Toxicological Impact of Azo Dyes and Their Microbial Degraded Byproducts on Flora and Fauna

14

Ambika Saxena and Sarika Gupta

Abstract

Azo dyes are the most versatile and immensely used class of dyes. They play an extensive role in industries such as leather, food, paper printing, pharmaceutical, textile dyeing and printing, plastics, cosmetics, etc. The estimated production of azo dyes is about 0.7 million tons annually. They can pass through municipal wastewater plants nearly unchanged due to their resistance to aerobic treatment, which potentially exposes humans and local biota to adverse effects. Approximately, 10–15% of synthetic dyes are dissipated throughout various processes in the textile dyeing and printing industry. Azo dyes cause adverse environmental impacts because of their color, bio-recalcitrance, and potential toxicity to the flora and fauna. The contaminated water takes the solvate (contaminants) to the fields in the vicinity and its consumers, thereby adversely affecting the quality of the agricultural produce, animal and human health causing chemosis, contact dermatitis, exophthalmos, lacrimation, permanent blindness, skin irritation, etc. These azo dyes are also the ones raising the biggest concern due to their mutagenic, genotoxic, and carcinogenic nature. These azo dyes have a distinctive one or more azo bonds (-N=N-) that link different aromatic structures, and the cleavage of this bond biologically or chemically often releases more mutagenic and toxic end products. Under anaerobic conditions, some azo dyes are cleaved by microorganisms forming potentially carcinogenic aromatic amines. The toxicity of an azo dye may be due to the dye itself or aryl amine derivatives generated during the reductive biotransformation of an azo linkage. Aerobic microorganism-based bioremediation is gaining importance as it is proved to have high efficiency in mitigating, detoxifying, and degrading these

A. Saxena · S. Gupta (✉)
Department of Bioscience and Biotechnology, Banasthali Vidyapith, Banasthali, Tonk, Rajasthan, India

S. Arora et al. (eds.), *Innovations in Environmental Biotechnology*,
https://doi.org/10.1007/978-981-16-4445-0_14

contaminants. It is thus important to explore the possibilities of isolating efficient microbial aerobic degraders for use in the bioremediation of textile effluents.

Keywords

Animal impact · Azo dyes · Bioremediation · Plant impact · Toxicity

14.1 Introduction

A tremendous decrease in the quality of air, soil, and water has been observed due to the industrial revolution that incurred as a result of increasing demands of the population (Sudha et al. 2014). Improving the quality of water and thereby the soil has been a major thrust for scientific research. The effluent/pollutant discharge from various industries poses a severe threat to the ecosystem (Bhainsa and D'Souza 2006). In India alone, 70% of the natural water sources like lakes, rivers, and streams are polluted due to the discharge of industrial effluents into them (Sriram and Reetha 2015). Textile dyeing and printing industries are one of the major sectors that release industrial effluents containing a wide variety of toxicants, including biodegradable organic matter, suspended solids, toxic organic compounds (phenol), synthetic dyes (such as azo, anthraquinone, phthalocyanine, and triarylmethane), heavy metal, and their conjugates that may cause alteration of the physical, chemical, and biological properties of water bodies (Satija and Bhatnagar 2017; Sharma et al. 2007). Azo dyes (acid dyes, basic dyes, direct dyes, disperse dyes, mordant dyes, reactive dyes, and solvent dyes) constitute a major portion of the dyes used in textile industries (Tang et al. 2019; Adebajo et al. 2017; Desai 2017; Yang et al. 2016; Lade et al. 2015; Karunya et al. 2014; Rani et al. 2014; Sudha et al. 2014). Over 1 lakh dyes have been generated worldwide with an annual production of over 7×10^5 metric tons, and approximately 28,000 tons of dyes are being discharged into the public drains without proper treatment that eventually go into the river (Sarker et al. 2019; Singh et al. 2015). Azo dyes are characterized by the existence of nitrogen-nitrogen (-N=N-) double bond, high solubility, and their vibrant color (Santos-Pereira et al. 2019). These may amass to lethal levels causing a vast variety of ecological damage under various environmental conditions.

Various methods are used for the decolorization and treatment of textile wastewater. Physiochemical methods (specific coagulation, filtration, chemical flocculation, use of activated carbon) and chemical methods (ozonation, irradiation oxidation, electrochemical oxidation, Fenton oxidation and ultrasonic chemical oxidation) are used for the treatment (Sarker et al. 2019; Singh et al. 2015; Esteves and Silva 2004; Pearce et al. 2003). These methods have limited usability due to excessive use of chemicals, large amount of sludge generation, production of secondary pollutants, low efficacies, high operational cost, high cost of the electricity, ozone, and radiation (Lade et al. 2015; Leelakriangsak 2013). Biotreatment offers a cost-effective and environmental friendlier alternative for decolorization of textile effluent. In biological treatment processes, various parameters, namely pH,

temperature, the degree of agitation, dye concentration and structure, oxygen, and supplementation of different carbon and nitrogen sources have a direct impact on the decolorization of the effluents (Modi et al. 2015). Thus, prior determination of these factors helps make the process more efficient, faster, and practically applicable.

Alternatively, bioremediation technology that employs the ability of microbes like bacteria, fungi, and/or their combination has emerged as an effective method for the treatment of textile dye effluent (Islam et al. 2017; Lade et al. 2015). Bacterial decolonization of azo dyes is found to be used under both aerobic and anaerobic conditions. However, the anaerobic degradation yields certain aromatic amines which are toxic and mutagenic; these byproducts cannot be further broken down under the conditions which generated those (Olukanni et al. 2006). Many bacteria that decolorize azo dye reportedly do not use the dye as the sole carbon source in aerobic conditions (Syed et al. 2009).

The mechanism of fungal decolorization mainly involves two aspects, biodegradation and biosorption. The biodegradation capability of fungi is due to their extracellular, nonspecific, and nonselective enzyme system (Yang et al. 2016). Fungal enzyme production depends on nutrient limitations, and their subsequent dye decolorization ability is achieved depending on the growth conditions.

In particular, the discharge of dye-containing effluents into the water environment is undesirable, not only because of their color, but also because many of the dyes released and their breakdown products are toxic, carcinogenic, or mutagenic to life forms mainly because of carcinogens, such as benzidine, naphthalene, and other aromatic compounds. The dye effluent may contain toxic substances that could be mutagenic, teratogenic, and carcinogenic to aquatic organisms and fish species. These are generally undesirable and toxic for animal and human at extreme minute quantities. The chemicals present in textile wastewater evaporate into the air, which cause adverse effect if inhaled or are absorbed through our skin may cause allergic reaction (Yadav et al. 2014; Kant 2012).

The color causes hindrance in light penetration, which subsequently inhibits the process of photosynthesis (Singh et al. 2015). Moreover, various research studies reveal that the toxic effects of dyes have a major influence over the germination rates and biomass of several plant species (Ghodake et al. 2009).

14.2 Impact of Azo Dye on Flora and Fauna

Azo dyes, except for their deteriorating effect on esthetic beauty and adverse impact in terms of chemical oxygen demand (COD) and biological oxygen demand (BOD), are also reported to adversely affect water resources, soil fertility, aquatic organisms, and ecosystem integrity. They pose lethal effect, mutagenicity, genotoxicity, and carcinogenicity to plants as well as animals.

14.2.1 Azo Dye Synthesis and Types

Azo dyes are one of the most important class of dyes and dominate 70% of the dye market. The elementary structure of azo dyes contains double-bonded nitrogen atoms (-N=N-), combining two identical and/or asymmetrical alkyl or aromatic radical. Mostly aromatic azo compounds are used as commercial colorants in textile industries. These dyes come in a wide range of colors, have better fastening capacity, cost effective in terms of their synthesis and availability of their starting materials, and therefore are preferred worldwide.

The most common procedure for azo dye synthesis is by diazotization of an aromatic primary amine, followed by coupling with one or more electron-rich nucleophiles such as amino and hydroxy. When hydrochloric acid is added to sodium nitrite, it produces nitrous acid. Nitrous acid under acidic conditions produces the nitrosonium ion and releases a water molecule (Fig. 14.1—Eq. (1)). The electrophilic nitrosonium ion attacks an aromatic amine to give a nitrosoamine, which undergoes tautomerism. Under acidic conditions, the OH group can be protonated to release water molecule. The diazonium salt is then resonance stabilized (Fig. 14.1—Eq. (2)) (Benkhaya et al. 2020). The final step for the synthesis of azo dye requires a diazonium salt and a coupling reagent (Fig. 14.1—Eq. (3)). The general synthesis of azo dyes is as follows (Table 14.1):

Others methods used for the synthesis of azo dye include: reduction of nitroso compounds by lithium aluminum hydride, reduction of nitro aromatic derivatives in alkaline medium, condensation of hydrazines and quinones, oxidation of primary amines by potassium permanganate or lead tetra acetate, condensation of primary amines with nitroso derivatives, etc.

