

Microbe-mediated remediation of dyes: Current status and future challenges

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ABSTRACT

An array of industrial dyes most often azo dyes ($-N=N-$) deployed for different staining purposes, consequently impacting the environment significantly. The increasing pace of dye production often produces enormous wastewater from textile processing. After processing steps, dyes concentration remains left in expelled wastewater, consequently causing water pollution, and triggers negative toxicological impacts. However, remediation or decolorization is necessitating minimizing its negative consequences. Improper treatment of dye-containing waste waters triggers pollution of soil, water bodies, and so on. Numerous biological, physical, and chemical approaches for dye degradation and wastewater decolorization have been established. However, the high cost and practical feasibility of such methodologies remain obstacles in dye-containing wastewater. Microbial-assisted remediation is predominantly resilient to transforming dye compounds and reducing toxicity from water matrices in the ability to cope and provide cost-effective and efficient solutions. To cover the literature gap, and highlighting recent update information on dye remediation, we outlined different azo dyes, and their remediation deploying different physicochemical and microbial-mediated systems. In addition, recent advances in dye degradation, together with concluding remarks and future perspectives, have been pointed out.

1. INTRODUCTION

Water is a key resource to sustain life on earth. Better access to clean water, sanitation services, and water management create tremendous strategies for the economic growth of the country. Water pollution due to various effluents is a cause of concern worldwide. There is a rising demand for adopting practices to minimize water pollution. Among all industries, the textile industry is one of the leading contributors to pollution by liquid effluents and the reason is the use of high quantities of water in the process of dyeing [1]. One of such industrial waste that is considered as hazardous pollutant is dye. Dye is an organic (natural or synthetic chemical), colored compound which has an

affinity towards the substrate to which it is being applied by giving it an everlasting color. These have been applied to products such as paint [2], plastic [3], textile [4] printing ink [5], pharmaceuticals [6], cosmetics [7], photographic, and paper [8]. According to estimates, more than 10,000 dyes and pigments are put to industrial use and over 7×10^5 tons of synthetic dyes are annually produced across the world and 10% of which is released into the water [9].

Azo dyes are most extensively used in the textile industry (60–70%) [10]. These have nitrogen-nitrogen ($N=N$) in their chemical structure. The azo group is generally connected to naphthalene and benzene rings and could also be attached to enolizable aliphatic groups or aromatic heterocyclic. These attached side groups to the ring are necessary for the dye to impart the color, with a variety of shades and intensity of color [11]. Azo dyes and their degradation products have been proven toxic and mutagenic to aquatic animals and humans [12]. The dyeing and dye industries do not take precautionary measures

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as per the legislation and freely release these toxic chemicals into the environmental sink. Color is one of the obvious water pollution indicators and is usually the first recognized contaminant [13]. The dye presence in water bodies interferes with the penetration of sunlight [14] and adversely affects the aquatic ecosystem. Some of the azo dyes are mutagenic and very toxic to living organisms [15]. Their discharge in water bodies could result in serious health issues and can have chronic and acute effects on aquatic life [16].

Several factors, including the type of dye, wastewater composition, environmental fate, and operation and handling costs of generated waste products, determine the economic and technical feasibility of each single dye removal technique. Different physicochemical methods such as membrane filtration, flocculation or coagulation, ion exchange (Dotto *et al.*, 2012), adsorption, electrolysis, activated carbon, Fenton's reagent, ozonation, and advanced oxidation process are utilized by industries to remove dye from the effluents. Such methods are often very costly, less efficient, and inapplicable to a wide dyes variety [17]. Moreover, these methods generate concentrated sludge which creates a disposal problem [18].

Bioremediation or utilization of microbial-based techniques to deal with pollution is a key area of research in the field of environmental sciences where naturally developed or acclimatized microbes are used to transform various toxic chemical compounds into less toxic forms. In the process of biological treatment, microorganisms follow two methods: adsorption on microbial biomass and dye biodegradation by the microbial cells [19]. Adsorption of dye may be on live cells of microbes or dead cells of microbes and this type of adsorption on biomaterial is also known as biosorption, where the dye original structure remains untouched, that is, not fragmented. On the hand in biodegradation, the structure of the original dye is fragmented or broken by microorganisms, thus achieving complete mineralization in certain cases, that is, conversion to some inorganic salts, CO₂ and H₂O. On studying the mechanism involved in the two processes (biosorption and biodegradation), biodegradation was more convincing. The process of biosorption does not remove or destroy the dye, instead entraps the dye in biomatrix (microbial cells). Dumping of these microbial cells having absorbed dye itself is a roadblock in their projected role in the bio-cleaning of colored water [20]. Thus, because of the disposal of adsorbed biomass, biosorption might not be the pragmatic approach for treating colored effluent from industries [21]. The effectiveness of microbial decolorization depends on the activity and adaptability of the selected microorganisms. Several microbiomes, including bacteria, fungi, and algae, are capable of biodegrading azo dyes, among which bacteria represent a most promising tool for the removal of the azo dye from textile effluents [22]. The environmentally friendly and efficient technologies development to decrease the content of dye in wastewater is of utmost important. An insight into the present review detailed the role of microbial systems in the bioremediation of environmental pollutants, their mechanism, and factors affecting biodegradation.

2. CLASSIFICATION OF DYES

The society of dyers and colorists (TDC) and the American Association of Textile Chemists and Colorists had classified the dyes based on their color, structure, and application method which are revised every 3 months since 1924. A C.I. (Color index number) generic name was given to each dye based on the color as well as its application and characteristics. Dyes based on their origin are classified into two types, namely, natural and synthetic.

2.1. Natural Dye

The dyes obtained from natural sources are called natural dyes. They are mostly applied to textiles using mordants. These mordants are metallic salts that have a high affinity for fiber as well as any coloring matter. These metallic mordants after mixing with dye in fiber form an insoluble precipitate, leading to both dye and mordant getting fixed [23]. On the basis of the chemical constitution, a natural dye can further be classified namely, indigoid dyes, alpha-hydroxynaphthoquinones, flavones, dihydropyrans, anthocyanidins, and carotenoids. Indigoid dye's most common examples are indigo and Tyrian purple [24]. This dye type is obtained from the leaves of a wood plant as it possesses indigo as the key component in dyeing. Oppositely, alpha-hydroxy-naphthoquinones is a lawsone also known as Heena. It is mainly cultivated in Egypt and India. This dye type is also found in the unripe walnuts shell which gives orange color shades [25]. Flavones are colorless organic compounds. In general, natural yellows are derivatives of methoxy and hydroxyl substituted isoflavones and flavones. Weld (containing luteolin pigment) is a common example, which gives bright and fast colors to silk and wool [26]. Dihydropyrans are similar in chemical structure to flavones. Some examples are hematin and its leuco form, hematoxylin. These categories of natural dyes give dark shades to wool, silk, and cotton. Some of the common sources of these dyes are brazilwood, sappan wood, and logwood. Another dye, anthocyanidins, naturally possesses orange dye for cotton, and these are extracted from *Bignonia chica* leaves. Carotene is derivative of orange pigment found in carrots. Carotenoids owe their color to the conjugated double bonds which are present in their structure. Annatto and saffron are examples of carotenoids [27].

2.2. Synthetic Dyes

Synthetic dyes are man-made petrochemicals that may consist of lead, mercury, copper, sodium chloride, toluene, chromium, and benzene. Synthetic dyes have a brighter and wider range of colors, longer color permanence, and are easier, faster, and cheaper to produce in comparison with natural dyes. There are over 10,000 different types of synthetic dyes. Synthetic dyes are categorized on the basis of their chemical composition and the method of their application in the dyeing process. Although natural dyes are important from the chemical and historical point of view, these dyes are very expensive, need purification, and do not bind well because they lack the chemical grouping required to react with the binding sites of a fabric. Therefore, for dyeing applications, synthetic colors are utilized in place of natural dyes. This is because synthetic dyes are generally purer, less expensive, and their colors bind faster to the fabric [28]. Synthetic dyes are classified into three categories including anionic, non-ionic acid, and azo dyes. Anionic synthetic dyes include acid and whereas non-ionic dyes are basic, dyes are also known as cationic. On the other hand, dyes can be classified based on their chemical structure into anthraquinone dyes and azo dyes [29].

Azo dyes are characterized by being strong, having good all-around properties, and being less expensive. This dye has at least one azo bond (-N=N-), along with one or more aromatic structures. Azo dyes are intended to transport resistance and high photolytic stability toward major oxidizing agents. They have wide variety of applications in textile, food making, and cosmetic industries [3]. After azo dyes, anthraquinone dyes are widely used textile dyes [30]. Anthraquinone dyes have a wide color range and it almost covers the whole visible light spectrum. Dyes based on anthraquinone are the most resistant to degradation because of their fused aromatic structures, which

retain the color for a duration [31]. Acid dyes are soluble in water and is used for silk, wool, nylon, modified acrylics, leather, paper, ink-jet printing, cosmetics, and food. The principal chemical classes of acid dyes are azo (including premetallized), anthraquinone, azine, triphenylmethane, nitro, nitroso, and xanthene [32]. Metal complex dyes consist of dyestuff and metal (usually chrome such as chromium, cobalt, nickel, and copper) and are being used since the 1940s. On the other hand, direct dyes are compounds of poly azo having some phthalocyanines, oxazines, and stilbenes. These dyes types are the anionic dye having water-soluble properties. They are utilized in dyeing rayon, cotton, nylon, leather, and paper [33]. Basic dyes are utilized on polyacrylonitrile, paper, cation dyeable polyethylene terephthalate modified polyesters, modified nylons, and in medicines. Dyes yield colored cations in a solution and they are known as cationic dyes. The chemical structure of basic dyes encompasses triarylmethane, aromatic methane, xanthene, thiazine, hemicyanine, oxazine, cyanine, and acridine [32,33].

Another dye, reactive dye is mainly used for dyeing cotton, silk, wool, nylon, and leather. This class of dyes makes a covalent bond with the fiber. It is so tightly fixed that they cannot be removed even in harsh conditions [34]. Sulfur dyes have a complex structure and it contains sulfur. This class of dyes is economically friendly as they are low priced and has good wash fastness properties. These dyes are not brightly colored and are used for dyeing cotton, rayon, polyamide fibers, paper, leather, silk, and wood [33]. Vat dyes are fast dyes and are used for coloring cellulosic fiber, mainly cotton fiber. These dyes belong to the chemical class comprising indigoids and anthraquinone derivatives (including polycyclic quinones) [32]. Disperse dyes are primarily used on polyester and to a lesser amount on nylon, acrylic fibers, cellulose, and cellulose acetate. They are insoluble in water and non-ionic in nature. Disperse dyes molecule is based on azobenzene or anthraquinone molecules having nitro, hydroxyl, and amine groups attached [32,33].

3. DYES: ENVIRONMENTAL CONCERN

Wastewater released from the textile industry has many substances which are polluting in nature, like organochloride-based pesticides, and heavy metals [35]. Synthetic dyes are modeled in such a manner that they are recalcitrant and resist fading on treatment with water, soap, sweat, light, or any type of oxidizing agent [18]. The textile industry is the main producer of wastewater because water is the key component in the process of dyeing [36]. It had been estimated that 100 L of water was being used for the processing of 1 kg of textile materials. Every year all around the world almost 280,000 tons of textile dyes get discharged into industrial wastewater [37]. The appearance of color in the effluent is the leading sign that water has been polluted and the release of this colored effluent affects negatively the main water stream [38]. These water streams, when utilized in agriculture, have an adverse impact on the environment which, in turn, affects the health of living organisms [39]. These colored effluents when mixed with water bodies reduce the light penetration capacity of the water body and thus have a negative impact on aquatic flora and fauna by affecting the food chain. Even at a 1 mg/L concentration some of the dyes could be observed in water [40]. Dye-containing effluent also has been shown to increase the contaminated water's biochemical oxygen demand [41]. Out of all the known dyes, azo dyes are the major group of synthetic dyes and are extensively used [42]. Textile effluent has been characterized by the presence of undefined organic pollutants, dyes, increased COD (organic compounds), and high conductivity due to a high amount of dissolved salt, sulfide, halogen, and heavy metals [43].

