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Review Article

A Review of the Biological Decolorization of Synthetic Azo Dye From Textile Wastewater by Bacterial Strains

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Abstract
Artificial dyes are regarded as one of the most problematic environmental pollutants. They are widely applied in the textile, print, paper, paint, pharmaceutical, food, cosmetics, and leather industries. The textile industry produces large volumes of colored wastewater, along with other pollutants such as salts, toxic substances (e.g., heavy metals, biocides, and oxidizing agents), high organic load, nutrients, and sulfur. These dyes adversely affect living organisms and ecosystems by inhibiting photosynthesis and causing health disorders such as skin irritations, allergies, cancer, vomiting, and weakened immune reactions. Thus, they should be removed using physical, chemical, and biological methods. Chemical and physical methods need regeneration processes and chemical agents, and they are expensive. In contrast, bio-decolorization by bacteria, fungi, algae, and plants is an environmentally friendly and cost-effective technique. Bacterial strains can adsorb, degrade, and flocculate dyes. Biodecolorization is positively or negatively affected by operational parameters such as agitation, pH, temperature, dye concentration, carbon and nitrogen sources, salinity, electron donors, and redox mediators. As stated, these parameters have positive (optimum concentration) and negative (beyond optimum) impacts on decolorization efficiency.
Keywords: Synthetic azo dyes, Textile industry, Bio-decolorization, Bacterial strains, Operational parameters

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1. Introduction

Dyes and colorants are inseparable substances of human life. They are ubiquitous and play vital aesthetic and functional roles (1). The earliest evidence of the application of colored substances by humans dates back to 15000-9000 BC, as seen in the cave paintings and drawings on the walls of the Altamira cave in Spain. Humans drew these paintings with ochre, hematite, soot, and manganese oxide (2). Today, dyes are widely applied for dying in different industries such as textiles, cosmetics, rubber, leather, and printing (3). They are typically used in aqueous solutions due to their affinity for substrates (e.g., fibers) (4). Dyes and colorants are categorized into natural and synthetic (artificial).

2. Materials and Methods

This review was carried out to assess the adverse impacts of textile industry and its treatment methods. In this review, the published manuscript were retrieved from Web of Science, PubMed, Google Scholar, and Scopus using keywords and phrases such as *natural dye*, *synthetic dye*, *textile wastewater*, *textile and living organisms*, *textile and human*, *textile and environment*, *textile wastewater and decolorization*, *fungi and decolorization*, *algae and decolorization*, *plant and decolorization*, *bacteria and decolorization*, *dye-biosorption and bacteria*, *dye-degradation and bacteria*, *and dye-flocculation and bacteria*. No time restriction was applied to the literature search.

3. Results and Discussion

3.1. Types of Dyes

Natural dyes are usually extracted from natural compounds and organisms. Vegetable dyes are extracted from bark, roots, wood, and leaves of plants. In addition, some natural dyes are extracted from lichens (*Rocella tinctoria*: Cudbear dye) and fungi (*Sarcodon squamosus*: blue, *Hydnellum geogenium*: green, and *Hypholoma*

fasciculare: yellow) (4). Red colorants are derived from plants such as *Sorghum bicolor* and from insects, while black dyes are derived from tannin-rich plants such as harda and logwood (5). The first synthetic dye was accidentally discovered by William Henry Perkin in 1856 (Mauveine: a cationic dye) (6). Organic synthetic dyes are composed of three main components: the chromophore, chromogen, and auxochrome (Fig. 1). Chromogens are aromatic compounds such as naphthalene, benzene, and anthracene (7). The chromophore is responsible for generating color and contains groups such as azo, nitro, anthraquinone, methine, phthalocyanine, triarylmethane (triphenylmethane), and indigo (8). Auxochromes influence the intensity of color, enhance solubility, and improve the adhesion of dyes to the fibers. They include carboxyl, methylamino, sulfonic, dimethylamino, amine, alkoxyl, hydroxyl, and electron-donating groups substituents (-NO_2 / -CO_2H / -SO_3H / (-OCH_3)Cl or I or Br or At) (9,10).

3.2. Classification of Synthetic Dyes

Approximately 10,000 commercial artificial dyes are utilized across various industries, including cosmetics, textiles, plastics, food, ink, leather, pharmaceuticals, paper, paint, and photography (11). Easy production and high resistance to heat, detergents, and sunlight are the main properties that make synthetic dyes more applicable compared to natural dyes (8,12,13). As presented in Fig. 2, synthetic dyes are categorized based on various criteria such as application, charge, and solubility. Cationic and anionic dyes produce positive and negative charges in the aqueous solution, respectively (14).

Azo dyes are the most popular group of synthetic colorants, accounting for about 70% of global dye production. They are generally grouped based on the number of azo bonds in their structure: mono-, dis-, tri-, tetra-, and poly-azo dyes contain one, two, three, four, and more than four azo bonds, respectively (17,18). Structurally, azo dyes comprise aromatic compounds (e.g., naphthalene and benzene) linked by azo groups (–N=N–) (19). Mono-azo dyes contain a single azo bond in three forms:

- Benzene–N=N–heterocyclic compounds or heterocyclic–N=N–benzene, producing yellow and orange shades.
- Benzene–N=N–naphthenic compounds, producing red and blue shades.

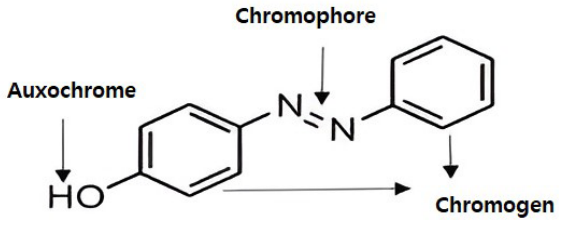

Fig. 1. The Component of Solvent Yellow-7 (or 4-Hydroxyazobenzene) (8)

- Naphthalene–N=N–naphthalene, producing black shades.

Dis-azo dyes (e.g., Matt blue, black, green, and brown) are usually derived from m-phenylenediamine and resorcinol compounds, while poly-azo dyes are used to produce dark shades of brown, red, and black (20).

3.3. Characteristics of Textile Wastewater

The textile industry plays a significant role in the global economy. Consequently, various textile factories have been developed worldwide. However, the textile industry involves various processes that generate different pollutants, as described below.

3.3.1. Pollutants in the Textile Industry

Different pollutants are generated in the textile industry.

Salts: Sodium chloride, sodium nitrate ($NaNO_3$), and sodium sulfate are extensively used in the textile industry. It is reported that approximately 40-100 g/L of $NaNO_3$ is applied during the dyeing process, as dyes are better fixed on the fibers in the presence of salts (21). When wastewater containing chlorine salts is discharged into the water environments, it reduces dissolved oxygen levels. Moreover, chlorine can react with other substances, producing resistant chlorine compounds.

Organic compounds: Different organic compounds, including enzymes, tallow, sizing materials, organic dyes, and organic acids, are applied in the textile processes, which can cause an increase in both biological oxygen demand (BOD) and chemical oxygen demand (COD) in wastewater (22).

pH variability: The pH of textile wastewater ranges from neutral to alkaline values, as dyeing processes with different dyes are carried out under various pH values (reactive dyes are treated under alkaline conditions) (21). In addition, the consumption of sodium hydroxide in the textile industry (e.g., mercerizing, washing, and scouring) leads to the generation of slightly alkaline wastewater (23).

