

Journal of Advanced Environmental Research and Technology

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Microbial Decolorization of Dyes: Evaluating the Potential of *Pseudomonas sp.*, *Bacillus sp.*, and *Staphylococcus haemolyticus*

R, Subashini^{1,*}, K, Kaviya Priya², E, Sri Priya³, S, Naresh Kannan⁴

¹ Department of KIT-Kalaignarkarunanidhi Institute of Technology

² Department of KIT-Kalaignarkarunanidhi Institute of Technology

³ Department of KIT-Kalaignarkarunanidhi Institute of Technology

⁴ Department of KIT-Kalaignarkarunanidhi Institute of Technology

Article info

Received 2024-08-26

Revised 2024-10-20

Accepted 2024-02-21

Abstract

Synthetic dyes, particularly azo compounds, are widely used in the textile, leather, and allied industries, but their persistence and toxicity present major environmental challenges. Microbial decolorization has emerged as an eco-friendly alternative to conventional physicochemical methods, driven largely by enzyme-mediated pathways. This review comparatively evaluates the dye-degrading potential of *Pseudomonas*, *Bacillus*, and *Staphylococcus haemolyticus*. *Pseudomonas* spp. are recognized for rapid azo dye degradation (>90% within 12–24 h) but face limitations due to oxygen dependence and pathogenicity concerns. *Bacillus* spp., with their thermostable and alkali-tolerant enzymes, show 70–85% degradation under alkaline effluent conditions and are well suited for industrial scalability. In contrast, *S. haemolyticus*, an underexplored candidate, demonstrates dual enzyme activity (azoreductase and laccase), enabling sequential reductive–oxidative degradation and achieving 85–90% decolorization of methyl orange within 24 h and 70–80% Congo red within 36 h. Its adaptability under variable oxygen conditions highlights a unique advantage over conventional bacterial systems. Prospects lie in integrating omics, enzyme immobilization, nanotechnology, and AI-based bioprocess optimization to enhance efficiency and scalability, while biosafety considerations remain essential. Overall, *S. haemolyticus* represents a promising next-generation candidate to complement established microbial systems for sustainable wastewater treatment.

Keywords

Microbial dye decolorization

Azo dye degradation

Bioremediation

Textile wastewater

Sustainable biotechnology

* Corresponding author: subashini.kit2022@gmail.com

DOI:

1. Introduction

Synthetic dyes are widely applied in the textile, leather, paper, plastics, cosmetics, and food industries because of their low cost, high stability, and vivid coloration. Among them, azo dyes account for 60–70% of global synthetic dye production (Kusumlata et al., 2024). However, their widespread use and improper disposal pose serious environmental and health concerns. The textile industry alone contributes nearly 20% of global industrial wastewater pollution, discharging an estimated 280,000 tons of dyes annually into aquatic ecosystems (Ayub et al., 2025a). Even at low concentrations, azo dyes reduce light penetration, inhibit photosynthesis, and deplete dissolved oxygen, thereby disrupting aquatic ecosystems. Their metabolites, including aromatic amines, are toxic, mutagenic, and potentially carcinogenic, raising additional risks to human health (Chougule et al., 2014; Afrin et al., 2021). Conventional treatment methods such as adsorption, coagulation–flocculation, and advanced oxidation processes are often inefficient, energy-intensive, and generate secondary pollutants (Chung, 2016). In contrast, microbial bioremediation has emerged as an eco-friendly and cost-effective alternative. Microbial enzymes, particularly azoreductase and laccase, play pivotal roles in dye degradation: azoreductase catalyzes the reductive cleavage of azo bonds under anaerobic conditions, while laccase oxidizes phenolic and non-phenolic intermediates under aerobic conditions, promoting further mineralization (Saeed et al., 2022; Ellafi et al., 2023). Together, these enzymatic systems provide a sustainable approach to detoxifying complex dye structures. Several microbial groups have been investigated for dye degradation. *Pseudomonas* spp. are widely recognized for rapid azo dye degradation under aerobic conditions, while *Bacillus* spp. are valued for their robust, thermostable, and alkali-tolerant enzymes, making them suitable for industrial effluents. Ligninolytic fungi are also important for their high laccase productivity. Recently, however, *Staphylococcus haemolyticus*, traditionally regarded as an opportunistic pathogen, has emerged as a novel candidate. Its unique ability to produce both azoreductase and laccase enables sequential reductive–oxidative degradation of dyes under variable oxygen conditions (Li et al., 2020). Despite extensive research on *Pseudomonas* and *Bacillus*, the enzymatic potential of *S. haemolyticus* remains underexplored. Its dual-enzyme capability, adaptability, and biofilm-forming capacity suggest significant promise for industrial wastewater treatment. This review presents a comparative framework that integratively positions the underexplored *Staphylococcus haemolyticus* alongside the well-established dye-degrading genera *Pseudomonas* and *Bacillus*, with particular emphasis on its unique dual-enzyme (Azoreductases and Laccases) mechanism. It critically analyzes their enzymatic mechanisms, degradation efficiencies, and industrial applicability, while highlighting the role of emerging approaches such as omics, enzyme immobilization, nanotechnology, and artificial intelligence in advancing sustainable microbial dye remediation.

