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Review Article

Microbial Biotechnology for Detoxification of Azo-Dye Loaded Textile Effluents: A Critical Review

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Abstract

Textile industries are generating a huge volume of wastewater having dye residues. Entry of these wastewaters into surface waters and their use for raising crops deteriorates these natural resources. Azo-dyes cause phyto-toxic, zoo-toxic and geno-toxic anomalies in the environment. Tightening government legislation is forcing textile industries to treat their effluent to minimize their harmful impacts in environment. During the last couple of decades, microbial biotechnology has emerged as an environment friendly and cost-effective approach for decolorization of textile effluents. Many microbial strains capable of decolorizing azo-dye effluents have been isolated and characterized. However, decolorization does not necessarily indicates detoxification of effluent. Recent scientific efforts are focused to identify microbes capable of simultaneously doing decolorization and detoxification of azo-dye effluents. Here we review (i) phyto-toxic, zoo-toxic and microbiocidal effects of azo-dye contaminated waters (ii) the effectiveness of bacteria, fungi and microbial consortium for treatment of textile effluents to reduce potential risks to plants and animals (ii) mechanisms of microbial detoxification of textile effluents and highlights the role of several azo-dyes degrading enzymes in detoxification of textile effluents. The review article suggests that microbial technology could be exploited for the treatment of textile effluents at large scale. © 2019 Friends Science Publishers

Keywords: Ecotoxicology; Geno-toxic; Phyto-toxic; Zoo-toxic; Bioremediation; Inducible enzymes

Introduction

Azo-dyes, characterized by the presence of one or more azo groups (HN=NH), *i.e*., a chromophoric color producing group (Cui *et al*., 2011; Mishra and Maiti, 2018) which generally belongs to benzene or naphthalene that causes toxicity to these dyes (Vijayaraghavan *et al*., 2013; Das and Mishra, 2019). According to an estimate, azo-dyes represent about 80% of the 100,000 commercial dyes synthesized in world with an annual production of 7×10^5 tons (Fu and Viraraghavan, 2001; Mishra and Maiti, 2018). The major reasons of their extensive use in textile sector include ease and low cost of synthesis, their stability and availability in variety of colors compared to natural dyes (Waghmode *et al*., 2011; Shah *et al*., 2014). However, due to inefficient dyeing process, large quantities of azo-dyes are directly released and lost into wastewater during dyeing and washing processes. This results into production of huge volume of wastewater. The amount of dyes lost in wastewaters ranges from 2–50% depending upon type of azo-dyes (O'Neill *et al*., 2000). Average concentration of dyes in effluents of textile industries is about 300 mg L^{-1} (Tony *et al*., 2009). Nevertheless, concentration as high as 1500 mg L^{-1} has also been observed (Pierce, 1994). In most of developing countries, treatment of colored effluents generated from textile units is not a common practice, and such effluents are directly released into soils or surface waters, thus, seriously polluting them (Das and Mishra, 2019). Occurrence of dyes in soil negatively affects nitrogen transformation processes *i.e*., urease activity, arginine ammonification rate, nitrification potential (Topac *et al*., 2009; Batool *et al*., 2015), and soil microbial community structure (Imran *et al*., 2015a). Under water shortage conditions, use of such wastewaters for raising vegetables and grain crops in peri-urban areas is becoming a common practice in some developing countries, resulting in production of contaminated poor-quality foods. The dye residues in textile effluents are undesirable because of their phyto-toxic, zoo-toxic, cyto- and geno-toxic effects (Phugare *et al*., 2011a; Przystas *et al*., 2012; Pokharia and Ahluwalia, 2016b). Thus, dye effluents must be treated for complete detoxification or at least to minimize biological toxicity level.

Several chemical (ozonation, coagulation-flocculation, Fenton oxidation, electrochemical, ultrasonic chemical and irradiation oxidation, chlorine disinfection) and physical (filtration, coagulation, bio-sorbents; activated carbon, chitosan, chitin, alumina, silica gel, clays, peat, sawdust, rice husk, maize cobs, orange peels, fly ash, red mud and bagasse pith) methods have been suggested and implied for treatment of azo-dye effluents, but these are not widely applied either because of high cost or secondary pollution due to sludge production and chemicals (Selcuk, 2005;

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Chacko and Subramaniam, 2011). For instance, Selcuk (2005) found that ferrous sulfate (500 mg L^{-1}) and aluminum sulfate (750 mg L^{-1}) did not reduce toxicity of dye effluent to *Daphnia magna* (*D. magna*) larvae. However, high concentrations of both chemicals reduced the toxicity, but are not economical due to high sludge production and cost of chemicals. Ozone treatment of Remazol black B resulted in the production of metabolites which increased toxicity to *D. magna* (Souza *et al*., 2010). Similarly, chlorine disinfection of water containing Disperse Red 1 was found ineffective in reducing geno-toxicity of azo-dye (Vacchi *et al*., 2013). Alternatively, biological treatment methods (microbial decolorization and degradation of dyes) have been proved to be effective in terms of decolorization and biotransformation of toxic dyes into non-toxic products and a lower amount of sludge generation with cost-effective operation and maintenance (Yang *et al*., 2011; Maqbool et al., 2018; Mishra and Maiti, 2018). A large number of bacterial and fungal strains capable of decolorizing synthetic and real dye effluents have been isolated, characterized and identified (Hussain *et al*., 2013; Anwar *et al*., 2014; Imran *et al*., 2014, 2015c, 2016; Abbas *et al*., 2016). However, measurement of only decolorization does not ensure that wastewater has been detoxified and is safe for discharge into environment (Selcuk, 2005). A few researchers reported that decolorization by biological means may not change toxicity level of dye effluent (Souza *et al*., 2007), while some others found even an increase in toxicity levels after microbial treatment (Ambrosio and Campos-Takaki, 2004; Anastasi *et al*., 2011; Przystas *et al*., 2012; Choi *et al*., 2014). Thus, research on azo-dyes has proven that decolorization does not ensure effluent detoxification. Previously published review articles mainly focus on the efficiency of microbes to decolorize azo-dye effluents. This review describes simultaneous removal of color and detoxification of textile effluents, confirmed by various biological assays. Moreover, detoxification mechanisms and role of different dye degrading microbial enzymes in detoxification of textile dyes have been elucidated in detail.

Eco-toxicity of Azo-dyes

Azo-dyes in textile wastewater are considered as a serious environmental pollutant due to their hazardous nature. However, azo-dyes vary in their toxicity level depending upon type and chemical structure (Zablocka-Godlewska *et al*., 2015). Various bioassays which measure phyto-toxicity, zoo-toxicity and microbicidal/microbiostatics effects have been employed for evaluating eco-toxicity of different azo-dyes commonly found in textile wastewaters (Zablocka-Godlewska *et al*., 2015; Bilal *et al*., 2016). Generally, eco-toxicity analysis of azo-dyes polluted wastewaters is carried out for two major reasons. Firstly, for risk assessment of discharge of azo-dyes polluted wastewaters into surrounding water and soil resources. Addition of such wastewaters in water and soils may have negative impacts on biological components of such environments (Topac *et al*., 2009; Imran *et al*., 2015a). Secondly, toxicity analysis is helpful to assess the effectiveness of different wastewater treatment technologies. Eco-toxicity of synthetic and real textile effluents is discussed in detail here.

Phyto-toxic Effects of Azo-dyes

The impacts of azo-dyes on plant growth are generally measured by soaking seeds in azo-dyes containing wastewater and distilled water as a control. After few days, germination, plumule and radical length are measured (Jadhav *et al*., 2013). Germination and growth analysis of synthetic and real azo-dyes effluents have been carried out by many researchers (Table 1 and 2). Lade *et al*. (2015a) reported that Reactive blue 172 at 50 mg L^{-1} inhibited germination of *Sorghum vulgare* and *Phaseolus mungo* up to 70 and 60%, respectively, compared to control seeds. Moreover, shoot and root length of both plants decreased. Likewise, Direct red 81 did a huge decrease in germination and growth of *S. vulgare* and *P. mungo* seedlings (Sahasrabudhe *et al*., 2014). In *Triticum aestivum*, Red HE7B and Brown 3RE did complete inhibition of seed germination (Kalme *et al*., 2007; Dawkar *et al*., 2008). The plant growth inhibition by structurally different azo-dyes including Basic red 46, Congo red, Methyl red, Remazol red, Remazol orange, Disperse red F3B, Acid red 27, Reactive orange 16, Reactive yellow-84A, Rubine GFL, Reactive levafix blue, Evans blue, Remazol brilliant blue R, Remazole brilliant violet 5R, Reactive blue 220, Trypan blue, Reactive red 120 and Green HE4BD is shown in Table 1.

Like synthetic azo-dyes polluted wastewaters, azo-dyes residues in raw textile effluents have also been found to suppress growth of different crop plants (Table 2). For Instance, Phugare *et al*. (2011b) reported that un-treated effluent from Ichalkaranji, India completely inhibited seed germination of *T. aestivum*. Lade *et al*. (2012) found that un-treated effluent of Mahesh Textile Processors, Ichalkaranji, India, inhibited seed germination of *S. vulgare* and *P. mungo* up to 60% and 50%, respectively. The shoot length of control plants of *S. vulgare and P. mungo* was 7.88±0.54 and 11±0.1 cm, respectively, which decreased to 1.60±0.32 and 4.10±0.13 cm, respectively. Similarly, radical length of plants was also lesser in case seeds of both crops soaked in un-treated effluent. Other researchers have also found that un-treated textile effluents suppress germination and growth of plants (Phugare *et al*., 2011b; Zhuo *et al*., 2011; Vijayalakshmidevi and Muthukumar, 2015).

Azo-dyes may suppress seed germination and plant growth due to their cyto-toxic and geno-toxic effects. Mostly for measuring cyto-toxic and geno-toxic properties of azo-dyes, *Allium cepa* have been used as a test plant. Cyto-toxic and geno-toxic effects of various azo-dyes and real textile effluent in plants are shown in Table 1, 2 and 3. Phugare *et al*. (2011a) exposed small bulbs of *A. cepa* to 500 mg L^{-1} Red HE3B for 48 h. Azo-dye inhibited root growth and did chromosomal aberrations and changes in DNA of root cells.

