The Biodegradation of Azo Dyes by Actinobacteria

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1 Introduction

Synthetic dyes have wide application in the textile, leather, pharmaceutical, cosmetic, paper and food industry. According to an estimate, global production of synthetic dyes is more than 700,000 tonnes and textile sector consumes about 60 % of the total production of dyes (Robinson et al. 2001; Shinde and Thorat 2013). Since dyeing process is not very efficient, production of highly colored wastewater is enormous. The amount lost in wastewater is a function of the class of dyes and in general, their loss through discharge in the wastewater can be 2 % of the initial concentration of basic dyes to as high as 50 % of a reactive dye (Tan et al. 2000; Boer et al. 2004).

Azo dyes are aromatic compounds containing one or more azo (−N=N−) chromophores. Such dyes are considered to be electron-deficient compounds, as they possess the azo (−N=N−) and sulfonic acid (SO_3^-) electron withdrawing groups, resulting in a deficit of electrons in the molecule which renders the compound more sensitive to oxidative catabolism by bacteria. Hence, azo dyes tend to persist under aerobic environmental conditions (Rieger et al. 2002). Because of the persistence nature, azo dyes have negative impact on the environment in terms of total organic carbon (TOC), chemical oxygen demand (COD) and biological oxygen demand (BOD) (Saratale et al. 2009). Many synthetic dyes and their metabolic intermediate products are found to be toxic, mutagenic and carcinogenic (Dafale et al. 2010; Poljsak et al. 2010; Sellamuthu et al. 2011; Yang et al. 2013). The treatment of dye-contaminated wastewater in an environmentally safe manner is essentially required prior to its disposal. Various physical, chemical and biological strategies can be used for the treatment of azo dyes (Qin et al. 2007;

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Onat et al. 2010). However, physico-chemical methods generally produce a huge amount of additional residues that make these methods non eco-friendly (Sharma et al. 2013). Complete mineralization of dyes with microorganisms is one of the most viable options for remediation of industrial effluents containing azo dyes. The versatility of microorganisms and their various metabolic pathways can be used to target the major chemical classes of dyes (Mohanty et al. 2006; Sadettin and Donmez 2007; Liu et al. 2011; Saratale et al. 2011; Khalid et al. 2012; Prasad and Aikat 2014). Although a variety of culturable bacteria, fungi and algae, capable of degrading dyes through the oxidoreductase enzymes, have been intensively studied (Table 1), but a group of microorganisms, such as actinomycetes, has not previously been focused much for their biodegradability potential.

Actinomycetes belong to Enterobacteriaceae family and now, they are known for their degradation capabilities. Actinobacteria are considered a very attractive option for the production of many secondary bioactive compounds which are metabolites (Nathan et al. 2004; Jensen et al. 2005; Suthindhiran and Kannabiran 2009). Actinobacteria are a group of filamentous Gram positive bacteria which are known as prokaryotes with high GC contents (Stackebrandt et al. 1997; Deepika and Kannabiran 2009; Deepika et al. 2009). They are widely distributed in terrestrial and aquatic ecosystems (Chronakova et al. 2009), *Streptomyces* spp. in soil consist of 50 % of the total microbial population and play a crucial role in the recycling of refractory biomaterials by decomposing complex mixtures of polymeric materials from dead plants, animals and fungi. Considering their diverse habitat and role in the environment, these microorganisms can be studied for their potential for biodegradation and bioremediation of contaminated soil and aquatic environment.

Very recently, *Streptomyces* species have been exploited for the degradation of synthetic dyes (Lu et al. 2013; Gousterova et al. 2014). Researchers have developed a great interest in actinomycetes because of their high potential in the treatment of industrial waste (Gousterova et al. 2011). Actinobacteria are particularly suitable for the wastewater treatment, because of their different metabolic pathways working in a wide range of environmental conditions and survival under extreme conditions. Microorganisms, isolated from extreme environments, were found to be producers of enzymes and metabolites with promising properties (Lee et al. 2012; Mahajan and Balachandran 2012). In this chapter, the role of actinomycetes in the degradation of azo dyes is discussed thoroughly.

