Bacterial Decolourization, Degradation and Detoxification of Azo Dyes: An Eco-friendly Approach

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Abstract Recent decades have noticed that the wastewater loaded with dyes is one of the most challenging to treat. There has been comprehensive research on azo dye biodegradation with the help of microorganisms. It is evolving as a promising substitute to the conventional treatment with physico-chemical methods. In this chapter, the use of various bacterial cultures, either in pure or mixed form, for their capability to decolourize dye wastewaters was described. Apart from microorganisms involved, this chapter focuses on various mechanisms involved in the dye biodegradation along with the impact of several factors influencing biodegradation of dye wastewater and the role of oxidoreductases involved in bacterial dye biodegradation. The various analytical techniques used for deducing the metabolic pathway for dye remediation and ensuring its detoxification through toxicity tests are described. The chapter also highlights the huge gap between the scientific progresses in this field and lack of commercialization of research. The information gathered here will be useful for the students, teacher, industrial persons and researchers to understand the state of the art of the subject and use it for removal of dye pollution by possible green technology for the sustainable environment.

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1 Introduction

The term dye is derived from old English word 'daeg' or 'daeh' meaning 'colour'. Colour can be imparted to a wide array of materials utilizing substances well known as dyes and pigments (Christie 2001). Dyes are coloured organic compounds, which strongly absorb visible light and can form physical as well as chemical bond with the textile fibre, thereby attaching firmly to it. Commercially important dyes should characteristically be colour fast and withstand rubbing and application of water (Mansoor 2008). According to the 'colour theory' proposed by O. Witt, a dye is composed of two vital components: one is the chromophores and the other one is the auxochromes. Chromophores (Gr, *Kuroma* = colour + *Phors* = carrier) have a delocalized system of electrons with conjugated double bonds and produce colour, and a molecule consisting such group is termed as a chromogen. Some groups do not produce colour themselves but are able to amplify the colour in association with chromophores, when present in a molecule. Such molecules are termed auxochromes (Gr, auxanein = to increase). –OH, –COOH, −SOH (acidic), (basic) NH, NHR and NR are some of the most effective auxochromes (Mansoor 2008).

1.1 Azo Dyes

Azo colourants contain an azo (−N=N−) bond as their common structural feature, which is attached on either side of the two sp^2 carbon atoms. The azo group is customarily diazotized to two aromatic ring systems. Majority of the commercially important azo colourants comprise of a single azo group and, hence, are denoted as monoazo dyes or pigments, but there are several dyes which contain two (diazo), three (triazo) or more such groups (Christie 2001). Dyes containing azo group are exceptionally stable and resistant to degradation by microbes, owing to their complex structure and electron-withdrawing tendency (Dave et al. 2015).

1.2 Present Scenario of Dye Production and Consumption in India

The dyestuff production capacity in India (Fig. 1) stood at around 200,000 tons of dye. Approximately, 700 different varieties of dyes and dye intermediates are manufactured in India (http://www.indiaenvironmentportal.org.in/content/573/united-colours-of-industry/). Currently, there are roughly 1000 small-scale units and 50 large organized units. Maharashtra and Gujarat make up 90% of dyestuff production in India because of the accessibility to the raw materials and dominance of textile industry in these states. Indian dyestuff industry meets more than 95% of

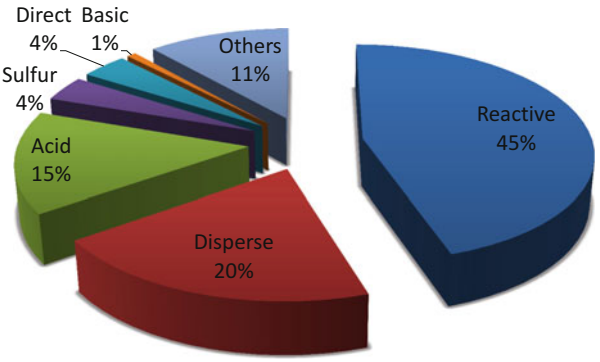

Fig. 1 Production capacity (tonnes per annum) of different major dyes by Indian dye industry

the domestic requirement, out of which approximately 60% is consumed by the textile industry and the remaining is collectively used by leather, paper and other consumer industries. The textile industries consume approximately 80% of the total dyes produced (Mangal 2010).

1.3 Discharge and Toxicity of Dyestuffs

Textile industry wastes consists of both inorganic and organic chemicals including finishing agents, sequestering and levelling agents, carriers and surfactants, etc. pH of the textile mill effluents is generally alkaline. Besides, it has high COD (3–1.4 g/L, approximately), BOD_5 (200–2000 mg/L, approximately), total solids (1000–10,000 mg/L, approximately), suspended solids (100–1000 mg/L, approximately), total phosphorus content (5–70 mg/L, approximately) and conductivity (1000–15,000 mS/cm) (Yonar 2011; Dave et al. 2015).

The three major problems associated with textile industry effluents are:

1. Textile industries use more than 8000 chemicals during several processes of textile manufacturing including dyeing and printing (Kant 2012). Approximately, 10–25% of the dyes are lost during textile dyeing and 2–20% is directly discharged as wastewater, without any treatment in diverse environmental bodies (Carmen and Daniela 2012). Several dyes are visible in water at an extremely low (1 mg/L) concentration. The average dye concentrations in the textile industry wastewaters have been found to be 300 mg/L. The release of dyes in the effluent may in consequence cause an ecotoxic hazard, potential threat of bioaccumulation as well as aesthetic problems (Agrawal et al. 2014a)
2. A textile industry of average size, with a daily manufacture of nearly 8000 kg of fabric, consumes approximately 1.6 million litres of water daily, 16% of which is used in dyeing and 8% in printing; 17–20% of the water pollution caused by the industries is contributed by the textile dyeing and finishing treatment given to fabric, as is estimated by the World Bank. Textile dyeing solely contributes to

some 72 toxic chemicals to wastewater, of which as many as 30 chemicals cannot be removed (Kant 2012).
3. Several dyes and their degradation products released to the environment are toxic, carcinogenic or mutagenic to life forms (Carmen and Daniela 2012).

1.4 Toxicity Considerations

ETAD, the Ecological and Toxicological Association of the Dyestuffs Manufacturing Industry), was established in 1974 with the objectives of reducing environmental hazards, safeguarding users and consumers and cooperation with government and public concerns with respect to the toxicological effect of their products (Chequer et al. 2011). Toxic effects of dyes may be categorized broadly into two ways. Acute toxicity denotes the consequences of short-term exposure to a substance, for example, in a single oral administration, while chronic toxicity denotes the consequences of regular exposure over a longer period of time (Christie 2001).

Dyestuffs usually exhibit low acute toxicity. LD_{50} values higher than 2000 mg/kg had been reported by ETAD for 4000 different dyes tested. Additionally, more than 90% of the dyes tested showed LD_{50} values beyond 2000 mg/kg, proving that exposure to azo dyes need not necessarily cause acute toxicity; however, in the context of systemic bioavailability, concern arises upon inhalation and skin contact of the azo dyes, owing to the likely formation of carcinogenic aromatic amines (Chequer et al. 2011). Various toxicological studies have reported that a lethal concentration value (LC_{50}) of more than 1 mg/L, for fishes, for the tested 98% of dyes, and 59% of the tested dyes have an LC_{50} value above 100 mg/L (Carmen and Daniela 2012).

Chronic and occupational hazards of dyes, particularly of azo dyes, have been investigated several times. The workers involved in dye manufacturing have showed increased rates in bladder cancer since 1895. Azo dyes can also be absorbed upon exposure to skin occurring either as an occupational hazard or during application as cosmetic products (Chequer et al. 2011). Evidently, some reactive dyes cause occupational asthma, allergic conjunctivitis, rhinitis, contact dermatitis or other allergic reactions in textile industry employees (Hunger 2003). In the same way, if dye-laden waste enters the water supply, probably by contamination of the groundwater, the common public may therefore be exposed to the azo dyes by the oral route (Chequer et al. 2011).

Evidently, reduction of azo dyes in addition leads to generation of aromatic amines, and a number of them are reported to be mutagenic and carcinogenic. In mammals, water-soluble azo dyes are predominantly metabolized through azo bond cleavage by azoreductase enzyme of the hepatic and extrahepatic tissue or by the microflora of the intestine in the body. The aromatic amines show acute toxicity by causing bladder cancer (Hunger 2003).

2 Dye Removal Techniques

Currently, majority of the practices under use for the dye wastewater treatment are chemical, physical or physico-chemical processes, which are generally expensive and of limited applicability (Jadhav and Phugare 2012). Along with the limitations associated with each technique, several factors such as the type of the dye, effluent composition, dosage and cost of the chemicals required for wastewater treatment, environmental fate and costs of treatment process and handling of generated waste products influence the technical and economic viability of each single technique (Van Der Zee 2002).

2.1 Physico-chemical Techniques

Physical techniques involve use of strictly physical phenomenon, without any gross chemical or biological changes to improve or treat the effluent. On the contrary, chemical processes use chemical reactions to improve water quality (http://envirosystems.net/effluent-treatment-plant/). Table 1 illustrates a detailed account of various physico-chemical techniques.

2.2 Biological Methods

The process in which biological systems are used to remove pollutants is known as bioremediation (Ali 2010). To date, many microorganisms including bacteria, yeast, fungi, actinomycetes and algae either in the form of pure or mixed cultures of microorganisms or their enzymes have been reported for azo dye degradation. The remediation of synthetic dyes from industrial discharges using microorganisms is beneficial as the process is comparatively economical, low operational costs in addition to mineralization of the pollutant so that the products are non-toxic (Jadhav and Phugare 2012).

Microbial decolourization of dyes may occur in two ways: either through biosorption, which is adsorption of the pollutant on the growing/living or dead microbial biomass, or through biodegradation by microbial metabolism. Biosorption of dyes is not a suitable solution to the problem because the dyes are not degraded but on the contrary gets entrapped into the biosorbent. The discharge of the bacterial biomass laden with adsorbed dyes is also a huge problem. Biodegradation is therefore preferred and seems to be promising (Ali 2010).

