Bioremediation of Textile Dyes: Appraisal of Conventional and Biological Approaches

23

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Abstract

The world is facing numerous environmental changes and challenges, which include generation of huge amounts of wastewater owing to several man-made activities. Use of man-made dyes has increased in the clothing and textile industries because of their bright colors and cost effectiveness in manufacturing. On the other hand, the use of natural dyes is not cost effective, and their preparation is a cumbersome process. Therefore, people are using synthetic colors and textile dye industries are producing huge volumes of wastewater, creating aquatic pollution all over the globe. Man-made dyes or imitation dyes are broadly employed in plastic toys, textile staining, printing of paper, food, various plastic wares, dye cinematography, pharmaceuticals, cosmetics, and other important industries. During the process of fabric dyeing, a huge quantity of colored wastewater is generated as polluted effluent.

23.1 Introduction

Textile industries generate huge amounts of contaminated water, which can lead to serious complications of environmental issues if not treated properly (Kunz et al. 2002; Hemapriya et al. 2010). Every textile industry uses harmful dyes and pigments to color its products, which include fabric, tannery, colored food, paper and pulp, printing, carpet, and mineral dispensation products (Asamudo et al. 2005).

Many studies have clearly demonstrated that the use of synthetic dyes has increased in textile industries due to their bright and stable colors, consequently

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also increasing the price of clothes. As a result, huge discharges of contaminated colored effluent are released into the ecosystem by different industries. Studies have reported that color can be noticeable in water at a concentration higher than 1 mg/L (Neill et al. 1999).

Wang et al. (2002) have stated that around 10–15% dyes are dumped into the environment during coloring practices. The effluent from textile industries thus contains a large number of dyes and other chemicals, which are added during the tinting process. Colored textile effluent can easily enter natural aquifers and pollute natural and pure water bodies or areas (Ezeronye and Ubalua 2005). The discharge of colored effluent into water bodies and the environment is detrimental not only because of the complexity of the color but also because of the breakdown products of those dyes, which are toxic and carcinogenic to life systems. These dyes contain mutagenic agents such as benzidine, naphthalene, and other aromatic compounds (Suteu et al. 2009). Without proper treatment, these dyes can persist in the environment for a greatly extended period of time. For example, the half-life of hydrolyzed Reactive Blue 19 is about 46 years at a pH of 7 and a temperature of 25 °C (Hao et al. 2000; Wijannarong et al. 2013; Gupta et al. 2014a). Certain nonbiodegradable dyes utilized by textile industries create serious environmental hazards. The color of wastewater becomes unappealing and unfriendly to aquatic life-forms, hampering the oxygenation of water; moreover, it also disturbs the whole aquatic ecosystem (Xu et al. 2005) and the food chain. Textile effluent comprises different dyes; therefore, it is becoming essential to encourage combination of existing techniques with new techniques to decolorize the mixture of dyes. Dyes are categorized on the basis of their uses and chemical structure. Basically, dyes comprise a group of atoms called chromophores, which are accountable for the color of the dye. These chromophore-comprising centers are made up of varied functional groups: azo, methine, nitro, anthraquinone, aril methane, carbonyl, and many other groups. There are certain electrons removing and adding substituents which change the stain of the chromophores; known as auxochromes. Commonly used auxochromes are amine, carboxyl, sulfonate, and hydroxyl (Christie 2001; Santos et al. 2007; Prasad and Rao 2010).

Numerous studies have suggested that microbes are proficient in biodegradation of lethal components (Aksu 2005). The bioremediation approach proposes several benefits in that it can be carried out on-site, it is cost effective and causes minimal problems, and it can be used in combination with physical and chemical approaches (Boopathy 2000; Dias 2000).

23.2 Textile Dyes and Their Types

On the basis of their usage or application, dyes are classified according to the Colour Index (CI) scheme, compiled by Zollinger (1987).

23.2.1 Reactive Dyes

Under appropriate conditions, these dyes bond to fibers by forming covalent chemical bonds, such as ether or ester bonds. Reactive dyes encompass azo groups that consist of metallized azo, triphendioxazine, phthalocyanine, formazan, and anthraquinone. The molecular arrangements of these dyes are different from and structurally simpler than those of direct dyes. They also yield brighter, more cheerful shades than direct dyes. Reactive colorants are principally used for staining and printing of cotton fibers.

23.2.2 Direct Dyes

These dyes are anionic and water soluble in the presence of electrolytes. They bind effectively to cellulose fibers. Dyes in this group mostly comprise polyazo compounds, as well as some stilbenes, phthalocyanines, and oxazines. Improvement in wash processing is regularly achieved by chelation with metallic group (such as copper and chromium) salts. They are also used with formaldehyde or a cationic dye–complexing resin.

23.2.3 Disperse Dyes

Dyes in this group are substantively water impermeable and are used to color polyester, polyamide, polyacrylonitrile, and polypropylene fibers. Such dyes are not used for dyeing of nylon, cellulose acetate, or acrylic fibers. Chemically, these dyes are chiefly prepared from azo and anthraquinonoid groups, with groups that support formation of stable aqueous solutions.

23.2.4 Sulfur Dyes

These dyes are not dissolved in water and are useful for dyeing cotton cloth using sodium salts by a reduction process. The chemical used for this process is sodium sulfide, which is used as a reducing agent under highly basic conditions. Owing to the low cost of these dyes, they are commonly used in industries.

23.2.5 Vat Dyes

Vat dyes are water insoluble. They are applied mainly to cellulose fiber by altering them to their leuco compounds. The leuco compound is formed by reduction and solubilization with $Na_2S_2O_4$ and NaOH solution, which is called a “vatting process.” The chief chemical/structural groups of vat dyes are anthraquinones.

23.2.6 Cationic (Basic) Dyes

The dyes in this group are cationic and soluble in water. They are used for dyeing paper, polyacrylonitrile, modified nylons, and modified polyesters. These dyes are also used to apply to silk fiber, wool, and tannin-mordant cotton when bright shades are more necessary than fastness to light and washing.

23.2.7 Acid Dyes

Dyes in this group are water soluble anionic dyes and are used to color nylon, wool, silk, and modified acrylics. Furthermore, they are used in inkjet printing and in the dyeing of paper, leather, food, greasepaints, and cosmetics.

23.2.8 Solvent Dyes

These dyes are not soluble in water but do dissolve in certain specific solvents. Their molecules are generally nonpolar or sometime polar; moreover, they do not undergo a process of ionization. They are used for coloring plastics, gasoline, oils, and waxes.

