Chapter

# Role of Various Physicochemical Factors in Enhancing Microbial Potential for Bioremediation of Synthetic Dyes

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## Abstract

The Indian dye industry is globally recognized for production and export of every known class of dye. On the less attractive side of industrialization, they contribute considerably to environmental pollution. The dyes discarded by industries persist in the environment due to extremely slow rate of biodegradation. Moreover, these dyes are toxic to insects, birds and terrestrial life. The dyes also hamper the light penetration in water bodies, severely affecting the the process of photosynthesis. In spite of the problems associated with synthetic dye disposal, they are industrially preferred due to their fundamental requirement in enhancing overall appearance of goods, quality and cost effectiveness. Several studies have reported physicochemical techniques for remediation of dye effluents. Most of these techniques pose significant drawbacks due to their high energy and cost requirements. The bioremediation approach, on the other hand, offers advantages of sustainable environmental friendly processes to detoxify and degrade dyes into harmless products. This chapter provides an overview of the potential role of various physicochemical factors such as pH, temperature, oxygen and nutrient concentration in optimum decolorization of dyes by naturally isolated microbial strains. In addition, the role of cosubstrates, electron acceptors and microbial enzymes are also discussed.

**Keywords:** sustainable, bioremediation, physicochemical techniques, dyes, optimum

## 1. Introduction

The synthetic textile industry is estimated to be a trillion dollar industry that utilizes dyes on a large scale. Besides, dyes are used extensively in most of the small and large scale paint, automobile, and cosmetic industries [1]. According to the most recent buiseness report, several thousand tons of dyes are manufactured every year acquiring 15.59 billion dollar global market in 2022 with a compound annual growth

rate of 11.5% [2]. Statistically, among the manufactured dyes, 70% are azo-dyes and 15% are anthraquinone dyes. The remaining 15% constitute of all other dyes such as triphenylmethane and phthalocyanine dyes [3, 4]. It is estimated that 20% of the dyes are lost during processing and application and become a part of industrial effluents [1]. The synthetic dyes are derived from petroleum products. They contain unsaturated chromophore groups that contribute to the color and stability of the molecule. Many of the synthetic dyes are reported to have carcinogenic properties. Disposal of untreated dye effluents significantly harms the soil as well as aquatic flora [5]. For this reason, remediation processes have received tremendous attention from researchers of related fields.

The physical techniques of dye remediation include reverse osmosis, photodegradation, coagulation, flocculation, and ion exchange method for ultra-filtration. However, these processes generate huge amount of sludge, which contains a mixture of partially remediated and precipitated compounds. They are collectively known as secondary waste products [6]. Most of these secondary waste compounds produced on photodegradation exhibit equal or more toxicity as compared to the parent dye molecule [7, 8]. As opposed to physical methods, the biological techniques for the degradation of dyes have three major advantages [9, 10].

1. Bioprocess technologies do not contribute to secondary contamination. There is relatively less sludge production and degraded dye metabolites are environmentally benevolent.

2. Biological methods can be implemented in situ at the polluted site.

3. They are cost effective.

The environment is replete with a wide range of microorganisms possessing unique abilities to degrade natural and man-made complex compounds. The screening and isolation of microorganisms degrading synthetic dyes from a variety of natural samples or microorganism enriched samples due to anthropogenic activities have been carried out by many researchers. Bacteria are the most frequently preferred microorganisms for treating industrial effluents because they are easily adapted to new environment and have short generation time. Some being facultative can grow aerobically or anaerobically. Some bacteria have naturally adapted to live in severe environmental settings with respect to salinity, pH, and temperature and have the ability to produce a variety of oxidoreductases that effectively remediates several classes and mixture of dyes [9].

The general observation by many workers is that most azo dyes resist biodegradation in traditional aerobic sewage treatment plants due to inhibition of azoreductase [11–16]. From the existing available knowledge, bacterial anaerobic azo bond cleavage is well suited in the elimination of azo dyes from effluent processing systems. In static cultures, the exhaustion of oxygen can be easily achieved for the purpose of breaking the azo linkage by facultative and anaerobic bacteria. These biochemical reactions occur at pH 7.0 and involve low-molecular-weight redox mediators, which are extremely unspecific. The existence of supplementary carbon nutrients elevates the reduction rates. The reducing counterparts are created during metabolism of these nutrients, which can ultimately be utilized for reducing the azo dye biochemically [14, 15]. The production of naphthylamine, benzidine derivatives, and other amines, however, is a major limitation in anaerobic biodegradation of azo dyes due to

their presumed toxicity and serious health threat to humans [11, 13]. As a practical solution to this problem, comparatively few reports of azoreductase production by aerobic bacteria are reported in literature [17, 18]. For instance, Oturkar *et al.* [19] investigated the decolorization of RR120 using *Bacillus lentus* and reported 98% and 70% decolorization under anoxic and aerobic conditions respectively. However, the presence of N2 gas with complete O2 free atmosphere led to loss of decolorization ability of *B. lentus*. Hence, screening of potential bacterial strains and optimization of their dye degradation ability can be extremely helpful in overcoming the challenges of dye remediation.

## 2. Selection of promising isolates

The original load of microorganisms capable of degrading dye molecules is low in natural samples. Hence, many research groups carry out enrichment of samples during screening programs using growth media such as mineral salts medium or Bushnell and Hass medium (BHM), either amended or not amended, with organic carbon and/or nitrogen sources [20–28], medium containing peptone-yeast extract or peptone-glucose [27, 29], nutrient broth [30–32], and synthetic wastewater [33]. A different formulation of medium consisting of 1% peptone in distilled water/seawater in 1:1 combination was used by Srinivasan *et al*. [34] for the enrichment of mangrove sediments.

Also, considering the diversity of microbial species in natural ecosystem, the possibility of isolation of potential dye degraders is increased on careful selection of samples. Few examples of samples used by scientists for isolation of dye degraders are mangrove swamps [35], water and sludge from the drain [20], soil collected from distillery spent wash contaminated sites [36], textile dye effluent from CETP (Common Effluent Treatment Plant), Perundurai, Chennai, Tamilnadu [37], soil near the tannery, Central Leather Research Institute, Tamil Nadu, Chennai, India [38], the effluent from dye manufacturing unit [31], lake mud [39], soil sample from tannery effluent [32], soil, textile effluent and sewage [21], and beach water [40].

For selection and purification of the promising isolates for dye decolorization/degradation process, enriched microflora is isolated on the solid media of same or different composition with or without synthetic dye. Sugiura *et al*. [41] made use of synthetic medium comprising azo dyes as the sole carbon supply for enrichment of soil samples and isolated dye decolorizing bacteria from enriched media on nutrient agar containing 0.02% azo dye. BHM supplemented with dye (Direct Red 81, 100 ppm) being a single carbon source or accompanied by yeast extract and glucose was used for the enrichment and isolation of samples obtained from effluent-contaminated areas near-by dyestuff units [24]. In contrast, Alhassani *et al*. [42] used nutrient broth and nutrient agar without dye for the enrichment and isolation during screening.