Table 1 Different physico-chemical techniques used for the dye decolourization and biodegradation

Process	Treatment stage	Methodology	Advantages	Disadvantages	References
Fenton's reagent	Pre/Main treatment)	Oxidation mainly using H_2O_2-Fe(II)	Effective decolourization of soluble and insoluble dyes No alteration in volume Simple equipment and easy implementation No energy input is necessary to activate hydrogen peroxide	Sludge generation and its disposal Expensive long reaction time	Lang (2009), Yonar (2011), Carmen and Daniela (2012), Zaharia et al. (2012)
Precipitation Coagulation/ flocculation	Pre/Main treatment	Use of coagulants, viz. lime, organic polymer, magnesium, iron and aluminium salts	Short detention time Economic Relatively good removal efficiencies This method was used to partly remove COD and colour from raw wastewater	Sludge production and its disposal Agglomerates separation and treatment Selected operating condition	Lang (2009), Carmen and Daniela (2012), Jadhav and Phugare (2012), Zaharia et al. (2012)
Sonication	Pre-treatment	Use of varying frequency of ultrasound waves	Complete decolourization and increase in rate of decolourization under spent dye bath conditions Simple to use Very effective in integrated system	Decolourization efficiency decreased with an increased dye concentration Comparatively new method and in anticipation of full-fledged application	Lang (2009), Carmen and Daniela (2012), Jadhav and Phugare (2012), Zaharia et al. (2012)
Ion exchange	Main treatment	Retention on ion exchange resins	Removal of soluble dyes. Adsorbent is not lost at regeneration Reclamation of solvent after use	Specific application; not applicable to all dyes Expensive	Lang (2009), Carmen and Daniela (2012), Zaharia et al. (2012)

Ozonation and ultrasound enhanced ozonation	Main treatment	Varying ozone, ultrasound and ultrasound enhanced ozone operational conditions. First-order rate constant increased to 200% using ultrasonic power inputs compared to ozonation alone	Effective azo dye removal Applied in gaseous state No alteration of volume	Generation of highly oxidizing reactive radicals Ozone generation process coupled with its very short half-life (20 min)	Lang (2009), Carmen and Daniela (2012), Jadhav and Phugare (2012), Zaharia et al. (2012)
Adsorption	Pre/Post-treatment	Adsorption of dyes using various adsorbent, viz. activated carbon, cellulosic bacteria, yeast, fungal chitin biomass, soil material, pulp mill waste, etc.	Equilibrium capacity varies with dyes and adsorbents Economically attractive Good removal efficiency	Requires long retention times High cost of organic solvents Huge quantities are required	Carmen and Daniela (2012), Jadhav and Phugare (2012), Zaharia et al. (2012)
Electrolysis	Pre treatment	Electric current is applied to the wastewater using electrodes.	Breakdown compounds are non-hazardous No additional chemicals required	Cost intensive Requires the high amount of energy	Lang (2009), Carmen and Daniela (2012), Jadhav and Phugare (2012), Zaharia et al. (2012)
Photocataysis	Post treatment	Oxidation using mainly H_2O_2-UV	No sludge production Process carried out at ambient conditions Inputs are nontoxic and inexpensive Complete mineralization with shorter detention times	Formation of by-products Effective for small amount of coloured compounds Expensive Mineralization with short detention	Lang (2009), Carmen and Daniela (2012), Zaharia et al. (2012)
Membrane filtration	Main treatment	Nanofiltration and reverse osmosis using membranes with a molecular weight cut-off below 10,000 Da	Low spatial requirements Removes all dyes Reuse of chemicals and water Chemicals and water can be easily recovered and reused	Flux decline Membrane fouling, necessitating frequent cleaning and replacement Concentrated sludge formation High capital costs The process fails to separate dissolved solids	Lang (2009), Carmen and Daniela (2012), Jadhav and Phugare, (2012), Zaharia et al. (2012)

2.3 Dye Decolourization Using Bacteria

Some examples of the biodegradation of textile dyes using pure and bacterial consortium/mixed cultures are given in Tables 2 and 3, respectively. Omnipotence, rapid growth, facultative nature and extraordinary adaptability are the qualities that make bacterial community suitable for the bioremediation (Gomare et al. 2009). Additionally, bacterial treatment system is eco-friendly and economical, produces less sludge and can lead to a higher degree of degradation and mineralization as well as it does apply to a wide spectrum of azo dyes (Saratale et al. 2011).

Reportedly, efforts for isolation of azo dye-degrading bacteria started in the late 1970s where species of *Bacillus* and *Aeromonas* were used. Several bacterial genera and species adapted for decolourization of dyes, either as pure cultures or as consortia, have been described to date (Saratale et al. 2011).

Reportedly, azo dyes cannot be degraded completely by individual bacterial strains, in general, and also the dye degradation products generated are usually aromatic amines that are often carcinogenic and need additional treatments. On the contrary, mixed/consortial cultures are especially useful in such situations, as different strains may attack the different bonds in the azo dye molecule or may use the dye intermediates generated by the symbiotic strains for additional degradation, and this synergistic metabolism of the microbial culture may lead to better degradation and mineralization. Moreover, isolation of a pure culture from dye-laden effluent samples followed by long-term adaptation processes is obligatory for efficient decolourization and biodegradation of azo dyes and is a time-consuming and tedious process. In spite of this, if pure culture is added it is not possible to sterilize huge volume of dye containing wastewater for treatment, and even if it is sterilized, it is not possible to run the treatment under aseptic conditions (Saratale et al. 2011).

3 Mechanism of Dye Biodegradation

3.1 Anaerobic Reduction of Azo Dye

Several bacteria, when encounter anaerobic conditions, degrade azo dyes by reducing its highly electrophilic azo bond with the help of non-specific enzymes. Anaerobic reduction of azo dyes leads to reductive cleavage of azo linkage, consequently leading to the generation of aromatic amines (Santos et al. 2007), which are usually colourless, and therefore, reduction of azo dyes is also denoted as dye decolourization. Anaerobic reduction of azo dyes includes different mechanisms, which may be broadly categorized into direct and indirect mechanisms (Fig. 2a, b). Direct enzymatic reduction of azo dyes and indirect reduction of azo dyes are catalysed by enzymatically generated/regenerated redox mediating compounds.

Table 2 Decolourization of azo dyes by isolated bacterial strains

Bacterium	Dye	Concentration (mg/L)	Conditions (pH, temperature, agitation)	Time (h)	Decolourization (%)	References
Acinetobacter calcoaceticus NCIM-2890	Direct Brown MR	50	7.0, 30°C, Static	48	91.3	Ghodake et al. (2009)
Aeromonas hydrophila	Reactive Black 5 Reactive Red 141 Reactive Red 198 Reactive Blue 171 Direct Yellow86 Reactive Green 19	300	7.5, 30°C, agitation (125 rpm),	–	80.9 66.5 60.2 36.0 30.9 23.2	Hsueh et al. (2009)
Bacillus sp. VUS	Navy Blue 2GL	50	7.0, 40°C, Static	18	94	Dawkar et al. (2009)
Citrobacter sp.	Azo and triphenylmethane dyes	5 mM	7–9, 35–40°C, static	1	100	An et al. (2002)
Bacillus sp. strain AK1	Metanil Yellow Amaranth Congo Red Fast Red Ponceau S Reactive Black	200	7.2 37°C	27	99 97 80 62 46 31	Anjaneya et al. (2011)
Brevibacillus laterosporus MTCC 2298	Golden Yellow HER;	50	7.0, 30°C, Static	48	87	Gomare et al. (2009)
Citrobacter sp. CK3	Reactive Red 180;	200	7.0, 32°C, anaerobic	36	96	Wang et al. (2009)
Escherichia coli JM109	Direct Blue 71	150	9.0, 30°C, anaerobic	12	100	Jin et al. (2009)

(continued)

Table 2 (continued)

Bacterium	Dye	Concentration (mg/L)	Conditions (pH, temperature, agitation)	Time (h)	Decolourization (%)	References
Lysinibacillus sp. strain AK2	Metanil Yellow Amaranth Congo Red Fast Red Ponceau S Reactive Black	200	7.2 37°C	12	100 99 98 71 58 43	Anjaneya et al. (2011)
Pseudomonas aeruginosa NGKCTS	Reactive Red BS	300	7.0 31°C static	5.5	91	Sheth and Dave (2009)
Providencia sp . SRS82	Acid Black 210	100	7.0 32°C static	90 min	100	Agrawal et al. (2014b)
Alcaligenes faecalis PMS-1	Reactive Orange 13	400	7.0 32°C static	16	100	Shah et al. (2012)
Micrococcus luteus	Direct Orange 16	100	8.0 37°C static	6	96	Singh et al. (2015)

Table 3 Decolourization of dyes by bacterial consortium

Name of strain constituting the consortium	Name of dye	mg/L	Condition (pH, Temp., agitation)	Time (h)	Decolorization (%)	References
Bacterial consortium (*Proteus vulgaris* NCIM-2027 *and Micrococcus glutamicus* NCIM-2168)	Green HE4BD Golden Yellow HE4R Violet 5R Orange 3R Red ME4BL Red M2BN	50	6.6 37°C Static	24	100 90.6 88.0 87.9 70.3 61.7	Saratale et al. (2010)
NAR (*Citrobacter freundii* A1, *Enterococcus casseliflavus* C1, and *Enterobacter cloacae* L17)	Amaranth	100	Not 45°C Static	0.5	100	Chan et al. (2012)
Two isolated bacterial strains (BF1, BF2) and *Pseudomonas putida* (*MTCC1194*)	Chrysoidine Procion Brilliant Red Procion Navy Blue, Procion Green, and Direct Blue Supranol Red	50	9.0 28°C 120	240 216	>90 >90 60 80 80 80	Senan and Abraham (2004)
Consortium with eight different bacteria	Congo Red	100	7.5 30°C Static	12	100	Lade et al. (2015)
Consortium DAS (3 different *Pseudomonas* species)	Reactive Orange 16	100	7.0 30 Static	48	100	Jadhav et al. (2010)
Consortium SDS (*Providencia* sp. SDS and *Pseudomonas aeruginosa* BCH	Red HE3B Red HE7B Scarlet GDR Brown 3 REL Green HE4BD Direct red 5B Ramzol black 5B	50	– – Static	1 1 3 7 2 1.5 1	100 97 98 99 100 98 100	Phugare et al. (2011)

(continued)

Table 3 (continued)

