Review paper

Role of Microbial Diversity in Textile Dye Degradation: A Comprehensive Review

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Abstract: About 80% of the dyes from the textile industry are directly dumped into the water bodies without prior treatment. This leads to the accumulation of these harmful compounds in the water bodies, which causes serious damage to the biota. The current physicochemical processes for effluent treatment have limitations due to high energy consumption, inaccurate technical optimization, and chemical cost. Moreover, the by-products of these processes are usually more harmful than the original dyes. Biotechnology offers a promising solution to this issue through the application of microbes for dye degradation. This approach allows cost-effective, eco-friendly detoxification without the excessive production of toxic by-products. Enzymatic degradation and biosorption are the two major strategies by which microbial-based dye degradation is carried out. This review describes the challenges posed by synthetic dyes and how various microbial species (bacteria, algae, fungi, and yeast) are involved in their degradation. Various emerging technologies, including microbial consortia, engineered microbes, material-assisted, and artificial intelligence (AI) based degradation strategies, are being developed for enhanced microbial efficiency. This review will demonstrate these cutting-edge dye degradation techniques, important bioreactor configurations used for this purpose, and the future directions towards the implementation of sustainable detoxification processes.

Keywords: Dye Degradation; Biodegradation; Textile Dyes; Microbial Degradation; Wastewater Treatment; Industrial Effluents

1. Introduction

Throughout the history of human civilization, dyes have been a crucial part of every industry. Whether it was art and fashion or trade and cultural expression, dyes have always played an important role. Historically, various natural products were utilized for the extraction of natural dyes, including plants, animals, and minerals [1]. Today, synthetic dyes extracted from chemical compounds are commonly used as they are more economical, stable, and versatile. The textile industry is one of the biggest producers of liquid pollutants because of the bulk quantities of liquid effluents produced from the dyeing process. About 80% of the dyes after being used in the textile coloring process, are directly dumped into the water bodies without prior treatment to reduce their harmful effects [2]. This results in a severe water pollution crisis. This wastewater produced from the textile industry carries a variety of toxic substances, including heavy metals, pigments, and organic chlorine compounds. This leads to the accumulation of these harmful compounds in the aquatic environment, causing serious damage to its biota [3]. Azo dyes account for the 70% of the entire organic textile-based effluent [4]. These azo dyes are composed of azo chromophores, which are light-absorbing functional components that produce a range of colors in these dyes. However, azo chromophores also cause severe damage to the aquatic systems by altering their chemical oxygen demand, color, salinity, and pH [5]. Only 47% of these dyes are biodegradable in nature, which poses a serious threat to the water-based habitats [6]. Moreover, some of these dyes are also known to be linked with various health problems in humans, like dermal irritations, allergies, respiratory problems, and even tumors and cancers [7]. Thus, in the last two decades, scientists have worked intensively to put forward different physicochemical and biological remediation strategies to deal with this major environmental threat.

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Currently, Advanced Oxidation Processes (AOPs) are one of the most primarily utilized physicochemical processes for the degradation of industrial dyes. Ozonation, Fenton's reagent, and photocatalysis have been observed to be most promising due to their ability to degrade even the smallest concentrations of dyes [8]. However, the energy consumption, precise technical optimization, and the cost of chemicals (H_2O_2, O_3) are the major limitations in these processes [9]. Adsorption techniques using active carbons have also been promising in the removal of colors from the industrial effluents, but they are also very costly. It has been estimated that the cost of traditional adsorption is >50% higher than the microbial-based adsorption due to the expensive adsorbent materials [10]. Other physicochemical processes include membrane separation, filtering, chemical oxidation, and coagulation, which are cost-effective but are energy-intensive and of limited efficacy [11]. Sometimes, the by-products produced by these processes are more harmful compared to the original dyes [12].

On the other hand, biological processes have been proven to be cost-effective and eco-friendly in nature with strong biosorption and dye mineralization [13-15]. In these methods, microbes like fungi, bacteria, and yeast are usually employed for the degradation purpose. The remediation strategy utilizes the microbial enzymatic pathways, including oxidoreductases like laccases, peroxidases, and azoreductases for the degradation purpose. For example, scientists were able to degrade Brilliant Green and Congo Red dyes up to 74-82% using bacteria (*Klebsiella aerogenes*, *Enterobacter sp.*) due to their ability to produce azoreductase enzyme [16]. Based on the microbial capability, degradation can either be complete or partial into compounds with milder toxic effects. The microbial dye degradation process involves microbes in various forms, including entire microbial cells, extracted enzymes and metabolites, nano-sized, and dried powdered forms. Dried or nanosized forms are generally employed as biosorbents [17]. Whereas, the entire microbe or enzymes are usually involved in the active breakdown of the chemical bonds and modification of complex dye molecules. Moreover, numerous studies have shown that microbial consortia consisting a mixture of different microbial cultures are more efficient as compared to the single microorganism [18]. In some cases, these approaches involving microbial consortia have reported a degradation efficiency of up to 98% [19]. These consortia are further engineered to enhance their environmental resilience and enzyme-producing capabilities. Another promising strategy is in-silico prescreening that allows the scientists to use techniques like molecular docking and homology modeling to select the most promising enzymes for degradation purposes [20, 21].

This review specifically aims to discuss various real-world microbes-based strategies for the sustainable bioremediation of environmentally toxic dyes.

1.1 Literature Search Strategy

A general narrative literature review approach was adopted in the writing of this article. Primarily, Google Scholar was used to identify the relevant studies. In addition, a general web search was also done and peer-reviewed studies were selected. A number of keywords, including "dye degradation," "microbial dye degradation," "fungal dye degradation," "phycoremediation," "bacterial dye degradation," "sustainable wastewater treatment," "nano-enabled," "AI-assisted bioremediation strategies" and related terms were searched. The time range was mainly limited to the articles from 2017 onwards. Special emphasis was given to the literature published between 2022 to 2026 in order to ensure the selection of studies reporting recent advancements. An iterative selection process was carried out while screening the studies, including reading the titles, scanning the abstracts, and skimming the full texts to select the relevant articles. Moreover, the reference list was also observed to locate and select additional relevant articles. The selection criteria were based on relevance. The studies on microbial-based dye degradation processes and their implications for environmental sustainability were identified. Both research and review articles were considered since this is a narrative review, so a formal systematic review protocol or meta-analysis framework was not adopted.

2. Textile Dyes: Classification & Challenges

The textile pigments are generally characterized into natural and synthetic dyes. Natural dyes, as the name indicates, are naturally produced pigments by natural sources like plants, insects, and minerals. Synthetic dyes, on the other hand, are man-made dyes synthesized from non-renewable organic sources, mainly petrochemicals and coal tar [1]. Due to their versatile color range, consistency and cost effectiveness, synthetic dyes are the preferred option in the market [22]. These dyes are different from one another based on their stability, molecular structure, and bonding capacity with the fiber. These dyes, on the basis of their solubility in aqueous solutions, are classified into soluble dyes and insoluble dyes. Soluble dyes generally contain a charge. The molecules containing a positive charge are called cationic dyes, which mainly include basic dyes. The negatively charged dyes are known as anionic dyes. These are further categorized into acid, reactive direct, and mordant dyes. The insoluble dyes are classified into disperse, sulphur, vat, and solvent dyes on the basis of their chemical structure and mode of application on the fiber [23]. In the textile industry, azo, anthraquinone, and triphenylmethane dyes are the most commonly utilized classes of dyes and are also the most difficult to degrade [24]. The major challenge in their degradation process arises from the aromatic nature of their structure, which makes them highly stable against light, water, and chemical treatments [25]. Thus, scientists are now looking forward to exploring microbes for targeted degradation purposes. In the microbes-based dye degradation process, mainly three types of enzymes are involved. Laccases utilize molecular oxygen to oxidize phenolic groups. Peroxidases (like LiP and MnP) employ hydrogen peroxide (H_2O_2) as an electron acceptor for the degradation of complex dye structures. The particular target of Azoreductases is the nitrogen-nitrogen double bonds of azo dyes, which produce colorless amines after undergoing a multi-electron transfer process (Figure 1). In short, via these biocatalytic reactions, toxic synthetic dyes are reduced into simpler and often innocuous metabolites.