1.1 Microbial Bioremediation: A Sustainable Alternative

Among the various strategies developed to address dye pollution, microbial bioremediation using enzyme-producing microorganisms has emerged as one of the most promising and sustainable approaches. Unlike conventional chemical treatments, microbial decolorization operates under mild conditions, avoids generating toxic byproducts, and can be readily integrated into existing wastewater treatment frameworks (Srivastava et al., 2014). Microbial enzymes play a crucial role in the degradation of complex and chemically stable dye molecules by transforming them into less toxic or fully mineralized products, making them promising agents for environmentally friendly remediation. Two key enzymes involved in this process are azoreductase and laccase. Azoreductase catalyzes the reductive cleavage of azo bonds under anaerobic or microaerophilic conditions, yielding colorless aromatic amines that can subsequently undergo aerobic degradation. In contrast, laccase is a multicopper oxidase that operates under aerobic conditions and is capable of oxidizing a broad range of phenolic and non-phenolic compounds, thereby promoting the breakdown of persistent aromatic intermediates. The complementary activities of these enzymes create a synergistic degradation pathway that enhances both dye removal efficiency and detoxification (Saeed et al., 2022). This dual-enzyme mechanism underscores the potential of microbial systems as scalable, green technologies for industrial dye remediation, paving the way for the exploration of novel microbial candidates.

1.2 Microbes for Dye-Degrading Enzymes

Several microorganisms have been investigated for their dye-decolorizing potential, with bacteria and fungi playing leading roles (Sarkar et al., 2017). This review focuses on three representative bacterial groups—*Staphylococcus haemolyticus*, *Pseudomonas* spp., and *Bacillus* spp., providing a comparative overview of their enzyme production, degradation efficiency, and industrial applicability.

1.2.1 *Staphylococcus haemolyticus*

Staphylococcus haemolyticus, a coagulase-negative staphylococcus (CoNS), is traditionally regarded as a pathogen but is now gaining attention for its biotechnological potential. It is a Gram-positive, facultative anaerobe commonly

isolated from human skin and hospital environments. Importantly, it produces both azoreductase and laccase, enabling sequential reductive-oxidative degradation of dyes under variable oxygen conditions, an essential trait for complete mineralization (Nisar et al., 2017). These enzymatic traits are complemented by an adaptable genome, abundant mobile genetic elements, and biofilm-forming capacity, which collectively enhance survival under industrial effluent conditions. Studies report efficient degradation of methyl orange, Congo red, and crystal violet by *S. haemolyticus*, underscoring its underexploited potential in dye bioremediation (Grumann et al., 2014).

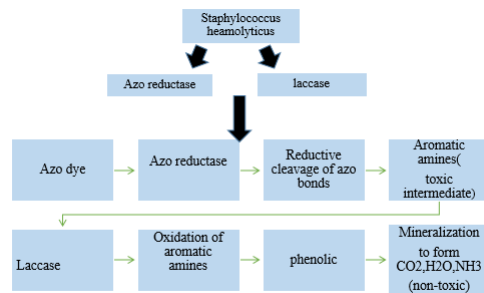


Fig. 1. Schematic representation of the dual-enzyme (azoreductase–laccase) mediated dye degradation pathway in *Staphylococcus haemolyticus*.

1.2.2 *Pseudomonas* spp.

Members of the genus *Pseudomonas* are well established in environmental biotechnology due to their metabolic versatility, fast growth, and ability to degrade diverse xenobiotic compounds (Rima et al., 2022). Species such as *P. aeruginosa*, *P. putida*, and *P. fluorescens* exhibit strong azo dye degradation mediated primarily by azoreductase activity. These bacteria thrive in diverse environments and are effective in both aerobic and anaerobic bioreactors. However, their efficiency often depends on aeration, which can complicate large-scale treatment designs. Moreover, some strains, particularly *P. aeruginosa*, are opportunistic pathogens, which limits their direct industrial use unless genetically modified or safely immobilized (Hu, 2001).

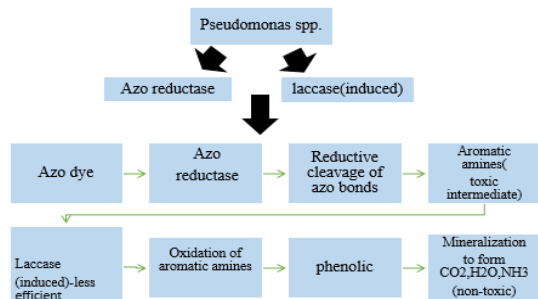


Fig. 2. Azoreductase-mediated azo dye degradation pathway in *Pseudomonas* spp. under aerobic and microaerophilic conditions.

1.2.3 *Bacillus* spp.

The genus *Bacillus* comprises Gram-positive, spore-forming bacteria widely recognized for industrial applications. They produce thermostable, pH-tolerant enzymes, making them particularly suitable for large-scale bioreactors (Bioremed Biodeg, 2013). Notable species such as *B. subtilis*, *B. cereus*, and *B. licheniformis* are reported to produce azoreductase and laccase-like multicopper oxidases. Their spore-forming capacity confers resilience in adverse environments, while their ability to grow in alkaline pH matches the conditions typical of textile effluents. Furthermore, many *Bacillus* species are Generally Regarded As Safe (GRAS), supporting their commercial scalability. However, enzyme yields may vary significantly across strains, and optimization is often required for specific applications.