Heavy metals: Heavy metals are individually applied in the dyeing process and are also present in certain structures, such as metal-complex or mordant dyes (24). These metals increase fixation and fastness of dyes on fibers (as a mordant). Chromate and dichromate salts (e.g., potassium dichromate and sodium dichromate) are the main salts for fixation in the dyeing process. Other heavy metals used in the textile industry include zinc (biocides, fixing agents, and impregnating agents), aluminum (impregnating and finishing agents), lead (mordant), copper (mordant), cadmium (biocide), and silver (biocide) are consumed in the textile industry (9). In wastewater, metals readily react with other compounds, resulting in the formation of complex metal salts (22). It should be noted that heavy metals have neurological and carcinogenic effects (25,26). Colorants and dyes are also important pollutants in textile wastewater. High temperature is considered an urgent parameter in dyeing performance. High temperature causes fiber swelling,

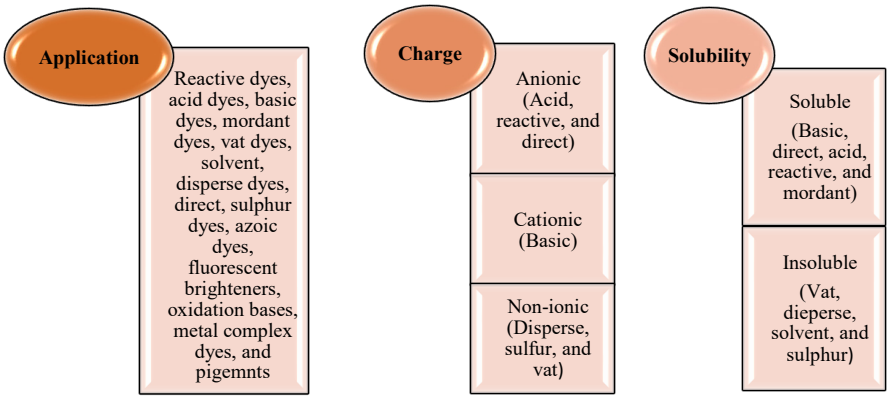

Fig. 2. Different Categories of Dyes (7,15,16)

which facilitates the penetration of dye auxiliary groups into the fiber's internal structure and enhances dye-fiber bonding (27).

3.3.2. Effect of the Textile Industry on the Environment and Living Organisms

Pollutants in textile wastewater exert adverse and harmful impacts on the environment and living organisms. The presence of dyes in the water bodies is particularly undesirable, as they remain visible in aquatic media even at low concentrations (1 mg/L) (12,28). These conditions lead to complicated problems, which are described in detail in Table 1.

3.4. Decolorization of Synthetic Dyes

Given the deleterious effects of textile wastewater containing dyes, it is essential to treat these effluents before their discharge into the environment. The aromatic structure and synthetic origins of artificial dyes make their decolorization process complicated. Various biological, chemical, and physical techniques have been applied for dye removal, each with its advantages and disadvantages (Table 2).

Removal of dyes by chemical techniques refers to the oxidation of these compounds. This approach is considered straightforward and transforms dyes into low-molecular-weight compounds such as nitrogen, water, carbon dioxide, sulfate, and aldehydes. The production of reactive radicals, which attack pollutants, is a key advantage of this method. Combined application of oxidants generates more radicals and higher removal efficiency compared to the use of individual oxidants. However, one of the main limitations of chemical oxidation is its high cost (33,34), which cannot be supplied in all areas and countries, especially in low-income countries.

Physical decolorization of dyes is generally implemented using membrane filtration and adsorption. Membrane techniques can effectively reduce BOD, COD, and colorants, while the adsorption process offers a simple approach. However, both methods produce secondary wastes that require additional treatment (35-37).

It should be noted that coagulation is a chemical process, whereas flocculation is a physical process, and both are most effective when applied together rather than individually. Therefore, a safe, cost-effective, and eco-friendly approach, such as bioremediation using potent microorganisms, should be applied to decolorize synthetic dyes from wastewater (38).

3.4.1. Biological Decolorization

Biological decolorization is an environmentally friendly technique that is generally less costly than other methods. In addition, dye decolorization can occur under different conditions, including aerobic, anoxic, anaerobic, and a combination of these environments (60). In anaerobic treatment, the degradation of organic compounds such as dyes occurs in the absence of oxygen. However, complete degradation and destruction of dyes do not happen through anaerobic treatment. Instead, dyes are transformed into colorless aromatic amine intermediates (monocyclic or polycyclic), which are toxic and require supplementary post-treatment such as aerobic or chemical oxidation techniques. Because of the non-biodegradable characteristic of the produced aromatic amine metabolites, they cannot be further degraded in the decolorized solution under anaerobic conditions, and this leads to persistent COD in the treated wastewater. Moreover, anaerobic microorganisms need longer adaptation periods. However, the major advantage of anaerobic treatment is the generation of methane gas, a renewable energy source.

Anoxic decolorization reduces dyes to simpler metabolites, but these reduced metabolites require additional treatment for mineralization. Aerobic post-treatment following anaerobic and anoxic conditions can completely degrade and decompose the generated aromatic amine metabolites, leading to mineralization. However, the aerobic treatment of colored wastewater occurs in the presence of oxygen and is not an efficient technique, especially for azo dyes because oxygen competes with azo bonds as an electron acceptor (24,60,61). The biological treatment of azo dyes can be carried out using various organisms, including fungi, algae, plants, and bacteria (described in detail in this review).

Table 1. Adverse Effects of Textile Wastewater on the Environment and Living Organisms

	Matrix	Effects	Reference
Environmental effects of textile wastewater	Water	Deterioration of water appearance and aesthetics, blockage of light infiltration, reduction of photosynthesis and oxygen production, formation of anoxic conditions, influence on microbial population and activity, increased turbidity of aquatic media due to suspended collidals in textile wastewater, production of low-quality water unsuitable for agriculture and human use due to salt discharge, influence on aquatic life due to pH alteration, and accumulation of heavy metals	(12,29,30)
	Soil and plants	Changes in productivity, fertility, and nutrient supply of plants due to surfactants in textile wastewater, changes in plant sensitivity to various pathogens, and detrimental impacts on chlorophyll content	(31,32)
Health effects of textile wastewater	Human	Abortion, hives, asthma, anaphylactic shock, reduced immune reactions, hyperactivity disorder in children, decreased intelligence quotient (IQ) in children, altered T3 and T4 generation, vitamin B6 deficiency, mitochondrial respiration issues, idiosyncratic behaviors, renal and hepatic disorders, brain and nervous system disorders, reduced lymphocyte and white blood cell → Synthetic colorants Respiratory problems, skin sensitivity and irritation, asthma, and nasal mucous membrane inflammation (rhinitis) in employees → Reactive dyes. Vomiting, altered heart rate, quadriplegic disorders, cyanosis, jaundice, and tissue necrosis → Cationic dyes. Mutagenic effects, allergies, and skin irritation → Basic dyes.	(6,28,29,33)

3.4.1.1. Fungi

The cell wall of fungi contains biomolecules such as proteins, polysaccharides (e.g., chitosan and chitin), lipids, and melanin. These biomolecules provide phosphate, amino, thiol, and carboxyl, which make them efficient in the bio-sorption process. One major advantage of using dead biomass for adsorption in dye decolorization is the reduced risk to human health (62). Dead biomass is not affected by toxicants and does not require supplements, optimal nutrients, or specific growth conditions (63). To enhance adsorption capacity, fungal biomass often undergoes pre-treatment processes, including autoclaving, organic treatment (e.g., formaldehyde), or inorganic treatment (H_2SO_4, NaOH, $CaCl_2$, and $NaHCO_3$). Live fungal biomass can simultaneously adsorb and degrade dyes. Fungal species produce intracellular and extracellular lignin-modifying enzymes, including manganese peroxidase (MnP), laccase (Lac), and lignin peroxidase (LiP), which contribute to dye decolorization. The application of fungi in batch-scale decolorization experiments is both efficient and widely popular. However, excessive fungal growth in continuous bioreactors causes reactor blocking, limiting their full-scale application in wastewater treatment plants (64).

3.4.1.2. Algae

The algal cell walls contain polysaccharides, proteins, and lipids with functional groups such as sulfate, phosphate, carboxyl, hydroxyl, and amino groups, which facilitate the removal of pollutants from wastewater. The adsorption process leads to the accumulation of dyes on the surface of algae biopolymers, after which the adsorbed dyes are dispersed into the solid phase of biopolymers (65). Extracellular polymers contain various functional groups capable of dye sorption. These biopolymers can form long chains that act as coagulant agents, allowing dyes to be adsorbed on the polymer surfaces and deposited through a bio-coagulation process (66).

