Bioremediation Technologies

for Decolorization of Effluent

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Abstract

Wastewater from the textile industry contains significant amounts of synthetic dyes that require treatment to prevent groundwater contamination. These synthetic dyes are stable and are highly persistent in nature. The search for innovative, cost-effective, and environment-friendly technologies has become the real challenge in recent years. In view of the need for a technical and economically satisfying treatment technology, a flurry of emerging technologies has been proposed and examined at different stages of commercialization. Appliance of biotechnological techniques in recent period emerged as a very promising area for decolorization of textile wastewater, i.e., targeted at breaking down the dye molecule to basic elements (mineralizing them), and has much less environmental impact than conventional methods. A lot of research in this field revealed the existence of a variety of microbial communities capable of decolorizing a wide group of dyes. This chapter reviews the usage of various microorganisms such as bacteria, fungi, algae, and microbial consortium as free cells or in immobilized form for the decolorization of different types of textile dyes. The performance and results of latest research studies with pure and mixed cultures in various reactors have been also compiled pertaining to the bioremediation of dyes and colorants from wastewater with the possible alternative emerging technologies.

Keywords

Bioremediation • Decolorization • Microbial consortia • Textile dyes

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6.1 Introduction

Due to rapid industrialization and urbanization, manufacturing and usage of synthetic dyes have been increased in various sectors. Dyes are the substances that impart color to the substrate. They adhere on compatible surfaces by mechanical retention, physical adsorption, and formation of covalent bond/complexes with metals. Dyes are used in the textile industry, leather tanning industry, paper production, food industry, photography, wood staining, biological and chemical research, pharmaceutical and medicine, light-harvesting arrays, photo-electrochemical cells, hair colorings, and cosmetics. The color of dye is combined effects of chromophores, delocalized electron system with conjugated double bonds, and auxochrome–electronwithdrawing or electron-donating substituent that enhance the color of chromophore by changing the overall energy of electron system. In addition to enhance the chromophore in production of color, auxochromes are also responsible for the solu-bility of dye and increase its reactivity toward fibers (Dos Santos et al. [2005\)](#page-24-0).

Textile industry is one of the greatest consumers of water uses about 100 L of raw water per kg of textile materials in dying process. So during dyeing and finishing operations, approx. 200,000 tons of these textile dyes are lost to effluent every year (Jin et al. [2007\)](#page-25-0). The discharge of highly colored synthetic dye effluent can be very damaging to the receiving water bodies since these dyes in the water strongly absorb sunlight that decrease the light intensity absorbed by the plants and phytoplankton-reducing photosynthesis and the oxygenation of water reservoir. Moreover, the presence of unnatural color is aesthetically unpleasant and tends to be associated with contamination. In addition, dyes used in the textile industries are toxic to aquatic organism and can be resistant to natural biological degradation.

During the last few years, stringent regulations coupled with increased enforcement concerning colored wastewater discharges have been established in many countries. Government legislation is becoming more and more stringent, especially in the more developed/developing countries, regarding the removal of dyes from industrial effluent (Robinson et al. [2001](#page-28-0)). Enforcement of this law will continue to ensure that textile and other dye-utilizing industries treat their dye-containing effluent to the required standards. So, traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. A wide range of methods has been developed for removal of synthetic dyes from aqueous solution to decrease their impact on the environment.

They are divided in following major categories:

- Physical method: Adsorption, (activated carbon, peat, bagasse, wood chips, fly ash and coal, silica gel), irradiation, ion exchange, electrokinetic coagulation
- *Chemical method*: Oxidative process, Fenton's reagent, ozonation, sodium hypochlorite photochemical
- Biological method: Activated sludge process, enzymatic treatment, anaerobic process
- Emerging technologies: Advanced oxidation process, membrane filtration, photocatalysis, sonication, redox mediators and engineered wetland systems, etc.

Biological and chemical methods involve the destruction of the dye molecule, while physical methods usually transfer the pollutant to another phase. Many of the conventional methods used for treating dye wastewater have not been widely applied on large scale as a result of the high operational cost and sludge disposal problems associated with them. Due to the complex nature of dye effluent, there is hardly any single method to treat the dye wastewater efficiently. So, combinations of different process are preferred to achieve the economical and desired level water quality (Saratale et al. [2010](#page-28-0); Loncˇar et al. [2013;](#page-27-0) Karthik et al. [2014\)](#page-26-0). Different combinations of treatment methods have been proposed in order to effectively manage the textile wastewater. Thus, chemical coagulation-flocculation, chemical oxidation, activated carbon adsorption, and anaerobic biological treatment usually combined with a activated sludge secondary treatment step are among the most well-known techniques (Hao et al. [2000;](#page-25-0) Robinson et al. [2001](#page-28-0); Forgacs et al. [2004](#page-25-0); Joshi et al. [2004](#page-26-0)).