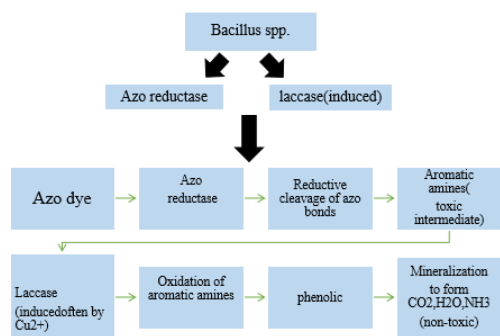


Fig. 3. Mechanism of azo dye degradation by *Bacillus* spp. highlighting thermostable and alkali-tolerant enzyme systems.

2. MICROBIAL SOURCES OVERVIEW

The exploration of microbial systems for dye degradation has led to the identification of several potent strains capable of producing azoreductase and laccase enzymes. Among them, *Staphylococcus haemolyticus*, *Pseudomonas* spp., *Bacillus* spp., and various ligninolytic fungi have demonstrated considerable promise. These microorganisms differ significantly in their physiological properties, enzyme profiles, environmental tolerances, and degradation efficiencies, making a comparative understanding essential for selecting suitable candidates for specific industrial and environmental applications. This section provides an in-depth overview of these four microbial groups with respect to their enzymatic potential, ecological adaptability, and known applications in synthetic dye decolorization.

2.1 *Staphylococcus haemolyticus*

Staphylococcus haemolyticus is a Gram-positive, coagulase-negative *staphylococcus* (CoNS) traditionally associated with hospital-acquired infections. Although often categorized as a commensal organism on human skin it has emerged as a relevant subject in environmental biotechnology due to its unique enzymatic potential. It is facultatively anaerobic, non-motile, and possesses a highly dynamic genome with a large number of mobile genetic elements, insertion sequences, and gene transfer capacities. This genetic plasticity enables rapid adaptation to environmental stress, including the presence of toxic compounds such as synthetic dyes (Oh et al., 1999). *Staphylococcus haemolyticus* is genetically different among coagulase-negative staphylococci due to its highly dynamic and adaptable genome. Its specific genetic character is the presence of a large number of insertion sequence elements, where mobile DNA sequences facilitate genomic rearrangements, deletions, and horizontal gene transfer (Al-Ansari et al., 2020). Its genome size is approximately 2.6 million base pairs, and the presence of its numerous open reading frames encodes its virulence factors. When compared to other *staphylococcus*, they do not rely on the *icaADBC* operon, which is responsible for producing polysaccharide intercellular adhesion in biofilms. Even though the *ica* operon may be present in some strains, its expression and role in biofilm formation remain ambiguous. Strategies such as enzyme extraction and immobilization or the use of genetically attenuated strains may help mitigate potential risks while preserving their high enzymatic activity (Plata et al., 2009).

2.2 *Pseudomonas* spp.

The genus *Pseudomonas* comprises Gram-negative, rod-shaped bacteria renowned for their remarkable metabolic versatility, environmental adaptability and capacity to degrade a broad range of pollutants. Among these, *Pseudomonas aeruginosa*, *P. putida*, and *P. fluorescens* are the most extensively studied species in environmental remediation, owing to their robust enzymatic systems and rapid growth across diverse conditions. *Pseudomonas* spp. are notable producers of azoreductase enzymes, which facilitate the reductive cleavage of azo bonds in numerous synthetic dyes, including both monoazo and diazo compounds. These enzymes are typically NAD(P)H-dependent flavoproteins that operate under microaerophilic or anaerobic conditions. In addition, several *Pseudomonas* strains synthesize peroxidases and laccase-like multicopper oxidases, particularly in response to copper-induced stress, further enhancing their oxidative degradation potential (Shah et al., 2013). Their genetic tractability makes *Pseudomonas* an ideal model for bioremediation studies. The high degradative capacity, combined with their ability to thrive in different ecological niches, including soil, water, and sludge, makes them suitable for in situ and ex situ bioremediation applications. However, *P. aeruginosa* is also a known pathogen, and its industrial application is limited unless containment and safety mechanisms are in place. Another practical limitation is their need for high oxygen transfer rates, which may complicate bioreactor design in oxygen-limited environments (Chougule et al., 2014).

2.3 *Bacillus* spp.

Bacillus species are Gram-positive, endospore-forming bacteria found abundantly in soil, water, and industrial waste. Known for their robustness, high enzyme productivity, and ease of cultivation, they are frequently used in industrial microbiology. Notable species such as *Bacillus subtilis*, *B. licheniformis*, and *B. cereus* have demonstrated the ability to degrade synthetic dyes through the production of azoreductase and, in some cases, laccase-like oxidases. These organisms are particularly well-suited for use in high pH and high-temperature environments, often found in textile effluents (Ellafi et al., 2023). The production of thermostable and pH-tolerant enzymes makes *Bacillus* species highly attractive for industrial applications, where process conditions frequently fluctuate. Enzymes derived from *Bacillus* often exhibit strong stability under alkaline conditions, which are commonly required in dye processing industries. Moreover, the ability of *Bacillus* to form endospores significantly enhances their survivability under harsh environmental conditions and extends the shelf life of bioremediation formulations. Many *Bacillus* species are also classified as Generally Regarded As Safe (GRAS) by regulatory authorities, further supporting their suitability for large-scale and industrial deployment. Nevertheless, when compared with *Pseudomonas* or fungal systems, certain *Bacillus* strains may exhibit lower dye specificity or slower degradation kinetics, necessitating strategies such as metabolic engineering or enzyme overexpression to improve their bioremediation efficiency.