Algae biomass can be modified using various physical (dry and wet heating) and chemical methods (application of salt, alkali solutions, organic solvents, and mineral acids) to increase porosity and surface area. Mineral acids protonate functional groups, increasing the adsorption of anionic dyes, while organic solvents esterify functional groups such as carboxyl, which can cause the solubilization of lipids and proteins in the cell wall, creating additional adsorption sites (67-69). Algae can also degrade dyes through an enzymatic process by azoreductase and laccase. Algae do not require external supplements for decolorization, while bacteria and fungi require additional supplements (34).

3.4.1.3. Plants

Bio-remediation of pollutants by plants (phytoremediation) is considered a sustainable and cost-effective technique. Plant roots filter and trap the suspended solids from aqueous media, while the production of extracellular enzymes by the root system contributes to pollutant elimination. Roots also adsorb pollutants and transport them into other plant tissues. Consequently, pollutants are eliminated in phytoremediation through enzymatic degradation, adsorption, and accumulation. Moreover, root surfaces can provide favorable conditions for microbial growth and biofilm formation.

Plants applied in phytoremediation should possess key characteristics such as being native species, fast-growing, perennial, non-invasive, having a wide root system, high pollutant tolerance, high adsorption capacity, and the ability to growth in aquatic environments for hydroponic culture applications (70-72).

However, phytoremediation has several limitations, including the accumulation of pollutants in the edible parts of plants, reduced plant biomass in the presence of pollutants, the need for continuous cultivation and harvesting to achieve efficient decontamination, long treatment duration, dependence on seasonal variations, and disposal and management of phytoremediators (73).

3.4.1.4. Bacteria

Bacterial strains are prokaryotic organisms commonly found in water and soil, often forming symbiotic relationships with other organisms in the world (74). Due to their widespread distribution, high activity, and

Table 2. Different Chemical and Physical Methods for the Decolorization Process (10,20,33,39-59)

Methods		Advantages	Disadvantages
Ozonation (O_3)		Strong and fast oxidants react with multiple bonds in dye structure (C=C, C=N, N=N): short reaction time	High cost, short half-life (20 minutes), instability in the presence of pH variations, salts, and temperature
Fenton		Effective for a variety of dyes, relatively low cost, high COD reduction	Requiring low pH (limiting for treatment of alkaline textile wastewater) and generating sludge
Photolysis	UV/H_2O_2	Generating highly active hydroxyl radicals (2.80 V), miscible ability of H_2O_2 with water, commercial accessibility and stability of H_2O_2; low investment cost, effective decomposition of dye chromophore groups	Sensitivity of UV + H_2O_2 to carbonate scavenging at high pH and to the scavenging effect of high H_2O_2 concentration
	UV/TiO_2	Effective dye removal, stable under different situations, high radical production ability	Decreasing the catalyst activity in the presence of high dye concentration
	UV/Fenton	Enhancing the activity of Fenton reagents through UV application, generating high-valence Fe intermediates with high capacity to attack organic compounds	Limited pH range (2-5), similar to the Fenton process, additional cost for sludge processing
	UV/O_3	Decreasing the required O_3 concentration through UV application, preventing bromate formation, and achieving higher removal capacity than O_3 and UV alone	Specific radiation output of UV lamp for effective photolysis of O_3
Electrochemical		High efficiency in removing a variety of dyes, BOD, COD, and TSS	High cost (high energy need), restricted electrode lifetime, uncontrolled radical reactions
Sodium hypochlorite		Initiating and accelerating azo bond cleavage via Cl	Generating aromatic amines, ineffective in removing disperse dyes
Sonolysis	US/TiO_2	Increasing the number of nucleation sites and generation of additional hydroxyl radicals, adsorbing anionic dyes at positively charged surface sites (pH < pH_{PZC})	Effective only in a specific range of pH (pH < pH_{PZC} of TiO_2)
	US/Fenton	Simple generation of hydroxyl radicals, more efficient than individual US or Fenton treatment, improving Fe^{3+} to Fe^{2+} conversion	Efficiency depends on the size of the iron powder or other particles in the medium (larger iron particles can scatter ultrasound waves and reduce cavitation performance)
	US/O_3	Synergetic effects on dye degradation, decomposing O_3 in the presence of US bubbles (cavitation) and producing more hydroxyl yields	Ineffective in the removal of high dye concentrations, scavenging effects from CO_3^{2-}, SO_4^{2-}, and PO_4^{3-}
Coagulation-flocculation		Low toxicity, high color removal ability, and simple process	Requiring process control, producing sludge
Adsorption	Activated Carbon	Effective for removing different types of dyes	Expensive, requiring regeneration
	Peat	Good efficiency due to cellular structure	Lower specific surface area than activated carbon
	Fly Ash and Coal	High surface area, inexpensive	Variable adsorption capacity depending on Al_2O_3, SiO_2, and combusted carbon content
	Nano Particles	High surface area, high activity	Agglomeration into the coarse particles reduces catalytic activity, surface area, and separation and detachment of fine nano particles after application
	Clays	Inexpensive, stable, high surface area, ion exchange characteristics, and broad accessibility	Lower efficiency for removing anionic dyes due to predominantly negative surface charge
Membrane filtration	MF	Effective as a pre-treatment for NF and RO, removing solids and colloids (0.1-1 µm)	Dye molecules can pass through due to the large pore size
	UF	Removing macromolecules and particles, serving as pre-treatment for RO	Pore clogging /short lifespan
	NF	Purifying and separating salts and bulk textile processing wastewaters, removing hydrolyzed reactive dyes	Requiring regular cleaning, high-performance cost, prone to fouling, high pressure
	RO	The best method for removing a wide variety of dyes	Prone to fouling, generating concentrated sludge, and requiring high operating pressure
Ion exchange		Regenerable	Not effective in removing all dyes

Note. Al_2O_3: aluminum oxide; BOD: Biochemical oxygen demand; CO_3^{2-}: Carbonate; COD: Chemical oxygen demand; Fe^{3+}: Ferric; Fe^{2+}: Ferrous; H_2O_2: Hydrogen peroxide; MF: Microfiltration; µm: Micro meter; min: Minute; NF: Nanofiltration; PO_4^{3-}: Phosphate; pHpzc: pH point of zero charge; RO: Reverse osmosis; SiO_2: Silicon dioxide; SO_4^{2-}: Sulfate; TiO_2: Titanium dioxide; TSS: Total suspended solids; UF: ultrafiltration; US: Ultrasound; UV: Ultraviolet; V: volt.

good adjustability, bacteria are extensively used in the decolorization process (64,71). Bacterial strains are particularly effective because they can treat dyes under aerobic, anoxic, and anaerobic conditions.

3.4.1.4.1. Mechanisms of the Decolorization Process by Bacterial Strains: Dye decolorization occurs through biosorption onto biomass and biodegradation within cells. Both dead and live cells are participate in biosorption, while live cells and enzymatic transformation are applied in biodegradation. The disposal of adsorbent-containing pollutants is the main challenge in biosorption, but in biodegradation, pollutants decompose into simpler compounds such as carbon dioxide and inorganic compounds (mineralization). However, incomplete decomposition may occur through biodegradation, which leads to the formation of more toxic compounds (75).

3.4.1.4.1.1. Bio-sorption: Biomolecules interact with ions and chemical substances, leading to the removal of compounds from aqueous solutions. This process is known as bio-sorption (76). In bacterial strains, bio-sorption is closely related to cell wall structure. According to the results, different functional groups such as hydroxyl,

carboxyl, phosphate, amino, and other charged groups are present in bacterial cell walls and play a key role in the bio-sorption process.

Reports indicate that cationic dyes are adsorbed by carboxyl and other negatively charged functional groups, while anionic dyes are adsorbed by amine functional groups through hydrogen and electrostatic bonding. Gram-positive bacteria have a thick peptidoglycan layer (approximately 90% of the cell wall), while gram-negative bacteria have a thinner peptidoglycan layer (10%-20%) and an outer membrane that consisted of lipopolysaccharides and phospholipids. The negatively charged functional groups in peptidoglycan, lipopolysaccharide, and phospholipid enhance adsorption of other compounds onto bacterial biomass. Under acidic conditions, functional groups of the biomass become protonated, which promotes adsorption of anionic compounds on the biomass surface (74). Bacterial biomass can also be treated with alkaline and acid solutions, acetone, or ethanol to increase bio-sorption capacity (66). Table 3 summarizes different bacterial strains and their adsorption ability.

