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# **Bioremediation and Detoxification Technology for Treatment of Dye(s) from Textile Effluent**

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Additional information is available at the end of the chapter

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## **Abstract**

The aim of this chapter is to demonstrate the technical and economic feasibility of an integrated process for microbial treatment of dye(s) containing wastewater from textile effluent that evaluates the efficiency and effectiveness to meet the dye(s)' maximum contaminant level. This chapter covers the whole process of microbial treatment methods that are adopted for dye removal to make an eco-friendly system. The purpose of this treatment technology includes process modifications and engineering approaches. It comprises existing technologies with new advancement technology at all stages of the process. This chapter evaluates the reliability of technologies for small and large systems to make the system cost-effective. It also demonstrates how genetically engineered micro-organism works and shows that the "microbial treatment platform for dye removal" can operate with positive economical balance to economize the bioprocess technology. Thus, future prospects of microbial treatment technology should be directed not only how to economically improve bioremediation but also how to effectively commercialize such economically sounded "bio-based" treatment methods in different industries.

**Keywords:** Dye(s), color, bioremediation, technology, genetic engineering

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## **1. Introduction**

The increasing demand for rapid urbanization, changing consumption, population growth, and fast socioeconomic development has inevitably led to an increased water pollution on the biosphere, which leads to environment pollution [1]. The effluents generated from domestic and industrial activities constitute the major sources of the natural water pollution load such as dye(s), heavy metal, cyanide, toxic organics, nitrogen, phosphorous, phenols, suspended solids, color, and turbidity. This pollution load is a great obligation in terms of wastewater

management, which not only increases treatment cost considerably but also introduces a wide range of chemical pollutants and microbial contaminants to water sources. Dye(s) and pigments used in textile industries are playing a crucial role in value addition, appearance, and fulfillment of customers' desire. It has been found that the use of synthetic dye(s) has been rapidly increased in textile industries due to cost-effectiveness in synthesis and high stability. In addition, various colors can be synthesized when compared to natural dye(s). In consequences, a huge discharge of polluted effluent was found in different industries. It has been reported that color can be visible at a concentration higher than 1 mg/L [2,3]. It is obvious that the dye(s) containing water interferes with penetration of sunlight, which retards photosynthesis. In addition, it also inhibits the growth of aquatic flora and fauna by interfering with gas solubility [4]. In addition, it was experimented that there are so many dyes that are carcinogenic in nature [5]. As the use of synthetic dye(s) has tremendously increased in the industrial process and humans are being exposed to them more, water pollution due to these dyes is a critical issue in terms of human health concerns and serious ecological consequences. Hence, there is an urgent need for removal of these dyes from the effluents.

There are several methods applied for the removal of these dyes from wastewater, which include physical, chemical, and biological. However, these technologies vary in terms of their efficiency, cost, and environmental impact. Hence, there is an urgent need for all researchers to find out an efficient, inexpensive, and environmentally friendly system to reduce dye content in wastewater at acceptable levels.

## **2. Background to dye(s)**

Colorants are chemicals that give color to the materials to which they are applied. Colorants can be classified into pigments and dye(s), and the pigments and dye(s) mainly differ from each other based on their solubility. Pigments retain their crystalline or particulate nature during the application. They are always combined in some medium that is applied to a surface. On the other hand, dyes are soluble and diffuse into the material and become an integral part. The former are usually used in paints, inks, and polymers, and the latter are applied not only mainly to textiles but also to paper, leather, food, and other products. For a colored substance to be regarded as a useful dyestuff, factors beyond solubility are required. A dyestuff must be substantive for a textile and thus be preferentially taken up by the fiber, usually from an aqueous solution [6].

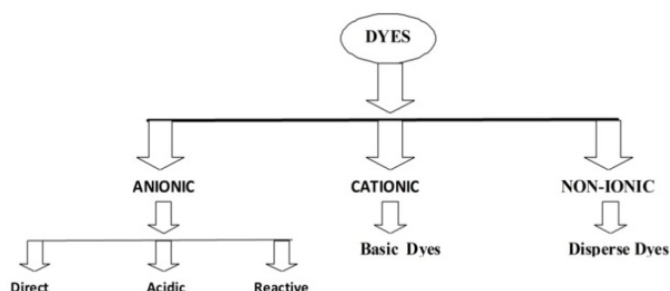
## **3. The physical basis of color**

Dyes possess color due to the absorbance of light in the range of 400–700 nm, i.e., in the visible spectrum; a dye possesses at least one chromophore (color-possessing group); it should have a conjugated system such as structure with alternate double bonds and single bonds. The dye molecule exhibits resonance of electrons, which is a stabilizing force in organic compounds [7]. When among these properties if anyone is not present, then the molecular structure of color