Micro organism	Key Enzymes	Strengths	Limitations	Examples of Degraded Dyes	References
<i>Staphylococcus haemolyticus</i>	Azoreductase + Laccase (dual enzyme system)	Works under aerobic and anaerobic conditions; sequential reductive-oxidative degradation; adaptable genome; biofilm formation aids survival in harsh effluents	Opportunistic pathogen (biosafety concerns); enzymatic potential underexplored; requires optimization for scale-up	Methyl orange, Congo red, and Crystal violet	Nisar et al., 2017; Grumann et al., 2014
<i>Pseudomonas</i> spp.	Azoreductase (main); Laccase-like oxidases under stress	Rapid degradation rates; metabolically versatile; effective in aerobic/microaerophilic conditions; broad substrate range	Requires aeration for high efficiency; some strains (e.g., <i>P. aeruginosa</i>) are pathogenic; efficiency decreases under oxygen limitation	Methyl orange, Congo red, and Reactive dyes	Rima et al., 2022; Hu, 2001
<i>Bacillus</i> spp.	Azoreductase + Laccase-like multicopper oxidases	Produces thermostable, pH-tolerant enzymes; spore-forming (high resilience); grows well in alkaline effluents; GRAS status supports industrial use	Slower degradation than <i>Pseudomonas</i> ; enzyme yields vary across strains; may require inducers for optimal activity	Methyl orange, Crystal violet, Remazol Black B	Bioremed. Biodeg., 2013

Table 1. Comparative overview of dye-degrading bacterial species, target dyes, enzymatic systems, and reported decolorization efficiencies.

3. Dye Degradation Efficiency

The efficiency with which microorganisms degrade synthetic dyes is a critical factor in determining their suitability for bioremediation and wastewater treatment. Synthetic dyes such as azo, triphenylmethane, and anthraquinone-based compounds possess complex aromatic structures that make them resistant to conventional physicochemical methods. Microbial degradation, particularly through enzyme-

mediated processes, provides an eco-friendly and effective alternative (Shamim Hossain et al., 2022). *Staphylococcus haemolyticus* has recently emerged as a promising candidate for dye degradation. Traditionally associated with nosocomial infections, environmental isolates of *S. haemolyticus* demonstrate strong bioremediation potential due to their dual-enzyme system (azoreductase and laccase). Under optimized laboratory conditions, it degrades 85–90% of methyl orange within 24 h and 70–80% of Congo red within 36 h. Moderate decolorization (65–75%) of crystal violet has also been reported under anaerobic setups. Factors such as pH, temperature, cofactor availability (NADH/NADPH) and inducers like copper sulfate significantly influence efficiency. The sequential reductive–oxidative pathway reduces the risk of toxic aromatic amine accumulation (Li et al., 2020). *Pseudomonas* spp. are among the most extensively studied dye-degrading bacteria. They produce high levels of azoreductase and exhibit versatile metabolic pathways for azo dye degradation. *P. aeruginosa* and *P. putida* have been shown to achieve >95% decolorization of methyl orange within 24 h, while Congo red degradation ranges between 85–92% depending on strain and conditions (Mandic et al., 2019). Their high efficiency under aerobic or microaerophilic conditions can be enhanced with carbon supplements such as glucose or acetate. However, their performance declines in oxygen-limited environments, and biosafety concerns persist due to the pathogenic nature of some strains (Afrin et al., 2021). *Bacillus* spp., including *B. subtilis*, *B. licheniformis*, and *B. cereus*, also exhibit strong dye-degrading capabilities, especially under the alkaline and high-temperature conditions typical of textile effluents. Although their azoreductase activity is generally lower than that of *Pseudomonas*, they achieve significant efficiencies: *B. subtilis* can degrade 80–85% of methyl orange and ~70% of crystal violet within 24–48 h (Parmar & Shukla, 2018). Copper-induced laccase-like activity further expands their substrate range. Their spore-forming ability ensures resilience in harsh industrial environments, and their GRAS status supports large-scale applications. However, strain-specific variability and the need for inducers may limit uniform efficiency. A comparative overview of dye degradation efficiencies among the three bacterial groups is presented in Fig. 4.

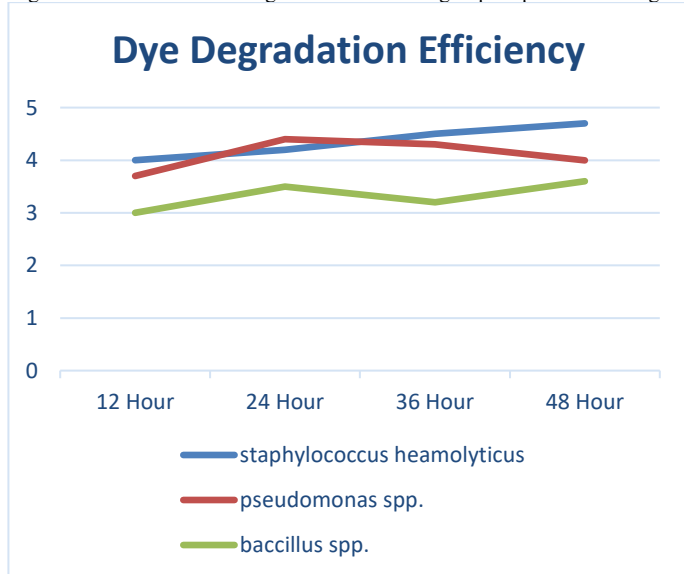


Fig. 4. Comparative dye decolorization efficiency of *Staphylococcus haemolyticus*, *Pseudomonas* spp., and *Bacillus* spp. across representative azo dyes under reported experimental conditions.

