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*Review*

# A Recent and Systemic Approach Towards Microbial Biodegradation of Dyes from Textile Industries

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**Abstract:** The textile industry generated a series of synthetic dyestuffs that threatened environmental protection. Azo dyes, widely utilized in textile, paper, fruit, leathers, cosmeceuticals and pharmaceutical fields, account for most of the dyestuffs made. Since they have colour fastness properties, stability, and susceptibility to oxidation, existing effluent treatment methods cannot entirely strip different dyes from effluents. Under certain environmental factors, bacteria decolourize and degrade dyes. The treatment process is cheap, environmentally safe, and can be used on various dyes. However, textile plant wastewater can produce many polluting chemicals and dyes. Environmental legislation is increasingly being enacted to regulate mainly azo-based dyes in the environment. The potential of the microbes for the decolourization of dyes and metabolizing them is long-known knowledge. The toxic components of dyes challenge a potential threat to all the living forms of life. Though both natural and synthetic dyes are used for the colourization of textiles, only synthetic ones are challenging to decolourize. Microbial-based bioremediation of dyes has been studied and reviewed primarily to accelerate dye degradation. The various piece of the literature revealed that the majority of these dye removal microbes belong to mainly white-rot fungi, a consortium of anaerobic bacteria. In addition to this, there are several (genetically engineered microorganisms) GEMs that remediate dyes efficiently. Here in the current review, the authors have tried to bridge the existing gap in the bioremediation of dyestuff. Moreover, the authors have also tried to provide the latest trend in this field. This study will surely benefit the industries and researchers related to dyestuffs by maintaining eco-friendly approaches.

**Keywords:** bioremediation; bacteria; fungi; microalgae; consortium; laccase; white-rot fungi



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## 1. Introduction

Over the last few decades, the utilization of dyes has increased drastically due to the rapid industrialization of dye-based industries and the increase in demand for textiles and

clothes [1]. However, due to rapid industrialization, the dyes remained the same synthetic ones, which are very difficult to degrade from the environment. These textile dyes lead to pollution of water and some of them are xenobiotic. Prolonged contamination of water bodies may lead to several diseases among aquatic and other living organisms. Some of the dyes are carcinogenic which will be very severe. Dyes are mainly used for colouring textiles, fabrics, etc. [2]. The classification of dyes could be carried out by various features such as their source of origin, function and chemical makeup, etc. Generally, based on the source of origin dyes could be classified into two categories, i.e., synthetic and natural. Synthetic dyes are generally manufactured by a chemical process in the industries whereas natural dyes are derived from natural materials, such as flowers, leaves, the bark of a tree, etc. [3]. Further, dyes could also be categorized based on their solubility in the aqueous medium for instance acid dyes, basic dyes (cationic), diffuse dyes (non-ionic), and direct and reactive dyes (anionic) [4]. All the dyes are generally soluble in an aqueous medium except non-ionic dyes. The degree of solubility of such dyes varies in the aqueous medium. The types of dyes based on their solubility are shown in Figure 1. Based on solubility, dyes could be broadly categorized into soluble and insoluble dyes. The soluble dyes could be further categorized into anionic and cationic dyes based on their charges. Cationic dyes mainly include basic dyes while anionic dyes could be further categorized into acid, reactive direct, and mordant dyes. Whereas the insoluble dyes can be divided further into azo dyes, disperse, sulphur, vat and solvent dyes. The members present in all these types of dyes are shown in detail in Figure 2.