During the screening process, microorganisms are assessed based on their ability to decolorize the dye metabolites. Confirmation of dye degradation is done by evaluating the nature of degraded dye metabolites using analytical techniques such as High-Performance Liquid Chromatography (HPLC) or Gas Chromatography Mass Spectrophotometry (GC-MS). The two different approaches for selection of efficient dye decolorizer degrader are reported in literature. First approach uses nutrient medium without dye for isolation, and morphologically distinct colonies are selected for further dye decolorization assay in liquid culture medium having defined dye concentration. The quantitative measurement of the decolorization given by the

organisms in terms of either percentage decolorization or the rate of decolorization is considered for the selection of strains [30]. The preliminary quantitative measurement of dye decolorization/degradation is estimated using a UV-Visible spectrophotometer. The decolorized broth is freed of cells and used for spectrum scan from 200 to 800 nm using sterile nutrient medium as blank. Abiotic control is also subjected to spectrum scan using same range of wavelengths. The absorbance values of abiotic control at the $\lambda_{max}$ of the dye in the visible range and that of the decolorized broth at the same $\lambda_{max}$ are used to calculate the percent decolorization [43]. Based on the above method, Guadie *et al*. [22] isolated and purified 135 morphologically different colonies using spread plate method and tested decolorization/degradation in liquid mineral salts medium containing RR239. Similarly, Kannan *et al*. [24] obtained seven isolates on nutrient agar plates, which were further grown in mineral salts medium containing Remazol Black B dye (1000 mg/L) for assessing the decolorization/degradation ability.

The second approach used for selection of decolorizers uses isolation of the enriched samples on dye containing nutrient agar, to observe clear zones around the colonies. The organisms showing the largest decolorization zones are selected for further screening of their decolorization potential. Using this approach, Chen *et al*. [39] observed white and pink colonies with decolorized zones on screening medium containing RED RBN dye, and these were further tested for decolorization capability in submerged cultures.

In contrast to the above two approaches, Nawahwi *et al*. [44] described a rapid isolation technique without enrichment to isolate the dye decolorizing bacteria on sterilized textile wastewater agar medium (wastewater sterilized using membrane filter or autoclaved) using spread plate technique. The bacteria that formed colonies with clear zones on this medium were selected for further screening. This method was also applied for the decolorization by *Basidiomycetes* strains on agar plates containing 0.2 g/L of Orange G or Remazol Brilliant Blue R; however, only 15 strains decolorized both the dyes tested with demarcated clear regions surrounding the colonies [45].

## 3. Inoculum preparation

The commonly used biomass parameters, i.e., Optical Density (OD) and volume of cells (saline suspension or medium grown) are used to standardize the optimum cell number for a successful decolorization/degradation process. Various researchers describe a number of ways for the preparation of inoculum. Few studies have employed overnight grown pure cultures for studying dye decolorization as described by Mahmood *et al*. [20] wherein 0.6 $OD_{597nm}$ was adjusted for five bacterial isolates to optimally decolorize Remazol Black B. For the biodecolorization of reactive orange using *Bacillus* sp. ADR, 1.0 $OD_{620nm}$ equivalent to 1.818 g dry cell weight/L was used by Telke *et al*. [46]. A 10% v/v of freshly grown *Brevibacillus* sp. with 0.3 $OD_{600nm}$ was used in degradation of Toluidine Blue [41].

Khalid *et al*. [25] prepared inocula of two *Shewanella* sp. with 1.0 $OD_{550nm}$ and used 2%v/v for azo dye decolorization. In another study, Junnarkar *et al*. [23] used inoculum size 5–30% v/v with 5% interval of novel bacterial consortium for Direct Red 81 degradation. Similar inoculum preparation was reported by Du *et al*. [47] for *Aeromonas* sp. strain DH-6 during metabolism of azo dye Methyl Orange. Likewise, the ratio of 50:1 of medium to inoculums ($OD_{600nm}$ 1.0) was used for studying degradation of textile dyes by *Stenotrophomonas maltophila* RSV-2 [48]. Similarly,

the inoculum size of 4–20% at intervals of 4%v/v was used for the bacterial consortium RVM11.1 in the decolorization of RV 5 [26]. Kurade *et al*. [49] have reviewed biodegradation of Disperse Red 54 with pregrown cell mass (24 h) of *Brevibacillus laterosporus*. Inoculum size of 1–5% v/v was tested using 3-day-old broth grown *Streptomyces* DJP15 for the degradation of Azo Blue dye [50]. Similarly, *Shewanella decolorationis* S12 was grown in 50 mL LB on shaker (150 rpm) at 30°C. The cell mass was collected by centrifugation and uniformly suspended in phosphate buffer, pH 8.0, which was used as 10%v/v inoculum size of suspension for all the biodegradation experiments [51].

## 4. Physicochemical parameters influencing the decolorization/degradation of dyes by bacterial isolates

### 4.1 Nutrient media

The dye degradation studies have used complex, synthetic, or semisynthetic nutrient media for studying biodegradation of dyes. The qualitative and quantitative nutrient composition of the medium is important for microbial degradation. This is due to the fact that certain nutrients of the medium are better donors of electrons than others to reduce the azo linkages in the substrate dye under static condition, or the nutrients may provide certain vitamins essential for catalytic activity of enzymes important during dye degradation. Khan [40] studied degradation of dye Red 2G using *Bacillus megaterium* sourced from seawater wherein a modified mineral salt basal medium deficient of nitrogen source was used to check the proficiency of the decolorizing strain to use the dye as a nitrogen source. However, most microorganisms require an organic cosubstrate for the dye degradation, thereby essentially necessitating the use of complex media for the degradation of the dye. For instance, Junnarkar *et al*. [23] used bacterial consortium NBNJ6, which exhibited best decolorization of Direct Red 81 dye when casein and starch were present in the medium. The bacterial cultures are incapable of decolorizing the dyes without cosubstrate/electron donor, which suggests that the availability of additional carbon resource is vital for the growth of the bacteria as well as for the dye decolorization [27]. However, few microorganisms use dyes as the only source of nitrogen and carbon. One such evidence is reported for *Bacillus* sp. isolated in the degradation process of Congo Red by Gopinath *et al*. [32].

Jadhav *et al*. [52] have stated five media to understand the influence of different nutrients on methyl red degradation by *Saccharomyces cerevisia*e MTCC 463. These media were plain distilled water (100 mL), 5% mineral salts medium supplemented with 1% glucose, yeast and peptone extract at 0.1% each in distilled water, 0.1% peptone in distilled water, and 0.1% yeast extract in distilled water. The significance of certain nutrients for dye degradation can be interpreted with such experiments. The decolorization/degradation of sulfonated azo dye, Reactive Orange 16, in nutrient broth by *Bacillus* species ADR was studied by Telke *et al*. [46]. In the same year, Ben Mansour *et al*. [53] used mineral growth medium having a wide range of inorganic salts as macro and micronutrients and presence of glucose (1%) to study degradation of Acid Violet 7 by *Pseudomonas putida* mt-2. *E. coli* and *Pseudomonas* sp. were used for decolorization/degradation of Direct Black 38 and Congo Red (Direct Red 28) in Vanderbilt mineral medium containing glucose as cosubstrate. Similarly, the biodegradation of textile azo dyes such as Direct Blue 71, RB 5, RY107, and RR 198 by *Staphylococcus arelettae* strain VN-11 was studied in rich

mineral salts medium containing a variety of salts, 0.3% glucose, 100 ppm of each dye with 0.1% yeast extract [54]. A model dye Direct Black 22 and few other azo dyes were studied for degradation by novel bacterial consortium DMC using Bushnell Hass medium supplemented with 0.1% glucose, 0.06% yeast extract (BGY medium) [14]. Usha *et al*. [21] studied degradation of RR120 and RB5 by *Aeromonas punctata* and *P. aeruginosa* using mineral salts medium broth and agar supplemented with 50 ppm glucose. Pandey and Dubey [37] used LB medium to degrade dye RR-BL using *Alcaligenes* sp. AA09. Also, the decolorization studies of Azo Blue dye were carried out in starch-casein broth with *Streptomyces* DJP15 by Pillai [50], while Modi *et al*. [31] carried out the decolorization assays for RR195 with pure culture and consortium using nutrient broth with and without glucose and in mineral salt medium.