6.2 Biological Methods

Bioremediation, or the use of microbial techniques to deal with pollution, is a key research area in the environmental sciences. Microbes acclimatize themselves to the toxic wastes, and new resistant strains develop naturally, which then transform various toxic chemicals into less harmful forms.

The ability of biological treatment for decolorizing of industrial effluent is ambiguous, different, and divergent. A number of biotechnological approaches have been suggested by recent research as of potential interest toward combating this pollution source in an eco-efficient manner, including the use of bacteria or fungi often in combination with physicochemical processes (Beydilli et al. [1998;](#page-24-0) Willmott et al. [1998](#page-29-0); Borchert and Libra [2001](#page-24-0); McMullan et al. [2001](#page-27-0); Robinson et al. [2001;](#page-28-0) Zissi and Lyberatos [2001\)](#page-30-0). Bioremediation of textile effluent using pure bacterial and fungal decolorization are represented in Table [6.1.](#page-3-0) Bioremediation systems were commonly applied in the treatment of industrial effluent using many microorganisms such as bacteria, yeasts, algae, and fungi that have capability to accumulate and degrade different pollutants. There were three principle advantages of biological technologies for the removal of pollutants; first, biological processes can be carried out in situ at the contaminated site; second, bioprocess technologies are usually environmentally benign (no secondary pollution); and third, ex situ method is cost-effective.

Recent fundamental work has revealed the existence of wide variety of microorganisms capable of decolorizing wide range of dyes. The use of microorganisms for the removal of synthetic dyes from industrial effluent offers considerable advantages, and the process was relatively inexpensive, running costs were low, and the end products were completely mineralized with no toxicity. Biodegradation is defined as biologically mediated breakdown of chemical compounds. When biodegradation is complete, the process is called mineralization, i.e., the total breakdown of organic molecules into water, carbon dioxide, and/or any other inorganic end products (Banat and Faison [1999](#page-23-0)). Degradation by mixed culture enhances the degradation process since individual strains attack the dye

molecule at different positions or uses decomposed products produced by one strain will be further decomposed by another strain (Mohana et al. [2008](#page-27-0)). However, it was stressed that the composition of mixed cultures may change during the decomposition process, which interferes with the control of technologies using mixed cultures. Moreover, the efficacy of decomposition considerably depends on the chemical character of the synthetic dye and biodegradation capacity of the microorganism consortium (Schliephake et al. [2000\)](#page-29-0). Decolorization of dyes with pure culture was found to be impractical, as the isolated culture would be dye specific, and their application in large-scale wastewater treatment plants with a variety of contaminant dyes was not feasible (Murugesan and Kalaichelvan [2003\)](#page-27-0). Efficient biodegradation of dyes can be accomplished when catabolic activity of individual strain was complement with each other in a mixed culture community. The other biological treatment method that includes bioaccumulation was defined as the accumulation of pollutants by actively growing cells by metabolism and temperature-independent and metabolism-dependent mechanism steps (Nigam et al. [2000](#page-27-0); Robinson et al. [2001;](#page-28-0) Ola et al. [2010;](#page-28-0) Tan et al. [2013](#page-29-0); Saratale et al. [2013\)](#page-29-0). These processes have potential to mineralize dyes to harmless inorganic compounds like carbon dioxide, water, and the formation of relatively insignificant amount of sludge.

6.3 Microbial Decolorization

The application of microorganisms for the biodegradation of synthetic dyes is an attractive and simple method by operation. However, the biological mechanisms can be complex. The large number of species has been tested for decolorization and mineralization of various dyes. Besides the traditional wastewater cleaning technologies, other methods have been employed in the microbial decolorization of dyes. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts, and algae capable of degrading azo dyes (Chen et al. [2003;](#page-24-0) Daneshvar et al. [2007](#page-24-0); Kalyani et al. [2009](#page-26-0)).