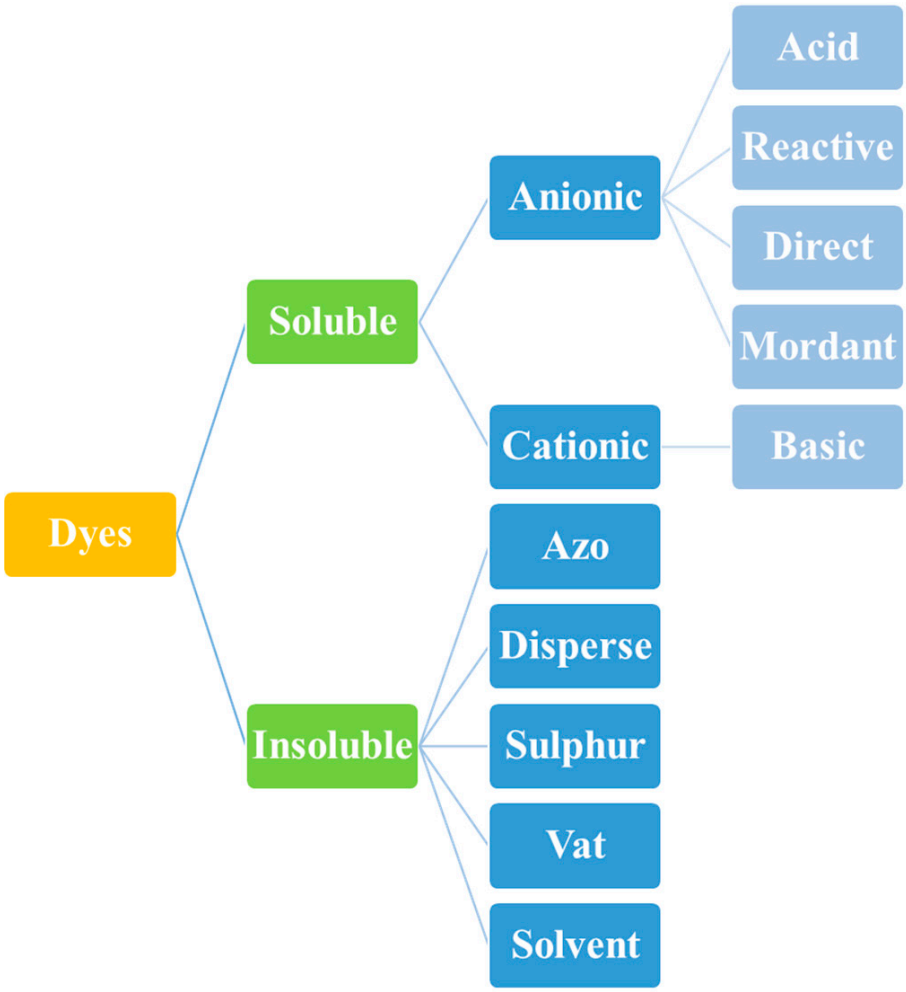


**Figure 1.** Types of dyes based on their adopted from Shamsani et al., 2020 [5] with permission.

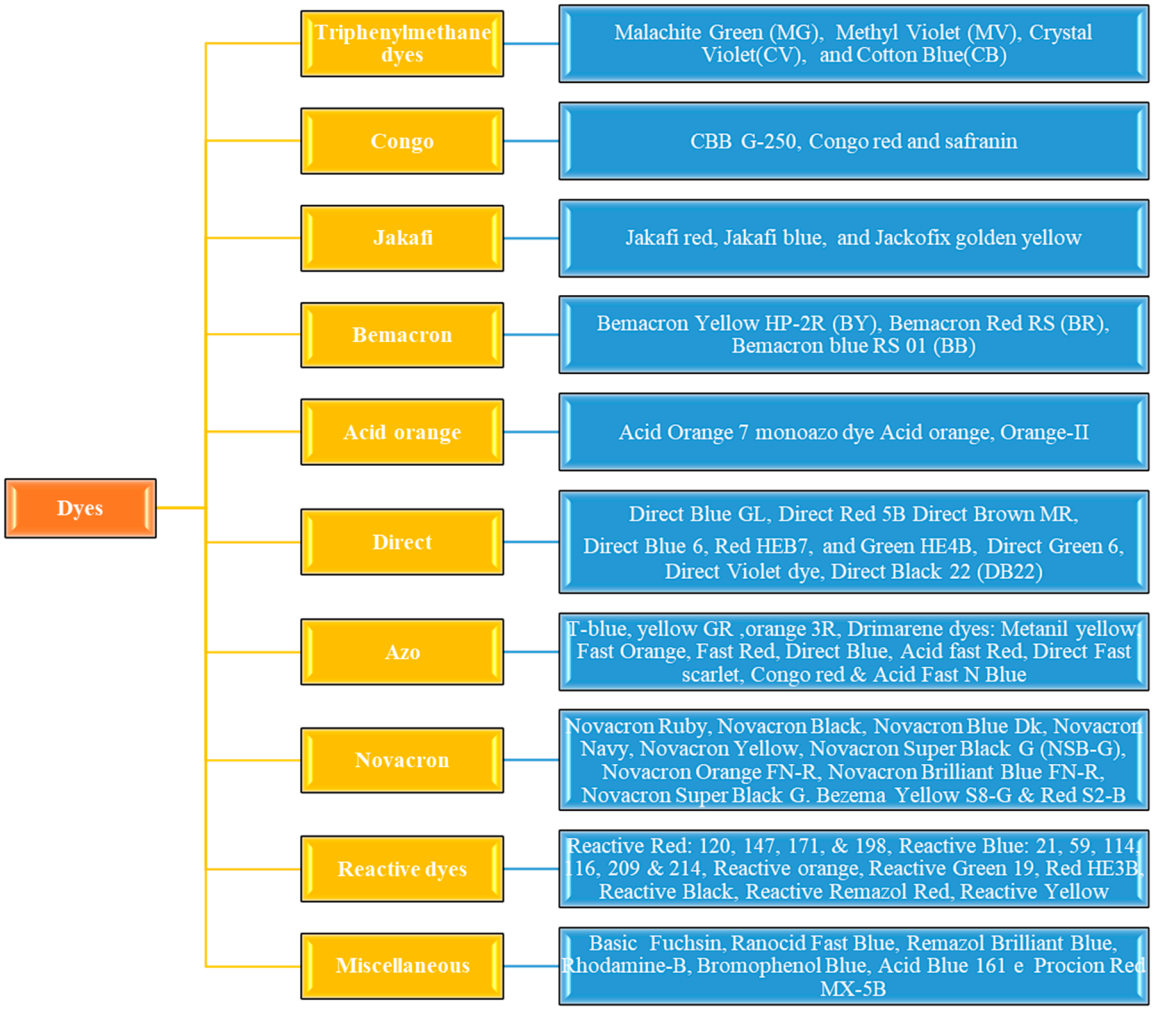


**Figure 2.** Dyes along with their representative members.

Dyes commonly contain anthraquinone, indigoid, and azo aromatic structures [6]. Dyes are mainly comprised of two parts, i.e., auxochrome and chromophore. The auxochrome is derived from the Greek word, where 'auxo' means 'to increase' and 'chrome' means to colour. Auxochrome is a group of atoms which imparts different colour to the material only when it is attached to a chromophore but when it is present alone it could not impart that colour. Auxochromes are also called 'colour helpers' or 'colour intensifiers. Chromophores are mainly a part of a dye that when exposed to visible light then it will absorb and reflect a certain colour [7].

Dyes are frequently applied to many substrates in edibles, cosmetics, paper, rubber, and products of textile industries [4]. Day by day the demand for dyes is increasing with the increasing population and industries. Therefore, there is more establishment of dye and textile industries. Due to the increased establishment of the dyes industry, there is a large amount of dye effluent production. These effluents are mainly disposed of in rivers or other water bodies leading to water pollution. Moreover, most of the dyeing process is inefficient at the time of dying of garments, which leads to the loss of the majority of the dyestuff in the water sources [8]. Once these dyes enter the water bodies, they are available in the environment. The number of dyes lost during a dyeing process totally depends on the type of dyes being used. If simple dyes are used, then the loss will be only 2% while if reactive dyes are used then the loss of dyes may reach up to 50% [9]. Therefore, both the process, i.e., disposal of dye effluent in the water bodies and the inefficient dying process deteriorates the quality of water in the environment. Due to these reasons the quality of water in terms of biological oxygen demand, chemical oxygen demand, total suspended solids, and optical

features are affected. Due to the dyes, the light could not penetrate into the water systems which affects the growth of flora and fauna of the aquatic system [10]. Dyes in the effluent are highly visible even at low concentrations, and oxidation products of these clothing dyes are often carcinogenic. In addition, these dyes lead to several toxic effects on living organisms including humans. For instance, there are several dyes which are neurotoxic, cytotoxic, genotoxic, mutagenic, hypersensitive and mitochondrial toxic [11]. Due to all these activities, various countries have adopted stricter environmental regulations to deal with the textile industries.

This dye effluent could be treated by various biological, chemical and physical methods. Biological methods include the utilization of microorganisms as such or their enzymes, phytoremediation, activated lagoons, trickling filters etc. [12]. The chemical approaches for dye removal include precipitation, absorption, oxidation, ozonation, electrolysis, reduction, advanced oxidation process, ion exchange etc. [13]. The physical methods include techniques such as ultrasonication, drying, sedimentation, reverse osmosis, and coagulation/flocculation membrane filtration such as microfiltration, nanofiltration, and ultrafiltration are very expensive [9]. Physical methods such as screening, sedimentation, and skimming are used to eliminate floating debris from the wastewater. Figure 3 shows all the biological, physical, and chemical approaches for the decolourization of dyes from wastewater.

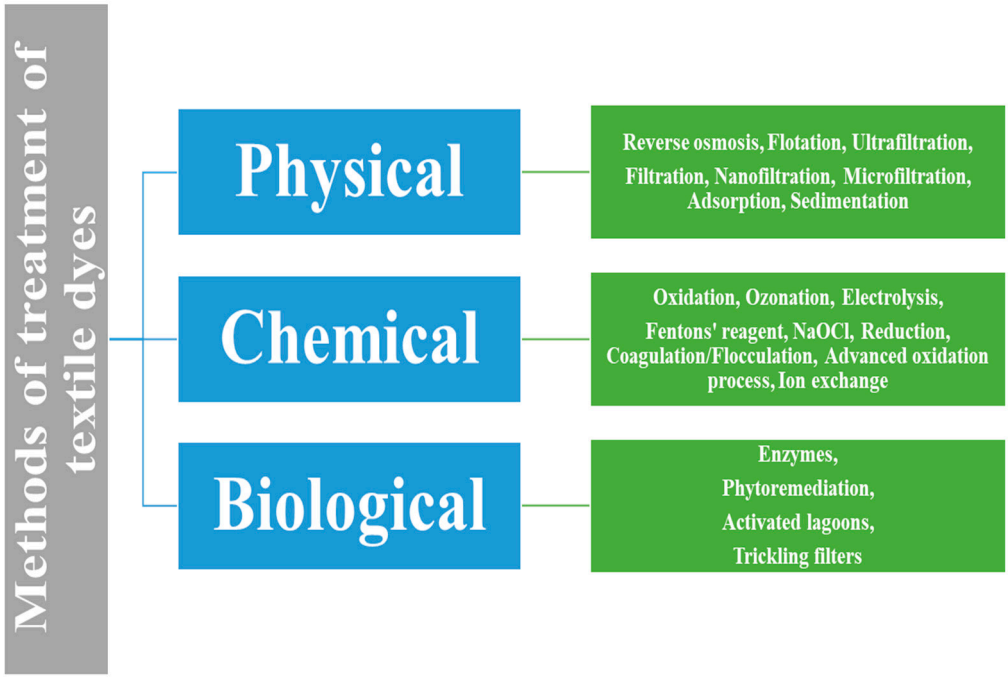


**Figure 3.** Various methods of treatment of textile dyes.

Out of the above-mentioned dye effluent treatment methods, majority of them are inefficient, expensive, energy intensive and leading to formation of by-products which are more harmful. Therefore, in all three types of approaches, biological one is comparatively eco-friendly, with no utilization of chemicals during the process and also no harmful metabolites by-products produced. In addition to all these features, biological materials used for dye removal are biodegradable in nature [14].

Even among biological approaches, there are choices to use plant-based remediation or microbial remediation. The selection of the two depends on the availability of the biological material, their cost and time consumption. The remediation by using plants is called phytoremediation while microbial remediation may be further categorized into mycoremediation (fungi) [15], phycoremediation (algae) [16], and bacterial remediation. Out of phytoremediation and microbial remediation, the former is time taking as the plant

has to grow firstly in those areas which will depend on the life span of the plant. Therefore, phytoremediation becomes a second choice when it comes to the rate of remediation of dye effluents from the site.