Maximum dyes pose health risks to all kinds of life forms because of their non-biodegradable nature.

Azo dyes have been known as potential health hazards. Several azo dyes have also been the cause of DNA damage which leads to malignant tumors [44]. When these compounds contacted the body of humans through skin or injection and ingestion, they are metabolized inside the mammalian liver and gastrointestinal tract by the azoreductases and converted into aromatic amines and free radicals. Azo dyes are known as relatively persistent pollutants as they are not easily degraded under aerobic conditions. Under limited oxygen conditions, these dyes could be reduced by intestinal bacteria and other microbes to colorless amines. The converted amines are toxic, mutagenic, and carcinogenic to humans and animals [40,45].

4. METHODS OF REMEDIATION OF DYES

Dye could be removed by three methods namely, physical, chemical, and biological. The conventional treatment methods, that is, physical and chemical have been ineffective in dealing with effluents containing synthetic dyes which are highly unstable chemical pollutants. There have been remarkable achievements in the use of biotechnological methods in recent years. Chemical and physical methods are used for the decolorization of colored effluent and were subjective to physiochemical factors such as dye interaction, particle size, temperature, sorbent surface area, pH, and contact time [46]. On the other hand, the biological method uses microbial cells for the bioremediation of dye.

4.1. Physical Method

There are several physical methods through which dye is biodegraded such as adsorption, membrane filters, coagulation/flocculation, ion exchange, and radiation. Adsorption is an effective and attractive method for dye elimination from wastewater, particularly if the adsorbent is not expensive and commonly available [47]. Activated carbon is mainly utilized for dye removal with great success due to its higher adsorption capacity [48]. The cellular structure of peat makes it a perfect choice as an adsorbent. It can adsorb polar organic compounds and transition metals from colored effluents. Unlike activated carbon, it requires no activation and is less expensive [49,50]. Wood chips have high-quality adsorption capabilities for acid dyes, although because of their hardness and longer contact times are required [51]. Many low-priced adsorbents have been studied on fly ash for dye adsorption [52]. Its adsorption ability depends on various properties of the adsorbent: Porous chemical structure, porous structure, and surface area [53]. Silica gel could be considered effective for removing basic dyes. However, side reactions such as air binding and air fouling with particulate matter prevent it from becoming effective for commercial use [18].

Membrane filter methods have several applications by improving the finished product quality, increasing the amount of yield generation, saving raw materials, or recovery of products from waste [54]. This method has some special features which are not found in other methods, namely, resistance to temperature, an adverse microbial attack, and chemical environment [18]. Concentrated sludge production is the main disadvantage. Ion exchange resins were used to decolorize the textile wastewater and to reduce the COD [55]. Most of the dyes are either anionic or cationic in nature; therefore, it was possible to remove them through ion exchange resins. These are not utilized extensively for the treatment of colored effluents, mainly because they couldn't accommodate a wide spectrum of dyes. The

whole process is possible only in the presence of organic solvents during regeneration time [18].

Coagulation/flocculation is the only cost-effective method used for the removal of color from the effluent. The primary wastewater treatment method proceeds with coagulation and flocculation by removing colloidal particles of turbidity, color, and bacteria. Coagulants such as Fe (III) or Al (III) salts are used in high concentrations for initiating precipitation and settling along with it [56]. Generation of the huge toxic sludge and disposal amount has been the major drawback of this method. Radiation, in general, is the emission of any rays or particles from a source. Radiation is classified into two main categories: Ionizing radiation and non-ionizing [57]. The rate of reaction was monitored by radiation dose and the amount of oxygen present in the solution. It is a proven effective method for the removal of the dye at the same time toxic organic compounds. Almost 90% of the color has been removed from the effluent. The whole process of radiation is very expensive which makes it less feasible for use. Finally, the drawbacks of all such processes have been mainly due to low efficiency, high cost, problems in the disposal, and limited versatility. Thus, most of the chemical and physical methods for the treatment of colored wastewater are not widely used in textile industry sites or plants [37].

4.2. Chemical Method

In the chemical method, various types of chemical reagents and treatments are applied to degrade the dyes such as ozone treatment, Fenton's reagent, photochemical oxidation, sodium hypochlorite (NaOCl), and electrochemical. Fenton's reagent is an effective decolorization chemical method of the textile wastewater which is unaffected to a biological method or is toxic to living biomass but not useful in reducing its COD if not combined with one more process such as coagulation [58]. In the acidic mixture, iron (II) acts as a catalyst which when acts on hydrogen peroxide, immediately leads to the formation of hydroxide radicals. These hydroxide radicals help in decolorizing dye wastes. This method can act on both insoluble and soluble dyes. Sludge generation is the major disadvantage of this process [59]. Ozone treatment is widely applied in the treatment of water; ozone either alone or in combinations (O_3 -UV or O_3 - H_2O_2) is used for treating industrial effluents [60]. Chromophore groups are accountable for color which can be fragmented by ozone either directly or indirectly through the formation of smaller fragments [61]. The only disadvantage is the short shelf life (20 min). Photochemical oxidation is the traditional technique used for industrial textile effluent treatment [62]. UV light activates the degradation of H_2O_2 into hydroxyl radicals which may attack and release hydrogen atoms from organic molecules capable of the oxidizing organic compound. The formation of byproducts is a major drawback [39,63].

NaOCl acts on the amino group of the dye molecule by the Cl^- which starts and speeds up azo bond cleavage [64]. NaOCl, when added to effluent may remove residual colors effectively. Moreover, this method is not favored much as it has been observed to lead to the generation of toxic chlorinated compounds which are dangerous for the environment as well as human health [65]. An electrochemical method in current years has been a growing interest in wastewater treatment. This type of method has been effectively functional in the detoxification of textile wastewater [66]. Electrochemical reactions are mainly used in sulfur and vat dyeing. Electrochemical oxidation leads to the generation of hypochlorite or hydroxyl radical. These electrogenerated species are used to degrade dyes present in wastewater. However, the electricity price used is high compared to the chemicals cost [18].

4.3. Biological Method

In the last few years, awareness among the scientific community about biological techniques has increased tremendously. These techniques have several benefits over conventional techniques such as low cost, environment-friendly, safe operation, and less sludge production. Bioremediation is now considered an upcoming treatment option for dye removal in diverse conditions. The bioremediation method can use natural and recombinant microorganisms to degrade toxic materials because of flexibility in operating conditions and design. Flexibility in this technique is understood by the fact that they could be employed *ex situ* (off-site) or *in situ* (on-site) and even plants can be used (phytoremediation). Biodegradation could be defined as the biologically facilitated dye molecule breakdown into several by-products by the action of a variety of enzymes. It is a process that is energy dependent [67]. Dye biodegradation results in decolorization and the breakdown of the dye molecules into smaller fragments. Diverse microbes such as bacteria, fungi, and algae are employed for the decolorization and degradation of synthetic dyes. Microbes have various capabilities for decolorizing a variety of dyes. Some groups of microorganisms have specific advantages over others in synthetic dyes biodegradation. The bioremediation of dyes effectiveness is subject to the activity and adaptability of the microbes [38].

4.3.1. Fungi

Fungi are most effective in breaking down or sometimes completing the mineralization of synthetic dyes [68]. These degradation properties are attributed to the presence of a powerful extracellular and intracellular enzyme system comprising laccase, manganese peroxidase, and lignin peroxidase, robust morphology, and various metabolic activity [69]. The mycelia of fungal species have an advantage over unicellular organisms that they solubilize the insoluble substance by enzymes. They have a high cell-to-surface ratio which helps them to have greater enzymatic and physical contact with pollutants. Extra-cellular fungal enzymes are also advantageous in tolerating high toxicants concentration [67]. Fungi initiate the process by adsorption of dye onto hyphae, followed by the breakdown of chemical bonds by enzymes [70]. However, fungi application for the removal of the dye from textile wastewater has some inherent drawbacks such as a long growth cycle and the need for nitrogen limiting conditions [71]. Gill *et al.* [72] reported Congo red decolorization by *Pha. chrysosporium* and *Dichomitus squalens*. Svobodová *et al.* [73] have reported maximum decolorization of Reactive Orange 16 up to 80% by fungal strain *Irpex lacteus*. Jayasinghe *et al.* [74] have reported *Ganoderma lucidum*, *Pycnoporus cinnabarinus*, *Pleurotus pulmonarius*, *Stereum ostrea*, and *Trametes suaveolens*, for their ability to decolorize Congo red (100 mg/L).

In a report, fungal species *Fusarium oxysporum*, *Penicillium lanosum*, and *Ganoderma resinaceum* was reported for Blue 21 dye decolorization [75]. Synthetic dye amaranth was reported to be degraded by *Bjerkandera adusta* [76]. White rot fungus *Armillaria* spp. was confirmed for degrading azo, anthraquinone, and triphenylmethane dyes. [77]. Noval species of *Alternaria alternata* was able to decolorize Congo red (600 mg/L) within 48 h of incubation [78]. *Ganoderma* spp. has also been reported to decolorize Reactive Orange 16 [79]. Chen *et al.* [80] reported *Corioloropsis* spp. for decolorizing triphenylmethane dyes. In a report, the novel fungal strain *Absidia spinosa* was reported for biotransformation of Cresol Red up to 65% [81]. Barapatre *et al.* [82] reported *Aspergillus flavus* for biodegradation of Malachite Green in which intermediate *N*-demethylated and *N*-oxidized metabolites were identified [Figure 1].

