# Potential of Thallophytes in Degradation of Dyes in Industrial Effluents

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**Abstract**

Diverse groups of microorganisms have inhabited this earth, which use different types of sources for energy and growth. Industries revolutionize the lifestyle of humankind, which affects negatively the ecosystem. Synthetic dyes impart fabulous colors to cloth, food, paper, and cosmetics. Due to their xenobiotic nature, they are mostly insurmountable for degradation and also toxic. Most of them are washed off during the various processes and mixed in the industrial effluents. Microorganisms have enzymatic system for the decolorization of dyes or simply they can adsorb them on their surface. Several genera of algae, bacteria, and fungi have developed a system to use these unwanted compounds in the water. They can also biotransform or degrade them into non-toxic products. Degradation of the dyes depends upon their toxicity and chemical structure and the type of strain used. Some species were found to be efficient against a variety of dyes at a high concentration level. The present review describes the diversity of three genera *Chlorella*, *Pseudomonas*, and *Aspergillus* of thallophytes for the degradation and decolorization of various dyes in industrial effluents and also the use of integrated approach of different consortia or other treatments for their application in wastewater treatment plants.

**Keywords**

Xenobiotic compounds · Industrial effluents · Azo dyes · Decolorization · *Chlorella* · *Pseudomonas* · *Aspergillus*

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## 13.1 Introduction

Dyes are synthetic or natural compounds used to color or change the shade of any substance. From the beginning natural dyes from plants were used, but the invention of synthetic dyes by the British chemist William Perkin (1856) from coal tar revolutionized the chemical industry. During the next few decades, production of synthetic dyes has been popularized due to their use in every sector of industries. Dyes are used in food products, paper and textile industry, tanning, cosmetics, pharmaceutical, etc. Commercial products use colors to attract the customers. Due to their high usage, they are concentrated in our environment as xenobiotic compounds. The major share of production goes to textile industry which uses more than 10,000 types of dyes, and most are used as excess levels with 1000 tonnes per annum. About 10–25% is lost at some stage in the dyeing process, and approximately 2–20% is discharged as effluents in water and soil (Carmen and Daniel 2012). They are highly toxic, if not disposed properly as most of them are washed off in the effluents of these industries and reach the water and soil bodies. Dyes and by-products cause environmental, esthetic, and health problems. Dyes can be categorized as disperse, basic, acid, direct, and reactive dyes (Asgher 2012). The breakdown of chromophore groups (azo or anthraquinone) from dyes leads to the formation of toxic compounds (Katheresan et al. 2018). They break down in the form of several carcinogenic or mutagenic forms (aromatic compounds, benzidine, naphthalene, etc.) and cause serious health problems in the food chain. With the time, xenobiotic compounds accumulate in Mother Nature and become problematic for every type of organism. They are mostly degraded or adsorbed by microorganisms, but sometimes become recalcitrant in nature because of insolubility, absence of transporting enzymes, and non-accessibility as substrates (Godheja et al. 2016).

The thallophytes are a group of non-mobile organisms which included algae, bacteria, fungi, and lichens. This group of organisms inhabited the earth in almost all types of conditions like hot springs, volcanoes, and Arctic and Antarctic regions. A variety of microorganisms can tolerate these conditions as well as adapt themselves for their survival. The xenobiotics or industrial effluents make the natural water bodies more acidic and also disturb the growth of biota. Some species of the group were found capable of removing the color from industrial effluents by adsorption or biodegradation or biotransformation or mineralization (Chang et al. 2001a). As compared to chemicophysical treatments, biological degradation of dyes is always cost-effective and also can remove the toxic amines in the effluents, and further the combination of both treatments can produce better results (Hai et al. 2007). The exploration of the diversity and deciphering the underlying mechanism of adaptability will be helpful to make the positive planning to transform the worst environmental conditions (Rampelotto 2010). In the present chapter, we have summarized three different genera, *Chlorella* (algae), *Pseudomonas* (bacteria), and *Aspergillus* (fungi), implicated in the natural degradation of dyes in industrial effluents and the underlying mechanism of decolorization.

## 13.2 Algae

Algae are a group of aquatic microorganisms having photosynthetic machinery and ca. 50,000 species adapted to various ecological conditions (Xu et al. 2006). They come under the group of thallophytes as due to undifferentiated roots, stems, and leaves. The major commercially available groups of microalga are Chlorophyta, Dinophyta, Haptophyta, Rhodophyta, and Stramenopiles (Heimann and Huerlimann 2015). The microalgal genera studied for the biotreatment of industrial wastewater are *Spirogyra*, *Oscillatoria*, *Spirulina*, *Scenedesmus*, *Cosmarium*, etc. (Fazal et al. 2018) Among these groups, *Chlorella* taxa have been majorly investigated for the treatment of various types of industrial effluents (Banat et al. 1997; Munoza and Guieysse 2006; Safi et al. 2014).

### 13.2.1 *Chlorella*

The genus is spherical shaped single cell green algae. It is widely used in the field of productions of biofuels, cosmetics, food, and pigments and wastewater treatments (de Andrade and de Andrade 2017). Industrial wastewater contains dyes and nutrients used by algal community for their growth, which can be used as a sustainable approach for biodiesel production and bioremediation (Fazal et al. 2018). The two species, i.e., *C. vulgaris* and *C. pyrenoidosa*, were well documented by various authors for the treatment of effluents of textile industry (Table 13.1).

The first report of degradation of azo dyes by *Chlorella* was given by Jinqi and Houtian (1992). They tested 30 azo compounds for the decolorization process and found removal percentage in the range of 5–100%. The most easily degradable dye was Direct Blue 71 (100%), and Methyl Red was not decolorized from the medium. The azoreductase enzyme was found to be responsible for the bioconversion of aniline intermediate into carbon dioxide. The same type of degradation product was confirmed by Acuner and Dilek (2004) while studying *C. vulgaris* for the decolorization of Tectilon Yellow 2G. Sinha et al. (2016) reported the degradation of many industrial pollutants by *C. pyrenoidosa* NCIM 2738-based photobioreactor. The organism was able to decolorize the dye completely within 2.16 days and also improved the water quality.

The dyes can be degraded into simpler products, or simply they can be adsorbed by the microalgae. Adsorption capacity of microalgae can vary for different dyes and their initial concentration (Aksu and Tezer 2005). The initial pH of the solution was a determining factor for the proper biosorption of the dyes, and it can also vary with the specific dyes. Aksu and Tezer (2005) found that the highest uptake of vinyl sulphone-type reactive dyes occurred at pH 2.0 by dried *C. vulgaris*, while Daneshvar et al. (2007) demonstrated that basic pH was more favorable for the decolorization of Malachite Green. Similar results were observed by Tsai and Chen (2010) by altering the pH from 3.0 to 11.0. To attain the highest uptake of cationic dyes, the surface should acquire more negative charge which is only possible at this pH. The functional groups, i.e., hydroxyl and carbonyl groups, present on the surface of