will be lost (dye chemistry). Chromophore undergoes  $\pi-\pi^*$  and  $n-\pi^*$  transitions. Auxochromes are also present in most dyes that will influence the solubility of the dyes, for example, carboxylic acid group, amino group, sulfonic group, and hydroxyl group. Auxochromes cannot undergo  $\pi-\pi^*$  transitions but can undergo transition of  $n$  electrons.

#### 4. Classification of dye(s)

Dye(s) are the organic compounds that will impart color to different substrates, which include paper, printing, textile, cosmetics, waxes, plastics, pharmaceuticals, and so on; there are two colorants present: dyes and pigments. Dyes are mostly soluble in water and diffuse into the material and are fixed for colorizing the material, whereas pigments are mostly insoluble in water and do not interact with the substrates. The preparation of the synthetic dyes involves the conversion of basic organic chemicals such as benzene, anthracene, and so on into dye intermediates through the addition of different functional groups such as nitro, amino, bromo, chloro, and so on [7]. Dyes are classified based on their chemical structure and color index (CI). Some common classes of dyes are mono-azo, di-azo, tri-azo, anthroquinone, triaryl methane, and phthalocyanines. Further, dyes can be classified based on the usage in textile industry such as anionic, cationic, and non-ionic (Fig. 1). On the basis of the color, dyes can be subclassified or subdivided into yellow, orange, red, violet, blue, green, and black [7].



**Figure 1.** Classification of dye(s) [8].

Some properties of dyes classified on their usage [9–11] are discussed elaborately as follows:

1. Acid dyes: They are water-soluble anionic dyes in nature and negative in soluble form. The functional groups that are present in acidic dyes are azo (including premetallized), triphenylmethane, anthroquinone, azine, xanthene, nitro, and nitroso. They are made for dyeing of nylon, wool, and silk. However, it can be used for paper, leather, ink-jet printing, food, and cosmetics.
2. Cationic (basic) dyes: They are also soluble in water after modification. However, they produced colored cations in solution; therefore, it is also called cationic dyes. The functional groups that are present in basic dyes are triaryl methane, diazahemicyanine, cyanine, hemicyanine, thiazine, oxazine, and acridine. Basic dyes are extensively used for dyeing of jute, cut flowers, dried flower, coir, and so on.

3. Disperse dyes: They are almost insoluble in water and remained as suspended microscopic particles. These dyes are only effective for dyeing of polyester. Some types are also used for nylon and acetate. The functional groups remained for disperse dyes are azo, anthroquinone, styryl, nitro, and benzodifuranone.
4. Direct dyes: They are anionic dyes and normally applied together with electrolyte, sodium chloride, and sodium sulfate for high affinity to cellulosic fibers. These dyes are molecules that can hold on cellulosic fibers without help from other chemicals. They are used for dyeing of cotton, rayon, paper, leather, and nylon.
5. Reactive dyes: They create a covalent bond between the dye and the fiber. They exhibit a reactive group that may be haloheterocycle or an activated double bond. They are normally used in alkali condition through the formation of chemical bond with a hydroxyl group on the cellulosic fiber. It has been found that during application the chromophore group is activated and allowed to react to the surface of the substrate. It is used as colorant for dyeing of cotton and cellulose.
6. Solvent dyes: They are normally soluble in organic solvents. They are used as colorants for organic solvents, hydrocarbon fuels, waxes, lubricants, and plastics. They are nonpolar and do not ionized in solution. The principal chemical groups are predominantly azo and anthroquinone, but phthalocyanines and triarylmethane dyes are also used.
7. Sulfur dyes: They are synthetic organic molecules used for the coloration of cellulosic fiber. These dyes contain sulfur group as chromophore. These are ionized through thionization or sulfurization of organic intermediates. They are not soluble in water and do not have any affinity for the cellulosic fiber. However, when treated with a weak alkaline solution of sodium sulfide or reducing agent to form a leuco compound that is soluble in water and has affinity to cellulosic materials. They are used for the coloration of cotton and rayon and have limited use with polyamide fibers, silk, leather, paper, and wood.
8. Vat dyes: They are normally insoluble in water. They are indigo and the anthraquinone derivatives, which are used for the coloration of cellulosic fibers. The dye is applied in a soluble or reduced form through the impregnation of fiber. It is further oxidized in the fiber back to its original insoluble form.

