



Role of Bacterial Consortia in the Decolorization of Azo Dyes

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ABSTRACT

Azo dyes, widely used in the textile industry, pose serious environmental and health risks due to their persistence, toxicity, and generation of carcinogenic aromatic amines during partial degradation. Conventional physicochemical methods for dye removal are often costly, energy-intensive, and produce secondary pollutants. Biological approaches, particularly using bacterial consortia, have emerged as sustainable, efficient, and cost-effective alternatives for azo dye decolorization and detoxification. This review explores the pivotal role of bacterial consortia in breaking the azo bond (-N=N-) primarily through reductive mechanisms involving azoreductase enzymes under microaerophilic or anaerobic conditions, followed by oxidative degradation of resulting intermediates under aerobic conditions. Consortia outperform single strains by offering synergistic interactions, broader substrate range, enhanced tolerance to extreme conditions (high salinity, alkalinity, temperature), faster decolorization rates (often >90–98% within hours to days), and superior mineralization and detoxification, as evidenced by reduced toxicity in bioassays (e.g., phytotoxicity, cytotoxicity, and aquatic organism tests). Key factors influencing performance—such as pH, temperature, carbon/nitrogen sources, oxygen levels, and dye concentration—are discussed, along with examples of effective consortia (e.g., involving *Bacillus*, *Pseudomonas*, *Halomonas*, and *Providencia* species) applied to reactive, direct, and disperse azo dyes. The review highlights the potential of bacterial consortia for real textile wastewater treatment and emphasizes future directions, including scale-up in bioreactors, metagenomic analysis of microbial interactions, and integration with advanced processes for complete mineralization.

Keywords: Azo dyes, Bacterial consortia, Decolorization, Biodegradation, Azoreductase, Textile wastewater, Detoxification, Synergistic degradation, Bioremediation, Halotolerant bacteria

Introduction

Azo dyes, characterized by the presence of one or more azo groups (-N=N-), represent the largest class of synthetic dyes used in industries worldwide. They account for approximately



60–70% of all dyes applied in textile processing, paper printing, leather tanning, food coloring, and cosmetics. These dyes provide vibrant colors, good fastness properties, and low production costs, making them highly popular in the global textile sector.

However, the extensive use of azo dyes has led to serious environmental concerns. During textile dyeing and finishing processes, a significant portion—often 10–50%—of applied dyes remains unfixed and is discharged into wastewater. Textile effluents containing azo dyes are highly colored even at low concentrations (10–50 mg/L), which reduces light penetration in water bodies, disturbs photosynthesis in aquatic plants and algae, lowers dissolved oxygen levels, and disrupts aquatic ecosystems. Moreover, under anaerobic conditions in the environment or in the human gut, azo dyes can be reduced to aromatic amines, many of which are known to be toxic, mutagenic, carcinogenic, and allergenic. These intermediates can bioaccumulate in food chains, posing risks to both aquatic life and human health. Conventional physicochemical methods such as adsorption, coagulation, oxidation, and membrane filtration are commonly used for dye removal, but they are often expensive, generate large amounts of sludge, require high energy, and may not achieve complete mineralization of the dyes.

Biological treatment offers a promising, cost-effective, and eco-friendly alternative for decolorization and degradation of azo dyes. Among biological agents, bacteria have received considerable attention due to their rapid growth, metabolic versatility, and ability to produce enzymes such as azoreductase, laccase, and peroxidases that can cleave the azo bond. While single bacterial strains can decolorize certain azo dyes, they frequently show limitations such as narrow substrate specificity, slow degradation rates, accumulation of toxic intermediates (e.g., aromatic amines), and poor performance under harsh conditions like high salinity, extreme pH, or fluctuating temperatures typical of real textile effluents.

Bacterial consortia—mixed populations of different bacterial species—address many of these limitations through synergistic interactions. In consortia, individual strains can perform complementary roles: one bacterium may initiate reductive cleavage of the azo bond under microaerophilic or anaerobic conditions, while others oxidize the resulting amines under aerobic conditions, leading to better mineralization, detoxification, and overall efficiency. Consortia often exhibit broader substrate range, higher tolerance to dye concentrations and environmental stresses, and faster decolorization rates compared to pure cultures. Recent



studies have demonstrated that bacterial consortia can achieve 90–99% decolorization of various reactive, direct, and disperse azo dyes within hours to days, with significant reduction in chemical oxygen demand (COD), biological oxygen demand (BOD), and total organic carbon (TOC), along with non-toxic end products confirmed by toxicity assays.