2 Environmental Concerns of Azo Dyes

Azo dyes and their intermediates are toxic, mutagenic and carcinogenic (Lu et al. 2010; Modi et al. 2010) and affect organisms in both aquatic and terrestrial systems (Puvaneswari et al. 2006). Among azo dyes, those produced from aromatic amines, such as benzidine and 4-biphenylamine, 4-aminobiphenyl, monoacetylbenzidine and acetylaminobiphenyl, pose a serious threat to the environment and human health (Manning et al. 1985; Cerniglia et al. 1986; Choudhary 1996; Chung 2000).

Table 1 Biodegradation of structurally different synthetic dyes by various microorganisms

Culture species	Dyes	Type of microorganisms	Comments	References
Pseudomonas aerogenosa	Direct orange, disperse brown, reactive green	Bacteria	About 94 % color removal of the dyes was observed after 7 days incubation under aerobic conditions	Ahmed (2014)
Bjerkandera adusta Dec1	Amaranth	Fungi	After 10 days, 98 % decolorization was achieved under aerobic conditions.	Gomi et al. (2001)
Enteromorpha sp.	Basic red 46 (BR46)	Algae	The maximum decolorization efficiency (83.45 %) was achieved with 4 g initial biomass of algae, after 5 h at temperature 25 °C	Khataee et al. (2013)
Aspergillus niger *Mucor racemosus*	Red HE7B, reactive red 141, yellow FN2R, reactive yellow 206	Fungi	Up to 94 % dyes degradation was achieved by fungi after 5 day incubation under static conditions	Balaji et al. (2012)
Aeromonas hydrophila	Crystal violet, Fuchsin green, brilliant green, malachite green	Bacteria	About 90 % color of dye was removed in 10 h under anaerobic conditions	Ren et al. (2006)
Trametes villosa	Cibacron blue 19, cibacron blue 20	Fungi	Both the dyes were degraded in 10 days of incubation under shaking conditions.	Machado et al. (2006)
Bacillus subtillis	Orange	Bacteria	More than 94 % decolorization was achieved in 23.7 h under static conditions.	Sha et al. (2014)
Pseudomonas fluorescen	Reactive orange-M2R, reactive blue-M58, reactive yellow-M4G, reactive black-B	Bacteria	The dye decolorization for the mentioned dyes was 59, 90, 77 and 79 % respectively within 16 days under shaking conditions.	Sriram et al. (2013)
Phanerochaete chrysosporium	aminobenzoic or aminosulphonic acids (diazo components)	Fungi	The decolorization was 80–89 % after 28 days under shaking conditions.	Martins et al. (2001)

(continued)

Table 1 (continued)

Culture species	Dyes	Type of microorganisms	Comments	References
Marasmius cladophyllus	Remazol brilliant blue R	Fungi	About 97 % of the dye was degraded in 16 days under static conditions.	Ngieng et al. (2013)
Entrobacter spp.	Congo red	Bacteria	Complete decolorization of the dye (200 mg L^{-1}) was observed within 93 h under static conditions	Prasad and Aikat (2014)
phanerochaete chrysosporium	Amido black B, procion blue-2G	Fungi	The dye decolorization was 20–100 % depending on the concentration of dye in 25 days under static conditions	Kumar et al. (2013)
Lyngbya sp. BDU 9001	Textile dye effluent	Cyanobacterium	The decolorization efficiency was 73 % at the 15th day of incubation	Henciya et al. (2013)
Bacillus cereus	Reactive red 195	Bacteria	97 % dye was degraded after 72 h under anaerobic conditions.	Modi et al. (2010)
Bacillus sp. VUS	Orange T4LL	Bacteria	Complete color removal of the dye was achieved within 24 h under static anoxic conditions.	Dawkar et al. (2010)
Enterococcus faecalis	Acid red 27, reactive red 2	Bacteria	Decolorization was between 95–100 % within 12 h under anaerobic conditions	Handayani et al. (2007)

A small amount of dye in the water (<1 mg l^{-1} for some dyes) can cause a very visible color change (Banat et al. 1996), which affects not only the aesthetic aspects of the water, but also raises environmental concerns (Lu et al. 2010; Modi et al. 2010). Previously, several researchers have reported the toxicity of synthetic dyes on ecosystems (Fraga et al. 2009; Osugi et al. 2009; Dafale et al. 2010). The effluents containing dyes affect the photosynthetic activity of aquatic plants and algae by changing the light and gas penetration into water bodies. Hence, wastewater is also considered very toxic to aquatic organisms, resulting in the disruption of the ecological balance (Modi et al. 2010). Also, azo dyes significantly increase the chemical and biological oxygen demand in water bodies, which also affects aquatic life indirectly (Sun et al. 2009).