23.2.9 Mordant Dyes

These dyes are capable of bonding strongly with metal residuals by forming covalent and coordinate bonds. A chelate compound is also involved. The salts of Al, Cr, Cu, Co, Ni, Fe, and Sn are used as fixatives of metallic salts. In the field of chemistry, chromophores and auxochromes are the principal constituents of dye molecules. Stain comprises an unsaturated group, basically accountable for color, and a known chromophore. Auxochromes ("auxo" means augment) are the distinguishing groups that strengthen color and/or develop the affinity of the dye for the substrate (Rangnekar and Singh 1980).

Table 23.1 lists common chromophores and auxogroups (Rangnekar and Singh 1980).

Table 23.1 Chromophore and auxochrome groups of dyes

Chromophore groups	Names	Auxogroups	Names
$-N{=}N-$	Azo	$-NH_2$	Amino
$-N{=}N^+-O^-$	Azoxy	$-NHCH_3$	Methyl amino
$-N{=}N-NH$	Azoamino	$-N(CH_3)_2$	Dimethyl amino
$-N{=}O$, $N-OH$	Nitroso	$-SO_3H$	Sulfonic acid
$>C{=}O$	Carbonyl	$-OH$	Hydroxy
$>C{=}C<$	Ethenyl	$-COOH$	Carboxylic acid
$>C{=}S$	Thio	$-Cl$	Chloro
$-NO_2$	Nitro	$-CH_3$	Methyl
$>C{=}NH$, $>C{=}N-$	Azomethine	$-OCH_3$	Methoxy

23.3 Bioremediation Methods

23.3.1 Ex Situ and In Situ Approaches

Bioremediation methods are broadly classified as in situ and ex situ. Ex situ methods are treatments that involve physical elimination of the polluted material for treatment processes, whereas in situ technologies involve the removal of the contaminants at the site itself. Details of in situ and ex situ bioremediation are given below:

1. *Land-dwelling*: A solid-phase treatment scheme for polluted soils may be used in both techniques.
2. *Bioreactors*: Biodegradation in a big reactor or container may be used to treat liquids or slurries.
3. *Composting*: This is an anaerobic and thermophilic treatment process in which contaminated substances are mixed with a bulking agent. This can be done using stationary piles or aerated piles.
4. *Bioventing*: This method treats contaminated soil by passing oxygen through it to stimulate microbial activity.
5. *Biofilters*: In this method, microbial stripping columns are used to treat air emissions.

23.4 Bioremediation of Dyes

Dyes are chemical substances that, when applied to a substrate, are responsible for providing such constituents with extensive coloring capability. Mostly they are used in the textile, plastics, therapeutics, food, cosmetics, graphics, and paper and pulp industries (Zollinger 1987;Carneiro et al. 2007). Dyes can stick to compatible surfaces by solution, with the help of formation of covalent bonds or complexes through salts or metals, by physical adsorption, or by mechanical retention (Kirk-Othmer Encyclopedia of Chemical Technology 2004; Bafana et al. 2011). Mauveine, the first artificial dye, was discovered by an Englishman, William Henry Perkin, in 1856. Rangnekar and Singh (1980) classified dyes into two groups on the basis of their structures, mainly consisting of chromophoric groups. Dyes can also be classified on the basis of their applications.

23.4.1 Dye Removal Techniques

Reports have described many approaches for the elimination of waste products from effluent. These approaches can be grouped into three classes: organic (biological), chemical, and physical (Robinson et al. 2001). Different methods have their own advantages and drawbacks.

Because of high costs and disposal problems, certain conventional approaches are not used to treat dye wastewater widely in the textile and paper and pulp industries.

Presently, no single process is sufficient to treat the effluent, because of its complex structure (Pereira et al. 2003; Marco et al. 1997). Nowadays, a combination of different approaches is frequently used to achieve the desired water quality in a less expensive way. A literature survey reported that research has been continuously carried out to study combined adsorption–biological treatments for biodegradation of dyestuffs and reduced sludge production. The different dye elimination approaches are discussed in the following sections.

23.4.1.1 Physical Methods

Various physical techniques are used worldwide—for example, membrane filtration processes (nanofiltration, reverse osmosis, and electrodialysis) and adsorption systems. The major shortcoming of membrane filtration methods is that they have a shorter life-span before fouling of the membrane occurs, and the cost of membrane replacement is too high. Liquid-phase adsorption is considered the most popular method to remove pollutants from wastewater. Adsorption is a popular equilibrium separation process and an effective technique for treatment of wastewater (Dabrowski 2001). Physical methods are also considered superior to other methods in terms of water reuse at low cost, flexibility, simplicity of design, and ease of operation. Adsorption also does not lead to formation of toxic or harmful substances. Physical methods include various processes, which are discussed in the following subsections.

23.4.1.2 Adsorption

Among all physical methods, adsorption is the method best suited to removal of pollutants that are very stable and not removed by any other method (Le Marechal 1998). Adsorption has an advantage over other techniques in that the pH of the discharged wastewater is not affected and no additional sludge is produced during the wastewater treatment. However, it also has some limitations (Weber et al. 1970).

23.4.1.3 Activated Carbon

For the removal of dyestuffs, activated carbon is the most widely used adsorbent (Nasser and El-Geundi 1991) and gives good results in adsorbing mordant, cationic, and acid dyes on a large scale. It has slightly poorer potential for removal of dyes such as disperse, direct, vat, pigment, and reactive dyes (Raghavacharya 1997; Rao et al. 1994).

23.4.1.4 Peat

Peat is considered a good choice for wastewater removal because of its cellular structure. It is highly effective in adsorbing transition metals (along with organic compounds with a polar nature) from textile mill effluent (Poots et al. 1976).

23.4.1.5 Wood Chips

The effectiveness of wood chips is limited to removal of acidic dyes.

23.4.1.6 Fly Ash and Coal Mixture

This method is not so effective, hence it is rarely used. In the mixture of fly ash and coal, if the concentration of fly ash increases, the efficiency of the adsorption increases. This is due to the availability of a large surface area for adsorption (Gupta et al. 1990).

23.4.1.7 Silica Gel

This method is limited to the removal of basic dyes but has good potential.

23.4.1.8 Other Materials

In this method, natural clay, corncobs, rice hulls, etc., are used to remove dye from wastewater. This method has the advantage of being inexpensive, and the compounds used in this technique are readily available (Nawar and Doma 1989).