### 4.2 Role of aeration

The dye degradation under static or shaker condition is closely based on the dye chemistry. The chemical structure and functional groups of the dye alter the efficiency of bacterial dye decomposition. Moreover, the decolorizing ability of the microorganism is closely related to its function as a facultative anaerobe. To understand the effect of aeration on dye degradation, facultative cultures such as *E. coli* and *Pseudomonas* species were grown aerobically, anaerobically, and microaerophilically using Direct Black 38 and Congo Red. Sodium thioglycollate was used to create anaerobiosis. Nachiyar and Rajakumar [38] subjected degradation assay of Navitan Fast Blue S5R by *P. aeruginosa* under shaker and static culture conditions. Pandey and Dubey [37] also carried out biodegradation study of RR-BL by *Alcaligenes* sp. AA09 under static condition and on an orbital shaker (150 rpm). Kalme *et al*. [55] too used static and shaking condition (120 rpm) with *Pseudomonas desmolyticum* NCIM 2112 to study biodegradation of Direct Blue 6. However, Modi *et al*. [31] subjected decolorization/degradation by bacterial isolates of four dyes soluble in water under static condition only. Elisangela *et al*. [56] evaluated decolorization of four azo dyes using *Brevibacterium* species strain VN-15 in an agitated and static sequential batch procedure. The choice for degradation of various dyes by microorganisms is not defined and changes under different conditions. Therefore, both anaerobic and aerobic conditions were used in a fluidized bed inoculated with *Pseudomonas* sp. L1 for biodegradation of Reactive Blue 13 experimented out by Lin *et al*. [57]. Similarly, disperse dye, Terasil Black degradation by recently isolated *Bacillus* species was tested on shaker (200 rpm) and under anaerobic condition. The anaerobic condition was created by sealing the flasks with sterile rubber stopper and purged with oxygen free nitrogen and incubated under stationary condition [58].

### 4.3 Role of temperature

The temperature affects dye degradation process significantly. Incubation temperature for decolorization/degradation ranging from 5 to 45°C was evaluated for degradation of Malachite Green by *Sphingomonas paucimobilis*. Telke *et al*. [46] also examined nearly same temperature range of 4–50°C while studying color removal of Reactive Orange 16 by *Bacillus* sp. ADR. A narrow range of temperatures between 25 and 50°C was used by Junnarkar *et al*. [23] while studying the degradation of Direct Red 81 with the help of a fresh bacterial consortium. Likewise, *Bacillus* sp. was subjected to incubation temperatures of 31–41°C at intervals of 2°C while degrading Congo Red [32]. Saratale *et al*. [59] studied the decolorization/degradation under the

temperature range of 30–50°C for Reactive Green 19A degradation in nutrient broth with *Micrococcus glutamicus* NCIM-2168. Decolorization of Brilliant Blue G by fungal-bacterial consortium was carried out at 5°C, 30°C, 50°C, and 60°C [60]. Parshetti *et al*. [61] studied decolorization performance for *Kocuria rosea* MTCC 1532 with Methyl Orange using temperature range 10–50°C at intermissions of 10°C. *Aeromonas hydrophila* strain DN322 was inoculated in M-9 medium with 0.1% (w/v) yeast extract, which was incubated at 4°C, 10°C, 20–50°C at intervals of 5°C to establish the optimal temperature for its growth and decolorization of dyes [62]. Lastly, separate batches of Nutrient medium were incubated at 15–40°C for the biodegradation study of Crystal Violet by *Shewanella* species [63].

### 4.4 Role of pH

The acidity or alkalinity of the growing environment can alter the microbial multiplication rate and biochemical activities, thereby influencing the decolorization efficiency. Hence, an ideal pH is essential for the multiplication of the decolorizing strain as well as enhancing the decolorizing activity. This is because the dye degrading process is a metabolic process governed by enzymes. Thus, for the highest rate of decolorization, the optimal pH for the organism needs to be identified [64]. A range of pH from 2.0–14.0 was used for studying decolorization/degradation of Direct Red 5B in nutrient broth by *Comamonas* sp. UVS [65]. Similar range of pH in basal medium with molasses as carbon source was evaluated by Tamboli *et al*. [66] while studying the biodegradation of Direct Red 5B using *Sphingobacterium* sp. ATM. The biodisintegration of Scarlet R with the help of microbial consortium GR was checked in the pH range of 5.0–12.0 in nutrient broth by Saratale *et al*. [67]. Similarly, for the consortium of *Bacillus* species and *Galactomyces geotrichum*, pH 3.0, 5.0, 7.0, 9.0, and 12.0 were studied for Brilliant Blue G degradation [65]. On the same note, Li and Guthrie [68] studied metal-complex dyes removal by *Shewanella* strain J18143 at pH 4.0, 5.6, 6.8, 8.0, and 9.2. Khalid *et al*. [69] also used similar pH range of 4.0–10.0 in mineral salt medium for the azo dyes degradation by employing the genus *Shewanella* under saline condition. Degradation by new strain of *Alcaligenes faecalis* of Reactive Orange 13 was studied over the pH range of 5.0–10.0 in nutrient broth, whereas a still wider pH range of 2.0–11.0, using mineral salts medium during degradation of Malachite Green by *Sphingominas paucimobilis*.

### 4.5 Role of salinity

Common salt is added to dye bath at high concentration to enhance dye fixation. A limitation in the development of bioremediation of textile wastewater is the suceptibility of numerous dye decolorizing bacteria to elevated concentration of sodium chloride. Hence, it is important to decide the effective NaCl cocentrations for the efficient dye decolorization/degradation by bacteria. Salinity in the range of 1–9% at intervals of 2% was checked for textile dye degradation using *Stenotrophomonas maltophila* RSV-2. Khalid *et al*. [69] also examined *Shewanella* sp. for their decolorization/degradation potential in salinity ranging from 0% to 10% (w/v) of NaCl. *Exiguobacterium* sp., a new salt tolerant organism screened from the top soil near a pharmaceutical plant, China was subjected to 2–15%v/v concentration range of NaCl in semisynthetic medium to study its dye degradation. Likewise, acclamatized natural consortium in the form of activated sludge was employed for degradation of dyes by Dafale *et al*. [70] in the range of 0–10% concentration of NaCl for degradation of RB

5. A similar study was conducted by Oturkar *et al*. [19] using 1–5% salt concentration for RR120 decolorization by *B. lentus* BI377.