So, due to these beneficial features, developed countries are now switching to microbial-based techniques for the remediation of dyes. The microbes are used either in the dried powder form, or their nanosized form. In addition to this microbe could be used directly as a whole organism or their extracted enzymes/metabolites used for remediation purposes. The dried or nanosized form mainly acts as a biosorbent whereas the whole microbe or its enzyme attacks the various bonds present in the dyes. The former two approaches simply adsorb the dyes (biosorption), whereas the latter two processes mineralize or metabolize the dye molecules and convert them into either mineral or less toxic products (enzymatic degradation) [11]. The microbial-based approaches have been used in either monoculture or a mixture of cultures called microbial consortiums. There are several pieces of literature where monoculture has been used for the remediation or decolourization of dyes. Some of the studies are discussed over here. Khadijah (2009) tested the ability of 1540 bacterial isolates to remove different azo dyes. Kochher and Kumar (2012) investigated the screening of bacteria that could decolourize textile dyes. The findings of this study suggested that bacteria could be used to remediate dye from wastewater. The bacterial population used over here for the remediation of dyes was isolated from waste effluent from the textile dye industry. Investigators have shown that bacteria are naturally adaptable and capable of degrading pollutants. Leena et al., 2008, investigated the remediation of Reactive Black-B dye (RBBD) from textile effluent using effluent-adapted bacteria (EAB) and effluent non-adapted bacteria (ENAB). The investigator found a group of five bacteria, i.e., *Alcaligenes* sp., *Pseudomonas aeruginosa*, *Bacillus* sp., *Eubacterium* sp., and *Arthrobacter* sp. For decolourization in ten days, investigators have utilized four ENAB, for say, *Kluyvara ascorbate*, *Bacillus* sp., *Pseudomonas* species, and *Pasteurella* sp. Effluent-adapted *Bacillus* sp. reduced colour by 35.68%, while non-adapted isolate of the same genus removed colour by 30.04%. *P. aeruginosa* adapted to effluent demonstrated 44.2% decolourization, comparable to non-adapted *Pseudomonas sps* (41.73%). A team of investigators led by Mutafov et al., 2007 revealed the potential of two bacterial species, *Alcaligenes faecalis* and *Rhodococcus erythropolis*, to remediate monoazo dye Acid orange (AO) at various initial dye concentrations. A diazo dye Reactive yellow 84A dye was effectively degraded by using a novel bacterial strain, *Exiguobacterium* sp. [17]. In addition, analytical techniques such as Fourier transform-infrared spectroscopy (FT-IR), gas chromatography-mass spectroscopy (GC-MS), and high-performance liquid chromatography (HPLC) showed that dye degradation resulted in a substantial decrease in phytotoxicity. Furthermore, there are several investigators who have tried to remove malachite green by using *Aeromonas hydrophila*, where the efficiency was about 96.8% [18], *Bacillus pseudomycoides* were used by Kumar et al., 2019 to remove acid black 24/40 mg/L where the efficiency was 96% [19]. Ayed et al., 2019 tried to remove brilliant orange 3R/750 mg/L by using *Lactobacillus acidophilus* with an efficiency of about 99.3% [20]. Similarly, a huge amount of work has been carried out by using monoculture but very less attempts were made by using mixed culture or by using microbial consortium.

Since the microbial consortium has different microbes which produce a variety of enzymes and metabolites so there is more effective removal of dyes with them. There are several reports where dyes remediated were carried out by microbial consortiums. Due to synergistic metabolic behaviour, it has been documented that these diverse microbial cultures can achieve effective dye degradation. Poddar et al., 2022 used a microbial consortium (MC) comprising mainly different Klebsiella sps. The authors have prepared 5 different consortiums from 19 strains. The developed MC was used to remove amaranth, sunset yellow, tartrazine, indigo carmine, and quinoline yellow dyes. The overall efficiency of about 96.5% was achieved for the most potential MC [21].

Algae, yeast, bacteria, and fungi are among the microorganisms that can mineralize and decolourize different dyes [22]. Microalgae have shown huge potential in the bioreme-

diation of dyes from wastewater. It has been used in various forms such as dry powder, live, extracts mostly in lipid-free form and nanoparticles. Mansour et al., 2022 developed nanoparticles of *Arthrospira platensis* NIOF17/003 microalgae and utilized them for the remediation of methylene blue (MB), from the synthetic aqueous solutions. The developed NPs were analysed by sophisticated instruments for detailed morphological and elemental properties. Further, the adsorption capacity of the *Arthrospira platensis* NIOF17/003 NPs was analysed against pH, temperature, contact time, and initial dose of MB dye. The authors concluded that the optimum remediation of MB dye was achieved when the NP's initial dose was about 0.4 g. Moreover, about 93% removal of MB dye was reached within 5 min at a stirring rpm of 150. Further, the authors have also analysed the recyclability of the adsorbent and found that it is possible to achieve MB remediation up to 65.5% after three recycles of the adsorbent [23]. Ashour et al., 2021 used the same strain of alga for the bioremediation of ammonia from aquaculture wastewater effluents. Here the investigators have used complete dry biomass and lipid-free biomass of the above alga. Further after acquiring a detailed investigation of both the adsorbents by sophisticated instruments, it was used to remove ammonium ions from aquaculture wastewater effluents. The investigators removed the ammonium ions from both the conditions, i.e., from the synthetic aquaculture solutions and from real aquaculture effluents. The investigators concluded that the ammonium ions removal percentage was higher in synthetic aquaculture water than in the real one. The removal percentage of ammonium ions by complete dry-biomass of alga was 64.24% and 25.7% from synthetic and real aquaculture wastewater, respectively. While in the case of lipid-free biomass, in synthetic aquaculture wastewater NH4+ ions were removed up to 98.68% and in real aquaculture wastewater was 37.85 [24]. Alprol et al. 2021 used complete dry biomass and lipid-free biomass of the above alga and assessed its potential for the removal of two organic dyes namely Ismate violet 2R, and IV2R from textile effluents. Both the adsorbents were thoroughly characterized by sophisticated instruments for detailed properties. By applying batch adsorption study of the dye removal, it was found that for complete dry biomass it was 75.7% and for lipid-free biomass, it was 61.1%. Therefore, it was found that the complete dry biomass of A. platensis was more effective for the said dye removal at optimized conditions [25].

While there are several enzyme-based dye removals that are found highly reliable for the removal of dyes. For instance, azo-reductase, laccase, lignin peroxidase, manganese peroxidase etc. remediate dyes very efficiently. Azo reductase is one such enzyme which specifically acts on the azo dyes. Azo dyes could be efficiently removed by using azo dye reductases and lactases. Azo-reductases catalyse reactions only in reducing equivalents such as FADH and NADH. The majority of Azo dyes have sulphate groups and have heavy molecular weight, making them difficult to move through [3]. Figure 4 is showing various types of dye biodegradation processes based on the types of microorganisms. The microbial-based dye removal process involves fungi, bacteria as a whole organism, microalgae, microbial enzymes, microbial consortium (MC), and dried or powdered forms of these microbes. While Figure 5 is showing types of microbial-based dye degradation in the presence or absence of oxygen. The oxygen-dependent microbial process generally depends on the nature of the used microorganism, i.e., whether it is aerobic or anaerobic. Therefore, the microbial process could be either aerobic, anaerobic or anoxic.

So, microbial-based bioremediation of dyes effluent from wastewater is the most economical and reliable globally. There are also good numbers of microorganisms that are easy to manage and need no planning. In addition to this periphyton (complex mixture of algae, cyanobacteria, heterotrophic microbes+ detritus or periphytic biofilm, methods may also be utilized for dye remediation. The microbial-based dye remediation has been carried out by various investigators either in natural conditions or in laboratory conditions either in aerobic or anaerobic conditions based on the nature of the microorganism [26].