Table 1: Phyto-toxicity analysis of microbially treated synthetic azo-dye wastewater

Microbial Strain	Azo-dye	Dye concentration (mg L^{-1})	Biological Treatment Time	Tested crop	Toxicity Analysis				Reference
					Parameter	Control	Azo-dye	Bio-treatment	
BACTERIA									
Bacillus fermus (Kx898362)	Direct Blue 14	150	72 h	*Allium cepa* root cells	No. of dividing cells	249	22	170	Neetha *et al.* (2019)
					MI (%)	24.9 ± 2.0	2.2 ± 2.66	17 ± 1.66	
					Aberrant cells (%)	1.4	2.0	1.8	
Enterobacter aerogenes PP002	Direct Blue 71	100	168 h	*S. vulgare*	Germination (%)	100	52	94	Sudha *et al.* (2018)
					PL (cm)	7.36 ± 0.7	2.06 ± 0.6	6.2 ± 1.05	
					RL (cm)	2.1 ± 0.7	2.06 ± 0.6	3.6 ± 1.06	
Acinetobacter baumannii MN3	Congo red	100	24 h	*V. radiata*	Germination (%)	100	30	90	Kuppusamy *et al.* (2017)
Staphylococcus epidermidis MTCC 10623	Basic red 46	100[a]	6 h	*T. aestivum*	Germination (%)	100	60	100	Pokharia and Ahluwalia (2016a)
					PL (cm)	15.74±0.50	6.50±0.50	16.25±0.05	
					RL (cm)	10.30± 0.36	3.53 ± 0.15	10.63±0.50	
Providencia rettgeri Strain HSL1	Reactive blue 172	50[a]	20 h	*S. vulgare*	Germination (%)	100	30	90	Lade *et al.* (2015a)
					SL (cm)	9.5 ± 0.5	4.5 ± 0.2	9.2 ± 0.4	
					Root length (cm)	3.8 ± 0.3	2.2 ± 0.1	3.6 ± 0.4	
				P. mungo	Germination (%)	100	40	90	
					SL (cm)	10.4 ± 0.4	5.8 ± 0.2	10.2 ± 0.3	
					Root length (cm)	4.5 ± 0.2	2.1 ± 0.3	4.1 ± 0.2	
Bacillus spp. strain UN2	Methyl red	100[a]	30 min	*T. aestivum*	Germination (%)	100	82	97	Zhao *et al.* (2014)
					SL (cm)	12.29 ± .40	7.33 ± 0.22	11.08± 0.26	
					Root length (cm)	8.42 ± 0.46	2.95 ± 0.21	5.10 ± 0.21	
				S. bicolor	Germination (%)	100	76	95	
					SL (cm)	6.55 ± 0.22	3.55 ± 0.44	5.29 ± 0.54	
					Root length (cm)	5.08 ± 0.34	3.11 ± 0.47	4.49 ± 0.38	
Enterococcus faecalis YZ 66	Direct red 81	50 (400)[a]	90 min	*P. mungo*	Germination (%)	100	70	100	Sahasrabudhe *et al.* (2014)
					SL (cm)	10.3 ± 1.91	8.18 ± 1.70	11.54± 1.11	
					Root length (cm)	5.11 ± 1.35	4.55 ± 0.87	7.23 ± 1.13	
				S. vulgare	Germination (%)	100	70	100	
					SL (cm)	10.46 ± 1.12	8.31 ± 1.44	10.8 ± 1.31	
					Root length (cm)	6.64± 0.512	5.04 ± 0.69	08.0 ± 1.01	
Lysinibacillus spp. RGS	Remazol red	300[a]	6 h	*P. mungo*	Germination (%)	100	40	90	Saratale *et al.* (2013)
					SL (cm)	3.52 ± 0.05	2.15 ± 0.03	3.68 ± 0.07	
					Root length (cm)	2.36 ± 0.05	1.17 ± 0.04	1.86 ± 0.05	
				S. vulgare	Germination (%)	100	40	90	
					SL (cm)	4.31 ± 0.11	2.25 ± 0.03	3.66 ± 0.10	
					Root length (cm)	1.67 ± 0.06	No roots	1.18 ± 0.03	
Pseudomonas aeruginosa BCH	Remazol orange	50 (500)[a]	5 h	*P. mungo*	Germination (%)	80	40	70	Jadhav *et al.* (2013)
					PL (cm)	6.35 ± 0.56	2.96 ± 0.24	5.41 ± 0.78	
					RL (cm)	4.83 ± 0.63	1.98 ± 0.82	4.51 ± 0.82	
				T. aestivum	Germination (%)	90	30	70	
					PL (cm)	10.0 ± 1.25	4.74 ± 1.72	8.73 ± 1.02	
					RL (cm)	5.13 ± 0.62	3.03 ± 0.74	4.01 ± 0.12	
				S. vulgare	Germination (%)	80	30	80	
					PL (cm)	8.28 ± 1.93	5.03 ± 0.92	7.56 ± 0.73	
					RL (cm)	5.01 ± 0.73	2.43 ± 0.51	3.98 ± 0.14	
Pseudomonas aeruginosa BCH	Remazol red	50	20 min	*Allium cepa* root cells	Cell viability (%)	-	86.5 ± 3.11	94.69±2.04	Jadhav *et al.* (2011)
					MI		12.2±1.304	10.4± 0.894	
					Chrom. Breaks		3	1	
					TCA		23	6	
Bacillus spp. VUS	Brown 3RE	50 (4000)[a]	8 h	*T. aestivum*	Germination (%)	100	0	100	Dawkar *et al.* (2008)
FUNGI									
Trichoderma tomentosum	Acid Red 3 R	86	72 h	*Glycine max*	Germination (%)	100	73	80	He *et al.* (2018)
Aspergillus flavus	Malachite Green	500	192 h	*Vigna radiata*	Germination (%)	100	77	100	Barapatre *et al.* (2017)
					PL (cm)	20.06± 3.77	14.56± 1.29	16.07±1.95	
					RL (cm)	8.37 ± 2.19	2.06 ± 0.76	5.02±1.57	
Ceriporia lacerata	Congo red	100	48 h	*Amaranthus mangostanus*	Germination (%)	76	62	55	Wang *et al.* (2017)
				Sesamum indicum		65	60	38	
Armillaria spp. F022	Acid red 27	30 (500)[a]	72 h	*S. vulgare*	Germination (%)	100	90	90	Adnan *et al.* (2015)
					PL (cm)	8.79 ± 0.27	2.54 ± 0.55	5.01 ± 0.54	
					RL (cm)	5.76 ± 0.42	2.38 ± 0.66	4.71 ± 0.46	
				T. aestivum	Germination (%)	100	60	90	
					PL (cm)	9.31 ± 0.97	4.11 ± 0.95	7.61 ± 0.77	
					RL (cm)	5.67 ± 0.33	2.01 ± 0.42	5.15 ± 0.63	
Ganoderma spp. En3	Reactive orange 16	2000[a]	96 h	*O. sativa*	SL (cm)	[b]1.8	[b]0.5	[b]1.6	Ma *et al.* (2014)
					Root length (cm)	[b]5.2	[b]0.3	[b]3.8	
Galactomyces geotrichum MTCC 1360	Rubine GFL	50 (1000)[a]	96 h	*P. mungo*	Germination (%)	100	80	100	Waghmode *et al.* (2012)
				S. vulgare	Germination (%)	100	10	90	
Irpex lacteus	Reactive levafix blue E-RA	250[a]	96 h	*B. juncea*	Germination (%)	100	100	100	Kalpana *et al.* (2012)
					PL (cm)	1.69	0.74 ± 0.24	0.98 ± 0.30	
					RL (cm))	1.83 ± 0.53	0.67 ± 0.34	1.13 ± 0.21	
Pleurotus ostreatus (BWPH)	Evans blue	80[a]	120 h	*L. minor*	OECD *Lemna* spp. growth inhibition test No. 221	-	Class IV (toxic)	Class-III	Przystas *et al.* 2012