## 5. The production and discharge of dye(s)

More than 10,000 dyes are commercially available [12] and currently used in various materials such as textiles, paper, plastic, leather, food, drugs, and cosmetics [13]. In 2005, about 108 tonnes of dyestuffs were produced in the world. As textile industries have long been the largest consumer of dyes, it was foreseeable that the demand of dyes in the world will continue to rise in the forthcoming years because the fiber consumption has generally increased at a rate faster than the growth of population [6].

The rapid growth in the use of reactive dyes is due to the increasing use of cellulosic fibers and the technical and economic limitations of other dyes used for these fibers [14]. It is estimated

that 2% of dyes produced annually are discharged in effluents from manufacturing operations, and 10% of dyes were discharged from textile and associated industries. Therefore, a large amount of dyes are lost into the effluents. The discharge of highly colored effluent is currently one of the major environmental problems. It can be seen that reactive dyes have rather low rates of fixation while the highest fixation rates are basic dyes. After the reactive dyeing process is complete, up to 800 mg/L of hydrolyzed dyes may remain in the bath. Therefore, a high concentration of reactive dyes is discharged into the effluent. In addition, reactive dyes are not easily biodegradable and thus may still remain in the effluent even after extensive treatment [15]. It has been found that large volumes of water and chemicals are normally required for textile industries. In consequence, huge unused dye(s) and auxiliary chemicals along with large amounts of water are found in wastewater streams from the textile dyeing operation. It has been reported that about 8–20% of the total pollution load was contributed due to incomplete exhaustion of the dyes. It is obviously true that the presence of dyes about as to 1 ppm in water is usually unacceptable [16].

## 6. The toxicity of dye(s)

Dye toxicity has been studied in many researches. The acute toxicity of dyes is generally low. Only a few dyes and pigments are considered to be carcinogenic by U.S. regulatory agencies. Among these are benzidine and benzidine congener dyes such as CI Direct Black 38, CI Acid Red 114, CI Direct Blue 15, and CI Direct Blue 218 [17]. Azo dyes contain one or more nitrogen–nitrogen double bonds ( $-N=N-$ ) called azo groups in the chemical structure. Azo dyes are seldom directly mutagenic or carcinogenic except for some azo dyes with free amino groups [18]. Under reductive condition, the azo groups can be cleaved to form two aromatic amines.

After cleavage of azo linkages, toxic amines are released to water. These intermediate products cause severe detrimental effects on human beings and aquatic life. For human being, these intermediates damage the vital organs such as the brain, liver, kidneys, central nervous system, and reproductive system. However, they can also prevent photosynthetic activity by reducing light penetration. In this way, the hazardous effects of dye(s) percolate from their discharge point to receive water. Therefore, it is urged for researchers to find out the way for removal of such toxic components in industrialized countries in the world [19]. Wastewater treatment is a difficult task by conventional methods such as physical, chemical, and biological due to its complex molecular structure of dyes. Some new technologies are being investigated by which decoloration of the problem could be solved.

## 7. Pollution from the dye-containing effluents

The colored effluent brings a negative esthetic effect on the wastewater because the color can also be observed by our eyes even when the concentration is less than 1 ppm. In addition, the absorption and reflection of sunlight by the colored effluents affect the water transparency and gas solubility of water bodies. The heavy-metal ions from textile effluents have also been



reported at high concentrations in both algae and higher plants [20]. Even worse, some of the dyes and their biodegraded products are also toxic, carcinogenic, and mutagenic [17].

Sometimes, it has been found that due to the accessibility of small-scale industries in the greater extent, it was started the decentralized treatment methods for individual industry. As the treatment process for such toxic pollutants in proper manner is an expensive, they are eventually discarded into the environment that is about 40% of the total industrial wastewater. As far the environmental protection is an urgent issue in connection with industrial development, it promotes to develop eco-friendly technologies that will reduce the consumption of freshwater and lower output of wastewater. The release of important amounts of synthetic dyes to the environment causes public concern and minimizes legislation problems [21,22].

