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Autochthonous Bacterial Community in Remediation of Azo Dyes: A Review

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ABSTRACT: Azo dyes are one of the most widely used chemical class of dyes and colorants of textile industry used to color natural and synthetic fibers. Structurally molecules of azo dyes contain two adjacent nitrogen atoms between carbon atoms. About 60-70% of dyes used in the food and textile industry are of azo dyes as they provide strong and variety of colours. Apart from holding a key position in textile industry, azo dyes create several problems as pollutants to living beings and environment. Their presence in textile effluent is a major problem due to their adverse effects. Several of the degradation products of the azo dyes have been found to be mutagenic and carcinogenic. There are number of proposed remedial measures for the treatment of waste water effluents containing azo dyes generated by various industries. Bioremediation is however, proved to be very effective which includes the use of living organisms, mostly microorganisms and plants, to degrade and reduce or detoxify waste products and pollutants. In addition, bioremediation is also effective in keeping living beings and the environment safe. Use of autochthonous bacterial communities in bioremediation of azo dyes has been initiated recently in which bacteria of indigenous origin are screened and utilized. The present review article summaries about azo dyes, their harmful effects on living beings and environment and bioremediation by autochthonous bacterial communities.

Keywords: Azo dyes; autochthonous bacterial communities; bioremediation; harmful effects and textile effluents.

INTRODUCTION: Dye is a natural or synthetic substance used to impart colour to textiles, paper, leather, and other materials. This process of colouring is known as dyeing in which colour of the material likely to be exposed, is not readily altered by washing, heat, light, or other factors. This process of dyeing was a revolutionary step in textile industries as it helps in achieving color of textile materials such as fibers, yarns and fabrics with desired fastness. These dyes may be natural in origin as derived from plant sources: roots, berries, bark, leaves, wood, fungi and lichens. However, synthetic dyes (man- made) in comparison to natural are of more in use. Textile industries are one of the largest users of different type of dyes. These dyes are classified according to their solubility and chemical properties as:

- **Acid dyes:** These are water-soluble anionic dyes that are applied to fibers such as silk, wool, nylon and modified acrylic fibers using neutral to acid dye baths.
- **Basic dyes:** These are water-soluble cationic dyes that are mainly applied to acrylic fibers, but also find used for wool and silk.
- **Vat dyes:** These dyes are water insoluble and incapable of dyeing fibers directly.
- **Reactive dyes:** These dyes utilize a chromophore attached to a substituent that is capable of directly reacting with the fiber substrate.
- **Disperse dyes:** These dyes were originally developed for the dyeing of cellulose acetate, however, mainly used to dye polyester. These are water-insoluble and can also be used to dye nylon, cellulose triacetate, and acrylic fibers.
- **Sulfur dyes:** These dyes are inexpensive dyes used to colour cotton with dark colors.
- **Azo dyes:** These are one of the most widely used chromophores in dye industries.

Azo dyes are one of the most widely used chemical class of dyes and colorants of textile industry used to color natural and synthetic fibers. However, these dyes are also used in colouring of food, candy, cosmetics and beverages. Azo dyes are the group of synthetic dyes whose molecules contain two adjacent nitrogen atoms between carbon atoms. Apart from the popularity of use in various industries, azo dyes create several problems as pollutants to living beings and environment. Their presence in textile effluent is a major problem due to their adverse effects. The nitro group containing azo dyes has been reported to cause mutations and produce toxic products after breakdown (Chung and Cerniglia, 1992; Rosenkranz and Kolpman, 1990).

As widely used in textile industries, their effluent causes problem of toxicity to water reservoirs with which they finally mixed and ultimately cause health problems among users and consumers depend upon water bodies. There are number of proposed remedial measures to get rid of this problem, however, bioremediation is proved to be very effective which includes the use of living organisms, mostly microorganisms and plants, to degrade and reduce or detoxify waste products and pollutants. In addition, bioremediation is also effective in keeping living beings and the environment safe. The present review article summarizes about azo dyes, their harmful effects on living beings & environment and bioremediation by autochthonous bacterial communities.