Table 1: Continued

Table 1: Continued

Galactomyces geotrichum MTCC 1360	Rubine GFL	50	96 h	*Allium cepa* root cells	Cell viability (OD_{600}) MI Chrom. Breaks TCA	-	0.142 15.67±0.417 6.0 10.0	0.076 13.72±0.672 3.0 4.0	Waghmode *et al*. (2012)
Galactomyces geotrichum MTCC 1360	Azo-dye mixture (7)	70 (1000)[a]	24 h	*P. mungo* *S. vulgare*	Germination (%) SL (cm) Root length (cm) Germination (%) SL (cm) Root length (cm)	100 10.70 ± 0.360 2.36 ± 0.263 90 1.96 ± 0.035 9.40 ± 0.091	70 2.22±0.182 1.09±0.105 40 0.58±0.018 1.25±0.072	90 8.29 ± 0.097 3.46 ± 0.032 90 1.90 ± 0.046 9.23 ± 0.066	Waghmode *et al*. (2011)
Pleurotus ostreatus MUT 2976 *Trametes pubescens* MUT 2295	Remazol brilliat blue R	2000[a]	6 days	*L. minor*	GI (%) Plant dry biomass Growth inhibition Plant dry biomass	- - 	24.4 ± 2.7 32.7 ± 5.0 24.4 ± 2.7 32.7 ± 5.0	11.5 ± 8.9 15.2 ± 4.7 7.8 ± 5.9 17.9 ± 2.1	Casieri *et al.* (2008)
MICROBIAL CONSORTIA									
Bacillus pumilus HKG212, *Zobellella taiwanensis* AT 1-3 and *Enterococcus durans* GM13	Remazol navy blue	300	7 days	*P. mungo*	Germination (%) PL (cm) RL (cm))	100 12.82 ± 0.51 3.35 ± 0.38	40 2.61 ± 0.39 0.81 ± 0.05	100 11.1 ± 0.72 3.24 ± 0.36	Das and Mishra (2019)
Acinetobacter baumannii MN3 and *Pseudomonas stutzeri* MN1	Congo red	100	24 h	*V. radiata*	Germination (%)	100	30	100	Kuppusamy *et al*. (2017)
Consortium VIE6: *Bacillus* spp. DMB1, *Staphylococcus* spp. DMB2, *Escherichia* spp. DMB3, *Enterococcus* spp. DMB4 and *Pseudomonas* spp. DMB5	Remazole brilliant violet 5R	200 (3000)[a]	48 h	*V. radiata*	Germination (%) PL (cm) RL (cm))	100 13.7 ± 0.9 1.9 ± 1.6	30 3.5 ± 1.5 0.8 ± 1.9	100 11.9 ± 0.4 1.3 ± 0.5	Shah *et al*. (2016)
Consortium VN.1: *Pseudomonas fluorescens* HM480360, *Enterobacter aerogenes* HM480361, *Shewanella* spp. HM589853, *Arthrobacter nicotianae* HM480363, *Bacillus beijingensis* HM480362 and *Pseudomonas aeruginosa* JQ659549	Reactive blue 220	2500[a]	8 h	*P. aureus*	Germination (%) PL (cm) RL (cm)	90 15.40 ± 0.73 7.56 ± 0.55	41 7.50 ± 0.16 2.45 ± 0.39	99 21.60 ± 0.39 10.80 ± 0.37	Patel and Bhatt (2015)
Microbial Consortium (15 bacteria)	Trypan blue	50[a]	24 h	*S. vulgare* *P. mungo*	Germination (%) SL (cm) Root length (cm)) Germination (%) SL (cm) Root length (cm)	100 9.8 ± 0.2 4.1 ± 0.2 100 10.2 ± 0.3 4.8 ± 0.3	20 3.5 ± 0.2 1.9 ± 0.1 30 4.9 ± 0.2 1.8 ± 0.2	90 9.5 ± 0.3 3.8 ± 0.2 90 10.1 ± 0.2 4.6 ± 0.2	Lade *et al*. (2015b)
Aspergillus niger and *Bacillus* spp.	Reactive red 120	50[a]	24 h	*V. radiata*	Germination (%) Root length (cm)	100 5.6 ± 0.4	90 1.2 ± 0.3	100 3.7 ± 0.9	Su and Lin (2013)
Microbial consortium SDS: *Providencia* spp. SDS (PS) and *Pseudomonas aeuroginosa* strain BCH (PA)	Red HE3B	50 (500)[a]	1 h	*P. mungo* *T. aestivum* *A. cepa* root cells	Germination (%) PL (cm) RL (cm) Germination (%) PL (cm) RL (cm) MI TA Chrom. Breaks Tail DNA (%) Tail length (µm)	100 9.14 ± 1.78 7.76 ± 1.53 100 10.10 ± 1.35 8.65 ± 1.23 11.10 ±0.2111 40.24 ± 4.21 26.64 ± 3.03 26.64 ± 3.03	60 4.66 ± 1.45 3.87 ± 1.32 40 5.16±1.46 3.35±1.01 13.36±1.16 8 3 58.51± 4.12 43.18± 3.02	80 8.51 ± 1.37 7.18 ± 1.89 70 7.21 ± 1.87 6.01 ± 1.09 10.98 ± 0.43 2 2 41.32 ± 3.43 27.63 ± 2.03	Phugare *et al*. (2011a)
Microbial consortium GR: *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168	Green HE4BD	50 (300)[a]	24 h	*P. mungo*	Germination (%) PL (cm) RL (cm)	100 14.63 ± 1.17 6.03 ± 0.74	40 1.28 ± 0.88 0.88 ± 0.56	100 10.32 ± 1.37 4.83 ± 0.84	Saratale *et al*. (2010a)

[a] Shows the concentration of azo-dye or metabolites (produced during azo-dye biodegradation) used in phyto-toxicity evaluation test
[b] Shows approximate values taken from figures
PL: plumule length, **RL**: radical length, **SL**: shoot length, **MI**: mitotic index, **TCA**: total chromosome alterations, **GI**: germination inhibition, **TA**: total number of alterations, **PRL**: primary root length, **CA**: chromosomal aberrations, **TMC**: total number of mitotic cells

Mitotic index (1–8), total alterations in chromosome (1–8), chromosome breaks (1–3), tail DNA (40.24 ± 4.21–58.51 ± 4.12%) and tail length (26.64 ± 3.03–43.18 ± 3.02 μm) were increased than control *A. cepa* root cells. Likewise, Waghmode *et al*. (2012) reported that Rubine GFL (1000 mg L^{-1}) reduced root cell viability and increased mitotic index (12.42 ± 0.517–15.67 ± 0.417), chromosome breaks (1–6) and total number of alterations (1–10). It has also been documented that Remazol red has cyto-toxic and geno-toxic effects (Jadhav *et al*., 2011). Cyto-toxic and geno-toxic effects of azo-dyes have been further confirmed by using real textile effluents having azo-dyes residues. *A. cepa* was exposed to the effluent of Textile Industry Ichalkaranji, India and much higher values of mitotic index, total number of alterations, tail DNA and tail length were found as compared to that of distilled water treatment. Viability of roots cells of *A. cepa* was also decreased by exposure to azo-dye effluent (Lade *et al*., 2012). Thus, most of the dyes occurring in textile wastewater inhibit germination and retard crop growth. The extent of inhibition is variable depending upon type of dye and its level in water. However, effect of textile dyes on yield of crops has not been assessed so far. The growth retardation of seedlings is caused by alterations in genetic material of plant cells. Therefore, before use of such textile waters on agricultural soils to raise crops, dye resides must be eliminated.

Table 2: Phyto-toxicity analysis of microbially treated azo-dye polluted real textile effluents

Microbial strain	Biological treatment time	Crop tested	Toxicity Analysis				Reference
			Parameter	Control	Untreated effluent	Treated effluent	
BACTERIA							
Trametes villosa SCS-10							
Pseudomonas spp. SUK1	24 h	*T. aestivum*	Germination (%)	90	40	70	Jadhav *et al.* (2015)
			PL (cm)	11.2 ± 1.62	5.12 ± 2.02	8.93 ± 1.64	
			RL (cm)	6.01 ± 0.31	3.53 ± 0.24	5.43 ± 0.73	
		S. vulgare	Germination (%)	80	30	60	
			PL (cm)	7.87 ± 1.32	4.03 ± 0.89	7.42 ± 0.93	
			RL (cm))	5.63 ± 0.61	2.41 ± 0.21	4.28 ± 0.54	
Lysinibacillus spp. RGS	24 h	*S. vulgare*	Germination (%)	100	20	80	Saratale *et al.* (2015)
			PL (cm)	12.56 ± 0.84	4.12 ± 0.65	10.06 ± 1.08	
			RL (cm)	6.42 ± 0.34	1.1 ± 0.08	4.72 ± 0.55	
		P. mungo	Germination (%)	100	20	90	
			PL (cm)	13.01 ± 0.85	3.5 ± 0.50	11.28 ± 1.11	
			RL (cm)	3.68 ± 0.22	0.95 ± 0.04	2.45 ± 0.24	
Exiguobacterium spp. RD3	60 h	*A. cepa* root cells	Root length (cm)	-	0.4 ± 0.018	4.3 ± 0.012	Dhanve *et al.* (2014)
Exiguobacterium spp. RD3	60 h	*T. aestivum*	Germination (%)	100	50	80	
		P. mungo	PL (cm)	9.5 ± 1.27	5.6 ± 1.33	8.5 ± 1.21	
			RL (cm)	6.83 ± 1.45	2.95 ± 1.10	5.33 ± 1.17	
			Germination (%)	100	90	100	
			PL (cm)	16.32 ± 1.26	8.14 ± 1.23	15.44 ± 1.37	
			RL (cm)	11.03 ± 1.34	1.42 ± 0.18	7.25 ± 1.41	
Proteus spp. SUK7	96 h	*P. mungo*	Germination (%)	100	60	100	Patil *et al.* (2012)
			PL (cm)	11.12 ± 0.50	7.12 ± 0.64	9.40 ± 0.45	
			RL (cm)	3.51 ± 0.42	2.04 ± 0.09	2.46 ± 0.65	
		S. vulgare	Germination (%)	100	70	90	
			PL (cm)	3.54 ± 0.74	1.33 ± 0.43	3.44 ± 0.36	
			RL (cm)	7.33 ± 0.62	1.12 ± 0.04	9.29 ± 0.46	
Pseudomonas spp. LBC1	72 h	*S. bicolor*	Germination (%)	80	20	60	Telke *et al.* (2012)
			SL (cm)	43 ± 5.0	6.0 ± 2.0	34 ± 6.0	
			Root length (cm)	48 ± 6.0	6.0 ± 1.0	55 ± 5.0	
		V. radiata	Germination (%)	60	20	45	
			SL (cm)	82 ± 6.0	11 ± 2.0	61 ± 7.0	
			Root length (cm)	60 ± 5.0	10 ± 2.0	40 ± 5.0	
		L. culinaris	Germination (%)	70	00	50	
			SL (cm)	4.80 ± 0.54	0.0 ± 0.0	2.95 ± 0.47	
			Root length (cm)	3.10 ± 0.55	0.0 ± 0.0	0.93 ± 0.11	
Bacillus spp. strain PS	20 days	*L. orientalis*	Germination (%)	100	00	100	Pourbabaee *et al.* (2006)
		T. boeoticum		100	2	90	
		T. aestivum		100	40	100	
FUNGI							
Trichoderma tomentosum	72 h						
fungal (*Ganoderma lucidum* IBL-05) ligninolytic enzymes	15 h	*T. aestivum*	Germination (%)	100	50	70	Bilal *et al.* (2016)
			PL (cm)	9.38 ± 4.02	4.26 ± 1.87	8.42 ± 2.37	
			RL (cm)	7.75 ± 1.29	4.13 ± 2.02	6.14 ± 2.41	
		Allium cepa root cells	Root length (cm)	-	3.74 ± 0.41	5.04 ± 0.41	
			MI		9.57 ± 1.11	11.74 ± 1.11	
Laccase of *Trametes* spp. strain CLBE55	20 h	*L. esculentum*	Germination (%)	100	0	24	Benzina *et al.* (2013)
Aspergillus spp. EL-2	48 h	*Trigonella-foenum-graecum*	Germination (%)	100	20	80	Gomaa *et al.* (2012)
			SL (cm)	4.5	0.5 ± 0.42	2 ± 0.42	
Ganoderma sp. En3	14 days	*T. aestivum*	SL (cm)	2.4	0.5	1.9	Zhuo *et al.* (2011)
			Root length (cm)	3.4	0.3	2.8	
Bjerkandera adusta (Willdenow) P. Karsten MUT 3060,	7 days	*C. sativus*	GI (%)	-	38.0 ± 3.9	99.8 ± 18.2	Anastasi *et al.* (2011)
Penicillium ochrochloron MTCC 517	10 days	*T. aestivum*	Germination (%)	100	50	100	Shedbalkar and Jadhav (2011)
			SL (cm)	19.35 ± 1.36	6.89 ± 2.58	15.8 ± 1.2	
			Root length (cm)	11.60 ± 0.92	2.92 ± 1.1	5.3 ± 0.23	
		Ervum lens Linn	Germination (%)	100	70	100	
			SL (cm)	19.80 ± 0.50	11.70 ± 2.6	15.76 ± 0.33	
			Root length (cm)	2.83 ± 0.24	1.35 ± 0.35	4.52 ± 1.1	
MICROBIAL CONSORTIA							
Bacterial consortium PMB11: *Bacillus odysseyi* SUK3, *Morganella morganii* SUK5, *Proteus* spp. SUK7	120 h	*P. mungo*	Germination (%)	100	50	100	Patil *et al.* (2015)
			PL (cm)	7.4 ± 0.80	3.1 ± 0.16	6.2 ± 0.18	
			RL (cm)	3.16 ± 0.24	2.4 ± 0.09	2.89 ± 0.13	
		T. aestivum	Germination (%)	100	40	100	
			PL (cm)	3.7 ± 0.74	2.17 ± 0.10	3.14 ± 0.17	
			RL (cm)	11.12 ± 0.58	6.8 ± 0.15	8.27 ± 0.29	
Consortium *Ochrobactrum* spp., *Pseudomonas aeruginosa* and *Providencia vermicola*	16 h	*V. radiata*	Germination (%)	100	45	90	Vijayalakshmidevi and Muthukumar (2015)
			PL (cm)	3.03 ± 0.11	0.20 ± 0	1.22 ± 0.13	
			RL (cm)	3.03 ± 0.15	0.23 ± 0	2.46 ± 0.01	
		P. mungo	Germination (%)	100	40	80	
			PL (cm)	10.69 ± 0.51	1.38 ± 0.22	8.0 ± 0.01	
			RL (cm)	6.24 ± 0.08	0.86 ± 0.3	3.98 ± 0.01	
Consortium TSR	24 h	*P. mungo*	Germination (%)	100	30	90	Patel *et al.* (2015)
			PL (cm)	11.67 ± 0.65	3.23 ± 0.20	10.77 ± 0.35	
			RL (cm)	5.97 ± 0.58	2.83 ± 0.12	4.5 ± 0.05	