## 8. Dye removal techniques

The strategy for removal of dyes and pigments from textile effluents is just a simple separation process from a purely engineering point of view. Theoretically, there is a great number of separation processes tailored to the removal of a specific compound. Dye-containing wastewater can be treated in different ways such as physical, chemical, and biological approaches. Physical treatment methods employ the application of physical forces for the separation of dyes from wastewater. Physical methods include different precipitation methods (such as coagulation, flocculation, and sedimentation), adsorption (on a wide variety of inorganic and organic supports), filtration, reverse osmosis, ultra filtration, and nanofiltration. However, chemical method is brought about by the addition of chemicals or by chemical reactions (such as reduction, oxidation, complex metric methods, ion exchange, and neutralization). However, biological treatments normally carried out aerobically or anaerobically that will depend on the presence or absence of oxygen to the system. Biological treatments happen in the presence of biological catalyst that stimulates the degradation are also called biodegradation. Chemical treatment methods are normally carried out by the addition of chemicals through chemical reaction such as reduction, oxidation, complex metric methods, ion exchange, and neutralization [23]. It may eventually generate toxic intermediate products. It is obviously true that the treatment of colored wastewaters depends not only on ecological parameters such as chemical oxygen demand (COD), biological oxygen demand, total organic carbon, absorbable organic halide, temperature, and pH but also on initial dye concentrations in wastewaters [23]. The entire process is considered environmentally friendly when the by-product stream has negligible environmental effects. Otherwise, additional treatment is required, and the problem of the removal of the hazardous compounds from the raw influent has been just relocated.

### 8.1. Physical treatment

#### 8.1.1. Coagulation–flocculation

Coagulation–flocculation methods were successfully applied for color removal of sulfur and disperse dyes, whereas acid, direct, reactive, and vat dyes presented very low coagulation–

flocculation capacity. Coagulant agents that are normally used for decolorization of colorants are aluminum sulfate, ferrous, ferric sulfate, ferric chloride, calcium chloride, and copper sulfate as well as several co-polymers such as penta ethylene, hexamine, and ethylenedichloride. The mechanism behind this coagulation process is by the formation of flocs with the dyes, which can be separated by filtration or sedimentation [23]. Polyelectrolyte can also be dosed during the flocculation phase to improve the floc settleability [24]. It is obviously advantageous that there is no question of decomposition of dyes, which can produce more potentially harmful and toxic compound. In addition, only separation of colorants has been taken place, which is economic. However, the production of sludge is only disadvantage for coagulation–flocculation processes [25].

In coagulation process, chemicals are rapidly dispersed in wastewaters, which can change the characteristics of the suspended particles such that they tend to coalesce and form flocs that sink rapidly. Conventional physical process is not efficiently separate the negatively charged colloidal suspensions. However, the decoloration of effluent stream can be made economically through the application of coagulation process. Coagulation process is normally carried out by the addition of positive ions that will reduce the electro kinetic repulsion between the particles. It has been reported by Marmagne and Coste that the color removal of sulfur dyes can be efficiently carried out by coagulation. They did their experiment in bench flocculators. The good-quality flocs were produced; therefore, minimum time was taken for settling. It was reported that the removal efficiency in terms of COD and color was found to be 83.9% and 96.1%, respectively [26,27]. Coagulation process is also affected by the chemicals, pH, and temperature of the system [27].

#### *8.1.2. Filtration technology*

The filtration technology is the prime module that is used in drinking water and wastewater treatment. This technology constitutes microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. For the removal of the color, this technology showed some promising results. Individual membrane is significant for the water treatment process. Microfiltration is not suited for the wastewater treatment due to its large pore size; nanofiltration and ultrafiltration are efficiently remove different sorts of dyes. Generally, the dye molecule clogged in the membrane and limits the separation process for the usage of dyeing effluent treatments. The limitation of this process is its high pressure, momentous energy consumption, cost of membrane is high, and life span is also short, and these properties are creating hindrance for the treatment of dyestuff or organic pollutant removal. The reverse osmosis process is better for rejecting salts. It gives better results in decolorizing and desalting against various dye effluents and can be applied for recycling. The treated wastewater is near to pure water.