6.3.1 Bacterial

Numerous bacterial strains isolated from the contaminated sites of textile dyes, having the ability to decolorize dyes, have been reported by various researchers (Table [6.2\)](#page-6-0). Bacterial cultures capable of degrading azo dyes are Bacillus subtilis (Mabrouk and Yusef [2008\)](#page-27-0), Aeromonas hydrophila (Ogugbue and Sawidis [2011\)](#page-27-0), and Bacillus cereus (Ola et al. [2010\)](#page-28-0). Klebsiella pneumoniae RS-13 and Acetobacter liquefaciens S-1 having the ability to decolorizing textile industrial effluent containing methyl red have been reported for bioremediation of azo dye (Wong and Yuen [1996](#page-29-0)). An efficient isolated species of Pseudomonas from soil degraded and decolorized dyes belonging to triphenylmethane and azo group. Malachite green, fast green, brilliant green, Congo red, and methylene blue were decolorized in the range of 30–70% under aerobic condition (pH 6–8 and temp.

Table 6.2 (continued) Table 6.2 (continued)

 $30-40$ °C) (Mali et al. [2000](#page-27-0)). Decolorization of Congo red and direct black 38 were carried out using E. coli and Pseudomonas sp. under anaerobic, aerobic, and microaerophilic conditions (Isik and Sponza [2003\)](#page-25-0). Color of the Congo red and direct black 38 was removed up to 98 and 72%, respectively, by E. coli at the end of anaerobic incubation, while no color was observed under aerobic incubation, whereas under microaerophilic condition, the azo dyes such as Congo red and direct black 38 were decolorized by E. coli up to 39 and 75%, respectively, 5-day incubation with anaerobic Pseudomonas sp., and showed 100% color removal. Further, out of the six bacterial strains which were isolated from sludge samples and mud lakes having ability of degrading textile dyes, Aeromonas hydrophila exhibited the higher color removal efficiency with various dyes (Chen et al. [2003](#page-24-0)) under optimal conditions (pH 5.5–10, temp. 20–35.8 °C). More than 90% of decolorization of red RBN was examined within 8 days at a dye concentration of 3000 mg/L. Pearce et al. ([2003\)](#page-28-0) presented an excellent review on the color removal from textile wastewater using whole bacterial cells. Mixed cultures of bacteria from a wide variety of habitats have also been shown to decolorize the diazo-linked chromophore of dye molecule in 15 days (Nagarathnamma et al. [1999](#page-27-0)). Ramya et al. [\(2010](#page-28-0)) reported 100% and 92% effective decolorization of indigo carmine by Paenibacillus larvae under shaking condition. Moosvi et al. [\(2007](#page-27-0)) also reported decolorization of Reactive Violet 5R (100 mg/L) by a microbial consortium consisted Paenibacillus polymyxa and Micrococcus sp. within 36 h, whereas individual isolates could not show decolorization even on extended incubation.

Similarly, Palamthodi et al. [\(2011](#page-28-0)) also revealed effective decolorization of green and blue dye by Paenibacillus azoreducers and further investigated 70% decolorization of textile wastewater by microbial flocs consisted Bacillus sp., Paenibacillus sp., Achromobacter sp., etc. and their ability to accumulate heavy metals present in the textile wastewater. Cetin and Donmez (2006) (2006) revealed that E. coli and Pseudomonas luteola had the ability to decolorize reactive black B, remazol Blue, and reactive red RB at pH 7.0 with constant decolorization rates up to pH 9.5. In contrast Klebsiella pneumoniae RS-13 completely degrades methyl red in pH range from 6.0 to 8.0 (Wong and Yuen [1996](#page-29-0); Mali et al. [2000](#page-27-0)). A pH from 7 to 8.5 has been reported as the optimum pH for the decolorization of reactive red 5 (Moosvi et al. [2005\)](#page-27-0) and reactive violet 5R (Moosvi et al. [2007](#page-27-0)).

6.3.2 Fungi

Fungi have proved to be a suitable organism for treatment of textile effluent and dye removal. Fungal mycelia have an additive advantage over single cell organisms by solubilizing the insoluble substrates by producing extracellular enzymes. Due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. The extracellular nature of the fungal enzymes is also advantageous in tolerating high concentrations of the toxicants. Many genera of fungi have been reported for the various types of textile dye decolorization (Table [6.3](#page-13-0)). White rot fungi are most efficient in breaking down synthetic dyes as

Table 6.3 Decolorization of dyes with fungi isolated from contaminated sites **Table 6.3** Decolorization of dyes with fungi isolated from contaminated sites

these constitute a diverse ecophysiological group comprising mostly basidiomycetous fungi capable of extensive aerobic lignin depolymerization and mineralization. This property is based on the WRF's capacity to produce one or more extracellular lignin-modifying enzymes (LME), which is due to their lack of substrate specificity are also capable of degrading a wide range of xenobiotics.

