency even in alkaline pH region. The extent of dye decolorization decreased with increasing dye concentration and required 16, 20, 36 h for 90% color removal of acid orange 10 at 100, 250 and 500 mg L^{-1} concentration under optimum conditions. Sawhney and Kumar[50] observed 85% decolorization of congo red azo dye (initial 50 ppm concentration) by *Bacillus* sp., VT-II isolate at optimum pH 7.0 and 40°C temperature.

Sivaraj *et al.*[13] reported the use of enriched aerobic cultures of indigenous microbes, *Streptococcus faecalis* and *B. subtilis* for decolorizing the textile dye effluent. The cultures exhibited maximum decolorization ability (72%) at pH 7.0-8.0, 30-35°C, 4.0% (v/v) inoculums and 1.5% (w/v) glucose concentration within 36 h incubation. Ghodake *et al.*[11] employed *Acinetobacter calcoaceticus* NCIM2890 to decolorize 20 different textile dyes of various classes. Decolorization of an azo dye was efficient (91%) at static anoxic condition, whereas, shake culture grew well, but showed less color removal (68%) within 48 h of incubation. Induction of lignin peroxidase, laccase, DCIP reductase and riboflavin reductase indicated their role in biodegradation of

amaranth mono azo dye. Liao *et al.*[51] inferred from their study that optimal conditions (25°C, pH 8.0, yeast extract 0.15 g L^{-1} and glucose 0.125 g L^{-1}) in culture medium and original river water could completely decolorize reactive black B (20 mg L^{-1}) aerobically by *B. cereus* Hj1 isolate within a period of 5 days incubation under static culture condition. Kabra *et al.*[52] attempted to develop a plant-bacterial synergistic system for efficient treatment of the textile effluents. *Glandularia pulchella* (Sweet) Tronc. and *Pseudomonas monteilii* showed decolorization of scarlet RR dye by 97 and 84% within 72 and 96 h, respectively, while their consortium exhibited 100% color removal within 48 h. In case of dye mixture, *G. pulchella*, *P. monteilii* and consortium-PG showed an ADMI removal of 78, 67 and 92%, respectively within 96 h incubation. During the decolorization of dye, induction of *G. pulchella* lignin peroxidase and DCIP reductase was evident, while *P. monteilii* showed induction of laccase, DCIP reductase and tyrosinase indicating their involvement in the dye metabolism.

Garg *et al.*[16] reported that *B. cereus* isolate was capable of decolorizing reactive orange 4 dye effectively under wide range of cultural and nutritional conditions. They optimized various process parameters using conventional and response surface methodology and suggested that dye concentration is the most important parameter for efficient decolorization. At bioreactor level, 100% dye decolorization was achieved within 60 h under optimum cultural (pH 8.0, 35°C, 40 mg dye L^{-1}, 4.0% *B. cereus* inoculum) and nutritional (1.0% glucose and 0.2% ammonium nitrate) conditions. Therefore, specific range of cultural and nutritional conditions is required for biodecolorization of textile dye effluent.

Actinomycetes: This group of bacteria is being recognized for degradation ability of highly recalcitrant compounds. The efficiency of *Streptomyces chromofuscus*, a soil actinomycete, was compared with *Phanerochaete chrysosporium* by Paszczynski *et al.*[53] and concluded that the extent of dye decolorization by soil bacterium was less than white-rot fungus. Zhou and Zimmermann[28] compared several other strains of actinomycetes for the decolorization of reactive dyes (viz., anthraquinone, phthalocyanine and azo dyes) through adsorption mechanism without biodegradation. However, other Cu-based azo dyes (e.g., formazan-Cu complex dyes) were decolorized through the biodegradation route by the same species of actinomycete. Mane *et al.*[7] reported complete decolorization and degradation of azo dye reactive blue-59 (reactive navy blue RX) by *Streptomyces krainskii* SUK-5 isolate at pH 8.0 of dye containing nutrient medium within 24 h under shaking/aerobic condition. This suggests that the precondition for reduction of azo dye is the presence and availability of a carbon/energy substrate which acts as an electron donor for azo dye reduction[38]. The induction in the activities of lignin peroxidase, NADH-DCIP reductase and methyl red reductase indicates the role of these enzymes in dye degradation[7].

Mixed bacterial culture/consortium: Microbial consortia are used as black boxes without analyzing the constituent microbial populations for environmental remediations. The complexity of microbial consortium enables them to act on a variety of pollutants. Mixed bacterial cultures from a wide variety of habitats have also been shown to decolorize the dye molecules containing diazo-linked chromophores[54]. Many researchers have reported that a higher extent of biodegradation and mineralization of dyes can be expected when cometabolic activities within a microbial consortium complement each other. Haug *et al.*[55] reported a bacterial consortium capable of mineralization of sulphonated mordant yellow azo dye. However, sequential anaerobic-aerobic conditions were required for complete degradation of azo dye, because different members of the consortium needed different conditions for optimum activity and decolorization of azo dye through main azo bond cleavage was possible only under anaerobic conditions[55]. The higher decolorization potential due to concerted activities of mixed bacterial culture was reported by Nigam *et al.*[38], wherein a consortium (PDW) based on two bacterial strains *Alcaligenes faecalis* and *Commamonas acidovoranas* decolorized eight azo dyes, whereas, individual strains were unable to decolorize any of the dyes. Several studies describing the use of mixed culture for decolorization/mineralization of textile dyes are presented in Table 1.