**Table 13.1** Removal of different dyes by *Chlorella* species

| Sr. no. | *Chlorella* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time (percentage) | By-product | References |
|---|---|---|---|---|---|---|
| 1. | *C. pyrenoidosa* *C. vulgaris* | Azo dyes | Biodegradation (azoreductase) – | (5–100%) | Aromatic amines, $CO_2$ | Jinqi and Houtian (1992) |
| 2. | *C. ellipsoidea* *C. kessleri* *C. vulgaris* | Tartrazine and Ponceau (5–20 ppm) | | 6 days (40–55%) | Aromatic amines | Hanan (2008) |
| 3. | *C. vulgaris* | Tectilon Yellow 2G (400 mg $L^{-1}$) | Bioconversion | 200 h (83%) | Aniline, $CO_2$ | Acuner and Dilek (2004) |
| | | Remazol Golden Yellow (200 mg $L^{-1}$); Remazol Red and Black B (800 mg $L^{-1}$); | Biosorption | – | – | Aksu and Tezer (2005) |
| | | G-Red, Orange II, and Methyl Red (20 ppm); basic cationic (10 ppm); basic fuchsin (5 ppm) | Biosorption and Biodegradation (azoreductase) | 7 days (4–91%) | Aromatic amines | El-Sheekh et al. (2009) |
| | | Malachite Green (6 mg $L^{-1}$) | Biosorption | 90 min (91.61%) | – | Kousha et al. (2013) |
| | | Congo Red (5–25 mg $L^{-1}$) | Biosorption and Biodegradation (azoreductase) | 96 h (83 and 58 %) | – | Hernández-Zamora et al. (2015) |
| 4. | *C. vulgaris* UMACC 001 | Lanaset Red 2GA (7.25 mg $L^{-1}$) | Biosorption | 10 days (48.7%) | – | Chu et al. (2009) |
| | | Supranol Red 3BW (20 mg $L^{-1}$) | | 10 days (50%) | | Lim et al. (2010) |
| 5. | *C.* sp. | Malachite Green (5 ppm) | Decolorization | 2.5 h (80.7%) | – | Daneshvar et al. (2007) |
| 6. | *C. pyrenoidosa* | Thioflavin T and Malachite Green | Biosorption | – | – | Horník et al. (2013) |

(continued)

**Table 13.1** (continued)

| Sr. no. | *Chlorella* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time (percentage) | By-product | References |
|---|---|---|---|---|---|---|
| | | Textile wastewater and Methylene Blue dye (10–60 mg $L^{-1}$) | | 60 min (40–90%) | – | Pathak et al. (2015) |
| | | Methylene Blue dye (100 mg $L^{-1}$) | | (98.20%) | – | Lebron et al. (2018) |
| 7. | *C. pyrenoidosa* NCIM 2738 | Direct Red-31 dye (40 mg $L^{-1}$) | Biodegradation (azoreductase) | 2.16 days (100%) | Aromatic amines | Sinha et al. (2016) |

microalgae help them for the biosorption of dyes (Horník et al. 2013). The optimal temperature range for the dye uptake by *Chlorella* lies between 25 and 35 °C; however, a wide range has little effect on the biosorption (Tsai and Chen 2010).

The continuous lighting conditions used in the case of mixed culture of algae (13 taxa including *Chlorella*) removed 80% color within 30 days as compared to 60% after 60 days of exposure under simulated field lighting conditions from the pulping effluent (Dilek et al. 1999). El-Sheekh et al. (2009) tested *C. vulgaris* among five taxa of microalgae for the removal of basic fuchsin, basic cationic, G-Red, Methyl Red, and Orange II. The most susceptible dyes were basic cationic and basic fuchsin. *C. vulgaris* removed 43.7 and 59.12% of Orange II and G-Red dyes. The G-Red dye acts as an inducer of the azoreductase enzyme and increases the activity up to 72.25%. Kousha et al. (2013) compared the biosorption activity for Malachite Green of the same species against *Scenedesmus quadricauda*. They considered the different parameters like dye concentration, contact time, algae amount, and pH. The maximum dye removal was done by *C. vulgaris* (91.61%) as compared to the latter one (73.49%). Similarly, Lebron et al. (2018) recorded maximum elimination of Methylene Blue by *C. vulgaris* (98.20%) as compared to *Spirulina maxima* (94.19%). Recently, Zhao et al. (2018) evaluated the effectiveness of wastewater treatment by *C. vulgaris*, *C. zofingiensis*, and *Scenedesmus* sp. in terms of the activity of photosystem II, nutrient loading, and lipid productivity. *C. zofingiensis* shows higher absorption capability, productivity, and efficiency as compared to the other two species, even in worse environmental conditions.

The immobilized form of microalgae has more advantages over the free cell suspension for the elimination of heavy metals and xenobiotics in wastewater (Luan et al. 2006). Chu et al. (2009) investigated the immobilized *C. vulgaris* UMACC 001 (1% κ-carrageenan and 2% sodium alginate) for the treatment of three dyes and textile wastewater. The algae immobilized on 2% sodium alginate has higher color removal efficiency for the textile wastewater and dyes. The immobilized form is more stable, easy to harvest, and protected from the direct exposure to toxicity as

compared to free cells. Later, Gao et al. (2011) also found the same results for the removal of nonylphenol using the same type of matrix. Horník et al. (2013) investigated the biosorption capacity of dried biomass of *C. pyrenoidosa* immobilized in polyurethane foam. The process of sorption of cationic dyes (Thioflavin T and Malachite Green) depends upon the preliminary concentration of dyes, flow rate of solution through the column, bed height, and biomass concentration. The simple or modified polyurethane-based adsorbent has been reported as an efficient sorbent for the elimination of dyes from wastewater (Sultan 2017).

Apart from the treatment of dyes, the genus has been also directly tested for the exclusion of xenobiotics directly from the textile wastewater. The organism utilizes textile wastewater for its growth and also removes the color in the range of 41.8–50.0% as reported by Lim et al. (2010). It also reduces phosphate, nitrate content, BOD, and COD from the effluents. The dried biomass was found more efficient as a biosorbent than wet algal biomass, due to its high binding affinity and large surface area. It can be cultured in the wastewater for color and COD removal and biomass production (El-Kassas and Mohamed 2014; Pathak et al. 2015; Tao et al. 2017). The integrated approach for the treatment of wastewater and production of biomass, lipids, biofuels, bioelectricity, etc. is the promising application of *Chlorella* in the industry (Logroño et al. 2017; Wang et al. 2017; Fazal et al. 2018). Malla et al. (2015) tested *C. minutissima* for biodiesel production and nutrient removal from primary and tertiary treated wastewater. The species removed TDS (90–98%), N (70–80%), P (60–70%), and K (45–50%) from the wastewater within 12 days. Zheng et al. (2017) demonstrated the enhanced production of biofuel by using kelp waste extracts combined with acetate in *C. sorokiniana*.

Seo et al. (2015) used oxidized dye wastewater composed of Methylene Blue and Methyl Orange for the harvesting of algae. The exposed amine groups of oxidized dyes act as amine-based coagulants. Daneshvar et al. (2018) investigated the feasibility of cultivation of *C. vulgaris* in a combination of aquaculture and pulp effluents. The carbohydrate, lipid, and protein percentage was very much high in the microalgae from the wastewater as compared to Bold's Basal Medium (BBM) solution. Another aspect of the use of microalgae and textile dyeing sludge was proved by Peng et al. (2015), as the combination of the duo improved char catalytic effect and increased the combustion process for the decomposition of textile dyeing sludge residue at high temperature (530–800 °C).

Undoubtedly, the discharge of the dyes into the aquatic ecosystem causes serious threats for the growth of many microorganisms. Toxicity studies of many dyes on *Chlorella* have been done by many workers (Hanan 2008; Qian et al. 2008; Hernández-Zamora et al. 2014; Kanhere et al. 2014; Xu et al. 2015). The deteriorated metabolic activity, growth rate, respiration, and photosynthesis efficiency of *C. vulgaris* were observed due to the direct exposure of Congo Red (Hernández-Zamora et al. 2014). After the bioremoval of the effluents by the species, the influents were less toxic to the primary consumer (*Daphnia magna*) of the aquatic ecosystem (Hernández-Zamora et al. 2015). Kanhere et al. (2014) observed genotoxic and cytotoxic effects of Malachite Green on *C. pyrenoidosa* in the form of altered cell morphology, high oxidative stress, DNA damage, and cell death. The

growth was inhibited in a dosage-dependent manner, and *D. magna* ingest the dye even at very low concentrations. Thus, there would be the same type of negative effects on the other aquatic organisms.