23.4.1.9 Membrane Filtration

This method is effective for continuous removal of color from dye effluent. It also concentrates the coloring molecules and finally separate them (Mishra and Tripathy 1993).

23.4.1.10 Ion Exchange

This method is not used on a commercial scale, as it has certain limitations and eliminates only those dye molecules that have charges (cations or anions) present on them. As maximum dyes have no charge, they cannot be eradicated by means of an ion exchange method (Slokar and Le Marechal 1997).

23.4.1.11 Irradiation

This method also has limitations, as it can be used only at the laboratory level. It removes only some dyes and organic compounds such as phenols (Hosono et al. 1993).

23.4.2 Chemical Methods

These approaches comprise coagulation or flocculation combined with filtration and flotation. Precipitation–flocculation is done through iron(II)/calcium hydroxide and conventional oxidation approaches by oxidizing agents (ozone), electrokinetic coagulation, irradiation, or electrochemical methods. These technologies are generally costly, and removal of colorants by these methods leaves concentrated sludge, which creates problems in its removal. These techniques also lead to secondary pollution due to the use of excessive chemicals. The big limitations of these methods are their high cost and high consumption of electric energy and chemical reagents. Chemical oxidation is achieved by strong oxidizing agents, leading to ring opening of aromatic dyes, which thus can be easily removed from wastewater (Raghavacharya 1997). Certain chemical methods are discussed in the following subsections.

23.4.2.1 Hydrogen Peroxide Iron(II) Salts (Fenton's Reagent)

H_2O_2_ Fe(II), or Fenton's reagent, is useful to remove dyes from wastewater where biological and physical treatments cannot be used because they show toxicity to living biomass (Slokar and Le Marechal 1997). For the removal of dissolved dyes from effluent, a chemical separation method is used, which involves bonding of dye molecules with chemicals. This method can also be used to remove insoluble dyes (Pak and Chang 1999).

23.4.2.2 Ozonation

Ozone is used as an oxidizing agent. This method is effective for the degradation of compounds of hydrogen and carbon with halogens and hydroxyl groups or without any substitutes (Lin and Lin 1993; Xu and Lebrun 1999). The amount of ozone to be used for the treatment depends upon the percentage of dyes present in the wastewater (Ince and Gönenç 1997). This method does not lead to any pollution (Gahr et al. 1994). Ozonation completely removes colors from effluent and produces less chemical oxygen demand (COD); hence, treated water can be directly discharged into water bodies (Xu and Lebrun 1999).

23.4.2.3 Photochemical Removal

This method uses ultraviolet light (UV) along with H_2O_2 to degrade dye molecules. During the degradation, water and carbon dioxide are formed as nontoxic by-products. In the reaction, the production of hydroxyl free radicals takes place, in turn causing the oxidation of dye molecules (Yang and Wyatt 1998; Peralto-Zamora et al. 1999).

$$\mathrm{H_2O_2} + \mathrm{h}\upsilon \rightarrow 2OH^{\circ}$$

23.4.2.4 Sodium Hypochlorite

This technique involve the breakdown of azo bonds and destruction of amino groups in dye molecules by the action of NaOCl, thereby aiding removal of dyes from effluent (Bannat et al. 1996).

23.4.2.5 Cucurbituril

Cucurbituril is a cyclic compound obtained from glycoluril and formaldehyde (HCHO) (Karcher et al. 1999). It has the ability to form complexes with compounds of azo groups with aromatic chains to remove dye molecules from textile effluent (Mock 1995).

23.4.2.6 Electrokinetic Coagulation

This is the least expensive method for the removal of dyes from textile effluent. It has limitations in that it can be used only for direct dyes. The removal of dyes is done by the use of Fe_2SO_4 and $FeCl_3$ (Gahr et al. 1994).

23.4.2.7 Electrochemical Destruction

This technique is economical and effective for dye decolorization. This approach can be used to degrade a wide range of dyes, even those with organic compounds that are resistant to degradation (Ogutveren and Kaparal 1994; Pelegrini et al. 1999).

23.4.2.8 Physicochemical Treatments

In addition to physical and chemical methods, there are numerous physicochemical approaches that are used for removal of colorants from textile effluent (Robinson et al. 2001). Physicochemical treatments transfer the contaminants present in effluent from one stage to another without eliminating them (Erswell et al. 1988), whereas biological method eliminate a variety of colors by aerobic and anaerobic microbial degradation (Aksu 2005). Advanced oxidation processes lead to the formation of reactive free radicals for oxidation of complex dye molecules present in effluent. This oxidation converts complex toxic contaminants to simpler and nontoxic degraded products. Generation of highly reactive free radicals can be attained by using UV, Fe + $2/H_2O_2$, TiO_2/H_2O_2, UV/O_3, UV/H_2O_2, etc. (Kestioglu et al. 2005). However, the use of physical or chemical techniques has certain shortcomings: it is economically unfeasible, as it requires more energy consumption and chemical use; it is incapable of degrading azo dyes and certain chemical compounds completely; and it leads to secondary pollution problems due to the release of large amounts of sludge (Zhang et al. 2004).

23.4.3 Biological Treatments

This method is the best alternative to physical and chemical processes used for bioremediation, as it is cost effective and eco-friendly. Biological degradation includes fungal decolorization, bacterial degradation, and adsorption of dyes by (dead and living) microbiological biomass and detoxification systems. This method can be used to degrade dyes present in textile effluent, as microbes such as yeasts, bacteria, fungi, and algae are able to accumulate in the effluent (Banat et al. 1996; McMullan et al. 2001). However, this treatment has certain limitations due to its technical constraints. Microbes such as fungi (Barr and Aust 1994) and bacteria are difficult to handle, as there is a risk of contamination, though their culturing is simple (Banat et al. 1997). Biological degradation is an economical procedure and is attracting wide attention (Van Der Zee and Villaverde 2005). Biological treatment can involve anaerobic and aerobic degradation of effluent. Anaerobic treatment of textile effluent utilizes numerous anaerobic bacteria, which produce powerful enzymes such as azoreductase. Van Der Zee (2005) have studied combined sequential or integrated anaerobic–aerobic treatment of textile effluent. The use of bacteria in degradation and decolorization of dyes has been studied by Santos et al. (2007). Aksu (2005) has reviewed the use of some algae for decolorization. Dye degradation by fungi has been widely investigated and studied (Brar et al. 2006; Kaushik and Malik 2009).