Azo Dyes, What? Azo dyes are used in dyeing textile fibres, particularly cotton but also silk, wool, viscose and synthetic fibres. These are organic compounds consists of two nitrogen atoms (-N=N-) linked with each other. The azo function is often bound to an aromatic ring and the dye can then be broken down to an aromatic amine, arylamine (Lacasse and Baumann 2004; Jin *et al.* 2007; Brüschweiler *et al.* 2014; Rawat *et al.* 2016). The easy in use, relatively cheaper and to provide clear, strong colours has been made azo dyes trendy in textile industries. There are approximately 3000 different varieties of azo dyes available in the market. The dyes 'azo' can be: acidic, basic, mordant, reactive, disperse, direct, solvents or food dyes. The manufacture of azo dyes involves transformation of an aromatic amine (also called diazo component) into a diazonium component which in turn reacts with a coupling component (e.g. Phenolor Naphthol) to form the dye (Singh *et al.* 2012).

The aromatic compounds in the azo dyes are the reason, they produce strong and variety of colors. The azo dyes are chiefly of red, brown and yellow in colors. About 60-70% of dyes used in the food and textile industry are of azo dyes (Asad *et al.* 2007; Rawat *et al* 2018).The major characteristic of these dyes is that they can provide almost all colors. These are synthetic colors and most of them contain only one azo group, however, some may contain more than one azo group. These dyes are not directly applied on the fabrics but, they are constructed within the fibers(Rahimi *et al.*, 2016). Here the fibers are soaked in one element of the dye and then another component of the dye is infused in the fiber. Because the dye is inbuilt in the fiber, the color is very fast and hence, this dye is widely used for coloring fabrics in the textile industry

The manufacturing of azo dyes is quite simple, easy and economic. The main reasons behind are: easy availability of materials, easy adjustment of each step used during processing, less energy requirements and formation of chemical structure happens at or below the room temperature. Due to all these reasons azo dyes are cheap, easily produced and widely used. They are steady and firm when tested and compared at the variable conditions and do not fade when exposed to light and oxygen. These are also found to be heat resistant. Moreover, their cleaning and disposing off is also very simple. All these factors have offered it a key position in textile industries.

Although, azo dyes are extensively used as coloring agent in most of the industrial processes, it has become a matter of debate regarding the toxic and carcinogenic components it contains (Sen *et al* 2016). All the chemical processes of azo dye required the use of water. The waste water released as effluent always contains variable amount of it which has posed a challenge for the textile industries for its removal and treatment.

Harmful Effects of Azo Dyes: Azo dyes present in textile effluent are of major concern due to their toxic and mutagenic nature. As most of the azo dyes are water soluble, it is very easy to mix with water released from industries as effluent into water bodies and surrounding environment. These azo dyes are not only be toxic to aquatic organisms and cause long-term adverse effects in the aquatic and terrestrial environment. The byproducts like arylamines emitted from the azo dye can be absorbed by the skin and accumulates in the body and can cause allergy on skin contact, irritating the eyes. Presence of dyes in the textile effluent causes an unpleasant appearance of water bodies and their breakdown products (colorless amines) make them unfit for use (Xu *et al.* 2005).

It has been found that about 80% of dyes used in the dyeing process of textile industries constitutes by azo dyes. Globally 2.8×10^5 tons of textile dyes discharged into water ecosystem every year which is one of the main sources of water pollution problems worldwide. Among textile dyes discharged into water ecosystem every during textile dyeing and finishing processes, azo dyes constitutes the main portion of produced effluents (Jin *et al.*, 2007). This generation of textile effluents containing azo dyes into water bodies and surrounding industrial areas is of major concern as it causes several adverse effects on life including decreased aquatic photosynthesis, ability to exhaust dissolved oxygen and toxic effect on flora, fauna and humans beings. It causes an unpleasant appearance of water bodies by imparting the color and also release of dye containing effluent derived from various industrial practices (Mugdha and Usha, 2012). It has been estimated that about 10-15% of the dyes used in dying process goes unbound with the textile fibers and are discharged into the environment (Chang *et al*. 2004; Xu*et al*. 2005; Asad*et al*. 2007).