Khehra *et al.*[65] compared the decolorization potential of individual cultures with that of consortium H-4 constituted by mixing four laboratory isolates identified as *B. cereus* BN-7, *P. putida* BN-4, *Pseudomonas fluorescens* BN-5 and *Stenotrophomonas acidaminiphila* BN-3. Six different azo dyes, viz., acid red 88, 119, 97, reactive red 120, acid blue 113 and acid brown 100 were used in this dye decolorization study. The individual bacterial isolates were able to decolorize only acid red 119 and acid blue 113 dyes, whereas, consortium HM-4 was able to decolorize all above dyes (at initial 20 mg dye L^{-1} concentration) at a significantly higher rate as compared to that achieved by individual isolates. The higher decolorization efficiency of consortium HM-4 was attributed to the concerted metabolic activities of the constituent strains. The individual culture(s) may transform the dye to intermediates, which can act as redox mediators for

efficient transfer of reducing equivalents from other strains, leading to an enhanced decolorization potential of the consortium. It is also reported in the literature that such metabolites of the dye may decrease the redox potential of the medium, thus making it more favorable for the reduction of azo bond for the dye decolorization[67]. Joshi *et al.*[68] reported that the natural bacterial consortium decolorized a mixture of eight textile dyes (89% ADMI removal) and textile industry effluent (67% ADMI removal) within 3.5 and 3 h, respectively, under static culture condition. Further, the rate of decolorization of individual dyes by natural consortium varied from 3-24 h, which may be attributed to structural differences in different dyes[53]. Significant induction of intracellular laccase, azo reductase, veratryl alcohol oxidase and DCIP reductase suggested their active role in dye decolorization[68].

Dye decolorization by fungi: The fungal biodegradation and biosorption of dyes has always proved efficient under aerobic conditions[69]. Among different fungi, white-rot are the most widely researched and are an effective tool in the removal of dyes. As such, enzymes like lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) of these fungi are actually meant for the transformation/degradation of lignin present in dead biomass of plants. However, same or similar enzymes due to their low substrate (lignin) specificity have also been employed quite successfully in the degradation of other aromatics, such as dyes[70]. White-rot fungal strains, being saprophytic in nature are found mainly in soil/sludge containing decaying organic material, specifically of plant origin. Different fungi employed for dye decolorization include: *Rhizopus arrhizus*[71] and different species of *Phlebia*[72]. Only a few studies have reported the bioremoval potential of brown-rot fungi for different dyes[73]. Qu *et al.*[74] studied decolorization of reactive dark blue K-R by the synergism of fungus and bacterium using response surface methodology.

Fungal biosorption of dyes: Biosorption is reported to be the primary dye removal process in wood rotting basidiomycetes[75]. Fungal biomass is considered as a good biosorbent for textile dyes because fungi can be cultivated economically in substantial amounts employing simple fermentation techniques and cheap growth media[76]. The main reasons of using macrofungi are (1) Availability of dry mass in large quantity, (2) Easily and economically available everywhere, (3) Chemical stability in most alkaline and acidic conditions and (4) Good mechanical strength. Furthermore, the fruiting bodies of macrofungi have a tough texture when dried and other physical characteristics are also suitable in the development of biosorbents. Biosorption is linked to electrostatic pull between the negatively charged dyes and positively charged cell wall components[71]. Reactive dyes typically contain azo-based chromophores substituted with different types of reactive group(s) which interact with the active groups present on the cell surface of fungal biomass, such as chitin, chitosan, acidic polysaccharides, lipids, amino acids and other cellular components.

Dye degradation properties of fungi tend to change with varying nutritional and cultural conditions. Therefore, optimization of operational culture conditions should always be considered as a prerequisite in bioremediation studies.

Kirby *et al.*[72] found that *Phlebia tremellosa* was capable of decolorizing eight synthetic textile dyes under static culture conditions. The researchers speculated that dye decolorization was due to the oxidative activity of laccase produced by the fungus, as color removal was inhibited in the presence of sodium azide (a laccase inhibitor) at 2.5 µM concentration. In contrast, Ali *et al.*[69] observed that decolorization of dyes (orange II, Sb and Db K_2RL) by *A. niger* SA1 strain significantly increased when shifted from stationary to shaking culture conditions.

Fungi, being aerobic organisms normally show relatively better dye removal activities under facilitated agitated cultures[29,70]. As such, shaking increases the mass (substrate) and oxygen transfer from the culture medium to the microbial cells, thereby facilitating optimum fungal growth and enzyme (oxidase) production[29]. Sometimes the production and stability of peroxidases gets compromised during agitation[72,70]. Therefore, the possibility of fungal dye degradation under static culture conditions should not be overlooked and such an idea will be more suitable for large-scale treatment of colored effluents at the industrial level[77].

Aksu and Tezer[71] reported maximum dye (vinyl sulphone type reactive dye) uptake capacity of *R. arrhizus* at 35°C, pH 2.0 and initial 800 mg dye L^{-1} concentration. Sam and Yesilada[78] employed *Coriolus versicolor* and *Funalia trogii* for orange II dye decolorization under varied culture conditions of agitation, temperature, initial pH and buffered medium. The most efficient color removal was evident at 30°C, pH 6.5-7.0 and pH 4.5 was the optimum in buffered cultures. High extent of orange II decolorization was obtained using immobilized fungal cultures under repeated batch mode. Robinson *et al.*[79] selected *Coriolopsis gallica* and *P. chrysosporium* for their capability to decolorize five dyes in an artificial effluent. The experiments were performed under N-rich and N-limited conditions at 100 mg dye L^{-1} concentration. *Phanerochaete chrysosporium* decolorized 53.6% dyes of the effluent in N-rich and 40% in N-limited conditions, whereas, *C. gallica* removed 80.7% color in

Table 2: Factors and mechanisms for textile dye decolorization by fungi

Microorganisms	Dyes	Conditions for decolorization	Mechanism	Decolorization (%)	References
Decolorization by fungi					
Aspergillus foetidus	Remazol red and remazol brown (50 mg L^{-1})	2 days	Adsorption	>98 >98	Sumathi and Phatak[86]
Rhizophus arrhizus	Vinyl sulphone type reactive dye (800 mg L^{-1})	35°C, pH 2.0	-	-	Aksu and Tezer[71]
Phanerochaete chrysosporium	Five dye mixture (100 mg L^{-1})	N-rich medium	-	53.6	Robinson *et al.*[79]
Trametes villosa *Pycnoporus sanguineus*	Drimaren brilliant blue	-	Biodegradation		Machado *et al.*[87]
Fusarium solani	Crystal violet and malachite green (at initial 2.5 mg L^{-1})	2 days under shaken culture condition	-	98 and 96	Abedin[81]
Pleurotus spp.	Malachite green	5 days	-	98	Yogita *et al.*[83]
Thermomucorindicae seudaticae	Azo-anthraquinone dye mixture (Azure B, Congo red, trypan blue and remazol brilliant blue R)	55°C	-	74.93	Taha *et al.*[85]

N-rich and 86.9% in N-limited conditions. Thus, nitrogen supplementation improved enzyme activities as well as dye decolorization by *P. chrysosporium*. Contrary to that, additional nitrogen enhanced enzyme activities, but did not improve effluent decolorization by *C. gallica*. It may be inferred from the above results that *C. gallica* was more efficient in mixed dye synthetic effluent decolorization as compared to *P. chrysosporium*.