Among all microorganisms, fungi have been considered the most suitable microbes for removal of dyes from industrial effluent. Fungal mycelium has an advantage over unicellular organisms as it solubilizes insoluble substrates by means of extracellular enzymes. Fungi have a greater cell to surface ratio; hence, they show better physical and enzymatic interactions with the environment. They also have a greater ability to degrade high concentrations of toxins. Biological treatment has limitations, as it needs a huge area and it is affected by day-to-day variations (Bhattacharya et al. 2003). Biological treatment sometimes becomes ineffective for color removal with the present conventional biodegradation processes (Robinson et al. 2001). Furthermore, numerous biological components are degraded and several others are recalcitrant because of their structural complexity (Ravi Kumar et al. 1998). Specifically, azo dyes are not completely degraded by biological methods, because of their xenobiotic nature. Textile effluent is difficult to treat; hence, combinations of biological treatment and physicochemical techniques have been used to decolorize textile effluent (Kannan and Sundaram 2001; Rai et al. 2005; Solmaz et al. 2009; Wojnarovits and Takacs 2008; Ince and Tezcanh 1999; Chaudhari et al. 2011).

23.4.3.1 Roles of Fungi in Bioremediation

Application of fungi has been mentioned in many reports on dye degradation and decolorization using various potential fungal strains.

The most effective and widely used fungi for removal of colorants are ligninolytic fungi (Bumpus 2004). Apart from them, fungi such as *Schizophyllum commune* IBL-062 (Asgher et al. 2013), *Aspergillus allahabadii* (30), *Aspergillus niger* (30), *Aspergillus sulphureus* (Namdhari et al. 2012), and white-rot fungi (e.g., *Pleurotus florida* (Krishnaveni 2011) and *P. eryngii* F032 (Hadibarata et al. 2013)) have been shown to be capable of degrading textile dyes in effluent. With regard to the potential of these dyes, numerous publications have shown that these fungi are able to oxidize nonphenolic, phenolic, soluble, and insoluble dyes (Padmanaban et al. 2013).

Fungi produce enzymes that degrade harmful dyes present in textile effluent or wastewater; for example, white-rot fungi produce enzymes such as lignin peroxidase, manganese peroxidase, and laccase, which degrade various aromatic compounds because of their nonspecificity (Toh et al. 2003) and can be used to degrade synthetic dyes (Reghukumar et al. 1996; Fu et al. 2001). The lignin peroxidase produced by *Phanerochaete chrysosporium* plays a crucial role in the degradation of azo dyes (Ollikka et al. 1993). However, the use of white-rot fungi for dye decolorization of wastewater has many problems, such as production of large amounts of biomass, the structure of the synthetic dyes involved, and management of the biomass (Stolz 2001).

Sathiya Moorthi et al. (2007) reported that two species of white-rot fungi have the capability to detoxify Blue CA, Black B133, and Corazol Violet SR dyes. *P. florida* and *Trametes hirsuta* show great potential in dye degradation. Laccase is the ligninolytic enzyme released by these fungi. Their decolorization activity was tested using several different concentrations of dyes such as 25, 50, and 75 mg/L. The maximum decolorization was seen with Blue CA and Corazol Violet SR dyes.

Wastewater from textile industries has been treated by using *T. hirsuta* and *P. florida* with different concentrations of glucose (1% and 2%) as an additional carbon source. Effective results were exhibited by *P. florida* with 2% of glucose.

Paszczynski et al. (1992) studied the comparative effectiveness of the soil actinomycete culture *Streptomyces chromofuscus* with *P. chrysosporium* and found that bacteria present in the soil were able to decolorize dyes present in effluent but to a lesser degree than white-rot fungi. Some actinomycete strains have shown the ability to degrade Cu-based azo dyes such as formazan (Zhou and Zimmermann 1993). Some other actinomycetes have the ability to degrade reactive dyes such as phthalocyanine, anthraquinone, and azo dyes through adsorption of colorants by the cellular biomass without their degradation.

The application of color removal of azo dyes by means of the white-rot fungus *P. chrysosporium* was first described by Cripps et al. (1990). It was shown that the colors of certain azo dyes such as Tropaeolin O, Orange II, and Congo Red could be removed by *P. chrysosporium*. Some other white-rot fungi such as *Bjerkandera adusta* and *Trametes versicolor* have also shown high efficiency in degrading azo dyes (Heinfling et al. 1997). Several other white-rot fungi such as *P. chrysosporium*, *P. tremellia*, *P. cinnabarinus*, *T. versicolor*, *Ceriporiopsis subvermispora*, *Cyathus stercoreus*, *P. ostreatus*, *Pleurotus oxysporum*, and *Phellinus pini* have also been tested for dye decolorization, and it was concluded that these fungi also degrade synthetic dyes efficiently (Cao 2000).

Vijaykumar et al. (2006) isolated a novel fungus, *Cladosporium cladosporioides*, from coal and assessed its efficiency in decolorization of five different azo and triphenylmethane dyes—Acid Black 210, Acid Blue 193, Crystal Violet, Reactive B \Black B(S), and Reactive Black BL/LPR—on solid media as well as in liquid media.

The effects of different factors such as the inoculum size, temperature, nitrogen source, and carbon source were investigated in degradation of industrial effluent using *P. chrysosporium*. The results showed that the efficiency of these fungi in decolorizing the industrial effluent was up to 97% (Shahvali et al. 2000).

White-rot fungi are considered superior dye decolorizers to prokaryotes. Decolorization efficiency depends on the components present in the medium and on the combination of the dye and microorganisms. Certain physical factors also influence dye decolorization, such as the temperature, pH, dye concentration, and use of agitators. Lignin-modifying enzymes also play an important role in dye decolorization by the action of white-rot fungus (McMullan et al. 2001).

It is possible to degrade Solar Brilliant Red 80 to the extent of 84.8% by using *S. commune* IBL-06 maintained at a pH of 4.5 and a temperature of 30 °C for 7 days (Asgher et al. 2013).

Namdhari et al. (2012) reported that *A. allahabadii*, *A. niger*, and *A. sulphureus* were capable of degrading Reactive Blue MR by up to 95.13% at a temperature of 25 °C maintained for 10 days.