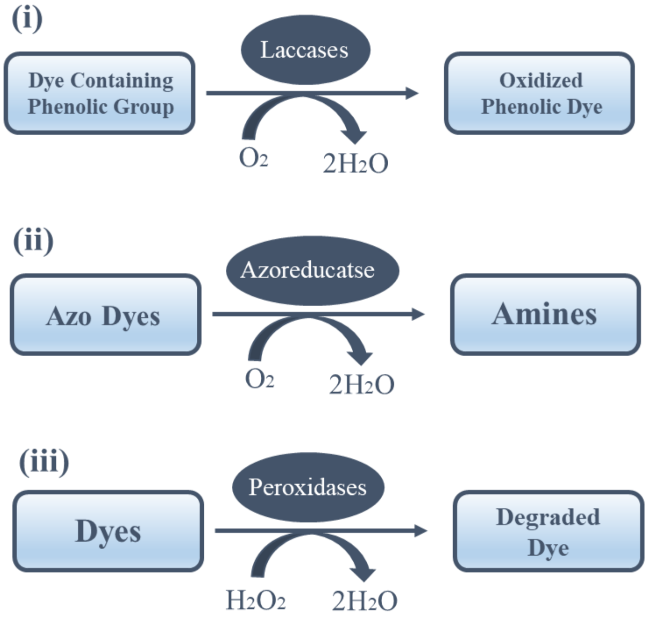

Figure 1. Enzymes responsible for dye degradation: (i) laccase, (ii) azoreductase, and (iii) peroxidases [26].

3. Mechanisms of Microbial Dye Degradation

The microbes-based degradation of dyes takes place via a multi-step procedure that involves enzymatic transformation, followed by the complete detoxification and mineralization of the dyes. In this procedure, the first step is usually the biosorption of the dye molecules. The dyes either get bound to the surface of the microbial cells or get absorbed within it. This allows the enzymatic interaction, which is crucial to initiate the degradation process. After this, the primary degradation step takes place, which is also referred to as the decolorization step. At this stage, enzymes directly target the chromophoric groups which are responsible for imparting the color. In azo dyes, this occurs under anaerobic conditions, resulting in the reductive cleavage of the azo bond (–N=N–) via azoreductase enzymes. However, in different dyes, the degradation pathways differ based on the structure of the dyes. In the anthraquinone dyes, there are fused aromatic rings and quinone groups, which make it difficult for the reductive cleavage to occur. In such cases, oxidative degradation occurs via enzymes like laccases and peroxidases. Similarly, in triphenylmethane dyes, oxidative mechanisms disrupt their stabilized structure, comprising a central carbon atom bonded to three aromatic rings. Despite the structural differences, a common principle is that the most reactive structural feature of each dye is targeted by the microbial enzymes. The primary degradation step results in the production of intermediate products like aromatic amines. These intermediate compounds are comparatively more toxic than the original dyes. Thus, secondary degradation becomes essential. This step takes place under aerobic conditions. Oxygen-dependent enzymes like oxygenases, laccases, and peroxidases, etc., transform the toxic intermediate into simpler aliphatic compounds via reactions like oxidation, deamination, and aromatic ring cleavage. This is followed by the final mineralization step, which results in the ultimate transformation of these products into inorganic end products like CO_2, H_2O, and NH_3. As this process requires varying environmental conditions in every step, treatment plants usually adopt a sequential anaerobic–aerobic process in which initial reductive decolorization is followed by oxidative detoxification (Figure 2).

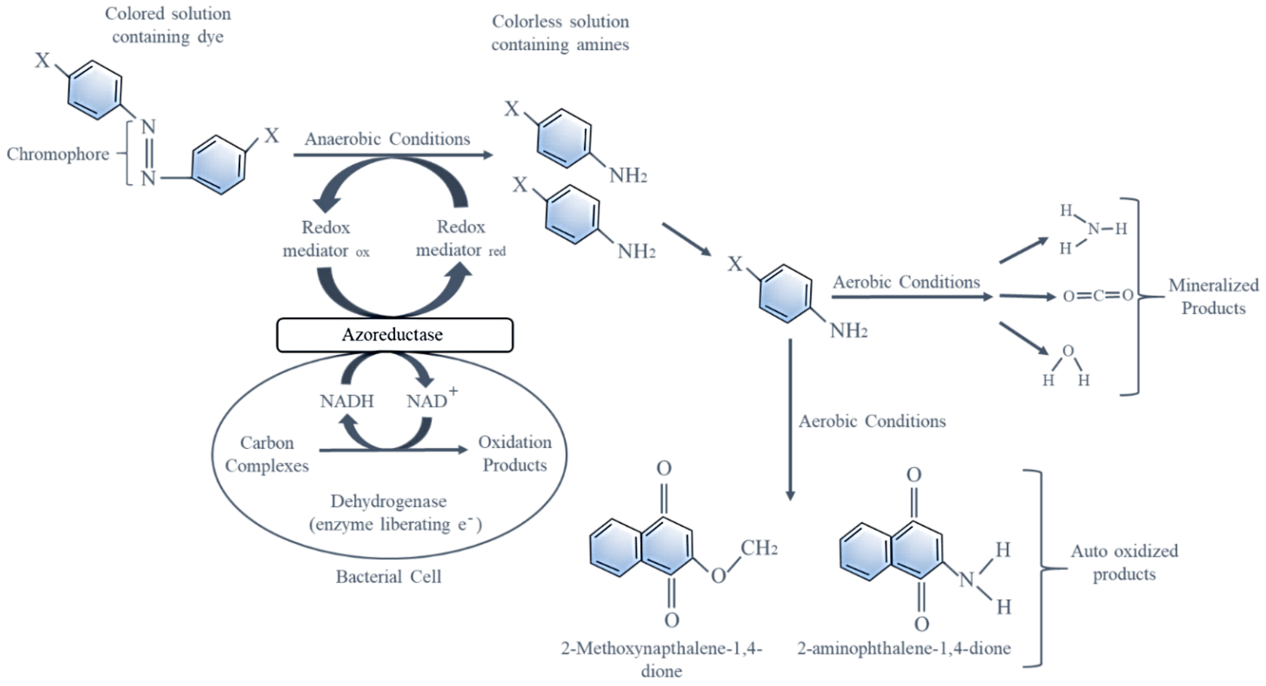

Figure 2. Flow diagram of dye degradation process [27, 28].

3.1 *Bacterial-Based Dye Degradation*

Bacteria are one of the most versatile and commonly utilized microbes for the degradation of textile dyes [29]. In the bacterial dye degradation process, the dye molecules are initially adsorbed by the bacterial cell, which is then subjected to intracellular or membrane-based enzymatic degradation. Oxidoreductases like azoreductases, oxygenases, peroxidases, etc., are generally involved in this step, which cleaves the chromophoric bonds like azo, C-N, and conjugated C=C bonds and decolorizes the molecules. These decolorized intermediate molecules are then further processed via ring-cleavage and metabolic pathways into less toxic and simpler end products [30]. To prevent the discharge of any residual toxic compounds, post-treatment steps are equally important. For this purpose, the biomass is typically separated via processes like sedimentation or coagulation-flocculation. This is followed by a filtration step, which generally involves membrane-based separation like microfiltration or ultrafiltration for better colloid removal. To further enhance the removal of any persistent recalcitrant intermediates, optional post-treatment steps like adsorption onto activated carbon or advanced oxidation are also applied [31, 32]. Bacterial dye degradation is particularly effective due to their metabolic diversity, adaptability, and ability to allow both reductive and oxidative degradation steps to occur under varying redox conditions. This makes them effective for a wide range of dye types, including azo, anthraquinone, triarylmethane [33]. As per the previous studies, a clear difference in the bacterial degradation efficiency was observed based on whether single strains, consortia, or engineered systems were utilized. For example, triphenylmethane dyes like malachite green and crystal violet were completely decolorized via Serratia sp. WKD within 12 h even under extreme pH (3-10) and temperature (5-60 °C) conditions. This degradation was primarily carried out via MnP (Manganese Peroxidase) and NADH-DCIP reductase (Nicotinamide adenine dinucleotide (NADH)-2,6-dichloroindophenol reductase) enzymes through