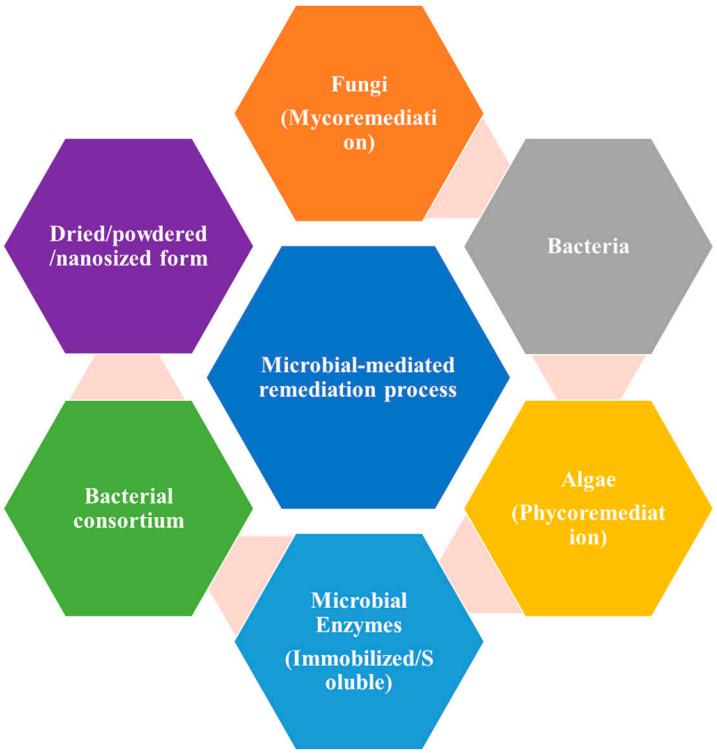


**Figure 4.** Various types of microbial methods for remediation of textile dyes.

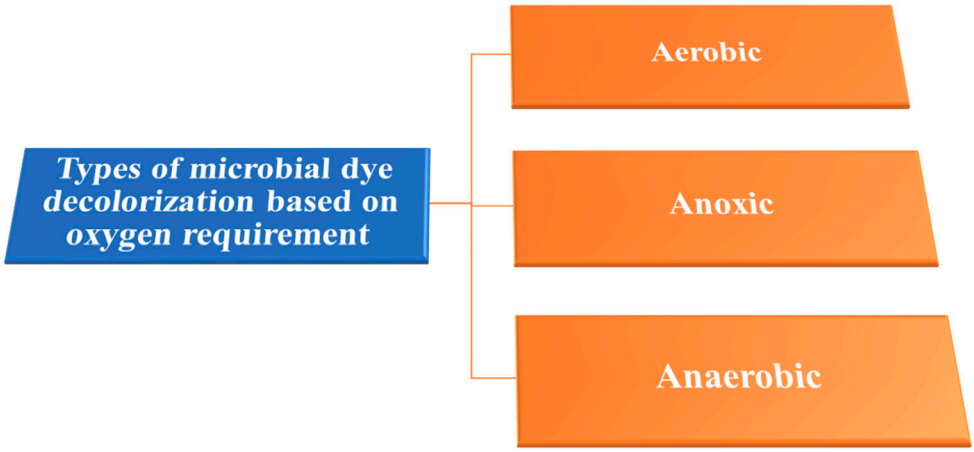


**Figure 5.** Various types of microbial degradation of dyes based on oxygen environment.

In the present review, the investigators have emphasized the microbial-based remediation of dye effluents from wastewater. The authors have focused on the existing gap in this field and try to provide a current state of the art. The authors have focused on the different microorganisms used for dye remediation purposes and recent trends. The authors have tried to provide a mechanism of dye removal from wastewater by using a microorganism. Finally, the authors have also focused on microbial enzymes and their role in the remediation of dyes in the environment. In addition to all, one of the aims was to focus on the decolourization and oxidation of dyes by both mixed and axenic bacteria and fungi cultures.

## 2. Microbial Biodegradation and Decolourization of Textile Dyes

Various microbes can be used to degrade various dyes; they have different structures and pathways for dye degradation. Azo dyes are a valuable family of dyes with the most significant colour diversity. Microorganisms degrade azo dyes in anaerobic conditions with

the assistance of azoreductase, resulting in colourless aromatic amines as a by-product. Benzidine is often utilized in the production of direct azo dyes and is reported to be associated with cancer. Natural dyes are low-cost and can be used to colour fabrics, leathers, and papers without the need for pre-treatment. Natural Black 38 (NB-38) is the most commonly used benzidine-based azo dye [27]. *Enterococcus gallinarum* can be used to degrade Direct Black 38 ink (DB 38) [28]. Active sites for azo-reductase are three azo bonds in Direct Black 38's structure which were converted to benzidine through metabolic reactions, which are then deaminated to produce 4-amino phenyl. Investigation revealed that those dyes with benzidine as a base are significantly more cancer-causing than dyes without Benzidine [29]. This is due to the contaminants such as 4-amino biphenyl and 2-4, diaminoazo-benzene, which have been linked to cancer. Microbial degradation of dyes involves a common approach towards a particular dye, the only thing which varies is their enzyme interacting with the dyes, intermediate and final products. Some of the microbes perform complete degradation of the dyes while some of them carry out incomplete degradation of dyes. Broadly, dye-degrading microbes could be classified as actinomycetes, bacteria, fungi, and algae. Moreover, based on the nature of microbes and the pathway followed for degradation it could be again aerobic and anaerobic degraders. Generally, bacteria could be classified further as aerobic degraders or anaerobic degraders. Here in Figure 6 a generalized scheme of degradation of dye is shown.

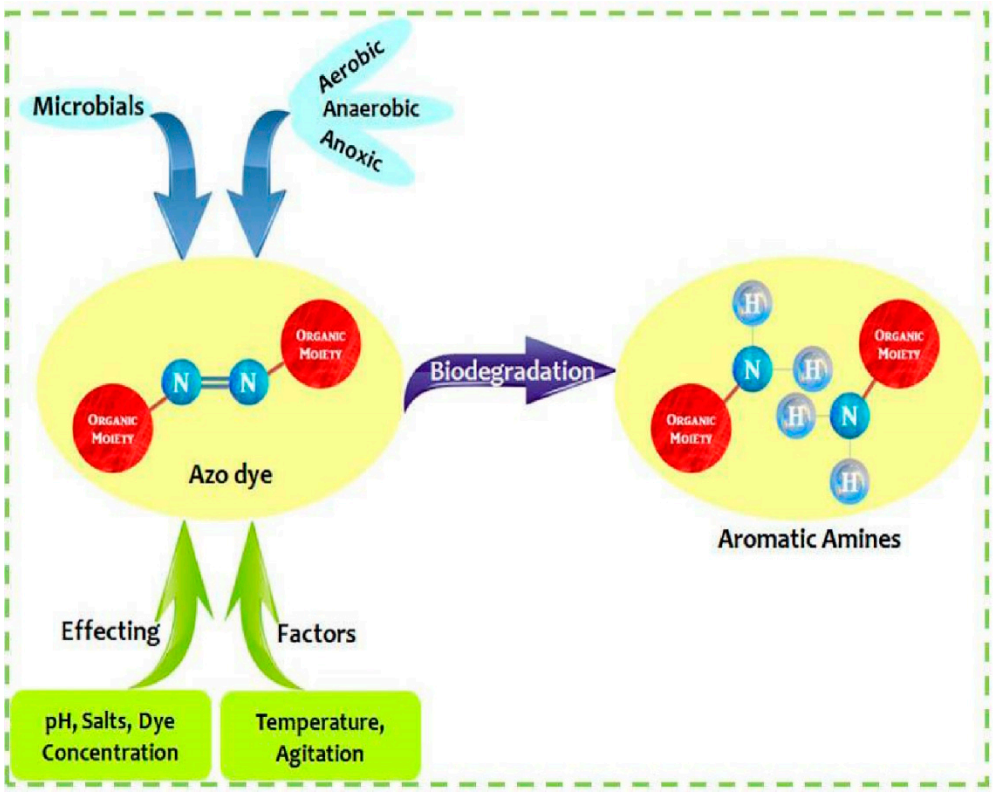


**Figure 6.** Basic mechanism for microbial biodegradation of azo dyes [30].

### *2.1. Bioremediation of Dyes by Actinomycetes*

Actinomycetes are fungi-like, filamentous bacteria with high GC content generally present in the soil. Extracellular peroxidases produced by actinomycetes, especially Streptomyces species, are known to play a role in lignin biodegradation [31]. The initial oxidation of lignin is carried out by the peroxidase released by the Streptomyces, into different water-soluble polymeric compounds. It has also been observed that the actinomycetes catalyse hydroxylation, oxidation, and dealkylation reactions in the presence of xenobiotics [32]. Three groups initially looked into the potential of actinomycetes to decolorize and mineralize textile dyes. Ball et al., tested 20 actinomycetes strains from various gen-

era for their potential to bleach polymeric dye Poly R in 1989. *Streptomyces badius 252*, *Thermomonospora fusca MT800*, and *Streptomyces* sp. *strain EC22* were the only three strains that greatly decolorized dye [6].

Following that, it embarked on a more extensive screening procedure that looked at the decolorizing ability of 159 actinomycetes. Investigators who utilized real textile effluents in the screening process were excited about this research. Five different effluents were used, each having a single dye of known concentration. Structurally each dye was different from azo compound RR147 to phthalocyanine Reactive Blue 116. Positive findings were obtained for 83 isolates, demonstrating the extensive potential of actinomycetes to cause dye decolourization. Given compounds' resistance to mineralization by other bacteria under similar conditions, a discovery that actinomycetes can aerobically decolourize and degrade azo dyes was important.

