

# Bioremediation of Dye Using Mesophilic Bacteria: Mechanism and Parametric Influence



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**Abstract** For centuries, dyes have been utilized in the tannery, textile, food, paper, cosmetic, and plastic industries. As a consequence of the fast urbanization and industrialization, the uncontrolled release of dyeing agents in the effluent is increasing. Such a release causes toxicity and pollution to the whole environment. These concerns become more critical due to the biomagnification phenomenon through various trophic levels resulting in severe toxicity in higher animals and plants including aquatic flora and fauna. Mitigation of this nuisance can be achieved by the economic application of biotechnology using safe biological agents to decolorize and degrade the dye in water bodies.

In this chapter, we reviewed the toxicity and harmful effects of various dyes along with different mechanisms and strategies of dye decolorization and degradation by biological agents while giving ample emphasis on the mesophilic type bacteria. Further, the effect of different physicochemical parameters on dye removal efficacy was explicitly discussed. Moreover, various techniques to investigate the harmful toxic effects of the produced post degradation metabolites were also enlightened. Thus, this present chapter will deliver a quintessential perception on the feasibility of the bioremediation technique using mesophilic bacterial strains to treat dye contaminated waste streams.

**Keywords** Mesophilic bacteria · Biodegradation · Wastewater · Dye · Aerobic bacteria · Anaerobic bacteria, · Biomagnification · Bioreactor · Biosorption · Enzymatic degradation

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## 1 Introduction

The rapid rate of industrialization, urbanization, and scientific developments has accelerated the discharge of several unwanted pollutants in the biosphere beyond safe permissible levels [55, 68, 90, 95]. Among these toxic chemicals, the dye is a major visible contaminant whose presence is highly objectionable in the water to be utilized for either domestic or industrial purposes [15, 36, 51]. The issue of water pollution because of the discharge of untreated or partially treated dye polluted effluent into natural aquatic bodies was initially observed in the nineteenth century [44, 50, 51, 66]). Since the mid-nineteenth century, all colorants used for various dyeing and printing purposes were of natural origin. Natural dyes were mostly safe for the environment and degradable in nature as they are either plant-derived or microorganism-derived. Sources of natural dyes include roots, bark, leaves, wood, berries, lichens, shellfish, and fungi [50]. However, because of their low availability, inefficient and expensive extraction process, there was an emerging requirement in the nineteenth century for the manufacturing of a bulk quantity of cheap synthetic pigments and dyes required in several textile industrial processes. As a consequence, the synthetic dye industry evolved as a “frontier technology industry” of Victorian times. English chemist, William Henry Perkin was acknowledged as the founder of the first synthetic dye named as “Mauve” [16, 47]. Any dye molecule is comprised of mainly two groups: (1) the chromophoric group, which is accountable for the visible color and (2) the auxochrome group, responsible for the dye’s solubility in aqueous phase and attraction towards the fiber materials.

Synthetic coloring agents are preferred over natural ones because of some attributes including their higher stability toward detergent, surfactant, temperature, microbial attack, and light, variation in color shades, firmness, easy and inexpensive synthesis process [70]. Nowadays, synthetic colorants are extensively utilized for leather dyeing, textile fiber dyeing, colored photography, food industry, paper printing, and as additives in various petroleum-derived products. More than ten thousand distinct pigments and dyes are being utilized in various industrial applications and their annual worldwide production is more than 0.7 MT [78]. Among the total dye production, approximately 70% are produced in India, China, Taiwan, Argentina, and Korea. In the complex industrial waste stream having different types of colorants, dye wastes are predominantly found [2]. These dyes are generally carcinogenic, teratogenic, and mutagenic in nature, which may induce chronic health hazards to both organisms and human beings [80]. Henceforth, it is obligatory to separate them from industrial wastewater before being discharged into terrestrial and aquatic environments.

To address this intricate pollutant, various conventional physico-chemical treatment methods are explored, which include flocculation, froth flotation, chemical coagulation, irradiation, ozonation, precipitation, photooxidation, adsorption, membrane filtration, ion exchange, and reverse osmosis [49, 87]. However, these techniques become limited because of operational problems, cost-expensiveness, and generation of a bulk quantity of secondary solid waste [49]. Moreover, due to the

complex aromatic chemical structure, persistent, and recalcitrant nature, treatment of dye contaminated effluent by conventional physico-chemical techniques is often not so effective [56]. In this context, the biological treatment route for decolorization is a promising, economic and environment-friendly alternative [5]. Several advantages of bioremediation technology are low running cost, minimum operational difficulties, on-site application, permanent waste elimination, minimum environmental impact, and opportunity to be used in integration with other physico-chemical treatment methods [10]. Through the biodegradation process, persistent pollutants are either completely degraded or are transformed into less hazardous components. Biodegradation involves the use of the biological agent for attaining at least one of the three consequences which include (a) a small alteration in an organic molecule keeping the primary chemical structure unchanged, (b) breaking of the multipart organic molecules in such a manner that the resulted components can be reorganized for the formulation of the initial molecule, and (c) complete mineralization.