Azo Dye Intake into the Food Chain Textile industries play a significant role in the economy of a nation; however, it is also one of the most polluting industrial sectors. The textile industry is responsible for a wide-ranging environmental pollution (Muthu 2017). This effluent prevents light from penetrating into the water bodies and also reduces its oxygen level, thereby reducing the rate of aquatic flora and subsequently its fauna (Imran et al. 2015; Hassan and Carr 2018).

A relatively small section of the chemical industry is represented by the dye industries, which globally produces approximately 800,000 tons of dyes in a year. About 10–15% of synthetic dyes are dissipated during various processes in the textile industry. Dyes are usually categorized into acid, basic, direct, disperse, mordant, metal complex, reactive, sulfur, and vat dyes. Out of the total dyes (approximately 10,000 dyes) exploited in the textile manufacturing unit, azo dyes that possess complex structure and are synthetic in origin contributes nearly 70% to overall produce. Primarily the main cause for the dye release in the environment is the fraction of residual dye in the textile effluent; this is one of the biggest concerns over the last few decades due to their esthetic damage and non-biodegradability (Hassaan and Nemr 2017; Hassan and Carr 2018).

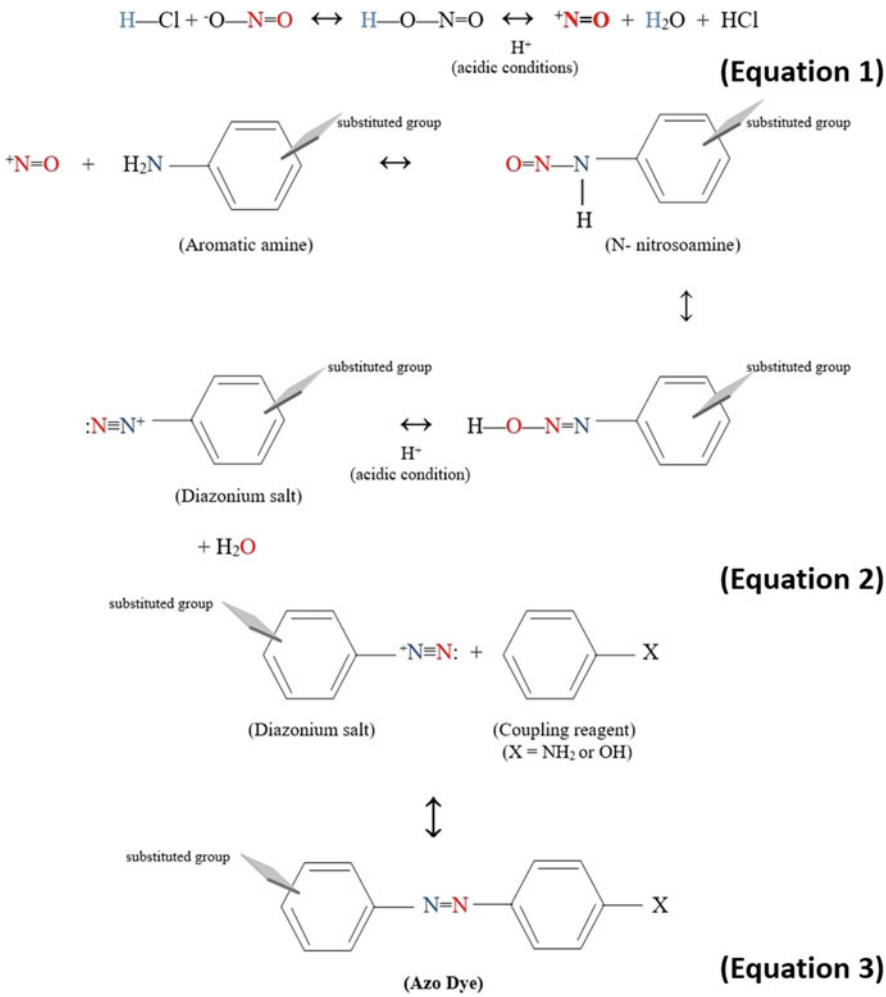

Fig. 14.1 Reactions involved in the synthesis of azo dyes

The solid waste that results from the fabric and yarn scrapings and packaging waste constitute textile sludge. Air pollution is caused by particulate matter, volatile organic compounds, oxides of sulfur and nitrogen. However, water pollution constitutes 80% of the total pollution caused by this sector that results due to discharge of untreated textile effluent into the nearby water bodies is the most harmful. Textile industries utilize large amount of dyes and chemicals. These dyes and chemicals are dissolved in water which is extensively used in various processes like dyeing, printing, bleaching, washing, etc. (Wang 2016). This untreated textile effluent contains dyes, heavy metals, other organic and inorganic dissolved solids,

Table 14.1 Types of azo dyes on the basis of color index

Type	Color index	Chemical formula	Example	Reference
Monoazo	11000–19999	Single (-N=N-) bond	Remazol Golden Yellow	Nigam et al. (1996)
Diazo	20000–29999	Double (-N=N-) bond	Reactive Black 5	Libra et al. (2004)
Triazo	30000–34999	Triple (-N=N-) bond	Acid Black 210	Agarwal et al. (2014)
Polyazo	35000–36999	Three or more (-N=N-) bond	Direct Red 80	Ogugbue et al. (2012)
Azoic	37000–39999	Insoluble ingrain dye, produced during dye process inside the fabric	–	Benkhaya et al. (2020)

which elevates the biological oxygen demand (BOD) and chemical oxygen demand (COD) of the adjoining river/surface water bodies.

These azo dyes are mutagenic and carcinogenic in nature. Since these dyes do not degrade easily, they enter the food chain and show biomagnification as organisms at higher trophic levels show higher levels of contamination compared to that on the initial levels (Newman 2015). When this effluent is used for irrigation purposes, these azo dyes are taken in by the plants where they affect its germination rate and plant growth. The use of these azo compounds also destroys the soil microbial biota (Imran et al. 2015).

14.2.2 Impact of Azo Dye on Flora

The most common methods employed to study phytotoxicity are monitoring of seed germination and plant growth. The study reported the toxicity of Procion Red MX-5B on the root growth in the seeds of *Lactuca sativa*. During the experiment, filter papers with 3 mL dye solution and 20 seeds were lined on petri plates at 21 ± 1 °C for 72 h in dark conditions with 0.05N zinc sulfate solution as positive control and water as negative control. This acute toxicity test showed inhibition of root growth rate in the seeds (Almeida and Corso 2014).

Lobiuc et al. (2018) used Lemna minor to test the toxicity of Congo Red from 5 to 5000 ppm concentration. The paper reported complete inhibition of plant growth, decrease in chlorophyll a content, and increase in carotenoid content at 2500 ppm dye; dye accumulation and necrosis formation. PS II efficiency decreased above 1000 ppm dye while chromosomal aberrations significantly increased at 5 and 1000 ppm dye.

Asses et al. (2018) performed phytotoxicity assays of Congo Red on *Zea mays* and *Solanum lycopersicum* seeds. Three milliliter of dye solution (200 mg/L) each day was used in petri dishes containing ten seeds with distilled water as control for 7 days. Significant reduction in the germination rate, shoot and root length of both

the plants was observed. Methyl Red dye at 750 ppm concentration was reported to exhibit 30% germination inhibition in *Sorghum bicolor*, whereas it was 55% in *Triticum aestivum* (Ayed et al. 2011).

Phytotoxicity of Direct Red 2B was tested on *Phaseolus mungo* (mung) plants, ten seeds were placed on a plastic petri dish and 5 mL of 300 ppm dye solution was daily poured on them. It was reported that germination and length of shoot and root were affected in the presence of pure dye (Desai 2017).

Jadhav et al. (2016) conducted a study using synthetic textile dye Blue GL, Blue 2RNL, Direct Red 5B, Golden Yellow, and Scarlet RR on five plant species, namely *Cicer arietinum*, *Vigna radiata*, *Triticum aestivum*, *Vigna sinensis*, and *Vigna aconitifolia*, concluded that increasing concentration of dyes had toxic effect on the development of shoot and root, and showed inhibition of seed germination and inhibition of α-amylase activity.