#### *8.1.3. Adsorption*

Adsorption is the process in which dissolved molecules are attached to the surface of an adsorbent by physical and chemical forces. Adsorption by activated carbon has been widely used for wastewater treatment. Activated carbon and other materials can remove the dyes in the wastewater, either by adsorption or by combined adsorption and ion exchange. Adsorption



gives good results and has gained favor recently due to its ability to remove different types of dyes, excellent adsorption ability [28,29], ease of operation, and insensitivity to toxic pollutants and can be used in fixed bed columns for treating the water continuously [30]. However, its widespread use is restricted due to high cost [31]. Therefore, researchers have been looking for low cost adsorbents as alternatives to activated carbon.

## 8.2. Chemical treatment

Chemical treatment of the wastewater is carried out with the help of coagulants and flocculants, and it gives quiet promising results. Chemicals that are being used in this treatment are aluminum, calcium, and ferric ions for the removal of the dye effluents and induce flocculation. For the betterment of the process, the combination of two might be used to augment the process. Sometimes, the process is economically sound, but, in some instances, it could be expensive due to the price of the chemicals. The process gives satisfactory removal of disperse, vat, and sulfur dyes. However, the limitation of this technique is the production of concentrated sludge in large quantity, and the removal of reactive, azo, and basic dyes by this technique is not at par or up to the mark.

### 8.2.1. Oxidation

Oxidation is the one of the most commonly used chemical decoloration process due to its simplicity of application. Oxidation processes can include oxidation through biological organisms, ozone, sodium hypochlorite, hydrogen peroxide, and even acids. The oxidative process will produce smaller molecules because the dyes are broken down. Conventional oxidation treatments are incapable to oxidize dyes (mainly for removing color) and toxic organic compounds completely from textile effluents. The above-mentioned limitation can overcome through the development of advanced oxidation processes (AOPs) where the generation of free hydroxyl radicals (OH) takes place. It is obviously true that these free radicals increase the rate of reaction with several folds when compared with normal oxidants. OHs can oxidize both the dyes and the toxic organic compounds that normally cannot be oxidized by conventional oxidants [25]. In AOPs, oxidizing agents such as ozone and hydrogen peroxide are used with catalysts (such as Fe, Mn, and  $\text{TiO}_2$ ) either in the presence or in the absence of an irradiation source [24]. Chemical oxidation removes the dyes from the dye-containing effluent by oxidation, resulting in aromatic ring cleavage of the dye molecules [32].

## 8.3. Biological techniques

Recent application of several physicochemical methods has been used for azo dye decolorization, but these methods are expensive and produce large amounts of sludge after treatment. Extensively used coagulation or flocculation techniques create or generate large amounts of sludge that requires safe disposal. Adsorption and, to a certain extent, membrane filtration techniques lead to secondary waste streams that need further treatment. There are many reports on the use of physicochemical methods for color removal from dye-containing effluents [33–35] apart from that present scenario biological treatment methods are most suitable and

widely used due to their cost effectiveness, ability to produce less sludge, and eco-friendly nature [36,37].

Bioremediation is normally carried out by the use of microorganisms to remove the pollution from the environment, which is a key research area in the environmental engineering [38]. In such approaches, microorganisms adapt themselves to the toxic wastes and develop into new resistant strains naturally, which then transform various toxic chemicals into less harmful forms. The mechanism behind the biodegradation of recalcitrant compounds in the microbial system is based on the action of the biotransformation enzymes [39]. Several reports demonstrate the degradation of complex organic substances that can be brought about by enzymatic mechanisms, such as those associated with laccase [40], lignin peroxidase [41], NADH-DCIP reductase [42], tyrosinase [43], hexane oxidase [39], and aminopyrine *N*-demethylase [44]. There are several treatment approaches successfully applied by the biotechnologists to remove the dyes from effluent streams with regard to tackling azo dye pollution in an eco-efficient manner. It is reported that the use of bacteria followed by physicochemical processes may be used for successful removal of azo dyes. As azo dyes are xenobiotic in nature and recalcitrant to biodegradation, the use of microbial or enzymatic treatment method may be useful for the complete removal or degradation of such dyes from textile effluent. In this approach, several advantages have eco-friendly, inexpensive, and less sludge production. In addition, the intermediate products that are formed are nontoxic due to complete mineralization, and the process requires less water consumption when compared with physicochemical methods [38]. The effectiveness of microbial decolorization depends on the adaptability and the activity of the selected microorganisms. Consequently, a large number of species have been tested for the decolorization and mineralization of various dyes in recent years [45]. The isolation of potent species and their degradation is one of the interesting biological aspects of effluent treatment [46]. A wide variety of microorganisms are capable of decolorizing a wide range of dyes, including bacteria [39], fungi [47], yeasts [48], actinomycetes [48,49], and algae [50].