3.4.1.4.1.2. Bio-degradation: Oxidoreductase enzymes, including azoreductase, NADH-DCIP reductase, laccase, lignin peroxidase, and manganese peroxidase, are responsible for the degradation and decolorization of azo dyes. Among these, azoreductase and laccase are the most effective enzymes in the degradation process. In addition, azo dye decolorization and degradation happen under conventional anaerobic, facultative anaerobic, and aerobic conditions, as complete degradation and decomposition of azo dyes occur through various individual or sequential aerobic or anaerobic processes to achieve mineralization of the treated effluent (75).

3.4.1.4.1.2.1. Azoreductase: Azoreductase (EC 1.7.1.6) is a specific enzyme involved in azo dye decolorization and is found in both eukaryotic and prokaryotic organisms (84). It acts as a catalyst in the presence of reducing agents such as NADH and NADPH, which serve as electron donors. Thus, electron transmission from NADH and NADPH to the azo bonds (electron receptors) leads to cleavage of the azo bonds. Azoreductases are categorized into two groups: Flavin-independent (Flavin-free) and Flavin-dependent types. Flavin-independent azoreductases utilize NADPH, while Flavin-dependent azoreductases utilize NADH. Reduction of azo dyes by Flavin-dependent azoreductase occurs through the Ping-Pong Bi-Bi procedure. In this process, azo dye is initially converted to hydrazine and then to constituent aromatic amines. The reduction of azo dyes occurs through the transfer of four electrons in two stages, with two electrons transmitted to the azo bonds in each step, which can lead to cleavage of the azo bonds and the formation of a colorless solution containing aromatic compounds. Azoreductase is sensitized to oxygen and acts efficiently under anaerobic or microaerophilic conditions (oxygen-sensitive azoreductase) (85,86). However, genes encoding azoreductase have also been identified under aerobic conditions nowadays (oxygen-insensitive azoreductase). For example, a Flavin-free oxygen-insensitive azoreductase from *Klebsiella oxytoca* GS-4-08 was isolated, characterized, and cloned for the Methyl Red substrate (84).

Textile effluents often contain high salt concentrations. Therefore, isolating and identifying azoreductase genes from halophile and halotolerant microorganisms is effective in textile wastewater treatment. NADH-dependent azoreductase and FMN-dependent NADH-azoreductase genes were isolated and identified in *Halomonas* sp. strain GT and *Staphylococcus* sp. strain MEH038S in the presence of salts, respectively (85,87).

Increasing the concentration of dye in the culture medium decreases azoreductase activity because electron transfer is restricted at high dye concentrations. Additionally, the active sites of the azoreductase enzyme can be blocked by higher concentrations of dye molecules (88). The optimal pH and temperature for azoreductase generally range from 5-9 and 25-85 ˚C, respectively (89). Maximum azoreductase activity was observed at pH 7.5 and 35 ˚C for *Pseudomonas aeruginosa* ASU3 and ASU6 (90), while Hua and Yu reported that the highest azoreductase activity at pH 7 and 50˚C for *Klebsiella*

Table 3. Bio-sorption Efficiencies of Bacterial Strains

Bacteria	Concentration of dye	Adsorption capacity (mg/g) or adsorption efficiency (%)	Optimum operational parameters	Reference
Corynebacterium glutamicum	500 mg/L of Reactive Red-4	104.60 (mg/g)	pH 1, 25 ˚C, 10 g/L of biomass, and 160 rpm	(77)
Bacillus cereus M116	400 mg/L of Malachite Green	485 (mg/g)	pH 5, 30 ˚C, 5 g/L of biomass, 120 rpm, and 260 minutes	(78)
Escherichia coli	500 mg/L of Reactive Yellow-2	543.78 (mg/g)	pH 2, 25˚C, 160 rpm, and 24 hours	(79)
Agrobacterium fabrum SLAJ731	200 mg/L of Methylene Blue	91 (mg/g)	pH 11, 25 ˚C, 160 rpm, and 60 minutes	(80)
Rhodopseudomonas palustris 51ATA	351 mg/L of Fast Black K Salt	89.43 (mg/g)	1 g/L inoculum size, pH 8, 35˚C, and 10 rpm	(81)
Corynebacterium glutamicum	500 mg/L of Reactive Black-5	195 (mg/g)	pH 1 and 35 ˚C	(82)
Corynebacterium glutamicum	200 mg/L of Methylene Blue	207.3 (mg/g)	pH 9, 25 ˚C, and 160 rpm	(74)
Bacillus gordonae NCIMB 12553	250 mg/L of Tectilon Blue 4R	13%	250 rpm, 2 days, pH 7, and 25 ˚C	(83)
Bacillus benzeovorans NCIMB 12555	250 mg/L of Tectilon Blue 4R	19%	250 rpm, 2 days, pH 7, and 25 ˚C	(83)
Pseudomonas putida NCIMB 9776	250 mg/L of Tectilon Blue 4R	18%	250 rpm, 2 days, pH 7, and 25 ˚C	(83)

oxytoca GS-4-08 (84).

The expression and activity of azoreductase are influenced by the chemical structure of the dye substrate. Azoreductase activity of *Enterobacter agglomerans* follows the sequence: Methyl Red > Disperse Yellow > Trypan Blue > Amaranth > Orange G (91). Similarly, gene expression in *Pseudomonas aeruginosa* ASU3 and ASU6 was higher for Methyl Red compared to Direct Blue-64 and Acid Yellow-17 substrates (90). According to the results, azoreductase exhibits greater expression and activity with lower molecular weight and simpler dye substrates.

3.4.1.4.1.2.2. Laccase: Laccase (EC 1.10.3.2) is a key non-specific multi-copper oxidative enzyme in the decolorization process. Its structure contains four copper ions: one type I copper, one type II copper, and two type III coppers. Type II and III copper ions form a cluster that reduces oxygen, while type I copper enhances electron transfer to the type II and III copper. Laccase can degrade different compounds, including aromatic hydrocarbons, phenols, and dyes (92,93). It acts as a catalyst in the oxidation of substrates under special situations (i.e., the presence of oxygen molecules).

The enzymatic degradation of azo dyes by laccase occurs in several steps. First, the asymmetric breakage of azo groups occurs through laccase activity, followed by subsequent reactions such as deamination, desulfonation, dihydroxylation, and demethylation of the degraded dye molecules (94). Laccase can be applied individually or in combination with chemical mediators for dye degradation. Recombinant LAC_2.9, LAC_2.9 + HBT (combined with 1-hydroxybenzotriazole, HBT) and LAC_2.9 + pHBA (combined with para-hydroxybenzoic acid, pHBA) from *Thermus* sp. 2.9, with an activity of 0.15 U/mL, was used for the decolorization of different dyes. Recombinant LAC_2.9 was expressed in *Escherichia coli*. Genomic DNA from *Thermus* sp. 2.9 was used to amplify the gene coding for LAC_2.9, after which the recombinant plasmid was transformed into *E. coli*. Maximum decolorization efficiencies were observed for xylidine (99.36% at pH 9) and Indigo Carmine (99.60% at pH 5) (95).

Sun et al investigated the activity of free and immobilized laccase from *Trametes versicolor* (≥ 0.5 U/mg) on polyacrylamide/chitosan (Lac-PAM-CTS) for the decolorization and degradation of Acid Orange-7. Immobilization of laccase on other materials enhanced optimization performance at industrial scales. They studied the effects of pH (3-8) and temperature on the activity of free laccase and Lac-PAM-CTS. Free laccase exhibited more than 60% efficiency only at pH 6-7, whereas Lac-PAM-CTS maintained over 60% efficiency at pH 3-7. Additionally, Lac-PAM-CTS showed lower deactivation than free laccase at 65 ˚C (96).

Li et al reported that free and immobilized laccase on Fe_3O_4/C-Cu^{2+} showed 100% relative activity at 55 ˚C and pH 4.5. The presence of metal ions influenced laccase activity. The relative activities of 98, 90, 87, 17, 54, and 56% were observed for free laccase in the presence of Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Pb^{2+} ions, respectively. The relative activities of 97, 93, 100, 67, 55, and 96% were detected for Lac-Fe_3O_4/C-Cu^{2+} in the presence of Zn^{2+}, Mn^{2+}, Cu^{2+}, Fe^{3+}, Cd^{2+}, and Pb^{2+} ions, respectively, indicating that Fe_3O_4/C-Cu^{2+} acts as a protective carrier for laccase against metals (97). These results suggest that immobilized laccase is an effective and promising approach for treating textile wastewater containing metals.