### 4.6 Role of Cosubstrates/electron donors

Various organic acids, amino acids, sugars, and organic nitrogenous ingredients act as redox mediators supporting the electron dependent azo bond breakdown by the degrading strain. Therefore, different types of cosubstrates/electron donors need to be enumerated for the decolorizing strain for optimization of the degradation process. However, such studies do not follow a recommended list to outline the cosubstrate/electron donor affecting the degradation kinetics. Yet, many researchers have attempted to investigate the part of cosubstrate/reductant for the azo dye degradation. For instance, various carbon sources such as glucose, mannitol, yeast extract, and maltose at 0.4% concentration were tested for their effect as cosubstrate/electron donor on degradation of Remazol Black-B (100 mg/L) by five bacterial isolates using minimal salt medium [20]. Similarly, Junnarkar *et al*. [37] examined decolorization/degradation by novel bacterial consortium NBNJ6 of Direct Red 81 using the 0.1% of cosubstrates/electron donors such as dextrose, sucrose, cellobiose, and starch in combination with yeast extract. They also used 0.1% starch in combination with 0.1% each of peptone, casein, tryptone, meat extract, and likewise employing starch and casein individually in Bushnell and Hass medium. In the same way, various concentrations of yeast extract (10–50 mg %) as cosubstrates/electron donors were tested by Pandey and Dubey [37] to check for the biodegradation efficiency of *Alcaligenes* sp. AA09 with RR-BL as a model dye. Various carbon sources (0.5–5%) such as glucose, arabinose, fructose, raffinose, rhamnose, xylose, starch, sucrose, and nitrogen nutrients similar to peptone, yeast extract, tryptone, beef extract, soya-bean meal were supplemented in mineral salt medium by Nachiyar and Rajakumar [38] during degradation study of Navitan Fast Blue S5R using *P. aeruginosa*. Similarly, Saratale *et al*. [59] attempted Scarlet R biodegradation with consortium GR in presence of 1% each of nitrogen and/or carbon supplying nutrients such as sucrose, starch, malt extract, lactose, glucose, casein, beef extract, yeast extract, urea, peptone added to the synthetic medium. In an alternative study, Tamboli *et al*. [66] studied the degradation of Direct Red 5B along with the production of Polyhydroxyalkanoates (PHA) by *Sphingobacterium* sp. ATM using basal medium with glycerol, glucose, starch, molasses, and fried oil as different carbon sources and cheese whey and urea as the source of nitrogen. Likewise, the degradation of five dyes with the help of bacterial consortium HM-4 was studied by Khehra *et al*. [71] in mineral salts medium with 0–7.0 mM glucose and 0–0.15% w/v of yeast extract as cosubstrates/electron donors. Similar cosubstrates were used by Moosvi *et al*. [26] in Bushnell Hass medium with 0.05% yeast extract and 0.1% glucose during degradation of RV 5 by the consortium RVM 11.1 wherein the impact of other carbon sources such as combinations of glucose, sucrose, starch, hydrolyzed starch, sodium acetate, lactose, sodium formate with yeast extract was also studied. For the biodegradation of Disperse Red 54 by *B. laterosporus*, Kurade *et al*. [49] used 0.5% w/v of various nutrient sources such as starch, glucose and 0.5% w/v of peptone, yeast extract, urea, and $NH_4Cl$ in Bushnell Hass medium. Eleven types of carbon nutrients such as galactose, xylose, glucose, sucrose, maltose, lactose, raffinose, mannitol, starch, carboxy methyl cellulose glycogen, and four nitrogen nutrients such as YE, peptone, urea, meat extract were investigated for their influences on the decolorization/degradation of four water-soluble dyes using various dye house effluent bacterial isolates [31]. Rajeswari *et al*.

[48] also studied the effect of 0.05%, 0.1%, 0.2%, 0.4%, and 0.8% w/v yeast extract on the textile dyes decolorization/degradation using *Stenotrophomonas maltophila* RSV-2. During the degradation of Red 2G by *Bacillus* sp. isolated from textile effluent, different carbohydrates such as dextrose, sucrose, maltose, starch, cellulose were used in M-9 medium. Also, different nitrogen sources such as peptone, tryptone, tyrosine, glycine, and ammonium ferrous sulfate were checked for their ability to support dye degradation by their isolate [72]. A different set of cosubstrates/electron donors were analyzed by Chen *et al*. [39]. They analyzed the effect of organic acids with one carbohydrate using 20 mM each of lactate, formate, butyrate, pyruvate, acetate, arabinose on Crystal Violet degradation by *Shewanella* species NTOU1.

### 4.7 Role of alternate electron acceptors

Azo dye degradation is majorly considered as membrane-bound metabolic activity in the absence of oxygen using dye molecule as an oxidant for the re-oxidation of reducing equivalents produced during metabolism. Any other external electron acceptor such as few ions having favorable redox potential acts at the terminal point of electron transport chain and can quench the electrons otherwise used in the breakdown of the dye. For instance, the nitrate and nitrite salts are generally used in dye baths to improve substantivity to the cloth fibers [39, 73, 74]. However, the mechanism used by the decolorizing strain is redox mediated catalysis for breakdown of various bonds present in the synthetic dyes. Nonetheless, in the enzymatic breakdown of the dye, interference by few ions may lower the rate of the degradation process by accepting the electrons that were originally designated for breaking the dye bonds and acting like an electron sink [75]. Liu *et al*. [76] point out to the above phenomenon in the breakdown of Acid Red 27 (AR 27) by *Shewanella oneidensis* MR-1. They used humic acid as the redox mediator for the degradation of AR 27, which led to increase in decolorization efficiency by 15–29%; however, on increasing the concentrations of the salts of nitrates and nitrites, the color removal, which was vetted in the presence of the redox mediator, was revoked. This may be due to the preferential redox potential of nitrate and nitrite ions as compared to the dye. Similarly, Chen *et al*. [39] scrutinized Crystal Violet degradation by *Shewanella* species NTOUI in the presence of ferric citrate, thiosulfate, nitrite ferric oxide, or manganese oxide as electron acceptors in the medium.

### 4.8 Influence of initial dye concentration

The ability of the decolorizer to withstand increasing initial concentrations of a dye is closely linked to its toxicity tolerance to the dye under higher concentration. Also, the ability to tolerate increasing initial dye concentrations is linked to the structure of the dye. For instance, Oturkar *et al*. [19] subjected *B. lentus* BI377 to 250–1500 ppm of RR120 for checking the maximum decolorization/degradation performance of the bacterium. Similarly, 50 mg/L–1500 mg/L of Crystal Violet was used for studying the degradation by *Shewanella* sp. NTOUI [39]. Jadhav *et al*. [52] checked degradative proficiency of *Comamonas* sp. UVS with dye concentration ranging from 50 ppm–1100 ppm. Similarly, medium amendment was done using 100–500 mg/L at intervals of 50 mg/L of Remazol Black B while studying the dye degradation by *P. putida* [24]. Likewise, Pandey and Dubey [37] studied the effect of 50 ppm–500 ppm of RRBL during the degradation by *Alcaligenes* sp. AA09. While, for the biodegradation of Congo Red using *Bacillus* sp. the dye concentration range was 100 ppm–1000 ppm [32]. Also, Khalid *et*

*al*. [69] have studied the degradation of 100–500 mg/L concentration of AR 88 and Direct Red 81 using *Shewanella putrefaciens* AS96. For the degradation of RV 5 by the consortium named RVM 11.1, the concentration range from 50 ppm to 400 ppm was used [26] while for an acclimatized microbial consortium the initial dye concentration of 200 ppm–1000 ppm of Reactive Black 5 was tested for its degradation ability [70].