N-demethylation and hydroxylation pathways, which highlights their enzymatic adaptability [34]. Conversely, *Paenibacillus dendritiformis* demonstrated up to 99% degradation of azo dye Acid Red 114 via azoreductase-based azo bond cleavage. Although it took a comparatively longer degradation duration (72 h), it also demonstrated biofilm formation and reduced phytotoxicity, which highlights its potential to be utilized as a sustainable biocatalyst [35]. Moreover, using bacterial strains in the form of consortia revealed a promising synergistic dye degradation potential. This was demonstrated by Elnabi et al. [36] in their comparative study. Under optimized conditions, their consortium achieved a much higher removal efficiency of 98.56% as compared to the individual strains (38.55-45.13%). Moreover, the consortium also demonstrated a reduction in the phytotoxicity from 66.38% to 21.38%, biotoxicity from 86.7% to 23.3%, and cytotoxicity from 55.31% to 14.45%. Immobilized bacterial cells have shown higher stability and usability than the free cell systems. Purnomo et al. [37] have demonstrated a significantly higher removal efficiency (up to 97%) as compared to planktonic cells for dyes like methyl orange. Thus, immobilized bacterial approaches can be very effective for continuous treatment applications. Overall, while the bacterial strains allow a rapid and condition-tolerant treatment process, incorporating the most robust strains into consortia and immobilized systems has been proven to be more effective for optimal dye removal [38]. The highly variable nature of dyes and their intermediate compounds has the potential to induce oxidative stress in bacterial cells. Thus, the application of extremophilic bacteria with the ability to endure harsh surrounding conditions is becoming increasingly popular in bioremediation strategies, including dye degradation (Table 1).

3.2 Algal-Based Dye Degradation

Algae are photosynthetic organisms that are mainly found in aquatic habitats. They are characteristically important for the dye degradation process because of their autotrophic nature. Unlike bacteria, which require an external substrate, algae utilize carbon dioxide (CO_2) dissolved in water as a carbon source. This significantly reduces the operational costs while also simultaneously fixing CO_2 [46]. This was demonstrated by a comparative life-cycle sustainability assessment of algal and bacterial wastewater treatment systems. In this study, the reduced energy requirements by the phycoremediation systems resulted in reduced operational cost (0.18 €/m3) compared to the bacterial systems (0.26 €/m3) [47]. The process of algal-based dye removal takes place via a combination of biosorption, enzymatic transformation, and photo-induced redox reactions (Figure 3). These processes are often sequential and synergistic and collectively degrade complex dye molecules. Firstly, the molecule is adsorbed by the algal cell wall via its functional groups (-OH, -COOH, $-NH_2$). This is followed by cleavage of those molecules via algal enzymes like azoreductases, laccases, and peroxidases, etc. For example, in the case of azo dyes, their -N=N- bond undergoes reductive cleavage, resulting in aromatic amines in low oxygen or anaerobic conditions. On the other hand, in phototrophic conditions, algae produce reactive oxygen species (ROS), which leads to the oxidative degradation of the conjugated aromatic structures within the dye molecules. This results in the partial or complete degradation. [48, 49]. *Chlorella*, *Scenedesmus*, and *Chlamydomonas* are most commonly utilized species due to their resilient nature and removal capabilities [50]. The post-treatment steps involve the valorization of the biomass for biofertilizers or biofuel production. Algae can also simultaneously remove nitrates and phosphates from the wastewater in addition to the dyes. The resulting biomass can then be processed into biofuels or fertilizer. This presents a promising model for the circular economy [51]. Another post-treatment strategy is photodegradation, in which light (usually solar or UV radiation) is utilized for the final breakdown of any persistent recalcitrant compounds [52, 53]. A contrast in the speed, efficiency, and operational complexity has been observed on the basis of the free-cell and immobilized algal systems. For example, in a study by Duong et al. [54], *Chlorella vulgaris* achieved 95% decolonization of wastewater under optimized conditions. This indicates a low-cost and eco-friendly solution for textile dye remediation. However, the treatment kinetics are extremely low as the process took 10 days, with just ~62% dye being removed in the first 5 days. This limitation is addressed by adsorption-based materials, which allow a faster removal rate by capturing and degrading the dye molecules. For example, in a comparative study by Majhi et al. [55], immobilized *Chlorella pyrenoidosa* removed up to 98% of dye from real industrial wastewater within a contact time of 180 minutes. This was much higher than the degradation achieved by the free algae (52–87%) within the same duration. The efficiency of the material-based algal dye degradation strategy is further enhanced by using modified materials. For example, a similar removal efficiency (95.92%) was achieved in a much shorter contact time (60 minutes) by algal-derived silver nanocomposite (nAgBC) [56]. In short, while free algal cells are promising, adsorption-based and engineered materials offer better treatment kinetics with operational feasibility. Nevertheless, there are challenges pertaining to the economic feasibility and large-scale implementation [57]. Thus, further research and development is crucial for bridging this gap between environmental sustainability and practical efficiency.

Table 1. Studies focusing on extremophiles for dye degradation

Extremophiles	Type	Target Dye(s)	Dye Class	Degradation Efficiency (%)	Enzyme(s) Involved	Time Taken	Isolation Site	Optimal pH	Optimal temperature (°C)	Reactor type	Industrial relevance	Ref
Salinivibrio kushneri HTSP	Halophilic	Coomassie brilliant blue G-250, Congo red, Safranin	Triphenylmethane, Azo dyes	>80%	FMN-dependent NADH azoreductase Clade III	48h	Saltpan	7.4	37 °C	Batch (shake flask)	Textile, biomedical research	[39]
Bacterial consortium (*Enterococcus*, *Enterobacteriaceae*, *Staphylococcus*, *Bacillus, Kosakonia*)	Halophilic	Congo Red, Direct Black G (DBG), Amaranth, Methyl Red, Methyl Orange	Azo dyes	100%	NADH-DCIP reductase, laccase, azo reductase, manganese peroxidase, lignin peroxidase	8-24 hours	Salt fields	7.0	37 °C	Batch (shake flask)	Textile, paper, rubber, plastics, laboratories	[40]
Bacillus licheniformis B3-15; *Bacillus* sp. S7s-1	Thermophilic	Methylene Blue (MB)	Thiazine dye	>50% (initial screening); Optimized: 96% (B3-15), 67% (s7s-1)	Laccase + Lignin peroxidase (B3-15); Laccase only (s7s-1)	48 h	Shallow hydrothermal vents	5.2 (B3-15); 4.0 (s7s-1)	45 °C	Batch (biofilm immobilized on polypropylene perforated balls)	Textile, paper, cosmetic, pharmaceutical	[41]
Consortium CJ (*Parageobacillus* and *Geobacillus* spp.)	Extreme-thermophilic	Acid Orange 7 (AO7)	Azo dye	96%	Azoreductase, Laccase (Lac), Manganese peroxidase (mnp); functional genes: FMN-dependent NADH-azoreductase, FMN reductase	36 h	Textile wastewater	7.0	75 °C	Not specified (flask-scale)	Textile, cosmetics	[42]
Salinicoccus roseus HPK1, *Bacillus altitudinis* HPK3, *Staphylococcus warneri* HPK4	Halophilic	Methyl Red; Methylene Blue	Azo dyes	67–75% (Methyl Red), 56–63% (Methylene Blue)	Azoreductase	5 days	Coastal marine sediments	8.0	40 °C	Batch (static)	Textile, printing, research	[43]
Marinospirillum alkaliphilum strain N (with Cu nanoparticles)	Alkaliphilic	Acid Red 88 (AR88)	Azo dye	100%	Intracellular and extracellular enzymes	11 h	Textile effluent	7.0	30-37 °C	Batch (shake flask)	Textile, leather, paper, ink	[44]
Marinobacter sp. Strain HBRA	Halophilic	Direct Blue-1 (DB-1)	Azo dye	100%	Azo reductase	6 h	Sea water	8.0	37 °C	Batch (static, flask)	Textile, paper, rubber, plastics	[45]