Ali *et al.*[69] reported that with the exception of *Aspergillus terreus* SA3, all other fungal isolates employed in their study exhibited better removal of azo dyes in Saboraud Dextrose Broth (SDB) than in Simulated Textile Effluent (STE). Greater removal of dyes in SDB might be associated with the availability of higher glucose (as C-source) level and low pH. Glucose not only acts as a reducing agent for dyes[80], but also promotes fungal growth and metabolism[77]. Higher glucose levels also create acidic condition in the culture medium, which supports better removal of dyes[77]. Abedin[81] reported 98% decolorization of crystal violet and 96% of malachite green (at initial 2.5 mg L^{-1}) within 2 days under shaken culture condition. Gou *et al.*[82] also reported azo dye decolorization by a new fungal isolate, *Penicillium* sp., QQ and fungal-bacterial cocultures. Yogita *et al.*[83] employed seven wild mushroom cultures, viz., *Pleurotus florida*, *P. sajorkaju*, *Grifola frondosa*, *Polyporous* sp., 1 and 2, *Jelly* sp. and *Schizophylum commune* for decolorization and degradation of malachite green and reported color removal of 98 and 99.75% by *Jelly* sp., 65.25 and 97.5% by *S. commune* and 26.25 and 68.5% by *Polyporous* sp., 2, within 5 and 10 days incubation, respectively. Przystas *et al.*[84] studied decolorization of mixture of brilliant green and evans blue by single and mixture of fungal strains *Pleurotus ostreatus*, *Gloeophyllum odoratum* and *Fusarium oxysporum*. A thermophilic fungus, *Thermomucorindicae seudaticae*[85] obtained from compost was successfully used for decolorization of azo anthraquinone dye mixture at 55°C (Table 2).

OPERATIONAL (PROCESS) PARAMETERS AFFECTING DECOLORIZATION OF TEXTILE DYES

The dye decolorization process is influenced by various factors which can be broadly grouped into two categories (1) Those concerned with microbial growth conditions and (2) Related to the characteristics of dye solution or wastewater (Fig. 2). The succeeding paragraphs dwell on some of these intricate features.

Oxygen: One of the factors that influences the microbial growth and dye decolorization is oxygen. The oxygen exerts a significant effect on the physiological characteristics of microbial cells during the cell cycle. Since oxygen is a high redox potential electron acceptor, it exerts inhibitory effect on dye reduction process. This is attributed to the fact that the electrons released from the oxidation of electron donors are preferentially used by the oxygen rather than the azo dye[88]. Thus, aeration and agitation should be avoided for the efficient microbial dye decolorization process.

Under anaerobic conditions, azo dyes are readily cleaved via four electron reduction at the azo linkage, generating colorless aromatic amines. The required electrons are provided by the electron donating carbon source, such as glucose, fructose, sucrose, starch, etc., which varies from microbial species to species. The aromatic amine residues resulting from anaerobic decolorization resist further anoxic degradation and they are also reported mutagens/carcinogens. Aerobic conditions are required for further degradation/mineralization of aromatic compounds via hydroxylation and ring-opening in the presence of oxygen. Thus, bacterial degradation of dyes is often initiated under anoxic/anaerobic conditions by an enzymatic transformation reaction[3] in which reductive cleavage of azo bond leads to the formation of colorless aromatic amines. In the second step, these toxic amines are further degraded (oxidized) to simpler non-toxic forms under