Kalyani et al. (2009) reported that the percent germination and *Sorghum vulgare* and *Phaseolus mungo* seeds decreased with Reactive Red 2 treatment. Similar inhibitory effects in length of plumule and radicle of *Sorghum vulgare* and *Phaseolus mungo* by Direct Red 5B dye (Khandare et al. 2013) and by Reactive Blue 172 were reported (Lade et al. 2015).

Seed germination test indicated toxicity of textile effluent and contaminated soil on mung bean by reduction in percent seed germination (86.67% and 95.28%, respectively) and drastic decrease in radicle-plumule growth (Khan and Malik 2017).

Another study showed phytotoxicity of R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL azo dyes using seed germination and plant growth bioassays in seeds *Guizotia abyssinica* Cass. The percent seed germination, shoot and root length as well as the protein and total carbohydrate content drastically reduced in the presence of pure dyes, indicating their toxic nature (Laxmi and Nikam 2015).

14.2.3 Impact of Azo Dye on Fauna

The dyes in the industrial effluent have been reported to cause several physiological and biochemical changes in the aquatic animals. Once they reach humans after entering the food chain, they cause various physiological abnormalities like hypertension, sporadic fever, renal damage, cramps, etc. (Puvaneswari et al. 2006) and were reported to be mutagenic (Umbuzeiro et al. 2005).

Sani et al. (2018) confirmed the toxicity of dyes through test animals, namely brine shrimps, *Artemia salina*. The study suggested that such compounds may pose serious risks to humans, plants, and other organisms (both soil and aquatic) through ecological interaction in the ecosystem. Asses et al. (2018) used *Bacillus cereus*, and *Escherichia coli* strains were used for microtoxicity evaluation of Congo Red. These strains were grown in nutrient broth (NB) with 200 mg/L CR at 30 °C and 37 °C, respectively, and their growth was assessed by OD at 600 nm recorded at 1 h interval

during 8 h, significant decrease in the density was observed in comparison to the control.

Histopathological effects of silk dye waste water in the intestine and stomach of Swiss albino mice showed atrophy of musculature, disintegration of mucosal epithelial cells characterized by cytoplasmic vacuolization, nuclear pycnosis and nuclear fragmentation (Khatun 2017).

Lade et al. (2015) used *Daphnia magna* for acute tests to evaluate lethal toxicity of Reactive Blue 172, which showed 100% mortality indicating the toxic nature of the dye to mammals and humans. The acute toxicity test of Procion Red MX-5B was performed on *Artemia salina* as it acts as a bioindicator of toxicity of industrial effluent since it is sensitive to the salinity and conductivity of textile effluent. Test tubes containing ten larvae in 3 mL of dye solution plus 2 mL artificial sea water and 5 mL artificial sea water as control were used in the experiment and the mortality rate was observed. After 48 h, the experiment concluded that the dye was non-toxic (Almeida and Corso 2014).

This acute toxicity was reported to be a result of dye accumulation up to a critical concentration. Disperse Orange 1 was reported to be genotoxic on the HepG2 cells until it reached the maximum concentration tested where the damage response had saturated despite cell viability being around 90%, which might be due to cytotoxic effects. This cytotoxic effect was confirmed as the dye-induced apoptosis in the cells (Ferraz et al. 2011).

Methyl Red biotoxicity test on *Artemia salina* at 24 h and 25 °C revealed an average lethal concentration (LC50) of 3.5% at 34 g/L (Ayed et al. 2011). Another study performed on *Daphnia magna* with Methyl Red reported water flea to be most sensitive to the dye (EC_{50} 6 ppm). The study also reported the effect of Methyl Red on two freshwater fish, *Poecilia reticulata* and *Gambusia affinis*. The EC_{50} value for erythrocyte counts was reported to be lower than the LC_{50} value for mortality; hence, the dye was concluded to be more toxic on animal physiology than the wholesome effect on the test organism. Since both these fishes belong to the same family *Poecilidae*, species-specific response to the dye was observed as *Poecilia* was found to be more sensitive to dye in comparison to *Gambusia*. This effect to *Daphnia*, *Poecilia*, and *Gambusia* was concluded to be the result of the order of their evolutionary level; hence, the invertebrate is more sensitive than the vertebrate (Sharma et al. 2009).

de Lima et al. (2007) studied mutagenicity of industrial effluent in *Salmonella*/microsome assay which tested positive. Carcinogenicity of industrial effluent was tested for in the aberrant crypt foci medium-term assay in colon of Wistar rats and confirmed as there was an increase in preneoplastic lesions in the colon of rats (Table 14.2).

Sharma et al. (2007) conducted serum biochemical and hematological studies on Swiss albino rats and stated that the values of white blood cells (WBC), red blood cells (RBC), packed cell volume (PCV), hemoglobin (Hb), and mean corpuscular hemoglobin concentration (MCHC) significantly decreased in wastewater exposed animals (12–46%) with respect to control animals (potable water). Further, reduction in RBC size (13–27%) and the shape modification (poikilocytosis) was observed.

Table 14.2 Effect of untreated dyes on plants and animals

Dye	Application	Environmental impact	Hazardous effect	Reference
Procion Red MX-5B	Dyeing cellulose, nylon, silk, and wool	Plant and animals	Inhibition of root growth rate in the seeds	Almeida and Corso (2014)
Congo Red	Dyeing cotton	Plants	Inhibition of plant growth, decrease in chlorophyll a content, increase in carotenoid content, dye accumulation, and necrosis formation. PS II efficiency decreased; chromosomal aberrations increased and reduction in the germination rate	Lobiuc et al. (2018), Asses et al. (2018)
Methyl Red	Textile dyeing	Plants and animals	Inhibition in germination rate	Ayed et al. (2011)
			Lethal to saline water animals	
Direct Red 2B	Dyeing of cotton, rayon, jute, coir, and linen	Plants	Negative impact on germination and length of shoot and root	Desai (2017)
Blue GL, Blue 2RNL, Direct Red 5B, Golden Yellow, and Scarlet RR	Textile dyeing	Plants	Adverse effect on shoot and root length, inhibition of seed germination and α-amylase activity	Jadhav et al. (2016)
Reactive Red 2	Dyeing of cellulosic fibers	Plants	Inhibition in seed germination	Kalyani et al. (2009)
Direct Red 5B	Dyeing of cotton, rayon, and linen	Plants	Reduction in length of plumule and radicle	Khandare et al. (2013)
Reactive Blue 172	Textile dyeing and printing	Plants and animals	Reduction in length of plumule and radicle	Lade et al. (2015)
			Toxic to freshwater animals, mammals and humans	
Textile effluent	–	Plants and animals	Inhibition in germination rate and length of root and shoot	Khan and Malik (2017)

(continued)

Table 14.2 (continued)

Dye	Application	Environmental impact	Hazardous effect	Reference
R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL	Textile dyeing and printing	Plants	Reduction in percent seed germination, shoot and root length, protein and total carbohydrate content	Laxmi and Nikam (2015)
Red, yellow, blue, and orange dye	Dyeing and re-dyeing in textile dyeing and printing industry	Plants and animals	Toxic to humans, plants, and other organisms	Sani et al. (2018)
Silk dye waste water	–	Animals	Atrophy of musculature, disintegration of mucosal epithelial cells, nuclear pycnosis, and nuclear fragmentation in stomach and intestine of Swiss albino mice	Khatun (2017)
Disperse Orange 1	Dyeing of polyester fiber, yarn, nylon, acrylic, and fabric	Animals	Genotoxic and cytotoxic	Ferraz et al. (2011)
Methyl Red	Textile dyeing	Plants and animals	Toxic on animal physiology	Sharma et al. (2009)
Industrial effluent	–	Plants and animals	Mutagenic and carcinogenic	de Lima et al. (2007)
Industrial wastewater	–	Plants and animals	Toxicity of untreated textile dye wastewater to the hemopoietic system of male albino rats	Sharma et al. (2007)

The serum biochemical parameters alanine transaminase (ALT), aspartate amino-transferase (AST), creatinine, urea, and bilirubin significantly increased (5–97%), while cholesterol, glucose, total protein, albumin, and globulin contents decreased (8–53%).

14.3 Pathway of Azo Dye Degradation

14.3.1 Physiochemical Pathway

Azo dyes cause major pollution in the environment; therefore, considerable research has been carried out on the removal of color from textile effluents. Azo dye treatment initially comprises physio-chemical treatment methods including coagulation, adsorption, precipitation, flocculation, oxidation, electrical destruction, ion exchange, ozonation, photocatalysis, etc. (Lade et al. 2015; Singh et al. 2015).