23.4.3.2 Roles of Algae in Bioremediation

Only limited studies on the role of algae in bioremediation have been carried out, though they showed great potential for removing dyes and other complex organic compounds (Semple et al. 1999). Certain species of *Chlorella* and *Oscillatoria* are sufficiently capable of degrading azo dyes into their aromatic amines and also breaking down the aromatic amines into simpler organic compounds. A few algae can even utilize azo dyes as core sources of nitrogen and carbon. The ability of such algae to remove aromatic amines means they can be used to stabilize ponds. Algae have a high surface area to volume ratio; hence, they have significant potential for adsorption (Rice and Sikka 1973; Tikoo et al. 1997).

Experimental results have shown that *Spirogyra* is efficient in degrading azo dye compounds. The decolorization rate depends upon the initial inoculum of the algae, the concentration of the dye, and its type. Algae take time to acclimatize to a new environment; hence, the initial elimination of dye is low but later on increases quickly prior to attainment of saturation (APHA 1995).

Hanan (2012) worked on 11 strains of green algae; his studies revealed that Cyanobacteria and diatoms were efficient for decolorizing a wide range of structurally different dyes. Selected isolates studied by him decolorized dyes either completely or to some extent. Maximum decolorization was achieved with lower concentrations of dye. The maximum degradation rate was observed in the first 3 days, after which the degradation rate until 6 days was slower. The decolorization percentage varied from 100% to only 13% after 6 days of incubation. Omar (2008) reported that variations in structural and functional groups in a monoazo dye (tartrazine) and a diazo dye (Ponceau) affected the decolorization process. The maximum degradation was observed at 5 parts per million (ppm) of tartrazine with *S. bijugatus* (68%) and *Nostoc muscorum* after 6 days of incubation.

23.4.3.3 Roles of Bacteria in Bioremediation

Studies have revealed that bacterial dye decolorization is generally nonspecific and rapid (McMullan et al. 2001). Various bacteria such as *Pseudomonas putida* (Tripathi and Srivastava 2011), *Bacillus subtilis* (Milikli and Ramachandra Rao 2012), *Bacillus* sp. (Abraham et al. 2014), *P. putida* (Wang et al. 2012), *Pseudomonas* spp. (Shah et al. 2013), *Tsukamurella* sp. J8025 (Wen-Tung and Jean 2012), *B. subtilis* SPR42 (Saharan and Ranga 2011), *P. fluorescens* (Saleh Al-Garni and Kubli 2013), *Georgenia* sp. CC-NMPT-T3 (Sahasrabudhe and Pathade 2013), and *Bacillus cereus* (Vidhyakalarani and Premaraj 2013) have the ability to degrade textile dyes. Some of these are aerobic bacteria and some are anaerobic. Bacterial decolorization is effective for anthraquinone and azo dyes, resulting in the manufacture of biogas. Sometimes the rate of decolorization becomes very slow (Bumpus 2004). Complete and effective dye decolorization has certain specific requirements such as specific oxygen-catalyzed enzymes and carbon and nitrogen sources (Hadibarata et al. 2013). Some strains of aerobic bacteria are able to utilize dyes as their basic sources of carbon and nitrogen, and some are capable of reducing azo dyes. Many researchers have reported that aerobic bacteria have the ability to mineralize azo dyes but not completely; they are just degraded into intermediate compounds. Complete degradation occurs only under coupled aerobic–anaerobic degradation (Sudha et al. 2014). In aerobic conditions, azo dyes are converted to their aromatic amines and mineralized by nonspecific enzymes through ring cleavage. Consequently, it has been suggested that for effective decolorization, couple treatment is used, followed by aerobic degradation. Feigel and Knackmuss (1993) and Mubarak Ali et al. (2011) found that appropriate growth of bacterial species took place in aerobic or agitation conditions, but the rate of decolorization was maximized in anaerobic culture.

23.4.3.4 Dye Decolorization by Microbial Consortia and Biofilms

Industrial effluent carries a diverse range of azo dyes. No single bacterial strain is capable enough to degrade aromatic amines. The difficulty of maintaining purity is the shortcoming that limits the application of pure strains in degradation of textile dyes. Pure cultures are considered less effective in dye decolorization and degradation; hence, use of a bacterial consortium may provide a good solution to this problem. Several studies have revealed that a consortium of microbes has an extra effect in removing colors from effluent as compared with pure cultures (Khouni et al. 2012; Aruna et al. 2015; Ndasi et al. 2011). In a consortium, higher efficiency of degradation is achieved because different bacteria attack different sites on dye particles and the by-products obtained after degradation can be used by other microbes as an energy source (Chang et al. 2004; Saratale et al. 2009).

23.4.3.5 Dye Decolorization by Molds

Filamentous fungi or multicellular molds have the capability to clean the environment. Their diversity of hyphal structures, large cell to surface ratios, presence of extracellular or intracellular enzymes, mixtures of secondary metabolites, and different modes of reproduction provide good scope for decolorization. Many researchers have studied the degradation efficiency of molds in decolorization of lignin-containing effluent from the paper and pulp industry. Two white-rot fungi (*P. chrysosporium* and *Tinctosporia* sp.) were studied and reported in 1980 (Eaton et al. 1980; Fukuzumi 1980); both organisms were capable of degrading polymeric lignin molecules. The mechanism behind the degradation of dyes includes enzymes such as lignin peroxidase and Mn-dependent peroxidase or laccase enzymes (Michel Jr et al. 1991). In all of the examples it was observed that limitation of nitrogen increased the ligninolytic activities of Mn-dependent peroxidase and lignin peroxidase, enabling efficient removal of color from wastewater (Perie and Gold 1991). However, Chao and Lee (1994) found that the rate of decolorization was higher when microbes were pregrown in a nitrogen-rich medium. Degradation of xenobiotic compounds was achieved in nonligninolytic conditions by laccase enzyme activity (Dhawale et al. 1992).

23.4.3.6 Roles of Yeasts in Dye Decolorization

Fewer studies have been done on removal of azo dyes by yeasts, as opposed to bacterial and mold species. In comparison with fungi and bacteria, molds have many advantages. Unlike bacteria, they do not have a rapid growth rate, but like filamentous fungi, they are able to grow in unfavorable conditions (Zhisheng and Xianghua 2005). Some yeasts have the capability to treat organic effluent such as wastewater and food industry effluent (Yang et al. 2003). Marco et al. (2005) isolated a phenolic acid–assimilating yeast, *Candida oleophila*, which could completely degrade up to 200 mg of Reactive Black 5 within 24 h of aerobic incubation. Zhisheng and Xianghua (2005) isolated two other species, *Pseudozyma rugulosa* and *Candida krusei*, which had the capability to decolorize the azo dye Reactive Brilliant Red K-2BP.