### 8.3.1. Decolorization and degradation of dyes by plants (phytoremediation)

Phytoremediation is an emerging technology that promises the effective and inexpensive approach for the remediation of soils and groundwater contaminated with heavy metals and organic pollutants [51]. As phytoremediation is an autotrophic system and requires little nutrient input, the main advantages of phytoremediation are easier to manage and accepted by public due to both of its esthetic appeal and environmental sustainability [38,52]. More specifically, narrow-leaved cattails have been studied in synthetic reactive dye wastewater treatment under caustic conditions [53,54], where approximately 72–77% can be reduced with coco yam plants. It has been reported that the three plant species namely *Brassica juncea*, *Sorghum vulgare*, and *Phaseolus mungo* from different agronomic consequences have been used to evaluate the decolorization efficiency by using azo dyes in textile effluent. It has been reported that *B. juncea*, *S. vulgare*, and *P. mungo* showed 79%, 57%, and 53% efficiency, respectively [38,55]. Similarly, an herb *Blumea malcommi* was found to degrade textile dyes (Reactive Red 5B). Extensive research has been undertaken to develop effective and efficient phytoremediation techniques. It was reported that hairy root cultures of *Tagetes patula* L.

(Marigold) are effective in the decolorization of Reactive Red 198, and the enzyme system in the plant responsible for this was determined [51]. However, there are several disadvantages related to large-scale phytoremediation process. It includes pollutants tolerance by the plant, the bioavailable fraction of the contaminants along with the transpiration of volatile organic pollutants and large areas to implant the treatment [38,56].

### 8.3.2. Microbial treatment

Microorganisms are already present in the wastewater treatment feed on the complex substances in the wastewater, converting them into simpler substances, improvement treatment. The biological treatment is nowadays common and extensive technique employed in dye wastewater treatment. There are several reports where a huge number of species have been used for the removal and complete mineralization of different sorts of dyes. The main advantage of this process is inexpensive, low running costs, and nontoxic end products. However, these process may be aerobic (in the presence of oxygen), anaerobic (without oxygen), or combined aerobic–anaerobic. Bacteria and fungi are normally used in aerobic treatment for their ability to treat dye wastewaters [11].

#### 8.3.2.1. Aerobic treatment

In aerobic treatment, enzymes secreted by bacteria present in the wastewater breakdown the organic compounds. The work to identify and isolate aerobic bacteria capable of degrading various dyes has been going on since more than two decades. A number of triphenylmethane dyes, such as magenta, crystal violet, pararosaniline, brilliant green, malachite green, and ethyl violet, have been found to be efficiently decolorized (92–100%) by the strain *Kurthia* sp. It was reported by the researchers that after biotransformation, the extent of COD reduction of the cell free extracts of triphenylmethane dyes was more than 88% in all dyes except in the case of ethyl violet (70%). Since last two decades, various researchers have investigated *Phanerochaete chrysosporium*, among various fungi, extensively for its ability to decolorize a wide range of dyes. Besides this, microorganisms including *Rhizopus oryzae*, *Cyathus bulleri*, *Coriolus versicolour*, *Funalia trogii*, *Laetiporus sulphureus*, *Streptomyces* sp., *Trametes versicolour*, and so on have also been tested for the decolorization of dyes. It has been found that different operating parameters such as initial concentration of pollutants, initial pH, and temperature of the effluent affect the removal process. Several strategies may be used after fungal treatment. It has been reported that the treatability of the effluent by other microorganisms can be improved for satisfactory removal of dyes. It is obviously true that these techniques are suitable for some dyes; however, most of the dyes are recalcitrant to biological breakdown or are non-transformable under aerobic conditions [11].