Inorganic coagulants such as polyaluminum chloride (PAC) or polyaluminum sulfate (PAS) have trivalent aluminum ions which react with hydroxyl and other alkaline ionic species. The hydroxides of aluminum are almost insoluble at neutral pH, which precipitate and settle down. In flocculation, coagulants are usually inorganic compounds that produce smaller and lighter flocs and require larger time to settle. It also reflects into the fact that the sludge volume is always greater with inorganic chemicals/coagulants (Ashtekar et al. 2014).

These physiochemical methods have been utilized for removing color especially for wastewaters, containing dissolvable solids. Textile industrial waste water contains large amounts of sludge volume which must be disposed of before further addition of chemicals through sedimentation, precipitation, filtration, etc. However, removal of dye requires high chemical dosage in other processes. In coagulation, trivalent cations have stronger coagulating tendency than divalent cations as cations in coagulants are responsible for neutralization of the negative charge in dye. Certain coagulants mainly used are ferric chloride/sulfate, aluminum sulfate, aluminum chloride, calcium/magnesium oxide, etc. Alum (aluminum sulfate) is one of the most widely used coagulant, and it produces insoluble precipitate of aluminum hydroxide and works better in the narrow pH range close to 5. Ferric compounds such as ferric chloride and sulfate are also some of the popular inorganic coagulants (Merzouk et al. 2011; Aziz et al. 2007).

Each of these methods has their merits and demerits. The chemicals used in these processes include alum, ferric or ferrous sulfate, lime and polyaluminum chloride (PAC), etc. (Saxena and Gupta 2020; Gogate and Pandit 2004).

Ozonation includes ozone bubbling through the effluent which degrades azo dyes. The degradation rate of azo dyes is first order with respect to both azo dye and ozone concentrations (Shu and Huang 1995). In photocatalysis, TiO_2 and ZnO semiconductors are majorly employed photocatalysts for the degradation of several environmental contaminants because of their stability, high photosensitivity, and large band gap. When illuminated with an appropriate light source, the photocatalyst generates electron/hole pairs with free electrons produced in the empty conduction band leaving positive holes in the valence band. These electron/hole pairs are capable of initiating a series of chemical reactions that eventually mineralize the pollutants in this case dyes (Sakthivel et al. 2003).

14.3.2 Microbial Pathway

These physio-chemical methods are non-ecofriendly as they tend to pollute the land and water sources. Microorganisms, however, completely degrade dyes and are environment friendly (Al-Tohamy et al. 2020; Ajaz et al. 2019; Hossen et al. 2019; Asses et al. 2018; Islam et al. 2017; Allam 2017; Lalnunhlimi and Krishnaswamy 2016; Cheng et al. 2016; Laxmi and Nikam 2015). These azo dyes are used as an energy source and a sole source of carbon and nitrogen by these microorganisms by mineralizing them into smaller compounds (Sudha et al. 2014). Bacteria, fungi, and algae are widely reported in literature to have azo dye degrading capabilities (Islam et al. 2017; Yang et al. 2016; El-Sheekh et al. 2009; Olukanni et al. 2006).

Fungal species like *Aspergillus niger*, *A. terreus*, *Alternaria alternate*, *Acrogenospora sphaerocephala*, *Ceriporia lacerate*, *Colletotrichum dematium*, *Corynespora cassiicola*, *Dictyosporium zhejiangensis*, *Fusarium thapsinum*, *F. oxysporium*, *Myrothecium verrucaria*, *Plectosporium tabacinum*, *Penicillium notatum*, etc. (Asses et al. 2018; Yang et al. 2016; Laxmi and Nikam 2015; Almeida and Corso 2014).

A wide range of bacterial species like *Bacillus cereus*, *Alcaligenes faecalis*, *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Micrococcus* sp., *Klebsiella* sp., *Sphingomonas paucimobilis*, *Rhizobium radiobacter*, *Arthrobacter soli*, *Citrobacter* sp., *Escherichia coli*, *Micrococcus* sp., etc. have been reported to biodegrade azo dyes (Hossen et al. 2019; Islam et al. 2017; Allam 2017; Khan and Malik 2017; Vimala et al. 2015).

Bacteria mineralize azo dyes through reductive cleavage, which results in the formation of amines. Anaerobic degradation results in the formation of aromatic amines which are nonbiodegradable. However, treatment of these aromatic amines under aerobic condition results in their effective biodegradation. It has been stated in literature that aerobic degradation post anaerobic treatment can be used to degrade recalcitrant and toxic dyes (Puvaneswari et al. 2006).

Other microorganisms like fungi, yeast, algae, and certain bacteria remove azo dyes through biosorption. The biosorption capacity of a microorganism is attributed to the heteropolysaccharide and lipid components of the cell wall, which contain different functional groups, including amino, carboxyl, hydroxyl, phosphate, and other charged groups, causing strong attractive forces between the azo dye and the cell wall. As biosorption is mainly a surface phenomenon, certain pretreatments like autoclaving, treatment with acids like sodium hydroxide, formaldehyde, and calcium chloride change the surface/surface area of the microorganism, therefore changing the capacity of binding sites and affecting the biosorption. The effectiveness of biosorption depends on the following conditions: pH, temperature, ionic strength, time of contact, adsorbent and dye concentration, dye structure, and type of microorganism (Solis et al. 2012).

14.3.3 Enzymatic Pathway

Enzymatic degradation of azo dyes can be done through bacteria, fungi, algae, plant, or other sources (Sarkar et al. 2017). However, enzymes secreted by microorganisms have several advantages compared to other sources due to low maintenance cost, cheaper culture, downstream processing, etc. Microbes reduce the azo dyes by secreting various enzymes such as azo reductase, laccase, oxidase, hydrogenase, and peroxidase. Enzyme systems for the decolorization and mineralization of azo dyes under certain environmental conditions are substrate specific, highly efficient, can be immobilized, and biodegradable and therefore have no adverse impact on the environment (Pandey et al. 2007).

Azoreductase, a reducing enzyme, degrades azo dye into colorless amines through reductive cleavage process with reducing equivalent like FADH or NADH as the electron donor. Azoreductase can be either membrane bound or cytoplasmic. The enzyme cleaves azo bond (-N=N-) and transfers four electrons as reducing equivalent. In each stage, two electrons are transferred to the azo dye (electron acceptor), which results in decolorization of the dye (Singh et al. 2015). Toxic aromatic amines are often formed as intermediates which is later degraded through aerobic processes or microaerophilically. Cell membrane-bound azoreductases utilize redox mediator as an electron shuttle under anaerobic condition. This mechanism that uses redox mediator-dependent reactions are different for both membrane-bound and cytoplasmic azoreductase. Non-sulfonated azo dyes can enter through the cell membrane; therefore, they are degraded by cytoplasmic azoreductase. Degradation in anaerobic condition is more efficient than the aerobic one, as azoreductase is an oxygen-sensitive enzyme. Thus, in aerobic condition, enzyme comes in contact with oxygen, and redox mediator is reduced instead of the azo dye (Sarkar et al. 2017).

Laccase, multicopper oxidase enzyme, oxidizes the aromatic amine using Cu^{2+} as the mediator. Several low molecular weight compounds act as the efficient redox mediator in electron transfer steps. It degrades azo dyes through a free radical-mediated mechanism which is highly nonspecific. The intermediates of this reaction are phenolic compounds rather than toxic aromatic compounds. The enzyme oxidizes the phenolic ring utilizing one electron to produce a phenoxy radical which is furthermore oxidized by the enzyme to generate carbonium ion. Nucleophilic attack of water produces 4-sulfophenyldiazene and benzoquinone; these are unstable in the presence of oxygen. Under aerobic condition, 4-sulfophenyldiazene gets oxidized to phenyldiazene radical, followed by loss of molecular nitrogen, producing sulfonyl radical and ultimately producing sulfophenyl hydroperoxide, scavenged by oxygen (Chacko and Subramaniam 2011).

14.4 Impact of Bacterial Degraded Azo Dye Byproducts on Flora and Fauna

14.4.1 Bacterial Degradation of Azo Dye

Desai (2017) concluded that *Klebsiella* spp. and *Staphylococcus* spp. degrade 98.83% and 98.72% of 200 ppm of Direct Red 2B in Bushnell Hass minimal salt medium at pH 7, 37 °C, and 1% glucose, respectively. *Arthrobacter soli* BS5 showed dye degrading capacity by degrading Reactive Black 5 textile dye with 98% degradation at pH range 5–9, 37 °C, and 120 h of incubation (Khan and Malik 2017). The bacterial strains *Enterobacter asburiae* and *Enterobacter cloacae* were identified to biodegrade (not absorb) up to 98% of 100 mL of textile effluent with 0.1 g biomass at 32 °C and pH 1.67 in 10 min under aerobic conditions (Singh et al. 2017).