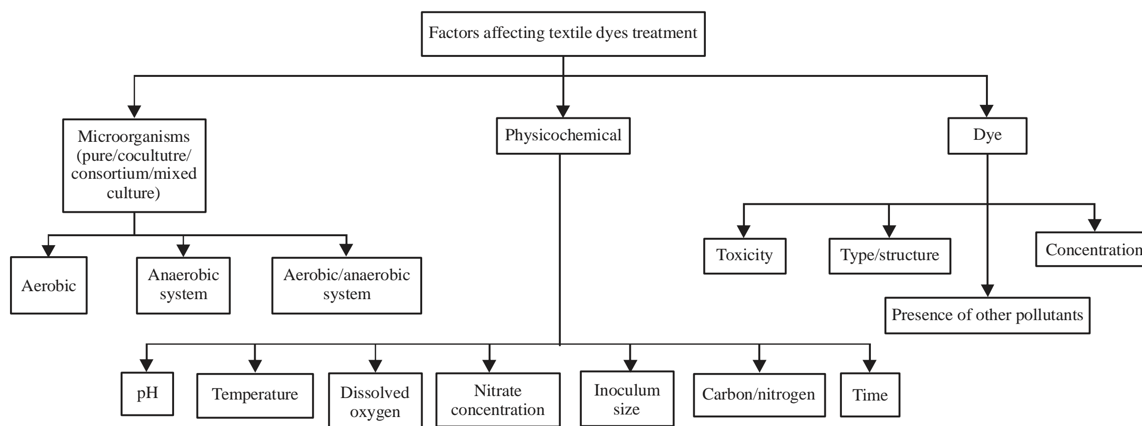

Fig. 2: Factors affecting the remediation of textile dyes effluent

aerobic conditions only[5]. So, for the most effective textile wastewater treatment, a two-stage process is necessary in which oxygen is introduced after the initial anaerobic decolorization of the azo dye. Hence, interest is now focused on microbes which can perform high rate of dye decolorization under anoxic/anaerobic/static culture conditions and detoxify the toxic aromatic amines aerobically. Most probably these microorganisms are facultative anaerobic bacteria, which are capable of metabolizing wide range of aromatic substrates under broad environmental conditions. However, the balance between anaerobic and aerobic conditions is required to be carefully monitored because there is a fear that aeration of decolorized dye solution might result in darkening of the solution, as the colorless aromatic amines produced during anaerobic stage are spontaneously unstable in the presence of oxygen. The oxidation of hydroxyl and amino groups of aromatic amines results in quinine and quinine imine formation. Such compounds can undergo di- and polymerization leading to the formation of dark colored chrompophores. However, when proper conditions are ensured in a sequential anaerobic/aerobic process, many facultative bacteria are capable of achieving high levels of decolorization and detoxification of dyes.

Several researchers have reported efficient dye decolorization under anoxic/static culture as compared to shaking (aerobic) conditions[89,90,9,11]. Isik and Sponza[91] observed complete (100%) decolorization of congo red dye in anaerobic culture than 76 and 45% color removal of direct black 38 by *Pseudomonas* sp., respectively under microaerophilic and aerobic conditions. Chen *et al.*[1] reported that although *Aeromonas hydrophila* displayed good growth in aerobic or agitated cultures, color removal was best (80% at initial 50 mg dye L^{-1} within 15 days) in anoxic/anaerobic cultures. Furthermore, shifting of agitated cultures to stationary conditions yielded good decolorization. Wang *et al.*[92] reported >96% decolorization of reactive red 180 by *Citrobacter* sp., CK3 under anaerobic conditions compared to only 13% in aerobic cultures at a shaking speed of 150 rpm. Ghodake *et al.*[11] reported 91% decolorization under static anoxic culture condition, whereas under shaking the culture grew well, but only 68% color removal of amaranth dye was evident within 48 h incubation. Tripathi and Srivastava[5] observed 90% decolorization of acid orange 10 by *P. putida* strain under static/anoxic conditions as compared to 32% in shake culture within 24 h incubation. Garg *et al.*[14] reported a direct correlation between the growth response of *P. putida* SKG-1 isolate and acid orange 7 dye (at initial 100 mg L^{-1} MSM) decolorization and observed 69, 57 and 36.5% color removal under static, intermittent and continuous shaking conditions, respectively at 96 h incubation. Likewise, *Bacillus cereus* RMLAU1 isolate also performed better in terms of growth response and dye (acid orange 7) decolorization under static culture (29% color removal) as compared to continuous shaking (10.7% decolorization) conditions within 72 h incubation[17].

Nevertheless, there are few reports on aerobic degradation of azo dyes[93,32,2] suggesting that microbes appear to possess more than one mechanisms for dye decolorization. Kulla[32] suggested that in aerobic degradation of methyl orange dye, despite the presene of oxygen, the initial step appear to be reduction of azo linkage by an oxygen-insensitive azoreductase. Under shaking (aerobic) conditions, the presence of oxygen deprives the azoreductase from receiving electrons required for azo bond cleavage. Whereas, under static culture conditions, these electrons are readily available to the enzymes from NADH to decolorize the azo dyes[94,95]. Hence, for efficient color removal, agitation and aeration, which increases the concentration of dissolved oxygen should be avoided.

Hydrogen ion concentration: The pH of the dye solution/textile effluent is another important factor that determines the growth response and the extent of dye decolorization, as it has marked effect on bacterial cell growth and production/activity of enzymes involved in the color removal/dye degradation process. The pH <3.0 and >9.0 or 10, as well as sudden change in the pH of treatment system matrix can significantly inhibit microbial growth and hence dye decolorization. The pH in the natural environment usually ranges between 5.0 and 9.0, which is more or less optimal for microbial metabolism as well as degradation/remediation of various pollutants. The optimum pH for color removal is around neutral or slightly alkaline. The color removal is adversely affected if the pH of wastewater turns strongly acidic or alkaline. However, the desired pH change is often maintained by a natural buffering capacity that exists in the wastewater matrix or colored effluent is externally buffered to enhance the color removal performance of the cell culture. Biological reduction of azo bond of dyes can result in an increase in the pH due to the formation of aromatic amines, which are more basic than the original azo dye compound. Altering the pH within a range of nearly 7.0-9.5 exerts very little effect on the dye reduction process.

Many researchers have reported the effect of pH on decolorization of different textile dyes by a variety of microorganisms[96-99,92,50]. The pH exerts significant effect on the efficiency of dye decolorization and that optimum pH varies[100,89,12] between 7.0-10.0. Chang *et al.*[101] found that the dye decolorization rate increased nearly 2.5 fold as the pH was raised from 5.0-7.0, while the rate was insensitive to pH in the range of 7.0-9.5. Dafale *et al.*[102] found pH 7.0-8.0 optimal for remazol black B decolorization by bacterial consortium containing *Pseudomonas aeruginosa*. Wang *et al.*[92] achieved maximum azo dye decolorization efficiency of *Citrobacter* sp., at pH 7.0. The natural bacterial consortium was able to efficiently decolorize the mixture of eight dyes in the wide range of pH (3.0-11.0) with optima[68] at pH 5.0. Ponraj *et al.*[12] also reported maximum dye decolorization efficiency of *Pseudomonas* sp. at pH 6.0 (86.72%) and 8.0 (89.06%). Likewise, Tripathi and Srivastava[5] found optimum pH 7.0 for acid orange 10 dye decolorization by *P. putida* MTCC 102, while *P. aeruginosa* NCIM 102 decolorized the dye with a good efficiency over a pH range of 7.0-9.0. Ponraj *et al.*[12] reported optimum pH range 6.0-8.0 for maximum decolorization of orange 3R dye. The researchers found that *Pseudomonas* sp., isolate removed 89.02 and 86.72% color at pH 6.0 and 8.0, respectively, while *Bacillus* sp., isolate achieved 86.72% decolorization at pH 7.0. Garg *et al.*[14] observed maximum growth response of *P. putida* SKG-1 and acid orange 7 dye decolorization (88.2%) at pH 8.0. They subsequently reported that *B. cereus* RMLAU1 isolate could grow and decolorize the same dye at a wide acidic-alkaline range of 6.0-8.0 and maximum degree of decolorization (60.8%) occurred at optimum pH 8.0 within 96 h incubation.