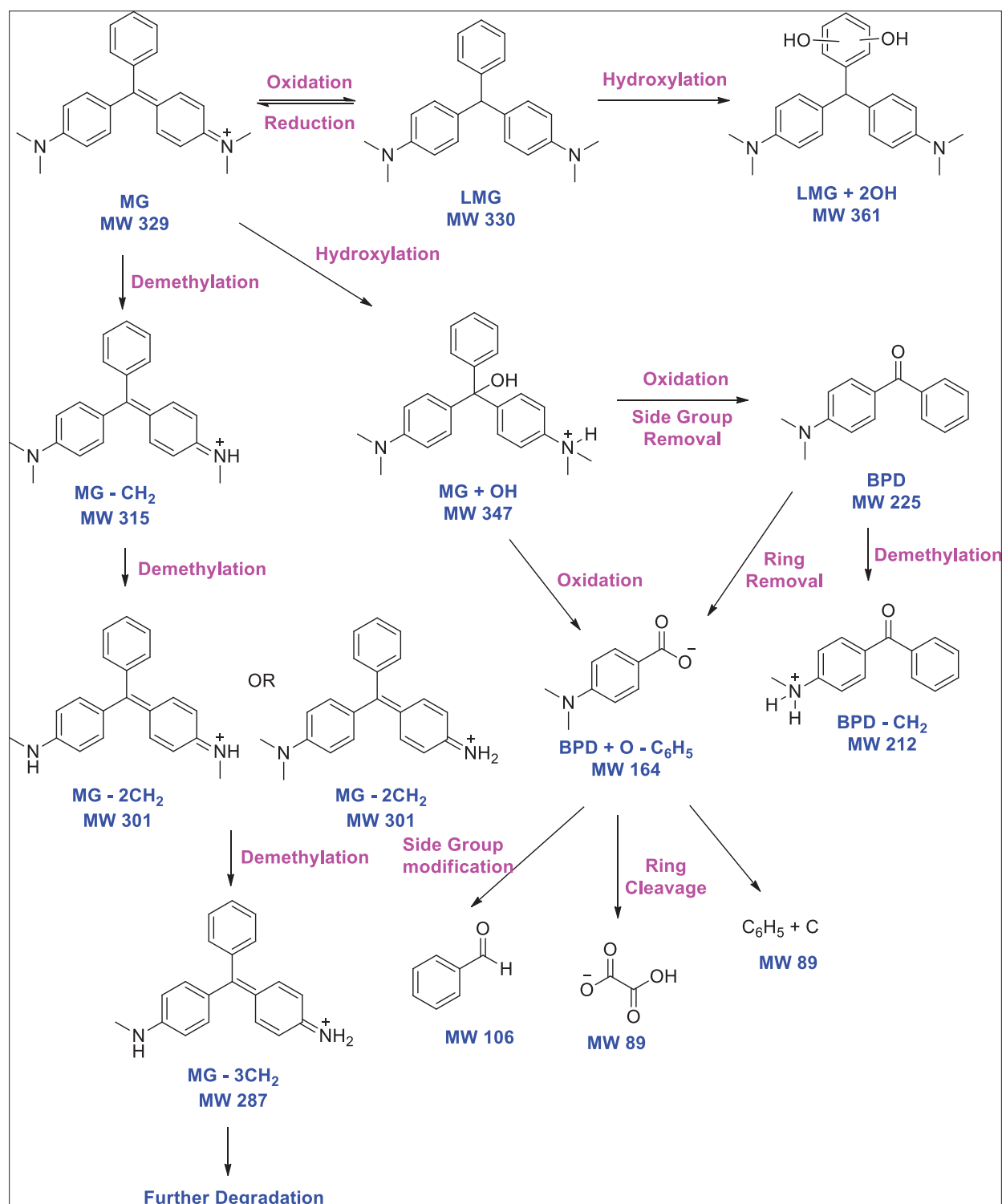


Figure 1: Biodegradation pathway of malachite green using *Aspergillus flavus*. Source: Adapted with permission from Barapatre *et al.* [82].

Fungal strain *Aspergillus bombycis* was reported for having the ability to degrade Reactive Red 31 [83]. Asses *et al.* [84] reported *Aspergillus niger* having the ability to decolorize Congo red (200 mg/L) within 6 days of incubation [Figure 2]. *Oudemansiella canarii*, the white rot fungi, was reported for degrading Congo Red dye [85]. In another report, *Aspergillus terreus* was reported for degrading Direct Blue-1 [86]. Krishnan *et al.* [87] reported *Fusarium equiseti* for the degradation of methylene blue dye.

4.3.2. Bacteria

Dyes decolorization through bacteria is faster than fungal decolorization as bacteria take less time to grow [88]. Bacterial cells represent a promising and inexpensive tool for the several azo dyes removal from the textile dye effluents. Bacteria have many advantages as compared to filamentous fungi such as higher hydraulic retention time, faster growth rate, and could be efficient in treating high strength organic wastewaters [89]. In general, the azo dyes decolorization occurs under

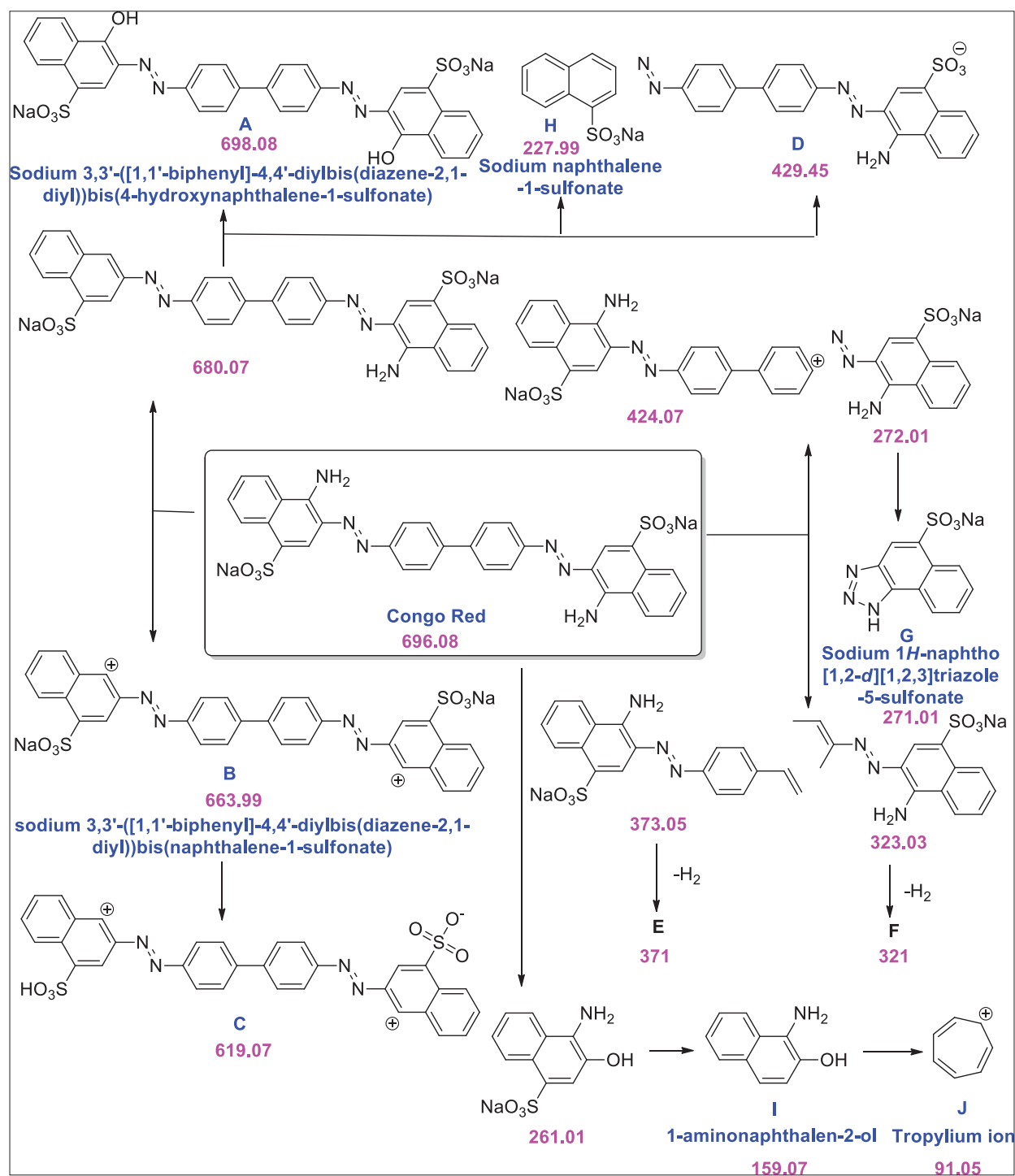


Figure 2: Decolorization of Congo red through *Aspergillus niger*. Source: Adapted with permission from Asses et al. [84].

conventional anaerobic, facultatively anaerobic, and aerobic conditions by different bacterial groups [90]. A bacteria like *Pseudomonas* spp. could decolorize Reactive Orange 16 (100 mg/L) to 98% within 24 h of incubation [91]. Park et al. [92] reported decolorization of Congo red (1 g/L) by *Staphylococcus* spp. up to 96%. In a report, *Pseudomonas* spp. SUK1 could decolorize methyl orange dye (300 mg/L) within 6 h of incubation [93]. On the other hand, strain *Kocuria rosea* exhibited complete decolorization [94]. In another study, *Nocardia* spp. *alba* could decolorize Reactive Orange 16–95% (1000 mg/L) dye within 24 h of incubation [95]. Ghanem et al. [96] reported decolorization

of methyl orange (38 µg/ml 10 µg/ml, 36 µg/ml, 32 µg/ml, and 40 µg/ml) by *Acinetobacter baumannii*, *Cytophaga columnaris*, *Corynebacterium* spp., *Escherichia coli*, and *Pseudomonas fluorescence* up to 76, 20, 72, 64, and 80%, respectively.

A study by Shah et al. [97] showed complete decolorization of methyl orange (2.9 mg/L) by *Bacillus* spp. complete methyl orange decolorization has also been reported for a much higher concentration of dye (50–200 mg/L) by several researchers. In a finding by Cui et al. [98] reported 100% decolorization of Methyl orange (100 mg/L) dye

by *Klebsiella* spp. strain Y3. Ng *et al.* [99] reported decolorization up to 96% for 200 mg/L of Methyl orange by *Shewanella xiamenensis*. Marine bacteria *A. baumannii* could decolorize Congo red (100 mg/L) to 99.1% within 30 h and 5 days of incubation under optimized conditions [100]. *Mycobacterium* could decolorize Reactive Orange 16 (250 mg/L and 100 mg/L) to 96% within 24 h [101]. *Bacillus cereus*, *Ochrobactrum* spp., and *Achromobacter xylosoxidans* could decolorize Congo red (25 mg/L) 93%, 62%, and 94%, respectively [102]. Bacterium *Alishewanella* spp. was reported for azo dyes degradation [103]. Mani *et al.* [104] reported *Shewanella oneidensis* for decolorization of acid orange 7 [Figure 3]. *Lysinibacillus fusiformis* was reported for degrading methyl red [105]. Saha and Rao [106] studied the degradation of Reactive Orange 16 up to 89% by *Bacillus flexus*. In a report, halo-alkaliphilic bacterium *Nesterenkonia lacusekhoensis* was reported for decolorization of Reactive Red-35 dye [107]. Akansha *et al.* [108] reported *Bacillus stratosphericus* for biodegradation of reactive orange 16 [Figure 4].

4.3.3. Yeast

Yeast can grow fast and withstand adverse environmental conditions [109]. *Trichosporon beigeli* was capable to decolorize Navy blue HER, Golden Yellow 4BD, Red HE7B, Green HE 4BD, Orange HE2R, and Malachite green [22] whereas *Candida krusei* was able to decolorize Reactive Brilliant Red K-2BP, Acid Mordant Yellow Reactive, Weak Acid Brilliant Red B, Reactive Black KN-B, Reactive Brilliant Blue X-BR, Acid Mordant Light Blue B Reactive, Acid

Mordant Red S-80, and Reactive Turquoise Blue KN-G [109]. Yeast *C. krusei*, isolated from textile wastewater, was reported for degrading Basic Violet 3 by 74% [110]. About 90% Acid Red B decolorization under aerobic conditions was reported by *Pichia* spp. [111]. In a report, Acid Brilliant Scarlet GR was detoxified by *Candida tropicalis* [112]. Tan *et al.* [113] reported *Magnusiomyces ingens* for degrading Acid Red B. In a report, Acid Red B was degraded by *C. tropicalis* [114]. Tan *et al.* [115] reported salt tolerant *Scheffersomyces spartinae* for the detoxification of Acid Scarlet 3R under anaerobic conditions. A recent study had reported complete decolorization of Reactive Orange 16 by *Pichia kudriarzenii* [116]. *Cyberlindnera samutprakarnensis*, the salt tolerant yeast, was reported for decolorization of Acid Red B by 97% within 18 h of incubation [117]. In a report, *Galactomyces geotrichum* was reported for degrading Acid Scarlet GR under aerobic conditions [118]. Similarly, halotolerant, *Sterigmatomyces halophilus* was reported for complete detoxification of Reactive Black 5 within 24 h of incubation [119]. In another report, *C. tropicalis* was reported for the Acid Red B degradation [120].