Extracellular production of ligninolytic enzymes by mycelium growing on solid malt extract/glucose medium supplemented with different dyes (malachite green, azure B, poly R-478, anthraquinone blue, Congo red, and xylidine), dye decolorization, and the relationship between these two processes were studied with 26 white rot fungi from Argentina (Levin et al. [2004](#page-26-0)). Only ten strains decolorized all the dyes and produced laccase, lignin peroxidase, and manganese peroxidase on solid medium. Comparing the isolates with the well-known dye degrader *Phanerochaete* chrysosporium, a new fungus, Coriolus versicolor, is potentially a candidate for use in biodecolorization processes. Eighteen-day-old cultures of P . *chrysosporium* were able to decolorize within hour up to 28, 30, 43, 88, and 98% of xylidine (24 mg/L), poly R-478 (75 mg/L), remazol brilliant blue R (9 mg/L), malachite green (6 mg/L), and indigo carmine (23 mg/L), respectively. Moreover, five species of white rot fungi were further evaluated for their ability to decolorize amaranth, remazol black B, remazol orange, remazol brilliant blue, reactive blue, and tropaeolin O in agar plates, Bjerkandera sp. BOS55, Phanerochaete chrysosporium, and Trametes versicolor. In static aqueous culture, the three cultures form fungal mats, which did not decolorize any dye beyond some mycelial sorption. When agitated at 200 rpm, the biomass grew as mycelial pellets. Bjerkandera sp. BOS55 pellets decolorized only amaranth, remazol black B, and remazol orange dye. Batch cultures of Bjerkandera sp. BOS55 and P. chrysosporium had a limited ability to decolorize repeated dye additions; however, T. versicolor rapidly decolorized repeated additions of the different dyes and dye mixtures without any visual sorption of any dye to the pellets (Swamy and Ramsay [1999](#page-29-0)). Similarly, decolorization and biodegradation of orange II, tropaeolin O, Congo red, and azure B with white rot fungus, Phanerochaete chrysosporium, were demonstrated by Cripps et al. [\(1990](#page-24-0)). Nyanhongo et al. ([2002\)](#page-27-0) examined the four ligninolytic fungi, namely, Trametes modesta, Trametes hirsuta, Trametes versicolor, and Sclerotium rolfsii, having the ability to produce fungal laccases which were screened for their ability to decolorize dyes such as anthraquinone, azo, indigo, and triarylmethane. The decolorization rate of this laccase increased with the rise in temperature to 50–60 °C. The decolorization efficiency of T . modesta laccase was improved remarkably in the presence of mediators like 1-hydroxybenzotriazole and 2-methoxyphenothiazine.

The strain Aspergillus fumigatus XC6 isolated from mildewing rice straw was found to be capable of decolorizing dyes effluent over a pH range 3.0–8.0 with the dyes as sole carbon and nitrogen sources (Jin et al. [2007\)](#page-25-0). The optimum pH was 3.0; however, supplemented with either appropriate nitrogen sources (0.2% NH₄Cl or $(NH_4)_{2}SO_4$ or carbon sources $(1.0\%$ sucrose or potato starch), the strain decolorized the effluent completely at the original pH of the dyes effluent. A new azo dye-decolorizing fungi strain identified as Penicillium sp. based on 26S rRNA

gene sequence analysis was isolated from activated sludge (Gou et al. [2009\)](#page-25-0). Penicillium sp. could aerobically decolorize 70% of reactive brilliant red X-3B at optimum pH 4–5, up to salinity 6% by the way of bioadsorption, and nutrient-poor medium was more beneficial for adsorption. Furthermore, the decolorization of azo dyes by fungal-bacterial co-cultures demonstrated that Penicillium sp. and Sphingomonas xenophaga QYY co-cultures performed better than any single strain.