Some dyes are reported to cause irritation allergy, dermatitis, eye irritation and respiratory tract problems in human beings (Keharia and Madamwar 2003; Kousha et al. 2012). Induction of bladder cancer in humans, splenic sarcomas, hepatocarcinomas and nuclear anomalies in some experimental animals were commonly observed (Rafii et al. 1997; Puvaneswari et al. 2006). Intestinal cancer in 1970's was of common occurrence in highly industrialized locations and linked to use of azo dyes (Wolff and Oehme 1974; Chung et al. 1978). Furthermore, the mutagenic activity of disperse azo dyes was also observed in *Salmonella* species (Ferraz et al. 2010). These studies suggest that the azo compounds in the water and the soil environment can affect the activity and composition of the microbial communities which are sensitive to these toxins. Similarly, Chen (2006) found that aromatic amines, which are common metabolic products of reductive cleavage of azo dyes, were found highly toxic to bacterium *Pseudomonas luteola*.

Recent studies have indicated that azo dyes also cause phytotoxicity. Ayed et al. (2011) reported a reduction of 55 and 30 % in the germination of *Triticum aestivum* and *Sorghum bicolor* respectively with a Methyl Red azo dye. However, degradation products of the dye were found to be non-toxic to the germination of the both plant species. On the contrary, Chaube et al. (2010) observed that even the degradation products of Direct Violet 51 and Tartrazine were toxic and a significant decrease in the germination and growth of *Triticum aestivum* and *Phaseolus mungo* was observed. Similarly, the application of dye-contaminated water inhibited the growth of peas and wheat plants in laboratory conditions, although the same water, after biological treatment, improved plant growth (Khalid et al. 2013; Saba et al. 2013).

3 Biodegradation of Azo Dyes by Actinobacteria

Actinomycetes were used mainly for the production of bioactive compounds, such as antibiotics and neglected earlier in relation to their biodegradation capacities. Now-a-days, they are becoming potential degraders of organic compounds, including azo dyes (Table 2). These bacteria have gained attention all over the world because of their versatile nature and enzyme system which is required for the

Table 2 Biodegradation of synthetic dyes by Actinobacteria via oxidation reduction processes

Actinomycetes sp.	Dyes	Conditions	Comments	References
Streptomyces krainskii SUK-5	Reactive blue-59	Shaking	Complete (100 %) degradation of the dye was observed within 24 h at pH 8 and temperature 30 °C	Mane et al. (2008)
Streptomyces sp. SS07	Xylidine ponceau-2R, direct black-38, direct brown-1	Reduced	Complete reduction of the dyes was achieved in 24 h at pH 9.2 and 37 °C	Bhaskar et al. (2003)
Streptomyces sp.	Reactive blue 160	Shaking	Actinomycetes had the potential to decolorize more than 98 % of dye in the presence of lactose and urea within 48 h.	Khobragade and Deshmukh (2013)
Streptomyces sp. C1	Indigo carmine diamond black PV	Oxidized	The 83.7 % decolorization was observed at pH of 8.0 and a temperature of 40 °C in 2 h with syringaldehyde as mediator	Lu et al. (2013)
Georgenia sp. CC-NMPT-T3	Reactive orange 16	Static anoxic	Decolorization of the dye was 94.2 % in 8 h at pH 6–8 and temperature 28-45 °C	Sahasrabudhe and Pathade (2013)
Actinomycetes sp. consortium	Reactive yellow	Static	The 90 % of dye was removed in 1 h	Bagewadi et al. (2011)
Streptomyces globosus	Acid fast red	Static/ shaking	Acid fast red was decolorized up to 82 % under static condition while 70 % dye removal was observed under shaking conditions.	El-Sersy et al. (2011)
Streptomyces psammoticus	Acid orange, methyl orange, and bismarck brown	Oxidized	The selected dyes were decolorized at the rates of 86, 71 and 75 %, respectively in the presence of 1-hydroxybenzotriazole as mediator.	Niladevi et al. (2008)
Streptomyces coelicolor	Acid blue, direct sky blue 6b, reactive black 5	Shaking	Decolorization was 94 % for acid blue 74, 91 % for direct sky blue 6b and 65 % for reactive black 5 in the presence of acetosyringone in 10 min.	Dube et al. (2008b)
Streptomyces cyaneus CECT 3335	Methyl orange and orange II,	Oxidized	Color removal was 90 % in the presence of acetosyringone (0.1 mM) redox mediator.	Moya et al. (2010)