Table 2: Continued

Table 2: Continued

		T. aestivum	Germination (%)	100	20	90	
			PL (cm)	9.56 ± 0.52	2.52 ± 0.28	8.35 ± 0.49	
			RL (cm)	6.23 ± 0.47	4.52 ± 0.32	5.98 ± 0.37	
Consortium-AP: *Aspergillus ochraceus* NCIM-1146 and *Pseudomonas* spp. SUK1	35 h	*S. vulgare*	Germination (%)	100	40	100	Lade *et al.* (2012)
			PL (cm)	4.99 ± 0.77	1.60 ± 0.32	4.15 ± 0.38	
			RL (cm)	2.29 ± 0.39	0.63 ± 0.09	1.65 ± 0.28	
		P. mungo	Germination (%)	100	50	100	
			PL (cm)	7.88 ± 0.54	4.10 ± 0.13	6.65 ± 0.42	
			RL (cm)	1.52 ± 0.26	0.70 ± 0.07	1.30 ± 0.06	
Consortium: *Providencia* spp. SDS and *Pseudomonas aeuroginosa* strain BCH	20 h	*T. aestivum*	Germination (%)	100	0.00	60	Phugare *et al.* (2011b)
			PL (cm)	10.10 ± 1.35	0.00	6.38 ± 1.54	
			RL (cm)	8.65 ± 1.23	0.00	4.89 ± 1.30	
		A. cepa root cells	MI	49.23 ± 1.23	54.41 ± 2.54	49.68 ± 1.11	
			Chromosome aberrations (%)	1.32 ± 0.81	10.13 ± 0.43	2.94 ± 0.21	
			Tail length (μm)	26.64 ± 3.03	53.62 ± 2.12	33.21 ± 2.87	
			Cell viability (%)	89	77	81	
Microbial consortium SDS: *Providencia* spp. SDS (PS) and *Pseudomonas aeuroginosa* strain BCH (PA)	20 h	*A. cepa* root cells	Chromosome aberrations (%)	-	10.1 ± 0.43	2.94 ± 0.21	Saratale *et al.* (2010b)
Consortium DAS: SUK1, LBC2 and LBC3	48 h	*A. cepa* root cells	Root length (cm)	-	3.534 ± 0.39	3.85 ± 0.26	Jadhav *et al.* (2010)
			MI		13.52 ± 1.1	11.26 ± 1.13	
			Chrom. Breaks		3	1	
			TA		7	2	

PL: plumule length, RL: radical length, SL: shoot length, MI: mitotic index, GI: germination inhibition, TA: total number of alterations

Zoo-toxic Effects of Azo-dyes

Daphnia magna is a commonly used bio-indicator test aquatic organism in acute and chronic toxicity studies of chemical compounds present in aquatic ecosystems (USEPA, 1985). Data regarding toxicity of different azo-dyes and real dye effluents to *D. magna* show that azo-dyes polluted waters are highly toxic to aquatic organisms (Table 3). Most commonly in this bioassay, mortality (%), EC_{50} (concentration of azo-dye effluent that causes 50% growth inhibition of tested organisms) and acute toxicity unit (TUa) of azo-dyes are calculated to assign toxicity class (I–V) to azo-dyes (Zablocka-Godlewska *et al*., 2014). According to ACE 89/BE 2/D3 final report commission of European communities, TUa<0.4 corresponds to class I (non-toxic), 0.4≤TUa<1.0 corresponds to class II (low toxicity), 1.0≤TUa<10 corresponds to class III (toxic), 10≤TUa≤100 corresponds to class IV (high toxicity) and TUa>100 corresponds to class V (extremely toxic). Zablocka-Godlewska *et al*. (2012) reported EC_{50}, TUa and toxicity class for Evans blue (100 mg L^{-1}) as 9.43 ± 0.22, 10.6 and class-IV, respectively. Przystas *et al*. (2012) measured toxicity to *D. magna* at 50 mg L^{-1} Evans blue. At this concentration, TUa and toxicity class were 13.20 and class IV, respectively. Franciscon *et al*. (2012) reported that exposure to Reactive yellow 107, Reactive black 5, Reactive red 198 and Direct blue 71 (100 mg L^{-1}) showed 100% mortality of *D. magna* larvae (Lade *et al*., 2015c). Nascimento *et al*. (2011) reported a high toxicity factor (TFD24h) of Reactive red 198 to *D. pulex*. Parrott *et al*. (2016) also observed chronic toxicity of Disperse Yellow and Sudan Red G to fish.

Colored effluents of different textile units have also been tested for measurement of their zoo-toxicity level worldwide (Table 3). Effluent released from textile industry, located in State of Santa Catarina caused 100% mortality of *Artemia salina* and *D. magna* (Souza *et al*., 2007). Bilal *et al*. (2016) reported that exposure of *A. salina* and *D. magna* to untreated effluent of six textile units located in Faisalabad, Pakistan, resulted in mortality ranging from 0–100%. Likewise, textile effluent collected from wastewater treatment plant at Keom-jun Dyeing Enterprise Cooperation (KDEC), Yang-ju, South Korea was found toxic to larvae of *D. magna*, 3.5 TUa was recorded (Choi *et al*., 2014). Azo-dyes have also been reported to alter genetic material of animal cells. For instance, Gomaa *et al*. (2012) observed that chromosome aberrations excluding gaps, chromosome deletion of mouse cells were increased from 21–154 and 2–5, respectively, by azo-dye effluent, whereas mitotic index value decreased from 190–85. Similarly, Fernandes *et al*. (2015) observed testicular toxicity in mouse caused by Disperse red 1. The increased frequency of sperm with abnormal morphology and an increased amount of DNA damage was also detected in testis cells. It concludes that presence of azo-dyes in aquatic systems is a high risk for aquatic life due to high level of zoo-toxicity as they cause damages to DNA of animal cells. Therefore, discharge of un-treated textile effluent to surrounding water bodies must be prohibited.

Microbicidal/Microbiostatic Effects of Azo-dyes

Various microbial strains have been employed as a bio-indicator of toxic chemicals/azo-dyes in environment. Table 3 shows the extent of microbicidal/microbiostatics effects of synthetic and real azo-dye effluents. For instance, Saratale *et al*. (2015) assessed toxicity of Green HE4BD (400 mg L^{-1}) to soil microorganisms: *Rhizobium radiobacter*, *Acinetobacter* sp., *P. desmolyticum* NCIM-2112 and *Proteus vulgaris* NCIM-2027. Exposure of soil microorganisms to Green HE4BD inhibited their growth. Zone of growth inhibitions were observed in the range of 5.0 to 7.5 mm for different microorganisms on nutrient agar medium.