## 13.3 Bacteria

The prominent genera of bacteria explored by different workers are *Aeromonas*, *Bacillus*, *Escherichia*, *Eubacterium*, *Citrobacter*, *Pseudomonas*, *Sphingomonas*, and *Staphylococcus* (Rafii et al. 1990; Bumpus 1995; Banat et al. 1997; Keck et al. 1997; Sugiura et al. 1999; Nakanishi et al. 2001; Coughlin et al. 2003). Several anaerobic bacteria produce azoreductase for the degradation of dyes and produced metabolites. Biochemical and molecular characterization has shown that the enzyme presumably a flavin reductase or FMN-dependent NADH-azoreductase or tetrameric NADPH-dependent flavoprotein, as found from *Sphingomonas*, *Escherichia*, and *Staphylococcus*, respectively (Nakanishi et al. 2001; Suzuki et al. 2001; Chen et al. 2005). Bacteria can degrade the xenobiotic compounds in either aerobic or anaerobic or both conditions. Many strains of *Pseudomonas* have degraded them into non-hazardous products and simultaneously utilized the dyes for their growth (Pandey and Upadhyay 2006). The next section of the chapter reviews the diversity of different species/strains of *Pseudomonas* capable of degrading dyes in industrial effluents (Table 13.2).

### 13.3.1 *Pseudomonas*

Several workers have isolated the azoreductase enzyme from different species of bacteria implicated in the deterioration of azo dyes (Michaels and Lewis 1985; Zhipei and Huifang 1991; Yatome et al. 1990; Hu 1994; Bumpus 1995; Banat et al. 1997). The bacteria utilize them as a source of carbon and nitrogen. However, in the case of $RP_2B$ dye, it only acts as an inducer rather than as a growth substrate in the case of *P. luteola* (Hu 1998). The enzyme was found to be substrate specific, and the susceptibility of the bacterial attack depends on the substitution of the chemical and charged group at specific positions (Zimmermann et al. 1982; Yatome et al. 1990; Ben Mansour et al. 2009a). The degradation reaction of azo dyes into aromatic amines was fully catalyzed by the enzyme under anaerobic conditions, but to produce complete inorganic compounds, aerobic conditions are needed (Zhipei and Huifang 1991; Idaka et al. 1987a, b).

Zimmermann et al. (1982) isolated oxygen-insensitive azoreductase from *Pseudomonas* KF46, able to degrade the aromatic amines and complete mineralization of carboxy-Orange II. Nachiyar and Rajkumar (2004, 2005) proposed the mechanism of systematic elimination of Navitan Fast Blue S5R by the oxygen-insensitive enzyme, purified from *P. aeruginosa*. The intermediate metabolites of the dye may have undergone further oxidative deamination/decarboxylation and further enter the TCA cycle to release carbon dioxide. One of the intermediates

**Table 13.2** Removal of different dyes by *Pseudomonas* species

| Sr. no. | *Pseudomonas* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time and percentage | By-product | References |
|---|---|---|---|---|---|---|
| 1. | *P. sp.* KF46 | Orange II | Orange II azoreductase | – | 4-Aminobenzoate and sulfanilic acid | Zimmermann et al. (1982) |
| 2. | *P.* sp. GM3 | Indigo Carmine, Reactive Blue 2, Acid Red 183, Acid Green 27, Acid Violet 7 (100 mg $L^{-1}$) | Decolorization | 72 h (18–97%) | – | Yu et al. (2001) |
| 3. | *P.* sp. PR41-1 | Methyl Red | Biodegradation (azoreductase) | – | Dimethyl *p*-phenylenediamine and *o*-aminobenzoic acid | Sugiura et al. (1999) |
| 4. | *P.* sp. S-42 | Diamira Brilliant Orange RR, Direct Brown M, Eriochrome Brown R | Biodegradation (azoreductase) | (70.5–95.3%) | Aromatic amines | Zhipei and Huifang (1991) |
| 5. | *P.* sp. | Direct Black 38 and Congo Red (100 mg $L^{-1}$) | Degradation | 5 days (83–100 %) | Benzidine | Işik and Sponza (2003) |
| 6. | *P.* sp. OX1 | Acid Orange 7 (800 mg $L^{-1}$) | Biodegradation | – | – | Lodato et al. (2007) |
| 7. | *P.* sp. SUK1 | Red BLI, Reactive Navy Blue RX, Reactive Red M5B, Reactive Red 6BI, Reactive Red HE, Reactive Red HE3B, Reactive Orange HE2R, and Reactive Orange M2R (50 mg $L^{-1}$) | Biotransformation (aminopyrine *N*-demethylase and NADH-DCIP reductase) | 80–360 min. (85.33–99.28%) | Nitroso compound, oximes, and imines | Kalyani et al. (2008) |
| | | Reactive Red 2 (5 g $L^{-1}$) | Biodegradation (lignin peroxidase and azoreductase) | 6 h | 2-Naphthol | Kalyani et al. (2009) |
| 8. | *P.* sp. DY1 | Acid Black 172 (100–300 mg $L^{-1}$) | Decolorization | 25 h (12.2–79.6%) | – | Du et al. (2010) |

| Sr. no. | *Pseudomonas* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time and percentage | By-product | References |
|---|---|---|---|---|---|---|
| 9. | *P.* sp. LBC1 | Direct Brown MR (100 mg $L^{-1}$) | Biodegradation | 14–42 h (90%) | *p*-Dihydroperoxybenzene, 2-hydroxy-7-aminonaphthol-3-sulfonic acid, and 3,6-dihydroxybenzoic acid | Telke et al. (2012) |
| 10. | *P. aeruginosa* | Navitan Fast Blue S5R | Azoreductase | 48 h | Metanilic acid, peri acid, aniline, and β-ketoadipic acid | Nachiyar and Rajkumar (2004, 2005) |
| 11. | *P. aeruginosa* CR-25 | Remazol Black B (50–500 mg $L^{-1}$) | Decolorization | 24 h (67–96%) | – | Joe et al. (2011) |
| 12. | *P. aeruginosa* KY284155 | Remazol Black B (200 mg $L^{-1}$) | Degradation (azoreductase) | 32 h (100%) | – | Hashem et al. (2018) |
| 13. | *P. aeruginosa* NCIM 2074 | Malachite Green (50 mg $L^{-1}$) | Biodegradation (MG reductase, laccase, and aminopyrine *N*-demethylase) | 5 h (100%) | Benzophenone | Kalyani et al. (2012) |
| 14. | *P. aeruginosa* 23N1 | Reactive Red 21 (50, 150 mg $L^{-1}$ ) | Decolorization | 48 h (91.5–93.5%) | – | Mishra and Maiti (2018) |
| 15. | *P. desmolyticum* NCIM 2112 | Red HE7B (100 mg $L^{-1}$) | Biodegradation (lignin peroxidase and aminopyrine *N*-demethylase) | | 2-Hydroxyl-6-oxalyl-benzoic acid and 8-amino-naphthalene-1,3,6,7-tetraol | Kalme et al. (2007a) |
| | | Direct Blue 6 (100 mg $L^{-1}$ ) | Biodegradation (lignin peroxidase, laccase, and tyrosinase) | 72 h (100%) | Aminonaphthalenesulfonic acid and 4-amino naphthalene | Kalme et al. (2007b) |

(continued)