Finally, but perhaps most importantly, a team led by Don Crawford at the University of Idaho began investigating the potential of lignin lytic microbes, including white-rot fungi and Streptomyces, to mineralize and decolourize textile dyes. Initially, the ability of 14 Streptomyces to decolourize two polymeric dyes, Poly B-411 and Poly R-478, as well as azo dye Remazol Brilliant Blue R, was investigated (RBBR). With two dyes, RBBR and Poly B-411, nearly similar findings were reported, indicating a close link between the isolate's ability to decolourize dyes and its ligninolytic ability. This investigation and the fact that extracellular $H_2O_2$ synthesis increased when actinomycetes sps, were allowed to grow in the presence of glucose indicated that peroxidases were involved in the decolourization process. Extracellular peroxidases were previously discovered in Streptomyces bacteria, and enzymes were exhibited to have substrate specificities identical to *P. chrysosporium's* Mn (II)-peroxidase [33]. Surprisingly, there was no association between decolorizing activity and ligninolytic activity with the 3rd pigment, Poly R-478 [34]. Enzymatic processes that take place during the decolourization of this dye are unknown. [8]. Recently Blanquez et al., 2019 reported 6–70% removal of AO-63 by using *Stp. Ipomoeae CECT 3341*, from the textile dyes [35]. Actinomycetes-based removal of various dyes is shown in Table 1, where the removal percentage of dyes varies from 3–100%.

**Table 1.** Degradation of dyes by using actinomycetes.

| Actinomycetes | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Streptomyces* sp. S27 | MR | 99% | [36] |
| *Streptomyces bacillaris* | Triphenylmethane dyes: MG, MV, CV, and CB | MG (94.7%), MV (91.8%), CV (86.6%), CB (68.4%) | [37] |
| *Streptomyces ipomoeae* CECT 3341 | AO63 | 6–70% | [38] |
| *Streptomyces sviceus* (marine) | CR-21 | | [39] |
| *Streptomyces sps* | RR 147 (azo)<br>RR 171<br>RB 114<br>RB 209<br>RB 116 | 3–100% | [40] |
| *Streptomyces sps* | MG,<br>MV,<br>CV<br>CB | (MG 95%, MV 92%, CV 87%, CB 68%). | [41] |

### 2.2. Bioremediation of Dyes by Aerobic Bacteria

It is very difficult to isolate bacteria that could aerobically decolorize and mineralize dyes, except for actinomycetes. Various studies claim that different azo dyes can be converted aerobically, out of which one study reveals the development of the green color of instant chocolate puddings. including one notable study on the greening of instant chocolate puddings. It has been found that these preadapted aerobic bacteria obtain their

energy by utilizing some carboxylated analogues of sulfonated azo compounds as their sole carbon and energy supply [9].

Orange-I azo-reductase [NAD(P)H:1-(4′-sulfophenylazo)-4-naphthol oxidoreductase] and Orange II azo-reductase [NAD(P)H:1-(4′-sulfophenylazo)-2-naphthol oxidoreductase] is distilled and characterized from *Pseudomonas.* Both of these enzymes are now categorized as the same enzyme, which is called azo-benzene reductase (EC 1.6.6.7). Aside from azo dyes, bacteria's ability to aerobically metabolize other dye groups has piqued interest, but with little success. Aerobic mineralization of triphenylmethane pigment, MV, by a strain of *Pseudomonas mendocina MCM B-402* was recently discovered. Isolate's sole carbon and energy source was MV, which has several industrial uses in addition to its well-known utilization as a regular bacteriological and histological stain. According to preliminary research, *Ps. mendocina* degraded dye to phenol by a variety of unspecified metabolites, which then joined the ketoadipic acid pathway [10].

Karim et al., 2018 and Mustafa et al., 2022 reported the decolourization of reactive dyes of textiles by using monoculture and consortium.

John et al., 2020 reported the utilization of a halophilic bacterium (*Salinivibrio kushneri HTSP*) for the biological decolourization of synthetic dyes (CBB G-250 and Congo red and safranin). Originally this strain was isolated from the saltpan for decolourization and bioremediation of dyes. The investigators obtained about 80% decolorization within 48 h. The further investigator reported that the rate of decolorization by using a particular bacterial strain was in the order of CR > CBBG-25 > Safranin [42].

Aktar et al., 2020 isolated *Bacillus* sp. and *Staphylococcus aureus* from the textile dye effluent and applied them for the decolorization and degradation of BY HP-2R, BR-RS, and BR RS 01 (BB) dyes. The removal efficiency of BR and BB reached up to 71% and 83% with *Bacillus sps*. While with *Staph. aureus* efficiency of BY dye reached 79% after five days [43].

Shete et al., 2020 isolated *Bacillus sps*, *Klebsiella*, *and Pseudomonas sps*, from textile effluent and applied them for the decolorization of JR, JF, and JGY dyes. The removal or decolorization of dyes was achieved from 45–50% for the following dyes under optimized conditions [44]. Khaled et al., 2022, applied *Bacillus cereus* (*B. Cereus*) and *Pseudomonas parafulva* (*Ps. parafulva*) for the decolorization of textile azo dyes (T-blue, yellow GR, and orange 3R,) and obtained the removal efficiency of up to 91.69 and 89.21% for orange 3R [45]. Table 2 shows summarized studies of bacterial-based dye removal from the wastewater. From all the studies it was found that in some of the studies bacteria was used directly, while in some cases their dry powder was used while in a few cases a bacterial consortium was also used. In some of the studies, the dye removal was achieved up to 100% either with monoculture or with the consortium. Some of the bacterial consortiums reached up to 97–99% dye removal within a short period of time.

**Table 2.** Microbial remediation of dyes by using aerobic and aerobic bacteria.

| Bacteria | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Aeromonas hydrophila*<br>*A. Hydrophila SK16* | RR141<br>MG<br>Acid fast yellow MR | 70–80%<br>96.8%<br>91.25% | [18,46,47] |
| *Comamonas sps UVS* | DB GL, DB5B | | [48] |
| *Bacillus* sp. and<br>*Staph. aureus* | BY HP-2R, BS-RS, and B. blue RS 01 (BB) | 71–83% | [43] |
| *Alcaligenes faecalis* AZ26,<br>*Bacillus cereus* AZ27 and *Bacillus* sp. | NSB-G | 90% | [49] |
| *Brevibacillus laterosporus* | DB-MR,<br>MO, Blue-2B,<br>Golden yellow (GY)<br>Brilliant blue | 100% | [50] |

**Table 2.** *Cont.*

| Bacteria | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Shewanella oneidensis (MFC)* | Acid orange (AO 7) | 80.4 | [51] |
| *Alcaligenes faecalis* and *Rhodococcus erythropolis* | Monoazo dye Acid orange | 80–95 | [52] |
| Consortium of *Ps. aeruginosa*, *B. pumilis*, *B. thuringiensis*, *Enterococcus faecium* in different combinations | NR, N Black, N Blue dk, N Navy, N Yellow | 82–97% | [53] |
| *Neisseria* sp., *Vibrio* sp., *Bacillus* sp., *Bacillus* sp. *and Aeromonas sps* | N Orange FN-R, N Brilliant Blue FN-R, N Super Black G, BY S8-G, and BR S2-B | 60–90% | [54] |
| *Acinetobacter* (ST16.16/164 and *Klebsiella* (ST16.16/034) | Monoazo dye RO 16 and diazo dye RG-19 | More than 80% | [55] |
| *Salinivibrio kushneri HTSP* | CBB G-250 and CR and safranin | More than 80% | [42] |
| *Pseudomonas* sp. (S3, S11 and S12), *Klebisiella* sp. (S4). and *Aeromonas* sp. (S2) | JR, JB and JGY | 45–50% | [44] |
| *Bacillus odyssey SUK3*, *Morganella morganii SUK5 and Proteus sps SUK 7* | Red HE3B, Reactive Blue 59 | 97–99% | [56] |
| *B. circulans NPP1* | MR and other dyes | 98% | [57] |
| *Ps. desmolyticum* NCIM 2112 | DB 6, Red HEB7, and Green HE4B | 70, 90% | [58] |
| *Acinetobacter calcoaceticus* | MR, MO | 90–98% | [59] |
| *Enterobacter* sp. CV–S1 | CV | | [60] |
| *B. subtilis* (E1), *Exiguobacterium acetylicum* (D1), *Klebsiella terrigena* (R2), *S. aureus* (A22), *Ps. pseudoalcaligenes* (A17), and *Ps. plecoglossicida* (A14) | Whale, mediblue, fawn and mixed dye, | 97.04%, 80.61%, 94.93% and 81.64% | [61] |
| *Sulfate-reducing bacteria (SRB)* | Orange II | 95% | [62] |
| *Sterigmatomyces halophilus SSA1 575* | RB-5, RB-5 | 100% | [63] |
| *Anoxybacillus ayderensis SK3-4* | DG 6 | 100% | [64] |
| *A. hydrophila SK 16* | AFY MR | More than 70 | [46] |
| *Serratia liquifaciens* | Azure B | 90% | [65] |
| *Bacillus cereus SKB12* | RB-5 | 88.7% | [66] |
| *B. cereus and Ps. parafulva* | Azo dyes: T-blue, yellow GR, and orange 3R | 91.69 and 89.21% | [45] |
| *B. circulans BWLI 061* | MO | 99.22% | [67] |
| Bacterial consortium-*Bacillus flexus TS8 (BF)*, *Proteus mirabilis PMS (PM)*, and *Ps. aeruginosa NCH (PA)* | Indanthrene Blue RS | 99% | [68] |
| *Bacillus pseudomycoides* | MG | 96.8% | [19] |
| *Citrobacter* sp. | CR | 93% | [69] |
| *Bacillus subtilis HAU-KK01* | CR | 92.8% | [70] |
| *Thiosphaera pantotropha* | RY | 100% | [71] |
| *Staphylococcus* sp. *K2204* | RBB | 100% | [72] |
| *Staphylococcus* sp. | Reactive blue | 97% | [73] |