Name of strain constituting the consortium	Name of dye	mg/L	Condition (pH, Temp., agitation)	Time (h)	Decolorization (%)	References
Consortium DMC (*Pseudomonas aeruginosa* PAO1, *Stenotrophomonasmaltophila*, and *Proteus mirabilis*)	Direct Black 22 Reactive Navy Blue HER Procion Red H7B Reactive Black B	100	7.0 45 Static	12	91 94 97 90	Mohana et al. (2008)
Consortium PMB11 (*Bacillus odysseyi* SUK3, *Morganella morganii* SUK5, and *Proteus* sp. SUK7)	Reactive Blue 59 Navy Blue HE2R Red HE3B Red HE7B Red HE8B Red 6BI Red BLI Orange 3RLI Green HE4B Green HE4BD 97	50	– 30 Static	3 6 12 12 12 12 10 8 24 24	92 91 99 87 87 86 83 85 81 83	Patil et al. (2008)
Consortium GR (*Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168)	Scarlet R	50	– 37 Static	3	100	Saratale et al. (2009)

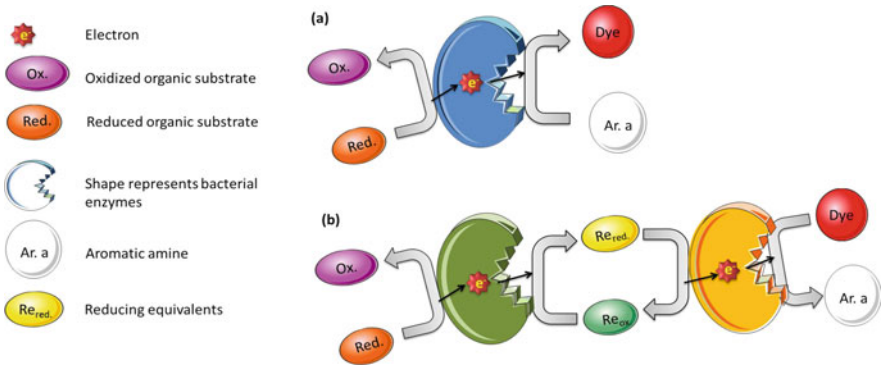

Fig. 2 Mechanisms of (**a**) direct enzymatic reduction of azo dye and (**b**) indirect biologically mediated azo dye reduction

Besides this, azo dyes can also be solely reduced chemically by biogenic bulk reductants like sulphide (Van Der Zee 2002).

Anaerobic reduction is usually non-specific, as various groups of azo dyes are decolourized by an assorted group of bacteria. Under static condition, depletion of oxygen is effortlessly achieved, consequently permitting obligate and facultative anaerobic bacteria azo dye reduction. Thus, anaerobic decolourization of azo dyes is a casual process, where the dye molecule probably serves as an electron acceptor for the electrons facilitated through the carriers of the electron transport chain. Contrastingly, dye decolourization may be accredited to non-specific reactions occurring extracellularly amongst reduced compounds formed by the anaerobic bacteria (Van Der Zee 2002).

3.1.1 Direct Enzymatic Azo Dye Reduction

Oxidation of organic substrates leads to generation of reducing equivalents which are then transferred, by the enzymes, to the azo dyes. The enzymatic reduction of azo dyes is usually categorized into different categories: one carried out by specialized enzymes termed as azoreductases, present in the bacteria utilizing azo dyes as sole source of carbon and energy, and second carried out by non-specific enzymes catalysing the reduction of a wide variety of electron-withdrawing substrates along with azo dyes (Santos et al. 2007). Specialized azo dye reducing enzymes, called as 'azoreductases', are reported with certain aerobic and facultative anaerobic bacteria that could utilize simple azo molecules as sole source of carbon and energy. Non-specific enzymes catalyse reduction of azo dyes and have also been isolated from aerobically grown bacteria like *Escherichia coli, Shigella dysenteriae* and *Bacillus* sp. These enzymes were characterized as flavoproteins. Presence of specific azoreductases in bacteria grown anaerobically is seldom reported. The azo bond reducing enzymes were reported to be dispersed in the bacterial cytoplasm devoid of any association with the cell membranes or other

organized cellular structures and are secreted before acting as an 'azoreductase' in vivo (Van Der Zee 2002).

3.1.2 Indirect Biologically Mediated Azo Dye Reduction

Indirect reduction of azo dyes is mediated by enzymatically reduced electron carriers like reduced flavins, for instance $FADH_2$, $FMNH_2$ and riboflavins, generated by flavin-dependent reductases (anaerobic azoreductases); NADH, NADPH and NADPH-generating system; and various artificial redox mediating compounds. For reducing azo dyes, the redox potential of a redox mediator should range, theoretically, between the redox potential of the azo dye and the primary electron donor. As estimated, the value of redox potential for the redox mediators catalysing azo dye reduction should lie between –430 and –100 mV (Van Der Zee 2002). Reductive decolourization of the azo dyes facilitated by the reducing equivalents occurs in two steps: a non-specific reduction of redox mediator by an enzyme is the initial step which is followed by a chemical reoxidation of the redox mediator by the azo dyes (Santos et al. 2007).

3.1.3 Site of the Reaction

Dye oxidation and the catalytic reduction of the electron carrier can occur intracellularly as well as extracellularly. Reducing cofactors like $FMNH_2$, $FADH_2$, NADH and NADPH, along with the dye-degrading enzymes are found in the cytoplasm. Intact cells require a membrane transport system for the reduction of azo dyes by these redox mediators. Additionally, as FAD and FMN cannot freely pass through the bacterial cell walls, this offers grave obstacle, particularly for the azo dyes comprising highly polar groups (Van Der Zee 2002; Solis et al. 2012). Cell lysis releases these cofactors in the extracellular environment, and therefore, cell extracts or starving or lysed cells exhibit higher reduction rates of azo dyes as compared to intact or resting cells. In addition to this, the absence of a direct relationship between the structure of a dye (size, molecular weight and degree of sulphonation) and the dye reduction rate suggests that the mechanisms of intracellular reduction of azo dyes are insignificant. Presumably, anaerobic biological reduction of azo dye ensues outside the cells, catalysed directly by reduced electron carriers regenerated with the help of these periplasmic enzymes (Van Der Zee 2002).

3.2 Aerobic Oxidation of Dyes

Even though azo dyes are usually resistant to bacterial degradation in aerobic conditions, few selected aerobic bacterial strains with specialized azo dye-degrading enzymes were noticed to degrade azo dyes under absolutely aerobic

conditions (Ola et al. 2010). Additionally, it is presumed that the initial step in the biodegradation of azo dyes is their reduction to the corresponding aromatic amines, a reaction catalysed by the enzyme azoreductase. The aromatic amines generated are then further mineralized aerobically (Sarayu and Sandhya 2010).

Sulphonated aromatic amines, except simple sulphonated amino benzene and amino naphthalene compounds, are specifically challenging to degrade because of the hydrophilic nature of their sulphonate group, which hampers membrane transport. Yet another additional transformation that the aromatic amines, substituted with ortho-hydroxy group, may undergo upon contact with oxygen is autoxidation. Several aromatic amines, like substituted anilines, amino benzidines and naphthyl amines, initially get oxidized to oligomers and in due course to dark-coloured polymers with reduced solubility and, therefore, are effortlessly removed from the aqueous phase (Van Der Zee 2002).

3.3 Combined Anaerobic–Aerobic Degradation of Azo Dyes

It is generally presumed that most azo dyes can be reductively decolourized under anaerobic conditions, while the biotransformation products produced during dye biodegradation, being insusceptible to anaerobic degradation, are readily metabolized under aerobic conditions. Consequently, anaerobic degradation followed by aerobic treatment is usually recommended for treating the dye containing effluent from textile processing and dye production industries. Two distinct strategies are usually detected: integrated treatment in a single reactor and sequential treatment in distinct reactors.

3.3.1 Sequential Anaerobic–Aerobic Treatment of Azo Dyes

It involves sequential treatment of dye-laden effluent under anaerobic conditions and aerobic conditions. Various investigators have studied this technique over the past few years and have pointed out that the anaerobic phase of the sequential system shows noticeable colour, COD and organic matter removal. Furthermore, it increases the biodegradability of azo dyes for subsequent aerobic treatment. However, evidence on complete biodegradation of azo dyes is not clearly available (Rai et al. 2005).

3.3.2 Integrated Anaerobic–Aerobic Treatment

The ability of aerobic as well as anaerobic microorganisms to coexist symbiotically in a biofilm underlies the success of this technique. Providing oxygen to an oxygen-tolerant anaerobic consortium or, instead, exposing a biofilm to low concentration of oxygen together with a co-substrate are some of the ways to generate an

integrated anaerobic–aerobic system. The integrated systems although showed transient accumulation of aromatic amines, generated from the bioreduction of the azo dyes. These compounds were ultimately mineralized, probably with the help of facultative aerobic bacteria already present in the anaerobic sludge or by subsequent addition of the aerobic enrichment culture to the anaerobic sludge (Rai et al. 2005). Although, an increase in colour during aerobic treatment has also been observed, in some studies, indicating autoxidation of aromatic amines (Van der Zee and Villaverde 2005). Koupaie et al. (2013) operated two fixed-bed sequencing batch biofilm integrated anaerobic–aerobic reactors (FB-SBBR) to estimate azo dye Acid Red 18 decolourization and biodegradation. FB-SBBR1 and FB-SBBR2 were packed with volcanic pumice stones and a type of plastic material composed of polyethylene, respectively. They observed that decolourization of the dye in both the reactors followed first-order kinetics in correlation to dye concentration. 1-naphthylamine-4-sulfonate was produced as main sulphonated aromatic amine during the anaerobic phase and more than 63.7% and 71.3% of which was successfully removed during the aerobic reaction phase of both the reactors.

As reported recently, the sequential anaerobic–aerobic system proved advantageous as compared to integrated system. Primarily, rapid azo dye reduction was detected under sequential anaerobic–aerobic states due to utilization of the co-substrate only for providing electrons for reduction of azo dyes; however, in an integrated system, the co-substrate is partly used in creating anaerobic micro niches and partly for azo dye reduction. Additionally, aerobic degradation of the co-substrate under integrated systems generally increases the unavailability of either oxygen or co-substrate, which ultimately is unfavourable for decolourization and degradation of azo dyes. These unfavourable effects certainly do not arise in the sequential anaerobic–aerobic conditions. Thus, a dynamic balance between co-substrate and oxygen is badly required, which was difficult to attain in the integrated anaerobic–aerobic systems (Rai et al. 2005).