4.3.4. Algae

Some microbial groups have not been studied extensively for their degradation abilities concerning pollutants such as synthetic dyes and xenobiotics. Cyanobacteria (blue-green algae) have distributed ubiquitously, but there is scant information about their ecosystem functioning role, including recalcitrant compounds degradation such as dyestuffs and dye [121]. *Chroococcus minutus*, *Gloeocapsa*

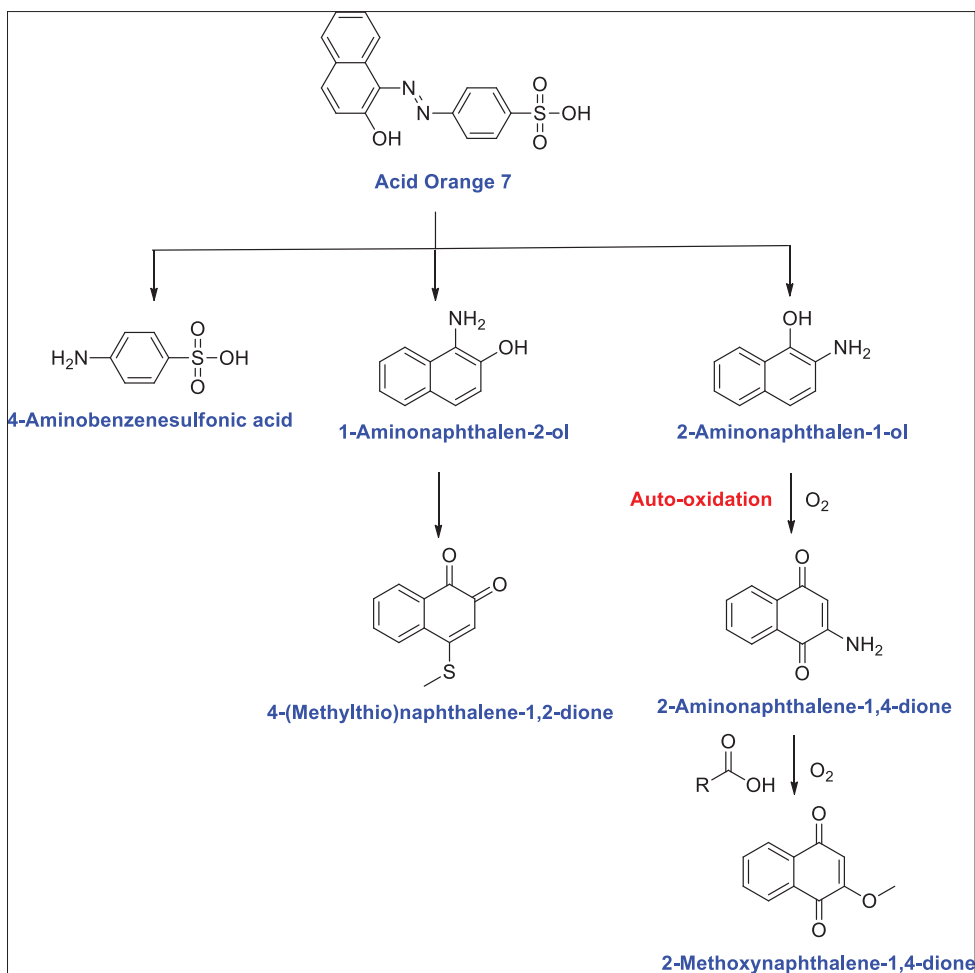


Figure 3: Acid Orange 7 biodegradation pathways by *Shewanella oneidensis*. Source: Adapted with permission from Mani *et al.* [104].

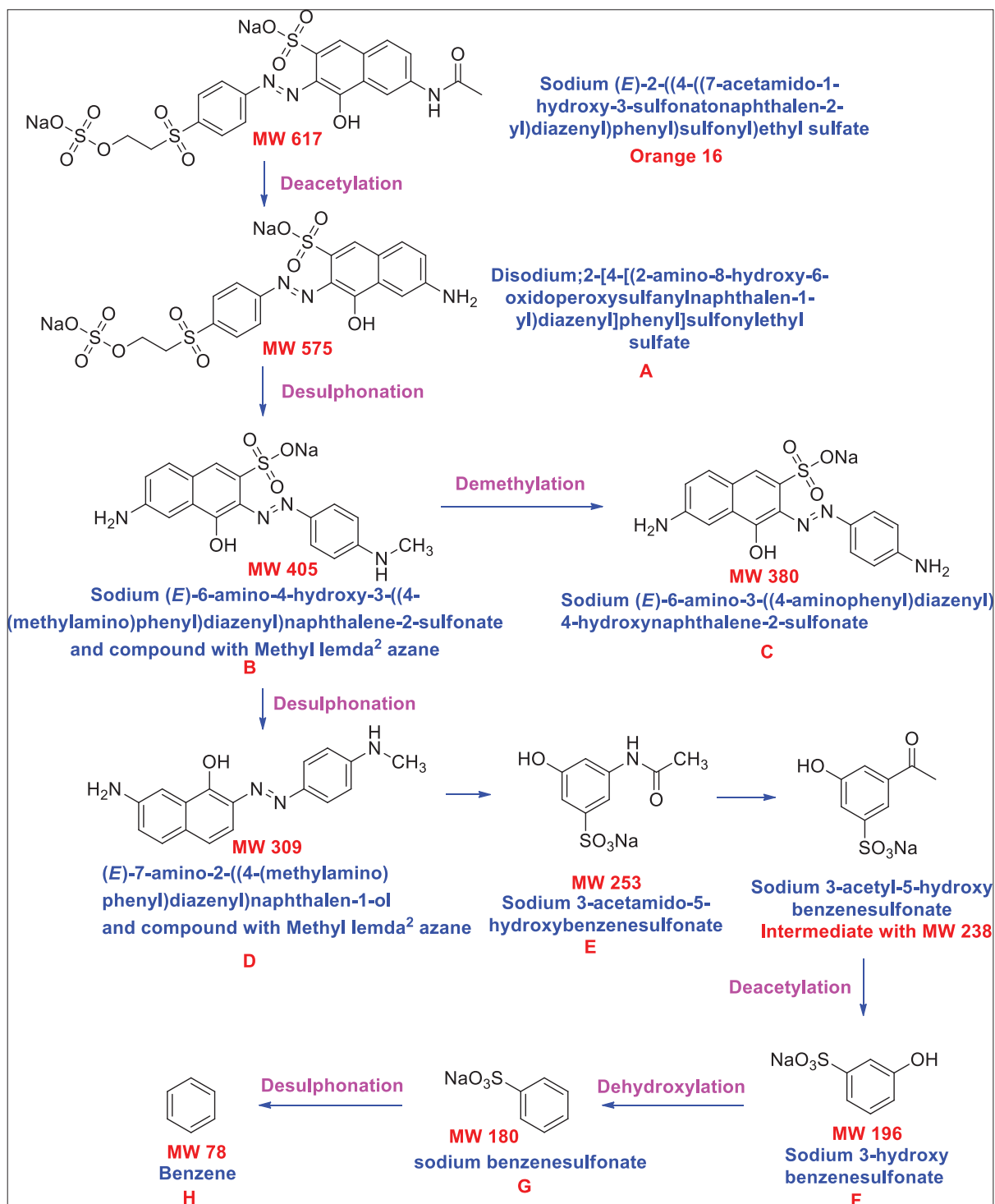


Figure 4: Biodegradation pathway of Reactive Orange 16 through *Bacillus stratosphericus*. Source: Adapted with permission from Akansha et al. [108].

pleurocapsoides, and *Phormidium ceylanicum* are some of the algae reported on dye decolorization [122]. In a report, detoxification of monoazo and diazo dye was reported by the *Nitzschia perminuta* and *Scenedesmus bijugatus* [123]. Khataee et al. [124] reported *Chara* spp., the macroalgae for the detoxification of Malachite Green solution. In a report, from a thermal spring environment, Methylene Blue and Malachite Green were removed by *Chlorella* spp. and *Chlamydomonas* spp. [125]. In another report, Orange G Dye was detoxified by the

microalgae *Acutodesmus obliquus* [126]. Chia et al. [127] reported *Scenedesmus quadricauda* for the degradation of Indigo Blue dye. Congo red dye detoxification was reported by green algae *Chlorella* spp., *Chlorella vulgaris*, *Haematococcus* spp., *Scenedesmus officinalis*, *Scenedesmus obliquus*, and *S. quadricauda* [128]. In similar report, Methylene Blue and Malachite Green were decolorized by the *Desmodesmus* spp. [129]. *C. vulgaris* was reported for Indigo Blue dye [130].

4.3.5. Microbial consortium

In various studies, microbial consortia have shown greater effectiveness as compared to pure strains [21]. The mixed microbial population has shown greater efficiency of synthetic dyes decolorization than pure culture because of synergistic metabolic activities of microbial communities [131]. Each strain in the consortium may target different sites in the dye molecules or may utilize degraded metabolites generated

by one strain followed by degradation by another strain [132]. Thus, using mixed microbial cultures dyes, biodegradation could be improved due to the synergistic effect [133]. Consortium development which can survive in the effluent by utilizing the components as a source of carbon, nitrogen, and energy would render the whole technique economically friendly [21]. Different microbial consortiums were reported for the remediation of various dyes [Table 1]. Tony et al.

Table 1: Dye remediation using microbial consortium.