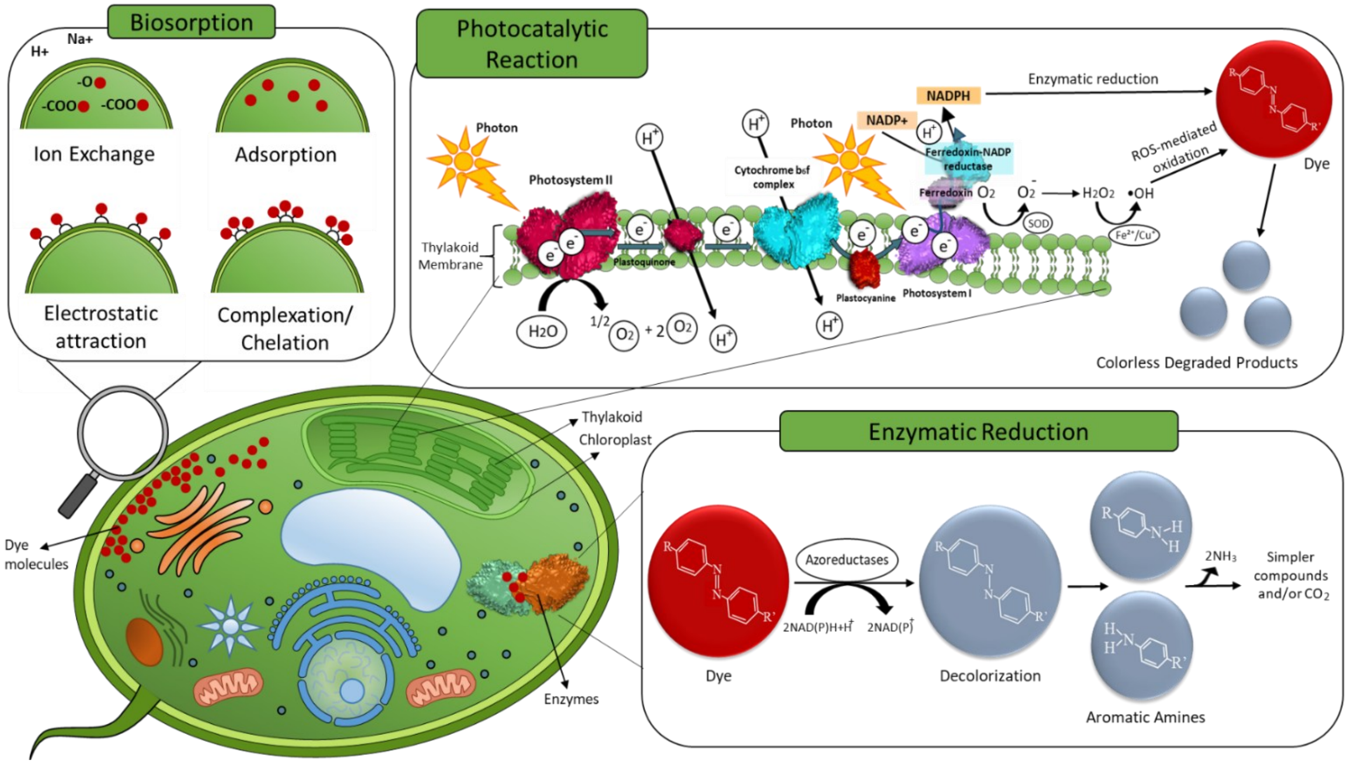

Figure 3. Mechanism of Algal Dye Degradation (concept taken from [58, 59]).

3.3 Fungal-Based Dye Degradation

Fungi have been studied for their application in the detoxification of dye-polluted water systems. The area that deals with using fungi for environmental remediation purposes is termed mycoremediation. Fungi possess the ability to survive extreme environmental conditions, including high concentrations of dyes (25 to 2000 mg L-1), heavy metals (up to 8000 ppm), and the presence of toxic aromatic intermediates (e.g., up to 50 mg L-1 o-tolidine), which are commonly present in textile effluents [60-62]. This makes them an ideal candidate for dye degradation [63]. The various mechanisms involved in fungal-based dye breakdown include biosorption, bioaccumulation, and biodegradation processes. Biodegradation is the primary mechanism, which is usually carried out by the production of lignolytic enzymes such as Laccase, Manganese peroxidase, and Lignin peroxidase [64]. These enzymes are capable of treating dyeing wastewater, particularly azo dyes, by attacking and cleaving their bonds. In this process, weaker bonds are usually cleaved to produce mildly toxic or atoxic compounds, which could then either be discharged into the water bodies after safety assessment or utilized for other purposes like biofertilizer or bioenergy production [65]. Some fungal species, like *Cunninghamella elegans* and *Trametes versicolor,* have demonstrated a superior efficiency to remove dyes as compared to traditionally employed activated carbon [66]. This is mainly due to the presence of diverse functional groups on the cell wall of the fungal cells (proteins, chitin, carboxylic acids), which allows them to capture dye molecules via various mechanisms, including electrostatic, complexation, and ion-exchange, etc. Moreover, fungal enzymes also carry out dye degradation in addition to the biosorption, making the mycoremediation process comparatively better than activated carbon, which only adsorbs molecules via physical adsorption, such as van der Waals forces. Moreover, its exorbitant cost also limits the general usage of activated carbon [67]. White rot fungi are one of the most promising fungal groups for industrial wastewater treatment due to their ability to degrade complex compounds, such as lignin, by multiple enzyme production. Their ability to produce enzymes that are non-selective and non-specific oxidants, such as laccase, lignin peroxidase, and manganese peroxidase, facilitates the removal of recalcitrant compounds (Figure 4) [68]. The ligninolytic activity of white rot fungi depends upon various factors, including process parameters and species. A well-known white rot fungal species, *Phanerochaete chrysosporium*, initially demonstrated an 84.8% decolonization efficiency in reactive dye wastewater. Upon carbon supplementation and optimization of environmental conditions (pH 5, temperature 30 °C, and inoculum size 4 mL), this efficiency reached up to 90%.

This highlights the role of environmental conditions on the enzymatic activity of these species [69]. Similar observations were made by Fayyaz et al. [70]. They opted for a two-step optimization protocol with initial single-factor optimization and the subsequent Response Surface Methodology. This significantly enhanced the manganese peroxidase activity from 145 to 194 IU/mL/min for pink B dye and 188.5 to 201.6 IU/mL/min for yellow BG dye. Ultimately, resulting in higher degradation of direct pink B from 58.9% to 93.29% and direct yellow BG from 54.6% to 87.42% [70]. While the major focus of these studies was on the enzyme-based biodegradation potential of fungi, Negi et al. [71] devised an approach beyond metabolic degradation. They reported that by using waste biomass of *Phanerochaete chrysosporium,* they were able to achieve a more than 95% dye removal mainly via adsorption within a time span of 30 min. In contrast to the monoculture systems, a mixed culture of *Pleurotus ostreatus* and *Coriolus versicolor* demonstrated complete degradation of dye-based textile effluent within 48 h. Moreover, it also led to a significant reduction in Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD). This was mainly due to the synergistic activity of ligninolytic enzymes (LiP, MnP, and laccase) produced by both fungal species that resulted in more effective breakdown [72]. Thus, according to these studies, when typical enzymatic degradation ranges from 70–90%, integrated or alternative fungal-based approaches can exceed 95%, based upon the operational and biological factors.