Over the last decade, numerous bacteria, yeasts, fungi, algae, and actinomycetes have been explored to bioremediate the hazardous dyes [75, 76]. Among these microorganisms, mesophilic bacteria are mostly used as the biological agent for biodegradation of dyes, because of their comparatively rapid multiplication rate under anaerobic, aerobic, facultative environmental conditions [18, 100]. The bioremediation of dyeing agents using mesophilic bacterial strains will be comprehensively reviewed in the current manuscript concerning the mechanism of biodegradation and parametric influence.

## 2 Types of Synthetic Dyes in Effluents

Dyes are usually categorized depending on their chemical moieties and applications. Basically, dyeing agents are comprised of two prime units (a) chromophores, which are responsible for giving the specific color to any dye, and (b) auxochromes, which complement the chromophores through intensification of color helping the water-soluble dye molecules. These chromophores are various functional groups such as carbonyl, azo, aryl methane, methine, anthraquinone, nitro, etc. Moreover, auxochromes are a group of atoms that do not have the ability to provide any color, but when are present in addition to the chromophores as substituents, they either alter or intensify the color of the chromophores. Some commonly utilized auxochromes are sulfonate, amine, carboxyl, aldehyde, hydroxyl, and methyl mercaptan [28, 73, 89]. The dyes' classification depending on chemical structure and applications is highlighted in Table 1.

**Table 1** Categorical classification of dyes on the basis of chemical structure, dyeing process, and applications

Type	Structural unit	Characteristics	Applications	Examples
Acid dyes	Azo, nitro, nitroso, triphenylmethane, anthraquinone, xanthene, and azine	Anionic; aqueous soluble	Dyeing of nylon, wool, modified acrylics, silk, leather, paper printing, inkjet printing, cosmetics manufacturing, food industry, etc.	Methyl orange, methyl red, congo red, orange II, orange I
Azo dyes	Azo	Insoluble dyes are synthesized in situ in the fiber through treatment with both diazoic and coupling components	Used for dyeing polyester, rayon, cellulose acetate, and cotton	Butter yellow, Disperse orange 1, aniline yellow
Basic dyes	Acridine, diazahemicyanine, cyanine, diphenylmethane, hemicyanine, thiazine, triarylmethane, oxazine, and xanthene	Cationic; water soluble	Coloring of paper, acrylic fiber, and medicine	Amine yellow, malachite green, butter yellow, methylene blue
Direct dyes	Phthalocyanine, polyazo, oxazine, stilbene,	Dyeing is performed at mild alkaline bath by adding sodium salts	Coloring of cotton, silk, rayon, wool, nylon, leather, paper. Also applied for biological staining and pH indicators	Direct black, congo red, violet 51
Disperse dyes	Azo, nitro, styryl, benzodifuranone, and anthraquinone	Non-ionic; water-insoluble	Dyeing hydrophobic fibers, polyester, nylon, cellulose acetate, synthetic polyamide, polyacrylonitriles fibers	Celliton fast pink B, disperse blue 3, violet 1
Mordant dyes	Azo and anthraquinone	A mordant is required	Applied for the appearance of black or navy shades in silks and wool	Alizarin

(continued)

**Table 1** (continued)

Type	Structural unit	Characteristics	Applications	Examples
Reactive dyes	Anthraquinone, azo, phthalocyanine, triarylmethane, oxazine, formazan	A chromophore is utilized for directly reacting with fiber substrate	Coloring of cotton, wool, silk, nylon, cellulose fibers at ambient pH and temperature in home or art studio	Reactive red 120, reactive green 19, reactive violet 2, brilliant blue
Sulfur dyes	Sodium sulphide, disulfide	Treatment of the fibers in a solution comprised of sulfide compounds and organic compounds	Cost inexpensive and are generally utilized for dyeing cotton to bring dark shades	Sulfur green 12, sulfur brown 12, sulfur black 1
Vat	Indigoids, anthraquinone	Insoluble in nature. It gets reduced in alkaline environment to produce aqueous soluble metal salts. These salts will subsequently bind to the textile fiber. Further, on oxidation, they will reform to the initial insoluble dye	Used to bring indigo color	Tyrian purple, indigo blue, indigo white, bezanthrone