**Table 13.2** (continued)

| Sr. no. | *Pseudomonas* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time and percentage | By-product | References |
|---|---|---|---|---|---|---|
| 16. | *P. fluorescens* | Acid Yellow-9 | Biodegradation | 4 days | 4-Aamino-2-hydroxybenzene sulfonic acid sodium salt, 2-amino-4-hydroxy-benzene sulfonic acid sodium salt, and 2,4-dihydroxybenzene sulfonic acid sodium salt | Pandey and Upadhyay (2006) |
| 17. | *P. luteola* | Azo Dye $RP_2B$ | Azoreductase | 5 days (95%) | Orthanilic acid | Hu (1998) |
| | | Reactive Acid Yellow (30–200 mg $L^{-1}$), Reactive Black B (29–252 mg $L^{-1}$); Reactive Red 22 (61–353 mg $L^{-1}$) | Biosorption | – | – | Chen (2002) |
| | | Reactive Red 22 (200 ppm) | Decolorization | (80–98%) | – | Chen and Lin (2007) |
| | | Congo Red (100, 210 ppm), Eriochrome Black T (100, 230 ppm), Methyl Orange (100–400 ppm), and Methyl Red (100–450 ppm) | Decolorization | 20–25 h (100 %) | – | Hsueh and Chen (2007) |
| | | Reactive Red 22 (200 mg $L^{-1}$) | Degradation (azoreductase) | 60 h (75–80%) | Aromatic amines | Chang et al. (2001a, b) |
| 18. | *P. mendocina* PM2 | Malachite Green (50–1800 mg $L^{-1}$) | Biodegradation (MG reductase, lignin peroxidase, and manganese peroxidase) | 24 h (73.5–99.5%) | – | Chaturvedi et al. (2013) |
| 19. | *P. nitroreducens* | Methyl Red | Decolorization | 15 h (80%) | – | Adedayo et al. (2004) |

| Sr. no. | *Pseudomonas* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time and percentage | By-product | References |
|---|---|---|---|---|---|---|
| 20. | *P. otitidis* WL-13 | Triphenylmethane dyes (Malachite Green, Brilliant Green, and Crystal Violet) (500 µmol/L) | Adsorption | 12 h (13–95%) | – | Wu et al. (2009) |
| 21. | *P. putida* | Crystal Violet (60 µmol/L) | Biodegradation | 7 days (80%) | *N*-Demethylation intermediates and pararosaniline | Chen et al. (2007) |
| 22. | *P. putida* SKG-1 | Orange II (100 mg $L^{-1}$) | Biodegradation | 96 h (92.8%) | Sulfanilic acid and 1,2-naphthoquinone | Kumar Garg et al. (2012) |
| 23. | *P. putida* mt-2 | Acid Yellow 17, Violet 7, and Orange 52 (100 mg $L^{-1}$) | Azoreduction and oxygen-dependent metabolization | 60 h (100%) | Sulfanilic acid, *N*,*N*-dimethyl-*p*-phenylenediamine, and 4′-aminoacetanilide | Ben Mansour et al. (2007) |
| | | Acid Violet 7 (200 mg $L^{-1}$) | Biodegradation (azoreductase) | | 4-Aminoacetanilide | Ben Mansour et al. (2009a, b) |
| 24. | *P. putida* MTCC 4910 | Basic Violet 3 and Acid Blue 93 (250 mg $L^{-1}$) | Biosorption | 8 h (50–100%) | – | Arunarani et al. (2013) |
| | | Direct Red 28 (10–250 mg $L^{-1}$) | Biosorption | 1 h (45–85%) | – | Deepa et al. (2013) |
| 25. | *P. stutzeri* IAM 12097 | 4′-Dimethylaminoazobenzene-2-carboxylic acid ($4.5 \times 10^{-5}$ mol $dm^{-3}$) | Biodegradation | (90%) | *o*-Aminobenzoic acid, *N*,*N*-dimethyl-*p*-phenylenediamine, and catechol | Yatome et al. (1993) |
| 26. | *P. alcaligenes* *P. mendocina* *P. putida* *P. stutzeri* | Methyl Violet (0.9–16.5 mg $L^{-1}$) | Decolorization | 7 days (33.3–86.7%) | – | Sarnaik and Kanekar (1995) |
| 27. | *P. aeruginosa* *P. fluorescens* *P. putida* | Navitan Fast Blue S5R (100–1200 mg $L^{-1}$) | Biodegradation (azoreductase) | 72 h (72–92 %) | Metanilic acid | Nachiyar and Rajkumar (2003) |

formed in this study, i.e., metalinic acid, was further degraded into aniline and β-ketoadipic acid (Nachiyar et al. 2007). Işik and Sponza (2003) used aerobic and anaerobic conditions to study the color removal efficiency of *Pseudomonas* sp. They found that decolorization of Direct Black 38 and Congo Red was 83% and 100% under anaerobic incubation while 74% and 76% under microaerophilic conditions. The aerobic degradation occurs by the action of lignin peroxidase, tyrosinase, and laccase as reported by Kalme et al. (2007b) in *P. desmolyticum* NCIM 2112. Further, they purified laccase enzyme from the species and demonstrated the asymmetric breakdown of azo bond and that the specificity depends on the position of amino, hydroxyl, and sulfonic group in a dye. The decolorization rate is less when hydroxyl group and sulfonic group are at *meta* position or charged carboxyl group at *ortho* position to the azo bond (Nigam et al. 1996; Chen 2006; Kalme et al. 2007b, 2009). The presence of electron-withdrawing groups or absence of charged groups also enhances the rate of decolorization as stated by Hsueh and Chen (2007, 2008) in *P. luteola*. The toxicity of dyes depends on the type of azo bond, molecular structure, functional groups, and types of intermediates or degraded products. The lesser the toxicity of the dye, the easier will be the decolorization. Chen (2002) tested the toxicity of three reactive dyes against *P. luteola* (Acid Yellow, Black B, and Red 22). The Reactive Red 22 was easily decolorized, while Reactive Black B was highly toxic as it contains two azo bonds. As in this study decolorization is not growth-associated, the viability of the cells is the important criterion for the metabolism and expression of enzymes. Alternatively the cells can go for biosorption rather than decolorization.

Various authors have also isolated the laccase enzyme from different strains/species of *Pseudomonas* and showed its applicability in the elimination of synthetic dyes in industrial effluents (Telke et al. 2009; Kuddus et al. 2013; Wang et al. 2012). Phugare et al. (2011) purified a highly active enzyme, i.e., veratryl alcohol oxidase, from *P. aeruginosa* BCH. The enzyme has specificity for wide varieties of substrates and decolorizes seven dyes (Methyl Orange, Rubine 3GP, Congo Red, Remazol Black, Red HE7B, Red HE8B, and Red HE3B) in the range of 85–100%. One of the dyes, i.e., Remazol Black, was decolorized completely within 6 h and degraded into 7-diazenyl-naphathalene-1-ol and naphthalene-1,2,7-triol. Kalyani et al. (2011) reported a heme-containing peroxidase enzyme isolated from *Pseudomonas* sp. for the symmetric cleavage of Methyl Orange into *N*,*N*-dimethyl-1,4-benzenediamine and an intermediate 4-aminobenzenesulfonic acid. The intermediate formed was further degraded into aniline.