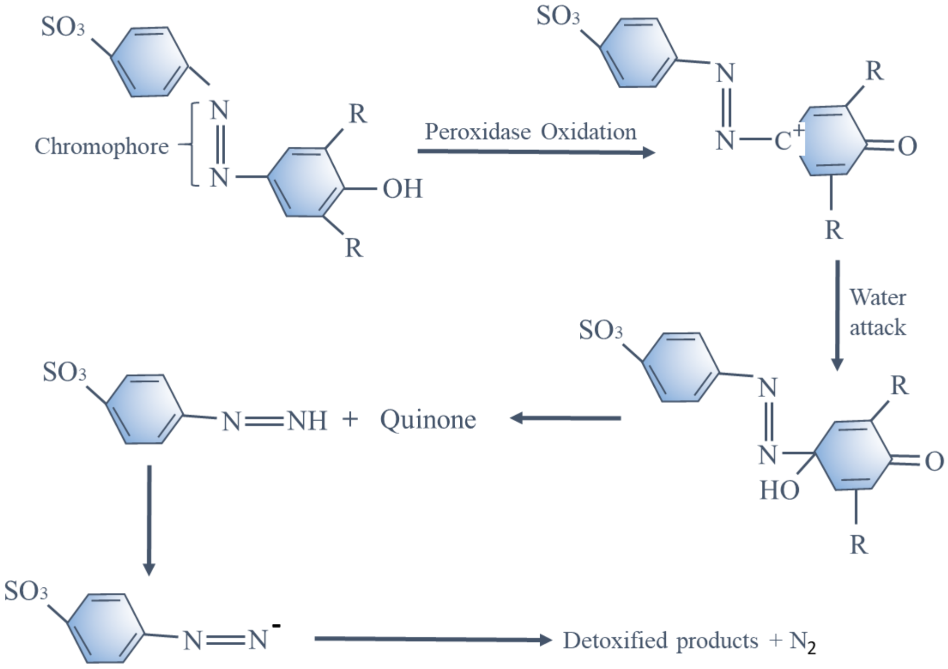

Figure 4. Proposed mechanism of peroxidase-based dye degradation [73].

3.4 Yeast-Based Dye Degradation

Yeast offers several benefits over other microbial classes when it comes to dye degradation. It combines the advantages of both bacteria and fungi by its rapid growth, handling ease, ability to withstand extreme conditions, including highly saline or acidic wastewater loaded with toxic pollutants, and genetic stability [74]. Moreover, several species have been considered as GRAS (Generally Regarded as Safe), which makes them a safer substitute for the treatment of dyeing wastewater. The process of yeast-based biodegradation involves a combination of biosorption and enzymatic degradation processes (Figure 5) [75]. Dye molecules, firstly, get attached to the surface of the yeast cells via electrostatic interactions, ion exchange, or chelation [76]. This is followed by the enzymatic degradation step in which intracellular and extracellular enzymes act upon and cleave the key bonds holding up the structure of the dye molecule. For instance, in the case of the breakdown of azo dyes, a redox reaction takes place in which electron donors like NADH transfer electrons via azoreductase enzymes to the chromophoric groups (like azo (-N=N-) linkages) of the dye molecules [77]. This results in the reduction of the dye molecule into colorless aromatic amines intermediates. In addition to the azo dyes, yeast can also be used for the breakdown of triphenylmethane and heterocyclic dyes. In this process, enzymes like laccases and peroxidases act upon their complex aromatic structures, leading to the formation of decolorized and less toxic intermediate compounds [78]. These intermediates are further metabolized by yeast enzymes like oxygenases and deaminases into harmless end products like CO_2 and H_2O [79]. Yeast cells have also demonstrated an evolving pattern in their application for dye degradation, i.e., from simple biodegradation to multifunctional and engineered systems. For example, *Rhodotorula mucilaginosa* demonstrated a complete (100%) degradation of malachite green (50 mg/L) within the time span of 12h. It also achieved complete detoxification of the effluent, confirming its potential as a promising model for dye degradation [78]. In another study, two yeast species, *Candida tropicalis* and *Pichia kudriavzevii,* exhibited comparatively lower degradation efficiencies (80 to 90%) but with a substantial metabolic advantage. In this process, lipid output in the presence of dyes ranged from 40% to 90%. Thus, it highlights the dual functionality of these species for dye removal along with the enhanced process valorization [80]. On the other hand, scientists are also developing engineered yeast-based systems to enhance the dye degradation via yeast cells. For example, *Saccharomyces cerevisiae,* when immobilized with $NiFe_2O_4$ nanoparticles, degraded up to 99% of methylene blue dye under Photo-Fenton conditions [81]. The flocculation potential of the yeast cells, as observed in the study by Tsilo et al., 2022 on *Pichia kudriavzevi,* was also promising, as about 81% of the anionic dyes were removed along with 73% BOD and 64% COD removal. This highlights the dual functionality of yeast-derived bioflocculants, which is useful not only for dye remediation but also for the overall wastewater treatment process [82]. Thus, while native yeast possesses good potential in dye remediation, engineered or immobilized approaches further enhance its functionality beyond the remediation of dyes.

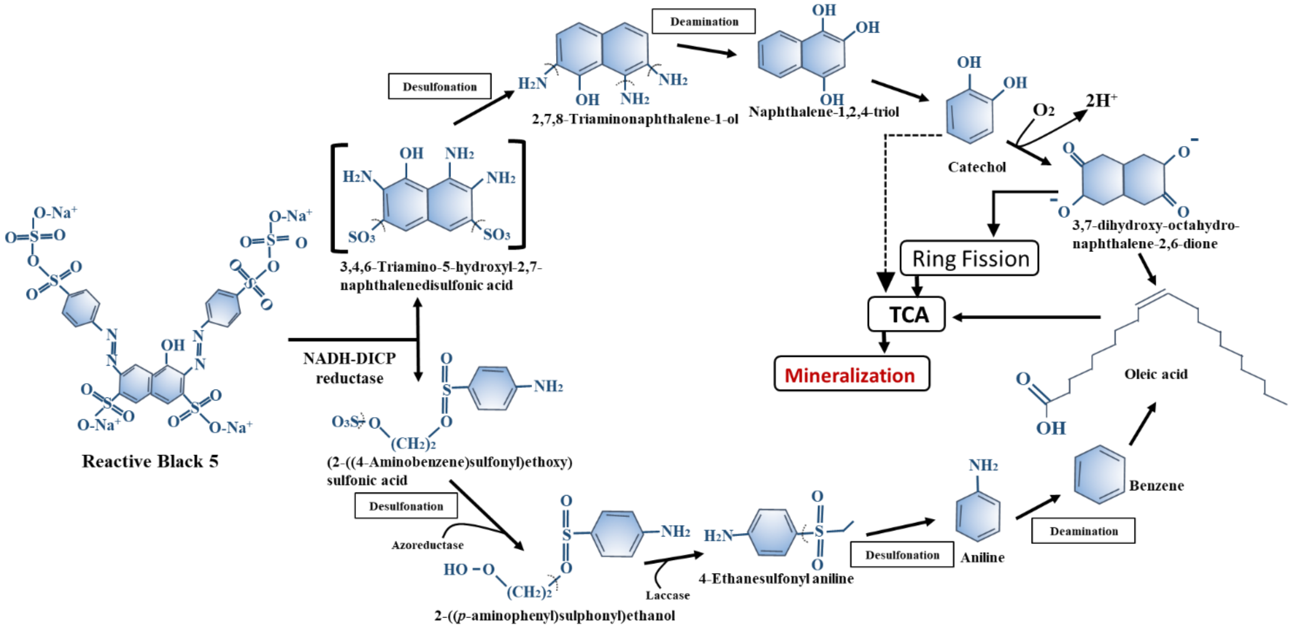

Figure 5. Yeast-based dye degradation mechanism proposed by Al-Tohamy et al [74].