### 3 Toxicity and Harmful Effects of Dyes

Discharge of bulk quantities of synthetic dyes in the effluent can cause severe toxicity and aesthetic nuisance, which are the prime environmental concerns [78]. Improper release of dyes enhances the biochemical oxygen demand (BOD) of the aqueous system and limits the penetration of sunlight through the water surface that reduces the photosynthetic activity followed by inhibition to the proper growth of photoautotrophic organisms. Again, the color of the effluent is inconvenient to the aquatic organisms, retarding the oxygenation of the water. Moreover, the acute toxicity imparted by these colored effluents completely disturbs the ecological balance of the fauna and flora in aquatic bodies [94].

Synthetic dyes are mostly organic aromatic compounds, containing different functional groups and heavy metals. Apart from imparting toxic effects, these dyes are also mutagenic, carcinogenic, and teratogenic (because of which normal embryonic development is disrupted) to the aquatic lives [1, 65]. The azo dyes embodied with the substituent aromatic amines can cause a higher risk of chemosis, bladder cancer, contact dermatitis, skin irritation, vomiting gastritis, vertigo, hypertension, permanent blindness, exophthalmos, lacrimation, rhabdomyolysis, acute tubular necrosis

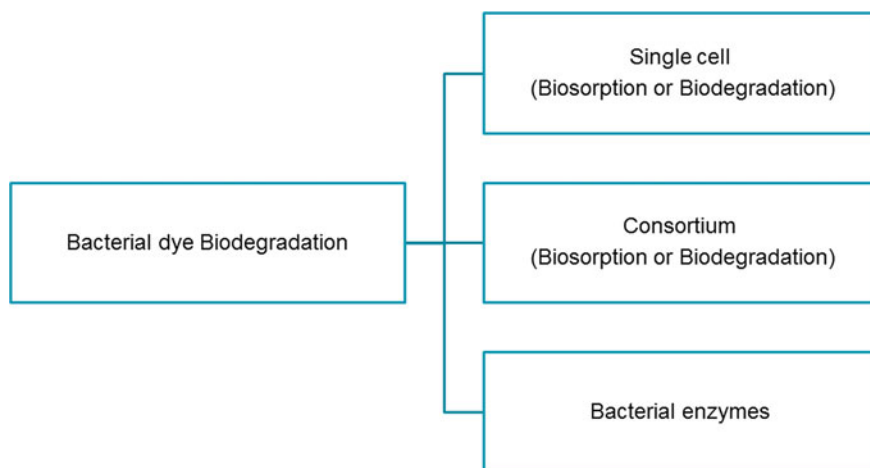
supervene, respiratory distress [62]. Basic dyes are potent clastogens, which can cause mutations, tumor growth, allergy, skin allergy, dermatitis, and also cancer [79]. Cationic dyes may also induce a heart attack, shock, cyanosis, jaundice, tissue necrosis, quadriplegia in human beings [60]. Moreover, heavy metals in the dye can induce chronic toxicity, resulting in kidney failure, ulceration of the mucous membranes and skin, etc. Thus, untreated or improperly treated dye polluted effluent can introduce extreme environmental and health complications once consumed through different food chains.

#### 4 Mechanisms of Dye Biodegradation by Biological System

The major mechanisms of dye decolorization from effluent through bioremediation route are (a) microbial biomass mediated adsorption (biosorption) and (b) inherent microbial enzyme system-mediated biodegradation [43]. In the case of the first mechanism, the adsorption of dyes can be done by either growing live microbial population or by the dead microbial cells. Adsorption of dye using biomass primarily occurs by ion exchange technique between the cell surface and the dye molecule [32, 64, 69]. When the effluent is carrying a relatively high amount of toxic pollutants or the environmental conditions are not promising or for proper cellular growth of microbes then the living microbial cells may not be much effective for dye degradation by using their inherent enzyme system. In such scenario, previously cultured microbial biomass can be applied to adsorb the dye by biosorption mechanism. On the contrary, the second mechanism involving biodegradation of recalcitrant dyes relies on the biotransformation enzymes present in various microbes, which are greatly dependent on the adaptability of microbes with the toxicity of the effluent.