Some of the harmful effects of azo dyes are listed below:

- Release of azo dyes from various textile industries into water decreased aquatic photosynthesis, ability to exhaust dissolved oxygen and impose toxic effects on higher organisms in both aquatic and terrestrial systems.
- Azo dyes can cause skin hypersensitivity and allergy. Although, azo dyes do not have any direct effect on the immune system and cause direct allergic reactions, however, may increase allergic reactions towards other substances (Brüschweiler, 2017). Some azo dyes especially tartrazine, may increase allergic reactions towards other substances like drugs. Azo dyes may cause increased allergic symptoms in people with asthma and similar disorders. However, the exact mechanism is still not fully understood.
- The polarity of azo dyes influences the metabolism and consequently the excretion.
- The generations of reactive intermediate like hydroxylamine are known to damage DNA and proteins.

As several of the degradation products of the azo dyes have been found to be mutagenic or carcinogenic and subsequently, some dyes were no longer permitted to use in industrial processes. Ministry of environment and forests (MoEF) has at last banned the use of azo dyes in India from June 23, 1997. The dyes were banned through a gazette notification issued by the Government of India to be applicable throughout the country. About 70 dyes specified in the schedule to the notification will be covered by the ban. List of azo dyes which has been banned since June 1997 is provided here in Table 1.

Table 1: List of Azo Dyes Prohibited from June 1997.

Sr. No.	Colour Index Generic Name	Sr. No.	Colour Index Generic Name	Sr. No.	Colour Index Generic Name	Sr. No.	Colour Index Generic Name
1.	Acid Red 4	19.	Acid Black 131	37	Direct Red 39	55	Direct Blue 151
2.	Acid Red 5	20.	Acid Black 132	38	Direct Red 46	56	Direct Blue 160
3.	Acid Red 24	21.	Acid Black 209	39	Direct Red 62	57	Direct Blue 173
4.	Acid Red 26	22.	Basic Red 111	40	Direct Red 67	58	Direct Blue 192
5.	Acid Red 73	23	Basic Red 42	41	Direct Red 72	59	Direct Blue 201
6.	Acid Red114	24	Basic Brown 4	42	Direct Violet 21	60	Direct Blue 215
7.	Acid Red 115	25	Developer 14 = Oxidation Base 20	43	Direct Blue 1	61	Direct Blue 222
8.	Acid Red 116	26	Direct Yellow 48	44	Direct Blue 3	62	Direct Blue 295
9.	Acid Red 128	27	Direct Orange 6	45	Direct Blue 8	63	Direct Black 91
10.	Acid Red 148	28	Direct Orange 7	46	Direct Blue 9	64	Direct Black 154
11.	Acid Red 150	29	Direct Orange 10	47	Direct Blue 10	65	Direct Green 85
12.	Acid Red 158	30	Direct Orange 108	48	Direct Blue 14	66	Disperse Yellow 7
13.	Acid Red 167	31	Direct Red 2	49	Direct Blue 15	67	Disperse Yellow 23
14.	Acid Red 264	32	Direct Red 7	50	Direct Blue 22	68	Disperse Yellow 56
15.	Acid Red 265	33	Direct Red 21	51	Direct Blue 25	69	Disperse Orange 149
16.	Acid Red 420	34	Direct Red 22	52	Direct Blue 35	70	Disperse Red 151
17.	Acid Violet 12	35	Direct Red 24	53	Direct Blue 53		
18.	Acid Brown 415	36	Direct Red 26	54	Direct Blue 76		