The pH of colored wastewater strongly affects the enzymatic processes and transport of compounds across the cell membrane. Since microbial azo bond reduction is enzyme-mediated, changes in pH will affect the degree of ionization of the enzyme(s) leading to alteration in protein conformation ultimately affecting the enzyme activity. However, the precise molecular basis of pH affecting bacterial metabolism in textile wastewater is obscure. Since proton-motive force in chemiosmosis is affected by the medium pH value, it is possible that under optimum pH range, the relative metabolic activity is high.

Temperature: In general, the temperature also plays a crucial role for the physiological performance of microbial cultures thereby affecting the rate of dye decolorization. The temperature affects the bacterial metabolism, microbial growth rates and the physiological state of the contaminants/pollutants. In many systems, the rate of color removal increases with increasing temperature, within a defined range that depends on the system, above which the rate of biochemical activity declines as enzyme denaturation occurs and organisms either die or become less active. Low temperatures below optimum seldom kill the microbes and with warming they recover. Temperature also affects gas solubility and must be considered while designing a remediation system. Even modest increases in temperature can significantly increase bioremediation rates. There appears to be a direct correlation between rate and extent of color removal and optimum microbial culture growth temperature of 35-45°C. The increase in temperature above optimum causes decrease in color removal which can be attributed to the loss/decrease in cell viability and/or to the denaturation of azoreductase enzyme[101].

The temperature exerts a major effect on the efficiency of dye decolorization and the optimum condition varies[100,89,12] between 30-40°C. Dafale *et al.*[102] found 37°C optimum for remazol black B decolorization by *P. aeruginosa* containing bacterial consortium. Wang *et al.*[92] reported maximum azo dye decolorization efficiency of *Citrobacter* sp. at 32°C. The natural bacterial consortium effectively decolorized the mixture of eight dyes in a broad temperature range of 10-70°C and the maximum color removal was observed at optimum 50°C. This might be due to triggering some genes of the consortium gene pool, which are stable and active under

environmental stress conditions[68]. Out of the four bacterial isolates, viz., *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas* sp., Ponraj *et al.*[12] found *Bacillus* sp. the best for maximum 78.56, 79.36 and 78.57% decolorization of orange 3R dye at 4, 27 and 37°C, respectively. Tripathi and Srivastava[5] found 37°C optimum for orange 10 dye decolorization by *P. putida* MTCC 102. Garg *et al.*[14] noted the best decolorization of orange 7 by *P. putida* SKG-1 isolate at 30°C within 96 h incubation. In their subsequent study, Garg and Tripathi[17] reported highest degree of the same dye decolorization by *B. cereus* RMLAU1 isolate at 33°C within 96 h, which was very close to color removal at 35°C.

Since the processes responsible for dye bioremoval are largely physicochemical in nature, enzyme-dependent mechanisms are more likely to be affected by temperature. Above the optimum temperature, cell degradation probably becomes dominant over the growth process and with sub-optimal temperature, the regulation of metabolism may fail. Hence, at optimum temperature, the bacterium could utilize the substrate better, in consonance with other optimum cultural and nutritional conditions. Further, the temperature is also known to affect the stability of microbial cell wall, its configuration and can cause ionization of chemical moieties.

Inoculum size: The dose of inoculum is another factor which should also be taken into consideration while developing dye decolorization strategy. Variable results have been reported regarding the effect of inoculum size on the dye decolorization process, which appear to vary with the microbial species employed. Pourbabaee *et al.*[2] observed that the rate of terasil black containing effluent decolorization enhanced with increase in inoculum size of *B. cereus* from 2.5-10% (v/v). However, further increase in inoculum size up to 20% did not cause any change in the intensity of color. Ponraj *et al.*[12] found 4.0, 6.0, 6.0 and 10.0% inocula of *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas* sp., optimum for 86.72, 67.19, 53.91 and 50% dye decolorization, respectively. Sivaraj *et al.*[13] also reported 4.0% inoculum of *B. subtilis* and *Streptococcus faecalis* optimum for textile wastewater decolorization and there was no significant difference in color removal at 6.0, 8.0 and 10.0% doses of inoculum. Contrary to that, Bayoumi *et al.*[9] could not find any distinct relationship between textile azo dyes (acid orange 7 and direct blue 75) decolorization and the inocula sizes of *Comamonas acidovorans*-TM1 and *Burkholderia cepacia*-TM5 strains. Similar to Sivaraj *et al.*[13] and Garg *et al.*[14] reported 4.0% inoculum of *P. putida* SKG-1 isolate optimum for maximum growth and acid orange 7 dye decolorization. In their another study, Garg and Tripathi[17] found 3.0% dose of *B. cereus* RMLAU1 isolate optimum for 68.5% decolorization of acid orange 7 within 72 h incubation.

Carbon and nitrogen supplements: Nutrients are generally supplemented in both *ex situ* and *in situ* bioremediation processes and the requirement of nutrient(s) largely depends on the nature of pollutants present in the wastewater. Biostimulation through substrate addition is commonly practiced to support co-metabolic biodegradation processes. Co-metabolism is a process in which microbes involved in the metabolism of a growth promoting substrate also transforms other organic contaminants which are called as co-substrates. The co-substrate(s) is/are not growth supporting if provided as the only source of carbon and energy. Such metabolic transformation of organic pollutants is an important process in both aerobic and anaerobic environments. Addition of stimulatory substrates to enhance bacterial growth and metabolic activity through electron transfer processes between electron donor and acceptor has also been used in bioaugmentation experiments involving environmental clean-up. The oxidation of organic electron donors is coupled to the color removal process.