Lade et al. (2015) used GC-MS analysis to identify the degraded product of Reactive Blue 172 obtained posttreatment with *P. rettgeri* which were 4-(ethenyl sulfonyl) aniline and 1-amino-1-(4-aminophenyl) propan-2-one based on fragmentation pattern and *m*/*z* values. FTIR and HPLC analyses confirmed the degradation of the dye. Khandare et al. (2013) showed FTIR spectral analysis between Direct Red 5B and its products post decolorization by *Pseudomonas putida*. FTIR spectrum of the dye had different peaks at 1754.7 cm^{-1} for C=O stretch, at 1619.3 cm^{-1} for N=N stretch for azo bond structure; other peaks at 1546.7 and 1486 cm^{-1} show N–O stretch and peaks at 1286.8, 1134.7, 1045.1 cm^{-1} show S=O stretch. FTIR analysis of the degraded dye products by *P. putida* showed few peaks of C–H deformation, indicating cleavage of the dye molecule. Peak at 2318.2 cm^{-1} represented NH_3, indicating the formation of amines, at 1703.4 cm^{-1} C=O for stretch. The decrease in peaks at 1229.5 and 1023.2 cm^{-1} indicated loss of sulfo groups. Further reduction in azo bond structure was also reported. Similarly, Lade et al. (2015) reported complete degradation of 50 mg/L textile azo dye Reactive Blue 172 using *Providencia rettgeri* strain at 30 ± 0.2 °C in 20 h under microaerophilic conditions with activities of azo reductase (159%) and NADH-DCIP reductase (88%). The decolorization was confirmed using HPLC, FTIR, and GC-MS analyses.

The strains *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* were reported to degrade consortia of four reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) up to 50–60% in 48–72 h at 2700 mg L and 2100 mg L, respectively (Rajeswari et al. 2014). *Sphingomonas paucimobilis* was reported to decolorize 99.63% toxic azo dye Methyl Red (750 ppm) in Mineral Salt Medium at 30 °C, pH 9 in 10 h confirmed using FT-IR analysis. The degradation of MR was possible through a broad pH (3–11) and temperatures (5–40 °C) range (Ayed et al. 2011). Kalyani et al. (2009) reported that *Pseudomonas* sp. SUK1 decolorizes sulfonated azo dye Reactive Red 2 at concentration ranging up to 5 g/L at 30 °C, pH ranging from 6.2 to 7.5 at static condition with 52% reduction in the COD within 24 h with respect to the activity of lignin peroxidase and azo reductase.

14.4.2 Impact of Bacterial Azo Dye-Degraded Byproducts on Flora

Phytotoxicity of Direct Red 2B conducted by Desai (2017) on *Phaseolus mungo* (mung) plants by petri dish method showed detoxification of Direct Red 2B by *Klebsiella* sp. hence better germination and length of shoot and root while *Staphylococcus* sp. isolate did not show any promising results for detoxification. Textile effluent biodegraded using *Enterobacter asburiae* and *Enterobacter cloacae* was reported to be less toxic for *V. radiata*, *T. aestivum*, and *P. mungo* as revealed after the phytotoxicity studies of percent germination and length of plumule and radicle (Singh et al. 2017).

The phytotoxicity of four mixed reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) and their degraded byproducts by *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* was carried out in *Triticum aestivum*. The degraded byproducts were extracted in ethyl acetate, dried and redissolved in water up to 1000 ppm concentration. The plant seeds were watered with 5 mL of dye mix and degraded metabolites, respectively, at 30 ± 2 °C; percent germination was more in degraded byproducts (80%) as compared to dye mix (30%). The phytotoxicity study showed that the length of plumule (3–4 cm) and radical (2–3 cm) was affected in case of the dyes, whereas with degraded metabolites, it showed significant growth (12–13 cm and 8–9 cm, respectively) (Rajeswari et al. 2014).

Sorghum vulgare and *Phaseolus mungo* seeds irrigated with 10 mL of degraded metabolites of Reactive Blue 172 (50 mg/L) using *P. rettgeri* showed 90% seed germination in comparison to 60–70% germination by original dye and better root and shoot elongation (Lade et al. 2015).

The degraded products of Direct Red 5B reported earlier were applied for toxicity analysis on seeds of *Sorghum vulgare* and *Phaseolus mungo* at room temperature. The seeds were soaked in 5 mL solution degraded metabolites at room temperature. The degraded metabolites had lower inhibitory effect on percent germination, shoot and root length of plants as compared to the original dye molecule (Khandare et al. 2013).

The metabolites of Methyl Red dye obtained post degradation using *Sphingomonas paucimobilis* at 750 ppm concentration was reported to exhibit no inhibition in germination of *Sorghum bicolor* and *Triticum aestivum* (Ayed et al. 2011).

The biodegraded products of Reactive Red 2 after degradation with *Pseudomonas* sp. SUK1 was monitored using UV–vis, IR spectroscopy, and HPLC. The final product was 2-naphthol characterized by GC-mass spectroscopy. The study conducted on *Sorghum vulgare* and *Phaseolus mungo* seeds revealed that the degraded metabolites generated after the biodegradation of Reactive Red 2 were less toxic as compared to original dye (Kalyani et al. 2009).

14.4.3 Impact of Bacterial Azo Dye-Degraded Byproducts on Fauna

Acute tests with *D. magna* used for the evaluation of lethal toxicity of Reactive Blue 172 to mammals and humans showed 100% mortality of *D. magna*; however, *P. rettgeri* treated dye completely detoxified the dye as there was no mortality of *D. magna*. The sample of Reactive Blue 172 dye treated with *P. rettgeri* strain was centrifuged and the supernatant was sterilized using 0.45-μm cellulose acetate syringe filter. The filtrate in Erlenmeyer flask was inoculated with five 24-h-old neonates of *D. magna* at 20 ± 0.2 °C for 48 h in dark. The mortality of *D. magna* was observed post exposure to light for 20 s, and no mortality was observed in the *P. rettgeri*-treated dye solution (Lade et al. 2015). Eczema, contact dermatitis, asthma, chronic bronchitis, tuberculosis, hematoma, bladder cancer, and irritation to eyes have been reported among the workers of textile industries (Rajeswari et al. 2014).

Rajeswari et al. (2014) conducted cytotoxicity study of degraded byproducts of four mixed reactive dyes (Black B, Blue RR, Red RR, and Yellow RR) by *Lysinibacillus sphaericus* and *Stenotrophomonas maltophilia* on human embryonic kidney cell line (HEK 293) using MTT assay to evaluate their toxicity. The assay at concentration of 31.25–500 μg/mL degraded metabolites showed percentage cell viability in the range of 79.41–98.42. The study concluded that degraded metabolites at high concentration had a mild interference in cell viability, therefore are nontoxic. *Erwinia* sp. bacterial strains decolorized brilliant green efficiently than Evans Blue. The decrease in the toxicity of brilliant green was interlinked to decolorization of brilliant green was connected with decrease of zootoxicity in *D. magna*. However, Evans Blue decolorization had no impact on its zootoxicity (Przystaś et al. 2012). Methyl Red biotoxicity test on *Artemia salina* revealed no significant change in the acute toxicity in relation to the test organism reported by Ayed et al. (2011) (Table 14.3).

14.5 Impact of Fungal Degraded Azo Dye Byproducts on Flora and Fauna

14.5.1 Fungal Degradation of Azo Dye

A. niger degradation of CR was correlated with lignin peroxidase and manganese peroxidase production, showing 97% degradation with 2 g mycelia incubated with 200 mg/L dye at 28 °C, pH 5 for 6 days under 120–150 rpm confirmed using LC-MS/MS spectral analyses (Asses et al. 2018).

Laxmi and Nikam (2015) reported that *Aspergillus flavus* degraded various azo dyes like R Red M8B, R Navy Blue M3R, R Orange M2R, R Green HE4B, Dt Black BT, Dt Orange RS, R RedM5B, Dt Sky Blue FF, and Dt Blue GLL in 3–7 days at pH 5, 30 °C, initial dye concentration of 40 mg/L as a result of biotransformating enzymes like lignin peroxidase, laccase, manganese peroxidase, and tyrosinase.