4. Next-Generation Remediation Strategies

4.1 Microbial Consortia

A microbial consortium comprises a variety of different microbial species that work in synergy for the degradation and detoxification of complex dye molecules. Microbial consortia have been observed to have superior degradation efficiency as compared to the single cells due to their synergistic metabolic pathways and high environmental stability, which allows them to completely mineralize the dye molecules [83]. This was proved by Mohanty & Kumar [84] in their comparative study in which a bacterial consortium demonstrated the degradation of Indanthrene Blue RS dye at about 11,088 $\mu g\ h^{-1}$ within 9 h. This efficiency was much higher than that of the individual strains under similar conditions. Recalcitrant organics and complex nitrogen compounds are highly complex in nature and thus resist the complete breakdown via single microbial activity. Whereas, the coordinated action of microbial consortium results in their complete degradation into mildly or non-toxic forms [85]. Afrin et al. [18], in their study, reported much faster and more efficient degradation of multiple Novacron dyes by a microbial consortium when compared with single strains. The degraded compounds were low molecular weight metabolites, which confirmed the detoxification of the effluent. These metabolites can then further be utilized by the other microbial strains present within the consortium [86]. To understand the synergistic mechanisms between different strains of a bacterial consortium, scientists sequenced the genomes of each of the strains. Results demonstrated a clear division of labor between each strain. The primary responsibility was carried out by the *Cellulomonas* sp. APG4 facilitated the glycerol co-substrate oxidation and enzymatic cleavage of the azo bonds. The resulting aromatic amines from this cleavage were further degraded by *Pseudomonas stutzeri* APG2 through its specific genetic degradation pathways. *Stenotrophomonas acidaminiphila* APG1 played a supporting role by producing electron-shuttle molecules to speed up the degradation process. This highlights the interdependencies within a bacterial consortium, which can be leveraged for enhanced bioremediation strategies [87]. In another study based on a sequential dye degradation procedure, the anaerobic microbes first degraded the recalcitrant compounds, which were then mineralized by the aerobic microbes. The results highlighted the importance of employing microbial consortia over single strains by demonstrating a removal of 97% of the dye and 86% COD [88]. Another study by Lade et al. [19] reported the reduction of 98-99% azo dyes by a microbial consortium within a duration of 12-30 hours. The use of microbial consortia for the detoxification of dye-laden wastewater has been studied immensely in recent years; however, keeping the consortium stable for large-scale units is a major challenge. Thus, there is a need of further research, particularly on optimizing parameters for the large-scale treatment systems. The focus of the research should be on the selection of the best-performing microbial strains, optimal conditions, system model, and the financial viability of the system [89]. Plants and microbes can also be utilized in synergy for this purpose. This process is known as phytoremediation [90]. In this system, microbial species are disseminated throughout the ground surface through plants' roots. The root system facilitates penetration into otherwise impenetrable subsoil. Moreover, metabolic by-products produced by the roots also support the microbial survival and activity in this environment. This results in enhanced contaminant breakdown with increased bioavailability of contaminants for the plants or lessened cytotoxicity. Thus, a synergistic approach with microbes and plants working together may result in better phytoremediation [91, 92]. A consortium of a plant (*Zinnia angustifolia*) and bacteria (*Exiguobacterium aestuarii*) has been reported to produce a superior combined degradation pathway which degraded Remazol Black B dye at a much faster pace with better removal as compared to either of their activity alone [90].

4.2 Engineered Microbes

Microorganisms have been proven to be a viable, cost-effective, and environmentally friendly solution for dye-laden industrial wastewater treatment. However, natural microorganisms exhibit some limitations [93]. Dye-containing wastewater is found in extreme environmental conditions like high alkalinity, salinity, and extreme temperature, with significant chemical and biological oxygen demand. It is extremely difficult for microorganisms to efficiently survive and function in such environments. Moreover, these effluents also contain complex dye molecules that possess resistant metabolites that are difficult to completely decolorize and mineralize [94, 95]. Some strains, although, possess the potential of degrading pollutants, scientists have suggested enhancing those traits for a more efficient degradation activity. This can be done by genetic engineering that will allow the bacterial genome to be altered so that it will be able to adapt to its extreme environmental surroundings and degrade the complex dyes more efficiently as compared to its unmodified counterparts [89]. Bioinformatics tools have allowed the modification of the genetic expression in microbes for higher degradation efficiency. For example, by overexpressing the azoreductase gene from *Bacillus* sp. into *E. coli*, scientists achieved a much higher degradation of Congo Red and Reactive Black-5 dyes as compared to the wild-type strains [96]. Similarly, overexpressing thermostable laccase genes from *Bacillus* sp. in *E. coli* significantly enhanced their stability. The recombinant strain demonstrated a half-life of ~16 h at 70°C and efficiently decolorized many synthetic dyes. [97]. In another study, scientists cloned an *Escherichia coli* to overexpress the azoK gene from *Klebsiella pneumonia*. Results demonstrated that the engineered strain performed reductive cleavage of azo bonds and decolorized methyl orange dye. Scientists further enhanced its efficiency with the help of redox mediators [98]. Thus, engineering microbes to enhance their survival and activity in extreme conditions against resistant compounds is a promising approach for the future of sustainable dye degradation.

4.3 Material-Assisted Dye Degradation

Another promising strategy that aids the microbial cells to resist and work efficiently in the presence of highly toxic dyes, slow reaction kinetics, and environmental instability is material-assisted dye degradation [99, 100]. Functional materials like nanoparticles, carbon-based supports, and magnetic biosorbents play a key role as both catalysts and protective shields, which optimizes the microbial mineralization process [101, 102]. Moreover, they also act as reusable systems. The physico-chemical properties of the material along with the microbe's biological activity cleaves the dyes, protects the microbe and facilitates the biochemical process which are the key factors for enhanced detoxification of the dye-laden effluent [103]. There are various techniques that are used to efficiently utilize materials in dye degradation including immobilization, adsorption, electron mediation, and catalysis (Table 2). For example, scientists created a multifunctional biocarrier system comprising a layer of lignocellulosic material (like soybean hulls or wheat straw) sandwiched between two polyurethane foam discs. A white-rot fungal specie *Peniophora laxitexta* was immobilized on this system. Via a combined material-based adsorption and fungal-based enzymatic degradation process, this system demonstrated the removal of a variety of different dyes including 30–50% azoic dye (Xylidine), over 50% anthraquinonic dye (RBBR), and nearly 90% triphenylmethane dye (Malachite Green) [104]. Many studies have reported an escalation in the microbial efficiency when any functional material was integrated into the process. In a comparative study conducted by Modi et al., [105], it was observed that zinc oxide nanoparticle assisted bacteria to achieve up to 95.46% methylene blue degradation. This was much higher than the 89.08% degradation by isolated bacteria and 76.3% microbial consortia based removal. This study demonstrated an increase in the metabolic activity and enzymatic break down pathways of the bacteria due to the nanoparticle integration. The synergistic action of cow dung biochar as immobilization matrix and adsorbent allowed *Lysinibacillus* sp. to achieve 49.4% higher removal of dyes as compared to free cells-based dye removal [106]. Thus, the application of functional materials in this process significantly aids the microbial immobilization and dye adsorption which results in better microbial stability and detoxification. However, despite its promises, some challenges remain with the material assisted dye degradation in terms of mass transfer limitations, material stability, and cost [107]. Scientist are currently focusing on the development of advanced immobilization techniques and material design to deal with these limitations. This will soon allow them to scale these laboratory studies to a pilot- and full-scale wastewater treatment plants [108, 109].