Jankowska et al reported that free laccase, ZrO_2-SiO_2-laccase, and ZrO_2-SiO_2/Cu^{2+}-laccase (from *Trametes versicolor*) exhibited approximately 100% relative activity at pH 4 and 30 ˚C, and then the relative activities declined at higher pH and temperature values for Remazol Brilliant Blue-R. The reduction in activity can be due to enzyme deactivation at elevated temperatures, while neutral and alkaline pH values affected the catalytic activity of laccase by altering the rearrangement of functional groups such as amino acids within its structure (98).

3.4.1.4.1.3. Bio-flocculation: Bio-flocculants are natural polymeric macromolecules, including proteins, lipids, glycoproteins, nucleic acids, and polysaccharides, produced by microorganisms. They can flocculate suspended particles such as colloids and cells. Compared to chemical flocculants, bio-flocculants are non-toxic and biodegradable. Due to these advantages, various fungi and bacteria with bio-flocculant activity have been isolated from different media, including wastewater, river, activated sludge, and soil. Bio-flocculation is a well-known process in activated sludge systems under aerobic conditions and can be effectively used to isolate bio-flocculant-producing microorganisms (99-102).

Natural flocculants are categorized into three groups based on molecular weight and functional groups: cationic, which carry a positive charge, such as chitosan with amino functional groups and high molecular weight, anionic, which carry a negative charge, such as tannin, starch, and cellulose with hydroxylic and carboxylic groups, and non-ionic, which are moderately neutral. Bio-flocculation occurs through different mechanisms. Initially, charge neutralization and patching take place, followed by hydrogen bonding and polymer bridging. The efficiency of bio-flocculation depends on the adaptability and charge of the polymer. Positively charged amino groups of bio-flocculants can adsorb negatively charged dyes through electrostatic interactions, neutralizing their charges. After that, the dyes are bridged together, forming flocs, which then aggregate and sediment (102). In addition, positively charged groups of dyes can be adsorbed onto negatively charged groups of bio-flocculant, such as carboxyl ($-COO^-$) (103). Various microorganisms with flocculating activity have been isolated and applied for dye decolorization. The flocculating activity of 96% was reported for the removal of Terasil Yellow-W4G

by *Chryseomonas luteola* at 50°C within one day (104). Maximum flocculation activity of 98.10% was obtained at 150 rpm and 28°C for *Aspergillus parasiticus* within 72 hours (101). *Kocuria rosea* strain BU22S exhibited more than 80% flocculation activity within four days (100), while *Paenibacillus xylanilyticus* BITSJ-11 reached 97.8% flocculation activity and was subsequently applied for the decolorization of Rhodamine B and Congo Red with efficiencies of 95% and 97%, respectively (105).

3.4.1.4.2. The Effective Factors of the Decolorization Process: Various parameters influence bacterial decolorization efficiency. Decolorization efficiency and speed are increased under optimal and ideal conditions for each bacterial strain. These optimum factors should be determined experimentally in the laboratory for each bacterial strain individually.

3.4.1.4.2.1. Agitation (Shaking and Static Conditions): Agitation (shaking) can reduce the decolorization efficiency of azo dyes. Shaking increases the concentration of dissolved oxygen, and both oxygen and azo bonds act as electron acceptors. Oxygen receives electrons as a preferred acceptor, preventing electron transfer to azo bonds, thereby reducing azo bonds and decreasing decolorization efficiency. The decolorization efficiencies of Acid Blue-113 by *Klebsiella* sp. strain AB-PR were 71% and 20% under static (microaerophilic and low oxygen concentration) and shaking conditions, respectively (106). Patel et al reported that *Klebsiella* strain Bz4 achieved decolorization efficiencies of 95.36%, about 90%, and 77.93% under static, semi-static, and shaking conditions, respectively (107). A consortium containing C1, C2, C3, and C4 strains showed higher decolorization efficiencies for Methyl Orange under static conditions compared to shaking (108).

According to the reports, higher bacterial growth occurs under shaking conditions. However, decolorization efficiency is greater under static situations. Wang et al observed that the decolorization efficiency of Reactive Black-5 by *Bacillus* sp. YZU1 decreased as the agitation speed increased from 0 to 250 rpm, while bacterial growth was enhanced, indicating that *Bacillus* sp. YZU1 is a facultative anaerobe (109). In addition, shaking provides ideal conditions for bacterial adsorption of dyes. The contact between bacterial cells and dye molecules increases under shaking conditions, which can influence the decolorization process (110). *Rhodopseudomonas palustris* 51ATA, *Corynebacterium glutamicum*, and *Corynebacterium glutamicum* exhibited adsorption capacities of 89.43, 350, and 207.3 mg/g for Fast Black K Salt, Reactive Black-5, and methylene Blue at 10, 160, and 160 rpm, respectively (81,82,111).

Mostafa et al reported that agitation improves supplement distribution (carbon and nitrogen sources) and facilitates oxygen transfer in the culture medium. These conditions lead to enhanced decolorization efficiency. For instance, *Cylindrocephalum aurelium* achieved removal efficiencies of 41%, 44%, 65%, and 85% (Mordant Orange-1) under 0 (static), 50, 70, and 100 rpm, respectively (112).

3.4.1.4.2.2. pH: The growth and metabolic activity of microorganisms, as well as enzyme activity, are affected by pH (113). pH values between 6 and 10 are considered more favorable for most microorganisms. The decolorization efficiency of *Enterobacter aerogenes* PP002 was 80% (100 mg/L of Direct Blue-71 and 100 mg/L of Direct Green-28) at pH values between 5 and 9, with maximum efficiencies for both dyes at pH 7 (114). *Alcaligenes aquatilis* 3c could decolorize 82% of Synazol Red 6HBN (50 mg/L) at pH 7 and 37 °C within four days (115). The isolation and characterization of pH-resistant microorganisms is a promising approach for textile wastewater treatment. Based on the results, reactive dyes are typically applied in alkaline dye baths because they bind with cotton under alkaline conditions. In this case, alkaline wastewater is generated (116). *Sphingomonas paucimobilis* could decolorize Methyl Red (750 ppm) with an efficiency of 99.63% at pH 9 and 30 °C (117). The maximum decolorization efficiency of Crystal Violet (97%) by *Aeromonas hydrophila* was observed at pH 7. In addition, 95% and 90% decolorization efficiencies were achieved at pH 8 and 9, respectively (113). The decolorization efficiency declines with increasing pH. For example, the decolorization efficiency of Acid Black-24 by *Bacillus pseudomycoides* decreased from 86% to 12.53% as the pH increased from 7 to 11, respectively (118). Cao et al reported that high pH values impact the transportation of dye molecules across the cell membrane (119). In addition, flocculation activity is influenced by pH. The flocculating activity of *Bacillus* sp. strain xn11 and xn7 for the decolorization of Basic Fuchsin decreased by increasing pH values from 3 to 11 (120). The flocculating activity of *Bacillus nitratireducens* for removing Eriochrome Black T was reduced from 86.25% to 0.14% as pH increased from 2 to 6. Alteration of pH influences the surface charge of bio-flocculants and the chemical properties of aqueous solutions (121). Removal of anionic dye Congo Red by bio-flocculants (*Paenibacillus xylanilyticus* BITSJ-11) was high under moderately acidic pH (i.e., pH 6). However, the removal of the cationic dye Rhodamine-B by the same bio-flocculant was low at pH 6, which may be attributed to repulsion forces between the positive charge of the cationic dye and the positive charge of bio-flocculants in the presence of H^+ions under acidic conditions (105).