Bras *et al.*[103] showed that the addition of electron donors such as glucose or acetate ions stimulates the reduction cleavage of azo bond(s). The rate of biochemical reaction is likely to be influenced by the type of electron donor, as the thermodynamics of various electron-donating half-reactions are different. The concentration of original electron donor (carbon/energy source or auxiliary substrate) determines the rate of intermediate electron donor (reduction equivalents) formation. The coenzyme reducing equivalents (that are part of normal electron transport system) produced by the oxidation of organic substrates may act as the electron donors for azo dye reduction[67]. Therefore, the rate of NADH formation would be rate-limiting and would determine the rate and extent of azo dye reduction. The products of cell lysis can function as electron donors for anaerobic dye reduction in which active live cells metabolize the lysis products[88].

Generally, a readily available substrate is required for the reduction of azo dyes[38], because it acts as an electron donor for azo bond reduction. Many substrates, viz., glucose[40,38], yeast extract[68], a mixture of acetate, butyrate and propionate[104,105] have been employed for azo dye reduction. Nigam *et al.*[38] observed varied extent of remazol black B decolorization with different substrates, which can be arranged as: Glucose (82%)>glycerol (71%) = lactose (71%)>starch (51%)>distillery waste (39%). Several researchers

have reported the requirement of glucose supplementation as a source of carbon and energy for azo dyes decolorization[2,106,4]. Hu[37] supplemented glucose for the decolorization of azo dyes by *Pseudomonas luteola*. Asad *et al.*[89] added glucose for the decolorization of remazol black B by halotolerant and halophilic bacterial isolates. Wang *et al.*[92] reported 96.2% decolorization of reactive red 180 anaerobically by *Citrobacter* sp., when added with glucose at 4.0 g L^{-1}. Similarly, maximum growth of *P. putida* SKG-1 isolate and acid orange 7 decolorization (89.9%) was achieved when glucose was supplemented at optimum 0.4% (w/v) concentration in dye containing minimal salt medium[14]. In their another study, Garg and Tripathi[17] reported 63.9% decolorization of acid orange 7 by *B. cereus* RMLAU1 isolate, when supplemented with 4.0 g glucose L^{-1} MSM within 96 h, which was only slightly higher than the results at 72 h incubation. Any deviation from optimum glucose concentration was inhibitory and reduced the growth response as well as the extent of dye decolorization. The low decolorization extent at lower than optimum glucose level could be due to insufficient concentration of glucose for meeting the growth requirement for color removal. Whereas, at higher than optimum glucose concentration, the bacterial cells could utilize glucose preferencially for other metabolic activities, thereby resulting in lower extent of dye decolorization[92]. Glucose is an easily metabolizable substrate, which acts not only as a reducing agent for dyes, but also promotes bacterial growth and metabolism, ultimately increasing dye decolorization[80]. In disagreement with other researchers, Chen *et al.*[1] found glucose inhibitory for decolorization of red BRN by *Aeromonas hydrophila*. This was attributed to production of organic acids, thus lowering the pH, which inhibited the bacterial growth and color removal process.

Contrary to above reports, Ponraj *et al.*[12] found sucrose (at 1.0%, w/v) as the best carbon and energy substrate for maximum 87.8% decolorization of orange 3K dye by *Bacillus* sp. They observed that orange 3R decolorization activity of *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas* sp. was 87.80, 72.36, 86.18 and 80.49% on sucrose; 81.83, 56.83, 83.33 and 75.76% on glucose and 87.09, 64.04, 86.84 and 73.68% on mannitol substrates, respectively, whereas, other researchers reported starch as the best main substrate for decolorization of different dyes by various microorganisms[107,9]. Alalewi and Jiang[108] also found starch as the best carbon and energy supplement for acid orange 7 and direct blue 75 dye decolorization by *C. acidovorans* and *B. cepacia* isolates. It appears that the preference for carbon/energy substrate varies from one microbial strain to another. In general, the increase in dye decolorization after supplementation of carbon/energy substrate is attributed to the fact that dyes are deficient in carbon content and biodegradation without any extra substrate is difficult[107].

Among various organic and inorganic nitrogen supplements, the choice depends and varies according to the organism employed for the dye decolorization process. Hu[37], Chen *et al.*[100] and Bayoumi *et al.*[9] found peptone as the best nitrogen source for dye decolorization by different bacterial strains. Likewise, Chen *et al.*[1] found peptone or yeast extract very effective for 90% decolorization of red BRN dye by *Aeromonas hydrophila*. The increasing yeast extract concentration from 0.0-8.0 g L^{-1} resulted in higher decolorization rates which reached a plateau at 8.0 g L^{-1} level. However, the extent of color removal did not enhance significantly (>90%) when yeast extract level increased from 8.0-10.0 g L^{-1}. Other researchers also reported that the color removal percentage of most dyes increased shortly after addition of yeast extract[66,109,89]. Whereas, Nachiyar and Rajakumar[110] reported that organic nitrogen sources did not support appreciable decolorization, but when combined with inorganic nitrogen (NH_4NO_3), there was an increasing effect both on the growth of *P. aeruginosa* as well as navitan fast blue SSR dye decolorization. Ponraj *et al.*[12] reported that the extent of orange 3R decolorization by *Bacillus* sp., *Klebsiella* sp., *Salmonella* sp. and *Pseudomonas* sp. were 84.67, 67.33, 70.67 and 87.33% on yeast extract; 85.29, 64.71, 42.16 and 78.43% on peptone and 82.81, 70.17, 53.91 and 81.25% on yeast extract nitrogen sources, respectively. Garg *et al.*[14] also found inorganic nitrogen supplement ammonium sulphate (at 0.1%, w/v) optimum for maximum 92.8% acid orange 7 dye decolorization by *P. putida* SKG-1 isolate. The performance of other nitrogen sources was in the order: Ammonium nitrate (70.6%)>ammonium chloride (39.4%)>peptone (30.1%). Likewise, when *B. cereus* RMLAU1 isolate was employed for acid orange 7 dye decolorization, 76% decolorization was achieved with ammonium sulphate as a nitrogen supplement at 0.1% (w/v) concentration. Therefore, it appears that certain C:N ratio is required for the maximum efficiency of azo dye decolorization by specific type of microorganisms.