### 2.3. Mechanism of Bioremediation

The use of biological processes to reduce emissions from atmospheric, aquatic, or terrestrial systems is known as bioremediation. Microorganisms and plants are most often used as biological structures for this purpose. Microorganism-mediated biodegradation is one of the most common bioremediation methods. Microbes may simplify many complex substances to meet their development and energy requirements. Air may or may not be needed for these biodegradation processes. In certain circumstances, the same metabolic pathways that cells utilize for growth and supply of energy are often utilized to simplify the components of pollutants. Microorganisms do not benefit directly in these situations, known as metabolism, but investigators have taken the benefit of this effect and used it for bioremediation. Mineralization, or complete oxidation, results in the production of water and either $CO_2$ or $CH_4$. Incomplete biodegradation can result in the cleaving of the product which is less toxic than the initial pollutant. As a result, bioremediation overcomes the drawbacks of traditional methods by causing physical degradation of specific organic chemicals at a lower rate. As a result, bioremediation has evolved from a nearly unknown technique to one that has been used to clean up a wide variety of toxins over the past two decades [74].

## 3. Fungi-Based Bioremediation of Dyes

### 3.1. White Rot Fungi (WRF)

WRF is the most well and extensively studied dye-decolorizing microbe because of its potential to mineralize the intricate polymeric composition of lignin (woody), the group of species that is critical to the global carbon cycle. WRF could mineralize a distinct variety of persistent organic compounds in addition to their natural substrate, which separates them from biodegradative bacteria, which are more substrate-specific. Since their ligninolytic enzymes, Lip, manganese peroxidase (MnP) (EC 1.11.1.13) laccase, are primarily nonspecific, these fungi can mineralize a wide variety of organic compounds. These enzymes and their catalytic properties are not described over here. Still, in a nutshell, LiP catalyses the oxidation of non-phenolic aromatic compounds such as veratryl alcohol, while MnP converts $Mn^{2+}$ to $Mn^{3+}$, which could oxidize a vast variety of phenolic compounds [75]. Laccase (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) is a Cu-containing enzyme that catalyses phenolic substrate oxidation by combining it with oxygen reduction in water [76]. Decolourization of dyes by WRF was first documented by researchers who devised a way to quantify *Phanerochaete chrysosporium*'s ligninolytic behaviour based on the decolorization of a variety of sulfonated polymeric dyes [77]. *Pleurotus ostreatus* [78] developed an uncommon enzyme capable of Remazol Brilliant Blue R (RBBR) decolorization during solid-state fermentation of wheat straw. This development was distinct from isolate's MnP, laccase, and manganese-independent peroxidase activities, as well as LiP and veratryl alcohol, oxidize actions, which were not detected in *P. ostreatus* but are common in other WRF. Since it had a catalytic metal centre and was inhibited by a variety of proven oxygenase inhibitors, activity was dubbed RBBR oxygenase [79].

### 3.2. Remediation by Non-WRF

Although degradation mechanisms used by non-WRF have not been identified in the literature, they are likely to be close to those found in the metabolism of other aromatic hydrocarbons. One such isolate, the strain of *Geotrichum candidum Dec1* isolated from soil and capable of a decolorizing variety of anthraquinone dyes, has been studied in depth. This isolate's broad substrate specificity led to Kim and revealed an extracellular peroxidase-like enzyme. DSP, glycosylated haeme-based peroxidase isolated from *G. candidum* with features different from LiP, MnP, horse-radish peroxidase, and other peroxidases, was obtained [80]. The robustness of this isolate in contrast to *P. chrysosporium* and other WRF makes it potentially useful for dye decolorization. In contrast with the action of *P. chrysosporium* ligninolytic enzymes, DyP is generated continuously and does not become affected by shear forces in shake flasks or stirred tank reactors. Such characteristics could allow the

development of a range of *G. candidum*-Dec1-based biological reactors for treating a variety of organic contaminants, including dyestuffs. To enable excellent protein development in the safe host, the recently cloning of a gene that encodes DyP into heterologous expression host *Aspergillus oryzae*. According to molecular research, DSP is different from other peroxidases in the plant peroxidase superfamily because it has a novel haeme-binding area [14]. Table 3 is showing summarized studies of mycoremediation of dyes under different experimental conditions. From all the myco-based remediation of dyes, it was found that some of the fungi have removed the dyes up to 100% from the wastewater. This is so because fungi have a large number of extracellular enzymes. Moreover, the presence of hyphae and mycelium might have been advantageous for the remediation of dyes.

**Table 3.** Dye degradation by using white rot and non-white rot fungi.

| Fungi | Dyes Used | Efficiency | References |
|---|---|---|---|
| *P. chrysosporium* | Rhodamine-B | 91% | [81] |
| *P. chrysosporium* | Bromophenol blue | 99.3% | [82] |
| *Pleurotus ostreatus* | Acid Blue 129 | 77% | [83] |
| *Trametes polyzona* KU-RNW027 | RB-B | 100% | [84] |
| *P. chrysosporium* | Ranocid Fast Blue | 83% | [85] |
| *Emmia latemarginata* (MAP03, MAP04, and MAP05) and *Mucor circinelloides* (MAP01, MAP02, and MAP06) | Azo, indigo, and anthraquinone dyes (Acetyl yellow G) | | [86] |
| *Cyberlindnera samutprakarnensis S4* | Acid Red | 97% | [87] |
| *P. chrysosporium* | Orange-II | 85% | [88] |
| *Phanerochaete sordida* | RR-120 | 90.6 | [89] |
| *Schizophyllum* sp. *F17* | Orange IV | 76% | [90] |
| *Penicillium chrysogenum* | DB22 | | [91] |
| *Trichoderma (T. virens, T. viride)* | CR and MG | 81–99% | [92] |
| *Trichoderma tomentosum* | AR-3R | 99.2% | [93] |
| *A. fumigatus* | Direct Violet dye | 51.38–93.74% | [94] |
| *Trametes hirsuta, Microporus xanthopus, and Ganoderma applanatum* | turquoise blue textile dye | 82.17%<br>78.50 and 85.84% | [95] |
| *Aspergillus niger, and Phanerochaete chrysosporium,* | BF (81.85%),<br>Nigrosin (77.47%), MG (72.77%) | *P. chrysosporium* maxium with Nigrosin (90.15%) > BF (89.8%), MG (83.25%) | [96] |
| *Aspergillus niger, Asp. terreus* and *Rhizopus oligosporus.* | Acid Blue 161 e Procion Red MX-5B | 58–68% | [97] |
| *Penicillium simplicissimum* INCQS 40211 | (RR198),<br>R B214 (RB214), RB21, and a mixture of all three | | [98] |
| *P. simplicissimum (n-WRF)* | Triphenylmethane Dyes: CV, MV, MG, CB | 96.1–98.7% | [99] |
| *Diutina rugosa* | Indigo | 100% | [100] |
| *Asp. lentulus* | VAT Novatic Grey (100%), RR-Red (98.47%), RY (91.55%), and Indanthrane Blue (99.28%)<br>Reactive S | 91–100%<br>55.33% | [101] |