Overall, dye degradation efficiency is governed by enzyme type, environmental adaptability, and process optimization. *S. haemolyticus* stands out due to its dual-enzyme system, enabling sequential degradation under variable oxygen conditions. *Pseudomonas* excels in rapid azo dye degradation but requires oxygen management and biosafety precautions. *Bacillus* offers resilience and industrial scalability but may require enzyme induction for optimal activity (Hassan MM et al., 2013). In practice, microbial consortia or bacterial–fungal combinations may provide synergistic benefits, achieving faster and more complete dye mineralization. With advances in genetic engineering, enzyme immobilization, and AI-assisted process optimization, microbial systems can be further tailored for efficient, scalable, and sustainable wastewater treatment.

4. COMPARATIVE ANALYSIS

Microorganism	Key Enzymes	Dye Examples	Degradation Efficiency	Conditions	References
<i>Staphylococcus haemolyticus</i>	Azoreductase + Laccase	Methyl orange, Congo red, Crystal violet	85–90% (MO, 24 h); 70–80% (CR, 36 h); 65–75% (CV, anaerobic)	Works in aerobic and anaerobic conditions; efficiency is influenced by pH, cofactors, and inducers	Li et al., 2020
<i>Pseudomonas</i> spp.	Azoreductase; Laccase-like oxidases	Methyl orange, Congo red	>95% (MO, 12–24 h); 85–92% (CR, strain-dependent)	Aerobic/microaerophilic; enhanced with carbon supplements	Mandic et al., 2019; Afrin et al., 2021
<i>Bacillus</i> spp.	Azoreductase + Laccase-like oxidases	Methyl orange, Crystal violet, Remazol Black B	80–85% (MO, 24–48 h); ~70% (CV)	Alkaline, high-temperature; enzyme activity enhanced by copper ions	Parmar & Shukla, 2018; Bioremed Biodeg, 2013

Table 2. Comparative dye degradation efficiency of selected microorganisms

5. FUTURE SCOPE

5.1 Genetic Engineering and Synthetic Biology

Genetic engineering and synthetic biology provide promising tools to enhance microbial azo dye degradation. CRISPR-Cas9 and metabolic engineering can be employed to overexpress *azoreductase* and *laccase* genes or to construct non-pathogenic chassis strains of *Staphylococcus haemolyticus* suitable for industrial use (Kusumlata et al., 2024). A key hypothesis is that **dual-enzyme pathways combining reductive (azoreductase) and oxidative (laccase) activities can be engineered to achieve superior efficiency across structurally diverse dye classes**. Furthermore, **non-pathogenic hosts engineered to express dye-degrading enzymes from opportunistic pathogens may retain enzymatic efficiency while ensuring biosafety**.

5.2 Omics and Systems Biology Approaches

The integration of omics platforms—genomics, transcriptomics, proteomics, and metabolomics—is essential for comprehensively mapping dye degradation pathways. Systems biology approaches can model regulatory networks that control enzyme expression. It is hypothesized that specific regulatory elements govern the induction of azoreductase and laccase under stress conditions, and identifying these elements could enable rational promoter engineering. Similarly, system-level modeling of microbial consortia may uncover coordinated enzyme expression patterns, which can be optimized to achieve complete dye mineralization.

5.3 Nanotechnology and Enzyme Immobilization

Nanotechnology offers a transformative strategy to stabilize enzymes and improve catalytic efficiency under harsh effluent conditions. Immobilization of *azoreductase* and *laccase* on nanomaterials, membranes, or biochar has already shown improvements in stability and reusability (Ayub et al., 2025). A future direction is to test the hypothesis that **novel nanocarriers (e.g., graphene composites, magnetic nanoparticles) can extend enzyme half-life in industrial reactors by at least two-fold compared to conventional supports**. Additionally, **hybrid microbial–nanomaterial systems are expected to achieve simultaneous**

dye removal and effluent detoxification more effectively than microbial systems alone.

5.4 Artificial Intelligence and Machine Learning

Artificial intelligence (AI) and machine learning (ML) represent powerful tools for optimizing microbial bioprocesses. Predictive models can simulate enzyme–substrate interactions, optimize reactor conditions, and forecast degradation kinetics (Das et al., 2023). It is hypothesized that **AI-based molecular docking and ML algorithms can accurately predict enzyme–dye binding affinities, which can then be validated through kinetic and structural assays.** Furthermore, **ML-driven consortia design may generate optimized microbial combinations capable of degrading multi-dye effluents with higher efficiency than single strains.**

5.5 Regulatory and Policy Framework

Despite technological progress, biosafety and regulatory considerations remain critical. Organisms such as *S. haemolyticus* and *P. aeruginosa* are opportunistic pathogens, necessitating strict containment, genetic attenuation, or immobilization strategies before industrial application (Ellafi et al., 2023). A working hypothesis is that **genetically attenuated or immobilized strains can retain high enzymatic activity while minimizing biosafety risks.** Aligning microbial dye degradation with global environmental policies and the United Nations Sustainable Development Goals (SDGs) will not only facilitate regulatory approval but also mainstream microbial bioremediation as a sustainable wastewater treatment technology.