The variation of pH also plays a significant role in the adsorption of dyes. In the presence of H^+ions and under acidic conditions, electrostatic interactions occur between negatively charged dye sites and positively charged biomass sites (protonated functional groups) (122). Vijayaraghavan and Yun reported that the maximum adsorption capacity (150 mg/g) of Reactive Black-5 (an anionic dye) by *Corynebacterium glutamicum* was

observed at pH 1, after which the capacity declined (to less than 50 mg/g) at pH 6 and 7. The p*K*a of amine is about 8.5, and thus the amino groups carry a positive charge at pH < 7. In this case, protonation of the cell wall enhances adsorption capacity. They also found that the decrease in adsorption capacity at pH 7 was due to the presence of carboxyl functional groups. Carboxyl groups, with a p*K*a of about 3.6-4.5, have negative charges at pH > 4. These negative charges can cause electrostatic repulsion with anionic dyes such as reactive dyes. Thus, the repulsion between the negatively charged carboxyl groups and anionic dye molecules reduces the overall adsorption capacity (82).

3.4.1.4.2.3. Temperature: High and low temperatures prevent microorganisms' growth and enzymatic activity involved in dye decolorization. In general, decolorization efficiency increases with temperature up to an optimum point, after which it decreases at temperatures beyond this optimum. The reduction in decolorization efficiency at higher temperatures is mainly due to the loss of cell viability. Moreover, the activity of enzymes responsible for decolorization (e.g., azoreductase) diminishes at higher temperatures because of enzyme denaturation.

For example, *Bacillus* sp. strain CH12 decolorized 40, 73, 99, 100, 99, 70, and 35% of Reactive Red-239 (100 mg/L) at 15, 20, 25, 30, 35, 40, and 45˚C, respectively. As a result, decolorization efficiencies were diminished with an increase in temperature (123). Similarly, the decolorization efficiencies of Metanil Yellow by *Bacillus* sp. strain AK1 and *Lysinibacillus* sp. strain AK2 declined with temperature increasing from 37 to 50˚C (124). Additionally, some bacterial strains maintain high efficiency even at higher temperatures, which may be due to the activity of azoreductase enzymes. Pearce et al and Misal and Gawai reported that azoreductase remains stable at 60 ˚C and 35-40 ˚C, respectively (89, 125).

The decolorization efficiencies of Azobenzene, Methyl Red, Orange G, and Congo Red by *Bacillus subtilis* increased from 9.61 to 17.68%, 62.41 to 78.49%, 6.83 to 22.54%, and 54.24 to 71.28%, respectively, as the temperature increased from 25 to 45 ˚C (88). Increasing temperature increases adsorption capacity because the kinetic energy of the solute and surface activity increase with temperature. However, higher temperatures can destroy the bio-sorbent structure (74). A halo-alkali-thermo-tolerant mixed culture decolorized Direct Red-81 with the efficiencies of > 90% at 60 ˚C, > 60% at salt concentration up to 5%, and > 60% at pH 9 and 10 (126). Similarly, a Halo-thermophilic bacterial consortium (HT1) could decolorize more than 80% of Metanil Yellow G at temperatures of 50 ˚C, pH 6-8, salinity of 1-15%, and a dye concentration of 100 mg/L (127). In contrast, the adsorption capacities of Methylene Blue by *Streptomyces rimosus* were 34.34, 32.98, and 24.35 mg/g at 20, 30, and 50˚C, respectively (128). Likewise, the bio-flocculation activity of *Alteromonas* sp. CGMCC 10612 reached its maximum at 25 ˚C but declined with increasing temperature at 30 ˚C, which can be attributed to reduced bacterial biomass activity (99).

3.4.1.4.2.4. Inoculum Size : The optimum inoculum dosage plays a crucial role in enhancing enzyme generation and decolorization efficiency. For instance, the decolorization efficiency of Reactive Blue EFAF by *Exiguobacterium profundum* strain CMR2 increased from 65% to 80% as inoculum size rose from 0.25 to 1 mL, but declined from 80% to 77% when inoculum size further increased from 1 to 1.5 mL (129). Similarly, the decolorization of Reactive Black-5 by *Shewanella* sp. IFN4 increased with inoculum sizes ranging from 1% to 15%, with faster decolorization rates observed between 1 and 4 hours of incubation (130).

Decolorization efficiencies of Congo Red, Direct Black, and Methylene Blue increased with higher inoculum dosage of *Alteromonas* sp. CGMCC 10612 bio-flocculants, as higher inoculum dosages provides a larger surface area and more adsorption sites (99). However, the flocculation activity of *Methylobacterium* sp. Obi and *Actinobacterium* sp. mayor consortium increased when the inoculum size increased from 0.5% to 1% v/v, and then reduced when the inoculum size rose from 1% to 2%. This decrease in flocculation activity is attributed to the inoculation effect, which refers to an insufficient ratio of microbial cell density to available nutrient supplies (131). The removal efficiency of Methylene Blue by *Streptomyces rimosus* increased when the inoculum size increased from 250 to 500 mg/100 mL, due to the expansion of surface area and adsorption sites (129). However, excessive inoculum size beyond the optimum causes agglomeration of bacterial cell biomass and scarcity of adsorption sites. *Bacillus cereus* M116 had adsorption capacities of 133.2 and 20.5 mg/g at inoculum sizes of 0.5 and 4 g/L, respectively (78).

3.4.1.4.2.5. Initial Dye Concentration: An increase in dye concentration in the culture medium generally diminishes decolorization efficiency, which can be attributed to the toxic nature of dye molecules and the inhibitory influence of intermediates and by-products formed during biological decolorization. High dye concentrations can suppress bacterial growth and block the active sites of key enzymes involved in decolorization, especially azoreducatse. The blockage of azoreductase active sites directly reduces decolorization efficiency (75). However, some studies suggest that a longer incubation time is needed under these conditions of high dye concentrations and high organic load (128,132).

The impact of dye concentration on decolorization efficiency has been investigated in various studies. *Staphylococcus hominis* subsp. hominis DSM 20328 decolorized 50-1000 mg/L of Reactive Blue-4 with 97-54% efficiency within 24 hours (133). The decolorization efficiency of Acid Blue-113 by *Staphylococcus lentus* decreased from 92% to 35% with an increase in dye concentration from 25 to 500 mg/L, respectively (134).

As shown, efficiency declines with increasing dye concentration. However, some investigations reported that decolorization efficiency increases with increasing dye concentration. The decolorization efficiency of Toluidine Red by the *Halomonas* strain GB increased with the concentration rising from 10 to 30 mg/L. In this case, bacterial strains utilize dyes as the sole carbon source for providing energy, which can influence the growth and activity of strains (135). Additionally, the biosorption of dyes declines at high concentrations. Insufficient biomass concentration and the unsuitable ratio of biomass (bacterial cells) to dye are key factors contributing to reduced decolorization efficiency (133). The adsorption efficiency of Fast Black K salt azo dye by *Rhodopseudomonas palustris* 51ATA declined from 72% to 49% as the initial dye concentration increased from 44 to 223 mg/L at 25°C, respectively (81).

3.4.1.4.2.6. Carbon Source: Carbon sources supply energy for the growth and survival of microorganisms. They also act as electron donor agents and are necessary for the reduction and cleavage of azo bonds (136). Bacterial consortium EDPA decolorized 92, 94, 95, 20, 85, 38, and 84% of Acid Maroon-V in the presence of glucose, xylose, sucrose, starch, fructose, lactose, and maltose, respectively (107). Khan et al reported that the decolorization efficiencies of Reactive Red-195 (100 mg/L) by bacterial consortium AR1 were 91%, 93%, and 100% in the presence of fructose, starch, and maltose, respectively. As shown, the highest efficiency for decolorization was observed with maltose. Catalytic breakage of maltose leads to the generation of electrons, which are then transferred to the azo bonds of dyes by the redox mediators. In this case, aromatic amines intermediates are produced during the breakage of azo bonds (137).

Starch is another important carbon source in the decolorization process. Since starch is generally used in the textile industry, using microorganisms capable of utilizing starch as a substrate is economical in textile wastewater treatment (138). Mixed bacterial strains demonstrated 86%, 95%, 91%, 85%, and 84% decolorization efficiencies for Red RB, Remazol Red, Remazol Blue, Remazol Violet, Remazol Orange, and Remazol Yellow in the presence of starch as optimum carbon co-substrate, respectively (139).