Source: http://textilescommittee.nic.in/faqlab.htm

Management of Azo Dyes: The byproduct of azo dyes produced as effluents with waste water of textile industries causes pollution in environment and pose harmful health impacts on living beings. There are several physical-chemical methods used for the treatment of colored effluents of dyes including azo dyes in wastewater. Removal of synthetic dyes is one of the main challenges before releasing the wastes discharged by textile industries. Because of the stability and solubility of disperse dyes in oxidizing agents, it very difficult to remove by traditional conventional methods .Several research papers have been published on combined, sequential or integrated, anaerobic–aerobic bioreactor treatment of azo dye-containing wastewater. The methods of treatments include adsorption, precipitation, chemical and photo-oxidation (Stolz, 2001; Shah, 2018). However, the applications of these physio-chemical methods for the treatment of waste water have been generally proved expensive and produce large amounts of sludge. More often these conventional modes of treatment lead to the formation of some harmful side products (Jadhav *et al.* 2016). Biological treatment either by bacteria, fungi or consortia of both, yeast, algae, plants and their enzymes, on the other hand received increasing interest due to their cost effective and eco-friendly nature. It has been reported that aromatic amines released during biological treatment of waste water aerobically does not removed ompletely and limited amount of toxicity remains available in treated effluent (Van der Zee and Villaverde, 2005; Shah, 2018). Similarly, Türgay *et al*. (2011) carried out the treatment of wastewater containing azo dyes by anaerobic biological method and chemical oxidation and suggested biological methods are safest in comparison to chemical ones. Biodegradation of azo dyes by alkaliphilic bacterial consortium has been studied by Lalnunhlimi and Krishnaswamy (2016). They found it as one of the environmental-friendly methods used for the removal of dyes from textile effluents. Likewise, Jadhav *et al*. (2016) also advocated micro-organism-based treatment of azo dyes and stated that biological treatment either by bacteria, fungi or consortia of both have been reported to reduce the toxicity of the dye to the permissible limit of discharge to the environment.

Bioremediation of Azo Dyes: Bioremediation is a process that uses living organisms, mostly microorganisms and plants, to degrade and reduce or detoxify waste products and pollutants to a safer limit as established by regulatory authorities (Mueller et al. 1996).This method is currently considered as cheapest and the least harmful method ofremoving azo dyes from wastewater released from textile industries. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The bacteria and fungi are quite popular in bioremediation processes. These microorganisms are only capable of degrading industrial effluents contain azo dyes, they significantly promotes simplification of bioremediation processes and its effectiveness and reduces the costs (Vitorand Corso 2008; Pajot *et al.* 2011). The treatment methods used by microorganisms in removal of colour of wide range of azo dyes include anaerobic, aerobic and sequential anaerobic-aerobic treatment. Biosorption, enzymatic decolorization and degradation are some major mechanisms adapted in bioremediation by microorganisms (Golab *et al.* 2005; Erden *et al.* 2011; Ambrosio *et al*. 2012; Singh *et al.* 2015, Pandya *et al.* 2018). Enzymes that mediate azo dye decolorization are grouped into two broad classes i.e. reductive and oxidative.

The advantages of use of microbes in bioremediation of dyes released with effluents of textile industries are that this process is useful for the complete destruction of a wide variety of dyes by transforming them in to harmless products. This process can be carried out on site without any requirement to transport quantities of waste off site. This reduces the potential threats to human health and the environment that can arise during transportation. Moreover, it can prove less expensive than other technologies that are used for clean-up of textile effluents.

Among microbial remediation of azo dyes, bacteria gained a prominent position to treat wide range of dyes efficiently, effectively, economically and eco-friendly (Verma and Madamwar, 2003; Khehra *et al.* 2006). Generally decolorization of azo dyes by bacteria occurred under conventional anaerobic, facultative anaerobic and aerobic conditions. In comparison to other microorganisms, focus on bacterial treatment of azo dyes in industries has been increased considerably since it can achieve a higher degree of biodegradation and mineralization. Bacterial communities have the ability to treat variety of azo dyes, are inexpensive and environment-friendly, and produce less sludge (Khehra *et al.* 2006; Rai *et al.* 2005; Saratale *et al.* 2009; Verma and Madamwar 2003). In addition, bacteria have many other advantages such as a fast growth rate and high hydraulic retention time, and thus they could be efficient in treating high-strength organic wastewaters.