Microbes such as, bacteria, fungi, and algae have a cellulosic cell wall that provides binding sites like carboxyl and hydroxyl groups essential for biosorption of dyes [93]. The dye molecules remain intact during biosorption, while during biodegradation, the primary dye structure is fragmented with the reacting enzymes, often achieving complete mineralization [71, 97]. Biosorption of dyes cannot eliminate the predicament because the dye remains adsorbed into the microbial biomass matrix. Henceforth, biosorption is specifically beneficial for such cases where dye biorecovery is a paramount concern. However, a combination of these two techniques (biomass-mediated biosorption and enzymes mediated biodegradation) is often appropriate to handle bulk quantities of dye-polluted industrial waste streams. Methods of bacterial dye biodegradation are schematically represented in Fig. 1. Extensive diversity of microorganisms which includes bacteria, algae, fungi, and yeasts are potent of biodegrading/decolorizing different dyeing agents. The isolation of new compelling microbial pure strains and understanding their dye degradation mechanism is an emerging biological research field for dye-containing effluent treatment [13].

In the case of enzymes mediated biodegradation, the oxidoreductive enzymes (reductive and oxidative) can generate reactive free radicals which can introduce complex sequences of cleavage reactions. These enzymes are most effective, where



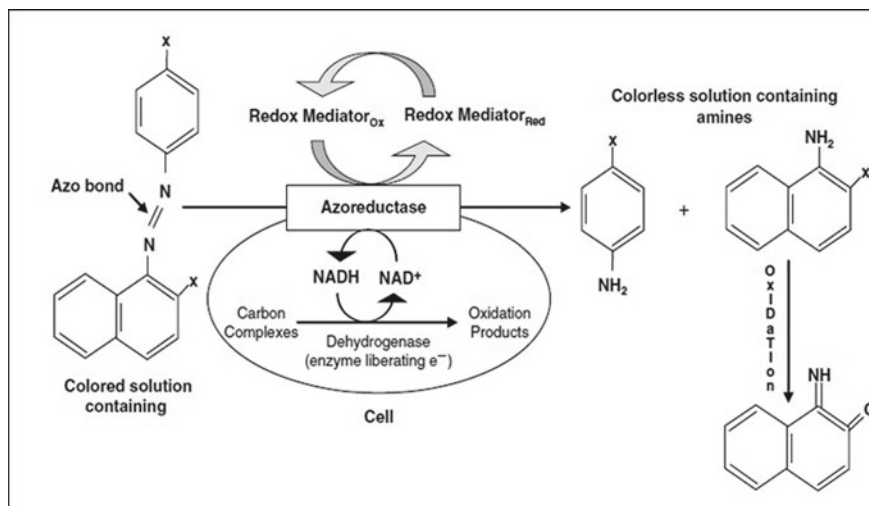
**Figure. 1** Methods of bacterial dye biodegradation

the presence of the target pollutant is maximum in terms of concentration compared to other competitive pollutants [67]. Several oxidoreductive enzymes have been used by the bacteria to decolorize and biodegrade dye molecules [76]. Azoreductase is one of the prevalent reductive enzymes responsible for dye degradation. Similarly, oxidative enzymes participating in dye biodegradation are tyrosinase, peroxidases, and laccases [25, 42]. Azoreductases are responsible for carrying out the reduction reaction of the chromophoric linkage ( $-N=N-$ ) present in azo dyes which helps to undertake the biodegradation of the dye and the formation of monochrome solutions as highlighted in Fig. 2 [41].

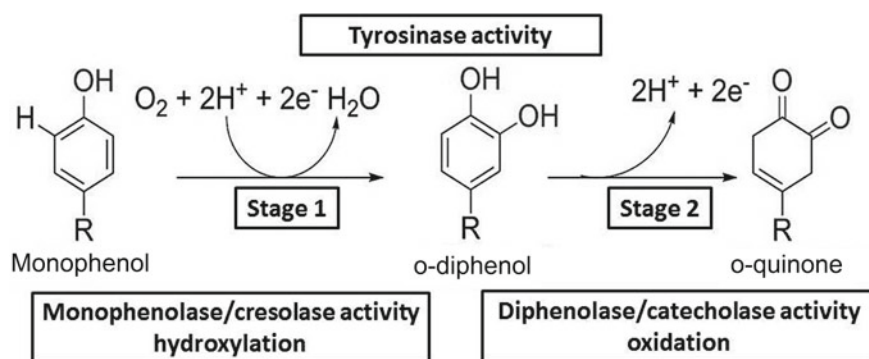
The intermediate metabolites are also degraded further aerobically or anaerobically [62]. Another enzyme named riboflavin reductase has been found to have the capacity to degrade the dyes by reducing various flavins [26, 76, 77]. The enzyme laccases (phenol oxidase) is responsible for the cleavage of the O–O bond of dioxygen to water. Peroxidases are heme-containing proteins capable of redox conversion processes, highly effective in degrading anthraquinone, a redox synthetic dye. Tyrosinase (monophenol monooxygenase) has the capability to degrade phenol group by oxidation using molecular oxygen as oxidant [33]. Monophenols are first converted to o-diphenols by hydroxylation and then o-diphenols are further oxidized to o-quinones (as shown in Fig. 3). However, the major concern here is the o-quinone, which can inhibit tyrosinase activity and regulates the reaction [76].