Consortium	Dye	References
<i>Bacillus vallismortis</i> , <i>B. cereus</i> , <i>B. pumilus</i> , <i>B. subtilis</i> , and <i>B. megaterium</i>	Blue BCC, Ranocid fast blue, Bordeaux, Congo red	Patil et al. [135]
<i>Brevibacillus laterosporus</i> and <i>Galactomyces geotrichum</i>	Golden yellow HER	Waghmode et al. [136]
<i>Klebsiella</i> , <i>Buttiauxella</i> , and <i>Bacillus</i>	Methyl red	Cui et al. [137]
<i>Citrobacter freundii</i> , <i>Moraxella osloensis</i> , and <i>Pseudomonas aeruginosa</i>	Mordant black 17	Karunya et al. [138]
<i>Providencia rettgeri</i> , and <i>Pseudomonas</i> spp.	Reactive Orange 16	Lade et al. [139]
<i>Escherichia coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus aureus</i> , <i>Proteus</i> spp., <i>Pseudomonas</i> spp., and <i>B. subtilis</i>	Congo red	Holey [140]
<i>Bacillus</i> spp., <i>B. subtilis</i> , <i>B. cereus</i> , <i>B. mycoides</i> , <i>Micrococcus</i> spp., and <i>Pseudomonas</i> spp.	Green dye, red dye, black dye, and yellow dye	Mahmood et al. [141]
<i>Pseudomonas stutzeri</i> and <i>Acinetobacter baumannii</i>	Congo red and gentian violet	Kuppusamy et al. [142]
<i>Aeromonas</i> spp., <i>Bacillus</i> spp., <i>Neisseria</i> spp., and <i>Vibrio</i> spp.	Novacron brilliant blue fn-r, bezema yellow s8-g, novacron super black G	Karim et al. [143]
<i>Barnettozyma californica</i> , <i>Sterigmatomyces halophilus</i> , and <i>Yarrowia</i> spp.	Remazol brilliant blue R	Ali et al. [144]
<i>Zobellella</i> , <i>Rheinheimera</i> , and <i>Marinobacterium</i>	Direct blue B, acid violet 7, acid black ATT	Guo et al. [145]
<i>Scenedesmus obliquus</i> , and <i>Oscillatoria</i> spp.	Reactive orange 122, reactive red 194	El-Sheekh et al. [146]
<i>Bacillus odyssey</i> , <i>Morganella morganii</i> and <i>Proteus</i> spp.	Red HE3B	Phugare et al. [147]
<i>Providencia</i> spp. and <i>Pseudomonas aeruginosa</i>	Red HE3B	Phugare et al. [147]
<i>Anoxybacillus</i> spp., <i>Clostridium</i> spp., and <i>Bacillus</i> spp.	Direct Black G	Chen et al. [148]
<i>B. subtilis</i> , <i>B. subtilis</i> , and <i>B. cereus</i>	Mixture of azo dyes	Thiruppathi et al. [149]
<i>Bacillus flexus</i> , <i>Proteus mirabilis</i> , and <i>Pseudomonas aeruginosa</i>	Indanthrene blue RS	Kumar and Mohanty [150]
<i>Lysinibacillus</i> spp., <i>Bacillus</i> spp., <i>Bacillus</i> spp., <i>Bacillus</i> spp., and <i>Ochrobacterium</i> spp.	Reactive violet 5R	Jain et al. [151]
<i>Galactomyces geotrichum</i> and <i>Bacillus</i> spp.	Brilliant blue G	Jadhav et al. [152]
<i>B. cereus</i> , <i>Pseudomonas putida</i> , <i>Pseudomonas fluorescence</i> , and <i>Stenotrophomonas acidaminiphila</i>	Acid red 88	Khehra et al. [153]
<i>Aeromonas caviae</i> , <i>Proteus mirabilis</i> , and <i>Rhodococcus globerulus</i>	Acid Orange 7	Joshi et al. [154]
<i>Enterobacter dissolvens</i> and <i>Pseudomonas aeruginosa</i>	Acid maroon V	Patel et al. [155]
<i>Stenotrophomonas rhizophila</i> , <i>Sphingomonas echinoides</i> , <i>Pseudarthrobacter oxydans</i> , and <i>Gordonia westfalica</i>	Reactive black-5	Eskandari et al. [156]
<i>Pseudomonas aeruginosa</i> , <i>Stenotrophomonas maltophilia</i> , and <i>Proteus mirabilis</i>	Direct black 22	Mohana et al. [157]
<i>Aspergillus ochraceus</i> and <i>Pseudomonas</i> spp.	Rubine GFL	Lade et al. [158]
<i>Pseudomonas aeruginosa</i> and <i>Bacillus circulans</i>	Reactive Black 5	Dafale et al. [159]
<i>Pseudomonas aeruginosa</i> , <i>Rhodobacter sphaeroides</i> , <i>Proteus mirabilis</i> , and <i>Bacillus circulans</i>	Remazol black-B	Dafale et al. [160]
<i>Enterococcus faecalis</i> and <i>Klebsiella variicola</i>	Reactive red 198	Eslami et al. [161]
<i>Alcaligenes faecalis</i> , <i>Sphingomonas</i> spp., <i>B. subtilis</i> , <i>Bacillus thuringiensis</i> , and <i>Enterobacter cancerogenus</i>	Direct blue-15	Kumar et al. [162]
<i>Anoxybacillus pushchinoensis</i> , <i>A. kamchatkensis</i> , and <i>Anoxybacillus flavithermus</i>	Reactive black 5	Deive et al. [163]
<i>Dichotomomyces cejpaii</i> and <i>Phoma tropica</i>	Congo red, methyl red, reactive blue	Krishnamoorthy et al. [164]
<i>Bacillus</i> spp., <i>Stenotrophomonas</i> spp., <i>Pseudomonas</i> spp., and <i>Alcaligenes</i> spp.	Procion red H-3B	Shah and Bera [165]
<i>Pseudomonas aeruginosa</i> , <i>Enterobacter</i> spp., and <i>Serratia marcescens</i>	Reactive red 120	Manogaran et al. [166]
<i>Penicillium</i> spp. and <i>Sphingomonas xenophaga</i>	Reactive brilliant red X-3B	Gou et al. [167]

B. cereus: *Bacillus cereus*, *B. pumilus*: *Bacillus pumilus*, *B. subtilis*: *Bacillus subtilis*, *B. megaterium*: *Bacillus megaterium*.

[134] showed 50–60% of biodegradation of dyes including Congo red, Bordeaux, Blue BCC, and Ranocid Fast Blue by bacterial consortium of *Bacillus cereus*, *B. megaterium*, *B. pumilus*, *B. vallismortis*, and *B. subtilis*. In a report, bacterial consortium of *Bacillus odysssei*, *Morganella morganii*, and *Proteus* spp. was reported for textile dyes decolorization [135].

The consortium of *Brevibacillus laterosporus* and *G. geotrichum* was reported for Golden Yellow HER decolorization [136]. In another report consortium, bacterial strains *Klebsiella*, *Buttiauxella*, and *Bacillus* were reported for the removal of Methyl Red under aerobic conditions [137]. Karunya et al. [138] developed a microbial consortium of *Citrobacter freundii*, *Moraxella osloensis*, *Pseudomonas aeruginosa*, and *P. aeruginosa* which helps in the Mordant Black 17 decolorization. Lade et al. [139] used microbial consortium consisting of *Providencia rettgeri* and *Pseudomonas* spp. for decolorization of Reactive Orange 16. The consortium could decolorize 1110 mg/L of Reactive Orange 16 by about 99%. Another study by Holey [140] reported a where consortium which comprised *E. coli*, *Salmonella* spp., *Staphylococcus aureus*, *Proteus* spp., *Pseudomonas* spp., and *B. subtilis* and had the ability to decolorize Congo Red (10 mg/L) up to 98% within 96 h of incubation. In another study, the results indicated that the consortium ability to decolorize the green, red, yellow, and black dyes was higher as compared to single strains application (*Bacillus* spp., *B. subtilis*, *B. cereus*, *B. mycoides*, *Micrococcus* spp., and *Pseudomonas* spp.). The consortium was capable to decolorize green (84%), red (84%), yellow (85%), black (85%), and mixed dyes (82%) within 24 h while individual strain required 72 h [141].

In an investigation, bacterial consortium of novel and indigenous strains, namely, *Pseudomonas stutzeri* and *A. baumannii* was reported for textile dyes decolorization [142]. In case of monoculture (*Aeromonas* spp., *Bacillus* spp., *Neisseria* spp., and *Vibrio* spp.), percentage of decolorization varies from no visible decolorization to highest 90% decolorization (Novacron Brilliant Blue FN-R) whereas the percentage decolorization of bacterial consortium varies from 65% (Benzema Yellow S8-G) to 90% (Novacron Super Black G and Novacron Brilliant Blue FN-R) [143]. Reactive azo dyes were also reported to be degraded by the consortium of yeast, that is, *Barnettozyma californica*, *S. halophilus*, and *Yarrowia* spp. [144]. Guo et al. [145] showed the detoxification of Acid Black ATT, Direct Blue B, and Acid Violet 7 by the consortium containing halotolerant microbes, namely, *Zobellella*, *Rheinheimera*, and *Marinobacterium*. Consortium of cyanobacteria and green algae, that is, *S. obliquus* and *Oscillatoria* spp. was reported for degradation of azo dyes including Reactive Orange 122 and Reactive Red 194 [146].

5. MECHANISM OF DYE BIODEGRADATION

The plant and microbial systems possess efficient enzymatic systems which could be used for bioremediation. Therefore, it is important to find the mechanism of biotransformation followed by the organism and probable enzymes which are involved in biochemical complex reactions. The enzymatic treatment methods positively affect the environment as they pose a low chance of biological contamination. Enzymes of both bacterial as well as fungal origin such as lignin peroxidase, laccase, and manganese peroxidase have the capacity to metabolize xenobiotic compounds [168]. Peroxidase enzyme purified from plant species which includes *Saccharum spontaneum* and *Ipomoea palmata* is used for efficient decolorization of various textile dyes [169]. Enzymes like horseradish peroxidase have been

immobilized and utilized for the treatment of effluents of textile mills and paper industries [170].

Laccase belongs to the multicopper oxidases group which has low substrate specificity and is highly capable of degrading the spectrum of xenobiotic compounds and aromatic as well as non-aromatic substrates [171]. These catalysts have good bioremediation potential at the same time. It does not require available oxygen as an electron acceptor, which makes them highly applicable in many biotechnological processes [172]. Enzymes can degrade phenolic compounds and aromatic azo compounds. Cu^{2+} is used as mediator to oxidize aromatic amines. First prokaryotic laccase has been reported by *Azospirillum lipoferum* [173]. Laccase basically catalyzes azo dyes decolorization by non-specific free radical without the mutagenetic and toxic aromatic amines formation. *Pseudomonas syringae* and *Pedomicrobium* spp. have shown laccase like activity [174]. Reports are available for purified laccase enzyme extracted from *Bacillus* spp. and *Pseudomonas desmolyticum* that can decolorize several textile dyes efficiently [175,176]. Fungal strain *Podoscypha elegans* can decolorize five azo dyes (Congo Red, Direct Blue 15, Orange G, Rose Bengal, and Direct Yellow) efficiently [171]. Similarly, fungal strains of *Aspergillus oryzae*, *Trametes versicolor*, and *Paraconiothyrium* variable can decolorize azo dyes through the laccase enzyme production [177].

Azoreductase also known as azobenzene reductase is a reducing enzyme. These catalysts can degrade azo dye into colorless amines through the process of reductive cleavage. The whole process requires NADH or FADH [178] which acts as an electron donor in a redox reaction [45]. Bio-treatment of azo dye containing effluent and microbial azoreductase has been observed to play a major role. Sometimes, under unfavorable environmental conditions, few usual cellular enzymes might get converted into dye degrading enzyme example flavin reductase produced by *E. coli* acts as azoreductase [179]. Microbes such as *B. subtilis*, *Pseudomonas* spp., and *S. aureus* have been found to decolorize azo dyes (Methyl Red, Disperse Blue, and Acid yellow) through the production of azoreductase [180-182]. Agrawal et al. [183] reported *Providencia* spp. for degrading Acid Black 210 through the production of azoreductase [Figure 5].

Peroxidase is related to the group of oxidoreductases, especially which act on peroxide acting as the electron acceptor. Knowledge of such factors will influence the degradation activity which will facilitate the bioreactor development for bioremediation of industrial waste. The fungus's efficiency to degrade azo dyes is related to the exo-enzymes formation such as peroxidases and phenol oxidases. Peroxidases can catalyze the breakdown of hydrogen peroxide into water and molecular oxygen [184]. These enzymes have a heme group attached to the active site [185]. Both manganese peroxidases and lignin have the same reaction mechanism (catalytic enzyme causes oxidation of H_2O_2 to an oxidized state). Basically, lignin peroxidases help in the oxidation of non-phenolic aromatic compounds whereas manganese peroxidases oxidize Mn^{2+} to Mn^{3+} and this Mn^{3+} is responsible for the oxidation of numerous phenolic compounds [186]. The first peroxidase was obtained from *Phanerochaete chrysosporium* [187]. Various microorganisms involved in dye decolorization with peroxidases activity include *Rhodococcus jostii* [188], *I. lacteus* [189], *Thermomonospora curvata* [190], *B. subtilis* [191], *Enterobacter ignolyticus* [192].

