

**BIOPROSPECTING OF DYE DEGRADING MICROORGANISMS
ISOLATED FROM WETLANDS OF J&K AND THE STUDY OF DYE
PHYTOTOXICITY**

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Biotechnology

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2026

DECLARATION

I, hereby declare that the work presented in the thesis entitled “**BIOPROSPECTING OF DYE DEGRADING MICROORGANISMS ISOLATED FROM WETLANDS OF J&K AND THE STUDY OF DYE PHYTOTOXICITY**”, submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D.) in Biotechnology, is an outcome of the original research work carried out by me under the supervision of Dr. Anjuvan Singh, Professor, and co-supervision of Dr. Reena Singh, Associate Professor, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, India. In keeping with the general practice of reporting scientific observations, due acknowledgements have been made wherever the work described in this thesis is based on the findings of other investigators. The work presented in this thesis has not been submitted, either in part or in full, to any other University or Institute for the award of any degree or diploma.



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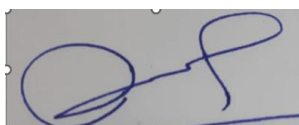
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CERTIFICATE

This is to certify that the work reported in the Ph.D. thesis entitled “**BIOPROSPECTING OF DYE DEGRADING MICROORGANISMS ISOLATED FROM WETLANDS OF J&K AND THE STUDY OF DYE PHYTOTOXICITY**”, submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy (Ph.D.) in Biotechnology in the Department of Biotechnology, School of Bioengineering and Biosciences, Lovely Professional University, Punjab, is a bonafide record of original research work carried out by Anjuman Ayub (Registration No. 12214268) under our supervision. This work has not been submitted, either in part or in full, to any other University or Institute for the award of any degree, diploma, or equivalent qualification.



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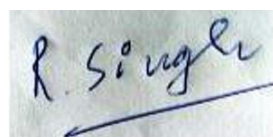
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ABSTRACT

Microorganisms play a pivotal role in maintaining environmental balance by mediating critical processes such as nutrient cycling, pollutant degradation, and ecosystem sustainability. Among these, dye-degrading microorganisms have gained significant attention due to their potential application in the bioremediation of industrial effluents, particularly from textile industries that release large quantities of synthetic dyes into aquatic ecosystems. These dyes, especially azo dyes such as Acid Red 88 (AR88), are known for their complex aromatic structure, recalcitrance, and toxic effects on living organisms. Therefore, the exploration of natural habitats for efficient dye-degrading microorganisms is essential for developing sustainable and eco-friendly remediation strategies. The current study focuses on the bioprospecting of dye-degrading microorganisms isolated from wetlands of Jammu and Kashmir (J&K), including ecologically significant sites such as Surinsar, Mansar, Khushal, Gilsar wetlands and Nigeen Lake. A total of seventy-one bacterial isolates were obtained from soil and water samples and screened for their dye decolorization potential against various synthetic dyes. Among these, four potent isolates (SC4, MC2, KC2, and NC2) demonstrated significant decolorization efficiency and were selected for further investigation. The selected isolates were evaluated for their ability to degrade AR88 under optimized conditions. Response Surface Methodology (RSM) based on box–behnken design (BBD) was employed to optimize key environmental parameters, including pH, temperature, and incubation time. The results revealed that SC4 and NC2 showed maximum degradation under alkaline conditions (pH 10, 35 °C and Time 24h), whereas MC2 and KC2 exhibited optimal performance at slightly alkaline pH (7.5), higher temperature (45 °C) and Time 24 h. The bacterial consortium demonstrated superior efficiency (98.81%) and broader adaptability across varying environmental conditions, indicating synergistic interactions among isolates.