This research explores the role of bacterial consortia in the decolorization of azo dyes, including the underlying mechanisms, key enzymatic pathways, influencing factors (such as pH, temperature, carbon/nitrogen sources, and oxygen levels), performance examples from diverse consortia, and advantages over single-strain approaches. By highlighting recent advances, this paper aims to underscore the potential of bacterial consortia as a sustainable biotechnological solution for treating textile wastewater and mitigating the environmental impact of azo dyes.

Literature Review

Azo dyes represent the largest class of synthetic dyes used in textile, paper, leather, and food industries, accounting for 60–70% of total dye consumption worldwide. Their complex aromatic structure with one or more azo ($-N=N-$) linkages makes them highly stable, resistant to biodegradation, and potentially mutagenic or carcinogenic when released into the environment (Saratale et al., 2011). Textile effluents containing these dyes cause serious ecological problems, including reduced light penetration in water bodies, oxygen depletion, and toxicity to aquatic life and humans.

Conventional physicochemical treatment methods (coagulation, adsorption, membrane filtration, advanced oxidation) are effective for color removal but suffer from high operational costs, generation of large amounts of sludge, and incomplete mineralization of toxic intermediates (Saratale et al., 2011). Biological treatment using microorganisms has emerged as a sustainable, cost-effective, and environmentally friendly alternative because it can achieve complete mineralization without harmful by-products under suitable conditions.

Among microorganisms, bacteria are particularly promising due to their rapid growth, metabolic versatility, and ability to produce azoreductase, laccase, peroxidase, and other dye-degrading enzymes. However, single bacterial strains often show limited substrate specificity, slow degradation rates, and poor tolerance to high dye concentrations, salinity, pH extremes,



or temperature variations commonly found in real textile wastewater (Khehra et al., 2005; Tony et al., 2009).

Bacterial consortia (mixed cultures) consistently outperform individual strains because of synergistic metabolic interactions: one bacterium may cleave the azo bond reductively, while others oxidize the resulting aromatic amines or utilize toxic intermediates as carbon/energy sources. Consortia also exhibit greater stability, broader dye-degradation spectrum, and higher tolerance to harsh environmental conditions (Saratale et al., 2011; Lade et al., 2015).

Several studies have documented the superior performance of bacterial consortia. Lalnunhlimi and Krishnaswamy (2016) isolated a moderately alkaliphilic consortium (four *Bacillus* species) from saline soil that achieved 97.57% decolorization of Direct Blue 151 and 95.25% of Direct Red 31 (200 mg/L) within 5 days at pH 9.5 and 36°C. Decolorization was enhanced by sucrose (carbon source) and yeast extract (nitrogen source).

Lade et al. (2015) developed a consortium consisting of *Providencia rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 that decolorized four structurally diverse azo dyes (Reactive Black 5, Reactive Orange 16, Disperse Red 78, and Direct Red 81 at 100 mg/L) by 98–99% within 12–30 h under sequential microaerophilic/aerobic conditions. The process resulted in 62–72% reduction in total organic carbon (TOC), complete absence of aromatic amines, and full detoxification (no mortality in *Daphnia magna* bioassays).

Guo et al. (2020) enriched a halo-alkaliphilic consortium (ZW1) dominated by *Halomonas*, *Marinobacter*, and *Clostridiisalibacter* that efficiently decolorized Methanil Yellow G (>90%) under extreme conditions (5–10% salinity, pH 8–10, 40°C). Metagenomic analysis revealed key functional genes involved in azo bond reduction and aromatic ring cleavage.

Earlier work by Khehra et al. (2005) compared a four-member consortium (*Bacillus cereus*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Stenotrophomonas acidaminiphila*) with its individual strains and found the consortium decolorized multiple azo dyes (60 mg/L) 2–3 times faster than pure cultures. Tony et al. (2009) reported an aerobic bacterial consortium that achieved high decolorization of various textile azo dyes under aerobic conditions.

The literature clearly establishes that bacterial consortia provide faster, more complete, and more robust decolorization of azo dyes than single strains, especially when applied in sequential anaerobic/aerobic or microaerophilic/aerobic systems. These consortia also achieve

mineralization and detoxification, making them highly suitable for real-world textile wastewater treatment. Future research should focus on scaling up these processes in bioreactors and exploring metagenomics for better understanding of community dynamics.

Mechanisms of Azo Dye Decolorization

Bacteria break the azo bond mainly under low-oxygen (microaerophilic or anaerobic) conditions using enzymes like azoreductase (which uses NADH or NADPH to reduce the –N=N– bond into amines). Under aerobic conditions, enzymes like laccase, veratryl alcohol oxidase, and peroxidases oxidize the amines into less toxic compounds. Consortia combine these steps: one bacterium reduces the dye, another breaks down the amines. This leads to better mineralization (measured as reduction in total organic carbon, often 60–70%).