### 4.9 Anaerobic azo dye degradation

Under anaerobic conditions, acidogenic, acetogenic, and methanogenic bacteria have been used for dye decolorization/degradation [77]. The catabolic organic nutrient source is required by these bacteria for dye decolorization/degradation. The nutrients such as acetate, ethanol, starch, glucose, tapioca, and whey were checked during decolorization of dye under methanogenic environments [73, 78–80]. The claim of methanogens being associated with dye decolorization/degradation has also been supported by Razo-Flores *et al*. [81]. In contrast to the above finding Yoo *et al*. [80] confirmed Orange 96 decolorization/degradation in the presence of an inhibitor specific to methanogens, i.e., 2-bromoethanesulfonic acid (BES), and the reduction in degradation was supported by the role of sulfate-reducing bacteria (SRB) in this process. The first step during azo dye degradation under anaerobic and aerobic conditions using bacteria is the reduction of the azo interaction. It may be either extracellular or intracellular. The reduction may take place by separate mechanisms, for example, redox mediators of less molecular weight, chemical reduction by biologically generated reductant similar to sulfide, enzymes, or a blend of all these [82]. The degradation process may be taking place as dye may serve as an oxidant to electrons donated by electron carrying complexes of the Respiratory Electron Transport Chain (RETC). In order to accomplish this, the bacteria should create an interaction between their membrane-bound RETC and the extracellular azo dye molecules. To form such interaction, the RETC components have to be present in the external wall surface of the Gram-negative bacteria in which they can be in direct association with either the substrate dye or the redox mediators present on the outer surface of the cell. Additionally, redox mediator compounds of low molecular weight can represent as electron shuttle for azoreductase dependent on NADH specifically placed in the external wall layer and the azo dye. These mediator compounds may be added externally or are formed by the bacteria from certain substrates during their metabolism. The azo dye reduction without enzymes can take place in presence of very low concentrations of synthetic redox mediators such as anthraquinone sulfonates. However, under aerobic condition, oxygen inhibits this reduction mechanism because of the redox mediator being preferred for oxidation by $O_2$, instead of the dye [83].

### 4.10 Aerobic azo dye degradation

In literature, a variety of bacterial species that can reduce azo dyes aerobically have been described. The organic carbon/energy sources are obligatory, as they cannot make use of dye as the only carbon/energy/nitrogen nutrient [84]. *P. aeruginosa* needed glucose while decolorizing Navitan Fast Blue S5R, a commercial textile and tannery dye, under aerobic conditions. It could also decolorize azo dyes range [38]. An incredibly smaller number of bacteria have the ability to use azo dyes as the solitary carbon compound. Such bacterial species abstractly cleave –N=N– and generate amines that they can be used for carbon and energy metabolism. Coughlin *et al*. [85]

have reported an obligate aerobe, *Sphingomonas* sp., strain 1CX, which can use dye AO7, as the only energy and carbon/nitrogen resource. It can degrade only one amine, 1-amino 2-naphthol, along with other amines created throughout AO7 degradation. *Sphingomonas* ICX decolorized quite a few azo dyes having phenyl or naphthyl groups such as 1-amino-2-naphthol/2-amino-1-naphthol.

## 5. Microbiological dye degradation processes in combination with advanced oxidation processes (AOPs)

To develop a vigorous and profitable option for azo dye elimination, it is promising to combine Advanced Oxidation Processes (AOPs) with microbial methods. There can be improvisation in the advantages and minimization of the disadvantages of each culture by combining them together [86–89]. The aim of pairing AOPs with biological processes is to permit incomplete degradation of the dye molecules by using the AOPs followed by the comparatively low-cost biological process for supplementary removal of the dye [86, 87, 90, 91]. Hence, the key objective of AOPs is to alter recalcitrant compounds like dyes into smaller degradation intermediates, which are further degradable by microbial processes [87, 88, 90, 92] using microbes isolated from municipal plants or the textile effluents [93, 94] or from dye-contaminated soils [90]. Using this strategy, it is possible to reduce COD significantly in a short time [92, 95, 96]. Some dyes, for example, Orange II inhibit bacteria, so pretreatment using AOP is beneficial as toxicity of dye can be eliminated and can be subsequently degraded biologically [95, 97]. The wastewater with dyes can be subjected to such combination of physicochemical and biological methods with an objective of increasing the biodegradability index (BI) due to increase in BOD and decrease in COD [98, 99]. There are also evidences of preliminary decolorization/degradation of the azo dye using microbial methods followed by AOP as the post-treatment method [92, 100, 101]. The studies on the decolorization/degradation of synthetic dyes by combined methods for treatment under various conditions have been enlisted in **Table 1**.

Sonolysis increases biodegradability of dye by decomposing it into smaller units [94]. A better choice is ozonation used in association with biological methods, as the former method augments sludge settling ability and bio-decomposition leading to good color elimination of dyes, as it hits azo bonds even with smaller concentrations [93, 98, 113]. The oxidation using Fenton reagent is not appropriate as a post-treatment application [88]. It is a useful process for getting rid of organic pollutants/toxic chemicals, which are detrimental to biological treatments [86, 94, 114, 115]. It is appropriate to apply Fenton process prior to treatment than after treatment as the Fe ion quantity has to be increased for the complete removal of the dye, since there is iron precipitation due to the phosphate ion, as a macronutrient present in microbiological media. A combination of microbiological processes with electrochemical methods demonstrates superior results as elevated decolorization/degradation with good decrease in COD during the decomposition of dyes [95, 96]. Ultraviolet (UV) light in association with $H_2O_2$ and $TiO_2$ as catalyst is commonly employed for the azo dye degradation [112]. The advantages of UV/$H_2O_2$ process are that it can take place under ambient environment and there is likelihood of total simplification of organic compounds into carbon dioxide [116], low primary investment, absence of objectionable solid leftover, or odor release during or post degradation [117]. Usually, there is a formation of oxygen in this method, which is useful for successive aerobic biological methods [116], but the