6.3.3 Algae

Algae have become significant organisms for biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic, inorganic toxic substances, and radioactive matters in their cells. Biological wastewater treatment systems with microalgae have particularly gained importance in the last 50 years, and it is now widely accepted that algal wastewater treatment systems are as effective as conventional treatment systems. These specific features have made algal wastewater treatment systems significant low-cost alternatives to complex expensive treatment systems particularly for purification of municipal waste-water (Table [6.4](#page-17-0)). The microalgae biomass production from textile waste effluent is a possible solution for the environmental impact generated by the effluent discharge into water sources. Pure and mixed algal cultures removed 50–70% of color within 3 months of incubation, and color reduction pattern showed a rapid removal rate phase followed by declining removal rate phase. Color removal by algae was due to three intrinsically different mechanisms of assimilative utilization of chromophores for production of algal biomass, $CO₂$ and $H₂O$ transformation of colored molecules to noncolored molecules, and adsorption of chromophore on algal biomass. A report of algae capable of degrading azo dyes, through an induced form of an azo reductase, showed good color removal (Jinqui and Houtian [1992](#page-25-0)). Several species of Chlorella and Oscillatoria were capable of degrading azo dyes to their aromatic amines and to further metabolize the aromatic amines to simpler organic compounds. Some were even capable of utilizing azo dyes as their sole carbon and nitrogen source. Use of such algae in stabilization ponds was proposed by Banat et al. [\(1996](#page-23-0)) as they play an important role in aromatic amine removal. The biodegradation of azo dyes by the algae (Chlorella pyrenoidosa, C. vulgaris, and Oscillatoria tenuis) has also been assessed (Liu and Liu [1992](#page-26-0)). In addition, the algae can play a direct role in degradation of azo dyes. Chlorella vulgaris which have biosorption capacity for several reactive dyes were reported by Aksu ([2005\)](#page-23-0). Dried Spirogyra rhizopus have the ability to decolorize acid red 274 dye by both biosorption and biocoagulation process, and the removal amounts decreased, while the removed concentration of AR 274 dye increased with increasing S. rhizopus concentration (Ozer et al. [2006](#page-28-0)). The potential of Cosmarium sp. belonging to green algae was investigated as a viable biomaterial for biological treatment of triphenylmethane dye and malachite green (Daneshvar et al. [2007\)](#page-24-0). Immobilized thermophilic cyanobacterial strain Phormidium sp. has good decolorization activity under thermophilic condition (Ertugrul et al. [2008\)](#page-25-0). Agitated batch sorption performed on

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algae Spirogyra 102 revealed the ability of test biosorbent to remove azo dye from the aqueous phase at acidic pH 2 at optimized temperature 30 \degree C and dye concentration 5 mg/L (Verkata Mohan et al. [2008\)](#page-29-0). C. vulgaris culture in the textile waste effluent demonstrated the possibility of using this microalga for the color and COD removal and for biomass production. The cultivation of C. vulgaris presented maximum cellular concentrations C_{max} and maximum specific growth rate μ_{max} in the wastewater concentration of 5.0% and 17.5%, respectively (El-Kassas and Mohamed [2014](#page-24-0)).

6.4 Enzymatic Treatment

Enzymes are able to break apart large sludge particles, creating more surface area for microbes to attack. This allows for a more complete and more efficient degradation of the sludge particles. Such particles are held together by extracellular polymeric substances that come from cell autolysis, bacterial metabolic reactions, and wastewater itself. Researcher has recognized the potential for enzymatic treatment systems. Hence, enzymes are the ultimate molecules which deal with the dye compounds and bring about cleavage and successive degradation. The initial step in degrading the azo dye is to cleave the electrophilic azo linkage, which immediately causes decolorization. Azoreductase brings about the cleavage of azo linkages in compounds containing azo bond to produce aromatic amines. A large number of enzymes from different plants and microorganisms have been reported to play an important role in array of waste treatment applications. The enzymatic decolorization of industrial dyes was a big challenge due to large diversity of chemical structures (Wesenberg et al. 2003; Akhtar et al. [2005\)](#page-23-0). Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products (Akhtar and Husain [2006\)](#page-23-0). Enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solutions (Duran and Esposito [2000](#page-24-0)). Oxidoreductive enzymes, polyphenol oxidases, and peroxidases are participating in the degradation/removal of aromatic pollutants from various contaminated sites (Husain and Jan [2000;](#page-25-0) Bhunia et al. [2001\)](#page-24-0). Polyphenol oxidases can act on a broad range of substrates such as substituted polyphenols, aromatic amines, benzene thiols, and a series of other easily oxidizable compounds. Thus, they can catalyze the decolorization and decontamination of organic pollutants. White rot fungi were able to degrade dyes using lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) (Murugesan et al. [2007](#page-27-0)). Other enzymes used for this purpose include H_2O_2 producing enzymes, such as glucose-2-oxidase along with laccase and phenol oxidase enzyme (Husain [2010\)](#page-25-0).