Autochthonous bacterial community: Role in bioremediation of Azo dyes: Autochthonous bacteria are native species of that particular indigenous region. There are autochthonous organisms in all parts of our earth. Right from our mucous associated bacteria in colon to soil, they are the native bacterial species which exist in respective locations and have adapted to live there peacefully and are not tempted by fast-nutrient diets. A new research about use of autochthonous bacterial communities in bioremediation of azo dyes has been initiated recently in which indigenous microorganisms are used. These microbes have the potential to degrade azo dyes both aerobically and anaerobically (Knapp and Newby 1995). The use of microbes in bioremediation of dyes is known as "bio bleaching" which appears to be the only eco-friendly and cost effective method to degrade dyes and reduce BOD and COD (Beydilli *et al.* 2000). Numbers of bacterial strains has been evaluated for their potential for bio bleaching of textile dyes. Olaganathan and Patterson (2009) has evaluated uncontaminated soil, Vat Blue 4 contaminated soil and Vat Blue 4 effluent for heterotrophic bacterial population and the bacterial density. Different bacteria like *Pseudomonas* spp., *Bacillus* spp., *Aeromonas* spp., *Achromobacter* spp. were isolated. All bacterial strains were screened for discoloration of textile dyes. Among all, free cells of *B. subtilis* decolorized Vat Blue 4 up to 92.30% after 24 hours of treatment. Total Dissolved Solids (TDS), Biological Oxygen Demand (BOD5) and Chemical Oxygen Demand (COD) were reduced upto 50.00, 79.60 and 75.40% respectively. The decolorization potential of two bacterial consortia developed from a textile wastewater treatment plant was evaluated by Tony *et al*. (2009). They found that consortium consisted of five different bacterial types as *Bacillus vallismortis, Bacillus pumilus, Bacillus cereus, Bacillus subtilis* and *Bacillus megaterium* were efficient to decolorize individual dyes and textile effluent using packed bed reactors. In a similar study carried out by Rajee and Patterson (2011), heterotrophic bacterial population isolated from soil and sediment samples obtained from Orange MR dye contaminated habitat screened for their potential rate and percentage of decolorization of textile dyes. The different bacterial types like *Poteus* sp., *Aeromonas* sp., *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. were able to utilize the dye as both nitrogen and carbon source. The indigenous bacteria isolated from textile dye effluent were evaluated for their ability to decolourize dyes by Hassan *et al.* (2013). Three bacterial isolates namely, *Micrococcus luteus, Listeria denitrificans and Nocardia atlantica* exhibited strong (up to 80%) decolourizing activity against Novacron dye, viz orange W3R, red FNR, yellow FN2R, blue FNR or navy WB. Recently, Srinivasan and Sadasivam (2018) studied docking and aerobic-microaerophilic biodegradation of textile azo dye by bacterial systems. Two non-adapted bacteria namely, *Aeromonas hydrophila* and *Lysinibacillus sphaericus* were analyzed for decolorization of 100 mg L^{-1} of a textile azo dye Drimaren Red CL-5B. It was suggested that *A. hydrophila* and *L. sphaericus* can be used for efficient decolorization and biodegradation of azo dye containing textile wastewater. A detailed list of autochthonous bacterial communities screened for treatment of textile dyes including azo dyes is listed in Table 2.

Table 2: List of bacterial communities or bacterial consortium used in bioremediation of azo dyes.

Sr. No.	Bacteria	Dye	References
1.	*Achromobacterspp.*	Azo dye	Olaganathan and Patterson 2009
2.	*Acinetobacter baumannii*	Congo Red	Ning *et al.* 2014
3.	*Aeromonas hydrophila, Aeromonas* spp.	Drimaren Red CL-5B	Olaganathan and Patterson 2009; Hassan *et al.* 2013; Srinivasan and Sadasivam 2018
4.	*Bacillus cereus, Bacillus* spp.,	Congo Red	Olaganathan and Patterson 2009; Sawhney and Kumar, 2011, Madhuri *et al.* 2018
5.	*Geobacillus stearothermophilus*	Orange II	Evangelista-Barreto *et al.*, 2009
6.	*Listeria denitrificans*	Drimaren Red CL-5B	Srinivasan and Sadasivam2018
7.	*Lysinibacillussphaericus*	Drimaren Red CL-5B	Srinivasan and Sadasivam 2018
8.	*Sphingomonas paucimobilis*	Methyl Red	Ayed *et al.* 2011
9.	*Bacillus subtilis*	Methyl Orange	Meenatchi *et al.*2018
10.	*Staphylococcus aureus*	Orange II, Sudan III	Pan *et al.* 2011
11.	*Staphylococcus hominis*	Acid Orange	Singh *et al.* 2014
12.	*Micrococcus luteus*	Congo Red	Srinivasan and Sadasivam 2018
13.	*Nocardiaatlantica*	Congo Red	Srinivasan and Sadasivam 2018
14.	*Iodidimonas spp*	Brilliant blue, amido black, indigo carmine	Taguchi *et al* 2018