6. FACTORS AFFECTING BIOREMEDIATION

The whole ecosystem consists of a dynamic environment with various abiotic factors such as temperature, metals, salts, pH, and the presence

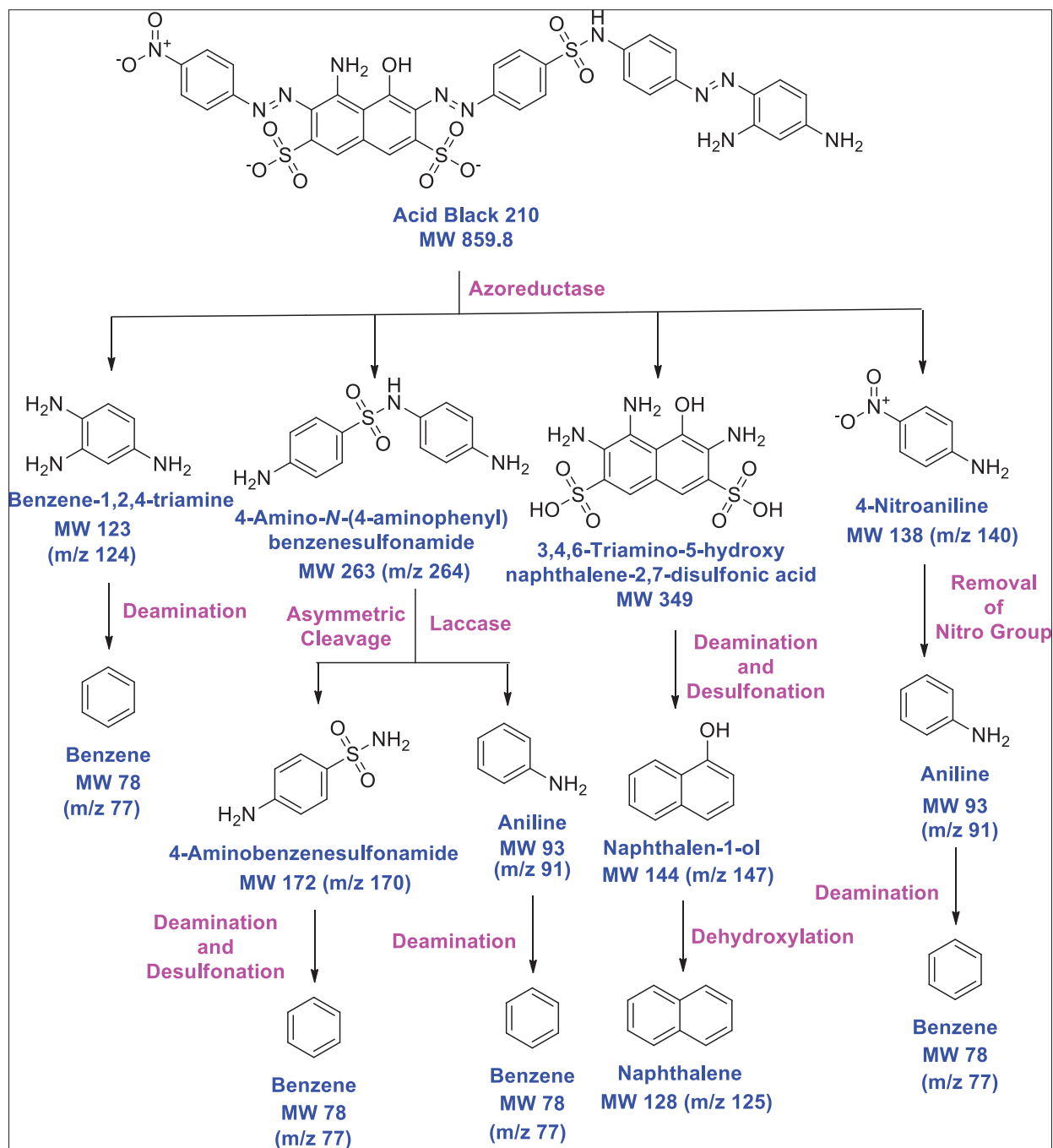


Figure 5: Azoreductase-mediated biodegradation of azo dye acid black 210 through *Providencia* sp. Source: Adapted with permission from Agrawal et al. [183].

of oxygen. Microbes play a key role in the carbon, nitrogen, and sulfur cycle and are greatly influenced by any change in these factors thus, affecting the decomposition process [Table 2]. Thus, it is very important to analyze the effect of these parameters on xenobiotics degradation. Knowledge of these factors which play a pivotal role in degradation activity will likely facilitate the development of bioreactors for the bioremediation of industrial waste.

6.1. pH

In general, effective decolorization or degradation of dyes using bacteria takes place at basic or neutral pH, while yeast and fungi at neutral or acidic pH [67]. At pH below 4, H^+ ions compete effectively

with cations of dye, causing a reduction in efficiency of color removal, while at pH higher above this point charge, the biomass surface gets negatively charged, which attracts the dye positively charged cations through electrostatic force of attraction [193]. The study on 27 different dyes by 21 various basidiomycetes reveals that optimum pH was found to be in the 3–5 range for dye decolorization [194]. A study demonstrated *Enterobacter* spp. decolorizes Reactive Black 5 at pH 7.0 [195]. The study revealed that decolorization and degradations of Scarlet R by *Proteus vulgaris* and *Micrococcus glutamicus* occurred in the range of pH 7–8 with optimum pH 7 [90]. In a study, bacterial mixtures consisting of total of six bacterial species, namely, *Bacillus* spp. (four strain), *Lysinibacillus* spp., and *Ochrobacterium*

Table 2: Factors affecting the microbes mediated remediation of dyes.

Microbes	Dye	pH	Temperature (°C)	Initial conc. of dye (mg/L)	Static/Agitation	References
<i>Acinetobacter baumannii</i>	Reactive red	7.0	37	500	Agitation	Unnikrishnan <i>et al.</i> [226]
<i>Acinetobacter</i> spp.	Reactive orange 16	7.0	40	500	Static	Meerbergen <i>et al.</i> [218]
<i>Aeromonas hydrophila</i>	Reactive Black 5	7.0	35	100	Static	El Bouraie and El Din [216]
<i>Aeromonas veronii</i>	Methyl orange	7.0	32	1000	Static	Mnif <i>et al.</i> [227]
<i>Alcaligenes aquatilis</i>	Synazol red 6HBN	7.0	37	10	Static	Ajaz <i>et al.</i> [219]
<i>Alcaligenes faecalis</i>	Novacron super black G	8.0	37	200	Static	Hossen <i>et al.</i> [203]
<i>Anoxybacillus</i> spp.	Direct black G	7.2	55	400	Static	Chen <i>et al.</i> [228]
<i>Aspergillus flavus</i>	Reactive red 198	4.0	3	50	Agitation	Esmaili and Kalantari [196]
<i>Aspergillus niger</i>	Crystal violet	5.5	30	-	Static	Ali <i>et al.</i> [200]
<i>Bacillus algicola</i>	Yellow azo dye	8.0	25	-	-	Chukowry <i>et al.</i> [217]
<i>Bacillus cereus</i>	Novacron super black G	8.0	37	200	Static	Hossen <i>et al.</i> [203]
<i>Bacillus fusiformis</i>	Acid orange 10	9.0	37	150	Static	Kolekar <i>et al.</i> [229]
<i>Bacillus</i> spp.	Reactive red 239	10.0	30	250	Agitation	Guadie <i>et al.</i> [201]
<i>Bacillus subtilis</i>	Reactive blue 160	7.0	35	500	Agitation	Barathi <i>et al.</i> [220]
<i>Bacillus vallismortis</i>	Aniline blue	6.0	70	-	-	Zhang <i>et al.</i> [212]
<i>Bacillus vietnamensis</i>	Malachite green	-	37	50	Static	Kabeer <i>et al.</i> [230]
<i>Bjerkandera adusta</i>	Lanaset grey G	6.0	40	150	Agitation	Daâssi <i>et al.</i> [231]
<i>Comamonas</i> spp.	Direct red 5B	6.5	40	1100	-	Jadhav <i>et al.</i> [232]
<i>Corioloropsis gallica</i>	Lanaset grey G	6.0	45	150	Agitation	Daâssi <i>et al.</i> [231]
<i>Enterobacter hormaechei</i>	Reactive yellow 145	7.0	37	100	Agitation	Thangaraj <i>et al.</i> [221]
<i>Enterobacter hormaechei</i>	Reactive yellow 145	7.0	37	100	Agitation	Thangaraj <i>et al.</i> [221]
<i>Enterobacter</i> spp.	Reactive black 5	7.0	37	200	Static	Wang <i>et al.</i> [195]
<i>Exiguobacterium</i> spp.	Navy blue HE2R	7.0	30	50	Static	Dhanve <i>et al.</i> [233]
<i>Geotrichum candidum</i>	Reactive blue 5	-	30	120	Agitation	Kim <i>et al.</i> [234]
<i>Halomonas glaciei</i>	Reactive red 2	8.1	30	1000	Static	Balamurugan <i>et al.</i> [235]
<i>Halomonas variabilis</i>	Reactive red 2	8.1	30	1000	Static	Balamurugan <i>et al.</i> [235]
<i>Issatchenkia orientalis</i>	Direct black 22	7.0	32	500	Agitation	Jafari <i>et al.</i> [236]
<i>Kocuria rosea</i>	Methyl orange	6.8	30	50	Static	Parshetti <i>et al.</i> [94]
<i>Lactobacillus paracase</i>	Acid black	6.0	30	100	Agitation	Huang <i>et al.</i> [199]
<i>Lysinibacillus fusiformis</i>	Methyl red	-	30	100	Agitation	Sari and Simarani [105]
<i>Lysinibacillus sphaericus</i>	Drimaren red CL-5B	8.0	37	100	Agitation	Srinivasan and Sadasivam [237]
<i>Lysinibacillus</i> spp.	C.I. Remazol red	7.0	30	250	Static	Saratale <i>et al.</i> [197]
<i>Micrococcus luteus</i>	Direct orange 16	8.0	37	100	Static	Singh <i>et al.</i> [215]
<i>Micrococcus yunnanensis</i>	Methyl orange	7.0	30	100	Agitation	Carolin <i>et al.</i> [205]
<i>Moraxella osloensis</i>	Mordant black 17	7.0	35	100	Static	Karunya <i>et al.</i> [214]
<i>Nesterenkonia lacusekhoensis</i>	Methyl red	11.5	30	50	Static	Bhattacharya <i>et al.</i> [238]
<i>Nesterenkonia lacusekhoensis</i>	Reactive violet 1	11.5	27	200	Agitation	Prabhakar <i>et al.</i> [239]
<i>Ochrobacterium</i> spp.	Reactive violet 5R	7.0	37	200	Static	Jain <i>et al.</i> [151]
<i>Ochrobactrum anthropic</i>	Reactive black 5	7.0	30	400	Static	Cheng <i>et al.</i> [240]
<i>Penicillium ochrochloron</i>	Cotton blue	6.5	25	50	Static	Shedbalkar <i>et al.</i> [209]
<i>Pichia kudriavzevii</i>	Acid red B	5.0	33	100	Agitation	Feng <i>et al.</i> [241]
<i>Pseudomonas aeruginosa</i>	Direct orange 39	7.0	60	50	Agitation	Jadhav <i>et al.</i> [225]
<i>Pseudomonas azoreducens</i>	Reactive green	7.0	30	500	Static	Meerbergen <i>et al.</i> [218]
<i>Pseudomonas putida</i>	Orange II	8.0	30	100	Static	Kumar <i>et al.</i> [242]
<i>Pseudomonas</i> spp.	Reactive red 195	8.0	40	100	Static	Khan <i>et al.</i> [198]
<i>Sphingomonas paucimobilis</i>	Methyl red	9.0	30	750	Agitation	Ayed <i>et al.</i> [211]
<i>Thiosphaera pantotropha</i>	Reactive yellow 145	7.0	-	100	Static	Garg <i>et al.</i> [243]
<i>Trametes trogii</i>	Lanaset gray G	5.0	45	150	Agitation	Daâssi <i>et al.</i> [231]
<i>Trametes versicolor</i>	Lanaset gray G	5.0	45	150	Agitation	Daâssi <i>et al.</i> [231]
<i>Trichoderma harzianum</i>	Cresol red	-	25	-	Agitation	Nor <i>et al.</i> [244]
<i>Trichosporon beigelii</i>	Navy blue	7.0	37	50	Static	Saratale <i>et al.</i> [22]
<i>Bacillus stratosphericus</i>	Reactive orange 16	7.0	35	150	Static	Akansha <i>et al.</i> [108]

spp. were reported for decolorization and removal of azo dye-Reactive Violet 5R at neutral pH i.e., 7.0 [151]. In another investigation, the bacterium *A. flavus* sorted out from the effluent disposal area soil was reported for degrading Reactive Red 198 effectively at low pH [196].