Dye concentration: The concentration of dye is another important critical factor that influences microbial textile dye decolorization. The dyestuff can influence the efficiency of its removal through a combination of factors including the toxicity of dye at higher concentrations and the ability of enzyme(s) to bind the substrate efficiently at very low concentrations that may be present in some textile

wastewaters. The effect of dye concentration on the rate of color removal by various microorganisms (bacteria and fungi) have been studied by a number of researchers[33,65,46,48].

Several researchers have reported that the extent of dye concentration in dye simulated minimal salt medium and textile effluent[111]. Wuhrmann *et al.*[31] observed that the rate of color removal remained rapid during initial incubation period and thereafter declined rapidly. This effect was attributed to the toxic effect of the metabolites formed during initial dye reduction and there was inverse relationship between dye concentration and the time required for its decolorization. Similarly, Sani and Banerjee[112] found that the dyes were decolorized easily when the concentration was in the range of 1.0-10.0 µM and color removal was reduced at 30 µM concentration. Khehra *et al.*[65] and Kalme *et al.*[46] observed that dye decolorization was strongly inhibited when a high concentration of dyestuff was used and it was due to the toxic effect of the dye on the degrading microorganisms. Grekova-Vasileva *et al.*[49] isolated a highly efficient *Pseudominas marginalis* AZ029 strain, which was capable of degrading up to 700 mg amaranth L^{-1} within 24 h incubation. Wang *et al.*[92] observed that the time required to reach a maximum dye decolorization extent were 24, 24, 36, 36 and 48 h, respectively when reactive azo 180 dye decolorization by *Citrobacter* sp. was performed at 25, 50, 100, 200 and 500 mg L^{-1} concentrations. Likewise, Tripathi and Srivastava[5] also reported *P. putida* MTCC 102 to tolerate and decolorize high level of acid orange 10 at 100-1000 mg L^{-1} concentration. However, the time required for decolorization proportionately enhanced with increase in dye concentration. Ghodake *et al.*[11] reported 92% color removal within 48 h at 50 mg dye L^{-1} and 71% decolorization of initial 100 mg amaranth L^{-1} by *Acinetobacter calcoaceticus* within 72 h incubation, further increase in dye concentration (150, 200 and 250 mg L^{-1}) was increasingly inhibitory for the decolorization activity exhibiting 56, 46 and 32% color removal, respectively at 120 h incubation. Garg *et al.*[14] reported that *P. putida* SKG-1 isolate could tolerate acid orange 7 concentration up to 1000 mg L^{-1} and the findings are in contrast to the toxic effect observed for acid orange 10 within 0.037-0.051 mM dye concentration[113]. The SKG-1 strain exhibited high extent of color removal (85.2%) performance at 100 mg L^{-1} MSM and was able to decolorize the dye at much higher initial concentration[14] as compared to other bacterial strains[79,43,114]. Garg and Tripathi[17] in their another study observed a direct correlation between the growth of *B. cereus* RMLAU1 strain and acid orange 7 decolorization and maximum color removal (63%) was evident at 100 mg dye L^{-1} MSM at 96 h incubation. The authors further reported that the increasing concentration of dye (100-500 mg L^{-1}) was inhibitory for the growth as well as dye removal at every incubation time (24-96 h). The inhibitory effect of dyestuffs could be attributed to toxicity on the bacterial growth and/or blockage of enzyme active site(s) involved in dye decolorization. Garg *et al.*[15] optimized the process variables for discoloration of reactive orange 4 dye by *P. putida* SKG-1 isolate. Optimization of various process parameters employing Box-Behnken design suggests that dye concentration is the most critical factor for efficient discoloration (98%) of reactive orange 4 dye.

DYE STRUCTURE

The rate of azo bond reduction depends on the chemical structure of azo dyes[115,105] and some of the dyes are accordingly more resistant to removal by microbial cells[103]. The dyes with simple structure and low molecular weight are more easily decolorized as compared to those which have complex (highly substituted) structure with high molecular mass[112]. Depending on the position (location) of azo bond in dye molecule and their number, some dyes are degraded more easily and rapidly than others. The color of monoazo dye is removed faster than diazo and triazo dyes. Hu[37] observed an enhanced turnover rate of monoazo dyes with their increasing concentration, while remained constant in case of diazo and triazo dyes. Fiber reactive azo dyes often contain solubilizing side groups as well as a reactive nucleophilic group. Depending on the structure of such groups, biodegradation rate of azo dyes may be altered. The effect of sulphonate groups on color removal depends on the mechanism of decolorization. If the dye reduction occurs intracellularly, the permeability of sulphonated dyes across the microbial cell membrane will be hindered. Thus, more are the numbers of sulphonate groups, the rate of dye reduction will proportionately decrease. Further, acid dyes exhibit low color removal rate due to number of sulphonate groups. However, if dye reduction occurs outside the cell (extracellular), the presence of sulphonate groups will have little effect on the rate of dye reduction. Thus, direct dyes exhibit high extent of color removal that is independent of sulphonate groups in the dye.

Lade *et al.*[59] reported that textile azo dyes were completely decolorized by the bacterial consortium under microaerophilic, sequential aerobic/microaerophilic and microaerophilic/aerobic processes with some differences in decolorization times depending on the dyes structures. The sequential microaerophilic/aerobic process was found to be very effective in azo dyes decolorization as no aromatic amines detected in dye-decolorized broths.