Table 2. Techniques for Material-assisted Microbial Dye Degradation

Technique	**Microorganism**	**Material Used**	**Target Dye**	**Degradation Time**	**Degradation (%)**	**Catalyst mechanism**	**Reusability cycles**	**Ref**
Immobilization	*Pseudomonas aeruginosa*	SA-PVA@UiO-66 beads	Methylene Blue (MB)	54.82 h	96.54%	Biosorption + enzymatic biotransformation + MOF adsorption	7 cycles	[110]
Electron mediation	*Aquaspirillum* sp. LM1 (cathodic); *Paralcaligenes* sp. KSB-10 (anodic)	Fe-based magnetic biochar (FMB)	Alizarin Yellow R (AYR)	90 days	>90%	Electron shuttle + enhanced extracellular electron transfer (EET)	Not specified (90-day continuous)	[111]
Immobilization	*Ralstonia pickettii* (live vs dead cells)	PVA-alginate-hectorite beads	Methyl Orange (MO)	30 Hours	24% (dead cells), 99% (live cells)	Adsorption (dead cells); Enzymatic degradation (live cells)	8 cycles	[112]
Catalysis	Bacterial Cellulose (BC)	Bacterial cellulose (BC) film with iron oxide nanoparticles (MIONPs)	Methylene Blue (MB)	120 min	73% (UV light), 68% (ambient light)	Photocatalytic degradation	-	[113]
Electron mediation	*Klebsiella oxytoca*	Peroxidized Vanillin or Peroxidized	Methyl Orange (MO)	12 h (vanillin RM), 24 h	95% (vanillin RM), 85%	Electron shuttle (quinone-mediated	5 cycles	[114]

		Syringaldehyde derived Redox Mediator (RMs)		(syringaldehyde RM)	(syringaldehyde RM)	extracellular electron transfer)		
Catalysis	*Microcystis aeruginosa*	Biosynthesized silver nanoparticles (AgNPs)	Congo Red	60 min	77.21%	Photocatalytic reduction	-	[115]

Abbreviations: SA, sodium alginate; PVA, polyvinyl alcohol; MOF, metal-organic framework; MIONPs, magnetic iron oxide nanoparticles; EET, extracellular electron transfer; "Continuous" = no discrete cycles.

4.4 AI-based Bioremediation

Recently, artificial intelligence (AI) has been introduced as an important tool in the microbial dye degradation process. Due to its multifunctional role, it allows a streamlined process by providing the scientists with AI-based insights for optimized dye removal. Predictive modeling is one of the most significant applications of AI in this process [116]. Scientists train the AI models based on the data about the effect of different environmental factors, like pH, temperature, nutrient levels, and dye concentration, on the microbial dye degradation efficiency. This significantly reduces the time and cost of the process [117]. For example, recently, scientists utilized an Artificial Neural Network (ANN) model for more efficient degradation of an anthraquinone dye, Remazol Brilliant Blue R, by optimizing the conditions for the laccase enzyme via a one-factor-at-a-time approach (OFAA). This allowed them to achieve a dye removal efficiency of up to 93% [118]. In a comparative study, SVR-FOA (Support Vector Regression optimized by Fruit Fly Algorithm) was observed to be the best model next to SVR, Gene Expression Programming, and Random Forest for the prediction of degradation in azo dyes and chromium by *Klebsiella* sp. [119]. AI-powered molecular docking simulations allow scientists to study how microbial enzymes interact with the dye molecules. This allows a better understanding of the degradation pathways and identifies the most effective enzymes specific to the dye structure [78]. AI also provides information about the development of potent functional materials for better dye degradation. For example, machine learning models can be used to predict how well a particular material will absorb dyes on the basis of its structure and production process. This will lead to the development of better engineered materials with simultaneous adsorption and biodegradation capabilities [120, 121]. Moreover, AI modes can also be used in the automatic adjustment of the operational parameters of the bioreactors for a precise wastewater treatment process [122]. The future of AI in dye degradation holds immense potential to scale up and automate dye degradation, which will pave the way for smart, large-scale wastewater processing plants. Next-generation approaches for microbial dye degradation are summarized in Figure 6.

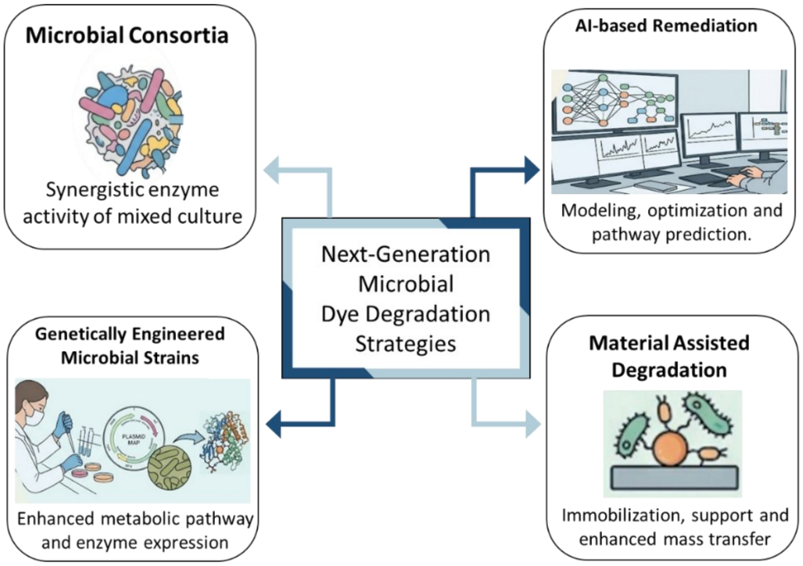

Figure 6. Next-generation microbial dye degradation strategies.

5. Process Configurations

Any biotechnological process with microbial or enzymatic activity for bioremediation or biotransformation purposes primarily involves bioreactors [123]. For the microbial bioremediation of toxic dyes, batch, continuous, fixed-bed, and photobioreactors are mainly involved [124]. There are many variables that influence the conditions and the processing via bioreactors, such as biotic and abiotic factors. Biotic factors include environmental factors, pollutants, and the nutrient concentrations, etc., whereas abiotic factors usually are the size and mode of operation of the bioreactor. These factors play an important role in the growth and functionality of the microbial cells [125]. A batch reactor is the most commonly employed type of bioreactor as it allows real-time monitoring, metabolite accumulation, and limited retention of the biomass [126]. A fed batch reactor is also beneficial, particularly when the highly toxic dyes are processed. The incremental addition of the substrate without the removal of effluent allows it to be processed without becoming toxic for the microbial cells [124, 127]. For the industrial applications, continuous bioreactors are typically employed due to their high efficiency (98% at 15 mg L−1) compared to other reactors like batch PBR (73 to 86%) under similar conditions. This efficiency can further be enhanced via packing materials. The bacterial cells embedded on the packing material develop stable biofilms and remain in contact with the dyes for long durations, which ultimately enhances the degradation efficiency [84]. In a study by Rai et al. [128], a continuous packed-bed bioreactor comprising *Bacillus tequilensis* on polyurethane foam achieved a degradation efficiency of 94.2% in Reactive Red 120 dye. A fluidized bed reactor is another effective bioreactor. In this configuration, the immobilization matrix is in the suspended form, which allows more uniform distribution of the substrate on the biofilm [129, 130]. The degradation process of the azo dyes involves two phases. First is the anaerobic phase, in which azo bonds get reduced to aromatic amines. These aromatic amines are then further degraded in the subsequent aerobic phase. A critical requirement for the complete and efficient biodegradation of the azo dyes is a sequential anaerobic and aerobic degradation process [131]. To fulfill this requirement, hybrid reactors have been introduced, which offer multiple redox zones within a single system [132]. Another important configuration used for the bioremediation of dyes is a photobioreactor. The microbial cells present in these bioreactors are generally photosynthetic bacterial or algal species that utilize light to photosynthetically produce oxygen and enzymatic activity for the degradation purpose [133]. In short, there are many important bioreactor configurations that can be employed for the detoxification of the dye-laden wastewater systems. The selection of the right type depends upon several factors, including cost, effluent volume, dye concentration, and required treatment quality (Figure 7).