The performance of sequential as well as integrated anaerobic–aerobic processes for the bioremediation azo dye Reactive Black 5-laden synthetic wastewaters was studied and compared. Both the processes showed colour removal to a significant extent during the anaerobic phase, while partial mineralization of the anaerobic dye metabolites, approximately to a similar degree, was achieved later during the aerobic stage. The major proportion of the COD was removed during the anaerobic stage for the sequential processes and during the aerobic stage in integrated process. Reportedly, both the processes lead to similar extent of overall COD removal (Bonakdarpour et al. 2011).

4 Factors Affecting Biodegradation of Dyes

Microbial metabolism is significantly affected by changes in abiotic parameters, such as pH, temperature, availability of oxygen, metal ions, salts, etc.; as a result, microbial dye degradation activities are also affected (Ali 2010). Hence, while

analysing the ability of various microorganisms pertaining to degradation of dyes reported to be as xenobiotic compounds, the effects of such parameters are considered. Optimization of these abiotic conditions will substantially facilitate advancements of industrial scale processes as well as bioreactors for bioremediation. A number of these components influencing the biodegradation of azo dyes are therefore reviewed here.

4.1 pH

The initial pH values of the textile industry dye wastewater vary widely. Enhanced decolourization and biodegradation activities are generally shown by fungi and yeasts at acidic or neutral pH (3.0–5.0) and by bacteria at neutral or basic pH, and the rate of dye decolourization tends to decrease under strongly acidic/alkaline pH conditions. Probably, pH affects solubilization and transportation of dye molecules through the plasma membrane as well as the growth of the microorganism, substrate-limiting stage for the dye decolourization (Agrawal et al. 2014b). Thus, organisms showing considerable dye degradation over a wide range of pH need to be preferred for actual waste treatment.

4.2 Temperature

Biodegradation activities associated with microorganisms are unquestionably altered significantly because of changes in temperature. The rate of reaction usually increases to two times with every 10°C increase in temperature, nevertheless, this relationship loses linearity beyond optimum temperature. The degradation activity of the microorganisms decreases beyond the optimum temperature probably, because of the reduced growth and reduced reproduction rate in conjunction with deactivation of enzymes accountable for dye degradation. Optimum growth and degradation temperatures vary amongst different microorganisms, with most of them growing at 25–35 °C (Ali 2010). The reduction in dye decolourization rate at elevated temperatures can be accredited to the denaturation of the enzymes and ultimately the loss of viability of the cells (Anjaneya et al. 2011).

4.3 Initial Dye Concentration

The time required for decolourization of the azo dye is directly proportional to the initial azo dye concentration in the system (Sheth and Dave 2009). The rate of dye decolourization usually slows down with escalating concentration of the dye. This decline in dye decolourization rate with escalation in initial concentration of dye is

caused due to the toxic nature of the dyes and its metabolites towards the multiplying microorganisms at elevated dye concentrations. Contrastingly, it has also been reported that the dye reduction rate was independent of dye concentration exhibiting compatibility with a reduction mechanism that is non-enzymatic and is regulated by processes that are independent of the dye concentration (Dave et al. 2015).

4.4 Dye Structure

Decolourization and biodegradation of azo dyes is extensively affected by the diversity in their structure, together with changes in their chemical structures like isomerism or the existence of diverse functional groups. Dyes having simple molecular structure and low molecular weights reportedly have higher rates of degradation as compared to those dyes having high molecular weight. Also, the degradation rate is faster with respect to monoazo dyes than with diazo or triazo dyes. Azo dyes possessing amino or hydroxyl groups are expected to degrade early as compared to those having methyl, sulpho, methoxy or nitro groups (Saratale et al. 2011).

Azo dyes are reduced to their anionic form by a rapid single-electron transfer reaction, leading to a subsequent second slower electron transfer reaction to generate a stable dianion. Therefore, functional groups with higher electron density might not favour dianion formation, leading to absence or reduced capability for decolourization. Additionally, permeation of azo dyes through the bacterial cell membrane is the rate-limiting step for the decolourization of sulphonated azo dyes by bacteria. Moreover, the electron-dense hydrogen bonds, present in the proximity of the azo bond, enhance the azo-hydrazone tautomerism of hydroxy azo compounds and therefore have a noteworthy impact on the azo dye reduction rate (Saratale et al. 2011).

4.5 Carbon and Nitrogen Content in the Medium

Azo dyes do not act as carbon sources, and therefore, the bioremediation of fabric dyes without supplementation of carbon or nitrogen sources is challenging. Decolourization of azo dyes by mixed or pure cultures usually necessitates complex organic substrates, for instance yeast extract, peptone or possibly a mixture of complex natural and organic substrates and carbohydrates. Reportedly, reducing equivalents from different carbon sources are transmitted to the dyes during azo dye decolourization by azo bond reduction. However, addition of carbon sources proved to be ineffective to enhance dye decolourization, almost certainly, because of the inclination of the cells for assimilation of the surplus carbon sources over utilization of the dye compound and also carbon source decolourization (Saratale

et al. 2011). By far the most readily utilizable carbon sources for almost all of the microorganisms can be glucose. On the other hand, glucose seems to be a costly carbon source, and therefore, other inexpensive carbon sources like starch, molasses and fructose are used in dye wastewater treatment decolourization (Kaushik and Malik 2009). Usually, the addition of the organic nitrogen sources like peptone, urea, yeast and beef extract, etc., can regenerate NADH, which serves as an electron donor for the azo dye reduction by microbes and hence effective decolourization has been witnessed. In making the approach economically viable and essentially applicable, certain researchers have utilized lignocellulosic agricultural waste as a possible supplement for successful decolourization (Saratale et al. 2011).

4.6 Agitation and/or Aeration

Contradictory results have been reported regarding the impact of shaking or agitation on microbial decolourization of textile dyes. It has been observed that azo dyes are usually recalcitrant under aerobic conditions, and therefore for efficient dye degradation, aeration along with agitation, which enhances the oxygen concentration in solution, ought to be avoided (Ali 2010). Bacterial azo dye reduction is strongly inhibited in the presence of oxygen, probably either due to the direct inhibition of the enzyme azoreductase or due to the favoured reduction of oxygen instead of the azo compounds (Pearce et al. 2003).

Bacteria generally degrade azo dyes to colourless toxic aromatic amines, under anaerobic environment, some of which will be readily metabolized under aerobic conditions. Apart from a few, the aromatic amines produced from azo dye decolourization are typically resistant to biodegradation under anaerobic conditions. Hence, anaerobic degradation of azo dyes is usually more suitable than aerobic biodegradation, but the intermediate metabolites (carcinogenic aromatic amines) generated after anaerobic degradation have to be degraded aerobically; therefore, a sequential anaerobic and aerobic treatment of textile industry wastewater containing aromatic compounds is essential before they are discarded to the environment (Rai et al. 2005).

4.7 Salts

Effluent from textile processing and dye producing industries usually comprises of 15–20% of salts along with azo dye residues. As a result, the characterization and development of halotolerant bacterial strains capable of degrading azo dyes may ease the establishment of biotreatment processes for the remediation of high salt containing azo dye wastewaters from textile industry (Khalid et al. 2008).

The dye decolourization rates initially increase with an escalation in salt concentration approximately up to 3–5 g/L of salt concentration, above which

decolourization rates started decreasing. This can be accredited to the variation of enzyme activity and molecular transportation across the plasma membrane of microorganisms. With elevated salt concentrations, the cell has increased osmotic pressure which in turn affects the membrane properties. The decrease in the substrate transportation reduces, generally, the decolourization rate of dyes. The presence of lower concentrations of salt might also stimulate the growth of some bacterial strains from non-marine environments; however, higher salt concentrations had lethal effects on their growth (Meng et al. 2012).

4.8 Electron Donor

The presence of an electron donor is a precondition for reduction of azo dyes. Hypothetically, only four reducing equivalents are required per azo linkage, indicating that the fewer amounts of electron donors are required. However, competition for electron donors by other reactions leads to an increase in the amount of the reducing equivalents required. Azo dyes in addition to other organic constituents of textile dyeing wastewater lack electron donors and therefore are not enough as substrate to support the growth of anaerobic bacteria. Therefore, the reductive cleavage of azo bond is apparently induced by supplementation of electron donors, for instance glucose, acetate or formate ions. In addition to this, the azo dye reduction possibly will also be kinetically benefitted by a higher electron donor concentration. Hence, it is crucial to deduce the physiological electron donors for every dye bioremediation process, because the electron donors not only stimulate the mechanism of azo dye reduction but additionally induce the particular cluster of enzymes accountable for the particular reduction process. It has also been noted that the presence of certain electron donors inhibits the process of electron transport probably because of the competition for electrons from the electron donors (Saratale et al. 2011).

5 Bacterial Enzyme Systems for Dye Biodegradation

Enzymatic reactions have proved their efficiency in textile processing for many years and now are progressively gaining importance as biocatalysts in textile waste processing. Bilirubin oxidase, laccase and azoreductase are some enzymes responsible for the biodegradation of dyes using non-lignolytic fungi. Likewise, various bacterial enzymes, viz. veratryl alcohol oxidase, tyrosinase, NADH-dependent dichlorophenolindophenol (NADH-DCIP) reductase, azoreductase and laccase, are also reported for dye decolourization. It was generally observed that because of the susceptibility of the enzyme to inactivation because of the presence of different chemicals, enzymatic treatment will probably be effective in the fields

having a maximum concentration of dye and minimum concentration of interfering substances (Saratale et al. 2011).

5.1 Laccase

Laccases, alternatively known as benzenediol oxygen oxidoreductases and with EC number EC 1.10.3.2, are principally polyphenol oxidases (PPO), belonging to a small category of enzymes called blue oxidase. Using molecular oxygen as the electron acceptor, they catalyse cross-linking of monomers, ring cleavage of aromatic compounds, degradation of polymers and oxidation of several substituted phenolic compounds (Sharma et al. 2007; Kunamneni et al. 2008). Genes encoding laccases have been reported in various gram-positive as well as gram-negative bacteria, which also includes extremophiles, e.g. in *Oceanobacillus iheyensis, Aquifex aeolicus* and *Pyrobaculum aerophilum* (Ferrer et al. 2010). Besides this, bacterial laccases overcome the drawbacks of in-process applications and instability of fungal laccases. They are extremely active and stable at high temperatures and pH values (Sharma et al. 2007).