Certain yeast species are also able to degrade anthraquinone dyes and other dyes. Studies by Yang et al. (2003) revealed that the manganese-dependent peroxidase (MnP) activity of the yeasts *Debaryomyces polymorphus* and *Candida tropicalis* enabled them to efficiently remove Reactive Black B and other azo dyes such as anthraquinone dye within 48 h. Martorell et al. (2012) reported that removal of dyes by yeasts had additional benefits in comparison with the use of fungi because of their fast growth and survival in harsh conditions. Reports have shown that for many yeasts, dye decolorization depends upon enzymatic action on the chromophore structure (Ramalho et al. 2004).

23.5 Mechanisms Behind Bioremediation of Textile Dyes

23.5.1 Biosorption

The accumulation of chemical substances present in dye molecules in microorganisms is known as biosorption. Biosorption can occur in both living and dead microbes. Dead microbes have the capability to adsorb compounds that contain the natural polysaccharide chitin or its derivative chitosan, which is present in the cell walls of fungi and shows great affinity for numerous groups of dyes because of its unique molecular structure (Joshi et al. 2004).

23.5.2 Biodegradation

The term “biodegradation” refers to a process in which complex organic compounds are decomposed into simpler substances by living microorganisms. This is a natural process performed by microbes. Biodegradation can be achieved by the action of enzymes secreted by microbes. Studies by many researchers have revealed that most white-rot fungi produce nonspecific enzymes such as lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase. These enzymes enable generation of free radicals during the biodegradation process (Pointing 2001; Knapp et al. 2001). The fungus *Flavodon flavus* releases laccase, which has the ability to decolorize synthetic dyes such as Azure B and Brilliant Blue R in scarcity of water (Soares et al. 2002).

Enayatizamir et al. (2011) reported that Azo Black Reactive 5 dye was degraded by up to 92% by *P. chrysosporium* within 3 days. Similarly, *P. chrysosporium*

URM6181 and *Curvularia lunata* URM6179 strains were shown to degrade indigo dye present in textile effluent by up to 95% within 10 days. Laccase obtained from the fungus *T. hirsuta* has the ability to degrade triarylmethane, indigo, azo, and anthraquinonic dyes present in industrial effluent (Annibale et al. 2000).

Sathiya Moorthi et al. (2007) reported that extracellular laccase was produced by *T. hirsuta* and *P. florida*. The oxidase activity of these microbes decolorized Blue CA, Corazol Violet SR and, to a lesser extent, Black B133. The degree of color elimination is not consistent for all dyes. The decolorization depends upon the amount of laccase produced, the type of media used, and the class of dye.

23.5.3 Mineralization

The action of fungi on mineralization or decolorization of textile dyes depends upon the chemical structure of the dyes. The rate of decolorization is higher in dyes with chains of aromatic rings than in those with unbranched rings. Better results have been obtained in media with a low nitrogen concentration. Studies have shown that that the dyes are used as carbon sources in certain cases, through cleavage of the bonds of the dye molecule, leaving the chromophore group unaffected. Mineralization occurs mainly in consortia, not in pure cultures (Knapp et al. 2001; Singh 2006).

23.5.4 Bioaccumulation

Many studies have been done on the use of living cells in bioaccumulation processes. Bioaccumulation of dyes can be done efficiently by growing cells with sufficient carbon and nitrogen sources. Use of living biomass for bioaccumulation has an advantage over use of dead biomass in that cell growth and bioaccumulation occur simultaneously, so there is no need to allow separate times for growth and bioaccumulation. However, this method has major limitations in that the microbes need nutrients for their growth and high dye concentrations can affect their growth rate (Aksu and Donmez 2005).

23.6 Factors Affecting Biodegradation of Dyes

There are several factors that affect the growth of microbes, such as certain harmful gases, pH, the presence of oxygen, temperature, metals, salts, and chemical compounds. Optimization of these abiotic factors is helpful in the development of industrial-scale bioreactors for bioremediation. Other than these abiotic factors, several factors such as the type of dye, the composition of the effluent, operating costs, environmental thrift, and handling of the produced sludge determine the technical and economic practicability of the methods used for treatment.

23.6.1 pH

Decolorization and biodegradation by fungi generate better results at either an acidic or neutral pH, whereas bacteria show optimal activity at either a neutral or basic pH. All microorganisms degrade textile effluent at their specific pH. Commonly, the optimal pH for decolorization of microbes is pH 6–10 (Ramachandran et al. 2013). At a neutral or slight basic/acidic pH the decolorization rate is high, but a strong acidic/basic pH decreases the percentage of decolorization. Nearly all fungal dye decolorization studies have found that the best results are obtained at an acidic pH. With a change in the pH range to 7–9.5, no variation or just a slight variation is observed in the decolorization rate (Ramachandran et al. 2013).

23.6.2 Temperature

Temperature also acts as an imperative factor that affects the dye decolorization activity of microbes. The rate of biodegradation increases with rises in the initial temperature (Ramachandran et al. 2013). The optimal temperatures for dye decolorization have been found to be 35–45 °C in the case of bacteria and 23–35 °C in the case of fungus. The degradation efficiency of microbes can be decreased by slow growth, their reproduction rate, secretion of enzymes, etc. (Adinew 2012).

23.6.3 Initial Dye Concentration

The dye concentration has a strong impact on the extent of dye decolorization and also influences the toxicity of dye molecules. In the case of bacteria, the decolorization rate increases with time irrespective of the initial dye concentration. An increase in the dye concentration has an adverse effect on the growth of bacteria; hence, the decolorization rate decreases. Growth of fungi is also affected by increased dye concentrations. Kapdan and Kargi (2002) reported that a 1200 mg/L dye concentration of Everzol Turquoise Blue G, a phthalocyanine-type reactive dyestuff, was degraded by *Coriolus versicolor*. The dye removal affinity was increased if the dye concentration was lower: a concentration of 100–250 mg/L was degraded within 3–5 days, and a concentration of 700–1200 mg/L required up to 9 days of incubation. Other researchers have also shown that decolorization of dye decreases with increasing dye concentrations (Parshetti et al. 2006).

23.6.4 Effects of Azo Dye Structure

Biodegradation of azo dyes by the action of enzymes is greatly influenced by the dye structure (Sawhney and Kumar 2011). Azo dyes comprising electron-withdrawing groups are more easily degraded than those consisting of electron-releasing groups (e.g., –NH–triazine). Therefore, it has been concluded that dyes with additional electron-withdrawing groups perform better decolorization.