#### 8.3.2.2. Anaerobic treatment

The anaerobic treatment is quiet promising for the degradation of an extensive range of synthetic dyes has been well demonstrated and established. From the literature, it has been reported that under anaerobic conditions, some dyes have been degraded or mineralized. Since last few decades, researchers reported that decolorization of azo dyes showed some positive

result in case of mordant orange-1 and azo-disalicylate could be reduced and decolorized under anaerobic conditions using methanogenic granular sludge. Another study proved the feasibility of the application of anaerobic granular sludge for the total decolorization of 20 azo dyes. An anaerobic pre-treatment step could be a cheap alternative compared with aerobic systems as expensive aeration is absent and problems with bulking sludge are avoided. It is reported that anaerobic treatment of effluent for dye removal can be efficiently carried out; however, heavy metals can be retained through sulfate reduction. In addition, due to foaming problems, associated for surfactants and high effluent temperatures along with high pH is the main limitation for degradation of dyes. Nevertheless, it is also mentioned that BOD removal can be insufficient; dyes and other refractory organics are not mineralized; nutrients (N and P) are not removed; and sulfates give rise to sulfides [11].

#### 8.3.2.3. *Combined aerobic–anaerobic treatment*

For the better removal of dyestuff from the wastewater of textile effluent, the combination of aerobic and anaerobic treatments may give promising results. It is advantageous because the complete mineralization is achieved due to the synergistic action of different organisms. It has been reported that the reduction of the azo bond can be achieved under the reducing conditions in anaerobic bioreactors. In consequence, colorless aromatic amines may be formed, which are mineralized under aerobic conditions. Therefore, the combined anaerobic–aerobic azo dye treatment system is an attractive approach for the researchers [11]. Thus, an anaerobic decolorization followed by aerobic post-treatment is generally recommended for treating dye wastewaters. Generally, the operating conditions such as initial concentration of dyes, initial pH of solution, and temperature of the effluent play an important role for decolorization of dyes. In addition, this technique is cost competitive and suitable for various dyes. However, the main limitation of the biological treatment is low biodegradability, less flexibility in design and operation, larger land area requirement, and longer times required for decolorization processes. Therefore, it is an urge for scientist for removal of dyes from effluent on a continuous basis in liquid-state fermentations [11].

#### 8.3.2.4. *Enzyme-mediated dye removal*

From the literature, it has been found that the white-rot fungi, which produce nonspecific extracellular ligninolytic enzymes are most efficient to remove synthetic dyes. These enzymes are lignin peroxidase (ligninase, LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13), and copper-containing laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2). Of these enzymes, laccases (EC 1.10.3.2) have great potential in bioremediation due to their ability to oxidize a broad range of substrates. Laccases belong to the group of oxidases, which contain four copper atoms in their catalytic site. The capability of laccases to degrade phenolic compounds makes them suitable for the degradation of xenobiotic compounds in the treatment of wastewaters [57].

There is an extensive research carried out for laccase-mediated dye removal using wild strain owing to their potential industrial applications. Screening of proper microorganism is important criteria to get the desired product. The microorganism that will be used for laccase



production should produce adequate yields and should not produce toxins or any other undesired products. The main challenges using wild strain are the availability of potent microbial strain and the application of this biocatalyst for industrial-scale dye removal [58]. It is also important to note that this strain should be robust under industrial conditions. It is advantageous that there is no batch-to-batch variation of laccase production found using wild strain. However, low yield is main drawback for laccase production using wild strain. In addition, the isolation of potent strain for removal of dyes is tedious and time consuming. The microbial diversity during the degradation of dyes under natural conditions needs to be evaluated, and the isolation, screening, and characterization of new well-adapted microbial strains are used to potentially improve enzyme production.

The main challenges for the engineered strategy include the availability of tools that can be modified by recombinant DNA technology and the application of these tools, so that a desired laccase will be produced with high yield and robustness under industrial conditions. In this strategy, the enzyme that is more suitable for industrial applications has been chosen [59]. The main challenge for recombinant DNA technology is to improve the fermentation characteristics of genetically engineered organisms by introducing genes. It has been found that the robustness of engineered laccase enzymes is often required for industrial applications. There are several reports where cloning of laccase gene, random mutagenesis, site-specific mutagenesis, or the combination of both have been frequently used to get robust engineered laccase enzymes for industrial applications [60]. Iterative saturation mutagenesis (ISM) is a directed evolution method to improve the favorable characteristics of enzymes. The repetitive cycles of saturation mutagenesis are applied in ISM at chosen sites of two or three amino acids of the protein and protein structure. Beneficial mutations were found by performing 3–4 rounds of ISM, and these beneficial mutations are systematically incorporated into the libraries [61]. The grafting of the above three enzymes [lignin peroxidase (ligninase, LiP, EC 1.11.1.14), manganese peroxidase (MnP, EC 1.11.1.13), and copper-containing laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2)] to chimeric enzymes could be the alternative path to improve the efficiency of the bioprocess and cost-effective dye removal. In addition, it also decreases the required cost of the applied enzymes. It is obvious that the primary goal is to decrease the process cost of the overall bioprocess. In the 21st century, the development of bioprocesses has been focused on enzyme mediated bioprocess, which is an attractive tool to reach the economical and ecological goals.