degradation of dye chemicals. Usually, the dye degradation occurs by the activity of lignin peroxidase, laccase, NADH-DCIP reductase or azoreductase enzymes (Sahasrabudhe and Pathade 2013).

Actinomycetes show a variable potential to degrade various synthetic dyes. Chengalroyen (2011) examined the decolorization behaviour of *Streptomyces* species against two structurally different dyes (Congo red and Orange II). *Streptomyces* species were able to decolorize structurally complex dye Congo red with two azo bonds and poly aromatic and sulfonated groups, while structurally simpler dye Orange II (with a single azo bond and sulfonated group) was not decomposed. Biodegradation of triphenyl methane dyes by two actinomycetes, such as *Nocardia corallina* and *Nocardia globerula*, was reported by Yatome et al. (1991).The Crystal Violet dye was completely decolorized within 24 h. *Nocardia coralline* was also able to decolorize four triphenyl methane dyes, such as Methyl Violet, Ethyl Violet, Basic Fuchsin and Victoria Blue, but maximum decolorization was observed in Crystal Violet dye. They suggest that the decolorization activity of actinomycetes is intracellular, as there was no activity in the culture filtrate. Similarly, the decolorization activity was not observed in washed cells of *N. corallina*, when the cells were incubated in a buffer, but the activity was recovered when the cells were incubated in LB medium. Bhaskara et al. (2003) reported that extracellular fluid protein (ECFP) of *Streptomyces* species could be used for the reduction of azo dyes soluble in water.

Sahasrabudhe and Pathade (2013), in a study, indicated that *Georgenia* sp. CC-NMPT-T3 could degrade the individual as well as mixture of five different dyes under static anoxic conditions (Sahasrabudhe and Pathade 2013). This suggests its potential to be used as inoculum in the bioreactor for the treatment of textile wastewater containing a variety of synthetic dyes. Under shaking condition, *Streptomyces krainskii* strain SUK -5 completely degraded textile dye Reactive blue-59 within 24 h (Mane et al. 2008). During the process of degradation of Reactive blue-59, the involvement of lignin peroxidase, and NADH-DCIP reductase and MR reductase enzymes was confirmed.

Consortium of different actinomycetes can also be used for the degradation of azo dyes. Bagewadi et al. (2011) developed a consortium having five actinomycetes strains. About 97.44 % of degradation of reactive yellow dye (5 mg 100 ml^{-1}) was observed by this consortium in 15 days. They concluded that degradation of the dye depends on the concentration of dye, as well as on the growth of actinomycetes. El-Sersy et al. (2011) studied the potential of five actinomycetes (*Streptomyces globosus, Streptomyces alanosinicus, Streptomyces ruber, Streptomyces gancidicus,* and *Nocardiopsis aegyptia*) for dye decolorization. *Streptomyces globosus* had the maximum potential for the degradation of acid fast red dye under static (81.6 %) and shaking (70.2 %) conditions. The authors suggested biosorption as dominant mechanism for removal of dye from the solution. A 1.14-fold more biosorption was observed with an increase in the size of the inoculum and a decrease in the concentration of starch. Studies conducted in 1990's, also revealed that the *Streptomyces* species can be used for the degradation of azo dyes (Pasti et al. 1991; Paszczynski et al. 1991; Burke and Crawford 1998).