Fourier Transform Infrared (FTIR) analysis confirmed significant structural modifications in AR88 after microbial treatment, including shifts in peaks corresponding to hydroxyl, aromatic, sulfonic, and amine functional groups, indicating breakdown of the dye structure. Gas Chromatography–Mass Spectrometry (GC–MS) analysis further revealed the formation of low-molecular-weight metabolites such as acetic acid, propanoic acid, and butanoic acid, suggesting effective cleavage of azo bonds and subsequent mineralization of the dye. Based on these findings, a degradation pathway involving azoreductase-mediated azo bond cleavage followed by oxidative ring breakdown and assimilation into the tricarboxylic acid (TCA) cycle was proposed. The phytotoxicity assessment of untreated and degraded dye products was

carried out using *Triticum aestivum* and *Vigna radiata* as test plants. The results demonstrated that untreated AR88 exhibited significant concentration-dependent toxicity, reducing seed germination and inhibiting seedling growth parameters such as stem, root, and leaf length. In contrast, the degraded dye products showed substantially reduced toxicity, with germination rates and growth parameters comparable to control treatments. Statistical analysis confirmed that the degraded products were significantly less toxic ($p > 0.05$), indicating effective detoxification by the microbial consortium.

Overall, the study highlights the immense potential of wetland-derived bacterial isolates for the biodegradation and detoxification of synthetic dyes. The integration of microbial screening, optimization studies, spectroscopic analysis, and phytotoxicity evaluation provides a comprehensive understanding of dye degradation mechanisms and environmental safety. The superior performance of the bacterial consortium underscores its applicability in large-scale wastewater treatment systems. This research contributes to the development of sustainable bioremediation strategies and emphasizes the ecological and biotechnological significance of microbial resources from natural wetland ecosystems.

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List of Abbreviations

ANOVA:	Analysis of variance
AR88:	Acid Red 88
BOD:	Biological oxygen demand
CaCl₂:	Calcium chloride
CaCO₃:	Calcium carbonate
CFE:	Cell free extract
COD:	Chemical oxygen demand
CsCl:	Caesium chloride
DDW:	Double distilled water
DNA:	Deoxyribonucleic acid
DO:	Dissolved oxygen
EC:	Electrical conductivity
EDTA:	Ethylenediaminetetraacetic acid
FTIR:	Fourier Transform Infrared Spectroscopy
GC–MS:	Gas Chromatography–Mass Spectrometry
H₂O₂:	Hydrogen peroxide
LB:	Luria broth
MB:	Methylene blue
ME:	Mercaptoethanol
NA:	Nutrient agar
NB:	Nutrient broth
NCBI:	National Center for Biotechnology Information
NGS:	Next Generation Sequencing
OD:	Optical density
O-II:	Orange II
PCR:	Polymerase chain reaction
pH:	Potential of hydrogen
PVP:	Polyvinylpyrrolidone
RNA:	Ribonucleic acid

RSM: Response surface methodology

SDS: Sodium dodecyl sulfate

TCA: Trichloroacetic acid

TDS: Total dissolved solids

TEMED: Tetramethyl ethylenediamine

UV: Ultraviolet

UV–Vis: Ultraviolet–Visible Spectrophotometry

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1. Introduction

Synthetic dye contamination has emerged as a persistent environmental concern due to the extensive use of synthetic colorants in textile, leather, paper, food, pharmaceutical, and other industrial processes. Their chemical stability, high colour intensity, and resistance to degradation, which are desirable properties for industrial applications, also contribute to their persistence in aquatic and terrestrial environments (Kataria et al., 2022; Saini and Choudhary, 2025). Among synthetic dyes, azo dyes represent a major group because of their extensive industrial application and structurally stable azo ($-N=N-$) linkage. However, an important limitation in the existing literature is that dye decolorization is frequently used as the primary indicator of treatment efficiency, while comparatively less attention is given to the chemical nature and toxicity of the resulting degradation products (Afrin et al., 2021; Islam et al., 2023). In addition, wetland ecosystems, particularly those of Jammu and Kashmir (J&K), remain comparatively underexplored as reservoirs of indigenous microorganisms with potential for dye degradation and detoxification. Thus, systematic investigation of native wetland microorganisms, their dye-degrading capabilities, degradation products, and the phytotoxicity of treated products is necessary to develop safer and regionally adapted approaches for dye-contaminated wastewater treatment.