Decolorization of eight synthetic dyes including azo, anthraquinone metal complex, and indigo was examined in white rot fungi by peroxidase-catalyzed oxidation (Young and Yu [1997\)](#page-30-0). The dyes were not decolorized by manganese-dependent peroxidase (MnP), and while above 80% color was removed by ligninase-catalyzed oxidation, further dye decolorization rate increased linearly with ligninase dosage

(Lip). Some azo and heterocyclic dyes were almost completely degraded by P. chrysosporium in ligninolytic solution but decolorized to different extent (0–80%) by crude ligninase (Cripps et al. [1990\)](#page-24-0). Peralta-Zamora et al. [\(1999](#page-28-0)) reported that enzymatic process promotes quick decolorization of the dye; nevertheless, maximum decolorization degree of about 30% is insignificant in relation to the decolorization degree achieved by the other processes. The enzymatic activity of four white rot fungi, viz., P. tremellosa, P. ostreatus, B. adusta, and C. versicolor, has the ability to produce ligninolytic enzymes, which decolorize dyes in artificial effluent (Robinson et al. [2001](#page-28-0)). Recently enzyme membrane reactors are emerging for wastewater treatment more specifically for dye decolorization (Lopez et al. [2002](#page-27-0)). In view of the potential of the enzymes in treating the phenolic compounds, several microbial and plant oxidoreductases have been employed for the treatment of dyes, but none of them has been exploited at large scale due to low enzymatic activity in biological materials and high cost of enzyme purification. In order to improve polyphenol oxidases activity and stability, enzyme immobilization technology has been applied. This technology is an effective means to make enzymes reusable and to improve its stability, which is considered as a promising method for the effective decolorization of dye effluents.

Further, immobilization of microbial biomass for dye removal in growthrestricted conditions is advantageous when the effluent has toxicity and does not promote cellular growth. Also, inactivated biomass does not require a continuous supply of nutrients and can be regenerated and reused in many cycles (Prigione et al. [2008](#page-28-0)). Immobilization can be of two types: entrapment and attachment. Entrapment means entrapment of microorganisms in the interstices of fibrous or porous material, whereas attachment means adherence of microorganisms on surfaces due to chemical bonding or self-adhesion (Couto and Toca Herrera [2007\)](#page-24-0). Prigione et al. ([2008\)](#page-28-0) immobilized the conidial suspension of Cunninghamella elegans in calcium alginate beads. This immobilized biomass (300 g beads corresponding to 50 g biomass on wet weight basis) was then inactivated through autoclaving and packed in a glass column for treatment of synthetic dye-containing effluent. After 30 min, 70% decolorization and nearly complete decolorization were obtained after 6 h.

6.5 Reactors Studied in Dye Decolorization

The widely used systems were stirred tank reactor (Linko [1988\)](#page-26-0), airlift and bubble column, fixed-bed bioreactor, rotating disk reactor (Kirk et al. [1986](#page-26-0)), packed-bed reactors (Feijoo et al. [1995](#page-25-0)), and silicone membrane reactor. Researcher also investigated continuous decolorization of an azo dye, orange II, in a packed-bed reactor (Zhang et al. [2009\)](#page-30-0) and pulsed flow bioreactor packed (Mielgo et al. [2001\)](#page-27-0), achieving high 97% and 90% decolorization efficiency, respectively, and up to 80% decolorization of a disperse dye (red-553) in a continuous (10–20 days) fixed-film bioreactor (Yang and Yu [2003](#page-30-0)).

Textile wastewater was treated by means of a fluidized-bed loop reactor and immobilized anaerobic bacteria at low hydraulic residence time of 6 h for a period of 3 months and achieved complete decolorization of the wastewater in addition to the production of methane-rich biogas. Furthermore, the effluent proved to be highly biodegradable by aerobic microbes (activated sludge), whereas Shah et al. [\(2012](#page-29-0)) reported the overall color, COD, and BOD removal in the stirred tank bioreactor system were 49.67%, 37.45%, and 33.89%, respectively, with 50 mg/L dye concentration, pH 6.6 \pm 1, and HRT of 24 h in a reactor with 2 L capacity. The color removal efficiency in activated sludge process was $75 \pm 10\%$, and around 50–70% of removed color was adsorbed on biomass or precipitated within the reactor. The color rejection of nano-filtration after biological treatment was almost complete and permeates color was always lower than 10 Pt–Co (Ayed et al. [2011\)](#page-23-0). The performance of a bench-scale submerged microfiltration bioreactor using the white rot fungus *Coriolus versicolor* NBRC 9791 for treatment of textile dye wastewater was investigated with an average flux of 0.05 m/d (HRT = 15 h) for a month at controlled temperature and pH of 29 ± 1 °C and 4.5 ± 2.0 , respectively (Hai et al. [2006](#page-25-0))