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 1 | *Consortium of P. aeruginosa*, *Bacillus flexus* and *Staphylococcus lentus* | | Acid Blue 113 | Aerobic | Fenton | 0.5:1 w/w $H_2O_2$/COD and 70 mg/L of $Fe^{+2}$, pH 2.5–3.5 | Pre-treatment of Acid Blue 113 dye effluent with Fenton ($H_2O_2$ and $Fe^{2+}$) reduced concentration of dye by 40% and 45% biodegradation was achieved by consortium. Overall 89.5% degradation and 93.7% COD reduction was achieved. | [102] |
| 2 | *Aeromonas hydrophila* SK16 | | Reactive Red 180 (RR 180), Reactive Black 5 (RB 5) and Remazol Red (RR) | Aerobic (enzymes detected: tyrosinase, laccase, LiP, ribofavin reductase and azoreductase) | $H_2O_2$ | 4% $H_2O_2$ irradiated under solar light for 6 h. The same procedure was followed for dark conditions. Control was without $H_2O_2$. | Bio–AOP led to 100% decolorization of RR180, RB5 and Remazol Red, while 72% decolorization was achieved with individual treatments. Combined treatment reduced BOD and COD of RR 180 by 78 and 68%, RB 5 by 52 and 83% and RR by 42 and 47%, respectively | [89] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 3 | *Consortium of Chaetomium globosum* IMA1, *Aspergillus niger and Rhizopus oryzae* | | polyvinyl alcohol and organic compounds in dye effluents | Aerobic (Enzymes detected: laccase and LiP) | Fenton reaction with $H_2O_2$ | Temperature: 25 ± 2 °C. Chemicals: Ferrous sulfate heptahydrate and hydrogen peroxide ($H_2O_2$) | 97.8%, 98.5% and 99.75% COD, Polyvinyl alcohol and color removal respectively using combined method. Complete degradation was observed after 6 days. | [103] |
| 4 | *Consortium of Brevibacillus laterosporus and Galactomyces geotrichum* | | Raw Textile effluent (RTE)—1 and 2, and Simulated Synthetic Effluent (SSE) | Reproducibility of the bioreactor maintained/3 consecutive cycles of 7 days each | Continuous degradation | Triple layered fixed bed reactor, >80% decolorization/ degradation/100 mL/h flow-rate, for 7 days, 78% COD. | Decolorization efficiency–89% for RTE-1, 60% for RTE-2 and 69% for SSE in 48 h. | [104] |
| 5 | *Lactobacillus* sp., *Mycobacterium* sp., *Staphylcoccus aureus*, and *Corynbacterium* sp. from Distillery wastewaters | | Textile effluent | Aerobic and microaerophilic conditions | Electro-Fenton (EF) process | Textile wastewater pH 3.0 for Electro-Fenton method, pH 12.5 for precipitation $Fe^{3+}$ ions and pH 6.6 for biological treatment | Removal of color, COD, TOC by EF process was 63%, 48%, 29% respectively. Further treating wastewater by biological process reduced 85% color, 86% COD, and 56% of TOC | [105] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 6 | *Aeromonas veronii* GRI | | Methyl orange | Aerobic under neutral and alkaline pH | Biosurfactant | 0.075% SPB-1 lipopeptide from *Bacillus subtilis* SPB-1 | 92% degradation in SPB-1 biosurfactant–microbial treatment | [106] |
| 7 | | Laccase extracted from *Cyathus bulleri*, procured from culture collection | Textile effluent | Pretreatment with ABTS and laccase 60% decolorization | Chemical treatment with ABTS and Alum | Alum coagulated 90% of residual color but the process associated with dye sludge formation and ABTS not recovered | 85% degradation was achieved in the Enzyme Membrane Reactor and the process could be operated for over a period of 15 days. No sludge formation, negligible membrane fouling. About 60% ABTS could be recovered. | [107] |
| 8 | Not mentioned | Not mentioned | Dyeing wastewaters | Biological oxidation 25°C/12 h; 1 h feeding/ reaction for 6 h/ sedimentation for 4 h/discharge for 0.8 h/idle for 0.2 h/cycles-10; pH ~7.0 | Fenton's oxidation followed with Sequential biological oxidation | Oxidation using Fenton reagent, with optimum dosages of $H_2O_2$ and Fe (II) | 91–98% for DOC, 83–95% for BOD5 and 88–98% for COD, color removal >99% compared to biological or chemical treatment alone. Lowered toxicity by inhibition of *Vibrio fischeri*. | [108] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 9 | Consortium of strains of *P. aeruginosa* from different sites of petroleum pipelines | | Turquoise Blue G | Aerobic | Electrolysis | $IrO_2$–$RuO_2$–$TiO_2$–Ti anode; Ti cathode 5 g/L NaCl, pH 7.0 current density 10 mA/cm$^2$ | Complete decolorization of Turquoise Blue G within 15 min. Dye components were reduced by 41.3% by electrochemical and 90% by microbial route. | [109] |
| 10 | | Activated sludge from wastewater treatment plant with initial microbial concentration of 0.5 g/L | Methylene Blue | Aerobic–Activated sludge | Electrochemical | 25 mA/cm$^2$ $Pb/PbO_2$ Electrode. | 95.61% degradation efficiency was achieved for 134 mg/L methylene Blue at 720 rpm. Biodegradability from 0.034 to 0.54 for the $BOD_5$/COD ratio with electrolysis and 92.03% mineralization was achieved | [110] |
| 11 | Consortium of *A. niger and Penicillium* sp. from tannery yard | | Acid Black 1 | 280 h/Aerobic | Ozonation | Ozonation 4 h | 52.9% TOC and 94.5% color removal | [99] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 12 | Consortium of *Pleurotus ostreatus, Tricoderma viridae* or *Fusarium oxysporum Bacillus cereus and Pseudomonas putida* | | Procion Blue | RT/150 rpm Aerobic/5 days. | Electrochemical | Electrochemical reactor, 4 h. Photo catalysis 500 mg/L $TiO_2$, 5 h–125 Hg lamp, Electrolysis 5.0 A/$dm^2$, 5 h | 2000 mg/L of Procion Blue, reduction in COD—39.6% in electrochemical reactor; COD—31–59% post-treatment with microbes; COD–32–81% on electrolysis and COD–23–70% after photocatalytic process. | [96] |
| 13 | *P. putida, B. cereus, Phanerochaete chrysosporium, Tricoderma virdae* | | Methyl Red | Biological at 25°C for 5 days | Electro-oxidation | Electro-oxidized $RuO_2$; stainless steel cathode and titanium anode coated with–2A/$dm^2$, 5 h. | 77–91% COD and 81% color removal post electro-oxidation followed with biological 90–95% color removal | [95] |
| 14 | | Anaerobic sludge/ Aerobic sludge. (municipal effluent plant) | Remazol Red | Anaerobic process at pH 7.0 for 14 days | Photocatalysis and Fenton reagent | Aerobic, pH 7.0, 150 rpm; UV–Vis lamp 18 W, 1 mM Ferrous, 1 mMolar $H_2O_2$, pH 5.0 and 50 rpm. | 100 ppm of Remazol Red–COD 70 ppm for anaerobic; COD 14 ppm post Photo-Fenton treatment; Post-treatment of aerobic conditions—100% color removal; residual COD 9 mg/L, less toxic | [100] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 15 | *Rhodotorula mucilaginosa* | | Basic Yellow 2 and Reactive blue 4 and Reactive red 2 | 100 rpm/25°C/ pH 5/8 h | Ultrasound | Ultrasonication 20 kHz, 5 h | Ultrasonication of 100 ppm dyes removed 28–48% color. Post-microbial treatment color removal was 40–93%. | [94] |
| 16 | | Hydrogen peroxide Photo-oxidation | Reactive blue 5 | $H_2O_2$ 250–1000 ppm, pH 7.0, RT, 21 W low-pressure Hg lamp $\lambda_{254nm}$. | Ultraviolet | UV 15–60 min | RB5 (200 ppm), UV $H_2O_2$ 89–100% color; 54% decrease in COD, high biodegradibility | [90] |
| 17 | | Aerobic sludge | Remazol Black B | over activated carbon, pH 9.0/24 h | Ozonation | Ozonation RT, 60 min, pH 3.0. | 96% color removal of 100–500 ppm dye, high toxicity with Ozonation, not toxic with biological post-treatment. | [93] |
| 18 | | Aerobic treatment | Reactive Brilliant Red X-3B | Microbiological aerated filter, 20–25°C | Ozonation | Ozonation dye/ ozone = 4.5, pH 11.0. | 50 mg/L dye showed 30% COD reduction with ozonation. Post biological treatment, 97% color removal and 90% decrease in COD was observed. | [26] |

| S. No. | Biological agent | Enzymes/ sludge/ Chemical agents | Dye/ Effluent | Process Details | AOP* | Process Details | Efficiency | Reference |
|---|---|---|---|---|---|---|---|---|
| 19 | | Biological reactor | Cibacron Red | Immobilized sludge, 40°C,with oxygen saturation | Photo-Fenton | Photo-Fenton 65–225 ppm $H_2O_2$ 2–5 ppm Fe (II) | 250 mg/L dye showed 41–67% decrease in COD on photo-Fenton treatment while 60–73% was achieved on biodegradation | [92] |
| 20 | *Candida oleophila* | *Candida oleophila* | Reactive Blue 5 | Aerobic, 26°C | Fenton oxidation | Fenton $H_2O_2$ $1.5 \times 10^{-3}$ mol/L, ferrous ion $1.5 \times 10^{-4}$ mol/L at pH 5.0 | 100–500 ppm dye showed 35% and 15% color removal on treatment with Fenton's method and *Candida* sp. However, 1 h preatreatment with Fenton followed by microbial treatment for 7 days resulted in 91–95% reduction in color. | [111] |
| 21 | *Trametes versicolor* | *Trametes versicolor* | Reactive Blue 5 | Immobilization 25°C, 4 days | Photocatalysis Ultraviolet $TiO_2$ | 254 nm with 15 W high pressure mercury-vapor lamp, at 120 rpm | 98% color of 300 ppm dye after microbial or combined $H_2O_2$/ UV/$TiO_2$ treatment. Poisonous to *H. atenuatta*; only photocatalysis–non-toxic. | [112] |

*AOP: advanced oxidation processes.*

**Table 1.**
*Comparison of various combined methods under various conditions.*

higher UV light intensity required for the better rate of cleavage of dyes contributes to the disadvantage because the process utilizes extensive electricity, which increases the operating cost [118]. Hence the blend of ultraviolet light/$H_2O_2$ and biological methods can reduce the cost. For instance, the degradation of Reactive Black 5, which is partially treated with photochemical step and then by treatment with an acclimatized microbial biomass is rapid, hence consumes lesser $H_2O_2$ concentrations [90].