## 5 Role of Mesophilic Bacteria in Dye Biodegradation

The studies on dye biodegradation are mainly focused on bacteria because they are found to be more efficient and effective than other groups of microorganisms [45].



**Figure. 2** Bacterial enzymatic actions for azo dye degradation. Adopted from [82] under Creative Commons Attribution License permitting unrestricted use and distribution



**Figure. 3** Mechanism of tyrosinase activity to degrade monophenolic compounds

In comparison to the conventional chemical methods, bioremediation of dyes by bacteria is a much more environment-friendly and cost-inexpensive technique [57]. Bacteria perform the degradation of dye molecules after cleaving them into fragments with various enzymes and thus achieving complete mineralization by producing  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , biomass along with other inorganics [48]. Bacterial species of *Sphingomonas xenophaga*, *Flexibacter filiformis*, *Agrobacterium tumefaciens*, *Alcaligenes faecalis*, *Ralstonia eutropha*, *Proteus mirabilis*, *Hydrogenophaga palleronii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Lactobacillus plantarum*, *Bacillus subtilis*, *Rhodococcus erythropolis*, *Bacillus licheniformis*, and *Serratia marcescens* were



found capable to reduce azo dyes. The majorities of these species is mesophilic type bacteria and grow best in a moderate temperature ranging from 20 to 45 °C.

It was previously observed that the mesophilic bacterial strain *Pseudomonas aeruginosa* was competent enough to biodegrade the Direct Orange 39 dye at a concentration of 1,000 ppm/day [84]. *Thermomonospora sp.* and *Streptomyces sp.* were able to decolorize Poly B-411, Poly R-478, and Remazol Brilliant Blue R dyes (anthraquinonic). The *Streptomyces sp.* was also found promising in degradation of benzene derivatives by catabolic pathways. Several Streptomyces species can also decolorize dyes such as Orange I, 3-methoxy-4-hydroxy-azobenzene-4'-sulfonic acid, and 4 (3-methoxy-4-hydroxy-phenylazo)-azobenzene-3,4'-disulfonic acid [98]. Eskandari et al. [18] showed that microbial consortia consisting of mesophilic bacterial genera such as *Pseudoarthrobacter*, *Gordonia*, *Stenotrophomonas*, and *Sphingomonas* were effective in biodegradation of Reactive Black-5 azo dye [18]. Again, [14] revealed that the *yhdA* gene of *Bacillus subtilis* can encode an oxidoreductase dependent on flavin mononucleotide, which can induce cleavage of the  $-N=N-$  bond present in the azo dyes with the aid of NADPH [14]. Highly promising decolorization efficiency of 96.9–99.5% for Congo red (CR) was achieved by *Vitreoscilla sp.*, *Acinetobacter lwoffii*, *Pseudomonas fluorescens*, *Escherichia coli*, *Bacillus thuringiensis*, *Enterobacter asburiae* *Enterobacter. Ludwigii*, and *Enterobacter asburiae* [31].

*Xenophilus azovorans*, *Staphylococcus aureus*, *Acinetobacter calcoaceticus*, *Bacillus sp. OY1-2*, *Escherichia coli*, *Enterococcus faecalis*, *Pigmentiphaga kullae K24*, and *Rhodobacter sphaeroides* were extensively explored for having azoreductase enzymes to carry out the biodegradation of azo dyes [9, 88]. *Galactomyces geotrichum* MTCC 1360, *Proteus vulgaris*, *Micrococcus glutamicus*, and *Bacillus sp.* have expressed riboflavin reductase activity for the degradation of Reactive Green 19A, Brilliant Blue G, Navy Blue GL, Mordant Yellow 10, and Scarlet R dye [20, 37]. *Bacillus sp.* and *Acinetobacter calcoaceticus* expressed Lignin peroxidase activity. The phenol oxidase activity was found in *Micrococcus glutamicus* and *Pseudomonas desmolyticum*. Tyrosinase activity was observed in *P. desmolyticum* against Direct Blue-6 dye [39].