Table 14.3 Effect of bacterial degraded dyes on plants and animals

Bacteria	Isolated from	Dye	Impact of degraded byproduct	Reference
Klebsiella sp., *Staphylococcus* sp.	Chemically contaminated sites	Direct Red 2B	Better germination and length of shoot and root in *Klebsiella*-treated dye	Desai (2017)
Enterobacter asburiae, *Enterobacter cloacae*	Textile effluent	Textile effluent	Less toxic for *V. radiata*, *T. aestivum*, and *P. mungo*. Increase in percent germination and length of plumule and radicle	Singh et al. (2017)
Lysinibacillus sphaericus, *Stenotrophomonas maltophilia*	–	Black B, Blue RR, Red RR, and Yellow RR	Increase in length of root and shoot of *Triticum aestivum*	Rajeswari et al. (2014)
			Degraded metabolites at high concentration had a mild interference inhuman embryonic kidney cell line (HEK 293) cell viability, hence non-toxic	
Providencia rettgeri	–	Reactive Blue 172	20% better germination and increase in length of root and shoot in *Sorghum vulgare* and *Phaseolus mungo*	Lade et al. (2015)
			100% mortality in original dye while no mortality posttreatment in *D. magna*	
Portulaca grandiflora, *Pseudomonas putida*	Root-associated bacteria	Direct Red 5B	Lower inhibitory effect of degraded metabolites on percent germination, shoot and root length of *Sorghum vulgare* and *Phaseolus mungo*	Khandare et al. (2013)
Sphingomona spaucimobilis	Textile wastewater	Methyl Red	Degraded metabolites had no inhibition in germination of *Sorghum bicolor* and *Triticum aestivum*	Ayed et al. (2011)
			No significant change *Artemia salina*	

(continued)

Table 14.3 (continued)

Bacteria	Isolated from	Dye	Impact of degraded byproduct	Reference
Pseudomonas sp. SUK1	Soil sample collected from the waste disposal site of textile processing and dye manufacturing units	Reactive Red 2	Degraded metabolites are less toxic as compared to original dye *Sorghum vulgare* and *Phaseolus mungo*	Kalyani et al. (2009)
Erwinia sp.	Wastewater treatment plant	Brilliant green and Evans Blue	Decrease of zootoxicity in *D. magna*	Przystaś et al. (2012)

Almeida and Corso (2014) reported in a study indicating *Aspergillus terreus* and *A. niger* to test the biodegradation and biosorption of Procion Red MX-5B. In the biodegradation study with the fungus *A. terreus*, in 336 almost 100% decolorization was achieved, and UV–Vis and FTIR spectroscopy revealed molecular degradation and the formation of secondary metabolites, such as primary and secondary amines, while biosorption was effective in both decolorization and reducing the toxicity of the solutions.

Another study used two fungi to conclude that fungi exhibit different tendencies in liquid medium and tube overlay method. *Aspergillus niger* recorded maximum decolorization of the dye in liquid medium Basic fuchsin (81.85%), Nigrosin (77.47%), Malachite green (72.77%), and dye mixture (33.08%) under shaking condition while maximum dye decolorization in tube overlay method was recorded in Malachite green and Nigrosin (92.85% and 93.33%, respectively), Basic fuchsin (90.05%) and then dye mix (10.4%). In *P. chrysosporium*, maximum decolorization occurred in Nigrosin (90.15%), Basic fuchsin (89.8%), Malachite green (83.25%) followed by dye mix (78.4%) in liquid medium while Malachite green (71.42%), Basic fuchsin (70%), dye mix (9.6%), and least in Nigrocin (8.33%) in tube overlay method (Rani et al. 2014).

14.5.2 Impact of Fungal Azo Dye-Degraded Byproducts on Flora

Asses et al. (2018) concluded that Congo Red degraded with *A. niger* showed less toxicity to *Zea mays* and *Solanum lycopersicum* seeds with almost no effect on their shoot and root length posttreatment. Azo dye degradation using *A. flavus* dye-decolorized samples, the percent seed germination, shoot and root length, and the protein and total carbohydrate content were reported similar to that of the control (Laxmi and Nikam 2015).

Almeida and Corso's (2014) experiment that used *A. terreus* for biodegradation of Procion Red MX-5B showed a significant increase in toxicity, inhibiting the growth of *L. sativa* seeds by 43%, and the acute toxicity tests confirmed lack of molecular degradation following biosorption with *A. niger*, as toxicity to *L. sativa* seed reduced from 5% to 0%.

Another study reported the effect of untreated dye solution and biodegraded dye on wheat seed germination. Sterilized wheat seeds kept soaked in untreated dye solution and biodegraded dye showed 90% higher seed germination percent in treated dye while original dye inhibited the seed germination after 4 days at 25 ± 1 °C (Rani et al. 2014).

Similar effects were reported in the phytotoxicity studies on *Triticum aestivum* and *Ervum lens* Linn. as high percent seed germination along with significant growth in plumule and radicle of both the plants occurred in malachite green decolorized sample using *Penicillium ochrochloron* (Shedbalkar and Jadhav 2011).

Kalyani et al. (2008) reported better germination rate and plumule and radicle growth in *Sorghum vulgare* and *Phaseolus mungo* when treated with Red BLI metabolites post decolorization as compared to the original dye.

14.5.3 Impact of Fungal Azo Dye Degraded Byproducts on Fauna

Asses et al. (2018) concluded that Congo Red posttreatment with *A. niger* was less toxic on *Bacillus cereus* and *Escherichia coli* strains. The experiment confirmed that Congo Red dye is detoxified by *A. niger*, indicating the degradation of the amines in the solution. The experiment concluded that the improper disposal of the dyes can exert a negative impact on aquatic ecosystems (Table 14.4).

Almeida and Corso's (2014) experiment that used *A. terreus* for biodegradation of Procion Red MX-5B showed a significant increase in toxicity, leading to a 100% mortality rate among the *A. salina* larvae. *A. niger* did not have any effect on the toxicity of Procion Red MX-5B.

14.6 Future Perspective

Azo dyes are the most widely used dyes and contribute to about 50% of the dyes produced annually. Approximately 70% of them are discharged of as residual dye in textile wastewater. As they are highly toxic to the ecosystem, their degradation from textile wastewater has become a significant challenge over the past few decades. As the norms are getting stricter with respect to the industries following all the waste disposal rules and regulation, there is a need for more appropriate methods that are not only effective, cheap, but also do not harm the environment in the long run. As we learn the three R's, we must also try to reuse and recycle the water and reduce the waste produced. Microbial degradation of azo dyes has been very promising as it is accessible, effective, environmentally friendly, and cheap. Microbial treatment not only decolorizes the dye but also results in its detoxification. Chemical treatment, on

Table 14.4 Effect of fungal degraded dyes on plants and animals

Fungi	Source	Dye	Impact of degraded byproduct	Reference
Aspergillus niger	Processing wastewater	Congo Red	Less toxicity to *Zea mays* and *Solanum lycopersicum* seeds with no effect on shoot and root length of degraded metabolites	Asses et al. (2018)
			Decreased toxicity on *Bacillus* cereus and *Escherichia coli* strains	
Aspergillus flavus	Textile dye-contaminated site	R Navy Blue M3R, R Red M8B, R Green HE4B, R Orange M2R, R RedM5B, Dt Orange RS, Dt Black BT, Dt Blue GLL, and Dt Sky Blue FF	Percent seed germination, shoot and root length, protein and total carbohydrate content were similar in both control and posttreatment metabolites	Laxmi and Nikam (2015)
Aspergillus terreus, *Aspergillus niger*	–	Procion Red MX-5B	Significant increase in toxicity, inhibiting the growth of L. sativa seeds by *A. terreus*	Almeida and Corso (2014)
			A. niger reduced toxicity to *L. sativa* seed from 5% to 0%	
			A. terreus increased toxicity post treatment (100% mortality rate in *A. salina* larvae). *A. niger* had no effect on toxicity	
Aspergillus niger, *Phanerochaete chrysosporium*	Dye effluent soil	Malachite green, Nigrosin, and Basic fuchsin	90% higher seed germination of wheat seed	Rani et al. (2014)

(continued)

Table 14.4 (continued)

Fungi	Source	Dye	Impact of degraded byproduct	Reference
Penicillium ochrochloron	–	Malachite green	High percent seed germination and significant growth in plumule and radicle *Triticum aestivum* and *Ervum lens* Linn.	Shedbalkar and Jadhav (2011)
Pseudomonas sp. SUK1	Soil sample collected from the waste disposal site of textile processing and dye manufacturing units	Red BLI	Increased germination rate, plumule and radicle growth in *Sorghum vulgare* and *Phaseolus mungo*	Kalyani et al. (2008)

the other hand, increases the chemical load in the environment worsening the pollution of water bodies and associated land masses. Since microbial degradation treats the wastewater in terms of both color and toxicity without any significant chemical load, it will increase the reusability of the water and reduce the water need of each industry and minimize toxic chemical discharge into the environment.