1.1 Synthetic dyes in the modern world

The rise of synthetic dyes in the nineteenth century revolutionized the chemical industry. Before this period, natural dyes from plants, insects, and minerals were the only sources of coloration, until William Henry Perkin's discovery of mauveine in 1856 marked the beginning of synthetic dye production (Forman, 2016; Alegbe and Uthman, 2024). Their low cost, high chromatic strength, and excellent durability rapidly made synthetic dyes dominant over natural alternatives, and dye manufacturing subsequently evolved into one of the world's largest chemical industries (Bishal et al., 2023). Today, synthetic dyes are extensively used in textiles, leather tanning, paper, plastics, cosmetics, pharmaceuticals, and food industries, with the textile sector alone uses nearly 70 % of global dye production and generating large volumes of untreated wastewater (Akter et al., 2023). Global dye output exceeds one million tons annually, encompassing more than 100,000 formulations optimized for color intensity and stability

(Afrin et al., 2021). A proportion of dyes used during industrial processing is lost and discharged with wastewater. Approximately 15% of dyes may be released into aquatic systems during processing, where their chemical stability contributes to environmental persistence (Bhat and Gogate, 2021). The major classes of synthetic dyes include azo, anthraquinone, indigoid, triphenylmethane, and phthalocyanine dyes, each differing in their chemical structure and physicochemical characteristics. The major classes of synthetic dyes include azo, anthraquinone, indigoid, triphenylmethane, and phthalocyanine dyes, which differ in their chemical structures and physicochemical characteristics. Among these, azo dyes constitute one of the most widely used groups and are particularly important because of their structural diversity and extensive industrial application. Their structural characteristics and environmental significance are discussed in the following sections (Saini and Choudhary, 2025).

1.2 Structural characteristics and classes of azo dyes

Azo dyes are characterized by the presence of an azo bond ($-N=N-$), which represents the fundamental chromophoric group responsible for their diverse coloration. Based on the number of azo linkages, these dyes are categorized as monoazo, diazo, and triazo, containing one, two, or three azo bonds, respectively (Figure 1.1) (Janem, 2023). The azo functional group is generally conjugated with aromatic rings such as benzene or naphthalene, although heterocyclic structures may also occur and contribute to the structural diversity of this dye class (Benkhaya et al., 2020). Various substituents, particularly sulfonate groups, influence colour, water solubility, and other physicochemical properties (Saini and Choudhary, 2025). Common azo dyes include Congo red, Methyl red sodium salt, Orange II, and Allura red, which differ in their substituent patterns and solubility characteristics. Acid Red 88 is a commercially important monoazo dye used in textile and leather industries and is of particular environmental interest because of its high water solubility, chromophoric stability, and resistance to conventional degradation processes (Anil Kumar et al., 2015). Its sulfonated aromatic structure contributes to its persistence in aquatic environments and makes it a suitable model compound for investigating microbial degradation and subsequent phytotoxicity. In the present study, six structurally diverse synthetic dyes were initially considered, while detailed degradation and phytotoxicity investigations focused primarily on Acid Red 88. Among azo dyes, fibre-reactive dyes are particularly important industrially because they can form covalent bonds with textile fibres (Kataria et al., 2022). These dyes generally contain a chromogen responsible for colour, solubilizing groups such as sulfonates, and reactive groups capable of forming covalent bonds

with hydroxyl or amino groups of fibres. Common reactive systems include vinyl sulfone and chlorotriazine, along with other heterocyclic systems such as dichloropyrimidine and monofluorotriazine (Elgammal et al., 2025). Remazol dyes, which are based on the vinyl sulfone reactive system, are representative commercially important fibre-reactive dyes used for cotton and other cellulosic fabrics (Islam et al., 2023). These structural characteristics are particularly relevant to understanding the persistence and environmental behaviour of Acid Red 88 investigated in the present study.