Carbon sources such as glucose increase the growth and biomass concentration of microorganisms, which leads to an increase in dye adsorption on the cell surface (140). Eskandari et al reported that glucose is the most efficient carbon source for decolorization due to easy metabolization by microorganisms (141). Molasses consumption as a carbon source is efficient in bioremediation. Molasses is easily accessible, inexpensive, and easy to store (142). However, the presence of an additional carbon source in the culture medium may prevent the decolorization process. In this case, the efficient and required genes for the utilization of secondary carbon sources are not expressed, leading to a decrease in decolorization efficiency. Moreover, microbial cells may adapt to external carbon sources in the culture medium instead of utilizing the carbon atoms of dyes, which can further decrease the decolorization process (143).

Kocuria rosea MTCC 1532 could decolorize 43, 33, 23, and 6% of Methyl Orange in the presence of lactose, maltose, sucrose, and glucose, respectively. A removal efficiency of 8% was observed in the culture medium without external carbon sources (144). Similarly, bacterial consortium GR decolorized 48 and 56% of Reactive Green HE4BD in the presence of sucrose and glucose, respectively (145). Kurade et al reported that decolorization efficiencies of 8 and 7% for Dispersed Red-54 were achieved by *Brevibacillus laterosporus* in the presence of glucose and starch, respectively. A decolorization efficiency of 19% was observed in the control blank (without any carbon source). The individual application of peptone and yeast extract (nitrogen sources) showed the best efficiency in the decolorization process, whereas efficiency declined with the combined application of carbon and nitrogen sources in the culture medium. This confirmed the inhibitory effect of carbon sources on the decolorization process. Carbon sources may also have prohibitive impacts on the enzymes involved (146).

Carbon sources play a considerable role in the bioflocculation activity of microorganisms. Chen et al reported that the flocculation activity of *Alteromonas* sp. CGMCC 10612 increased in the presence of glucose compared with other carbon sources (sucrose, fructose, starch, lactose, glycerol, and sodium citrate) for treating colored textile wastewater. Carbon sources are essential for cell growth and bio-flocculant production. Flocculation activity increased from 0 to 1300 U/mL as glucose concentration rose from 0 to 30 g/L, but decreased at higher glucose concentrations (above 30 g/L) (99).

3.4.1.4.2.7. Nitrogen Source: Azoreductase is the most important enzyme in the degradation and decolorization of azo dyes. It catalyzes the reduction and breakage of azo bonds by consuming reductive factors (NADH and NADPH). These reductive factors are produced through the metabolism of organic compounds, which may include dye molecules themselves or supplemental co-substrates such as nitrogen and carbon sources (147).

Nitrogen sources are classified into organic (yeast extract, beef extract, and peptone) and inorganic ($NaNO_3$, ammonium sulfate, and ammonium chloride) (141). Among these, yeast extract is the most efficient nitrogen source in the decolorization process. Riboflavin, thiamine, and pyridoxine are the main components of yeast extract, and they impact the growth and activity of microbial strains. Experimental results demonstrated that the individual application of these components was not effective in the decolorization process.

Shewanella sp. strain IFN4 could decolorize 99.2, 89.7, 93.9, and 95% of Direct Red-81, Reactive Black-5, Acid

Red-88, and mixed dyes, respectively, in the presence of 4 g/L yeast extract. In contrast, efficiencies 10.9, 9.31, 11.8, and 9.6% were observed for Direct Red-81, Reactive Black-5, Acid Red-88, and mixed dyes, respectively, by *Shewanella* sp. strain IFN4 in the absence of yeast extract. Yeast extract plays a crucial role in the generation of reducing agents (NADH and NADPH), which can act as electron carriers in the reduction of dyes and accelerate azoreductase activity (147).

Bacillus subtilis 35A bio-flocculant exhibited maximum flocculation activity (66%) in the presence of yeast extract (5 g/L). Vitamins and amino acids from organic nitrogen sources are critical factors in bio-flocculant production. However, increasing yeast extract from 9 to 15 g/L did not remarkably increase flocculation activity, which ranged from 70 to 78%. In addition, decolorization efficiencies by microbial flocculant *Bacillus subtilis* 35A for Methylene Blue and Toluol Blue were 94-73% and 95-36% at concentrations of 10-100 mg/L, respectively (148).

The presence of co-substrate (carbon and nitrogen sources) in the microbial culture medium enhances bacterial growth and respiration, leading to a decline in oxygen concentration (optimum condition for reducing azo dyes by azoreductase enzymes) (107). Some microorganisms utilize agricultural by-products (wood shavings, rice husk, bagasse, coconut husk, and wheat bran) as carbon and nitrogen substrates. Bacterial consortium GR could completely decolorize Reactive Green HE4BD by consuming rice straw and husk within 36 and 30 hours, respectively. In contrast, efficiencies of 30 and 25% were obtained with bagasse and wood shavings within 48 hours, respectively (145).

Lignocellulosic agricultural products increase decolorization efficiency because they stimulate the production of lignolytic enzymes. The application of agricultural products instead of commercial and pure substrates like beef extract, peptone, and yeast extract is low-priced, economical, and environmentally friendly (149). Excessive concentrations of nitrogen sources have no significant effect on decolorization efficiency, but their use in wastewater treatment can adversely affect the environment. High nitrogen concentrations in the treated wastewater cause eutrophication in the aquatic environment. Therefore, using an appropriate nitrogen concentration for decolorization is essential (150).

In addition, the use of $NaNO_3$ and sodium nitrite ($NaNO_2$) as the nitrogen source in microbial culture decreases decolorization efficiency. Nitrate and nitrite act as electron receptors and compete with azo bonds for electron transfer. For example, the decolorization efficiency of Reactive Red-184 by *Halomonas* sp. strain A55 in the presence of $NaNO_3$ and $NaNO_2$ was less than 50%, whereas complete decolorization (100%) was achieved in the presence of yeast extract and peptone within 96 hours (151). Moreover, some microorganisms utilize dyes as the sole nitrogen and carbon sources (138).

3.4.1.4.2.8. Salinity: High concentrations of salts impact osmotic pressure, plasmolysis, microbial growth, and enzymatic metabolisms. Salinity causes dehydration of microbial cells. In the presence of salts, water molecules inside the structure of microbial cells move into external environments, leading to the prohibition of microbial growth and cell death (143). Consequently, removal efficiency through microbial methods decreases under saline conditions. In such cases, pre-treatment processes such as dilution with fresh water, electrodialysis, and reverse osmosis are indispensable and effective in providing favorable conditions for the growth and activity of non-salt-tolerant species. However, these methods are costly and increase the volume of wastewater. Conversely, salt-tolerant microorganisms can treat saline textile wastewater directly at industrial sites without pre-treatment, making the process more economical and beneficial.

Microorganisms are categorized as moderately halophilic (0.5-3.5 M NaCl) or extremely halophilic (>3.5 M NaCl) (85). The mechanism of salt tolerance in microorganisms is often associated with the accumulation of osmolytes. These molecules are produced under salinity (osmotic pressure) and heat-shock stress for osmotic adjustment. Osmolytes interact with the polar head groups of phospholipid membranes, which can maintain the liquid crystalline structure during cell dehydration, thereby protecting the cells (152).

Textile wastewater often contains high salt concentrations (about 60-100 g/L). Sodium hydroxide, commonly used to enhance dyeing performance in the dye bath, increases the pH (almost up to 10), sodium concentrations, and overall salinity in wastewater. Thus, the isolation and application of salt-tolerant microorganisms is an effective approach for textile wastewater treatment.

Consortium bacterial cultures BDN decolorized more than 90% of Reactive Blue-160 in the presence of 0-8 g/L of NaCl. However, at higher salt concentrations (40 g/L), about 60% efficiency was achieved, indicating that consortium BDN could not tolerate high salinity (153). Similarly, Liu et al reported that *Shewanella oneidensis* MR-1 achieved >97% decolorization efficiency of Congo Red at 0-20 g/L NaCl, but efficiency decreased to 70% at 40 g/L NaCl (154). Complete decolorization of Methyl Orange (50 mg/L) by *Bacillus circulans* BWL1061 was obtained under 60 g/L of NaCl within 12 hours, while efficiency fell below 20% at 80 and 100 g/L NaCl (155).