Upflow Anaerobic Sludge Blanket (UASB) reactor, considered as high rate reactor, are generally more resistant to toxic compounds as a result of structure of formed granular sludge with good settling velocities and mechanical strength, and suitable for the treatment of wastewater containing xenobiotic and recalcitrant compounds, and it promotes adaptation of bacteria to the presence of toxic compounds, and as well as it can be used for treatment of wastewater previously considered unsuitable for anaerobic treatment (Jantsch et al. [2002;](#page-25-0) Harada et al. [1996;](#page-25-0) VanLier et al. 2001; Donlon et al. [1997\)](#page-24-0). Synthetic textile wastewater containing three acid dyes was treated in UASB reactor system and achieved decolorization up to $89 \pm 1.86\%$ at 300 mg/L dye concentration.

Immobilized Phanerochaete chrysosporium decolorized 94% of the maxilon red dye in the trickle-bed reactor over a period of 4–5 days (Afzal et al. [2009\)](#page-23-0), using the basal nitrogen-limited growth medium. Moreover, it continuously decolorized three different mixed azo dye effluents by greater than 90% in rotating tube bioreactor system over the 38-day operating period (Alleman et al. [1995;](#page-23-0) Kirby [1999](#page-26-0)) to investigate the remediation of actual textile effluent by P . *chrysosporium*. However, Phanerochaete sordida decolorized 80% of the phthalocyanine dye basic blue 22 in a rotating disk reactor operating with a retention time of 48 h (Yang et al. [2004\)](#page-30-0) and 90.3% in 72 h for an initial reactive black 5 concentration of 100 mg/L on nylon sponge and sunflower seed shells (SS) in laboratory-scale bioreactors (Enayatizamir et al. [2011\)](#page-24-0). Similarly, Blánquez et al. ([2004\)](#page-24-0) reported the biodegradation of Grey Lanaset G, a mixture of metal complex dyes, was studied in a reactor with the fungus *Trametes versicolor*. The ability to achieve 80% decolorization with *Irpex lacteus* to decolorize the remazol brilliant blue R and reactive orange 16 was reported by Pocedic et al. [\(2009](#page-28-0)).

Moreover, a fixed-bed bioreactor packed with Trametes pubescens was able to decolorize, for four successive cycles, 200 ml of a solution of the dye reactive black 5 at a concentration of 60 mg/L (Enayatzamir et al. [2009\)](#page-25-0), and Casieri et al. [\(2008](#page-24-0))

have shown the efficiency of degradation of Bacillus adusta and Pseudomonas ostreatus against successive cycles of solutions containing 200, 1000, and 2000 mg/ L of one model and two industrial dyes. Sponza and Isik (2005) (2005) reported the 96% color removal efficiencies of direct red 28 azo dye in a sequential upflow anaerobic sludge blanket reactor systems. The decolorization efficiency for malachite green was found to be 85.2% at pH in the range of 7–10, with increasing initial MG concentration up to 100 mg/L with immobilized P. pulmonicola YC32 continuous column system (Chen et al. [2009](#page-24-0)).

6.6 Microbial Consortia for Treatment of Textile Wastewater

In nature there is a diverse range of microorganisms and energy sources that makes it possible to break down a large number of different organic chemicals. Basically, microorganisms cannot mineralize most hazardous substances individually. So, the target pollutant being a complex molecule/mixture of compounds can only be broken down by a very specific combination of microorganisms (a "consortium") and pathways. Therefore, the use of microbial consortia offers considerable advantages over the use of pure cultures in the degradation and decolorization of synthetic dyes. The individual strains may attack the dye molecule at different positions or may use the decomposition products produced by another strain for further decomposition. However, the composition of mixed cultures may change during the decomposition process interfering with the control of the system. The most commonly used consortium in activated sludge system is mainly constituted by bacteria in addition to the presence of fungi and protozoa.