Toxicity analysis of the decolorized dyes should be done either by elucidating the structure of the degraded products by FTIR, GC-MS, HPLC, and NMR techniques or by using different organisms or cell lines. Several authors have checked the genotoxicity/cytoxicity/mutagenic potential of the metabolites formed by *Pseudomonas* during the remediation of industrial effluents (Adedayo et al. 2004; Pandey and Upadhyay 2006; Kalme et al. 2007a; Kalyani et al. 2009). Perei et al. (2001) isolated an aerobic bacterium called *P. paucimobilis* from the contaminated sites for the effective degradation of mutagenic metabolite sulfanilic acid. During the degradation of Orange 52, Violet 7, and Acid Yellow 17 by *P. putida* mt-2,

genotoxic metabolites were found high in static cultures as compared to shaken conditions (Ben Mansour et al. 2007). Later on the authors demonstrated that the amines were mutagenic formed under static conditions, which later on vanished during shaken incubation. Further, the metabolite 4′-aminoacetanilide exhibited maximum mutagenicity, while 5-acetamido-2-amino-1-hydroxy-3,6-naphthalene disulfonic acid shows less effect due to presence of sulfonic groups (Ben Mansour et al. 2009b). Telke et al. (2012) tested the toxicity assays of *p*-dihydroperoxybenzene, 2-hydroxy-7-aminonaphthol-3-sulfonic acid, and 3,6-dihydroxy benzoic acid, metabolites formed during biodegradation of Direct Brown MR by *Pseudomonas* sp. LBC1. The textile effluents and the dye were more toxic to *Vigna radiata* and *Sorghum bicolor* as compared to the biodegraded metabolites.

In the case of Methyl Orange, there wasn't any kind of removal under aerobic conditions by *P. putida* mt-2 (Thao et al. 2013). So an immobilized bacterial system can solve the problem for oxygen-sensitive decolorization by creating miniature anoxic environment and complementarily increasing the biomass concentration and providing mechanical strength, feasibility of continuous processing, low-cost recovery, and reusability of biocatalyst (Stormo and Crawford 1992; Park and Chang 2000; Chang et al. 2001a). Puvaneshwari et al. (2002) studied the effective role of immobilized *P. fluorescens* on sodium alginate for the degradation of Direct Blue (71%) and Direct Red (82%). Chen and Lin (2007) used silicate/alginate sol-gel beads of *P. luteola* for the decolorization of Reactive Red 22. The rate of decolorization of the free cells decreased, while the immobilized system was static after five repeated batch cycles. Tuttolomondo et al. (2014) reported the biodegradation of Methyl Orange, Benzyl Orange, and Remazol Black by immobilized *Pseudomonas* sp. in sol-gel silica matrices due to higher expression of extracellular enzymes. The encapsulation directly protects the bacteria from toxic conditions and consecutively increases the production of enzymes involved in degradation. *Pseudomonas* sp. DY1 immobilized in the fungi (*A. oryzae*) cellular mass shows 96% decolorization in the batch cycle, still after 16 days. Inhibition test confirmed that the activity of the pellets was mainly due to the bacteria, demonstrating their stable and long-term usability for the dye treatment (Yang et al. 2011a, b). Recently, Roy et al. (2018) used immobilized *Pseudomonas* sp. in fly ash for the biodegradation of Reactive Yellow. The highest removal percentage (98.72%) was recorded in *Pseudomonas* sp. on fly ash as compared to sorption by fly ash (88.51%) and degradation by species (92.62%).

The activated carbon in combination with *P. luteola* was found to be very much effective for the adsorption and biodegradation of Reactive Red 22 (Lin and Leu 2008). Selvakumar et al. (2010) use electro-oxidation and bio-oxidation by *P. aeruginosa* for the removal of color from textile effluent having Procion Blue 2G dye. Later the treated effluents have been treated with photo-oxidation to remove the bacteria, so that water can be recycled. Similarly, Srinivasan et al. (2011) combined the sonolysis pretreatment with post-biological treatment by the mutant strain of *P. putida* in the case of Tectilon Yellow 2G.

The studies on the optimization of the conditions like temperature, pH, presence of organic compounds, carbon and nitrogen source, concentration range of dyes,

and aerobic or anaerobic or both conditions are very much necessary, depending on the nature of the dye to be treated by *Pseudomonas*. Yu et al. (2001) observed that presence of nitrate at concentration 1000 mg/L inhibits the process completely, while increase in the temperature from 10 to 35 °C enhances the decolorization rate of *Pseudomonas* strain GM3. Chang et al. (2001b) found that tryptone and yeast extract enhances the decolorization process of Reactive Red 22, while retarded by the added glucose concentration and dissolved oxygen. The activity of azoreductase enzyme isolated from cell-free extract also depends upon the growth phase of bacteria. Lodato et al. (2007) proved that depletion of dye can be achieved irrespective of the initial concentration by changing the aerobic-anaerobic operating conditions. In the aerobic conditions, growth of *Pseudomonas* sp. OX1 can be achieved, while in the anaerobic conditions, depletion of dye takes place. Similarly, Lin et al. (2010) observed complete mineralization of Reactive Blue 13 by *Pseudomonas* sp. L1 in the same conditions. Joe et al. (2011) investigated the optimal conditions needed for Remazol Black B dye by *P. aeruginosa* CR-25. The maximum rate of removal occurs at 37 °C, pH7 with supplementation of peptone, yeast extract, glucose and fructose as nitrogen and carbon sources under static conditions. The same results have been observed under the above-said conditions by other workers using different species of *Pseudomonas* (Kalyani et al. 2008; Telke et al. 2009; Thao et al. 2013). Kumar Garg et al. (2012) showed that supplementation of ammonium sulfate (0.1%, w/v) and glucose (0.4% w/v) improved the decolorization of Orange II. Mishra and Maiti (2018) demonstrated that yeast extract has positive effect, while peptone and glucose have negative effect on the decolorization of Reactive Red 21 by *P. aeruginosa* 23N1. This may be due to the fact that species must have utilized peptone and glucose as primary sources of nitrogen and carbon rather than the dye molecule. Recently, Hashem et al. (2018) isolated a pH-tolerant *P. aeruginosa* KY284155 with high decolorization rate for Remazol Black B. With the addition of iron, magnesium, and yeast extract in the medium, the degradation rate was further accelerated. The heavy metals and salts at high concentrations in the medium have inhibitory effects on the decolorization of dyes (Gopinath et al. 2011). Some strains of *P. aeruginosa* were very effective in the degradation of reactive azo dyes even in the presence of heavy metals like lead, zinc, cadmium, and chromium (Maqbool et al. 2016; Hafeez et al. 2018).

The majority of the studies done in *Pseudomonas* were related to biodegradation of the dyes, but few authors have also studied the adsorption phenomena for the management of industrial effluents. Du et al. (2012) compared the adsorption capacity of live and heat-treated *Pseudomonas* sp. strain DY1 biomass for Acid Black 172. The heat-treated cells have high adsorption due to increased permeability and denatured intracellular proteins. Deepa et al. (2013) showed that 4 to 9 pH and 1 to 1000 mM NaCl concentrations have insignificant effect on the adsorption rate of Direct Red by *P. putida*. Later on, Arunarani et al. (2013) proved the same type of effect on the adsorption of Acid Blue 93 and Basic Violet 3 by the same taxa due to pH and salts. Liu et al. (2017) extracted a biosurfactant from *P. taiwanensis* L1011 and utilized it to accelerate the chemical and biological decolorization of Congo Red and Amaranth, respectively. Recently, Iqbal et al. (2018) developed a novel

biosorbent using *P. aeruginosa* USM-AR2 cells immobilized on mesoporous rice husk ash silica (RHA-SiO2).