5.2 Lignin Peroxidase

Lignin peroxidases (abbreviated as LiP and recognized with the EC number, E.C. 1.11.1.14), the enzyme first reported in the year 1983, are also referred to as ligninase or diaryl propane oxygenase. This enzyme is a member of the category oxidoreductases, having hydrogen peroxide as an electron acceptor (peroxidases), and is broadly categorized as ligninases (Husain 2010). LiP catalyses a variety of reactions, including hydroxylation, ortho-demethylation, benzylic oxidation, cleavage of bond between two carbon atoms, phenol dimerization, hydroxylation, oxidation of non-phenolic aromatic lignin moieties and oxidation of azo dyes and pesticides. Bacterial lignin peroxidases have been utilized for dye bioremediation through *Brevibacillus laterosporus* and *Acinetobacter calcoaceticus* (Gomare et al. 2008; Ghodake et al. 2009). Innumerable investigations have revealed a noteworthy induction in the cellular activity of lignin peroxidase in a diverse variety of bacteria during decolourization of azo dyes, symptomatic of their probable role in decolourization of dyes.

5.3 Azoreductase

Azobenzene reductase also referred to as azoreductase (EC 1.7.1.6) (Maier et al. 2004) is an enzyme, which is a member of the family of oxidoreductases,

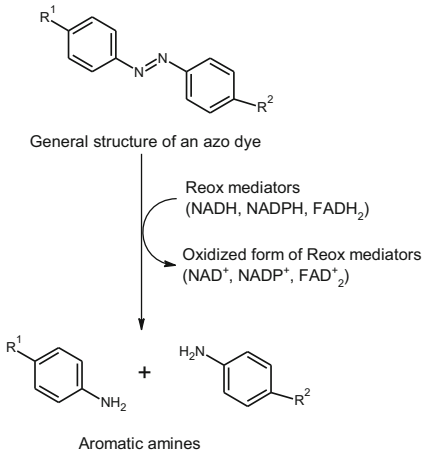

Fig. 3 Reaction catalysed by bacterial azoreductases

specifically catalysing oxidation–reduction reactions only with the help of reducing equivalents FADH, NADH and NADPH as acceptor. Several azo bond reducing enzymes have been recognized as FMN-independent reductases, FMN-dependent reductases, NADH-dependent reductases, NADH-DCIP reductases and NADPH-dependent reductases (Saratale et al. 2011).

Azoreductases are present in bacteria, algae and yeast. Activity of the enzyme azoreductases has been detected in a number of bacteria, such as *Pseudomonas luteola*, *Rhodococcus* sp., *Xenophilus azovorans* KF46F, *Shigella dysenteriae* Type I, *Klebsiella pneumoniae* RS-13, *Clostridium perfringens*, *Bacillus laterosporus* RRK1 (Sandhya et al. 2008), *Caulobacter subvibrioides* C7-D (Mazumder et al. 1999), *Bacillus badius* (Misal et al. 2011), *Staphylococcus aureus* ATCC 25923 (Chen et al. 2005), *Pigmentiphaga kullae* K24 (Chen et al. 2010) and *Rhodobacter sphaeroides* AS1.1737 (Bin et al. 2004). It is localized either to the interior or to the exterior of the bacterial plasma membrane (Saratale et al. 2011). Azoreductases in bacteria can be broadly categorized into two different types: anaerobic and aerobic azoreductases (Solís et al. 2012). Azoreductase as well as the flavin reductase is the crucial enzyme accountable for the azo dye decolourization; conversely, they lead to generation of toxic aromatic amines upon reductive hydrolysis of azo linkage (Saratale et al. 2012; Telke et al. 2015). The reaction catalysed by the enzyme azoreductase is shown in Fig. 3.

5.4 Polyphenol Oxidase or Tyrosinases

Polyphenol oxidase (PPO) enzymes also referred as tyrosinases (EC1.14.18.1) are copper-dependent enzymes. They catalyse the hydroxylation at ortho position of monophenols to o-diphenols (often known as monophenolase) and further catalyse the oxidation of o-diphenols to produce o-quinones (also known as o-diphenolase),

in the presence of molecular oxygen. The enzyme remains in an inactive deoxy state and undergoes a conformational change to the active oxy state upon binding of a new molecule of oxygen. The enzyme also serves as an indicator of the oxidative enzymes accountable for azo dye degradation (Saratale et al. 2011). Activity of the enzyme polyphenol oxidases has been detected in a wide range of bacteria, viz. *Streptomyces glaucescens*, *S. antibioticus*, *Bacillus licheniformis*, *B. sphaericus* and *B. natto* (Telke et al. 2015).

5.5 NADH-DCIP Reductase

This enzyme with EC number (EC 1.6.99.3) catalyses reduction of 2,6-dichlorophenol (DCIP) to its leuco form, with the help of NADH as an electron donor. The enzyme is usually known as diaphorase (Nishiya and Yamamoto 2007). NADH-DCIP reductases are considered as marker enzymes for the mixed function oxidase system of bacteria and fungi and are responsible for azo bond reduction and detoxification of xenobiotic compounds. DCIP is converted from blue colour to colourless, upon reduction (Saratale et al. 2011).

The substantial induction of non-specific reductase was observed during the biodegradation of the dye Malachite green and the non-specific reductase was termed as MG reductase. It reduced the Malachite Green to Leucomalachite Green with the help of NADH as an electron donor (Parshetti et al. 2006).

6 Characterization of Dye Metabolites

With the aim of understanding the environmental fate of the dyes and to reveal the possible mechanism of dye decolourization, study of the metabolic products of azo dyes is crucial. Several analytical techniques are utilized to deduce the degradation products produced upon biodegradation of azo dyes and some of them are described in the following section.

6.1 UV–Visible Spectroscopy

UV–vis spectroscopy (UV–vis) is the principal technique used to ascertain whether dye decolourization is due to absorption or biodegradation (Wilson and Walker 2010). The characteristic peaks in the absorption spectrum of a molecule usually decrease in comparison to each other along with deep colouration of bacterial cells because of dye removal due to adsorption. Contrastingly, in case the dye remediation is due to biodegradation, the main absorbance peaks in the visible region will either completely disappear or some new peaks towards the UV region will appear

as well as the cells retain their original colour (Wijetunga et al. 2007). The true colour of the mixture of dyes and industrial effluents, independent of the hue, is measured with the help of ADMI 3WL, the tri-stimulus filter method of the American Dye Manufacturers Institute (Saratale et al. 2011).

The UV–Vis spectra of the Navy blue 2GL, a textile azo dye, upon treatment by *Bacillus* sp. VUS showed decolourization and reduction in the concentration of the dye over time without any shift in λ_{max} until complete decolourization of the medium (Dawkar et al. 2009). Similarly, noteworthy changes appearing in both the ultraviolet and visible spectra of the dye Acid Black 210, upon treatment by the bacterium *Providencia* sp. SRS83, point out that molecular structure of AB210 changed manifestly upon decolourization undoubtedly owing to the biodegradation (Agrawal et al. 2014b).

6.2 Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FTIR) spectroscopy involves absorption of infrared light, including the wavelength range of about 700–25,000 nm, by a molecule resulting in transition to higher levels of vibration. An infrared spectrum arises because of absorption of incident light of a specific wavelength by a molecule which in turn will then disappear from the transmitted light. The observed spectrum will demonstrate an absorption band. The bonds between atoms can be contemplated as flexible springs, and bond vibrations can therefore be either stretching or bending (deformation) actions.

A comprehensive analysis of the structure of a molecule is possible, because the wavenumber associated with a specific functional group differs marginally, due to the influence of the molecular environment. For instance, it is possible to differentiate between C–H vibrations in methylene (–CH–) and methyl groups ($–CH_3$). FTIR is increasingly used for the analysis of peptides and proteins (Wilson and Walker 2010). The FTIR spectrum facilitates estimation of type as well as strength of interactions amongst different functional groups of the azo dye molecule upon bacterial treatment, and consequently it serves as a precious analytical tool (Saratale et al. 2011).

FTIR spectrum of the azo dye and its metabolites formed upon biodegradation, upon comparison, clearly reveals dye degradation by bacterial species. The FTIR spectra of dye metabolites indicated disappearance of specific peaks, in the range of 1575.0–1630.0 cm^{-1} for azo compounds, thereby confirming breaking of azo bonds present in the dye (Lade et al. 2015). Rest of the peaks indicate the bending and/or stretching vibrations of different groups of the dye and hence support dye degradation (Agrawal et al. 2014b)

6.3 High-Performance Liquid Chromatography

A chromatogram is a pictorial record of the detector response as a function of retention time or elution volume, consisting of a series of peaks or bands, ideally symmetrical in shape, demonstrating the elution of individual analytes. This time is characteristic of the analyte and helps in its identification (Wilson and Walker 2010). High-performance liquid chromatography (HPLC) technique can be utilized for confirming dye degradation, through the advent of novel peaks in the chromatogram with altered retention times in comparison to the original dye. These metabolites generated upon dye biodegradation are represented by the formation of new peaks.

6.4 Mass Spectrometry

Mass spectrometry (MS) assists in obtaining the mass spectra of the mixture of compounds, offering an extremely effective qualitative tool for the analysis of the dye biodegradation. The technique is known as liquid chromatography–mass spectrometry (LC–MS) when a liquid is used as mobile phase and gas chromatography–mass spectrometry (GC–MS) when the mobile phase employed is a gas. Both the techniques have proved to be of help for the estimation of molecular weights as well as the structural details of metabolites of dye biodegradation formed after bacterial treatment and can help in elucidating the microbial pathways of azo dye degradation (Saratale et al. 2011). GCMS analysis was utilized to deduce the metabolites generated upon biodegradation of triazo dye Acid Black 210 with the help of *Providencia* sp. SRS83; the retention time and *m*/*z* values helped in predicting the metabolites generated and probable pathway (Fig. 4) followed by the organism for dye biodegradation (Agrawal et al. 2014b). The dye metabolites generated during anaerobic phase are further mineralized during the aerobic phase, which was deduced by Chan et al. (2012). The dye degradation metabolites, their molecular weights generated from LCMS/MS and the biodegradation pathways can be correlated and hypothesized with reference to the metabolic pathways illustrated on the MetaCyc database at http://metacyc.org/ and KEGG Pathway database at http://www.genome.jp/kegg/pathway.html. The hypothetical pathway for the mineralization of an azo dye is represented in Fig. 5.