**Table 3.** *Cont.*

| Fungi | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Aspergillus foetidus* | Azo reactive dyes, Drimarene dyes | >95% | [102] |
| *Aspergillus oryzae* | Reactive dyes PR-HE7B and PV-H3R | | [103] |
| *Aspergillus* sp., | Fast Red A | 99.5% | |
| *Aspergillus flavus* | Azo textile dye, RR198 | 84.96% | [104] |
| *Aspergillusoryzaedead cells* | RB, MB, and MO | 100% | [105] |
| *Trichoderma harzianum* | AR and anthraquinone (Basic blue) | 70% and 51% | [106] |
| *Saccharomyces cerevisiae* | Ramazole Blue | 100% | [107] |
| *Mucor circinelloides* | Ramazole Blue | 95% | [108] |

## 4. Algae-Based Bioremediation of Textile Dyes

There are several reports in the literature where algal systems have been used for the decolorization or bioremediation of textile dyes. Algae also have numerous enzymes and other molecules which play a role in the decolorization of dyes from textile wastewater. Algae are reported to utilize the azo dyes as a source of carbon and energy and degrade them into aromatic amines, followed by their subsequent conversion into simpler organic and inorganic compounds [109]. To date, *Chlorella sps*, *Oscillatoria sps*, *Synechocystis*, and *Phormidium sps* have been used widely by various investigators for the decolorization of dyes from textile samples [110] and a detailed one is shown in Table 4. From the various studies of phycoremediation of dyes, it was found that the highest efficiency of dye removal was achieved up to 99–99.5%. Though all the studies were carried out with different dyes, different concentrations, and under different reaction conditions, the mixed culture of algae showed maximum removal, i.e., 99.5%, which shows the effectiveness of MC.

**Table 4.** Algae-mediated decolourization of textile dyes.

| Algae | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Chlorella vulgaris* | MB and AO7 | 83.04 ± 2.94% | [111] |
| *C. vulgaris* | Azo dye: Metanil yellow, FO, FR, DB, AF Red, Direct Fast Scarlet, CR, and Acid-Fast N Blue | Up to (75.68%) | [112] |
| *C. vulgaris* UMACC 001 | Supranol Red 3BW (SR 3BW) | 41.8% to 50.0%. | [113] |
| *C. vulgaris (Immobilised)* | SB 3BW, Lanaset Red 2GA and Levafix Navy Blue EBNA | 44.0–49% | [114] |
| *Chlorella vulgaris* | Blue dye<br>Green dye<br>Reactive black | Blue: 63.89% (1 mg/L)<br>Green: 45.71% (1 mg/L)<br>80% | [115,116] |
| *Sphaerocystis schroeteri* | Blue dye<br>Green dye | Blue: 63.87% (1 mg/L)<br>Green: 60.00% (1 mg/L) | [115] |
| *Anabaena flos-aquae UTCC64, Phormidium autumnale UTEX1580 and Synechococcus* sp. *PCC7942* | Indigo, RBBR and Sulphur Black) | 80–90% | [117] |
| *Haematococcus* sp., *Chlorella* sp., *Chlorella vulgaris*, *Scenedesmus obliquuss*, *S. officinalis*, *and S. quadricauda Arthospira maxima (Blue green algae)* | | Up to 98% | [118] |
| *Phormidium valderianum*, a marine cyanobacterium | AR, AR-119 and DB-155, (at Ph > 11) | >90% | [119] |

**Table 4.** *Cont.*

| Algae | Dyes Used | Efficiency | References |
|---|---|---|---|
| *Chlorella pyrenoidosa* | MB<br>Rhodamine B | 84% | [120] |
| *Gloeocapsa pleurocapsoides and Phormidium ceylanicum* | AR 97 and FF Sky Blue dyes | 80% after 26 days | [121] |
| *Achaetomium strumarium* | Acid red | 99.5% | [122] |
| *Anabaena flos aquae*, *Nostoc elepsosporum*, *Nostoc linkia*, *Anabaena variabilis and Chlorella vulgaris* | Azo dyes | *N. elepsosporum* (100%), *C. vulgaris* (96.16%), *A. variabilis* (88.71%), *N. linkia* (88.71) and *A. flos aquae* (50.81). | [123] |
| *Arthrospira platensis NIOF17/003* | Methylene Blue | 93% | [23] |
| *A. platensis* complete drybiomss and Lipid-Free Biomass | Ismate violet 2R, IV2R | 75.7% and 61.11% | [25] |
| *A. platensis* complete drybiomss and Lipid-Free Biomass | Ammonium ions ($NH_4^+$) | In synthetic aquaculture:<br>ACDW (64.24%)<br>LFB (89.68%)<br>In real aquaculture:<br>ACDW (25.7%)<br>LFB (37.8%) | [24] |
| *Oedogonium subplagiostomum* | MO | 97% | [124] |
| *Cyanobacterium phormidium* | Indigo | 91% | [125] |

## 5. Enzyme-Based Bioremediation of Dyes

To date mainly two types of enzymes are widely used for the remediation of textile dyes. One is laccase and the other one is hydrogen peroxidases. Both enzymes are efficient and widely used by several investigators for the removal or decolourization of dyes from wastewater. The source of enzymes for these purposes are mainly microbial and even from microbes' fungi are more widely exploited. Laccase is a type of copper-containing polyphenol oxidase which is produced by several bacteria and fungi. Its potential has been exploited for the decolourization of Azo dyes. While peroxidase is an enzyme that degrades $H_2O_2$ and uses oxygen to oxidize other substances. Earlier lignin peroxidase has been used for the removal of Congo red dye. Furthermore, it has also been used for the decolorization of Reactive Orange 16 and remazol Brilliant Blue R (RBBR) [110].

Dong et al., 2019 reported the utilization of azoreductases extracted from *Streptomyces sps*. for the removal of methyl red from the wastewater [126].

Sherifah et al., 2019 used *Kluyveromyces dobzhanskii DW1* and *Pichia manshurica DW2* bacterial laccase for the enzymatic degradation of MG and MR [127]. Darvishi et al., 2018 used yeast laccase extracted from *Yarrowia lipolytica* and applied them for the enzymatic degradation of Bromocresol Purple, Safranin, Bromothymol Blue and Phenol Red [128]. In 2017, Afreen et al., reported the remediation of reactive Blue-4, from wastewater by using algal laccase isolated *Spirulina platenis*. In another study, a team led by Afreen used algal laccase from *Arthrospira maxima* and utilized them for the removal of RB-4, RBBR. A team led by Othman et al., 2018 reported the extraction of laccase from the edible mushroom (*Agaricus bisporus*) and used them for the enzymatic degradation of Acid Blue [129]. Dai et al., 2021 extracted laccase from a novel bacterium (transformed *E. coli BL21(DE3)* and applied it for the decolorization of acid violet 7, bromophenol blue (BpB), and Coomassie brilliant blue. Investigators obtained a mutant laccase (mut lac 2-9) which was reported to have enhanced decolorization of dyes [130].

Furthermore, these investigators have also used lignin and manganese peroxidase for the enzymatic degradation of dyes. For instance, Zhang et al., 2018 performed enzymatic degradation of MO and BpB by using fungal MnP [131].

Shaheen et al., 2017 and Bouacem et al., 2018 reported the enzymatic degradation of a group of dyes by using fungal lignin peroxidase extracted from *Ganoderma lucidum IBL 05* and *Bjerkandera adusta CX-9*, respectively [132,133].

Here Figure 7 is showing a mechanism of degradation of dyes (indigo dye) by the laccase enzymes. Here, firstly the laccase enzyme attacks the indigo dye and removed the hydrogen. Once the hydrogen is eliminated the indigo dye becomes converted into dehydrindigo. This dehydroindigo goes under several chemical changes leading to the formation of different intermediates. The dehydroindigo becomes converted into isatin which again forms an intermediate structure and finally becomes converted into anthranilic acid which is quite stable in the environment and less harmful than the original dye itself.