## 6 Strategies of Bacterial Dye Degradation

Effluents containing a cluster of structurally complex dyes are toxic for most aquatic organisms (flora and fauna) when discharged into the aquatic bodies manifesting anoxic conditions by reducing the dissolved oxygen concentrations. Several mesophilic bacteria species like *Aeromonas hydrophila*, *Bacillus cereus*, *Proteus mirabilis*, *Bacillus subtilis*, *Pseudomonas sp.*, and *Pseudomonas luteola* have showcased promising dye degradation efficacy as a single strain [38]. However, it is often tough to accomplish complete decolorization with a pure bacterial culture. Owing to the cooperative approach of the mixed bacterial cultures (consortium), an enhanced decolorization effect is often observed showing better result than single strains in

decolorization and biodegradation of dyes [81]. However, it is difficult to interpret the results of mesophilic consortium-based dye degradation because the mixed bacterial cultures do not bestow the meticulous understanding of the dye metabolism mechanisms and the experimental findings are often obscure to reproduce.

It was observed that the biodegradation rate of a mesophilic bacterial consortium is generally superior to a single bacterial strain due to versatile enzymes from multiple bacterial strains that can attack the target dye molecules at distinct positions or linkage [29]. Moreover, the co-existing strains are often utilizing the metabolites produced by the decomposition of the dye molecules and achieve complete mineralization [22, 76]. However, isolation of new adaptable and formidable pure bacterial strains with multiple dye degradation capability from the wastewater and environment is very crucial for achieving greater biodegradation efficiency. It is an exciting research opportunity for future industrial effluent treatment but it takes a prolonged time and hard labor to isolate such pure cultures from textile wastewater.

Nowadays utilization of bacterial biofilms for effluent treatment is popular. Biofilms are substantially organized surface-associated microbial cells that can be comprised of both single and multiple species of bacteria. The association of the cells is mainly attributed to their self-produced extracellular polymeric substances rich in cellulose, lipids, and polysaccharides [21, 30, 59]. Biofilms are highly resistant to various environmental stress factors, including an extremely elevated concentration of toxic contaminants, temperature, pH, and salinity than their planktonic single-cell counterparts [21, 30]. Hence, dye degradation using biofilm-producing bacteria such as *Bacillus subtilis*, *Pseudomonas fluorescens*, *Acinetobacter lwoffii*, *Bacillus thuringiensis*, *Enterobacter asburiae* are a very lucrative technique for dye polluted industrial effluent treatment [31, 54].

Numerous researches have revealed that a combination of an aerobic and an anaerobic system is a particularly reasonable approach for dye biodegradation [53]. It was found that the aerobic process with agitation is appropriate for bacterial growth but maximum dye decolorization/degradation is achieved in an anaerobic system [3, 89]. Under aerobic conditions, most mesophilic bacterial species are not competent to utilize the dye as a sole source of carbon. These bacteria generally require a secondary carbon source for growth and survival. Aerobic bacteria have oxidoreductive enzymes that can disrupt the dye molecules asymmetrically or symmetrically by deamination, desulfonation, and hydroxylation process. However, the enzyme azoreductase has shown proficiency at anaerobic environment. Anaerobic degradation of different azo bonded colorants achieved with facultative anaerobic and aerobic microbes were narrated in several previous studies [19, 23, 58, 61, 99]. Most of these microbial cultures are capable of growing in aerobic atmosphere although the degradation process was accomplished only under complete anaerobic environment [38]. Adequate decolorization and removal of the recalcitrant dyes can be accomplished in the anaerobic stage and the remaining auxiliary substrates may be mineralized in a subsequent aerobic step. Thus, the integrated approach involving anaerobic and subsequent aerobic treatment is predominantly proposed for degradation of dye polluted effluents [83]. In combination treatment process color removal efficiency varies from 75 to 96% [63].

Utilization of anoxic conditions, where the concentration of dissolved oxygen is lower than 0.5 ppm has also been found promising for the effective decolorization of several colorants using both facultative anaerobic and aerobic bacteria. However, this process requires other complex nutrient sources, which amplify the operating cost [76]. Several types of bioreactor (batch and continuous mode) configurations have been used for anaerobic systems using single/mixed bacterial species for dye-containing effluent treatment [86]. These include upflow anaerobic baffled reactors, anaerobic sludge blanket, trickle-bed reactor, rotating biological contactors, and activated sludge process [6, 73].