DYE TOXICITY CONSIDERATIONS

The treated effluents from the textile industries are generally released into the nearby agricultural farms for the purpose of irrigation. Therefore, it becomes essential to test the toxicity of untreated and treated textile effluents on the agricultural seeds. Most common methods employed to study phytotoxicity are monitoring seed germination and plant growth. Phytotoxicity studies of dyes and textile effluents using plant seedlings is the main and primary toxicity study to assess the toxic nature of dye molecules.

The potential toxic effects to the environment and humans resulting from the exposure to dyes and their metabolites, is not a new concern. As early as 1895, high rates of bladder cancer were evident in workers involved in dye manufacturing. Since then, several studies have been performed exhibiting the toxic potential of azo dyes[116], which reveal that the toxicity is mainly due to dye metabolites. Azo dyes are primarily composed of aromatic amines. Substituted benzene and naphthalene rings are common constituents of azo dyes and have been identified as potentially carcinogenic agents. While, most of the azo dyes are non-toxic by themselves, a significantly larger proportion of their metabolites are toxic. Other concerns are the impurities associated with commercial dye compounds and the additives used during the dyeing process. Many textile effluents contain heavy metals that are complexed in azo dyes. High salt concentrations are often used to force fiber-reactive dyes out of solution and onto substrates[117]. These compounds can cause high electrolyte concentration and conductivity in the dye wastewater, leading to acute and chronic toxicity concerns. An investigation of several hundred textile dyes revealed that nearly 10% of them were mutagenic in Ames test. Another study performed on 45 combined effluents from textile finishing plants revealed that 27% of wastewater samples were mutagenic in Ames test.

Pourbabaee *et al.*[2] studied the relative sensitivity of three plant seeds against the untreated and treated real textile effluents. Untreated real effluent at 30 ppm (v/v) caused complete inhibition of *Lens orientalis* and 50-60% inhibition of *Triticum boeoticum* seeds germination. However, the latter seed variety experienced 100% inhibition at the real effluent concentration range of 50-150 ppm. *Triticum aestivum* was noted to be less sensitive than other above two seed species (30% inhibition at 30 ppm and 50% inhibition at 50-150 ppm). On the other hand, bacterially (*Bacillus* sp., strain PS) treated (completely decolorized) effluent exerted only 10% inhibition on the germination of *T. boeoticum* seeds and no inhibitory effect on the germination of *L. orientalis* and *T. aestivum* seeds. The researchers finally concluded that the biodegradation products formed after 20 days treatment of terasil black containing real effluent by *Bacillus* sp. did not interfere with the germination of three seed types, which was attributed probably to consumption or at least transformation of terasil black to non-toxic metabolites by PS strain of *Bacillus* sp., isolate.

Liao *et al.*[51] evaluated the toxic effects of Reactive Black B (RBB) azo dye, before and after decolorization with the freshwater unicellular green algae *C. vulgaris*. The results revealed that untreated RBB exhibited toxicity and media effective concentration (EC_{50}) for *C. vulgaris* was 48 mg L^{-1}. Kabra *et al.*[52] examined the toxic effect of untreated and plant-bacterium (*Glandularia pulchella-Pseudomonas monteilii* ANK) consortium treated dye mixture (B3RFL, RGFL, SRR, GHE4B) and textile effluents A and B on the germination and root/shoot growth of *Phaseolus mungo* and *Sorghum vulgare* seeds. The seeds showed a higher percentage of germination in distilled water and treated textile effluents and dye mixture. At the same time, the length of plumule and radical was found to be lower in seeds germinated in the untreated effluents and dye mixture than those germinated in distilled water and treated textile effluent and dye mixture. It is clearly inferred from the findings that the untreated samples were toxic to these plants, while the treated samples were almost as non-toxic as distilled water[52].

Brown and De Vito[116] have suggested biological mechanisms thought to be responsible for carcinogenic activation of azo dye compounds. The examples of aromatic amines and other potential dye metabolites which may be considered as potential carcinogens are cited in Table 3. The researchers postulated that (1) Azo dyes may be toxic only after reduction and cleavage of the azo linkage, leading to release of aromatic amines, (2) Azo dyes with structures containing free aromatic amine groups that can be metabolically oxidized without azo reduction may cause toxicity and (3) Azo dye toxic activation may occur following direct oxidation of the azo linkage producing highly reactive electrophilic diazonium salts.

The textile dye effluents are recalcitrant to biodegradation and cause acute toxicity to the receiving water bodies due to the presence of various toxic dyes. Therefore, decolorization and detoxification of textile wastewater is very important to degrade the toxic chemicals present in textile wastewater before releasing it into the nearby local waterbodies/environment.

Table 3: Toxic intermediates formed during azo dye degradation

Azo dye	Dye degrading bacteria	Intermediate metabolites	References
Reactive green 19 A	*Micrococcus glutamicus* NCIM 2168	Naphthanlene	Saratale *et al.*[99]
Direct brown MR	*Acinetobacter calcoaceticus*	Biphenyl amine, 3-amino 6-hydroxybenzoic acid and naphthanlene	Ghodake *et al.*[11]
Direct black 22	Bacterial consortium DMC	1-Naphthol	Mohana *et al.*[118]
Acid red 22	*Stenotrophomonas* sp., *Pseudomonas* sp. and *Bacillus* sp.	6-amino naphthalene sulfonic acid	Khehra *et al.*[65]
Reactive blue 172	*Pseudomonas aeruginosa* NBAR12	Aromatic amines	Bhatt *et al.*[64]
Methyl red	*Vibrio logei* and *Pseudomonas nitroreducens*	2 amino benzoic acid and N-N, dimethyl 1-4 phenylenediamine	Adedayo *et al.*[119]

CONCLUSION

The discharge of textile azo dye into the environment is of great concern due to its color, toxicity, mutagenicity and carcinogenicity. Various physico-chemical treatment methods in the removal of color from textile effluent are now used or have been suggested, but often aren't implemented due of the excessive cost involved in the method and generation of secondary pollutants. More recently, the research employing eco-friendly microbial based methods are offering promise for even greater development in the future. The ultimate objective should be zero toxicants or color emission through discharge of the decolorized and detoxified textile effluent into the environment.

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