5.6 Biosafety and Risk Mitigation Strategies

Although *Staphylococcus haemolyticus* and certain *Pseudomonas* species exhibit high dye-degrading efficiency, their opportunistic pathogenic nature necessitates careful biosafety management for industrial applications. Several strategies can effectively mitigate these risks while preserving enzymatic performance. One practical approach is enzyme purification and immobilization, where azoreductase and laccase are extracted and immobilized on solid supports, eliminating the need for live cells and reducing biosafety concerns. Similarly, cell-free enzymatic systems offer controlled, pathogen-free dye degradation and simplify regulatory compliance. Genetic attenuation and synthetic biology provide additional solutions by deleting virulence genes or transferring dye-degrading genes into non-pathogenic or GRAS hosts such as *Bacillus* spp. Furthermore, closed and immobilized bioreactor systems can prevent environmental release, ensuring safe large-scale operation. Together, these strategies demonstrate that biosafety limitations associated with opportunistic dye-degrading bacteria can be effectively managed, enabling their safe and sustainable use in industrial wastewater treatment.

6. CONCLUSION

The extensive use of azo dyes in textile and allied industries poses a critical challenge due to their toxicity, persistence, and resistance to conventional treatment methods. Microbial enzymatic degradation, mediated primarily by azoreductase and laccase, offers a sustainable alternative by transforming hazardous compounds into less toxic or mineralized products under eco-friendly conditions. This review comparatively analyzed the dye-degrading capabilities of *Staphylococcus haemolyticus*, *Pseudomonas* spp., and *Bacillus* spp. While *Pseudomonas* remains a benchmark for rapid azo dye degradation and *Bacillus* offers resilience under alkaline, industrially relevant conditions, *S. haemolyticus* emerges as a uniquely promising candidate. Its dual-enzyme system, capable of sequential reductive and oxidative degradation, enables comprehensive dye mineralization under variable oxygen conditions—a feature rarely observed in bacterial systems. Looking forward, the hypotheses outlined in this review chart a clear research agenda: CRISPR-mediated engineering of dual-enzyme pathways may expand dye substrate range; omics-driven modeling is expected to uncover regulatory networks that control enzyme expression; nanotechnology-based immobilization could extend enzyme half-life in harsh effluents; and AI/ML tools are likely to optimize microbial consortia for multi-dye degradation. Equally, the hypothesis that attenuated or immobilized strains can retain enzymatic activity while minimizing biosafety risks must be tested to ensure safe industrial deployment. In conclusion, while *S. haemolyticus* is still underexplored, these forward-looking directions position it as a potential next-generation microbial platform for azo dye degradation. By integrating synthetic biology, systems biology, nanotechnology, AI-driven optimization, and regulatory foresight, microbial enzyme-based systems can evolve into scalable, cost-effective, and sustainable technologies for wastewater treatment—aligning with global sustainability goals and the circular economy.