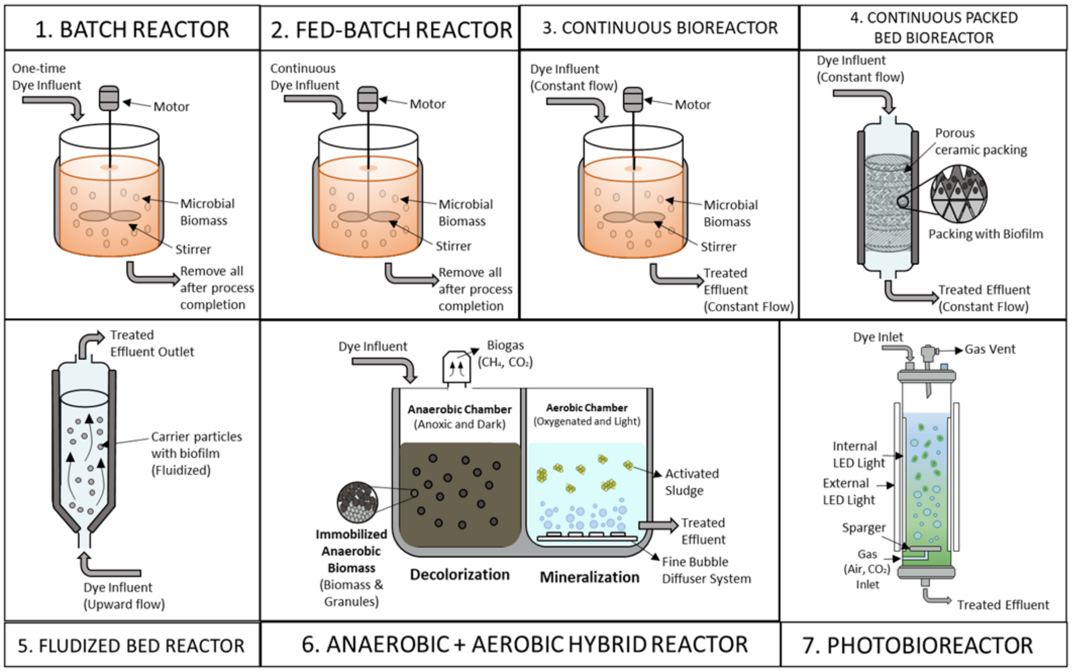

Figure 7. Comparison of bioreactor systems for dye degradation.

6. Analytical Approaches to Monitor Dye Degradation

There are a number of analytical tools that allow scientists to not only visually monitor the dye removal but also track the entire degradation process. Platinum–Cobalt (Pt–Co) scale (also known as the Hazen method or APHA Standard Methods) is a widely accepted quantifying technique for routine monitoring purposes. This method usually compares the apparent color of treated effluent with reference to the platinum cobalt solution. The color produced by 1mg platinum cobalt dissolved in 1L water is considered as 1 unit of color on the platinum-cobalt scale [134]. Usually, ≤ 50-200 Pt-Co units are permissible in the industrial or treated effluent [135]. However, since this method only demonstrates the change in color, it doesn't confirm the complete degradation. In contrast, ultraviolet-visible (UV-Vis) absorption provides insights about the real-time reaction progress and the decolorization kinetics in a variety of conditions, including photocatalysis or advanced oxidation processes (AOPs) [136]). However, decolorization alone is not the indication of complete degradation due to the formation of colorless toxic intermediates like aromatic amines. This issue is addressed by techniques like high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC MS), which allow compound-specific resolution and identification of intermediate products [137]. Fourier transform infrared (FTIR) spectroscopy is another spectroscopic technique that allows scientists to track the specific bond cleavages and compound transformations wherein the degradation process [138]. Finally, Total Organic Carbon (TOC) analysis assists the scientist in the quantification of the remaining organic carbon, which ultimately provides insights about the true mineralization and formation of CO_2 and H_2O [139]. Thus, these techniques not only allow the scientists to assess the dye degradation potential but also provide the complete picture of the underlying process, including molecular transformation and the extent to which the toxic intermediates and recalcitrant compounds persist.

7. Future Perspective

The shift from laboratory-scale experimentation to large-scale application of microbial-based dye degradation requires strategic innovation in order to tackle the current bottlenecks that limit the real-world implementation. A key priority should be to design experiments that completely elucidate the toxic transformation products, as the current research majorly characterizes the decolorization step as a success metric [140]. To completely mineralize the dye molecules, it is important to systematically implement advanced tools like high-resolution metabolomics and pathway reconstruction. It will particularly assist in tracking the persistent intermediates like aromatic amines. On the other hand, it is equally important to separate the catalytic activity from the whole cell. This will allow the scientists to address the limitations linked with using microbial cells due to their sensitivity to environmental conditions like high salinity and the presence of toxic pollutants. This will result in better dye degradation via systems like immobilized enzyme consortia or cell-free systems, even under constrained environments like the presence of high dye loads, surfactants, or heavy metals [29].

Moreover, the non-sterile and fluctuating industrial conditions also hinder the operational workflow. Thus, the future systems must be designed in such a way that they don't lose the process efficiency under variable pH, temperature, and influent composition. Moreover, during the degradation of hydrophobic or structurally complex dyes, challenging mass transfer due to low solubility and slow diffusion also hinders the process. This can be resolved by integrating nanostructured carriers or improving the reactor hydrodynamics [141].

In addition, for the scale-up of microbial dye degradation systems, it is essential to work on pilot-scale validation using actual textile wastewater. Equally important is to align regulatory frameworks with these technological advancements to set toxicity metrics and assessment protocols specific to the hybrid microbial-nanomaterial systems [142]. Thus, applying these innovative approaches in the wastewater treatment models will further enhance the feasibility of the microbial dye degradation process.

8. Conclusions

The pollution from synthetic dyes is one of the major environmental challenges the world faces today. Its devastating effects on the environment and human health demand urgent remediation strategies. Biotechnology offers a promising and sustainable solution by offering microbial dye degradation, which allows cost-effective, eco-friendly detoxification without the excessive production of toxic by-products. However, most of the current bioremediation strategies are still at the laboratory scale. The integration of these strategies for commercial usage at the industrial level is one of the major technological challenges. This could be addressed by working towards developing integrated bioreactor systems, which would allow multiple treatment processes to take place within a single system. This is usually done via a sequential process involving microbial, photocatalytic, and nanoparticle-based treatment steps, which results in complete dye degradation. Moreover, optimization of these bioreactors by the integration of cutting-edge sensor technology and data analytics will further enhance the efficiency of these reactors. The future research should also focus on genetically engineering microbial strains with enhanced dye degradation potential. By focusing on the in-silico docking studies, scientists can have better insights about the interaction patterns between the enzymes and the dyes, which will ultimately facilitate the selection of the best-performing strains specific to each dye molecule. In short, the future of the dye-laden wastewater treatment not only depends on the selection of the best-performing microbial strains but also on the development of practical biological systems for the complex and variable wastewater toxins. This could be achieved via an integrated approach involving synthetic biology, bioreactor engineering, and an AI-assisted control system. Thus, via interdisciplinary collaboration, technological optimization, and developing sustainable approaches, scientists can resolve this environmental challenge and pave the way towards a sustainable future.

Conflict of Interest

The author declares no conflict of interest related to the publication of this research.

Data Availability Statement

No new data were generated or analyzed in this study. All information presented is derived from previously published studies, which have been appropriately cited in the manuscript.

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During the preparation of this manuscript/study, the author(s) used ChatGPT and Gemini for the purposes of language editing, grammar checking, and creation of a few pictures. The authors have reviewed and edited the output and take full responsibility for the content of this publication".

Author Contributions

Author 1 H.I.: Writing–review and editing. Author 2 J.A.: Writing−original draft preparation. Author 3 U.U.: Conceptualization. Author 4 Y.N.: Writing−original draft preparation. Author 5 A.M.: Writing−original draft preparation. Author 6 H.Y.: Writing−original draft preparation. Author 7 J.M.: Writing−original draft preparation. Author 8 A.A.: Supervision, Project Administration.

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