Another report has reported bacterium *Lysinibacillus* spp. isolated from the textile industrial area which was degrading and decolorizing the toxic sulfonated azo dye C.I. Remazol Red at pH 7.0 [197]. In a study by Khan et al. [198], Reactive Red 195 dye was degraded by the bacterial consortium consisting of, *Bacillus* spp., *Pseudomonas* spp., and *Ochrobactrum* spp. at pH 8.0. In an investigation, the bacterium *Lactobacillus paracase* isolated from the deep sea sediments were reported for removal of Acid Black azo dye between the pH range from 5.0 to 7.0 [199]. In another study, the most effective pH for the Crystal Violet decolorization by the fungi *A. niger* was reported 5.5 [200]. *Bacillus* spp., isolated from an alkaline lake, was reported for degradation of Reactive Red 239 dye. The most efficient pH for dye degradation by the bacterium was 10 [201]. In another investigation, *Enterobacter* spp. was degrading Crystal Violet at pH 6.5 [202]. In a report, bacteria obtained from textile industry released effluent were identified as *Alcaligenes faecalis*, *Bacillus* spp., and *B. cereus* which were reported for biodegradation of Novacron Super Black G dye at pH 8.0 under static conditions [203]. Javadzadeh and Asoodeh [204] isolated *Bacillus* spp. from the gut of termite which was having indigo dye biodegrading properties. The bacterium was reported for biodegrading the dye at pH 8.0. In an investigation, the microbial species *Micrococcus yunnanensis* was reported for degrading Methyl Orange at pH 7 [205].

6.2. Temperature

Temperature is a vital factor and plays an imperative role in the environment during the process of biodegradation. The metabolic activity of microorganisms is greatly affected by temperature. It is commonly experiential that the decomposition process is faster in summer as compared to winter as a warmer climate favors the growth and multiplication of various microorganisms [206]. However, the process is not the same after a certain temperature. Beyond this optimum temperature, there is a reduction in growth, metabolic activity, and deactivation of enzymes which ultimately lead to a decline in the decolorization process [193]. Thus, various studies conclude that biodegradation of dye by the microorganism is possible at ambient temperature which is responsible for their metabolic activities and reproduction [207]. Various microorganisms require different ambient temperatures for growth, with most growing at a temperature range of 25–35°C [208]. In a study by Shedbalkar et al. [209], the ambient temperature required to decolorize Cotton Blue by *Penicillium ochrochloron* was found to be 25°C. *Enterobacter* spp. could decolorize Reactive Black 5 at an optimum temperature of 37°C and on further increasing the temperature to 42°C there was a drastic decrease in decolorization activity [195]. In a study, *Pseudomonas* spp. was reported for degrading Congo red dye along with textile industry released effluent at 40°C temperature [210]. An isolate *Sphingomonas paucimobilis* was reported for bioremoval of Methyl Red dye between a broad range of temperatures i.e. 4–40°C [211]. In another report, *B. vallismortis* isolated from the disposal site of the textile industry was reported for degrading triphenylmethane dyes, including Aniline blue, Malachite Green, and Brilliant Green at high temperature range of 70°C [212].

A study has reported, Reactive Black-5 dye was reported to be degraded by the halotolerant bacterial strain *Pseudomonas* spp. at a temperature 25°C [213]. Similarly, the bacterium *M. osloensis* was

reportedly degrading Mordant Black 17 at 35°C temperature [214]. In a report, Direct Orange 16 was degraded at 37°C temperature by bacterial isolate *Micrococcus luteus* [215]. Reactive Black 5 dye was reported to be biodegraded by the *Aeromonas hydrophila* at 35°C temperature [216]. In a study, the bacterial strain *Bacillus algicola* was reported for decolorizing red, yellow, and blue dye at temperatures 25°C, 35°C, and 45°C. The highest degradation was observed at 25°C temperature [217]. In a similar report, two bacterial strains, namely, *Acinetobacter* and *Klebsiella* were decolorizing the azo dyes, that is, diazo dye Reactive Green 19 and monoazo dye Reactive Orange 16 at a temperature range of 20–40°C [218]. Ajaz et al. [219] reported *Alcaligenes aquatilis* for decolorization of Synazol Red 6HBN dye at 37°C temperature in 4 days [Figure 6]. In a report, *B. subtilis* was reported for decolorizing Reactive Blue 160 dye at 35°C temperatures [220]. *Enterobacter hormaechei* isolated from textile effluent was reported to degrade Reactive Yellow 145 and Reactive Red 180 at a temperature 37°C [221].

6.3. Initial Dye Concentration

The impact on microbial insisted decolorization of the dye initial concentration was investigated. Studies show that there is a lowering in the efficiency of dye decolorization by microbes with an initial concentration increase of dye. This is because the dye toxicity increases at a higher concentration which inversely affects the growth of the microbial cells [193]. A similar report was demonstrated by Parshetti et al. [222] which indicated that higher concentration Malachite Green (100 mg/L) was toxic to *K. rosea*. Jirasripongpun et al. [223] found that Reactive Red 195 at concentrations 50 and 100 mg/L had a lethal effect on *Enterobacter* spp. and was not able to grow. A report on Congo red decolorization by *Bacillus* spp. reported that the decolorization rate declined with the dye concentration increase [224]. In a report, *P. aeruginosa* obtained from the dyestuff contaminated sediments was reported for 93.06% decolorization of Direct Orange 39 with 50 mg/L concentration within 45 ± 5 min and the maximum concentration degraded by the strain was 1.5 g L⁻¹ with 60% decolorization [225]. The consortium of *Providencia* spp. and *Pseudomonas aeruginosa* was reported for 100% bioremediation of dye Red HE3B at 50 mg/L initial concentration and as the dye concentration rises to 200 mg/L and 250 mg/L the decolorization decreases to 85% and 70%, respectively [147].

A study reported *Pseudomonas putida* was decolorizing dye Orange II at maximum up to 1000 mg/L initial concentration and maximum decolorization was achieved at 100 mg/L concentration [242]. Shah et al. [53] reported that as the concentration of methyl orange was increased (3, 4, and 5 g/L), the incubation time required for decolorization was varied from 66 to 90 h. In another report, *Pichia kudriavzevii* was reported for degrading Acid Red B dye with maximum initial concentration 400 mg/L within 40 h and maximum decolorization was achieved with 100 mg/L initial concentration within 40 h [241]. Saroj et al. [245] concluded, the fungal consortium containing *Penicillium oxalicum*, *A. niger*, and *A. flavus* strains was able to degrade three different azo dyes, namely, Direct Blue 15, Direct Red 75, and Acid Red 183, with the initial concentration range 200–400 mg/L. All these three dyes were degraded at lower initial concentrations by the fungal consortium. Bacterium *Aeromonas veronii* sorted out from acclimated textile effluent was reported to decolorize the azo dye up to 1000 mg/L initial concentration [227].

Reactive Blue 4, the anthraquinone dye, was degraded by the bacterial granules up to 1000 mg/L initial concentration [246]. In a report,

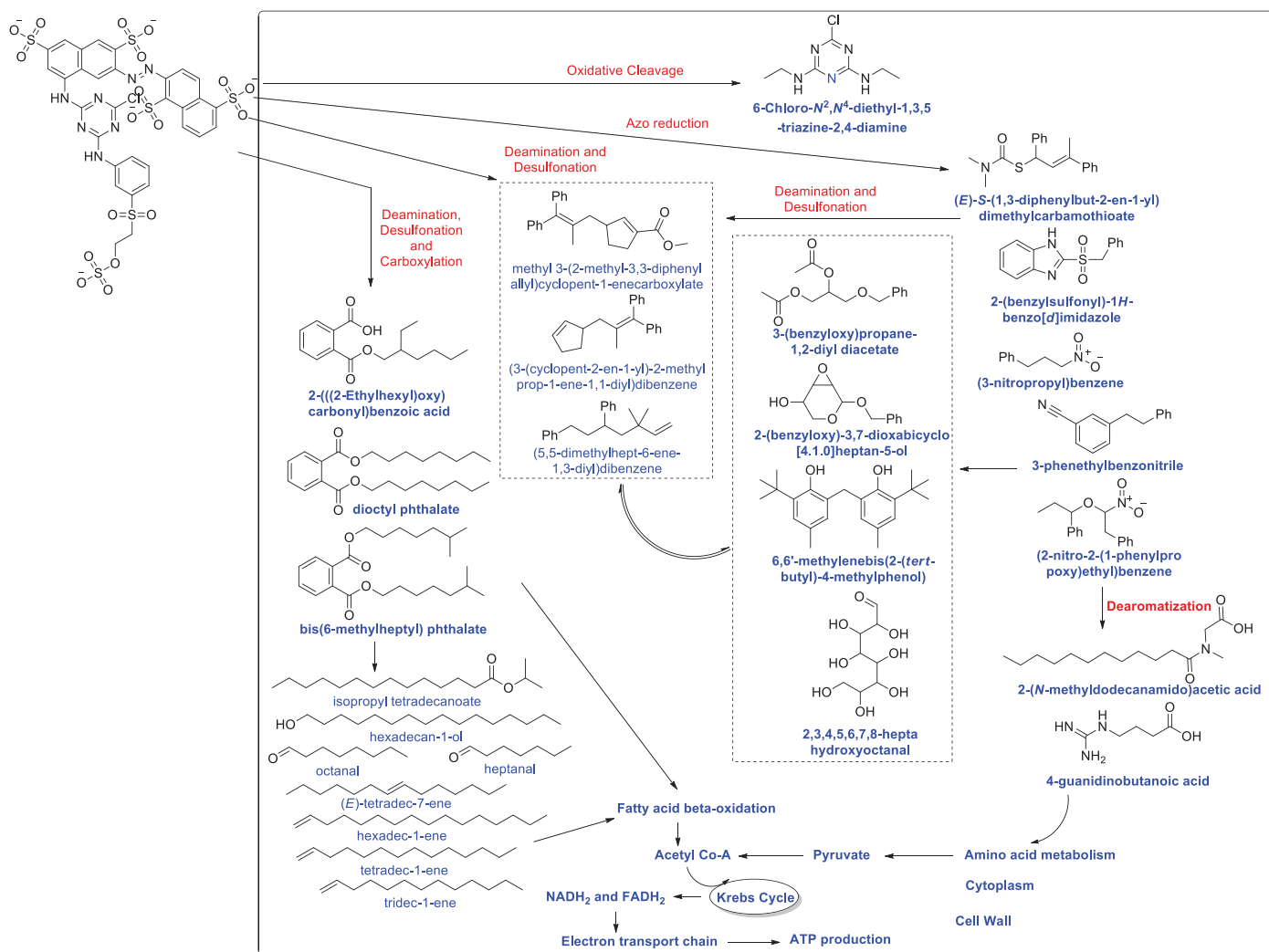


Figure 6: Biodegradation of Synazol Red HF 6BN dye *Alcaligenes aquatilis*. Synazol Red HF 6BN enters the cell of *A. aquatilis* through unknown process. After the entrance dye is processed enzymatically into several end products. First, the azo group of the dye is reduced which is followed by cleavage reaction to form various end products. Second, the desulfonation and oxidative deamination results in synthesis of pyrrolo[1,2-a] pyrazine-1,4-dione derivative which can be used as substrates in amino acid metabolism. The amino acid catabolism can synthesize pyruvate (3C compound) which can be converted into acetyl-CoA. The acetyl-CoA undergoes Krebs cycle to produce NADH_2 and FADH_2 (substrates of electron transport chain). Moreover, dye desulfonation, oxidative deamination, and carboxylation lead to produce phthalate derivatives, which can be transformed into different fatty acids and aldehydes. The phthalate, fatty acids, and aldehydes can directly/indirectly enter into fatty acid oxidation reactions (β -oxidation) to produce acetyl-CoA, NADH_2 and FADH_2 . Source: Adapted with permission from Ajaz et al. [219].

thermophilic microflora was reported for detoxification of azo-dye Direct Black G with an initial concentration 600 mgL^{-1} [247]. In another report, Reactive Black-5 dye decolorization through bacterial (*Gordonia*, *Pseudoarthrobacter*, *Sphingomonas*, and *Stenotrophomonas*) consortium was tested with three different initial concentrations, that is, 25, 50, and 100 mg/L . The best dye decolorization by the consortium of bacterial strains was found at 50 mg/L initial concentration [156]. Amin et al. [248] reported *Bacillus* spp. to detoxify the diazo dye at an initial concentration of 100 mg/L . In a study, *Ochrobactrum anthropic* from textile wastewater was reported for biodegradation of Reactive Black 5 at the initial concentration of 400 mg/L [240].