6.5 COD

Additionally, the degree of azo dye decolourization in addition to degradation can be estimated by calculating the percentage of degradation through the measurement

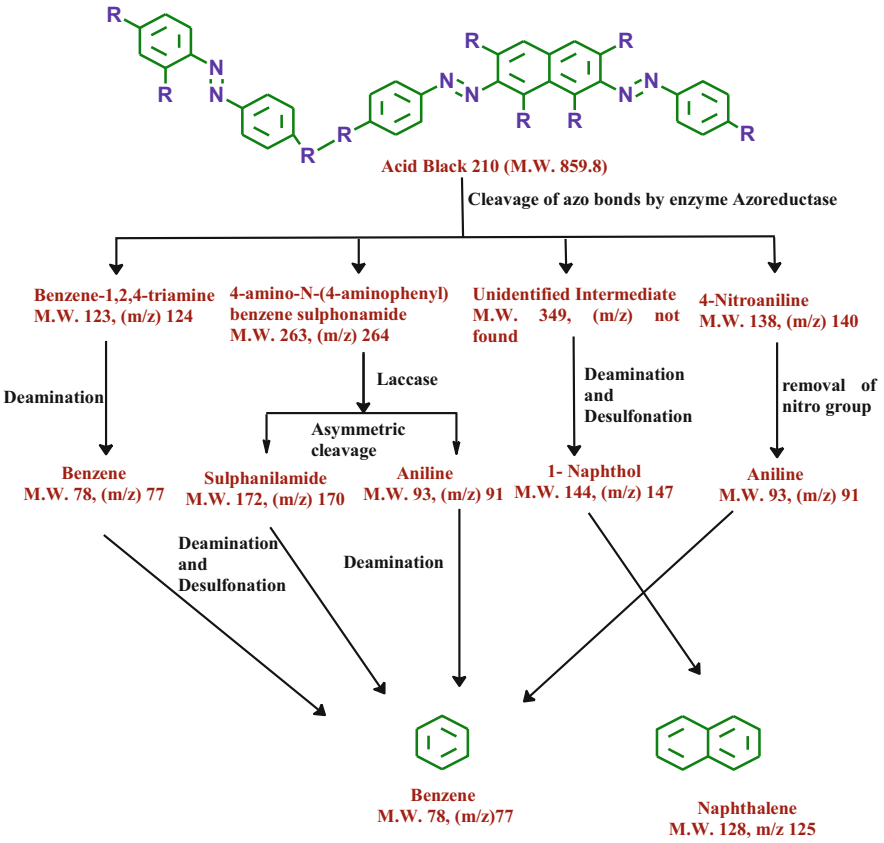

Fig. 4 Proposed pathway for the biodegradation of a model azo dye by *Providencia* sp. SRS82

of the decrease in the ratio of both the chemical oxygen demand (COD) and biochemical oxygen demand (BOD), before and after the treatment.

Analysis and interpretation of dye biodegradation by sophisticated techniques mentioned above are not much helpful for dye containing effluent, as with individual dye biodegradation by bacteria, because composition of industrial wastewater is very complex and practically unknown. So, parameters such as COD, BOD, ADMI values and total organic carbon are estimated to estimate the degree of mineralization and biodegradation of dye-laden effluent. A reduction in values of COD, ADMI and TOC values indicates mineralization of complex dye molecule (APHA 1998).

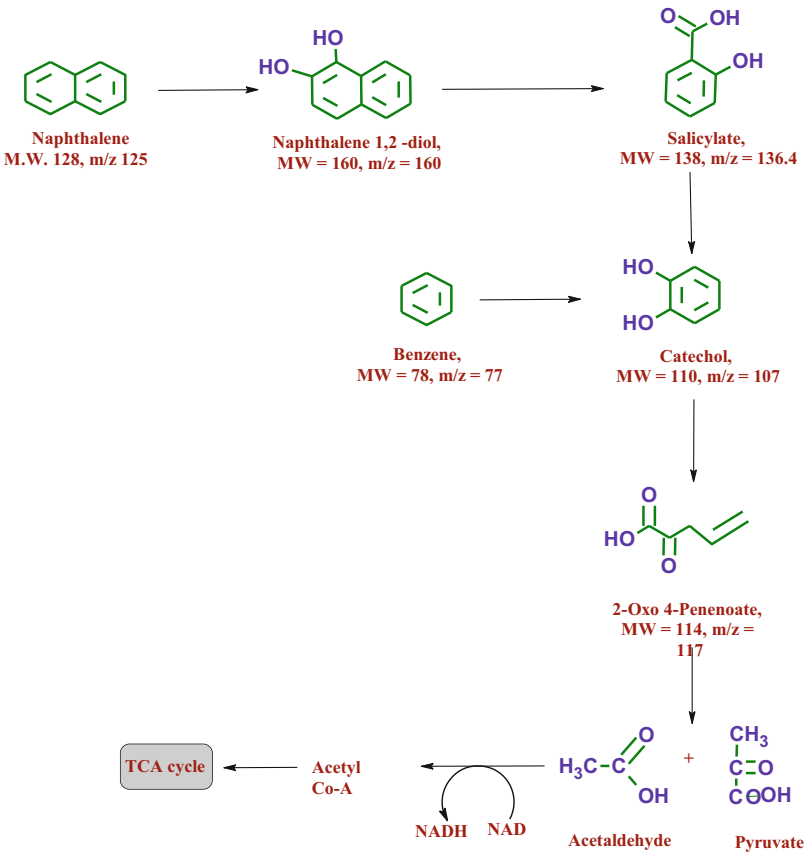

Fig. 5 Proposed pathway for the aerobic biodegradation of dye metabolites

7 Toxicity Study

The release of textile industry and dyestuff manufacturing industry effluent may lead to unusual colouration of the surface water which causes the ultimate ecological distress pertaining to water quality as well as directly disturbs the aquatic biota. The synthetic dyestuffs and their degradation products released by the textile industry are mutagenic and carcinogenic. Certainly, lack of adequate statistics related to toxicity of dyes with reference to cell populations makes bioremediation unpredictable and undependable for on-site processes. Thus, it is very essential to find out phytotoxicity and microbial toxicity of the treated dyeing effluent.

7.1 Phytotoxicity

Phytotoxicity studies involve treatment of the seeds of model plants with the parent dye and its biodegradation products at a specific concentration. The impact of this treatment on the seed germination percentage and length of the plumule and radicle can be estimated and can then be compared with the control (treated with water). Various groups of researchers have treated the seeds of model plants with water, the dye and its extracted metabolite separately and subsequently compared percentage of seed germination along with the lengths of plumule in addition to the radical. Reportedly, seed germination (%), shoot length and root length for the tested plants were less upon dye treatments as compared to those treated with metabolites obtained after its decolourization confirming detoxification of the dye molecule upon microbial treatment (Saratale et al. 2011).

7.2 Microbial Toxicity

The toxicity of the azo dyes and their metabolites can be analysed by evaluating the toxicity of the dye sample before and after bacterial treatment towards the known microbial cultures. This is a sensitive method producing reproducible and comparable results. Furthermore, if the biodecolourizer itself is the test organism, then the results indicate the performance of the dye degradation process and suitability of the bio decolourizer for this process too (Saratale et al. 2011). The toxicity of the dye sample and its metabolites can also be compared by counting the changes in microbial cells count per millilitre as compared to that in nutrient broth (Ali 2010).

Correspondingly, some researchers have investigated the toxic effect of a variety of dyes and their biodegradation products generated upon bacterial treatment on plate assay by measuring their zone of inhibition at equal concentrations and used common laboratory microorganisms along with cellulose, nitrogen and phosphate solubilizing bacteria, owing to their significance in agriculture. The outcomes suggest that upon treatment with bacteria the extract of dye metabolites proved to be less toxic than the dye itself, indicating azo dye detoxification (Saratale et al. 2011).

7.3 Other Toxicity Tests

Higher plants are considered as outstanding genetic models to estimate mutagenicity of toxic compounds; therefore, cytotoxicity and genotoxicity tests conducted upon *Allium cepa* (onion) are nowadays generally studied. *A. cepa* species also have the advantage of being cheap and simplicity in handling. Mitotic index (MI) and chromosomal aberrations in the cells of roots of *A. cepa* are utilized to detect

genotoxicity. MI of sample-treated dye is usually higher as compared to those treated with biodegraded metabolites. MI, cell death assay and certain nuclear abnormalities are evaluated to estimate cytotoxicity of dyes. Analysis of the increase or decrease in MI provides a useful insight for the estimation of the cytotoxicity of a test compound. Reportedly, total numbers of cells with chromosomal aberrations are considerably higher in dye-treated samples as compared to those treated with water and dye metabolites, indicating genotoxicty of the dyes (Dave et al. 2015). The different forms of chromosomal aberrations induced under the influence of dyes are laggards, chromosomal breakages and anaphase bridges and micronuclei formation. Additionally, the frequencies of total alterations (TA) were also detected to be higher in dye-treated samples (Jadhav et al. 2011)

Environmental pollutants can lead to induction of the oxidative stress which can subsequently bring about lipid peroxidation and/or protein oxidation or even damages to DNA in plant and mammalian cells. Antioxidants or scavenging enzymes, for instance superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase and peroxiredoxins, are present in the plant systems as their defence against such oxidative stress. Consequently, to analyse the toxicity status of dye and its degraded metabolites, various researchers assayed the status of antioxidant enzymes, protein oxidation and lipid peroxidation. Reportedly, the activities of these enzymes in *A. cepa* root cells were elevated in dye-treated samples as compared to those samples that were exposed to dye metabolites which showed virtually similar levels of these with respect to control set, clearly indicating the relatively less toxic nature of the dye metabolites generated upon its treatment with bacteria (Jadhav et al. 2011).

The Ames test or the *Salmonella* mutagenicity assay is extensively utilized to deduce mutagenicty and carcinogenicity of azo dyes and their metabolites. Similarly, toxicity of the certain dyes has been studied using bioluminescent marine bacterium *Vibrio fischeri*. *Pseudokirchneriella subcapitata, a green unicellular alga,* has also been used as test organism to analyse the toxicity of the azo dyes (Dave et al. 2015).