As noted, decolorization efficiencies of bacterial strains generally reduce with increasing salt concentration, although all strains maintain acceptable activity under low salt concentrations. Maqbool et al reported that low salt concentrations can stimulate decolorization efficiency. They observed that 12.5 g/L of salts enhanced the decolorization efficiency of four reactive dyes by *Pseudomonas aeruginosa* strain ZM130 (156).

The stimulatory concentration of salt differs among

various microorganisms. Therefore, an appropriate salt concentration should be determined under experimental conditions in the laboratory. The application of salt-tolerant decolorizer microorganisms is a promising approach for improving conventional biological treatment performance. It should be noted that the maximum flocculating activity of *Alteromonas* sp. CGMCC10612 for treating colored wastewater was observed at 30-40 g/L of salt, after which flocculation declined with further increase in salt concentration (99).

3.4.1.4.2.9. Electron Donors, Redox Mediators, and Ions in the Culture Medium: The presence of electron donor groups such as sodium formate, sodium citrate, sodium acetate, sodium succinate, sodium pyruvate, volatile fatty acids, glucose, and lactose enhances decolorization by facilitating electron transfer to the receptor groups of dyes. Methanol is broadly applied as a cost-effective electron donor in wastewater treatment plants (157).

Due to the toxicity of dyes, microbial growth and activity are often limited. Consequently, microorganisms cannot utilize dyes, but the presence of a co-substrate such as electron donor groups in the colored culture medium, promotes the catabolism of chromophore groups (62). Apostol et al investigated the effect of three electron donor compounds: glucose, lactose, and volatile fatty acids (acetic acid: propionic acid: butyric acid, 1:10:10) on the decolorization efficiency of 0.3 mM Erythrosine B by anaerobic granular sludge. The decolorization efficiencies were 38% (lactose), 36% (glucose), and 17% (volatile fatty acids).

Redox mediators facilitate the transmission of reducing equivalents from the co-substrate or electron donor to the electron receptor (158). Redox mediators can be either soluble or insoluble. Soluble mediators, such as quinone compounds including anthraquinone-2,6-disulfonate (AQDS) and anthraquinone-2-sulfonate (AQS), can be discharged. Therefore, they should be immobilized on various insoluble materials, such as metal oxide nanoparticles, alginate, activated carbon, graphite, or anion exchange resins, to avoid discharging (158,159).

Apostol et al reported that the decolorization efficiencies of Erythrosine B were 10, 46, 20, 66, 26, 56, 72, and 80% in the presence of 0.1 g/L activated carbon, 0.5 g/L activated carbon, 0.94 g VSS/L anaerobic granular biomass, 3.77 g VSS/L anaerobic granular biomass, 0.1 g/L activated carbon/0.94 g VSS/L anaerobic granular biomass, 0.5 g/L activated carbon/0.94 g VSS/L of anaerobic granular biomass, 0.1 g/L of activated carbon/3.77 g VSS/L anaerobic granular biomass, and 0.5 g/L activated carbon/3.77 g VSS/L anaerobic granular biomass, respectively. These results indicate that higher concentrations of activated carbon (as a redox mediator) combined with increased anaerobic granular biomass resulted in the maximum decolorization efficiency (158). Activated carbon has a quinone structure on its surface, which can enhance the decolorization efficiency of dyes (160).

The culture media of microorganisms contain different compounds and ions that influence bio-flocculation activity. Cationic ions are commonly applied as additives in the bio-flocculation process. Abbas et al noted that divalent cationic ions were more effective in enhancing the bio-flocculation activity of *Bacillus nitratireducens* for removing Eriochrome Black T compared to mono- and trivalent cationic ions. Specifically, the maximum and minimum flocculation activities of 89.63% and 87.14% were observed in the presence of calcium and aluminum ions, respectively. Divalent cationic ions destabilize the negatively charged sites on the microorganisms' structure through neutralization and bridging processes. The bridging of bio-flocculants generates long chains that can adsorb additional chains in the medium, forming larger flocs that rapidly sediment, thereby enhancing bio-flocculation activity (121).

Based on this review, the biological treatment of textile wastewater is highly promising. Due to factors such as the lack of chemical substance consumption (e.g., oxidants), no need for regeneration as in adsorbent-based methods, and fewer controversial operational difficulties, such as fouling, seen in membrane technologies, attention has been drawn to the use of biological methods. This approach is cost-effective and involves fewer operational difficulties if managed properly. The biological approach treats wastewater under both aerobic and anaerobic conditions (unlike other methods) and is also considered a source of energy generation under anaerobic conditions, which reduces energy production costs. Thus, it is a cost-effective technique that can be applied in various countries (157). However, bioremediation by bacteria, algae, fungi, and plants as a green technology has some disadvantages that can restrict the application of bioremediators under full-scale conditions. Bacterial decolorization (as the most popular applied microorganisms) of azo dyes by pure strains is efficient, but it is specific to each dye. Hence, the application of pure strains for treating real wastewater with complex dye mixtures is impractical on a large industrial scale. In addition, some carcinogenic intermediates may be generated during the bioremediation of dyes using bacterial strains. In such cases, the co-culture of bacterial strains for detoxifying and mineralizing intermediates is essential (161). Environmental and operational conditions, such as pH, temperature, and dye concentration, should be optimized to improve bioremediation efficiency regarding different microbial strains. Pathogenic strains are another challenging complication in bioremediation. It should also be noted that the cost and technical feasibility of strain isolation for decolorization remain controversial, in contrast to conventional biological treatment (162). These factors may limit the application of biological processes at full-scale, but they can be minimized by an engineering-based approach. This approach focuses on potent inoculum, biotechnological tools (genetic engineering and omics technology), hybrid systems (combining biological with

physical and chemical decolorization methods), and AI-driven bioremediation for enhancing bioremediation capacity.

4. Conclusion

Synthetic dyes are extensively applied in different industries due to their stability against light and detergents, a wide range of colors, and ease of production. The textile industry is one of the largest consumers of dyes among all industries. The textile industry contains toxic substances (dyes, heavy metals such as Cr, As, Cd, Pb, Ni, and Hg, biocides, surfactants, starch, and oxidizing agents) and has high BOD, COD, total dissolved solids, total suspended solids, and electrical conductivity. Azo dyes are the most widely used group of dyes in the textile industry. The presence of these dyes has adverse effects on water quality (e.g., reduced photosynthesis and anoxic conditions) and can cause serious health complications in humans (e.g., cancer, allergy, and irritation). Thus, they should be removed using appropriate physical, chemical, and biological approaches. Physical methods such as RO and NF nanofiltration are costly and generate secondary pollutants that need additional treatment. Chemical treatment methods require large amounts of chemicals, leading to high costs. Due to the restrictions of physicochemical methods, bioremediation has emerged as an efficient alternative for textile wastewater treatment. Biological treatment is considered cost-effective and environmentally friendly. Bio-decolorization can be carried out by different living organisms (bacteria, yeast, algae, fungi, and plants). Bacterial strains are extensively applied for biodecolorization due to their broad distribution and high adaptability. Since conventional wastewater treatment systems cannot remove dyes and other complex organic substances, the isolation, identification, and application of potent bacterial strains for textile wastewater treatment is a promising and efficient technique.

Bacterial decolorization can occur under aerobic, anoxic, and anaerobic conditions. At first, azo dyes should be treated under anaerobic and anoxic conditions for the breakage of azo bonds, followed by further degradation and detoxification (oxidative mechanisms) under aerobic or advanced oxidation processes. Reductive and oxidative degradation of azo dyes occurs through the action of azoreductase and laccase enzymes, respectively. It should be noted that bio-decolorization is influenced by operational factors such as agitation, pH, temperature, dye concentration, inoculum size, carbon source, nitrogen source, salinity, redox mediator, and electron donor compounds. These operational factors have positive impacts at their optimum levels, but beyond these levels, negative impacts may be observed. These factors affect the growth and activity of bacterial strains, particularly enzyme production and function, including azoreductase and lacasse. Therefore, identifying the optimum range of these factors is essential to improve the effectiveness of bacterial decolorization.

Authors' Contribution

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Competing Interests

The authors declare no conflict of interests.

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