Table 3: Zootoxicity and microbicidal/microbiostatic effects of microbially treated synthetic and real textile effluents

Microbial Strain	Type of Azo-Dye	Azo-Dye Conc. (mg L^{-1})	Biological treatment time	Tested organism	Toxicity Analysis			Reference
					Parameter	Before treatment	After treatment	
SYNTHETIC WASTEWATER								
Zoo-toxicity								
Pleurotus ostreatus (BWPH)	Evans blue	100	96 h	*D. magna*	TUa Toxicity Class	169.22 Class V	4.6 Class III	Przystas *et al.* (2018)
Klebsiella spp. (Bz4)	Evan blue	100	144 h	*D. magna*	EC_{50} TUa Toxicity Class	9.43 ± 0.22 10.62 Class IV	30.03 ± 2.07 3.33 Class III	Zablocka-Godlewska *et al.* (2015)
Consortium: *Providencia rettgeri* strain HSL1 and *Pseudomonas* spp. SUK1	Reactive black 5	100	30 h	*D. magna*	Mortality (%)	49 ± 4.0	0 ± 0.0	Lade *et al.* (2015c)
Microbial Consortium (15 bateria)	Trypan blue	50	24	*D. magna*	Mortality (%)	60	0	Lade *et al.* (2015b)
Pseudomonas fluorescens (Sz6)	Evan blue	100	120 h	*D. magna*	EC_{50} TUa Toxicity Class	9.43 ± 0.22 10.6 Class IV	4.69 ± 1.70 21.3 Class IV	Zablocka-Godlewska *et al.* (2014)
Pseudomonas fluorescens (SDz3)					EC_{50} TUa Toxicity Class	9.43 ± 0.22 10.6 Class IV	18.87 ± 3.20 5.35 Class III	
Bacillus spp. ETL-1979	Direct blue 71	100	168 h	*D. magna*	Mortality (%)	47	0	Shah *et al.* (2014)
Brevibacterium spp. strain VN-15	Reactive yellow 107 Reactive red 198	100 25*	168 h	*D. magna*	Mortality (%)	40 47	0 0	Franciscon *et al.* (2012)
Pleurotus ostreatus (BWPH)	Evans blue	50	120 h	*D. magna*	TUa Toxicity Class	13.20 Class IV	2.40 Class III	Przystas *et al.* (2012)
Mixed fungal culture (CE)	Reactive red 198	100	7 days	*D. pulex*	$TF_{D24\,h}$	14.0	Non-toxic	Nascimento *et al.* 2011
Pycnoporus sanguineus MUCL 41582	Acid blue 62	1.75 m*M*	14 days	Caco-2 cells (human intestinal cells)	Detoxification (%)	- -	99 ± 5 45 ± 3	Vanhulle *et al.* (2008)
Microbicidal/microbiostatic effects of azo-dyes								
Aspergillus flavus	Malachite Green	500	192 h	Pseudomonas aeruginosa Pseudomonas aeruginosa *Pseudomonas aeruginosa*	Zone of growth Inhibition (cm)	1.8	ND	Barapatre *et al.* (2017)
Lysinibacillus spp. RGS	Reactive orange 4	50 (400)	5 h	*Bacillus circulans* *Pseudomonas aeruginosa* *Azotobacter* spp. Lysinibacillus spp. *Cellulomonas biazotea* NCIM-2550 *P. aurugenosa* *A. vinelandii*	Zone of growth Inhibition (cm)	0.53 0.54 0.32 0.65 0.42 0.3 0.9	0.17 ND ND ND ND ND ND	Saratale *et al.* (2015)
Bajerkandera adusta (Willdenow) P. Karsten MUT 3060	Mixture of AY49, AR266 & Abu62	100	7 days	*Pseudokirchneriella subcapitata* (Korshilov)	Inhibition of cellular growth (%)	89.5 ± 10.3	0.9 ± 0.2	Anastasi *et al.* (2011)
consortium GR: *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168	Green HE4BD	300	24 h	*Rhizobium radiobacter* *Acinetobacter* spp. *Pseudomonas desmolyticum* NCIM-2112 *Proteus vulgaris* NCIM-2027	Zone of growth Inhibition (cm	0.55 0.75 0.50 0.60	0.10 0.10 0.15 NI	Saratale *et al.* (2010a)
Kocuria rosea (MTCC 1532)	Methyl orange	50 (500)	72 h	*K. rosea* *P. aurugenosa* *A. vinelandii*	Zone of growth Inhibition (cm)	0.5 0.5 0.7	NI 0.2 0.1	Parshetti *et al.* (2010)
Perenniporia ochroleuca MUCL 41114	Acid blue 62	1.75 mM	14 days	VITOTOX assay [*Salmonella typhimurium* strain (TA 104 recN2)]	DNA damage	+	ND	Vanhulle *et al.* (2008)
Cunninghamella elegans UCP 542	Oragne II Reactive black 5	-	-	*Escherichia coli*	Respiration inhibition (%)	≈50 ≈30	≈63 ≈20	Ambrosio and Campos-Takaki (2004)
REAL TEXTILE EFFLUENTS								
Microbial Strain	Biological Treatment Time			Tested organism	Parameter	Before treatment	After treatment	Reference
Zoo-toxicity								
Fungal (*Ganoderma lucidum* IBL-05) ligninolytic enzymes	15 h			*D. magna* Human red blood cells	Mortality (%) RBC lysis (%)	67 ± 3.9 74 ± 3.6	31 ± 2.4 23 ± 1.2	Bilal *et al.* (2016)
Consortium: *Aspergillus terrus* and *Aspergillus* spp.	48 h			*Mus*	Chromosome aberrations excluding gaps chromosome deletion	154 5	39 2	Gomaa *et al.* (2012)
***Bjerkandera adusta* KUC9065**	3 weeks			*D. magna*	TUa	≈3.5	≈2.8	Choi *et al.* (2014)
Horseradish peroxidase (HRP)	24 h 48 h			*A. salina* *D. magna*	Mortality (%)	40 ± 0.41 100 ± 0	30 ± 1.0 100 ± 0	Souza *et al.* (2007)

Table 3: Continued

Table 3: Continued

Microbicidal/microbiostatic effects of azo-dyes						
Lysinibacillus spp. RGS	120 h	*Bacillus circulans*	Zone of growth Inhibition (cm)	81	0.1	Saratale *et al.*
		Pseudomonas aeruginosa		0.63	0.14	(2015)
		Azotobacter sp.		0.74	0.21	
		Lysinibacillus sp.		0.72	NI	
		Cellulomonas biazotea NCIM-2550		0.46	0.15	
Trametes spp. laccase	20 h	*Saccharomyces cerevisiae* BY4741	Growth inhibition (%)	59%	11%	Benzina *et al.*
		B. cereus		38%	25%	(2013)
Consortium:	48 h	*Escherichia coli*	Zone of growth Inhibition (cm)	+ve	-ve	Gomaa *et al.*
Aspergillus terrus and *Aspergillus* spp.		Hepatocellular carcinoma (Hep-G2) cells	Cell Viability (% of control)	<75	>90	(2012)

Class V is more toxic than class VI, III-

Values in parenthesis in azo-dye Concentration column show the dose of azo-dye used for measurement of detoxification

EC_{50}: The effective concentration of a wastewater sample that causes 50% mortality of tested organisms, **TUa**: Acute toxicity unit, **TA**: total number of alterations, **TCA**: Total number of cells with alterations

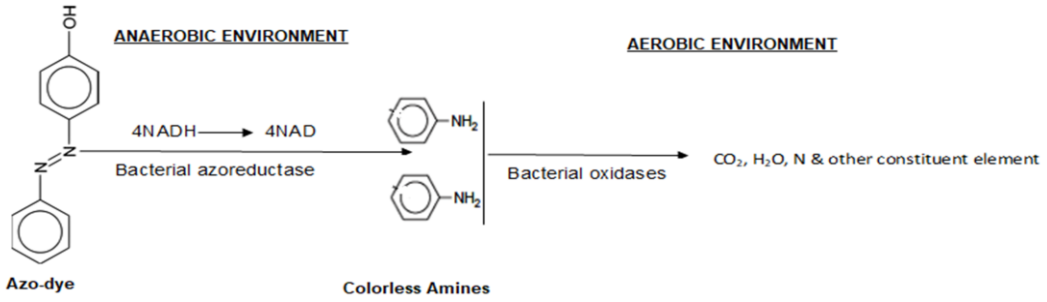

Fig. 1: Bacterial degradation pathway of azo-dyes

Respiration inhibition of microorganism is another indicator of stressful environment. Orange II and Reactive black 5 caused respiration inhibition of *Escherichia coli* up to 50% and 30%, respectively (Ambrosio and Campos-Takaki, 2004). Like synthetic azo-dye wastewaters, textile effluent of Egyptian company for textile dyeing and printing suppressed growth of *Escherichia coli* and zone of inhibition was detected (Gomaa *et al*., 2012). Similarly, textile effluent from Ksar Helal (Tunisia) suppressed 59% growth of *Saccharomyces cerevisiae* BY4741 and 38% of *Bacillus cereus* compared to control (Benzina *et al*., 2013). Thus dye residues in distilled water and/or textile effluent suppress growth and multiplication of various beneficial soil microorganisms.

Overall it is concluded that un-treated textile effluents having dye residues should not be used for irrigating crops as such wastewaters seriously inhibit crop growth and affect beneficial microorganisms present in agricultural soils. In addition, discharge of such effluent to aquatic systems will seriously disturb the aquatic life as azo-dyes do mutations to animal cells. Hence, textile effluent must be treated with suitable technology to improve the quality of such waters and then released into environment.

Microbial Detoxification of Azo-dye Polluted Wastewater

High biological toxicity of azo-dye polluted wastewaters shows high needs of treatment prior to their discharge into environment. Over last two decades, use of microorganisms for decolorization of synthetic and raw textile effluents have been much investigated, and many promising azo-dye degrading microbes have been identified (Imran *et al*., 2014; Chaturvedi and Verma, 2015; Jadhav *et al*., 2015; Mahmood *et al*., 2017; Chen *et al*., 2018; Das and Mishra, 2019). Mostly the process of microbial treatment of azo-dyes contaminated water is environment friendly and reduces their biological toxicity, however, sometimes secondary metabolites produced during their biodegradation may be more toxic in nature. In the following sections, potential of microbial biotechnology for the detoxification of synthetic and real azo-dyes effluents is discussed.

Bioremediation of Synthetic Azo-dyes Effluents

Several microbial isolates including bacteria, fungi, yeast and algae have been reported to be capable of degrading azo-dyes (Jinqi and Houtian, 1992; Waghmode *et al*., 2012; Jadhav *et al*., 2013; Jafari *et al*., 2013). However, this review focused primarily on the role of bacteria and fungi that have been applied predominantly for azo-dye degradation and detoxification.