4 Enzymes Involved in the Biodegradation of Azo Dyes by Actinobacteria

Possible mechanisms of microbial removal of azo dyes include biosorption, bioaccumulation, reduction and oxidation. However, bioremediation by biosorption and bioaccumulation are slow and dyes are often not completely mineralized (Gadd 2009; Wang and Chen 2009). In this scenario, role of actinomycetes in the degradation of azo dyes or toxic by-products through oxidation-reduction processes is very important.

4.1 Reductive Degradation of Azo Dyes

Azo dyes are generally decomposed by microorganisms in a two-step process (Dawkar et al. 2010; Khalid et al. 2010; Liu et al. 2011). In the first step, microbes breakdown the azo bond (−N=N) of azo dyes with the help of azoreductase enzyme in low oxygen condition. This process is also called decolorization, resulting in the formation of colorless aromatic amines, which are mineralized in the oxidation process. So far, a little is known about the azo dyes degradation pathways of actinomycetes. Azoreductase enzyme has been reported in a few species of actinomycetes. Usually, the azoreductase enzyme is not easily identified in actinomycetes, due to its low concentration. However, the surfactant can be used to improve the production of the enzyme in the microorganisms (Reese and Maguire 1969). For this purpose, Chengalroyen (2011) used two surfactants, Tween-80 and Tween-20, to improve the production of azoreductase in *Streptomyces*. A closer monitoring of different enzymes in the same host showed that Tween-80 might result in the selective inhibition or enhancement of certain enzymes, suggesting that a complex interaction is involved. The identification of decolorizing strains in the presence of Tween-20 suggests the release of superior stabilized-protein into culture media (Chou et al. 2005). Chengalroyen (2011) also identified azoreductase in *Streptomyces coelicolor* by genome sequencing. This study provides an evidence that the azoreductase is also widespread in Actinobacteria.

4.2 Oxidative Degradation of Azo Dyes

In the case of actinomycetes, oxidation process is the most dominant mechanism for the degradation of azo dyes (Lu et al. 2013; Priyaragini et al. 2013). Usually, peroxidase or lignin-degrading enzymes are involved in the degradation process; either a single enzyme is being involved in the process or a group of enzymes act synergistically. Dye degrading peroxidases are reported to degrade hydroxyl free anthraquinone dyes (Sugano et al. 2006; Marchis et al. 2011). A combination of

lignin peroxidases and veratryl alcohol was found to enhance the decolorization of azo and anthraquinone dyes (Joshi et al. 2010). The role of lignin-degrading enzymes in the treatment of contaminated effluent is very critical, as it demands a thorough understanding of lignin degrading organisms and their enzyme systems. The laccases produced by *Streptomyces* were also found to be very effective for the decolorization of textile dyes (Dube et al. 2008a; Molina-Guijarro et al. 2009; Lu et al. 2013). Gottlieb et al. (2003) demonstrated the usefulness of a laccase enzyme produced by *Streptomyces cyaneus* CECT 3335 for the decolorization of azo dyes.

Often laccases are coupled to the reduction of oxygen to water along with oxidation of various substrates (Kurniawati and Nicell 2007, 2009; Morozova et al. 2007).The role of redox mediators is very important in a system for oxidative degradation, since it mediates the movement of electrons in the system and increases the degradation rate indirectly (Gonzalez-Gutierrez and Escamilla-Silva 2009). Lu et al. (2013) demonstrated the degradation of indigo carmine and diamond black PV with syringaldehyde as mediator. In this study, eight strains with laccase activity were isolated from composting samples in different phases, among which a novel strain was identified as *Streptomyces* sp. C1. The purified laccase-like multi copper oxidases (LMCO) of strain C1 showed a single protein band on SDS-PAGE gel with a molecular mass of about 38 kDa. The novel laccase showed alkaline resistance and moderate thermostability. The enzyme activity was activated by some metal ions, such as Cu^{2+}, Co^{2+} and Fe^{3+}at the concentration of 1 mM, while it was strongly inhibited in the presence of Hg^{2+}.