Acknowledgments The authors are grateful to Professor Aditya Shastri, Vice-Chancellor Banasthali Vidyapith, Rajasthan. The authors also give thanks to DST-CURIE for providing financial assistance in conducting the research work. The authors wish to acknowledge the support from the Department of Science and Technology for providing INSPIRE fellowship for the project.

References

Adebajo SO, Balogun SA, Akintokun AK (2017) Decolourization of vat dyes by bacterial isolates recovered from local textile mills in Southwest, Nigeria. Microbiol Res J Int 18:1–8

Agarwal S, Tipre D, Patel B, Dave S (2014) Optimization of triazo acid black 210 dye degradation by *Providencia* sp. SRS82 and elucidation of degradation pathway. Process Biochem 49:110–119

Ajaz M, Rehman A, Khan Z, Nisar MA, Hussain S (2019) Degradation of azo dyes by *Alcaligenes aquatilis* 3c and its potential use in the wastewater treatment. AMB Express 9:1–12

Allam NG (2017) Bioremediation efficiency of heavy metals and azo dyes by individual or consortium bacterial species either as free or immobilized cells: a comparative study. Egypt J Bot 57:555–564

Almeida EJR, Corso CR (2014) Comparative study of toxicity of azo dye Procion Red MX-5B following biosorption and biodegradation treatments with the fungi *Aspergillus niger* and *Aspergillus terreus*. Chemosphere 112:317–322

Al-Tohamy R, Kenawy ER, Sun J, Ali SS (2020) Performance of a newly isolated salt-tolerant yeast strain *Sterigmatomyces halophilus* SSA-1575 for azo dye decolorization and detoxification. Front Microbiol 11:1163. https://doi.org/10.3389/fmicb.2020.01163

Ashtekar VS, Bhandari VM, Shirsath SR, Sai Chandra PLVN, Jolhe PD, Ghodke SA (2014) Dye wastewater treatment: removal of reactive dyes using inorganic and organic coagulants. J Ind Pollut Control 30:33. ISSN: 0970-2083

Asses N, Ayed L, Hkiri N, Hamdi M (2018) Congo red decolorization and detoxification by *Aspergillus niger*: removal mechanisms and dye degradation pathway. Biomed Res Int 2018:1–9

Ayed L, Mahdhi A, Cheref A, Bakhrouf A (2011) Decolorization and degradation of azo-dye methyl red by an isolated *Sphingomonas paucimobilis*: biotoxicity and metabolites characterization. Desalination 274:272–277

Aziz HA, Alias S, Adlan MN, Faridah, Asaari AH, Zahari MS (2007) Colour removal from landfill leachate by coagulation and flocculation processes. Bioresour Technol 98:218–220

Benkhaya S, M'rabet S, Harfi AE (2020) Classifications, properties, recent synthesis and applications of azo dyes. Heliyon 6:e03271

Bhainsa KC, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigates*. Colloids Surf B: Biointerfaces 47:160–164

Chacko JT, Subramaniam K (2011) Enzymatic degradation of azo dyes – a review. Int J Environ Sci 1:1250–1260

Cheng WN, Sim HK, Ahmad SA, Syed MA, Shukor MY, Yusof MT (2016) Characterization of an azo-dye-degrading white rot fungus isolated from Malaysia. Mycosphere 7:560–569

Desai SA (2017) Isolation and characterization dye degrading bacteria for detoxification of dark red 2B. Biosci Discov 8:426–431

El-Sheekh MM, Gharieb MM, Abou-El-Souod GW (2009) Biodegradation of dyes by some green algae and cyanobacteria. Int Biodeterior Biodegradation 63:699–704

Esteves MF, Silva JD (2004) Electrochemical degradation of reactive blue 19 dye in textile wastewater. In: Autex, World textile conference, Roubaix, vol 4, pp 1–6

Ferraz ERA, Grando MD, Oliveira DP (2011) The azo dye Disperse Orange 1 induces DNA damage and cytotoxic effects but does not cause ecotoxic effects in *Daphnia similis* and *Vibrio fischeri*. J Hazard Mater 192:628–633

Ghodake GS, Telke AA, Jadhav JP, Govindwar SP (2009) Potential of *Brassica juncea* in order to treat textile effluent contaminated sites. Int J Phytoremed 11:297–312

Gogate PR, Pandit AB (2004) A review of imperative technologies for wastewater treatment II: hybrid methods. Adv Environ Res 8:553–597

Hassaan MA, Nemr AE (2017) Health and environmental impacts of dyes: mini review. Am J Environ Sci Eng 1:64–67

Hassan MM, Carr CM (2018) A critical review on recent advancements of the removal of reactive dyes from dyehouse effluent by ion-exchange adsorbents. Chemosphere 209:201–219

Hossen MZ, Hussain ME, Hakim A, Islam K, Uddin MN, Azad AK (2019) Biodegradation of reactive textile dye Novacron Super Black G by free cells of newly isolated *Alcaligenes faecalis* AZ26 and *Bacillus* spp. obtained from textile effluent. Heliyon 5:1–11

Imran M, Crowley DE, Khalid A, Hussain S, Mumtaz MW, Arshad M (2015) Microbial biotechnology for decolorization of textile wastewaters. Rev Environ Sci Biotechnol 14:73–92

Islam T, Rahman MS, Hussain MS (2017) Heavy metal tolerance pattern of textile dye degrading native bacteria: a bioremediation viewpoint. Ann Med Health Sci Res 7:67–73

Jadhav UU, Dhawale RN, Dawkar VV, Chougale AD, Padul MV (2016) Phytotoxic effect of synthetic textile dye effluent on growth of five plant species. Trends Biotechnol Res 5:1–6

Kalyani DC, Patil PS, Jadhav JP, Govindwar SP (2008) Biodegradation of reactive textile dye red BLI by an isolated bacterium *Pseudomonas* sp. SUK1. Bioresour Technol 99:4635–4641

Kalyani DC, Telke AA, Dhanve RS, Jadhav JP (2009) Ecofriendly biodegradation and detoxification of Reactive Red 2 textile dye by newly isolated *Pseudomonas* sp. SUK1. J Hazard Mater 163:735–742

Kant R (2012) Textile dyeing industry an environmental hazard. Nat Sci 4:22–26
Karunya A, Rose C, Valli Nachiyar C (2014) Biodegradation of the textile dye Mordant Black 17 (Calcon) by *Moraxella osloensis* isolated from textile effluent-contaminated site. World J Microbiol Biotechnol 30:915–924
Khan S, Malik A (2017) Toxicity evaluation of textile effluents and role of native soil bacterium in biodegradation of a textile dye. Environ Sci Pollut Res 25:4446–4458
Khandare RV, Kabra AN, Awate AV, Govindwar SP (2013) Synergistic degradation of diazo dye Direct Red 5B by *Portulaca grandiflora* and *Pseudomonas putida*. Int J Environ Sci Technol 10:1039–1050
Khatun S (2017) Toxicity impact of Silk dye waste effluent induced Histopathological changes in the Stomach and Intestine of Swiss albino mice Mus musculus and their mitigation using *Moringa oleifera* leaf powder. J Med Sci Clin Res 5:19306–19313
Lade H, Govindwar S, Paul D (2015) Low-cost biodegradation and detoxification of textile azo dye C.I. reactive blue 172 by *Providencia rettgeri* strain HSL1. J Chem 2015:1–10
Lalnunhlimi S, Krishnaswamy V (2016) Decolorization of azo dyes (Direct Blue 151 and Direct Red 31) by moderately alkaliphilic bacterial consortium. Braz J Microbiol 47:39–46
Laxmi S, Nikam TD (2015) Decolorisation and detoxification of widely used azo dyes by fungal species isolated from textile dye contaminated site. Int J Curr Microbiol App Sci 4:813–834
Leelakriangsak M (2013) Molecular approaches for bacterial azoreductases. Songklanakarin J Sci Technol 35:647–657
Libra JA, Borchert M, Vigelahn L, Storm T (2004) Two stage biological treatment of a diazo reactive textile dye and the fate of the dye metabolites. Chemosphere 56:167–180
de Lima ROA, Bazo AP, Salvadori DMF, Rech CM, Oliveira DP, Umbuzeiro GA (2007) Mutagenic and carcinogenic potential of a textile azo dye processing plant effluent that impacts a drinking water source. Mutat Res 626:53–60
Lobiuc A, Olaru S, Hancu EI, Costica N, Fortuna ME, Zamfirache MM, Constantinescu G (2018) Toxicity and removal of Direct Red 28 diazo dye in living polymeric systems. Revista de Chimie – Bucharest 69:1628–1635
Merzouk B, Yakoubi M, Kone M, Leclerc JP, Paternotte G, Pontvianne S, Lapicque F (2011) Effect of modification of textile wastewater composition on electrocoagulation efficiency. Desalination 275:181–186
Modi S, Pathak B, Fulekar MH (2015) Microbial synthesized silver nanoparticles for decolorization and biodegradation of azo dye compound. J Environ Nanotechnol 4:37–46
Muthu SS (2017) Sustainability in the textile industry. In: Textile science and clothing technology. Springer Nature Singapore Pte Ltd, Cham. ISBN: 978-981-10-2639-3, pp 1–147
Newman MC (2015) Fundamentals of ecotoxicology: the science of pollution. CRC Press, Boca Raton, FL, ISBN: 978-1-4200-6704-0, pp 3–63
Nigam P, Banat IM, Singh D, Marchant R (1996) Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes. Process Biochem 31:435–442
Ogugbue CJ, Morad N, Sawidis T, Oranusi NA (2012) Decolorization and partial mineralization of a polyazo dye by *Bacillus firmus* immobilized within tubular polymeric gel. 3 Biotech 2:67–78
Olukanni OD, Osuntoki AA, Gbenle GO (2006) Textile effluent biodegradation potentials of textile effluent-adapted and non-adapted bacteria. Afr J Biotechnol 5:1980–1984
Pandey A, Singh P, Iyengar L (2007) Bacterial decolorization and degradation of azo dyes. Int Biodeterior Biodegrad 59:73–84
Pearce CI, Lloyd JR, Guthrie JT (2003) The removal of colour from textile wastewater using whole bacterial cells: a review. Dyes Pigments 58:179–196
Przystaś W, Zabłocka-Godlewska E, Grabińska-Sota E (2012) Biological removal of azo and triphenylmethane dyes and toxicity of process by-products. Water Air Soil Pollut 223:1581–1592
Puvaneswari N, Muthukrishnan J, Gunasekaran P (2006) Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol 44:618–626