There is a lot of variability for the potential of degradation of dyes within the different genera of bacteria. Hu (1996) compared the adsorption efficiency of *Aeromonas*, *Bacillus*, *Escherichia*, *Pseudomonas*, and *Staphylococcus* for four reactive azo dyes. The dead biomass of the three genera exhibits higher adsorption capacity in the order of *Aeromonas* > *Pseudomonas* > *Escherichia*. Nachiyar and Rajkumar (2003) tested three species (*P. aeruginosa*, *P. fluorescens*, and *P. putida*) for the decolorization of Navitan Fast Blue S5R and found that *P. aeruginosa* exhibited maximum efficiency (72–92%) within 72 h. Silveira et al. (2009) compared 4 species (*P. oleovorans*, *P. putida*, *P. cepacia*, *and P. aeruginosa*) for the efficiency of decolorization of 14 commercial textile dyes. Among them, *P. aeruginosa* and *P. oleovorans* were more capable to decolorize ten textile dyes. The mixed consortia of *Pseudomonas*, *Acinetobacter*, *Escherichia*, *Enterobacter*, *Aspergillus*, and *Actinobacteria* were also found to significantly decolorize or degrade different kinds of azo dyes (Kadam et al. 2011; Yang et al. 2011a, b; Patel et al. 2012; Khan et al. 2014; Isaac et al. 2015; Kuppusamy et al. 2017; Sathishkumar et al. 2017).

*Pseudomonas* genus was also studied for the biotreatment of triphenylmethane dyes, used extensively as biological or dermatological agent, and in various processes in the food, medical, and textile industry (Sarnaik and Kanekar 1995, 1999; Yatome et al. 1981, 1990; Lin et al. 2004; Wu et al. 2009). Malachite Green and Crystal Violet dyes were extensively studied by several researchers (El-Naggar et al. 2004; Chen et al. 2007; Li et al. 2009; Huan et al. 2010; Kalyani et al. 2012; Chaturvedi et al. 2013). Enhancement of degradation of triphenylmethane dyes can be attained by adding glucose and sucrose as cosubstrates and heavy metals in the medium (Oranusi and Ogugbue 2005). Kalyani et al. (2012) showed that aminopyrine *N*-demethylase, MG reductase, and laccase enzymes were induced in *P. aeruginosa* NCIM 2074 and degraded Malachite Green into a non-toxic product. The same category of enzymes was also found to degrade heavy amounts of the dye (1800 mg/L) in *P. mendocina* (Chaturvedi et al. 2013). Li et al. (2009) isolated a strain of *Pseudomonas* sp. MDB-1 from water of an aquatic hatchery, capable of degrading various triphenylmethane dyes. Later on, *tmr2* gene encoding the enzyme (triphenylmethane reductase) was also fully characterized responsible for the biodegradation (Huan et al. 2010; Li et al. 2009). Zabłocka-Godlewska et al. (2014) compared SDz3 and Sz6 strains of *P. fluorescens* for the biodegradation of mixture containing triphenylmethane (Brilliant Green) and azo (Evans Blue) dyes. The strain Sz6 was able to degrade the dyes faster in shaken/semistatic conditions, and maximum removal (95.4%) was achieved in the case of Brilliant Green.

Various species of *Pseudomonas* were also reported for the removal of other xenobiotic compounds used for the preparation of dyes. The compounds include phenol by *P. putida* DSM 548, *Pseudomonas* CF600, and *P. stutzeri* (Sá and Boaventura 2001; Moharikar and Purohit 2003; Pazarlioğlu and Telefoncu 2005; Nowak and Mrozik 2018; Singh et al. 2018); 4-aminophenol by *Pseudomonas* ST-4 (Afzal Khan et al. 2006); pyridine by *Pseudomonas* sp. PI2 (Mohan et al. 2003); naphthalene and *p*-cresol by *P. putida* and *P. gessardii* LZ-E (Huang et al. 2016a, b;

Izmalkova et al. 2013; Surkatti and El-Naas 2014); chloroanilines by *P. putida* T57 (Nitisakulkan et al. 2014); polycyclic aromatic hydrocarbons by *P. stutzeri* (Álvarez et al. 2015); polynuclear aromatic hydrocarbons by *P. plecoglossicida* PB1 and *Pseudomonas* sp. PB2 (Nwinyi et al. 2016); and phenanthrene by *P. stutzeri* JP1 and P. *mendocina* NR802 (Mangwani et al. 2014; Kong et al. 2017).

## 13.4 Fungi

Many genera of fungi were also explored for the color removal from industrial effluents, especially actinomycetes and basidiomycetes (Chivukula and Renganathan 1995; McMullan et al. 2001). These organisms produce extracellular enzymes (laccase, peroxidases, and azoreductase) to catalyze dealkylation, oxidation, and hydroxylation reactions for the metabolism of dyes (Goszczynski et al. 1994). Most of the work was done for white rot fungus (*Phanerochaete*), as they are capable to degrade the majority of the azo dyes (Bumpus 1995; Banat et al. 1997; Cripps et al. 1990). The other fungal genera reported for the biodegradation of xenobiotic compounds are *Streptomyces*, *Lenzites*, *Coriolopsis*, *Neurospora*, *Penicillium*, *Pleurotus*, *Trichoderma*, and *Trametes* (Paszczynski et al. 1992; Chao and Lee 1994; Knapp and Newby 1999; Saparrat et al. 2014; He et al. 2018; Naraian et al. 2018; Pandey et al. 2018). The brown rot fungus (*Aspergillus*) has also shown potential to biodegrade a variety of toxic xenobiotic compounds and for the biotreatment of wastewater (Ali et al. 2010; Abd El-Rahim et al. 2017; Gomaa et al. 2011). Recently, Ning et al. (2018) reported biodegradation of 15 dyes by *Aspergillus flavus* A5p1 in a range of 61.7–100.0%. So there is always a need to explore the different strains/species of the *Aspergillus* for the degradations of the wide varieties of dyes (Table 13.3).

### 13.4.1 *Aspergillus*

The genus is composed of 340 species, widespread in diverse habitats, and reported as a pathogen, spoils food materials, and produces mycotoxins (Bennett and Klich 2003; Houbraken et al. 2016). They reproduce by asexual reproduction via conidiophores. The key to identify or classify various species of the genus is based on the size, color, and arrangement of asexual spores of conidiophores. Some species are associated with serious health problems like allergic bronchopulmonary aspergillosis, liver cancer (consumption of food containing mycotoxins), etc. (Hedayati et al. 2007). Most of the species are also used to produce beneficial products (enzymes, food fermenters, antibiotics, etc.) in biotechnology industry (Samson et al. 2014). To mention some of the species with beneficial/harmful effects are *A. flavus* (aflatoxin), *A. fumigatus* (cellulose, xylanase), *A. niger* (homologous or heterologous proteins), *A. oryzae*, *A. sojae* (food fermentation), *A. tamari* (Japanese soya sauces), and *A. terreus* (lovastatin, terrein) (Park et al. 2017). The present section reviews the diversity found within the *Aspergillus* species for the elimination of hazardous dyes from the industrial effluents (Table 13.3).