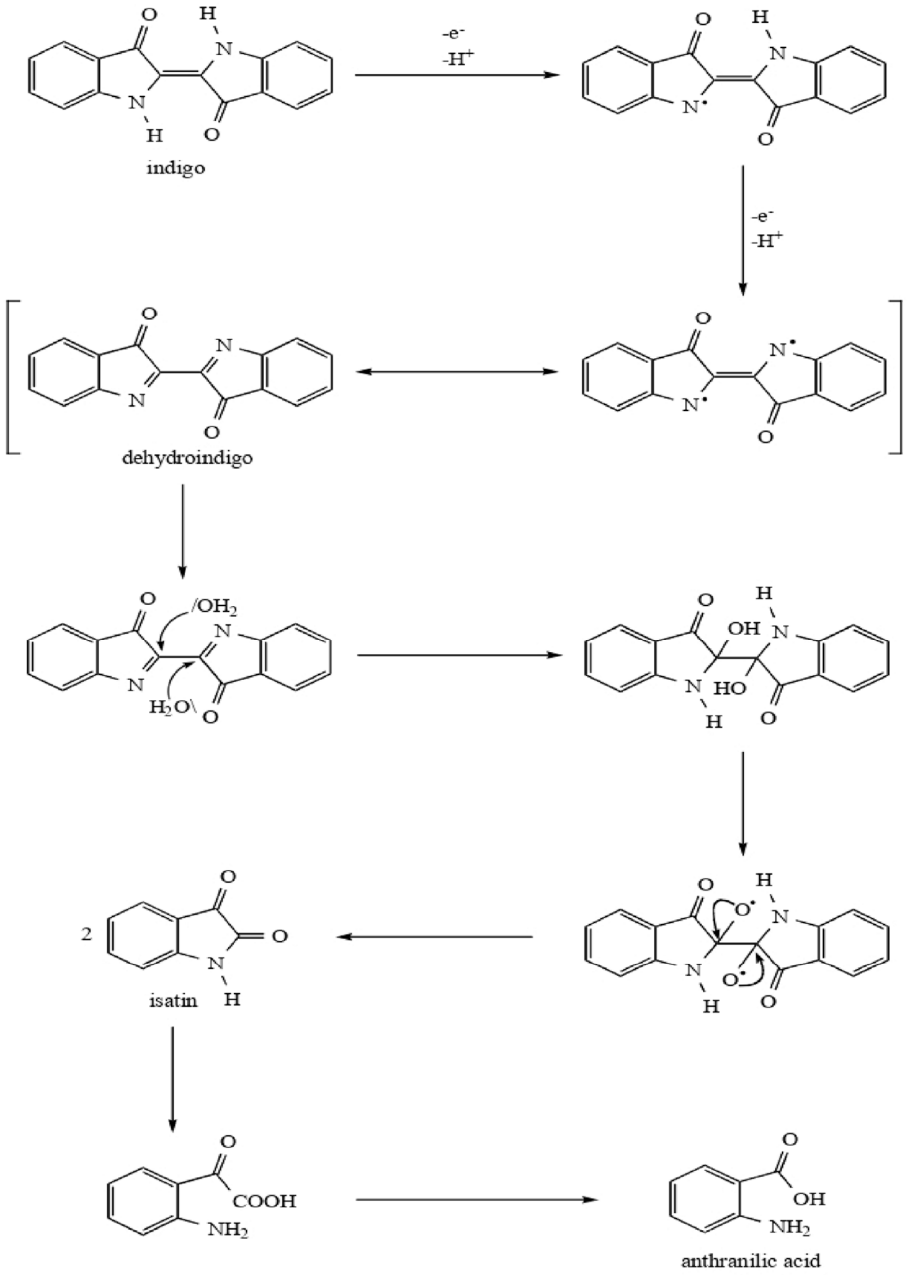


**Figure 7.** Mechanism of degradation of Indigo dye by using laccase enzyme adopted from [134].

Figure 8 is showing image where a genetically modified laccase from *Bacillus amyloliquefaciens* have been used for the enhanced enzymatic degradation of indigo carmine dye. The image shows reduction of colour intensity of the dye after enzymatic treatment.

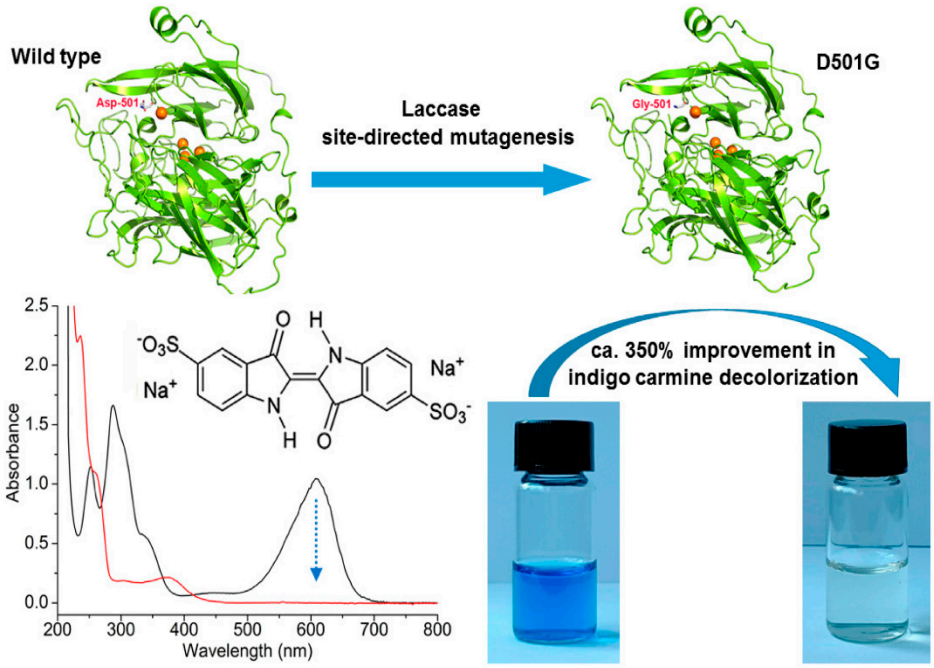


**Figure 8.** Genetically modified laccase from *B. amyloliquefaciens* for improving indigo carmine decolorization [135].

Few of the investigators have also reported the utilization of bacterial polysaccharides, i.e., intra and extra both for the removal of dyes from the wastewater [136]. Mustafa et al., 2022, reported the utilization of bacterial polysaccharides mainly exopolysaccharides for the decolorization of dyes from textile water [53].

## 6. Gaps and Future Prospects and Advantages of Microbial Degradation of Dyes

In order to acquire good efficiency and outcomes in dye biodegradation, further experimentation is required on (a) responsible microorganisms, (b) experimental factor limitations, (c) bioremediation site, and (d) degradation pathways before using microorganisms in the area. It will be critical to find out the type of degradation materials and determine their (none) toxicity to marine or plant species. While dyes have defied many microbial degradation strategies, there is an alternative approach to degrade dyes using genetic engineering, opening up a new domain for investigators working in this area. Advanced molecular biology techniques can investigate genes responsible for dye degradation. Dye degradation can result in the production of by-products, sources of nutrition, and energy, both of which can be utilized as commodities. However, researchers face a difficult task in attaining complete degradation of dye. Various scientific trials must be conducted to apply biodegradation of dye wastewater successfully. Future dye degradation research should focus on reducing factors that inhibit microbial activity. Recent and early promising experiments must be re-examined to increase their effectiveness. The removal of contaminants in the biodegradation process depends on the pathways, rate, and mechanisms of degradation pathways and environmental conditions. It must be essential to ensure that degraded materials do not harm marine organisms or plants. Integration of dye pollution treatment systems is desirable for successful industrial translation. The exploration of bacterial degradation kinetics will benefit from the study of pathways and hypotheses for bacterial degradation of dye wastewater. There are no additives used in this treatment. No toxic substances are released into the atmosphere by the consortium. The procedure,

therefore, reduces the need for chemical refining and vapour. No oil is used, resulting in carbon-free operation. Therefore, a carbon credit is also valuable, and no complicated plants are needed.

## 7. Conclusions

The remediation of dye effluent is possible with all the three approaches, i.e., chemical, physical and biological. Biological approaches especially microbial biodegradation of dyes has shown potential and interest in the recent times. The reliability of microbial-based dye biodegradation is mainly based on its biodegradable nature, eco-friendly, easy to use and faster action. The microbes have been used either as a biosorbent when it is used as a dried powder or has been used for enzymatic degradation as a whole organism. The bacteria, algae, fungi and actinomycetes have shown huge potential for dye removal in the direct form. Indirectly the enzymatic degradation of dyes in the soluble form or the immobilized enzymes has gained a tremendous response. Microorganisms are the warehouse of natural enzymes which mineralizes dyes effectively in the environment without producing a toxic product. The utilization of fungi is more economical and efficient as the enzymes produced by them are extracellular in nature and produce a wide range of enzymes. The mixed culture has shown more potential for dye removal than the monoculture. Some of the microbial-based approaches especially enzymatic degradation has shown 100% removal of dyes. Such approaches for the remediation of dyes from the environment make the technique environment-friendly, and sustainable.

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