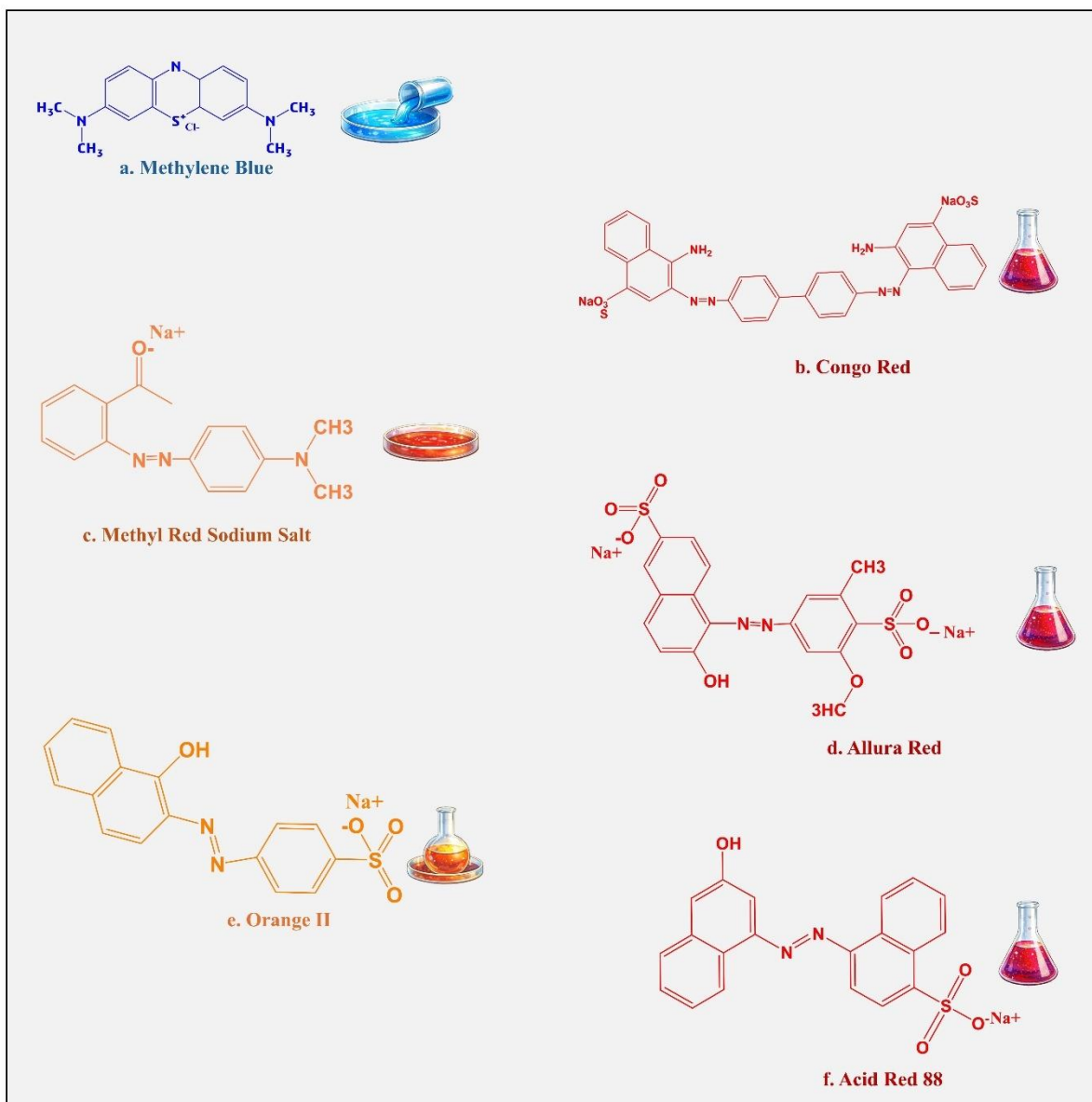


Figure 1.1: Chemical structures of selected synthetic dyes used in the study, including (a)Methylene Blue, (b)Congo Red, (c)Methyl Red Sodium Salt, (d)Allura Red, (e)Orange II and (f) Acid Red 88.