Rajeswari K, Subashkumar R, Vijayaraman K (2014) Degradation of textile dyes by isolated *Lysinibacillus sphaericus* strain RSV-1 and *Stenotrophomonas maltophilia* strain RSV-2 and toxicity assessment of degraded product. J Environ Anal Toxicol 4:222–226

Rani B, Kumar V, Singh J, Bisht S, Teotia P, Sharma S, Kela R (2014) Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability. Braz J Microbiol 45:1055–1063

Sakthivel S, Neppolian B, Shankar MV, Arabindoo B, Palanichamy M, Murugesan V (2003) Solar photocatalytic degradation of azo dye: comparison of photocatalytic efficiency of ZnO and TiO_2. Sol Energy Mater Sol Cells 77:65–82

Sani ZM, Abdullahi IL, Sani A (2018) Toxicity evaluation of selected dyes commonly used for clothing materials in Urban Kano, Nigeria. Eur J Exp Biol 8:1–4

Santos-Pereira GC, Corso CR, Forss J (2019) Evaluation of two different carriers in the biodegradation process of an azo dye. J Environ Health Sci Eng:1–11. https://doi.org/10.1007/s40201-019-00377-8

Sarkar S, Banerjee A, Halder U, Biswas R, Bandopadhyay R (2017) Degradation of synthetic azo dyes of textile industry: a sustainable approach using microbial enzymes. Water Conserv Sci Eng 2:121–131

Sarker MR, Chowdhury M, Deb AK (2019) Reduction of color intensity from textile dye wastewater using microorganisms: a review. Int J Curr Microbiol App Sci 8:3407–3415

Satija A, Bhatnagar M (2017) Environmental assessment of textile wastewater of Sanganer Area in Jaipur. Int J Innov Res Sci Eng Technol 6:10779–10786

Saxena A, Gupta S (2020) Bioefficacies of microbes for mitigation of azo dyes in textile industry effluent: a review. Bioresources 15:9858–9881

Sharma S, Kalpana A, Shweta, Suryavathi V, Singh PK, Ramesh S, Sharma KP (2007) Toxicity assessment of textile dye wastewater using Swiss albino rats. Australas J Ecotoxicol 13:81–85

Sharma S, Sharma S, Upreti N, Sharma KP (2009) Monitoring toxicity of an azo dye methyl red and a heavy metal Cu, using plant and animal bioassays. Toxicol Environ Chem 91:109–120

Shedbalkar U, Jadhav JP (2011) Detoxification of malachite green and textile industrial effluent by *Penicillium ochrochloron*. Biotechnol Bioprocess Eng 16:196–204

Shu H, Huang C (1995) Degradation of commercial azo dyes in water using ozonation and UV enhanced ozonation processes. Chemosphere 31:3813–3825

Singh AL, Chaudhary S, Kayastha AM, Yadav A (2015) Decolorization and degradation of textile effluent with the help of *Enterobacter asburiae*. Indian J Biotechnol 14:101–106

Singh AL, Chaudhary S, Yadav A (2017) Decolourization, degradation and removal of heavy metals of textile effluent with the help of mixed bacterial consortium. Indian J Biotechnol 16:258–264

Solis M, Solis A, Perez HI, Manjarrez N, Flores M (2012) Microbial decolouration of azo dyes: a review. Process Biochem 47:1723–1748

Sriram N, Reetha D (2015) Isolation and characterization of dye degrading bacteria from textile dye effluents. Cent Eur J Exp Biol 4:5–10

Sudha M, Saranya A, Selvakumar G, Sivakumar N (2014) Microbial degradation of azo dyes: a review. Int J Curr Microbiol App Sci 3:670–690

Syed MA, Sim HK, Khalid A, Shukor MY (2009) A simple method to screen for azo-dye-degrading bacteria. J Environ Biol 30:89–92

Tang W, Xu X, Ye BC, Cao P, Ali A (2019) Decolorization and degradation analysis of Disperse Red 3B by a consortium of the fungus *Aspergillus* sp. XJ-2 and the microalgae *Chlorella sorokiniana* XJK. R Soc Chem Adv 9:14558–14566

Umbuzeiro GA, Freeman HS, Warren SH, de Oliveira DP, Terao Y, Watanabe T, Claxton LD (2005) The contribution of azo dyes to the mutagenic activity of the Cristais River. Chemosphere 60:55–64

Vimala G, Jeyakumar P, Devi CA, Singh A, Iyer P (2015) Azo dye degrading bacteria from textile effluent. Int J Curr Microbiol App Sci 4:199–210

Wang DM (2016) Environmental protection in clothing industry. Sustainable development. In: Proceedings of the 2015 International Conference on sustainable development (ICSD2015). World Scientific Publishing Co Pte Ltd, pp 729–735. https://doi.org/10.1142/9789814749916_0076

Yadav AK, Jain CK, Malik DS (2014) Toxic characterization of textile dyes and effluents in relation to human health hazards. J Sustain Environ Res 3:95–102

Yang P, Shi W, Wang H, Liu H (2016) Screening of freshwater fungi for decolorizing multiple synthetic dyes. Braz J Microbiol 47:828–834

Ambika Saxena, DST INSPIRE JRF, Department of Bioscience and Biotechnology, Banasthali Vidyapith, has been pursuing her Ph.D. in Applied Microbiology and Biotechnology. She was a gold medalist in her postgraduation. Her interest lies in the area of environmental biotechnology, nanotechnology, animal and plant biotechnology. She is an environment enthusiast and wants to do research that would benefit the nature and all its being.

Sarika Gupta, Senior Assistant Professor, Banasthali Vidyapith, Rajasthan, has been involved with the planning and execution of a number of research and teaching programs in biotechnology. She is Director, Greenathon Technologies Pvt. Ltd., with a clean technology mission, and is a bootstrap and academic spinoff to serve the society to meet sustainable development goals. For the last 20 years, she is actively engaged in teaching, training, and research. Her research interests are in the area of medical microbiology, molecular biology, and plant biotechnology. Dr. Gupta is also coordinating as an integral part of the organizing team of various biotechnology programs in collaboration with various government funding agencies such as DST, DBT, ISCA, MSME, RMOL, etc. She has various publications in journals of national and international repute. She has presented papers at various national and international platforms. She has completed her Ph.D. in Seed Technology and Microbiology from the University of Rajasthan, Jaipur, and M.Sc. in Biotechnology from Banasthali Vidyapith, Banasthali.