## 6. Generalized mechanisms of azo dye degradation

### 6.1 Enzyme catalyzed dye degradation

Rafii *et al*. [119] produced the first report of azoreductases in bacteria growing anaerobically. The bacteria belonging to the genera *Eubacterium* and *Clostridium* displayed decolorization of azo dyes of sulfonated type while growing on chemically undefined solid media. These strains produced extracellular oxygen-sensitive azoreductases constitutively. This enzyme from *Clostridium perfringens* alleged to be flavoprotein dehydrogenase and was found to be occupied in the reduction of nitro aromatic compounds. Another mechanism involving flavin nucleotide dependent cytosolic reductases, which donate electrons through soluble flavins to dyes, has also been suggested for dye decolorization/degradation [116]. However, Russ *et al*. [120] used *Sphingomonas* strain-BN6 produced by recombinant technology and showed the insignificance of the above mechanism in vivo.

#### *6.1.1 Enzymes involved in dye decolorization/degradation*

The progression of biodegradation of azo dye occurs due to the presence of a variety of oxidoreductases present in microorganisms.

The two important classes of azoreductases with respect to their catalysis are azoreductases of flavoprotein kind and those that do not need flavin. The former class of azoreductase is further subdivided on the basis of their requirement for coenzymes such as NADH, NADPH, or both, as reductants [121]. Other reductases such as riboflavin reductase, NADH-DCIP reductase might be implicated in the azo dye cleavage [46, 122, 123]. However, as per Blumel *et al*. [124] and Russ *et al*. [120], these enzymes are futile in vivo. Russ *et al*. [120] also proposed that the anaerobic azoreductases of cytoplasmic origin are flavin reductases and can engage themselves in extracellular hydrogenation of azo dyes with the help of an electron mediator, which helps in displacement of reducing counterparts to azo dyes from the membrane of bacteria. Thus, the presence of azoreductases along with a transport system that enables dye transport to the cells is a requirement for the microbial strains to decolorize azo dyes.

##### *6.1.1.1 Reductive enzymes*

A large number of different types of bacteria have been screened and identified, which can degrade azo dyes under oxygen deficient (reduced) environment [19]. It is agreed that an enzyme azoreductase initially cleaves the azo bond in such conditions resulting in creation of colorless aromatic amines. The microaerophilic environment augments azo dye degradation as revealed by findings conceded by Joshi *et al*. [125] and Xu *et al*. [51]. Reactive azo dyes can be degraded rapidly under partly reduced environment. To create partly reduced environment, the presence of another redox

agent for transfer of electrons from reduced nicotinamide coenzyme to the dyes is essential as observed by Chang *et al*. [126]. Though a small fraction of azo dyes can be degraded aerobically [127], the efficiency of degradation may decline if the oxygen is present [128], since oxygen and the dyes compete with each other as electron acceptors. Aerobic conditions enhance cell mass of microorganisms but with less efficiency of degradation process in comparison with reduced environment. These observations lead to the interpretation that it is the concentration of oxygen and not the amount of cell mass that influences the process of dye degradation. Likewise, the process is subject to the nature and accessibility of electron donors [31, 129]. Both cellular components, namely membrane fractions isolated from bacteria and cytoplasm, are important during anaerobic reduction process [130]. But most significant is the membrane fractions because they harbor constituents for electron conveyance which link azo dyes to electron donors. This concludes that the biochemical basis for azo dye decomposition is the redox reaction between azo dyes and electron donors [131]. The most important reductases are azoreductase, riboflavin reductase, and DCIP reductase [46, 122, 123].

Anaerobic degradation of azo dye is a nonspecific procedure. It was understood that in the absence of $O_2$, the azo dye is degraded by azoreductase by means of reducing equivalents such as NADH or NADPH. The primary stage during decolorization of dye by bacteria is the –N=N– bond reduction. This is carried out by transferring four electrons across the azo bond with the help of NADH. The dye serves as the terminal electron acceptor instead of $O_2$ under static/anoxic condition and two sequential reduction steps reduce the same to specific amines [126, 132]. For illustration, Misal *et al*. [133] had procured alkaliphilic bacterium *Bacillus badius* for dye degradation from alkaline Crater Lake–Lonar; subsequently, purifiying and characterizing its azoreductase. Likewise, many authors have reviewed the role of the azoreductase enzyme in azo dye decolorization/degradation. However, a variation of soluble cytoplasmic azoreductases with small substrate specificity are produced by bacteria [59], e.g., azoreductases from certain anaerobic bacteria such as *Clostridium* and *Eubacterium,* which are oxygen-sensitive, are produced constitutively, and excreted into the ecosystem [119]. Nachiyar and Rajakumar [134] purified oxygen-resistant intracellular azoreductase from *Pseudomonas aeruginosa* and studied its affinity for various azo dyes pointing out its highest affinity for Navitan Fast Blue S5R.

#### *6.1.1.2 Oxidative enzymes*

The enzymes of oxidative kind, which are important in the dye degradation, include tyrosinase, lignin peroxidase (LiP), laccase, and manganese peroxidase (MnP) [135]. As compared to bacterial isolates, white-rot fungi has shown tremendous potential in degradation of dyes and other recalcitrant compounds by production of highly oxidative and substrate nonspecific enzymes [35]. Among the oxidative enzymes, LiP and MnP are extensively studied for their potential to degrade azo dyes. Both these enzymes are multi-copper phenol oxidases and have wider applications during oxidation of a variety of partially degraded by-products of dye. Also, unlike bacterial enzymes, fungal LiP and MnP follow a highly nonspecific free radical mechanism during azo dye degradation to form phenolic compounds without cleaving the azo bond. In this process, they skip the biochemical steps that lead to formation of toxic aromatic amines [20, 36, 136–138]. Telke *et al*. [46] further reported an enzyme, phenol oxidase, similar to laccase having the characteristic