## 7 Parametric Influence on the Dye Biodegradation

Several environmental and operational parameters, including dye concentration, the structure of dye, pH, temperature, supplementation of different nitrogen and carbon sources, oxygen, level of agitation, greatly influence the dye biodegradation performance. For making the treatment process highly efficient, rapid and practically feasible, prior optimization of each parameter for the bacterial remediation of dye is necessary.

### 7.1 *Effect of Dye Concentration*

The dye degradation rate progressively declines with the enhancement in the dye concentration probably because of the toxic effect of hazardous dyes to microbes or/and deficient dye to microbial cells ratio, along with the obstruction of the enzyme (azoreductase) at its active site by dye molecules [91, 92, 24]. Though, the toxicity to microorganisms is primarily related to the concentration of dye and the type of dye. Reactive and metal-complex dyes (such as Acid Black 172, Irgalan Black RBLN, Irgalan Blue 3GL, Irgalan Grey GLN) are found to exhibit exaggerated toxicity on bacterial bioremediation process [17, 52, 53].

### 7.2 *Effect of Dye Structure*

Dye possessing a simple chemical structure and less molecular weight exhibits a higher decolorization rate. Moreover, the characteristics of substituents on the aromatic ring present in the dye molecules have portrayed a significant impact on the oxidation phenomenon. For instance, with the presence of electron-giving methoxy and methyl substituents, the enzyme-mediated biodegradation of dyes is facilitated. On the contrary, the presence of electron-receiver substituents ( $-\text{SO}_3\text{H}$ ,  $-\text{SO}_2\text{NH}_2$ , fluoro, chloro, nitro) at the phenyl ring's para position with reference to azo bond will

restrict oxidation process and reduce the dye removal rate [34, 85]. Moreover, the susceptibility of azo bond for degradation is promoted if the substituent is present at the para position of the phenyl ring compared to the ortho and meta positions [34]. Additionally, in case of monoazo dyes, the dye degradation rate is faster than diazo and triazo dyes [35]. Metal-ion-containing dyes may impose intricacy in the biodegradation process, and eventually lowers the degradation efficacy [11].

### ***7.3 Influence of Nitrogen and Carbon Sources***

The dyes remain mostly inadequate in nitrogen and carbon sources, because of which dye biodegradation without additional supplement of these nitrogen and carbon sources is challenging. Single bacterium as well as consortium generally demands either or both carbohydrates and multipart organic sources (yeast extract, peptone) for effective degradation [45, 72]. Since among different carbon sources, glucose is readily available and highly effective for microbial metabolism, its inclusion enhances the efficiency of biodegradation [4]. Peptone, yeast extract, and urea are good nitrogen sources from organic origin that can be supplemented to restore the NADH, that plays the role of an electron donator to undertake the reduction of dyes using microbial agents, and thereby higher dye degradation can be achieved [12].

### ***7.4 Effect of pH***

The pH level controls the transport of dye molecules across the membrane of microorganisms' cells. This is contemplated as the rate-controlling step of the degradation process [7]. Microbial dye degradation rate is greater at optimized pH and follows a declining trend at extreme alkaline or acidic pH 6–10 pH is optimum for bacterial decolorization of dye [27, 46]. However, as most of the textile industrial processes are undertaken at alkaline (high pH) conditions, the sustainability or tolerance of mesophilic bacterial strains to high pH condition is recommended markedly.

### ***7.5 Effect of Temperature***

Temperature is another vital parameter for any processes related to microbial vitality [8], including the remediation of dye polluted wastewater by mesophiles. Studies related to microbial decolorization reported that the decolorization rate enhances up to a certain optimum temperature range (25–40 °C). However, enhancing the temperature beyond the optimum value will drastically reduce the dye biodegradation rate. This is probably attributed to the denaturation of azoreductase enzyme or the damage of cellular integrity at extreme temperatures. In case of dye degradation at

extremely high temperature, utilization of thermophilic bacterial strains shows better performances compared to the mesophilic ones [74, 75].