Bacteria

Various bacterial strains isolated from different environments have been employed to degrade and detoxify azo-dyes in azo-dye contaminated wastewaters

(Table 1 and 2). Findings of researchers show that bacteria are highly efficient in detoxification of textile effluents confirmed by bioassays *viz*., phyto-toxicity, zoo-toxicity and microbicidal/microbiostatic analysis. Pokharia and Ahluwalia (2016a) found that treatment of Basic red 46 (100 mg L^{-1}) with *Staphylococcus epidermidis* MTCC 10623 for only 6 h reduced its toxicity. In case of un-treated azo-dye water, only 20% seeds of *Vigna radiata* were germinated, whereas 60% seeds were germinated after bacterial treatment that was close to germination rate (70%) of control seeds. Moreover, root and shoot lengths were substantially reduced by seed soaking in un-treated water, whereas, root and shoot lengths were statistically at par with control after bacterial treatment. Likewise, Jadhav *et al*. (2013) observed an increase in germination rate, radical and plumule length of *P. mungo*, *T. aestivum* and *S. vulgare* by using treated Remazol orange (500 mg L^{-1}) contaminated water with *Pseudomonas aeruginosa* BCH. According to Kumar *et al*. (2013), germination inhibition of *Brassica nigra* caused by Disperse red F3B (100 mg L^{-1}) can be removed by *Enterococcus faecalis* treatment. The germination inhibition and growth suppression impact of other azo-dyes were also observed to be reduced after bacterial treatment (Table 1). Microbial treatment of azo-dye contaminated wastewaters also reduces their cyto-toxic and geno-toxic effects on plant cells, thus decreases dye induced growth retardation of crop plants. Jadhav *et al*. (2011) found that treatment of Remazol red by *P. aeruginosa* BCH increased cell viability of *A. cepa* root cells from 86–95%, and noted reduction in mitotic index (from 12.2±1.304–10.4±0.90), chromosome breaks (from 3–1) and total number of cells with alterations (from 23–6) in *A. cepa* root cells. Likewise, Phugare *et al*. (2011a) observed removal in cyto-toxic and geno-toxic effects of Red HE3B by bacterial consortium (*Providencia* spp. SDS and *P. aeurginosa* strain BCH).

Table 3 shows that bacterial treatment is also highly effective in reducing zoo-toxicity and microbicidal/ microbiostatic effects of synthetic azo-dye polluted wastewaters. For instance, Franciscon *et al*. (2012) recorded 40% mortality of *D. magna* larvae by un-treated Reactive Red 198-contaminated water, whereas, no death of larvae was noticed after treatment with *Brevibacterium* spp. strain VN-15. Decrease in mortality rate and growth inhibition of *D. magna* by bacterial treatment of synthetic azo-dye wastewaters have also been observed by other researches (Franciscon *et al*., 2009; Zablocka-Godlewska *et al*., 2012; Przystas *et al*., 2013; Zablocka-Godlewska *et al*., 2014; Shah *et al*., 2014; Lade *et al*., 2015a). In contrast, treatment of Evan blue with Erwinia sp. S12 did more toxicity of neonates of *D. magna* than parent dye molecule (Przystas *et al*., 2012). Since use of colored textile effluent for growing crops is a very common practice in developing countries, therefore, the efficacy of bacterial treatment has also been evaluated by microbicidal/microbiostatic test. For instance, Saratale *et al*. (2015) reported that Reactive orange 4 inhibited growth of soil microorganisms including *P. aeruginosa*, *Azotobacter* spp., *Cellulomonas biazotea* NCIM-2550 and *Lysinibacillus* spp. RGS on nutrient agar plates. However, metabolites produced during biodegradation of azo-dye by *Lysinibacillus* spp. RGS did not suppress growth. Opposed to these observations, treatment of AY49, AR266 and Abu62 dyes containing wastewater by *Bjerkandera adusta* MUT 3060 resulted in an increase in growth inhibition of green unicellular alga (Anastasi *et al*., 2011). These findings suggest that azo-dye induced inhibition in germination and growth of different crops, aquatic animals and beneficial soil microorganisms could be minimized or eliminated after treatment with bacteria. In addition, treatment of dye-contaminated water reverts dye induced alterations in genetic materials of organisms.

Fungi

Fungal strains have also been found effective in removing azo-dye residues and reducing biological toxicity of azo-dyes because of their effective enzymatic system and large mycelial biomass for adsorption of azo-dyes. Treatment of synthetic azo-dyes by various pure cultures of fungi has been found to reduce phyto-toxicity, zoo-toxicity and microbicidal/microbiostatic effects, thus making them safe for discharge into surrounding water bodies and soils (Table 1 and 3). Laxmi and Nikam (2015) found that bioaugmentation of Reactive navy blue M3R (40 mg L^{-1}) polluted water with *Aspergillus flavus* for 7 days improved germination of *T. aestivum* from 54 to 97%. Moreover, plumule and radical lengths were also statistically at par with control seedlings, indicating mitigation of negative/toxic impacts of azo-dye on growth. Similar mitigation of Rubine GFL was observed by Waghmode *et al*. (2012). They found that only 10% germination of *S. vulgare* seeds by soaking in 1 g L^{-1} Rubine GFL solution, however, metabolites produced during biodegradation of Rubine GFL by *Galactomyces geotrichum* MTCC 1360 were almost non-toxic as 90% seeds were germinated. Przystas *et al*. (2012) observed that toxicity level of un-treated Evan blue (80 mg L^{-1}) contaminated wastewater was class-IV using *Lemna minor* as test plant, it reduced to class-III by treatment with *Pleurotus ostreatus* (BWPH) within 120 h. Many other fungal strains including *Dichomitus squalens*, *P. ostrealus*, *Trametes pubescens*, *G. geotrichum* MTCC 1360, *Polyporus picipes* (RWP17), *Irpex lacteus*, *G. geotrichum*, *Ganoderma* spp. En3 and *Armillaria* spp. F022 have potential to mitigate deleterious effects of azo-dyes on germination and growth of crop plants (Casieri *et al*., 2008; Waghmode *et al*., 2011; Kalpana *et al*., 2012; Przystas *et al*., 2012; Govindwar *et al*., 2014; Ma *et al*., 2014; Adnan *et al*., 2015). The fungal treatments have also been found to reduce cyto-toxic and geno-toxic

impacts of azo-dyes (Table 1 and 3). For instance, Waghmode *et al*. (2012) reported that treatment of Rubine GFL (50 mg L^{-1}) for 96 h by *G. geotrichum* MTCC 1360 reduced mitotic index, chromosome breaks and total number of cells with alterations in *A. cepa* root cells compared to un-treated water. Moreover, cell viability of *A. cepa* root cells increased after treatment with *G. geotrichum* MTCC 1360.

Fungal strains have also been tested to reduce zoo-toxicity and microbicidal/microbiostatic effects of different synthetic azo-dyes. For instance, effect of un-treated and treated Evans blue water was evaluated on *D. magna* larvae using acute toxicity unit (TUa) as indicator. Un-treated Evans blue synthetic wastewater had 13.20 TUa, whereas it decreased to 2.40 TUa after treatment with *P. ostreatus* (BWPH) (Przystas *et al*., 2012). Likewise, Zablocka-Godlewska *et al*. (2015) reported 13.20 TUa and 3.33 TUa for un-treated and treated Evans blue synthetic wastewater. Moreover, EC_{50} increased from 9.43±0.22 to 30.03±2.07, clearly showing a significant reduction in toxicity of Evans blue to *D. magna* larvae. In contrast, Congo red and Orange II (100 mg L^{-1}) containing water treated with *I. lacteus* KUC8958 showed higher mortality of neonates of *D. magna* (Choi *et al*., 2014). The metabolites of Evan blue produced during biodegradation of the dye also exhibited more toxicity to neonates of *D. magna* than parent dye molecule (Przystas *et al*., 2012). Respiration inhibition of microbial strains is used as indicator of their exposure to certain stress. While, elimination of stress normalizes their respiration rate and activities in environment. Respiration inhibition of *Escherichia coli* caused by Orange II and Reactive black 5 was reduced by treatment of these azo-dyes with *Cunninghamella elegans* UCP 542 (Ambrosio and Campos-Takaki, 2004). *Laccases* from *P. ostreatu* reduced growth inhibition potential of Remazol brilliant blue R to *B. cereus*, strain 6E/2 by 95% in just 3 days (Palmieri *et al*., 2005). Fungal treatment of azo-dyes also reduces their cyto-toxic and geno-toxic properties (El-Fakharany *et al.*, 2016). Vanhulle *et al*. (2008) reported that treatment of Acid Blue 62 by *Pycnoporus sanguineus* MUCL 41582 and *Perenniporia ochroleuca* MUCL 41114 minimized cyto-toxic and geno-toxic properties of Acid Blue 62. Treated synthetic wastewater had no damage to DNA of *Salmonella typhimurium* strain (TA 104 recN2), whereas, untreated synthetic wastewater caused high DNA damage. Thus, similar to bacteria, fungi also simultaneously decolorize and detoxify azo-dyes, however fungi are slow degraders of dyes than bacteria.