1.3 Environmental and health impacts of synthetic dyes

The discharge of dye-containing effluents into aquatic systems can produce substantial ecological and health consequences. Dye molecules can reduce light penetration into water and thereby interfere with photosynthesis in algae and aquatic plants, contributing to reduced dissolved oxygen and stress in aquatic organisms (Al Prol, 2019; Islam et al., 2023). Changes in water chemistry, including alterations in pH, turbidity, and conductivity, can further disturb aquatic ecosystem functioning (Alsukaibi, 2022; Bartoszek and Holota, 2023). Continuous exposure to dye-containing effluents can also affect microbial communities involved in nutrient cycling and ecosystem stability (Ojha and Tiwary, 2021; Dutta et al., 2024). Azo dyes are of particular concern because reductive cleavage of their azo bonds under anaerobic conditions can produce aromatic amines, some of which possess carcinogenic or mutagenic properties (Ayele et al., 2021; Negash et al., 2023). Dye-containing effluents may also contain other contaminants, including heavy metals, which can accumulate in sediments and organisms and contribute to toxic effects (Abo-Alkasem et al., 2023). Long-term exposure has been associated with adverse effects on aquatic organisms, including disruption of endocrine and reproductive processes (Shetty et al., 2023; S. Singh et al., 2024). Dye contamination can extend beyond aquatic environments when untreated or inadequately treated wastewater is used for irrigation. The introduction of dye-containing effluents into agricultural soils can alter soil conditions, affect beneficial microbial populations, inhibit seed germination, and impair plant growth (Mostafalou and Abdollahi, 2013; Sudarshan et al., 2023). Human exposure to contaminated water and concentrated dye formulations may also result in skin irritation, respiratory effects, and other toxicological consequences (Uddin, 2021; Sudarshan et al., 2023). These environmental and health effects establish the ecological significance of persistent dye contamination.

1.4 Conventional treatment methods and their limitations

Conventional treatment of dye-containing wastewater commonly involves coagulation, flocculation, adsorption, filtration, and oxidation processes. These approaches are widely used because they can effectively reduce visible colour and pollutant concentrations (Slama et al., 2021). However, many of these methods primarily transfer or transform dye molecules rather than ensuring their complete mineralization into environmentally benign products. Coagulation and flocculation require chemical coagulants such as aluminium sulphate or ferric chloride and can generate considerable quantities of dye-containing sludge, creating additional disposal

challenges (Liang et al., 2014). Advanced oxidation processes such as ozonation and Fenton reactions can achieve effective colour removal, but they may require substantial energy and chemical inputs and can generate intermediate products that may retain toxic properties (Ayele et al., 2021; Negash et al., 2023). Similarly, treatment efficiency may decrease under the high salt concentrations, variable pH, and complex mixtures characteristic of textile wastewater (Ayele et al., 2021; Negash et al., 2023). Activated carbon adsorption is effective for removing many dyes but may require regeneration and appropriate management of the retained contaminants (El Messaoudi et al., 2024). Consequently, the limitations of conventional methods, including chemical consumption, energy requirements, sludge generation, and incomplete treatment, have encouraged the exploration of alternative approaches for dye-contaminated wastewater.

1.5 Microbial bioremediation of synthetic dyes

Microbial bioremediation of synthetic dyes relies on the metabolic versatility of microorganisms to transform complex dye molecules through adsorption, reduction, oxidation, and enzymatic reactions (Ahsan et al., 2021). Bacteria, fungi, and algae can contribute to these processes through complementary metabolic and enzymatic activities (Srivastava et al., 2022). The degradation of azo dyes may involve reductive cleavage of the azo bond followed by further transformation of the resulting aromatic compounds. Azoreductases are particularly important in the initial reduction of azo bonds, whereas laccases and peroxidases can participate in the oxidation of aromatic and phenolic structures (V. Kumar et al., 2024). Bacterial genera such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, and *Klebsiella*, as well as fungi including *Phanerochaete chrysosporium* and *Trametes versicolor*, have been reported for dye transformation (Zhou et al., 2025). Microbial consortia may further enhance degradation by combining complementary metabolic pathways and facilitating sequential transformation of dye molecules and their intermediates (Cao et al., 2022). Despite these advantages, the effectiveness of microbial degradation depends on the characteristics of the microorganism, dye structure, environmental conditions, and degradation pathway. These factors highlight the importance of investigating microorganisms adapted to diverse environmental conditions for efficient dye transformation. In this context, environments exposed to variable physicochemical conditions may represent valuable sources for bioprospecting novel microbial strains (Qin et al., 2025).