23.6.5 Effects of Nutrients (Carbon and Nitrogen Sources)

Sudhakar et al. (2002) reported that nutrients play significant roles in dye removal processes; larger amounts of nutrients significantly increase the growth of microorganisms and hence improve the rate of degradation. *Pseudomonas* sp. BSP-4 isolated from azo dye–polluted soil has the ability to decolorize and degrade the azo dye Black E by consuming it as a nitrogen source at up to 300 ppm within 36 h. The nutrient constituents of the culture medium play an important role in color removal. Natural supplements have been shown to have a positive impact on dye decolorization by the fungus *Aspergillus fumigatus* XC6; supplementation with high carbon and nitrogen sources, predominantly in the form of salts such as ammonium sulfate, had a significant effect on effluent degradation (Jinqi and Houtian 1992).

23.6.6 Effects of Biological Structures Involved in Decolorization of Azo Dyes

Various microbial systems have been tested for decolorization and degradation of azo dyes, including algae, yeasts, molds, filamentous fungi, actinomycetes, and bacteria. All microbes have their own mechanisms and act differently in dye decolorization. The mechanisms involved are biosorption, enzymatic degradation, bioaccumulation, etc.

23.6.7 Effects of Electron Donors

Studies have revealed that addition of an electron donor (e.g., glucose or acetate ions) leads to the breakdown of azo bonds. The cleavage depends upon the type and availability of the electron donor, which plays an important role in attaining optimal dye decolorization in fermenters working under anaerobic situations (Razia Khan et al. 2013).

23.6.8 Effects of Redox Mediators

Razia Khan et al. (2013) have reported that redox mediators (RMs) improve the degradation processes of azo dyes under anaerobic conditions.

23.6.9 Effects of Agitation

Rohilla et al. (2012) reported that agitation favored the decolorization of certain dyes such as Orange M2R, with a maximum decolorization rate of 89.3% being achieved by a fungal consortium, whereas *Aspergillus flavus* and *A. niger* degraded dye concentrations by up to 60–70% in stationary conditions within 10 days. It was observed that shaking of the medium suppressed the functioning of ligninolytic enzymes in *P. chrysosporium* (Swamy and Ramsay 1999). Usually, better results were achieved under shaking conditions than under stationary conditions because of increases in mass and the oxygen transfer rate between cells and the medium. Jarosz Wilkołazka et al. (2002) concluded that shaking had a positive effect on dye decolorization. Shaking of cultures of various strains such as *Bjerkandera fumosa*, *Stropharia rugosoannulata*, and *Kuehneromyces mutabilis* promoted greater dye removal than that achieved in stationary cultures (Kirby et al. 2000). Yet, certain microbes such as *Phlebia tremellosa* gave better results in stationary conditions (Rohilla et al. 2012).

23.6.10 Effects of Aeration

Aeration means supply of oxygen to microbes growing in culture media. This is an important factor for the growth of microorganisms. The limitation of aeration is that oxygen has low water solubility (8 mg/L at 20 °C). To overcome this problem and make oxygen more soluble in water, aeration and agitation are required. This may affect the morphology of fungi and may lead to a decrease in the production of enzymes (Žnidaršič and Pavko 2001). Nowadays, bioreactors are designed to have static and agitated configurations to provide plenty of oxygen. Selection of the bioreactor depends on the specific system; however, proper agitation provides better results than static conditions (Knapp et al. 2001).

23.7 Other Technical Factors Affecting Bioremediation

There are several other factors that affect the biodegradation of textile dyes. These factors are categorized as environmental, microbial, substrate, mass transfer limitation, aerobic and aerobic processes, etc., as listed in Fig. 23.1.

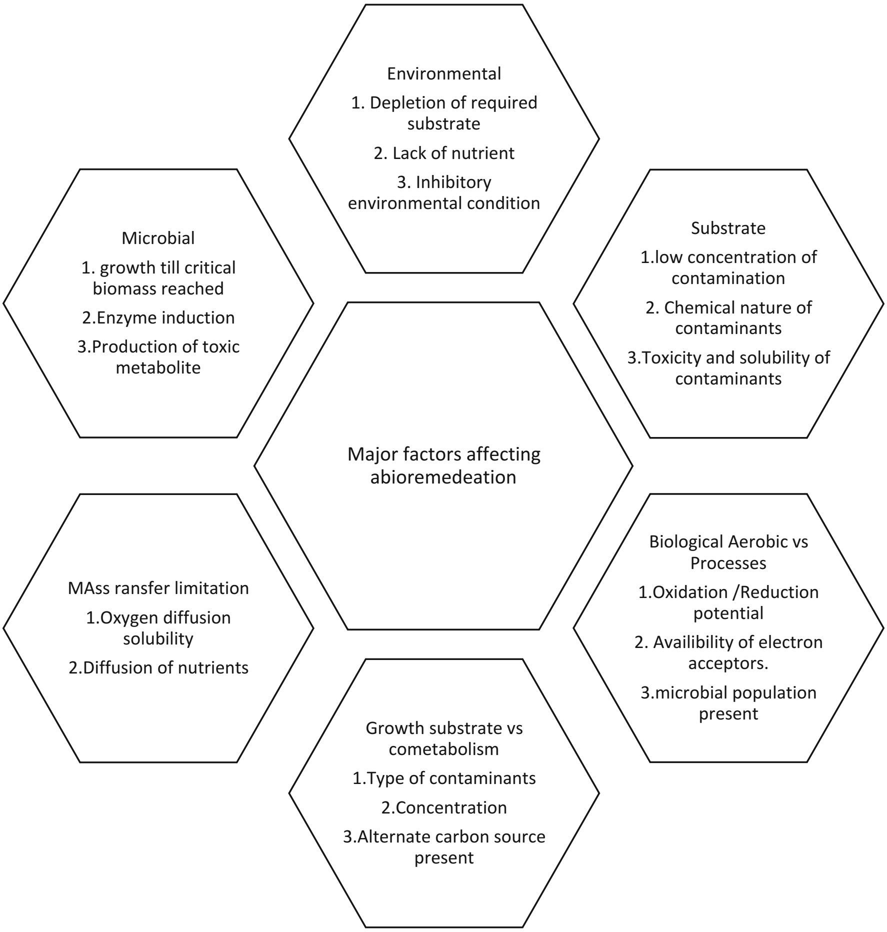

Fig. 23.1 Factors affecting bioremediation

23.8 Environmental Concerns

Environmental issues related to the residual dye content of textile effluent from fabric industries are considered a big challenge. Direct discharge of effluent directly into water bodies contaminates them (Zaharia et al. 2011). It is not known exactly what amounts of dyes are produced worldwide, nor what amounts are discharged into the environment (Forgacs et al. 2004). As dyes are used on a large scale, their discharge into the environment remains high, which in turn leads to serious health risk concerns. The growing impact of environmental protection on industrial development is resulting in the introduction of new eco-friendly technologies (Forgacs et al. 2004).