Microbial Consortia

Bacterial, fungal and myco-bacterial consortia have also been tested to degrade synthetic azo-dyes at accelerated rate. Although pure cultures of bacteria and fungi can decolorize/detoxify azo-dyes, sometimes mixed microbial cultures perform better than pure cultures due to synergistic metabolic activities (Tony *et al*., 2009; Su and Lin, 2013). Su and Lin (2013) demonstrated that fungi-bacteria synergism enhanced decolorization of Reactive red 120. The consortium consisting of *Aspergillus niger* and *Bacillus* spp. decolorized Reactive red 120 (50 mg L^{-1}) up to 90% after 24 h, whereas, pure cultures of both decolorized less than 65% under similar conditions. In addition, myco-bacterial consortium completely detoxified Reactive red 120 as degradation products did not inhibit seed germination of mung beans. They proposed that this might happened because degradation products produced by reductive enzymes of bacteria did not inhibit fungi activities and were further degraded by oxidative enzymes of fungi. Likewise, *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 cooperation decolorized Green HE4BD more effectively and metabolites produced were non-toxic to *P. mungo* germination and its seedlings (Saratale *et al*., 2010a). The bacterial consortium BMP1/SDSC-01: *B. subtilis, B. cereus, B. mycoides, Bacillus* spp., *Micrococcus* spp. and *Pseudomonas* spp. reduced phyto-toxicity of Red dye (200 mg L^{-1}) to *S. vulgare* and *Zea mays* in 24 h (Saratale *et al*., 2010b). The microbial consortium consisting of *Providencia* spp. SDS and *P. aeuroginosa* strain BCH was assessed to reduce cyto-toxic and geno-toxic effects of Red HE3B. It was found highly effective as within 1 h cyto-toxicity and geno-toxicity to *Allium cepa* root cells was substantially reduced (Phugare *et al*., 2011a). Other researchers have also demonstrated bioremediation of synthetic azo-dyes by microbial consortium (Saratale *et al*., 2009; Phugare *et al*., 2011a; Lade *et al*., 2015b; Shah *et al*., 2016).

The effectiveness of microbial consortium has also been checked in reducing zoo-toxicity and microbicidal/microbiostatic effects of synthetic azo-dyes (Table 3). Lade *et al*. (2015c) recorded 49% mortality of *D. magna* larvae by untreated Reactive black 5 solution, whereas no mortality was observed after treatment with *Providencia rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 together. Similarly, Trypan blue (50 mg L^{-1}) caused death of 60% larvae of *D. magna*, but no mortality was noticed after treatment with microbial consortium consisting of 15 strains (Lade *et al*., 2015b). The consortium GR: *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168 was also found effective in reducing toxicity of azo-dyes to soil microbes (Saratale *et al*., 2010a). The most of identified microbial strains are effective in minimizing phyto-toxic, zoo-toxic and geno-toxic impacts of dyes in water. However, there are a few reports indicating that microbes may cause bio-activation of dye molecule. So, use of microbial consortia with ability to mineralize azo-dyes is preferred than pure cultures of bacteria and fungi in order to achieve mineralization.

Microbial Detoxification of Real Azo-dyes Effluents

Real textile effluents are difficult to decolorize and detoxify due to presence of other pollutants in addition to azo-dyes (Imran *et al*., 2015b). However, some microbial strains have been isolated which can reduce toxicity of textile effluents to permissible limits. The following section describes potential of bacteria and fungi to lessen harmful effects of textile effluents to different biological lives.

Bacteria

Bacteria have been found not only effective in removing azo-dyes from synthetic wastewaters, but also from real textile effluents of diverse composition. Various bacterial strain *viz*., *Pseudomonas* spp. SU-EBT, *Proteus* spp. SUK7, *Bacillus* spp. strain PS, *Pseudomonas* spp. SUK1, *Comamonas* spp. UBL 27, *Exiguobacterium* spp. RD3, *Aeromonas salmonicida*, *Lysinibacillus* spp. RGS and *Pseudomonas* spp. LBC1 have been found highly efficient in degrading azo-dyes in factory effluent and reducing phyto-toxic level of such waters (Table 2). Jadhav *et al*. (2015) evaluated germination and growth inhibition of *T. aestivum* and *S. vulgare* seedlings by untreated real effluent and metabolites produced during decolorization by *Pseudomonas* spp. SUK1. It was found that 80–90%, 30–40% and 60–70% seeds of *T. aestivum* and *S. vulgare*, were germinated by distilled water, untreated real effluent and metabolites extracted after effluent treatment, respectively. It clearly demonstrates less toxic nature of metabolites as compared to factory effluent. In another study, *Pseudomonas* spp. LBC1 showed 90% decolorization of effluent within 72 h. This color reduction also improved the germination of *S. bicolor* and *V. radiate*. About 60–80%, 20%, 45–60% germination of *S. bicolor* and *V. radiata* was recorded by distilled water, real effluent and treated effluent, respectively (Telke *et al*., 2012). According to Pourbabaee *et al*. (2006), seed germination of *T. boeoticum* improved from 02–60% by treatment of factory effluent with *Bacillus* sp. strain PS for 20 days. The effectiveness of bacterial treatment of real effluent can also be seen from Table 3, clearly showing that treatment of factory effluent by bacterial strains reduced its microbicidal/microbiostatic effects. Saratale *et al*. (2015) observed high microbiostatic effect of untreated dye effluent to soil microbes. However, growth inhibition caused by effluent to *P. aeruginosa* and *Azotobacter* spp., was eliminated after bioaugmentation of effluent with *Lysinibacillus* spp. RGS for 120 h, whereas, negligible growth inhibition was recorded in case of *B. circulans*. To measure cyto-toxic and geno-toxic effect of dye effluent, Dhanve *et al*. (2014) exposed roots of *A. cepa* to textile effluent for one week. Untreated effluent inhibited root elongation of *A. cepa* and decreased mitotic index. However, the treatment of the effluent with *Exiguobacterium* sp. RD3 reverted its adverse effect on root growth and mitotic process. Thus, like synthetic dye-contaminated water, phyto-toxic, zoo-toxic and microbicidal properties of real textile effluents are reduced after bacterial treatment. However, in case of real wastewater extent of eco-toxicity removal efficiency of bacteria is low than synthetic wastewaters that might be due to presence of other contaminants.

Fungi

Retrieval of plant growth inhibitory effect of real factory effluents by different fungal strains indicates that fungi could also be an effective bio-resource for treatment of textile effluents (Table 2). A brown rot fungus, *Aspergillus* sp. EL-2, was found capable of reducing plant growth inhibition effect of Egyptian factory dye effluent as germination of *Trigonella foenum-graecum* improved from 20–80% after 48 h aerobic treatment. Combined treatment by *Aspergillus* spp. EL-2 and gamma radiations further detoxified dye effluent as 90% seeds of *T. foenum-graecum* were germinated. Shedbalkar and Jadhav (2011) observed complete detoxification of factory effluent by treating water with *P. ochrochloron* MTCC 517 for 10 days. The germination of *T. aestivum* improved from 50–100%, and similar response was observed for shoot and root length of seedlings. Bilal *et al*. (2016) found that ligninolytic enzymes of *Ganoderma lucidum* IBL-05 detoxified wastewater of Sitara textile factory, Pakistan. The enzyme treatment increased germination of *T. aestivum* from 50–70% and decreased mortality of *D. magna* larvae by 36%. Likewise, laccase of *Trametes* spp. strain CLBE55 improved germination of *L. esculentum* from 0–24% (Benzina *et al*., 2013). Although pure cultures of fungi are capable of degrading a large number of azo-dyes in real effluents, data on decrease in zoo-toxicity of dye effluent after fungal treatment are very limited. Choi *et al*. (2014) measured toxicity of treated and untreated effluent from Keom-jun Dyeing Enterprise Cooperation (KDEC), Yang-ju, South Korea to *D. magna* larvae. It was observed that TUa value decreased from 3.5 to 2.8 after treatment with *B. adusta* KUC9065. Microbiostatic assay has also shown that fungal treatment is efficient enough to minimize growth suppressive effects of factory effluent to beneficial microbes. For instance, *Trametes* spp. laccase treatment of dye effluent reduced microbiostatic effect of effluent to *Saccharomyces cerevisiae* BY4741 and *B. cereus* (Benzina *et al*., 2013). In another study, it was found that cyto-toxicity test performed on *A. cepa* root cells showed that treatment of dye effluent by *Exiguobacterium* spp. RD3 reduced cyto-toxicity level of effluent (Dhanve *et al*., 2014). Hence, treatment of textile wastewaters by different fungal strains helps to simultaneously reduce color and eco-toxicity to organisms.

Microbial Consortia

Remediation of azo-dyes effluent using different microbial consortia is shown in Table 2 and 3. Patil *et al*. (2015) prepared a three-member bacterial consortium and evaluated to decolorize and detoxify textile dye effluent. The pure cultures of *B. odysseyi* SUK3, *Morganella morganii* SUK5 and *Proteus* spp. SUK7 decolorized the effluent 67–84% in 120 h, whereas, the consortium removed 91% color under similar conditions. The effluent after treatment did not inhibit seed germination of *P. mungo* and *T. aestivum*, whereas only 40–50% seed germination was observed before treatment. Similarly, Lade *et al*. (2012) observed elimination of inhibitory effect of factory effluent on seed germination of *S. vulgare* and *P. mungo* after treatment with myco-bacterial consortium (*A. ochraceus* NCIM-1146 and *Pseudomonas* spp. SUK1). Saratale *et al*. (2010b) reported cyto-toxic and geno-toxic impacts of treated and un-treated dye effluents on *A. cepa* root cells. It was found that chromosome aberrations were reduced from 10.1 ± 0.43 to 2.94 ± 0.21 by treatment of dye effluent using consortium SDS: *Providencia* spp. SDS (PS) and *P. aeuroginosa* strain BCH (PA) for 20 h. Jadhav *et al*. (2010) found decrease in mitotic index, chromosome number and TCA in *A. cepa* root cells by consortium DAS: SUK1, LBC2 and LBC3. The extent of remediation of effluent after microbial consortia treatment using microorganisms as test organisms is shown in Table 3. Gomaa *et al*. (2012) observed no growth inhibition of *Escherichia coli* on agar plates after dye effluent treatment with a mixture of *Aspergillus* spp. Thus, bacteria, fungi and their consortia reduce phyto-toxicity, zoo-toxicity and geno-toxicity of real textile effluents. It is concluded that pure culture and consortium of bacteria and fungi helps in minimizing photo-toxic, zootoxic and microbiocidal impacts of textile effluents.