Some studies showed that two-domain small laccase isolated from *Streptomyces* species could tolerate high temperature and alkaline conditions (Steger et al. 2007; Dube et al. 2008a; Molina-Guijarro et al. 2009) and also caused degradation of pigment and phenolic compounds along with antibiotic formation (Nakamura et al. 2003; Molina-Guijarro et al. 2009). The ability of a laccase (EC 1.10.3.2), produced by *Streptomyces cyaneus* CECT 3335, to decolorize and detoxify azo dyes was also assessed by Moya et al. (2010). Acetosyringone was used as the redox mediator in this study. A significant decrease in the toxicity of the dyes, New Coccine and Chromotrope 2R, was observed after the decolorization process. Laccases had a large catalytic versatility due to their low substrate specificity against phenolic compounds and aromatic amines. In the presence of redox mediators, these enzymes can extend their oxidative action even to non-phenolic compounds (Bourbonnais and Paice 1990; Call and Mücke 1997). A large number of phenolic compounds were produced by the selected *Streptomyces* strains, when grown under solid state fermentation conditions on wheat straw (Hernandez-Coronado et al. 1998). These compounds can act as natural mediators. Usually these compounds are produced by microorganisms during their growth or lignin depolymerisation (Camarero et al. 2005), whereas Maneet al. (2008) isolated lignin peroxidase from *Streptomyces krainskii* SUK-5. In the presence of this enzyme, reactive blue-59 dye was completely degraded in less than 24 h. Recently, titanium oxide nano-particles (TiO2 NPs) were synthesized from the marine Actinobacteria (Priyaragini et al. 2013). Actinobacteria crude extract and NP synthesized TiO2 were found very effective for the degradation of azo dyes, such as acid red 79 and acid red 80.

Immobilized nano-particles and bacterial cells have shown promising results in the dye degradation (Lachheb et al. 2002; Daneshvar et al. 2005).

From these studies, it may be concluded that oxidative enzymes, produced by actinomycetes in the presence of some redox mediators, may have a practical application in the degradation of azo dyes.

5 Factor Affecting Biodegradation of Azo Dyes

The effectiveness of any biological treatment depends on the environmental conditions. The thermal stability and functioning of the enzyme in the Actinobacteria strain is very important for practical application. The isolation of *Streptomyces* spp. from unusual environment may produce a variety of bioactive compounds (Chronakova et al. 2010; Gousterova et al. 2014).Therefore, it is urgent to isolate and identify strains that can survive and maintain their activities under varying environmental conditions.

Thermostable laccases have been isolated from various strains of actinomycetes (Endo et al. 2003; Suzuki et al. 2003; Lu et al. 2013). Thermostable laccases have been also reported in Actinobacteria, such as *Streptomyces lavendulae* REN-7, *Streptomyces griseus* and *Streptomyces* sp. C1, which retained their original activity even at 70 °C (Endo et al. 2003; Suzuki et al. 2003; Lu et al. 2013). Recently, Sahasrabudhe and Pathade (2013) reported degradation of reactive orange dye using *Georgenia* sp. CC-NMPT-T3 at 28–45 °C. The actinobacteria could decolorize 94.2 % reactive orange dye (50 mg l^{-1}) within 8 h. In general, most laccases have an optimum enzymatic reaction at an acidic pH and the optimum temperature about 30 °C (Baldrian 2006). Lu et al. (2013) reported optimum activity of laccase enzyme isolated from *Streptomyces* sp. C1 at pH 8.0. Moreover, this enzyme was capable of retaining 70 and 50 % of its initial activity after 3 h incubation at pH 11.0 and 12.0, respectively. This demonstrated that the enzyme showed a moderate resistance to alkaline conditions. Mane et al. (2008) reported pH 8.0 as the best suitable condition for *Streptomyces krainskii* strain SUK-5 for optimal decolorization of reactive blue-59 dye. Similarly, *Georgenia* sp. CC-NMPT-T3 was able to degrade the azo dye reactive orange at pH 6–8 in static anoxic conditions (Sahasrabudhe and Pathade 2013).