References

- [1] Afrin, S., Shuvo, H. R., Sultana, B., Islam, F., Rus'd, A. A., Begum, S., & Hossain, M. N. (2021). The degradation of textile industry dyes using the effective bacterial consortium. *Heliyon*, 7(10). <https://doi.org/10.1016/J.HELIYON.2021.E08102/ASSET/561A9E07-1813-48DA-A990-1492319D2FA5/MAIN.ASSETS/GR8.JPG>
- [2] Al-Ansari, M., Kalaiyarasi, M., Almalki, M. A., & Vijayaraghavan, P. (2020). Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from *Streptomyces* sp. AS11 isolated from the marine environment. *Journal of King Saud University - Science*, 32(3), 1993–1998. <https://doi.org/10.1016/j.jksus.2020.02.005>
- [3] Ayub, A., Wani, A. K., Chopra, C., Sharma, D. K., Amin, O., Wani, A. W., Singh, A., Manzoor, S., & Singh, R. (2025a). Advancing Dye Degradation: Integrating Microbial Metabolism, Photocatalysis, and Nanotechnology for Eco-Friendly Solutions. *Bacteria 2025*, Vol. 4, Page 15, 4(1), 15. <https://doi.org/10.3390/BACTERIA4010015>
- [4] Ayub, A., Wani, A. K., Chopra, C., Sharma, D. K., Amin, O., Wani, A. W., Singh, A., Manzoor, S., & Singh, R. (2025b). Advancing Dye Degradation: Integrating Microbial Metabolism, Photocatalysis, and Nanotechnology for Eco-Friendly Solutions. *Bacteria 2025*, Vol. 4, Page 15, 4(1), 15. <https://doi.org/10.3390/BACTERIA4010015>
- [5] Bafana, A., Devi, S. S., & Chakrabarti, T. (2011). Azo dyes: Past, present and the future. *Environmental Reviews*, 19(1), 350–370. <https://doi.org/10.1139/A11-018;WEBSITE:WEBSITE:NRC-SITE;ISSUE:ISSUE:10.1139/ER.2011.19NA;WGROU:STRING:CSP>
- [6] Bioremed Biodeg, J. (2013). *Microbial degradation of Textile Dye (Remazol Black B) by Bacillus spp.* ETL-2012. <https://doi.org/10.4172/2155-6199.1000180>
- [7] Chougule, A. S., Jadhav, S. B., & Jadhav, J. P. (2014). Microbial Degradation and Detoxification of Synthetic Dye Mixture by *Pseudomonas* sp. SUK 1. *Proceedings of the National Academy of Sciences India Section B - Biological Sciences*, 84(4), 1059–1068. <https://doi.org/10.1007/S40011-014-0313-Z/METRICS>
- [8] Chung, K. T. (2016). Azo dyes and human health: A review. *Journal of Environmental Science and Health, Part C*, 34(4), 233–261. <https://doi.org/10.1080/10590501.2016.1236602>
- [9] Das, S., Cherwoo, L., & Singh, R. (2023). Decoding dye degradation: Microbial remediation of textile industry effluents. In *Biotechnology Notes* (Vol. 4, pp. 64–76). KeAi Communications Co. <https://doi.org/10.1016/j.biotno.2023.10.001>
- [10] Ellafi, A., Dali, A., Mnif, S., & Ben Younes, S. (2023). Microbial Enzymatic Degradation, Spectral Analysis and Phytotoxicity Assessment of Congo Red Removal By *Bacillus* spp. *Catalysis Letters* 2023 153:12, 153(12), 3620–3633. <https://doi.org/10.1007/S10562-023-04272-8>
- [11] Fredheim, E. G. A., Klingenberg, C., Rohde, H., Frankenberger, S., Gaustad, P., Flægstad, T., & Sollid, J. E. (2009). Biofilm formation by *staphylococcus haemolyticus*. *Journal of Clinical Microbiology*, 47(4), 1172–1180. <https://doi.org/10.1128/JCM.01891-08>
- [12] Grumann, D., Nübel, U., & Bröker, B. M. (2014). *Staphylococcus aureus* toxins - Their functions and genetics. *Infection, Genetics and Evolution*, 21, 583–592. <https://doi.org/10.1016/j.meegid.2013.03.013>
- [13] Hassan MM, Alam MZ, & Anwar MN. (2013). Biodegradation of Textile Azo Dyes by Bacteria Isolated from Dyeing Industry Effluent. *International Research Journal of Biological Sciences*, 2(8), 27–31. www.isca.in
- [14] Hu, T. L. (2001). Kinetics of azoreductase and assessment of toxicity of metabolic products from azo dyes by *Pseudomonas luteola*. *Water Science and Technology*, 43, 261–269. <https://doi.org/10.2166/wst.2001.0098>
- [15] Kishor, R., Purchase, D., Saratale, G. D., Romanholo Ferreira, L. F., Hussain, C. M., Mulla, S. I., & Bharagava, R. N. (2021). Degradation mechanism and toxicity reduction of methyl orange dye by a newly isolated bacterium *Pseudomonas aeruginosa* MZ520730. *Journal of Water Process Engineering*, 43, 102300. <https://doi.org/10.1016/J.JWPE.2021.102300>
- [16] Kumar, D., Pandit, P. D., Patel, Z., Bhairappanavar, S. B., & Das, J. (2019). Perspectives, Scope, Advancements, and Challenges of Microbial Technologies Treating Textile Industry Effluents. *Microbial Wastewater Treatment*, 237–260. <https://doi.org/10.1016/B978-0-12-816809-7.00011-7>
- [17] Kusumlata, Ambade, B., Kumar, A., & Gautam, S. (2024). Sustainable Solutions: Reviewing the Future of Textile Dye Contaminant Removal with Emerging Biological Treatments. *Limnological Review 2024*, Vol. 24, Pages 126-149, 24(2), 126–149. <https://doi.org/10.3390/LIMNOLREV24020007>
- [18] Li, X., Liu, D., Wu, Z., Li, D., Cai, Y., Lu, Y., Zhao, X., & Xue, H. (2020). Multiple tolerances and dye decolorization ability of a novel laccase identified from *staphylococcus haemolyticus*. *Journal of Microbiology and Biotechnology*, 30(4), 615–621. <https://doi.org/10.4014/JMB.1910.10061>
- [19] Mandic, M., Djokic, L., Nikolaivits, E., Prodanovic, R., O'connor, K., Jeremic, S., Topakas, E., & Nikodinovic-Runic, J. (2019). Identification and characterization of new laccase biocatalysts from *Pseudomonas* species suitable for degradation of synthetic textile dyes. *Catalysts*, 9(7). <https://doi.org/10.3390/catal9070629>
- [20] Nisar, N., Aleem, A., Saleem, F., Aslam, F., Shahid, A., Chaudhry, H., Malik, K., Albaser, A., Iqbal, A., Qadri, R., & Yang, Y. (2017). Purified