Mechanisms of Detoxification of Azo-dye Loaded Wastewater

Bacteria and fungi remove dyes from wastewater either by adsorption on their biomass or through enzymatic degradation or a combination of both (Solís *et al*., 2012). Biodegradation of azo-dyes is either co-metabolic (require co-substrate) or growth linked process. Bacterial degradation of dyes is initiated by cleavage of azo-bond mediated by reductases with the generation of aromatic amines, which is the key step for decolorization of azo-dyes (Fig. 1). The most widely reported reductase that causes decolorization of dyes is azoreductase, however flavin reductase, NADH-DCIP reductase have also be identified (Choi *et al*., 2014). However, reducing co-factors (NADH, NADPH) *etc*. are required for catalyzing the enzymatic reduction of azo-dyes by azo reductases. In contrast, flavin reductases oxidize reduce flavins which cause azo reduction through a chemical reaction (Russ *et al*., 2000; Liu *et al*., 2004). The subsequent breakdown of the amines is achieved through different oxidases which convert the dye molecule to mineral elements (Garg and Tripathi, 2017). Generally complete degradation of dyes is achieved by enzymes coming from different individual strains. Fungi possess strong ability of producing extracellular ligninolytic enzymes including laccase, manganese peroxidase and lignin peroxidase which are predominantly involved in dye degradation and detoxification. Laccases use O_2 to oxidize various aromatic and non-aromatic compounds by abstracting protons with the production of free radicals. These radicals are capable of further proton abstraction and dye degradation process progress (Sen *et al*., 2016). Unlike laccases, peroxidases (MnP, LiP) need hydrogen peroxide as terminal electron acceptor instead of O_2. The mechanism of peroxidases is similar to that of for laccases and leads to dye degradation (Imran *et al*., 2015b). The following section describes modifications in activities of different enzymes during dye degradation process.

Bacterial Enzymes

During degradation of azo-dyes, activities of several enzymes (Lac, LiP, azoreductase, riboflavin reductase, NADH-DCIP reductase, tyrosinase and VAO) are induced. For instance, Saratale *et al*. (2013) reported that activities of bacterial azoreductase, Lac, riboflavin reductase, NADH-DCIP reductase and VAO were 10.0, 2.9, 7.0, 1.1 and 1.43-fold, respectively, higher in the cells of *Lysinibacillus* spp. RGS obtained after complete decolorization of Remazol Red than control cells. Likewise, azoreductase, Lac and NADH-DCIP reductase activities were induced in cells of *E. faecalis* YZ by Direct red 81) (Sahasrabudhe *et al*., 2014). Zhao *et al*. (2014) found that intracellular activities of Lac, NADH-DCIP reductase and azoreductase in cells of *Bacillus* sp. UN2 were induced by the presence of Methyl red as enzyme activities increased up to 1.59, 2.11 and 2.87-fold, respectively, after complete decolorization. No significant difference ($P > 0.05$) in enzymatic activity was found for LiP. Jadhav *et al*. (2013) reported the enzymatic activities of various bioremediation enzymes to check their role during Remazol orange degradation. The intracellular activities of Lac, NADH-DCIP reductase, VAO and tyrosinase were induced after dye decolorization. Other researchers also reported similar increase in the activities of Lac, LiP, azoreductase, riboflavin reductase, NADH-DCIP reductase, tyrosinase and VAO in bacterial cells after decolorization of synthetic dyes (Kalme *et al*., 2007; Dawkar *et al*., 2008; Telke *et al*., 2012; Lade *et al*., 2015b). Activities of various azo-dye degrading enzymes have been studied before and after decolorization of real factory effluents to understand mechanism of azo-dye degradation by

bacterial strains and enzymes involved. For instance, Patil *et al*. (2012) measured the enzyme activities before and after decolorization of dye effluent by *Proteus* spp. SUK7. The activities of aminopyrine N-demethylase and Lac increased from 3.140 ± 0.044 to 15.7 ± 0.011 moles of formaldehyde produced (mg of protein)$^{-1}$ min^{-1} and 0.005 ± 0.001 to 0.006 ± 0.011 enzyme units: min^{-1} mg protein^{-1}, respectively. However, activities of Lip and DCIP reductase were lower after decolorization. Jadhav *et al*. (2015) observed an induction in the activities of both intracellular and extracellular Lac, NADH-DCIP reductase, tyrosinase and VAO from *Pseudomonas* spp. SUK1 after effluent treatment. Lip, Lac, riboflavin reductase, azoreductase and NADH-DCIP reductase were also induced in the cells of *Lysinibacillus* spp. during dye effluent treatment (Saratale *et al*., 2015). Choi *et al*. (2014) reported that decolorization of dye effluent was caused due to production of Lac and MnP by *B. adusta* KUC9065. In wastewater, Lac and MnP activities were maximum at 6 days (4.2 U mL^{-1}) and 12 days (48.3 U mL^{-1}), respectively, while in liquid medium (control), enzyme activities were remarkably lower than those in dye effluent. Thus, activities of most of the bacterial enzymes reported for azo-dye degradation are increased during degradation process, however in a few bacteria activities were lowered by the dye.

Fungal Enzymes

Lac, MnP, LiP, tyrosinase, NADH-DCIP reductase and flavin reductases are predominant enzymes studied during dye decolorization of synthetic and real dye effluents by fungi. *D. squalens* produced Lac with maximum activity of 0.012 U mL^{-1} and MnP with maximum activity of 0.0042 U mL^{-1}. Maximum activity of Lac and MnP was measured after 10 and 7 days, respectively, however activities of both enzymes were substantially reduced after 14 days. Ma *et al*. (2014) found that Lac activity of *Ganoderma* spp. En3 increased from 38.7 ± 4.2 to 375.8 ± 9.8 U L^{-1} during decolorization of Reactive orange 16 (2000 mg L^{-1}). Casieri *et al*. (2008) measured decolorization of Remazol brilliant blue R by *P. ostreatus* MUT 2976 and enzyme activities (Lac, MnP, LiP) before and after each of 5 decolorization cycles in a bioreactor. Lac activity increased from 417–1500, 168–1633, 350–1667, 1100–2833 and 750–7000 nkat L^{-1} after 1–5 decolorization cycles, respectively. Likewise, strain had MnP and LiP activities which were further induced by Remazol brilliant blue R, clearly showing involvement of these enzymes in dye degradation. Waghmode *et al*. (2012) reported that at start of decolorization of Rubine GFL by *G. geotrichum* MTCC 1360, Lac activity of fungal culture was only 0.07 ± 0.05 U mL^{-1}, which increased to 0.29 ± 0.00 U mL^{-1} after decolorization (6 h). Fungi also had activities of other azo-dye degrading enzymes including tyrosinase (intracellular), tyrosinase (extracellular), riboflavin reductase, DCIP-reductase and azoreductase, but specific enzyme activities were lower after decolorization. Govindwar *et al*. (2014) demonstrated that, after decolorization of Reactive yellow-84A by *G. geotrichum* MTCC 1360, induction of Lac (210%) and intracellular tyrosinase (78%) enzyme activities were observed, whereas, tyrosinase (extracellular), NADH-DCIP reductase and riboflavin reductases showed reduction in activity after decolorization as compared to control (before decolorization). In another study, Lac activity by white rot fungus *I. lacteus* was recorded for three days. Maximum amount of Lac was recorded after 48 h and then decreased gradually at 72 h. Moreover, enzyme activity increased as concentration of dye increased (Kalpana *et al*., 2012). Lac activity before and after decolorization of real dye textile effluent by *Ganoderma* sp. En3 was 5.12 ± 0.59 U L^{-1} and 38.89 ± 2.61 U L^{-1}, respectively. The fungi did not carry MnP and LiP activities and decolorization occurred probably due to Lac activity (Kalpana *et al*., 2012). Bilal *et al*. (2016) detected ligninolytic enzymes in crude extract of *Ganoderma lucidum* (MnP 717.7, LiP 576.3, and Lac 323.2 U mL^{-1}). Most of the fungal strains synthesize more than one type of azo-dye degrading enzymes; a few actively participate in decolorization process while others are passive. Fungal enzymes do decolorization of different types of dyes and activities of the most of enzyme involved in dye degradation are induced during decolorization process. However, in a few fungal strains an increment in activities of one type of enzymes was observed, while activities of other types of enzymes were lowered. Thus, enzymes with high activities are involved in dye degradation process.

Limitations of Biological Treatment of Azo-dye Polluted Wastewaters

Even though microbial bio-resources have been widely applied for detoxification of various synthetic and real textile effluents, there are few drawbacks which limit their large-scale application for treatment of real textile wastewaters. For example, in most of the studies, the microbial bio-resources were tested for treatment of synthetic wastewaters containing only one specific dye. However, this is not the situation in real textile wastewaters which may contain diverse types of organic and inorganic pollutants which reduce the potential application of tested bio-resources. This problem can be overcome either by isolating bio-resources having potential for simultaneous removal of multiple pollutants or by preparing the consortia of different microbial strains having diverse capabilities for removal of different pollutants existing in textile effluents. Another drawback is, sometimes the intermediates of dye biodegradation are even more toxic than parent dye molecule. Therefore, bio-resources capable of mineralizing textile dyes are

suggested to be identified and used in bioreactors. Likewise, another observation is that microbial activities are suppressed at high levels of azo-dyes in wastewaters, which may increase the time to get rid of dye resides from wastewater.

Conclusions and Future Prospects

Azo-dyes exhibit serious toxicity to plants, animals and non-dye degrading beneficial soil microorganisms. This toxicity is caused by alterations in genetic material of organisms in response to dyes exposure. Biodegradation of dyes in textile effluents using bacteria, fungi and microbial consortia reverts the adverse effects of these wastewaters to plants, animals and beneficial soil microbes. Mechanisms involved in this detoxification are adsorption on microbial biomass or enzymatic biotransformation or both. Both oxidative and reductive microbial enzymes take part in dye detoxification at different stages of biodegradation. Activities of most of these enzymes are induced during dye degradation process.

The success stories related to detoxification of azo-dye loaded textile effluents through microbes at laboratory scale direct that these microflorae must be scrutinized at large scale for treatment of textile effluents at industry sites. For this purpose, along with microbes dye degrading purified enzymes should be used for accelerated degradation of azo-dyes in effluents. It is also directed to assess retrieval of yield reduction of crops through microbial treatment of textile effluents. In addition, changes in plant nitrogen content should be determined in plants irrigated with un-treated and microbial-treated dye-contaminated water. Moreover, economic feasibility of the microbial treatment should also be assessed to make it more adoptable by the industry.

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