Previously, azo reductase activities have been reported in static conditions in several species of Actinobacteria, such as *Streptomyces coelicolor, Nocardia corallina* and *Nocardia globerula* (Yatome et al. 1991; Bhaskara et al. 2003; Chou et al. 2005; Chengalroyen 2011). However, agitation (aerobic) is more effective in case of laccase enzyme and lignin-degrading enzymes. Actinobacteria *Georgenia* sp. CC-NMPT-T3, *Streptomyces cyaneus* CECT 3335, *Streptomyces krainskii* SUK-5 and *Streptomyces* sp. C1 have been reported to perform their activities best under aerobic conditions (Mane et al. 2008; Lu et al. 2013; Sahasrabudhe and Pathade 2013).

The presence of metals in the medium may also affect the performance of the microbial enzyme system. For example, the presence of Cu^{2+} in the medium increased the activity of laccase of *Streptomyces* sp. (Lu et al. 2013). Enhanced activity can be due to filling of the type-2 copper binding sites with copper ions (Bao et al. 1993). Similarly, the presence of some metalions Co^{2+}, Ba^{2+}, Mn^{2+} and Fe^{3+} at low concentrations also stimulate the activity of the enzyme from *Streptomyces* species (Nagai et al. 2002; Baldrian and Gabriel 2006). However, high concentration of these ions decreases the performance of the microbial enzyme systems. This decrease can be attributed to interference of these metals with proteins or enzymes, which ultimately form complexes with molecules of protein which may render it inactive or even completely inactivate the enzyme (Mills and Colwell 1977; Jadhav et al. 2012).

Carbon source is required by the microorganism as a source of energy and the electron donor for the removal of color of azo dyes (Moosvi et al. 2007; Perumal et al. 2007; Yemendzhiev et al. 2009). Among the various carbon sources, such as glucose, lactose, maltose, xylose, fructose, galactose, mannitol and sucrose, Khobragade and Deshmukh (2013) found lactose as a carbon source for the strongest decolorization of Reactive Blue 160. They also tested the efficacy of different nitrogen sources such as urea, peptone, yeast extract, ammonium nitrate, ammonium chloride and potassium nitrate, for color decolorization. Among all, urea was found to be the best source of nitrogen for the decolorization of dye. Sahasrabudhe and Pathade (2013) found yeast extract and sucrose as the best carbon sources for *Georgenia* sp. CC-NMPT-T3 to degrade Reactive orange 16 dye. However, contrary to the findings of Khobragade and Deshmukh (2013), they observed a very low degradation of the dye in the medium containing urea as a nitrogen source.

6 Conclusions

Actinobacteria are new options for the treatment of azo dye wastewater for safe disposal into the environment. These bacteria are widely distributed in the ecosystem and can operate in a broad range of environmental conditions. In particular, under extreme environmental conditions, they can have a better survival due to the presence of different adaptation mechanisms. These bacteria typically have polyphosphate reserves, which not only act as reserves of phosphorus, but also space for metal chelation, which can reduce the toxicity of the metal ions present in the industrial wastewater. Presence of superoxide dismutase genes in Actinobacteria may also play an important role in their resistance against environmental stresses. This can result in the production of biosurfactant comprising haemolytic, drop collapsing and activity of lipase production. Biosurfactant increases the surface area of hydrophobic substances, which enhance the bioavailability of these substances and modify the properties of the surface of the bacterial cell. Spore formation is another very important feature in actinomycetes which can be exploited in a stressed environment for its long-term application in the treatment system.

Actinomycetes degrade azo dyes by enzyme involved in reduction and oxidation process. However, further work is still needed for the isolation and selection of strains carrying the effective enzyme systems for bioremediation of wastewater containing dyes. Besides, there is an urgent need to study the source of low carbon like potatoes peel or other organic waste for the production of low-cost actinomycetes biomass. The adaptability and long-term survival of degrading Actinobacteria in the bioreactor system should also be taken into account to develop an effective treatment strategy that can be used across the field. Maintaining high levels of specific microorganisms in the treatment system is a great challenge. Advanced molecular techniques should be applied to monitor the microbial community structure, the persistence of the inoculum added, and their interactions with indigenous populations.

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