Various researchers have examined the microbial consortium JW-2 (Moosvi et al. [2005](#page-27-0)) consisting of Paenibacillus polymyxa, Micrococcus luteus, and Micro*coccus* sp. completely decolorizes reactive violet 5R (100 mg L^{-1}) within 36 h, and aerobic bacterial consortium SKB-II [Tony et al. [2009\]](#page-29-0) comprised of Bacillus sp. decolorizes the azo dyes such as Congo red, Bordeaux, Ranocid fast blue, and blue BCC of 10 mg/L concentration each. Joshi et al. ([2008\)](#page-26-0) have investigated bacterial consortium that decolorizes acid orange 7 and consists of A. caviae P. mirabilis and R. globerulus. Further, a microbial consortium (comprising of two spp. of Bacillus and six spp. of fungi, viz., Aspergillus flavus, A. niger, A. fumigates, Cladosporium cladosporioides, Trichoderma harzianum, Fusarium oxysporum) isolated from the textile wastewater polluted habitats of Sanganer showed the 95% decolorization of 100 mg/L methyl red (Kumar et al. [2006\)](#page-26-0). Similarly, significantly a higher reduction in color (90.14%) and COD removal (77.47%) from textile wastewater in less time (96 h) were achieved with the consortium comprises of Sphingomonas paucimobilis, Bacillus sp., and Staphylococcus epidermidis (Ayed et al. [2011\)](#page-23-0). The variance in the microbial communities in these consortia might involve different mechanisms for dye decolorization.

Waghmode et al. [\(2012](#page-29-0)) reported the enhanced decolorization and degradation of azo dye rubine GFL (50 mg/l within 30 h) using defined consortium GG-BL of Galactomyces geotrichum MTCC 1360 yeast and Brevibacillus laterosporus

MTCC 2298 bacterium, whereas individual cultures fail to completely decolorize the dye. The rate of decolorization of consortium AP was significantly higher than that of individual cultures. The increased decolorization rate might be due to the synergistic enzymes actions of both the organisms in the consortium. As researcher reported, the degradation of intermediates metabolites by bacteria could decline the fungal inhibition and thus enhances the decolorization efficiency of consortium (Gou et al. [2009](#page-25-0)). It is also known that the degradation products of one culture in the consortium may act as inducer for another co-culture, which results in the further mineralization of dye and metabolites (Chang et al. [2001;](#page-24-0) Forgacs et al. [2004\)](#page-25-0). Similar findings were reported by Kurade et al. ([2011](#page-26-0)), who observed higher decolorization rate of azo dye navy blue HE2R in solid state fermentation by developed consortium PA of Aspergillus ochraceus NCIM-1146 and Pseudomonas sp. SUK1.

6.7 Conclusions

This chapter concluded the effective decolorization of a wide variety of commercial textile dyes from simulated and real textile wastewater by the utilization of various types of microorganisms (bacteria, fungi, algae, and yeast) isolated from textile wastewater, sludge, and soil contaminated with textile effluent. Such microbes acclimatized themselves against the highly toxic dyes and make use of them for their growth with or without supplementing additional nutrients. This was also observed that a combination of different microorganisms (viz., bacterial-fungal, bacterial-algal and bacterial yeast, etc.) were more potent decolorizer than single pure cultures. Decolorization performance in reactor studies also confirmed the complete decolorization and degradation of toxic synthetic dyes with a small hydraulic residence time (HRT) and enhancing the efficiency of continuous reactor system. No doubt, bioremediation is considered to be one of the green approaches to clean the planet. Although advances in bioremediation techniques seem to be highly attractive, these technologies need scale-up trials in order to increase its market potential. Broader validation of these techniques and integration of different methods in the current treatment schemes will most likely, in the near future, render both efficient and economical viability.

In most cases, single technology fails to work in field due to various environmental factors associated and the toxicity of targeted compounds. Hence there is a need to develop hybrid technologies which can fit to ever-changing environmental conditions and toxicity of the compound. Researchers and scientists have been trying to develop a single and economical method for the treatment of dyes in the textile wastewater, but economical removal of color from effluent remains a big challenge. Thus, there is a necessity to develop better integrated technique to decolorize and completely mineralize the textile industrial effluent in spite of various successful systems of physicochemical techniques.

Most of the microbes are unculturable in laboratory condition, but their role in the particular niche cannot be neglected particularly in the process like bioremediation. In order to trace the microbial population and their role in the environment, molecular techniques prove to be a boon in this field. The genes involved in the bioremediation of the targeted compound and their respective enzymes can be traced, and hence in turn the microbial floras involved in the bioremediation process are traceable. It also helps to record the metabolic pathway followed by the organism to degrade the particular compounds.

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