15.	*Pseudomonas* spp.	Remazol Red, Reactive Black 5	Olaganathan and Patterson 2009; Jadhav *et al.*2011; Khan and Malik, 2016
16.	*Klebsiella* sp.	Acid Blue 25	Aruna *et al.*2015
17.	*Methylobacterium populi* VP2	Organic pollutants in aqueous media	Sannino *et al.*2016
18.	*Bacillus pumilus, Zobellella taiwanensis, Enterococcus durans*	Azo dyes	Das, 2016
19.	*Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa*	Removal of soluble chemical oxygen demand	Ardeshir *et al.*2017
20.	*Nostoc carneum*	Methyl Orange	Hussein *et al.*2018
21.	*Bacillus brevis, seudomonas aeruginosa*	Industrial Oil-Polluted Wastewater	El-Borai *et al.*2016
22.	*Bacillus licheniformis* U1	Disperse blue DBR	Sunil *et al* 2018
23.	*Aeromonas hydrophila, Lysinibacillus sphaericus*	Reactive Yellow F3R, Joyfix Yellow 53R, Remazol Red RR, Drimaren Black CL-S and Disperse Red F3BS	Srinivasan *et al.*2017
24.	Alpha, beta and gamma *Proteobacteria*	Reactive Blue 59	Kolekar *et al.*, 2012
25.	*Galactomycesgeotrichum, Brevibacillus laterosporus*	Golden Yellow HER	Waghmode*et al.*2011
26.	*Pseudomonas, Arthrobacter, Rhizobium*	Acid Orange 7	Ruiz-Arias *et al.*2010
27.	*Providencia*sp., *Pseudomonas Aeuroginosa*	Red HE3B, Remazol Black 5B, Red HE7B	Phugare*et al.*2011
28.	*Enterococcus casseliflavus, Enterobacter cloacae*	Orange II	Chan *et al.*2011

Current status and future perspective: The present review clearly revealed that textile industries are largest producer of dye containing effluent into the environment, which is of great concern due to their toxicity, mutagenicity and carcinogenicity. The removal of dyes from effluents prior to their disposal is only measure to get rid form this problem. Presently numbers of physical and chemical methods are in use to manage and treat the industry based effluents containing dyes. However, bioremediation based on natural attenuation, the public considers it more acceptable than other technologies. Bioremediation generally include the use of microbes for treatment of wastewater effluents which makes them inexpensive and environment friendly. Among various microbes, bacteria are the most frequently applied microorganisms for the removal of dyes from textile effluents. The reasons behind their popularity are being applicable to different structural varieties of dyes, easy to cultivate, adapted to survive in extreme environmental conditions. The bacteria have been reported to have faster rate of decolonization the azo dyes as compare to other microbes. The roe of enzymes microbial enzymes is now clearly understood in treatment of azo dyes and textile effluents. The adaptability and the activity of microorganisms are now well recognized as they play important role in effective bioremoval of azo dyes.

Bioremediation, like other technologies has its limitations. There are certain contaminants released along with textile effluents found resistant to microbial attack. To find out new microbes as well new advanced technologies to achieve complete degradation by bioremediation is still a matter research. Some azo dyes are either resistant to microbial degradation or degraded slowly. In such cases the exact rate of degradation is very tedious to predict. If it happens with slow rate, this process became a long, time consuming and expensive. Azodyes used as colouring agents in the textile industry, especially in developing countries, have banned due to their harmful effects. Since the 1990s, when legislation was introduced restricting certain azo dyes, there has been much confusion and misunderstandings concerning azo dyes. There are no set rules or criteria for selection of microbes to be used for bioremediation.

Nanotechnology, a science based on the use of nanoparticles, can be future solution to solve the problem of azo dyes and wastewater generated by textile dyeing industries. The combination of nanotechnology with conventional biological processes will hopefully prove as more efficient method of bioremediation. Although, research is continuously going on find out more new microbes having the better potential of bioremediation and to understand their possible mechanisms for decolonization and degradation of dyes. However, lot of research is still needed to explore more efficient technologies which will play a critical role in increasing environmental protection.

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