There are several papers published on cellular recognition of dyes through genetic regulation and expression of laccase enzyme in presence of such complex organic compounds. It has been found that laccases are synthesized when microorganisms are cultured on dyes because it induced the activity of enzyme complexes in microorganisms [62]. It is interested to note that dyes did not directly enter inside the cell to influence the regulation of gene and the expression of laccase enzyme. As enzyme secretion is an induction process, there should be a physical contact between part of the regulatory machinery of the cell and the inducer. The inducers have some recognition site on the surface of cell that regulates the process. The expressed enzyme will secrete extracellularly and hydrolyze the complex structure that can be easily transported inside.



A fermentation process involving microbial cells requires the investigation of raw materials, biomass, and how they are treated and mixed with other ingredients required for cells to grow well. A pure strain of a microorganism is normally introduced into the vessel. The bioreactor supports the natural process by providing suitable conditions such as optimum temperature, pH, nutritional elements, enabling cells to grow and form metabolites and enzymes. The cells will start to multiply exponentially after a certain period of lag time and reach a maximum cell concentration as the medium is depleted. In addition, the fermentation process may be constituted anywhere between 5% and 50% of the total fixed and operating costs of the process that is basically different depending on the type of product, the concentration level it produces, and the purity desired [63]. Therefore, optimal design and operation of a bioreactor frequently dominate the overall technological and economic performance of the process. To carry out a bioprocess on a large scale, it is necessary to investigate and develop three principle areas. To obtain a potent biocatalyst (such as microorganisms, animal cell, plant cell, or enzyme) along with medium, optimization is a primary criterion for a fermentation process. In addition, it is required to create the best possible environment for the catalyst to perform by designing the bioreactor and operating it in the most efficient way. However, low stability and high production cost are the key factors for the application of free enzyme for dye removal. However, the immobilized laccase-based system can overcome the limitations such as low stability and high production cost. The stability of enzyme in extreme environment condition or in the presence of chemical is the main advantage of immobilized system. It is also obvious that immobilized laccase can be separated easily from the reaction, allowing the enzymes can be used in continuous manner [64,65].

Recently, Kaushik et al. (2014) experimented with *Aspergillus lentulus* for the production of xylanase through solid state fermentation. In this experiment, various low-cost agro residues were used as substrate. The maximum xylanase production (158.4 U/g) was reported on the 4th day of incubation where wheat bran was used as the substrate. However, 153.0 U/g, 129.9 U/g, and 49.4 U/g of xylanase production were achieved in presence corn cob, sugarcane bagasse, and wheat straw as substrate, respectively. It was experimented to solve the problem associated with pulp and paper industries to control pollution that is due to pulp bleaching and release of colored wastewater [65]. The enzyme showed good stability at high pH and temperature (>75% activity retained at pH 9 and 70°C). They also experimented to remove anionic (>85.0% removal) and cationic (>96.0% removal) dyes. It was revealed that dye removal can be possible significantly to meet the requirements of pulp and bleaching industries through an effective and sustainable approach [66].

## 9. Conclusion

There are, however, various technological and economic obstacles that have to be overcome before industrial-scale enzyme-mediated dye removal can take place. The selection and successful large-scale cultivation of strain for maximum enzyme production remain a major upstream challenge. On the other hand, the development of an effective genetic-engineered strain is critical for the successful fermentation processes. Despite the routine use of laboratory-

scale cloning, the variables affecting recombinant strain are not well understood, and no method for industrial scale is currently established. This manuscript attempts to address the knowledge gap surrounding enzyme-mediated bioprocess technology for development of commercial dye removal process from textile effluent.

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