Initial studies for the wastewater treatment were mainly focused on the white rot fungus group, as they have lignin-degrading enzymes for the oxidation of organic compounds (Bumpus and Aust 1987). *Aspergillus* genus (brown rot fungi) was also explored for the removal of dyes in the industrial effluents. Ryu and Weon (1992) analyzed four species of *Aspergillus* (six strains) and one species of *Phanerochaete* (two strains) for the biodegradation of three azo dyes and stated that the former genus was much more effective in the process. Mainly two processes for the treatment of dyes in the solution or synthetic effluents were studied extensively, either biosorption or biodegradation (Conatao and Corso 1996; Fu and Viraraghavan 2000, 2002a; Sumathi and Manju 2000; Zope et al. 2007; Esmaeili and Kalantari 2011; Almeida and Corso 2014). The biosorption of dyes was influenced by their chemical structure and functional group on the surface of fungus (Fu and Viraraghavan 2002b, 2003). Parshetti et al. (2007) observed faster adsorption rate in *A. ochraceus* in the shaking conditions. The treatment of *Aspergillus* species with immobilization beads, autoclaving, and specific compounds also accelerates the process of decolorization (Wang and Hu 2007; Wang et al. 2008; Patel and Suresh 2008). Yang et al. (2011a, b) demonstrated higher biosorption capacity in the CDAB (cetyldimethylammonium bromide) modified biomass of *A. oryzae*. The same type of result was seen by Huang et al. (2016a, b) while investigating the effect of heavy salts, metals, and SDS on the adsorption kinetics of chemically modified (cetyltrimethylammonium bromide) *A. versicolor*. They found a close relationship between low pH (2.0) and heavy metals on the biosorption rate. The chemical modification increases the surface area and functional groups. Naskar and Majumder (2017) used response surface methodology for *A. niger* and demonstrated that adsorption rate depends upon the concentration of biomass, temperature, and pH of the solution. Further, they also revealed that amine and carboxyl groups play an important role in dye sorption along with electrostatic interactions. The same type of phenomena was observed by the authors using different dyes and the same species (Xiong et al. 2010; Mahmoud et al. 2017). The high temperature and low pH range (1–3) in the solution speed up the uptake of the dyes, as the biosorption is mostly endothermic (Akar et al. 2009). This type of condition increases the kinetic energy and diffusion rate (Ramya et al. 2007; Aksu and Karabayır 2008; Abdallah and Taha 2012). Contradictory to this, other authors reported optimal temperature (28–30 °C) and pH (5) as much more favorable condition for the biodegradation of azo dyes (Ali et al. 2007a, b; Ameen and Alshehrei 2017; Sharma et al. 2009) by four *Aspergillus* spp. The nutritional condition needs to be standardized as sources of nitrogen and carbon in the medium, as they are also a detrimental factor for the rate of dye removal (Kaushik and Malik 2010, 2011). Gomaa et al. (2017) demonstrated the role of calcium chloride as stress response in *A. niger* and high removal efficiency for commercial dye Malachite Green.

The live fungal strains were extensively studied for the decolorization of dyes from industrial effluents; however, some workers used pellets and dead biomass for the process and found promising results as compared to the living strains (Abdallah and Taha 2012; Abdel Ghany and Al Abboud 2014; Lu et al. 2017). The formation of bioflocculants and silver and zinc oxide nanoparticles using different *Aspergillus*

**Table 13.3** Removal of different dyes by *Aspergillus* species

| Sr. no. | *Aspergillus* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time/ percentage | By-product | References |
|---|---|---|---|---|---|---|
| 1. | *A.* sp. CB-TKL-1 | Methyl Violet (5–30 mg $L^{-1}$) | Biosorption and biodegradation | 24 h (100%) | *N*-Demethylated compounds | Kumar et al. (2011, 2012) |
| | | Brilliant Green (5–20 mg $L^{-1}$) | | 72 h (99.24%) | | |
| 2. | *A.* sp. TS-A CGMCC 12,964 | Mordant Yellow 1 | Degradation (lignin oxidases) | 1 h | – | Kang et al. (2017) |
| 3. | *A.* sp. | Reactive Red, Yellow, Black, Blue, Coloron Violet, and Black | Decolorization | 24 h (9–99%) | – | Ramya et al. (2007) |
| 4. | *A. carbonarius* M333 | Congo Red (25–125 mg $L^{-1}$) | Biosorption | – | – | Bouras et al. (2017) |
| 5. | *A. flavus SA2* | Drimarene Blue K2RL (50 mg $L^{-1}$ ) | Biosorption and biodegradation | 24 h (71.3%) | 1,4-Dihydroxyanthraquinone, 2,3-dihydro-9,10-dihydroxy-1,4-anthracenedione, phthalic acid, and benzoic acid | Andleeb et al. (2012) |
| 6. | *A. flavus* | Reactive Red 198 (25–100 ppm) | Decolorization | 24 h (84.96%) | – | Esmaeili and Kalantari (2011) |
| 7. | *A. flavus* A5p1 | Direct Blue 71, Direct Blue 86, and Reactive Blue (100–1000 mg $L^{-1}$ ) | Biosorption and biodegradation | (61.7–100.0%) | | |
| 8. | *A. foetidus* | Drimarene Red BF F3B1, Drimarene Navy Blue BF F2G1, and Drimarene Black HFGR1 (50, 100 mg $L^{-1}$) | Decolorization | 72 h (85–95%) | – | Sumathi and Manju (2000) |
| | | Drimarene Red (50 mg $L^{-1}$) | Decolorization | 24 h (50%) | – | Bidisha et al. (2006) |

| Sr. no. | *Aspergillus* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time/ percentage | By-product | References |
|---|---|---|---|---|---|---|
| | | Reactive Black 5 (100 mg $L^{-1}$) | Biosorption | 2 h (97%) | – | Patel and Suresh (2008) |
| 9. | *A. fumigatus* | Methylene Blue (5 mg $L^{-1}$) | Biosorption | 90 min. (80%) | – | Abdallah and Taha (2012) |
| | | Reactive Brilliant Red (96.6 mg $L^{-1}$) | Biosorption | 48 h | – | Wang et al. (2008) |
| 10. | *A. fumigatus fresenius* | Acid Red 151 (100–200 mg $L^{-1}$) | Biosorption | 5 days (41.62–84.77%) | – | Sharma et al. (2009) |
| 11. | *A. lentulus* FJ172995 | Acid Blue 120 (100–300 mg $L^{-1}$) | Decolorization | 24 h (84.48–99.97%) | – | Kaushik and Malik (2011) |
| 12. | *A. niger* | Basic fuchsin, nigrosin, and Malachite Green | Biosorption | 6 days | — | Rani et al. (2014) |
| | | Procion Blue MX-G (100 µg $mL^{-1}$) | Biosorption | 120 min. (99%) | – | Conatao and Corso (1996) |
| | | Basic Blue 9 | Biosorption | 30 h | – | Fu and Viraraghavan (2000) |
| | | Congo Red (50 mg $L^{-1}$) | Biosorption | 42 h | – | Fu and Viraraghavan (2002a) |
| | | Acid Red 27, Acid Red 151, Reactive Blue 19, and Reactive Blue 81 (20–100 mg $L^{-1}$) | Biosorption and biodegradation | 9 days (70–80%) | – | Ali and El-Mohamedy (2012) |

(continued)

**Table 13.3** (continued)

| Sr. no. | *Aspergillus* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time/ percentage | By-product | References |
|---|---|---|---|---|---|---|
| | | Direct Blue 199 ( 400 mg $L^{-1}$) | Biosorption | 4 h | – | Xiong et al. (2010) |
| | | Direct Red (100–1000 mg $L^{-1}$) | Biosorption and biodegradation | 7 days (58.6–99.69%) | – | Mahmoud et al. (2017) |
| | | Synozol Red HF6BN and Synozol Yellow HF2GR | Biosorption | 18 h (88%) | – | Khalaf (2008) |
| 13. | *A. niger grp* | Reactive Red 195 and Reactive Green 11 (50–200 ppm) | Decolorization | 72 h (75.4, 62.9%) | – | Zope et al. (2007) |
| 14. | *A. niger* SA1 | Acid Red 151, Orange II, Drimarene Blue K2RL, and Sulfur Black | Biosorption and Biodegradation | 8 days (9.33–68.64%) | – | Ali et al. (2007a, b) |
| 15. | *A. niger* ZJUBE-1 | Congo Red | Biosorption and biodegradation | (>98.5%) | – | Lu et al. (2017) |
| 16. | *A. ochraceus* | Reactive Blue 25 (100 mg $L^{-1}$) | Biosorption and biodegradation (lignin peroxidase, laccase, and tyrosinase) | 7 h (100%) | Phthalimide and diisobutyl phthalate | Parshetti et al. (2007) |
| 17. | *A. parasiticus* | Reactive Red 198 (100–300 mg $L^{-1}$) | Biosorption | 50 min. (98.57%) | – | Akar et al. (2009) |
| 18. | *A. sojae* B-10 | Amaranth, Sudan III, and Congo Red | Decolorization | 4–8 days (100%) | – | Ryu and Weon (1992) |
| 19. | *A. versicolor* | Reactive Black 5 (200 mg $L^{-1}$) | Biosorption | 420 min. (98%) | – | Huang et al. (2016a, b) |
| 20. | *A. wentii* | Brilliant Blue G (119.3–544.8 mg $L^{-1}$) | Biosorption | 3 h | – | Khambhaty et al. (2012) |