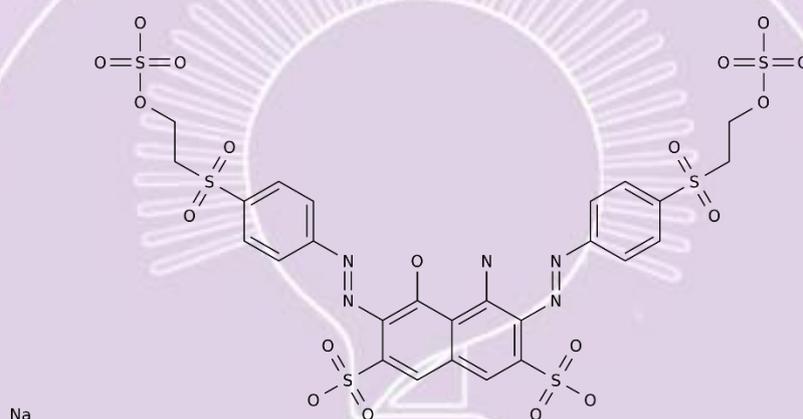


Figure 1: Chemical structure of Reactive Black 5 (a common azo dye used in studies). The –N=N– bond is the target for bacterial breakdown.

Advantages of Bacterial Consortia

Consortia are better than single strains because:

- They handle a wider range of dyes and concentrations.
- They tolerate extreme conditions (high salt, alkaline pH, high temperature).
- They show synergy: one strain produces metabolites that help another.
- They achieve faster and higher decolorization (often 10–30% better).
- They detoxify harmful intermediates.



Examples of Bacterial Consortia and Their Performance

Table 1: Selected examples of bacterial consortia for azo dye decolorization

Consortium (strains)	Dyes (concentration)	Conditions	Decolorization (%) / Time	Reference
Bacillus flexus, B. cereus, B. cytotoxicus, Bacillus sp.	Direct Blue 151 & Direct Red 31 (200 mg/L)	pH 9.5, 36°C, saline soil origin	95–98% / 5 days	Lade et al. (2016)
Providencia rettgeri HSL1 + Pseudomonas sp. SUK1	Reactive Black 5, Reactive Orange 16, Disperse Red 78, Direct Red 81 (100 mg/L)	Sequential microaerophilic/aerobic, 30°C, pH 7	98–99% / 12–30 h	Phugare et al. (2015)
Halomonas, Marinobacter, Clostridiisalibacter	Metanil Yellow G (various)	5–10% salt, pH 8–12, 40°C	>90% / few hours	Guo et al. (2020)
Various (e.g., Bacillus, Pseudomonas, Ochrobactrum)	Multiple reactive dyes	Microaerophilic, pH 8, 40°C	94–100% / <24 h	Khan et al. (2014); Shah et al. (2016)

Factors Affecting Decolorization

- pH: Most consortia work best at pH 7–9.5 (alkaliphilic ones at pH 9–11).
- Temperature: 30–40°C is common; some tolerate 50°C.
- Carbon and Nitrogen sources: Sucrose, glucose, yeast extract greatly improve rates.
- Dye concentration: Up to 200–300 mg/L works well; higher levels slow the process.



- Oxygen: Microaerophilic or sequential microaerophilic/aerobic gives best results (complete detoxification).

- Salinity: Halotolerant consortia handle 5–10% salt.

Table 2: Optimization example from one study (Lade et al., 2016)

Parameter	Optimal value	Decolorization (%)
pH	9.5	87
Temperature	36°C	87.5
Carbon source	Sucrose (1%)	90.6
Nitrogen source	Yeast extract (0.5%)	92.7
Combined C+N sources	Sucrose + yeast extract	94

Detoxification and Mineralization

Many consortia not only remove color but also reduce toxicity. In sequential microaerophilic/aerobic processes, aromatic amines (toxic intermediates) are further broken down, leading to 60–70% TOC reduction and non-toxic metabolites (tested with *Daphnia magna* or plants—no mortality or growth inhibition).

Conclusion

Bacterial consortia are powerful tools for treating azo dye wastewater. They are efficient, adaptable, and environmentally safe. Future work should focus on scaling up in bioreactors, using real industrial effluent, and understanding community interactions through metagenomics. This approach can help industries meet strict environmental regulations while being cost-effective.

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