## ***7.6 Impact of Oxygen and Shaking***

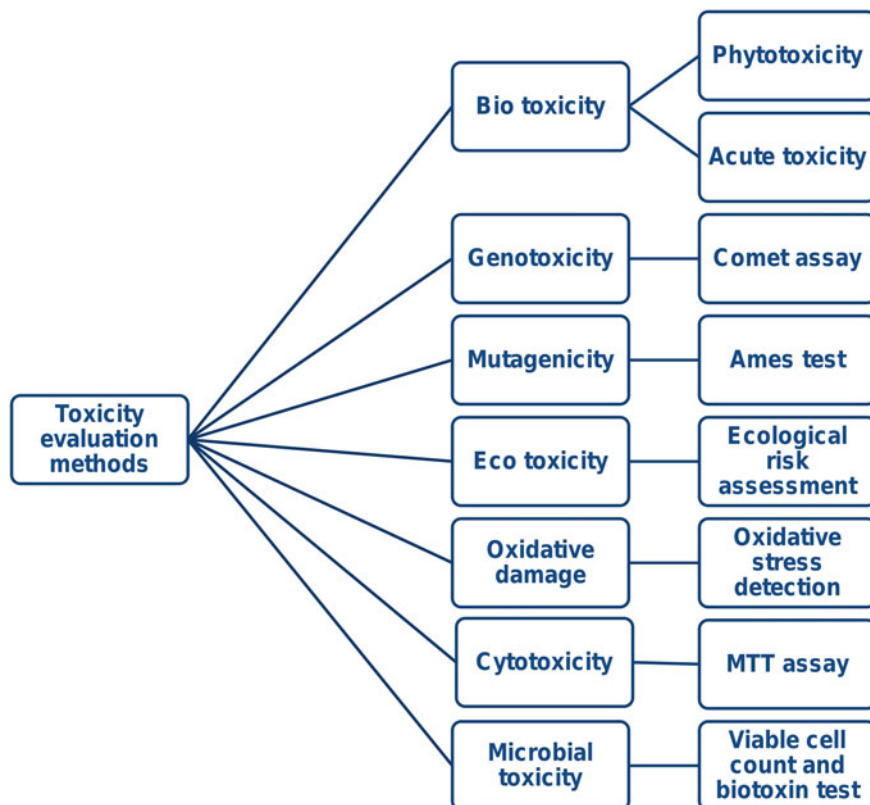
The degradation of dyes was more proficient under strict anaerobic conditions, though it can also be performed in semi-anaerobic environment [96]. When dye degradation process is operated at anaerobic conditions, activities of reductive enzyme are higher, which facilitates to break the complex dye structures. Dissolved oxygen acts as an inhibiting agent to the reduction process of dye. This inhibition effect of oxygen can be indirectly validated by comparing the efficacy of decolorization process performed under shaking and static environment [40]. Inefficient decolorization and degradation were evidenced at shaking/agitated environment, as improved oxygenation was provided through shaking. This indirectly manifests that the oxygen imposes inhibition effect to microbial-induced degradation mechanism.

## **8 Toxicity of Dye Degradation Products**

In some particular cases, the degraded products of dyes are found hazardous, mutagenic, and carcinogenic type. The anaerobic bacterial population present in the lower gastrointestinal tract of mammals can reduce the ingested dye molecules. In the intestinal tract, the reduction of azo dyes by anaerobic bacteria can generate acyloxy amines as a dye degradation product, which is carcinogenic and often leads to bladder cancer. The acyloxy amines are converted to carbonium and nitrenium ions that can attach with RNA and DNA of somatic cells provoking the mutations to form malignant tumors [47]. Several other moieties like benzidine, 1-amino-2-naphthol, o-tolidine, and 1, 4-phenylenediamine are also harmful [43]. Similar compounds may be generated during dye-contaminated effluent treatment and may cause toxic health hazards to both plants and animals. Hence, it is advisable to inspect various toxicity levels (Fig. 4) of the degraded dye products after bioremediation and before effluent discharge.

## **9 Future Prospective**

Accumulation of dye in wastewater creates environmental pollution and health-related problems to plants and animal kingdom present in the biosphere. Biodegradation of dyes present in effluents using diverse group of mesophilic bacterial strains has evolved as a promising strategy. As environmental policies are becoming stringent by the regulating authorities, a compelling requirement to develop ecofriendly,



**Figure. 4** Various types of toxicity assessment methods for the degraded dye products

cost-effective, and technically efficient treatment methods is paramount. Bioremediation using single and mixed bacterial consortium are environmentally benign and cost-effective strategy for the decolorization of effluents discharged by industrial facilities.

Further improvement of the degradation potential of mesophilic bacteria may be achieved through divulging them gradually to elevated concentration of dyeing agents, where they will adapt and evolve. Also, genetic modification of mesophilic bacteria is another interesting tool for improving the dye degradation potential. Hence, research on the regulation of genes and proteins present in various bacteria and the critical analysis of their effects may be further explored for selecting the microbial agent with greater biodegradation proficiencies. Presently, many esteemed laboratories worldwide including sophisticated laboratories in developing countries like India are actively involved in the progressive research for better dye biodegradation using various mesophilic bacterial strains.

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