8 Research to Implementation

Earlier reviews on biodegradation of dyes have shown that a lot of literature is already available on the usage of different bacteria for dye removal; however, detailed economic and market studies are yet not available. However, imposition of strict regulations regarding pollution has pressurized dye manufacturing and dye using industries towards usage of advanced and technologically sophisticated methods for effluent treatment. Industrialists are still reluctant to use the microbial or bacterial reactors. The challenge with bioremediation of dyes is to implement this process to an industrial scale.

The overwhelming publications in the arena of the remediation of azo dyes from wastewaters have been exploring the different applicable facets of the microbial

techniques, with simultaneous hunt for novel microorganisms giving efficient dye degradation along with an insight into the elucidation of the key biochemical and biophysical processes essential to azo dye decolourization and degradation.

However, decrease in the dye-laden waste and water recycling along with development of effective, accessible, cost-effective and eco-friendly treatment processes are the need of the hour. Therefore, the future research activities should emphasize on the following facets:

1. Application of molecular biology techniques to acquire knowledge regarding the dye biodegradation pathway, which in turn can be used to develop genetically modified bacterial strains with enhanced biodegradation capacities along with tolerance to alkaline and high-temperature conditions might increase the economic feasibility and viability of the process.
2. Advancements in techniques for enzyme immobilization or development of microbial fuels cells, which can produce electricity during the bioremediation process, may help in increasing the efficiency of the process.
3. On the basis of successful laboratory results, efforts should now be directed towards application and scale-up of bacterial decolourization techniques on real industrial effluents. It is relatively easy to demonstrate it at lab scale, but it is a herculean task to demonstrate it at a pilot scale, but to actually scale it up to a large scale requires considerable financial and technological effort and therefore multidisciplinary researches should be conducted in this field.
4. Besides this, multifaceted researches can also be directed towards process design leading to reduction in use of water, reduction in discharge of toxic chemicals, and use of dyes with modified structures that can be degraded easily.

9 Problem Delineated

The microbial degradation of azo dyes has drawn significant attention, as a cost-effective process for dye bioremediation. Pure, mixed or consortial bacterial cultures can degrade a variety of azo dyes, discharged in textile industry effluent, under aerobic, microaerophilic or anaerobic conditions. The initial step in the dyes biodegradation is usually the reduction of the azo dye to its corresponding aromatic amines that are then degraded aerobically. The effectiveness of the process for the degradation of azo dyes is determined by the type of dye in addition to its structure and the physico-chemical parameters of the effluent, such as temperature, pH and salt concentration, availability of nutrient sources, oxygen and electron donors. The dye biodegradation process is mediated by different oxidoreductive enzymes. A variety of sophisticated analytical techniques help in ascertaining the degradation of dye as well as in elucidating the probable pathway followed by the bacterium for dye degradation. The detoxification of the dye after bacterial treatment is confirmed by less toxicity of dye metabolites to tested microbes, plants, cells and enzymes. The development of innovative processes, such as advanced oxidation process and microbial fuel cells combined with recombinant microbiological strains and the

addition of novel efforts and methodologies in this area, will play a crucial role in increasing efficiency of dye bioremediation and therefore consequently environment protection.

References

Agrawal S, Tipre D, Dave SR (2014a) Isolation, characterization and study of microorganisms capable of decolourizing triazo dye Acid Black 210. Ind J Environ Protect 34:540–546

Agrawal S, Tipre D, Patel B, Dave S (2014b) Optimization of triazo Acid Black 210 dye degradation by *Providencia* sp. SRS82 and elucidation of degradation pathway. Proc Biochem 49:110–119. doi:10.1016/j.procbio.2013.10.006

Ali H (2010) Biodegradation of synthetic dyes—A review. Water Air Soil Pollut 213:251–273. doi:10.1007/s11270-010-0382-4

An SY, Min S, Cha IH, Choi Y, Cho YS, Kim CH, Lee YC (2002) Decolourization of triphenylmethane and azo dyes by *Citrobacter* sp. Biotechnol Lett 24:1037–1040. doi:10.1023/A:1015610018103

Anjaneya O, Souche YS, Santoshkumar M, Karegoudar TB (2011) Decolourization of sulfonated azo dye Metanil Yellow by newly isolated bacterial strains: *Bacillus* sp . strain AK1 and *Lysinibacillus* sp. strain AK2. J Hazard Mater 190:351–358. doi:10.1016/j.jhazmat.2011.03.044

APHA, Standard Methods for the Examination of Water and Wastewater (1998) APHA-AWWA-WEF, 20th edn. Washington DC, USA. Method 2120 E. isbn: 0-875530788

Bin Y, Jiti Z, Jing W, Cuihong D, Hongman H, Zhiyong S, Yongming B (2004) Expression and characteristics of the gene encoding azoreductase from *Rhodobacter sphaeroides* AS1. FEMS Microbiol Lett 236:129–136. doi:10.1016/j.femsle.2004.05.034

Bonakdarpour B, Vyrides I, Stuckey DC (2011) Comparison of the performance of one stage and two stage sequential anaerobic-aerobic biological processes for the treatment of reactive-azo-dye-containing synthetic wastewaters. Int Biodeter Biodegr 65:591–599. doi:10.1016/j.ibiod.2011.03.002

Carmen Z, Daniela S (2012) Textile organic dyes—characteristics, polluting effects and separation/elimination procedures from industrial effluents—A critical overview. In: Puzyn T, Mostrag-Szlichtyng A (eds) Organic pollutants ten years after the Stockholm convention—environmental and analytical update. InTech, Croatia, pp 55–86. isbn: 978-953-307-917-2

Chan GF, Rashid ANN, Chua LS, Nasiri R, Roslan M, Ikubar MRM (2012) Communal microaerophilic–aerobic biodegradation of Amaranth by novel NAR-2 bacterial consortium. Bioresour Technol 105:48–59. doi:10.1016/j.biortech.2011.11.094

Chen H, Feng J, Kweon O, Xu H, Cerniglia CE (2010) Identification and molecular characterization of a novel flavin-free NADPH preferred azoreductase encoded by azoB in *Pigmentiphaga kullae* K24. BMC Biochem 11:13–22. doi:10.1186/1471-2091-11-13

Chen H, Hopper SL, Cerniglia CE (2005) Biochemical and molecular characterization of an azoreductase from *Staphylococcus aureus*, a tetrameric NADPH-dependent flavoprotein. Microbiology 151:1433–1441. doi:10.1099/mic.0.27805-0

Chequer FMD, Dorta DJ, De Oliveira DP (2011) Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks. In: Hauser PJ (ed) Advances in treating textile effluent. InTech, Croatia EU, pp 28–48, isbn: 978-953-307-704-8

Christie RM (2001) Colour chemistry, vol 27. The Royal Soiety of Chemistry, Cambridge, UK. doi:10.1039/9781847550590

Dave SR, Patel TL, Tipre DR (2015) Bacterial degradation of azo dye containing wastes. In: Singh SN (ed) Microbial degradation of synthetic dyes in waste water. Springer, Cham, Switzerland, pp 57–83. doi:10.1007/978-3-319-10942-8_3

Dawkar VV, Jadhav UU, Ghodake GS, Govindwar SP (2009) Effect of inducers on the decolourization and biodegradation of textile azo dye Navy blue 2GL by *Bacillus* sp. VUS. Biodegradation 20:777–787. doi:10.1007/s10532-009-9266-y

Ferrer M, Beloqui A, Golyshin PN (2010) Screening metagenomic libraries for laccase activities. In: Wolfgang RS, Rolf D (eds) Metagenomics: methods and protocols. Humana Press, London, pp 189–202. doi:10.1007/978-1-60761-823-2_13

Ghodake G, Jadhav S, Dawkar V, Govindwar S (2009) Biodegradation of diazo dye Direct brown MR by *Acinetobacter calcoaceticus* NCIM 2890. Int Biodeter Biodegr 63:433–439. doi:10.1016/j.ibiod.2008.12.002

Gomare SS, Jadhav JP, Govindwar SP (2008) Degradation of sulfonated azo dyes by the purified lignin peroxidase from *Brevibacillus laterosporus* MTCC 2298. Beiotechnol Bioprocess Eng 13:1–8. doi:10.1007/s12257-008-0008-5

Gomare SS, Tamboli DP, Kagalkar AN, Govindwar SP (2009) Eco-friendly biodegradation of a reactive textile dye Golden Yellow HER by *Brevibacillus laterosporus* MTCC 2298. Int Biodeter Biodegr 63:582–586. doi:10.1016/j.ibiod.2009.03.005

Hsueh CC, Chen BY, Yen CY (2009) Understanding effects of chemical structure on azo dye decolourization characteristics by *Aeromonas hydrophila*. J Hazard Mater 167:995–1001. doi:10.1016/j.jhazmat.2009.01.077

Hunger K (ed) (2003) Industrial dyes chemistry, properties, applications. Wiley-VCH Verlag GmbH and Co. KGaA, Weinheim. isbn: 978-3-527-30426-4.

Husain Q (2010) Peroxidase mediated decolourization and remediation of wastewater containing industrial dyes: a review. Rev Environ Sci Biotechnol 9:117–140. doi:10.1007/s11157-009-9184-9

Jadhav JP, Kalyani DC, Telke AA, Phugare SS, Govindwar SP (2010) Evaluation of the efficacy of a bacterial consortium for the removal of color, reduction of heavy metals, and toxicity from textile dye effluent. Bioresour Technol 101:165–173. doi:10.1016/j.biortech.2009.08.027

Jadhav JP, Phugare SS (2012) Textile dyes: general information and environmental aspects. In: Nemr A El (ed) Non-conventional textile waste water treatment. Nova Science, New York, pp 1–345. isbn: 978-1-62100-228-4.