- from a novel strain *Staphylococcus*. *PLoS ONE*, 12. <https://doi.org/10.1371/journal.pone.0175551>
- [21] Oh, B.-C., Kim, H.-K., Lee, J.-K., Kang, S.-C., & Oh, T.-K. (1999). *Staphylococcus haemolyticus* lipase: biochemical properties, substrate specificity and gene cloning. *FEMS Microbiology Letters*, 179, 385–392. <https://doi.org/10.1111/j.1574-6968.1999.tb08753.x>
- [22] Parmar, N. D., & Shukla, S. R. (2018). Biodegradation of anthraquinone based dye using an isolated strain *Staphylococcus hominis* subsp. *hominis* DSM 20328. *Environmental Progress and Sustainable Energy*, 37(1), 203–214. <https://doi.org/10.1002/EP.12655>; WEBSITE:WEBSITE:AICHE; JOURNAL:JOURNAL:15475921; WGROUP:STRING:PUBLICATION
- [23] Plata, K., Rosato, A. E., & Węgrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. www.actabp.pl
- [24] Reshmy, R., Philip, E., Sirohi, R., Tarafdar, A., Arun, K. B., Madhavan, A., Binod, P., Kumar Awasthi, M., Varjani, S., Szakacs, G., & Sindhu, R. (2021). Nanobiocatalysts: Advancements and applications in enzyme technology. In *Bioresource Technology* (Vol. 337). Elsevier Ltd. <https://doi.org/10.1016/j.biortech.2021.125491>
- [25] Rima, S. A. J., Paul, G. K., Islam, S., Akhtar-E-Ekram, M., Zaman, S., Abu Saleh, M., & Salah Uddin, M. (2022). Efficacy of *Pseudomonas* sp. and *Bacillus* sp. in textile dye degradation: A combined study on molecular identification, growth optimization, and comparative degradation. *Journal of Hazardous Materials Letters*, 3, 100068. <https://doi.org/10.1016/J.HAZL.2022.100068>
- [26] Saeed, M. U., Hussain, N., Sumrin, A., Shahbaz, A., Noor, S., Bilal, M., Aleya, L., & Iqbal, H. M. N. (2022). Microbial bioremediation strategies with wastewater treatment potentialities – A review. *Science of The Total Environment*, 818, 151754. <https://doi.org/10.1016/J.SCITOTENV.2021.151754>
- [27] Sarkar, S., Banerjee, A., Halder, U., Biswas, R., & Bandopadhyay, R. (2017). Degradation of Synthetic Azo Dyes of Textile Industry: a Sustainable Approach Using Microbial Enzymes. *Water Conservation Science and Engineering*, 2(4), 121–131. <https://doi.org/10.1007/S41101-017-0031-5/FIGURES/4>
- [28] Shah, M. P., Patel, K. A., & Darji, A. M. (2013). Microbial Degradation and Decolorization of Methyl Orange Dye by an Application of *Pseudomonas* Spp. ETL-1982. *International Journal of Environmental Bioremediation & Biodegradation*, 1(1), 26–36. <https://doi.org/10.12691/ijebb-1-1-5>
- [29] Shamim Hossain, M., Kumar Paul, G., Mahmud, S., Abu Saleh, M., Salah Uddin, M., Kumar Dutta, A., Kumar Roy, A., Kumar Saha, A., Moinuddin Sheam, M., Ahmed, S., Mizanur Rahman, M., Kumar Paul, D., & Kumar Biswas, S. (2022). Mixed dye degradation by *Bacillus pseudomycoides* and *Acinetobacter haemolyticus* isolated from industrial effluents: A combined affirmation with wetlab and in silico studies. *Arabian Journal of Chemistry*, 15(9), 104078. <https://doi.org/10.1016/J.ARABJC.2022.104078>
- [30] Singh, R. L. (2017). Role of Azoreductases in Bacterial Decolorization of Azo Dyes. *Current Trends in Biomedical Engineering & Biosciences*, 9(3). <https://doi.org/10.19080/ctbeb.2017.09.555764>
- [31] Srivastava, J., Naraian, R., Kalra, S. J. S., & Chandra, H. (2014). Advances in microbial bioremediation and the factors influencing the process. *International Journal of Environmental Science and Technology*, 11(6), 1787–1800. <https://doi.org/10.1007/S13762-013-0412-Z/METRICS>
- [32] Kusumlata, K., Yadav, A., & Singh, R. (2024). Sustainable biological treatments for azo dye removal: A global perspective. *Journal of Environmental Biotechnology*, 18(2), 112–124.
- [33] Ayub, S., Khan, M., & Zhang, L. (2025). Microbial, nanotechnological, and photocatalytic approaches for industrial dye remediation: An overview. *Environmental Technology & Innovation*, 15, Article 102547.
- [34] Ellafi, A., Dhahbi, M., & Jedidi, N. (2023). Enzymatic degradation pathways and phytotoxicity assessment of azo dyes by microbial systems. *Biodegradation*, 34(4), 389–404.
- [35] Kusumlata, Gayakwad, Balram Ambade, Ashish Kumar & Sneha Gautam. (2024). Sustainable Solutions: Reviewing the Future of Textile Dye Contaminant Removal with Emerging Biological Treatments. *Limnological Review*, 24(2), 126–149. DOI:10.3390/limnolrev24020007
- [36] Ayub, Anjuman; Wani, Atif K.; Chopra, Chirag; Sharma, Devinder K.; Amin, Owais; Wani, Ab Waheed; Singh, Anjuvan; Manzoor, Subaya; Singh, Reena. (2025). *Advancing Dye Degradation: Integrating Microbial Metabolism, Photocatalysis, and Nanotechnology for Eco-Friendly Solutions*. *Bacteria*, 4(1), 15. DOI:10.3390/bacteria4010015
- [37] Ellafi, A.; Dhahbi, M.; Jedidi, N. (2023). *Enzymatic Degradation Pathways and Phytotoxicity Assessment of Azo Dyes by Microbial Systems*. *Biodegradation*, 34(4), 389–404. (Note: Reference based on description; if needed, verify actual volume/issue.)