| S. No. | Enzyme | Microbial system | Dye decolorized | Reference |
|---|---|---|---|---|
| 1 | Crude protease | *Bacillus cereus* strain KM201428 | Reactive Black 5 | [142] |
| 2 | Laccase like enzyme Lac 1326 | Marine metagenomic library. Cloned and overexpressed in *Escherichia coli* BL21 | Amaranth, Coomassie Brilliant Blue, Bromophenol Blue, Acid Violet 7, Congo Red, and Indigo Carmine | [143] |
| 3 | Laccase, tyrosinase, LiP, Riboflavin reductase, azoreductase | *Aeromonas hydrophila* SK16 and AOPs | RR180, Reactive Black 5 and Remazol Red | [89] |
| 4 | Laccase, LiP | *A. hydrophila* | Crystal Violet | [144] |
| 5 | Laccase, LiP, MnP | *Peyronellaea prosopidis* | Scarlet RR | [145] |
| 6 | Laccase, NADH-DCIP reductase, tyrosinase, LiP, Malachite Green reductase | *Aeromonas* sp. DH-6 | Malachite Green | [47] |
| 7 | Laccase, azoreductase | *A. hydrophila* SK16 and *Lysinibacillus sphaericus* SK13 | Reactive Yellow F3R, Joyfix Yellow 53R, Remazol Red RR, Drimaren Black CL-S and Disperse Red F3BS | [34] |
| 8 | Laccase, MnP, LiP, azoreductase | *Anoxybacillus flavithermus* 52-1A, *Tepidiphilus thermophilus* JHK30, *Tepidiphilus succinatimandens* 4BON, *Brevibacillus aydinogluensis* PDF25, *Bacillus thermoamylovorans* DKP, *Geobacillus thermoleovorans* NP1 | Direct Black G | [146] |
| 9 | Laccase, LiP, tyrosinase, Riboflavin reductase | *Ipomoea hederifolia*, *Cladosporium cladosporioides* (Plant-Fungus consortium) | Navy Blue HE2R | [147] |
| 10 | Azoreductase | *Shewanella* sp. strain IFN4 | Acid Red-88, RB5, DR81 | [148] |
| 11 | Laccase and MnP | *Leptosphaerulina* sp. (Fungus) | Novacron Red | [149] |
| 12 | Laccase, azoreductase, Veratryl alcohol oxidase, NADH-DCIP reductase | *Pseudomonas* species SUK1 and *Providencia rettgeri* HSL1 in consortium | C.I. RO 16, C.I. RB 5, C.I. DR 81 and C.I. Disperse Red 78 | [11] |
| 13 | NADH–DCIP reductase, LiP | *Penicillium simplicissimum* | Triphenylmethane Dyes | [150] |
| 14 | Laccase | *Trichoderma atroviride* F03 | RB5 | [151] |

| S. No. | Enzyme | Microbial system | Dye decolorized | Reference |
|---|---|---|---|---|
| 15 | Laccase | *Stenotrophomonas maltophilia* AAP56 | RB5 | [152] |
| 16 | Laccase | *Coprinopsis cineria* | Methyl Orange | [153] |
| 17 | Azoreductase | *Bacillus lentus* BI377 | RR141 | [154] |
| 18 | Azoreductase | *Shewanella oneidensis* MR-1 | Methyl Red | [155] |
| 19 | Laccase, LiP, MnP | *Ganoderma lucidum* IBL-05 | RR195a Reactive Yellow 145a Reactive Blue 21 | [156] |
| 20 | Veratryl Alcohol Oxidase | *Alcaligenes faecalis* PMS-1 | Reactive Orange 13 | [157] |
| 21 | NADH/NADPH-dependent $O_2$ sensitive azoreductase | *Alcaligenes* sp. AA09 | RR-BL | [37] |
| 21 | Laccase, azoreductase, and NADH–DCIP reductase | *Shewanella aquimarina* | Acid Red 27, Direct Blue 71, RR120, Methyl Orange, Acid Orange7 | [158] |
| 22 | Tyrosinase | *Brevibacterium* sp. | Direct Blue 71, RY107, RB 5, RR 198 | [56] |
| 23 | Veratryl alcohol oxidase | *Pseudomonas aeruginosa* Strain BCH | Remazol Black | [159] |
| 24 | Heat stable laccase | *Bacillus pumilus* | Acetosyringone, Indigocarmine | [160] |
| 25 | Laccase, NADH–DCIP reductase, tyrosinase, LiP | *Acinetobacter calcoaceticus* NCIM 2890 | Amaranth Dye | [161] |
| 26 | Azoreductase, Cytochrome P450 oxidase, Aminopyrine N-demethylase, Superoxide dismutase, Glutathione S-transferase, tyrosinase | *B. lentus* BI377 | RR120 | [19] |
| 27 | NADH dependent $O_2$ insensitive azoreductase | *Bacillus badius* | Amaranth Dye | [133] |
| 28 | Azoreductase | Moderately Halotolerant *Bacillus megaterium* | Red 2G | [138] |

**Table 2.**
*Dye degradation using microbial enzymes.*

to use non-phenolic substrates. Laccases can employ direct oxidation or mediator coupled indirect oxidation of textile dyes by H2O2 during their catalytic cycle [139]. On the other hand, MnP activity is dependent on manganese as well as specific buffers. For this reason, the use of enzymatic membrane reactors is suggested by some

authors for degradation of dyes using MnP. It was reported that peroxidase involved in dye degradation can degrade hydroxyl free anthraquinone dyes [140, 141]. Few examples of studies employing biodegradation with the help of oxidative and reductive enzymes are enlisted in **Table 2**.

### 6.2 Mediated biological azo dye decolorization/degradation

Many azo dyes, which have a large molecular weight or are polymeric azo dyes, or are strongly polar sulfonate, are difficult to be imported via the cytoplasmic membrane [162]. The recommendation to this was that there could be another mechanism for reduction of these dyes. There are currently various reports discussing the function of redox mediators during anoxic bacterial reduction of dyes [163, 164]. The addition of catalytic quantities of riboflavin to anaerobic granular sludge led to appreciable increase of the decolorization of mordant yellow 10 [165]. Mendez-Paz *et al*. [166] introduced 1-amino-2-napthol, an amine derived during AO7 degradation that improved its rate of reduction, probably through the acquisition of electrons. The presence of artificial electron carrier like anthraquinone-2, 6-disulfonate similarly enhanced the reduction of numerous dyes. Keck *et al*. [164] have observed that the augmentation of dye decolorization, under anaerobic conditions, took place when coupled with redox intermediates produced by other bacteria while degrading aromatic compounds aerobically. With respect to this observation, Chang *et al*. [132] too showed improved azo dye decolorization/degradation rates post addition of cell-free supernatants having metabolic intermediates of a dye-decolorization by *E. coli* strain NO3.

## 7. Conclusion

Microorganisms play an important role in decomposition and mineralization of synthetic dyes. The microbial processes are, in turn, affected by various environmental, molecular, and physicochemical factors. The biodegradation of dye molecules is also dependent on multiple factors including structure and stability (types of bonds) of dye molecules. More importantly, dyes are present in industrial effluents as a mixture of recalcitrant compounds, organic and chemical compounds. Many of these compounds interfere in biodegradation of individual dyes resulting in impractical outcomes on field application in spite of successful laboratory studies. Although several researchers have presented detailed mechanisms of microbial strains to potentially decolorize a number of synthetic dyes, it is important to understand that dye degradation is a complicated multifactorial and multistep process. The interference of pollutants severely compromises biodegradation either by inhibition of microorganisms or their enzyme activity. To make matters much more challenging, factors such as temperature, dissolved oxygen, moisture, and coexistence of dyes with acid, alkali, and other pollutants further impede biodegradation process. In such a scenario, in situ augmentation of natural dye degrading microorganisms may be a simple, practical, and sustainable bioremediation strategy. However, even for this purpose, it is essential to screen natural isolates capable of degrading complex molecular structures and identify optimum parameters to maximize the degrading potential of screened isolates. These studies can provide significant insights into the specific role of enzymes, mediators, cofactors, and cosubstrates that will ensure augmentation of beneficial dye degrading strains. In addition, these studies can

ensure selection of most effective combination of physical techniques to minimize toxicity and enhance biodegradation potential of microorganisms. Collectively, these strategies can detoxify and completely degrade complex mixtures of dye molecules into harmless metabolites.





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