6.4. Sodium Chloride Concentration

Effluents from the textile industry contain various salts or metal ions, acids, and alkalis as impurity in addition to dyes [67]. The salt

concentration varies from 15 to 20% and has been calculated in dye industry wastewater. Thus, a microbial strain that can tolerate high concentrations of salt facilitates the degradation of dye wastewater. The biological treatment of the dye effluent containing various components, and identification of salt tolerant microorganisms is important. Rudakiya, Pawar [249] have shown the decolorization efficacy of a bacterial strain using salt concentrations up to 10%. In their study, the result come out to be that 6% salinity was effective in promoting both cell biomass and degradation of Reactive Orange 16. A similar study was done on *Shewanella marisflavi* and *S. algae algae* which were able to degrade single or mixed azo dyes at lower concentrations of NaCl (2–3%). It has been concluded that a lower level of salinity induces the activities of azoreductase, laccase, and NADH-DCIP reductase enzymes [250]. Cui et al. [98] reported that *Klebsiella* spp. was able to decolorize Methyl red, Orange I, Congo red, and

Methyl orange efficiently over the salinity range (1–4%). In a study, *M. luteus* bacterium was reported for the detoxification of the Direct Orange 16 dye with 3% NaCl concentration in 6 h of incubation [215]. In another report, halotolerant *S. marisflavi* reported for decolorization of Xylidine Ponceau 2R under the 20% concentration of NaCl [251]. Song et al. [252] reported a yeast *Pichia occidentalis* for the Acid Red B dye biodegradation in 16 h with 30 g L⁻¹ of NaCl. In another report, *Bacillus* spp. was biodegrading 96% of sulfonated dye, that is, methyl orange with 5–20 g/L of sodium chloride concentration [253]. Salt tolerant bacterium *Halomonas* was reported for the biodegradation of Toluidine Red dye where the concentration of NaCl was 5% [254]. Zhuang et al. [255] reported Methyl Orange and Reactive Yellow 84 decolorization by the *Shewanella indica* and *Oceanimonas smirnovii*. These strains were decolorizing the dye in the presence of NaCl (0–70 g L⁻¹). Similarly, *A. baumannii* was reported for the 87% and 90% degradation of Reactive Black 5 and Reactive Blue 221, respectively, with 5% NaCl concentration [256].

6.5. Agitation/Static Condition

There are conflicting reports on azo dye decolorization through microbes under shaking/static conditions. According to studies, the decolorization rate increases by shaking culture conditions while other reports suggest static conditions. A higher rate of decolorization was observed during shaking due to the easy transport of oxygen and nutrients over static conditions [257,258]. On the other hand, decolorization by *Pseudomonas* spp. under agitation showed no decolorization while static culture conditions showed 96% Reactive Red 2 decolorization [88]. Similarly, Direct Red 81 and Reactive Red 120 decolorization by *A. niger* were more efficient under static conditions [259]. The rate of enzymatic activity was higher in the still condition [67]. Complete Navy Blue dye decolorization by *Trichosporon beigelii* was observed under static conditions whereas it declined to 30% under shaking conditions [22]. Another study concluded *K. rosea* for 100% Methyl Orange decolorization under the static conditions [94]. In a report, halophilic and halotolerant *Halomonas variabilis* and *Halomonas glaciei* were reported for degrading reactive textile dye in batch mode static condition [235]. Shah et al. [260] reported *A. faecalis* for degradation of Reactive Orange 13 under static anoxic condition. In a study, white rot fungi, that is, *B. adusta*, *Coriolopsis gallica*, *Trametes trogii*, and *T. versicolor* were reported for decolorizing Lanaset Gray G dye under the static conditions [231]. In agitation condition, *Issatchenkia orientalis* was reported for the five azo dyes decolorization, that is, Reactive Red 198, Reactive Orange 16, Direct Yellow 12, Direct Black 22, and Direct Blue 71 [236]. Nor et al. [244] reported *Trichoderma harzianum* for degrading Cresol Red dye under agitation conditions. In a study, *Chaetomium globosum* was tested for dye detoxification under stirred and static conditions and the report concluded that fungi were efficiently degraded the dye under agitation conditions in comparison with static conditions [261]. Bhattacharya et al. [238] reported under static conditions, halotolerant and alkalophilic *N. lacusekhoensis* was efficiently detoxifying Methyl Red. Similarly, *A. baumannii* from the sea sediments was reported for Reactive Red dye degradation at both agitation and static conditions [226]. In a comparative study, *L. fusiformis* was experimented for azoreductase degradation under both static and agitation conditions and results showed that under agitation conditions, dye degradation was better [105]. In a study, under static conditions, *P. aeruginosa* and *Thiosphaera pantotropha* were reported for decolorization and Reactive Yellow 145 dye detoxification [243].

6.6. Aerobic/Anaerobic Culture Conditions

Azo dyes are usually resistant to the attack of bacterial species under aerobic conditions [4]. Anaerobic degradation of dye has been more effective than aerobic, but the intermediates formed are carcinogenic and toxic in nature and must be degraded before being discharged to the main water stream [262]. Apart from few, all aromatic amines produced after azo dyes decolorization has recalcitrant properties under oxygen limiting condition [152]. However, the process when combined shows much more efficient output as reported by many authors [262]. They reported that the dye degradation by bacteria under anaerobic conditions is usually accompanied by the colorless aromatic amine production which is easily metabolized under aerobic conditions. The breakdown products are non-toxic in nature [139]. Bacterium *P. aeruginosa* was able to degrade, Navitan Fast Blue SSR, under an aerobic condition in the presence of glucose. The organism was also able to discolorate various other textile dyes [263]. Aerobic bacteria consisting of *B. cereus*, *B. megaterium*, *B. pumilus*, *B. subtilis*, and *B. vallismortis* were found to be efficient in decolorizing microbial strains individually as well as the mixture of dyes. Almost 80–90% decolorization was noticed in four out of six azo dyes (Congo red, Bordeaux, Blue BCC, and Ranocid fast blue) when present in mixture [134]. In a study, thermophilic microbial strains *Anoxybacillus pushchinoensis*, *A. kamchatkensis*, and *A. flavithermus* blend were reported for detoxifying 80% of Reactive Black 5 in aerobic conditions [163]. A comparative study has reported, microbial strain *A. hydrophila* was efficiently degrading the synthetic dye basic fuchsin, Crystal violet, Solophenyl red 3BL, Polar brilliant red B, Safranin, and Nigrosine under microaerophilic conditions in comparison to aerobic conditions [264]. Aerobic detoxification of Acid Scarlet GR was reported by a halotolerant yeast strain *G. geotrichum* [118]. A study has reported efficient degradation of dye Acid Red 14 by microbial strain *Oerskovia paurometabola* under anaerobic conditions [265]. In another report, *Pseudomonas* spp. and *Clostridium* spp. were reported to degrade the dye under aerobic conditions [266].

7. PHYTOTOXICITY AND MICROBIAL TOXICITY OF DYES AND THEIR BIODEGRADATION PRODUCTS

It is essential to determine whether dye degradation leads to dye detoxification. Microbial toxicity and phytotoxicity studies on dye and its biodegraded metabolites can confirm this. In phytotoxicity studies, experimental plant seeds are treated with the dye and its degraded metabolites. Effect on germination percentage, length of root, and shoot is measured, to compare with control (without dye and its biodegraded metabolites). Results are enumerated to determine whether the degraded metabolites are less toxic to growing seedlings than the dyes being studied. Likewise, the antimicrobial activity (toxicity) of the dye being studied can be compared with its degraded metabolites. Reactive Red 2 and its degraded metabolites were studied using *Phaseolus mungo* and *Sorghum vulgare* as model plants by Kalyani et al. [88]. The result observed concluded that the product formed after degradation was less toxic than Reactive Red 2. A similar result was observed when Malachite Green phytotoxicity and its degraded metabolites using *P. mungo* and *Triticum aestivum* as a model plant was performed [222]. Phytotoxicity study of Navy blue 2GL and its biodegraded product by *Bacillus* spp. on *Sorghum bicolor* and *Triticum aestivum* have shown similar results when compared with dye as control. There was 2–5% reduction in growth (root and shoot length) in the presence of degraded products than in distilled water [267]. The microbial toxicity study on *Azobacter vinelandii* showed a growth inhibitory zone of 1.2 cm against dye while no zone of inhibition was found around metabolites formed. The toxicity

of crystal violet dye against *E. coli* was checked. The study reveals the toxic nature of crystal violet, but the product formed after degradation with *Shewanella decolorationis* NTOU1 was non-toxic to *E. coli* [268].

8. FUTURE PERSPECTIVES

The rampant industrialization has proliferated the huge amount of effluent containing mixture of dyes, metals and other hazardous materials. The degradation of dyes and other pollutants has hazardous impact on the environment and its removal is one the urgent need. The recent advancement have suggested microbial-based remediation is an efficient method of the removal of dyes, but unfortunately dye removal through the use of microbes still depends on the environmental changes. In this regard, many reported have suggested the proper oxygen transfer, operational stability, homogenization, less operational time, and suitability in hybrid bioreactors. The studies have provided the rationale for the application of microbial consortium of different microbes including bacteria, fungi, and algae. The microbial consortium has found to be more stress resistant with stability and enhanced adaptability over the single inoculation. The genome engineering of microbes may leads to the development of exceptionally adaptive techniques of bioremediation for high degradation of dyes. Hence, it is preferable to develop an alternative technology that could upgrade the microbial-based removal of dyes.

9. CONCLUSION

In times, bioremediation through microbes is gaining much important to because of its effectiveness, cost, and eco-friendly nature. Microbes have been also known to remediate the textile released dyes. Dyes and organic colored compounds are known to be hazardous pollutants which are categorized under xenobiotics. These are known to have several deleterious effects on aquatic and human life. Various types of microbes are known to remediate the dyes including fungi, bacteria, algae, and yeast. In the future, new microbial strains could be researched and could be used as bioremediation.

10. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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This study does not involve experiments on animals or human subjects.

14. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

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