Jadhav SB, Phugare SS, Patil PS, Jadhav JP (2011) Biochemical degradation pathway of textile dye Remazol red and subsequent toxicological evaluation by cytotoxicity, genotoxicity and oxidative stress studies. Int Biodeter Biodegr 65:733–743. doi:10.1016/j.ibiod.2011.04.003

Jin R, Yang H, Zhang A, Wang J, Liu G (2009) Bioaugmentation on decolourization of C. I. Direct Blue 71 by using genetically engineered strain *Escherichia coli* JM109 (pGEX-AZR). J Hazard Mater 163:1123–1128. doi:10.1016/j.jhazmat.2008.07.067

Kant R (2012) Textile dyeing industry an environmental hazard. Nat Sci 4:22–26. doi:10.4236/ns.2012.41004

Kaushik P, Malik A (2009) Fungal dye decolourization: Recent advances and future potential. Environ Int 35:127–141. doi:10.1016/j.envint.2008.05.010

Khalid A, Arshad M, Crowley DE (2008) Decolourization of azo dyes by *Shewanella* sp. under saline conditions. Appl Microbiol Biotechnol 79:1053–1059. doi:10.1007/s00253-008-1498-y

Koupaie EH, Moghaddam MRA, Hashemi SH (2013) Evaluation of integrated anaerobic/aerobic fixed-bed sequencing batch biofilm reactor for decolorization and biodegradation of azo dye Acid Red 18 : Comparison of using two types of packing media. Bioresour Technol 127:415–421. doi:10.1016/j.biortech.2012.10.003

Kunamneni A, Plou FJ, Ballesteros A, Alcalde M (2008) Laccases and their applications: a patent review. Recent Pat Biotechnol 2:10–24. doi:10.2174/187220808783330965

Lade H, Govindwar S, Paul D (2015) Mineralization and detoxification of the carcinogenic azo dye Congo Red and real textile effluent by a polyurethane foam immobilized microbial

consortium in an upflow column bioreactor. Int J Environ Public Health 12:6894–6918. doi:10.3390/ijerph120606894

Lang AR (ed.) (2009) Dyes and pigments: new research. Nova Science, New York. isbn: 978-1-60876-195-1

Maier J, Kandelbauer A, Erlancher A, Cavaco-Paulo A, Gubits GM (2004) A new alkali thermostable azoreductase from *Bacillus* sp. strain SF. Appl Environ Microbiol 70:837–844. doi:10.1128/AEM.70.2.837-844.2004

Mangal VP (2010) The future of Indian dyes and dye intermediates. Textile Rev www.fibre2fashion.com, accessed on June 6, 2014

Mansoor I (2008) Textile dyes. Rahber Publishers, Karachi

Mazumder R, Logan JR, Mikell JAT, Hooper SW (1999) Characteristics and purification of an oxygen insensitive azoreductase from *Caulobacter subvibrioides* strain C7-D. J Ind Microbiol Biotechnol 23:76–483

Meng X, Liu G, Zhou J, Shiang FQ, Wang G (2012) Azo dye decolourization by *Shewanella aquimarina* under saline conditions. Bioresour Technol 114:95–101. doi:10.1016/j.biortech.2012.03.003

Misal SA, Lingojwar DP, Shinde RM, Gawai KR (2011) Purification and characterization of azoreductase from alkaliphilic strain *Bacillus badius*. Proc Biochem 46:1264–1269. doi:10.1016/j.procbio.2011.02.013

Mohana S, Shrivastava S, Divecha J, Madamwar D (2008) Response surface methodology for optimization of medium for decolourization of textile dye Direct Black 22 by a novel bacterial consortium. Biresour Technol 99:562–569. doi:10.1016/j.biortech.2006.12.033

Nishiya Y, Yamamoto Y (2007) Characterization of a NADH: dichloroindophenol oxidoreductase from *Bacillus subtilis*. Biosci Biotechnol Biochem 71:611–614. doi:10.1271/bbb.60548

Ola IO, Akintokun AK, Akpan I, Omomowo IO, Areo VO (2010) Aerobic decolourization of two reactive azo dyes under varying carbon and nitrogen source by *Bacillus cereus*. Afr J Biotechnol 9:672–677

Parshetti G, Kalme S, Saratale G, Govindwar S (2006) Biodegradation of Malachite Green by *Kocuriarosea* MTCC 1532. Acta Chim Solv 53:492–498

Patil PS, Shedbalkar UU, Kalyani DC, Jadhav JP (2008) Biodegradation of Reactive Blue 59 by isolated bacterial consortium PMB11. J Ind Microbiol Biotechnol 35:1181–1190. doi:10.1007/s10295-008-0398-6

Pearce CI, Lloyd JR, Guthrie JT (2003) The removal of colour from textile wastewater using whole bacterial cells: a review. Dye Pigment 58:179–196. doi:10.1016/S0143-7208(03)00064-0

Phugare SS, Kalyani DC, Patil AV, Jadhav JP (2011) Textile dye degradation by bacterial consortium and subsequent toxicological analysis of dye and dye metabolites using cytotoxicity, genotoxicity and oxidative stress studies. J Hazard Mater 186:713–723. doi:10.1016/j.jhazmat.2010.11.049

Rai HS, Bhattacharyya MS, Singh J, Bansal TK, Vats P, Banerjee UC (2005) Removal of dyes from the effluent of textile and dyestuff manufacturing industry: a review of emerging techniques with reference to biologocal treatment. Crit Rev Environ Sci Technol 35:219–238. doi:10.1080/10643380590917932

Sandhya S, Sarayu K, Uma B, Swaminathan K (2008) Decolorizing kinetics of a recombinant *Escherichia coli* SS125 strain harboring azoreductase gene from *Bacillus latrosporus* RRK1. Bioresour Technol 99:2187–2191. doi:10.1016/j.biortech.2007.05.027

Santos B, Cervantes FJ, Lier JB (2007) Review paper on current technologies for decolourisation of textile wastewaters: perspectives for anaerobic biotechnology. Bioresour Technol 98:2369–2385

Saratale RG, Gandhi SS, Purankar MV, Kurade MB, Govindwar SP, Oh SE, Saratale GD (2012) Decolorization and detoxification of sulfonated azo dye C.I. Remazol Red and textile effluent by isolated *Lysinibacillus* sp. RGS. J Biosci Bioeng 115:658–667. doi:10.1016/j.jbiosc.2012.12.009

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2010) Decolourization and biodegradation of reactive dyes and dye wastewater by a developed bacterial consortium. Biodegradation 21:999–1015. doi:10.1007/s10532-010-9360-1

Saratale RG, Saratale GD, Chang JS, Govindwar SP (2011) Bacterial decolourization and degradation of azo dyes: a review. J Taiwan Inst Chem Eng 42:138–157. doi:10.1016/j.jtice.2010.06.006

Saratale RG, Saratale GD, Kalyani DC, Chang JS, Govindwar SP (2009) Enhanced decolourization and biodegradation of textile azo dye Scarlet R by using developed microbial consortium-GR. Bioresour Technol 100:2493–2500. doi:10.1016/j.biortech.2008.12.013

Sarayu K, Sandhya S (2010) Aerobic biodegradation pathway for Remazol Orange by *Pseudomonas aeruginosa*. Appl Biochem Biotechnol 160:1241–1253. doi:10.1007/s12010-009-8592-1

Senan RC, Abraham TE (2004) Bioremediation of textile azo dyes by aerobic bacterial consortium aerobic degradation of selected azo dyes by bacterial consortium. Biodegradation 15:275–280

Shah PD, Dave SR, Rao MS (2012) Enzymatic degradation of textile dye Reactive Orange 13 by newly isolated bacterial strain *Alcaligenes faecalis* PMS-1. Int Biodeter Biodegr 69:41–50. doi:10.1016/j.ibiod.2012.01.002

Sharma P, Goel R, Capalash N (2007) Bacterial laccases. World J Microbiol Biotechnol 23:823–832. doi:10.1007/s11274-006-9305-3

Sheth NT, Dave SR (2009) Optimisation for enhanced decolourization and degradation of reactive Red BS C.I. 111 by *Pseudomonas aeruginosa* NGKCTS. Biodegradation 20:827–836. doi:10.1007/s10532-009-9270-2

Singh S, Chatterji S, Nandini PT, Prasad ASA, Rao KVB (2015) Biodegradation of azo dye Direct Orange 16 by *Micrococcus luteus* SSN2. Int J Environ Sci Technol 12:2161–2168. doi:10.1007/s13762-014-0588-x

Solis M, Solis A, Perez HI, Manjarrez N, Flores M (2012) Microbial decolouration of azo dyes: a review. Proc Biochem 47:1723–1748. doi:10.1016/j.procbio.2012.08.014

Telke AA, Kadam AA, Govindwar SP (2015) Bacterial enzymes and their role in decolourization of azo dyes. In: Singh SN (ed) Microbial degradation of synthetic dyes in waste water. Springer, Cham, Switzerland, pp 57–83. doi:10.1007/978-3-319-10942-8_7

Van Der Zee FP (2002) Anaerobic azo dye reduction. Ph.D. Thesis, Wageningen University, Wageningen, Netherlands.

Van Der Zee FP, Villaverde S (2005) Combined anaerobic-aerobic treatment of azo dyes—a short review of bioreactor studies. Water Res 39:1425–1440. doi:10.1016/j.watres.2005.03.007

Wang H, Qiang J, Wei X, Tian Y, Jing X, Ling T (2009) Bacterial decolorization and degradation of the reactive dye Reactive Red. Int Biodeter Biodegr 63:395–399. doi:10.1016/j.ibiod.2008.11.006

Wijetunga S, Xiufen L, Wenquan R, Chen J (2007) Removal mechanisms of acid dyes of different chemical groups under anaerobic mixed culture. Ruhuna J Sci 2:96–110

Wilson K, Walker JM (2010) Principles and techniques of biochemistry and molecular biology. 7th Ed England: Cambridge University Press. 10: 0521731674.

Yonar T (2011) Decolorisation of textile dyeing effluents using advanced oxidation processes. In: Hauser PJ (ed) Advances in treating textile effluent. InTech, Croatia EU, pp 28–48. isbn: 978-953-307-704-8

Zaharia C, Suteu D, Muresan A (2012) Option and solutions for textile effluent decolourization using some specific physico-chemical treatment steps. Environ Eng Manag J 11:493–509

http://envirosystems.net/effluent-treatment-plant/, Accessed on October 20, 2015

http://metacyc.org/

http://planningcommission.gov.in/aboutus/committee/wrkgrp12/wg_chem0203.pdf, Accessed on November 23, 2015.

http://www.genome.jp/kegg/pathway.html

http://www.indiaenvironmentportal.org.in/content/573/united-colours-of-industry/, Accessed on June 18, 2014.