| Sr. no. | *Aspergillus* species | Dyes (concentration) | Mechanism (enzyme(s)) | Removal time/ percentage | By-product | References |
|---|---|---|---|---|---|---|
| 21. | *A. flavus*<br>*A. terreus* | Acid Red 151 and Orange II (20 mg $L^{-1}$) | Biosorption and biodegradation | 96 h (48–97%) | α-Naphthol, sulfanilic acid, and aniline | Ali et al. (2010) |
| 22. | *A. lentulus*<br>*A. fumigatus* | Reactive Remazol Red, Reactive Blue, and Reactive Yellow dyes | Biosorption and bioaccumulation | 4 h | – | Mathur et al. (2018) |
| 23. | *A. niger*<br>*A. terreus*<br>*A. oryzae*<br>*A. flavus*<br>*A. fumigatus*<br>*A. alabamensis* | Reactive Red, tartrazine, Direct Blue, Naphthol Blue Black, Direct Red, Trypan Blue, Direct Violet, Janus Green, Reactive Blue, Alizarin Yellow, Reactive Orange, Evans Blue, Fast Green, Brilliant Green, Methyl Red, Safranin, Crystal Violet, pararosaniline, Allura Red, and Ponceau S (100 µg/mL) | Biosorption and biodegradation | 5 days (0.2–93.3%) | – | Abd El-Rahim et al. (2017) |
| 24. | *A. niger*<br>*A. terreus* | Procion Red MX-5B (200 µg/mL) | Biosorption and biodegradation (azoreductase) | 336 h (98%) | Primary amines | Almeida and Corso (2014) |
| 25. | *A. niger*<br>*A. terreus*<br>*A. flavus*<br>*A. fumigatus* | Reactive Red 120 (100 ppm) | Biodegradation | 7 days (84–86%) | Sodium 2-aminobenzenesulfonate | Ameen and Alshehrei (2017) |
| 26. | *A. flavus*<br>*A. fumigatus*<br>*A. ochraceus*<br>*A. puniceus*<br>*A. sulphureus*<br>*A. versicolor* | Reactive Blue 19, Poly R-478, Poly S-119, Acid Blue 113, Acid Blue 225, Acid Red 111, Reactive Blue 214, Reactive Blue 41, Reactive Blue 49, Reactive Red 243, Direct Blue 81, and Direct Red 80 (200, 1000 ppm) | Biosorption | 2 h (90%) | – | Anastasi et al. (2009) |

spp. has also the potential for the color removal from industrial effluents (Deng et al. 2005; Muthu Kumara Pandian et al. 2016; Kalpana et al. 2018a, b). Copete-Pertuz et al. (2019) demonstrated that *A. terreus* in combination with *Trichoderma viride* can act as a co-inducer for *Leptosphaerulina* sp. ligninolytic enzyme activity and improved removal of Reactive Black 5 dye.

Survey of literature reveals that most of the studies were related to the biosorption mechanism rather than the degradation. The metabolites formed during degradation process are shown in Table 13.3. The enzymes involved in the biodegradation were laccase, manganese peroxides, and lignin-modifying enzymes, which mineralize synthetic lignin of dyes (Ali and El-Mohamedy 2012; Hasanin et al. 2019). Azoreductase is one of the key enzymes found in the degradation pathways of the organism. Ameen and Alshehrei (2017) found laccase and azoreductase to be involved in the degradation of Reactive Red 120 into sodium 2-aminobenzenesulfonate. Tamayo-Ramos et al. (2012) characterized three forms of laccase-like multicopper oxidase enzymes having high catalytic activity for several phenolic compounds and synthetic dyes. The optimization process for the high production and activity of laccase enzyme has been done for several *Aspergillus* species. The factors associated are pH, temperature, carbon and nitrogen sources, inoculum size, etc. (Jin and Ning 2013; Benghazi et al. 2013; Kumar et al. 2016). Recently, Abd El-Rahim et al. (2017) isolated 18 strains belonging to 6 species from the wastewater sample and evaluated them against 20 azo dyes. The most resistant dye was Fast Green azo dye, and easily degradable dyes were Direct Violet and Methyl Red. The decolorization process was enhanced by glucose supplementation, and the limiting factor was a nitrogen source, as in its absence the strains were unable to produce lignin peroxidase enzyme. The high pH has been also shown to be related to the low formation of residual products (Ali et al. 2007a, b).

The different *Aspergillus* species have shown very much diversity in the biodegradation of various dyes. Anastasi et al. (2009) compared five species of mitosporic fungi (*Penicillium*, *Cladosporium*, and *Aspergillus*) for the removal of nine industrial and two model dyes. They found that *A. ochraceus* and *A. flavus* were efficient for the decolorization of all the dyes tested and one species, i.e., *A. ochraceus*, causes over 90% decolorization against simulated effluents. Similarly, other workers found the maximum potential of *Aspergillus* as compared to *Penicillium* (Ali et al. 2010; Gomaa et al. 2011; Ali and El-Mohamedy 2012). Khalaf (2008) tested the effectiveness of *Spirogyra* sp. (green algae) and *A. niger* against the reactive dye (Synozol) in textile wastewater. The autoclaved biomass of the both species exhibited 88% and 85% dye removal, respectively. Some species have higher absorption capacity, but still they lack the ability to degrade them into non-toxic metabolites (Almeida and Corso 2014).

The degraded products should be checked for the toxicity assays, as decolorization does not always lead to the absence of toxicity, rather forming incomplete toxic metabolites (Almeida and Corso 2014). The extracellular enzymes were found to degrade triphenylmethane dye by stepwise demethylation into non-toxic *N*-demethylated products (Kumar et al. 2011, 2012). Andleeb et al. (2012)

investigated the toxicity of degraded products formed during biodegradation of Drimarene Blue dye by *A. flavus*. As compared to dye treatment, the germination and morphological characteristics in *Lolium perenne* were somewhat near to the untreated. Similarly, Parshetti et al. (2007) observed that germination of *Phaseolus mungo* was high or near to control in comparison to the Malachite Green treatment.

## 13.5 Conclusion

The treatment of industrial effluents with cost-effective methods is the urgent need of the society. The literature shows that aerobic and anaerobic conditions were well utilized by algae, bacteria, and fungi for the management of dyes. The effluents also serve as a growth substrate or also can be used to extract biomass. The integrated approach of remediation as successive treatment along with extraction of enzymes, lipids, and biofuels seems to be the best practice for sustainable development. The mixed consortium of best strains of algae, bacteria, and fungi should be tested for the degradation of toxic dyes. Genetically engineered strains may be used for the degradation of toxic amines in the severe environmental conditions. Toxicity assays clearly show which strain is best for the future applications to clear the water for recycling.

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