Robinson et al. (2001) reported that most dye effluent was not completely degraded by conventional wastewater treatment, because of its high stability to light, temperature, water, chemicals, soap, detergent, and certain additional parameters. Water is essential for life and is required in various household activities. Water is also used as a chief component in all industries. It is the most essential natural resource, but unfortunately it is the most polluted for reasons such as increased population, urbanization, lavish lifestyles, and industrialization.

23.8.1 Harmful Effects to Living Bodies

The deadly effects of textile dyes may be due to the presence of azo dyes or aryl amine derivatives produced during breakdown of azo bonds (Rajaguru et al. 1999). These dyes enter organisms by consumption and are converted into aromatic amines by the enzymes present in the intestinal tract. Nitro dyes are metabolized by nitroreductases produced by the same microorganisms (Umbuzeiro et al. 2005). Mammalian liver enzymes catalyze the reductive breakdown of azo bonds and nitroreduction of nitro groups. In both cases, if N-hydroxyl amines are produced, they can cause destruction of DNA (Umbuzeiro et al. 2005; Arlt et al. 2002). Moreover, the effects produced by other contaminants greatly disturb the quality and transparency of water bodies such as ponds, rivers, and lakes, damaging aquatic life (Ibrahim et al. 1996; Wijetunga et al. 2010).

As noted by Hassani et al. (2008), textile effluent consists of a blend of contaminating substances comprising dyes and organic substances. Ninety-three percent of water intake comes out contaminated by dyes containing large amounts of organic complexes and heavy metals (Wijannarong et al. 2013; Gupta et al. 2014b). The nonbiodegradable nature of some dyes and organic compounds leads to serious ecological hazards. Colored wastewater is unhealthy for aquatic bodies, reducing the oxygenation ability of the water and disturbing the life of the aquatic ecosystem and the food chain (Xu et al. 2005).

23.8.2 Effects on Humans

Research in 1992 found that occupational exposure to some azo dyes with aromatic amines—principally benzidine, 2-naphthylamine, and 4-aminobiphenyl—increased the risk of bladder cancer (Chequer et al. 2011;Puvaneswari et al. 2006). These dyes are toxic and also contain chemical compounds that are carcinogenic, mutagenic, or teratogenic to humans (Novotný et al. 2006; Mathur and Bhatnagar 2007). Carcinogenicity caused by the presence of azo dyes in textile effluent is well known (Weisburger 2002;Umbuzeiro et al. 2005). Studies done by Srivastava et al. (2004) have shown that Malachite Green not only destroys the human immune system and reproductive systems but also acts as a probable genotoxic and cancer-causing agent. The dye CI Disperse Blue leads to frameshift mutations and base pair substitutions in *Salmonella* (Umbuzeiro et al. 2005). The genotoxic and cytotoxic effects of CI Disperse Blue on human cells was studied by Tsuboy et al. (2007).

23.8.3 Effects on Water Bodies

Chen (2006) has reported that nitro and azo dyes are degraded into harmful carcinogenic amines and deposited in water bodies. These derivatives lead to water pollution and affect aquatic life.

Table 23.2 shows the various advantages and disadvantages of different conventional and bioremediation processes.

Table 23.2 Advantages and disadvantages of conventional and bioremediation processes

Methods	Advantages	Disadvantages
Fenton's reagent	High-grade removal of both soluble and insoluble dyes	Huge sludge production
Ozonation	Applied in a gaseous state: no alteration of volume	Short half-life (20 min)
Photochemical	No sludge production	Generation of toxic chemical compounds as by-products (e.g., NaOCl)
Cucurbituril	Good sorption potential for a large variety of dyes	Economically unfavorable
Electrochemical destruction	Decomposition products are nontoxic	High cost of electricity
Activated carbon	High-grade potential to adsorb a wide variety of dyes	Very expensive
Peat	Efficient absorbent	Less effective than activated carbon
Wood chip/ wood sawdust	Good sorption capacity for acid dyes; effective adsorbent because of cellular structure; good adsorption capacity for acid dyes	Requires long retention times
Silica gel	Applied successfully for basic dye removal	Less suitable on a commercial scale because of side reactions
Membrane filtration	Good potential to remove all dye types	Concentrated sludge generation
Ion exchange	Regeneration: no adsorbent loss	Less effective
Irradiation	Effective oxidation at the lab scale	Requires a lot of dissolved O_2
Electrokinetic coagulation	Good economic feasibility	Sludge production
Sonication	Simple to use; very effective in integrated systems	Relatively new method, awaiting full-scale application
Enzymatic treatment	Effective for specifically selected compounds	Enzyme isolation and purification is tedious
Photocatalysis	Process carried out in ambient conditions; inputs are nontoxic and inexpensive; complete mineralization with shorter detention times	Effective for only small amounts of colored compounds; expensive process
Single cell (fungal, algal, and bacterial)	Good removal efficiency for low volumes and concentrations; very effective for specific color removal	Culture maintenance is cost intensive; cannot cope with large volumes of wastewater

Data sources: Anjaneyulu et al. (2005), Babu et al. (2007), Robinson et al. (2001), and Joshni and Subramaniam (2011)

23.9 Conclusions

Various physical and chemical approaches have been used for degrading dye effluent. These approaches commonly have many limitations, such as high costs, low efficiency, and inadequate resources; they also release secondary pollution (sludge, etc.). On the other hand, bioremediation is an economical, efficient, eco-friendly, and biologically benign technique for textile dye and wastewater treatment. Microbial degradation of textile effluent includes use of bacteria, fungi, yeasts, algae, and plants. Studies have shown that diverse microbes are proficient in detoxifying various dyes. Use of microbes for the elimination of synthetic dyes from textile effluent is an alternative to make remediation processes economically viable. Thus, bioremediation is considered the method best suited to treatment of textile effluent.

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