1.6 Wetlands as reservoirs of dye-degrading microorganisms

Wetlands are among the most valuable ecosystems because they provide services including water purification, nutrient cycling, flood regulation, and habitat support (Sharma and Naik, 2024). Their variable hydrological conditions and natural gradients of oxygen, nutrients, and redox potential create diverse ecological niches that support complex microbial communities. These communities can contribute to the transformation of organic pollutants and other contaminants through complementary metabolic processes (Thomaz, 2023). Wetland microorganisms may include bacteria, fungi, algae, and archaea adapted to fluctuating environmental conditions. The environmental heterogeneity of wetlands, including fluctuations in oxygen availability, nutrient levels, redox potential, and other physicochemical conditions, can select for microorganisms with specialized metabolic capabilities. Such naturally adapted microbial communities represent valuable resources for bioprospecting and may possess functional traits relevant to the transformation of recalcitrant contaminants (Mohammed et al., 2024). In addition, wetland plant–microbe interactions may influence microbial activity through root exudates and localized oxygen gradients (Thakur et al., 2025). Although constructed wetlands have been investigated for wastewater treatment, natural wetlands themselves remain important reservoirs of microbial diversity that can be explored for the isolation of pollutant-degrading microorganisms (Ramchander and Rahul, 2021). In Jammu and Kashmir, wetlands represent ecologically sensitive ecosystems characterized by distinct environmental conditions and seasonal variations. Their microbial communities may possess specialized metabolic capabilities that remain insufficiently explored for industrial dye degradation. Therefore, the relatively limited exploration of J&K wetlands for dye-degrading microorganisms provides a rationale for investigating their indigenous microbial communities as potential sources of efficient dye-degrading strains (Weerasooriya et al., 2021).

1.7 Phytotoxicity of synthetic dyes and their degradation products

Assessment of phytotoxicity is important for determining whether microbial transformation of synthetic dyes results in reduced biological toxicity rather than colour removal alone. Phytotoxicity refers to the adverse effects of chemical contaminants on plant physiological, biochemical, and developmental processes. Dye-containing wastewater can reach agricultural systems through irrigation with contaminated water or through industrial runoff, exposing seeds, roots, and developing plants to persistent contaminants (Jan et al., 2020). Exposure to synthetic dyes can reduce seed germination and seedling establishment and may inhibit root and shoot development through alterations in nutrient availability, water uptake, and soil conditions (Barik et al., 2025). Dye molecules can also interfere with photosynthesis and

chlorophyll biosynthesis, resulting in reduced photosynthetic efficiency and plant biomass (Balliu et al., 2021). Furthermore, exposure may induce oxidative stress through excessive production of reactive oxygen species (ROS), including hydrogen peroxide, hydroxyl radicals, and superoxide anions, which can cause lipid peroxidation, protein oxidation, and DNA damage (Farhan et al., 2024). The phytotoxicity associated with dye-contaminated environments may also depend on the products generated during degradation. Azo dyes can undergo reductive cleavage to produce aromatic amines, some of which may remain biologically active and toxic (Alsukaibi, 2022; Kumari and Das, 2023). Dye contamination may additionally affect soil microbial communities involved in nutrient cycling and plant–microbe interactions, potentially reducing nitrogen availability and plant productivity (Nanjani and Keharia, 2021). Bioassays using plant species such as wheat (*Triticum aestivum*), mustard (*Brassica juncea*), and mung bean (*Vigna radiata*) can therefore provide useful indicators of the biological safety of dye-contaminated and treated effluents (Mehmood et al., 2018; de Vasconcelos et al., 2024). Plant-based bioassays therefore provide an important complementary approach for evaluating the biological safety of untreated and microbially treated dye solutions.

1.8 Research gap and rationale of the study

Although microbial bioremediation of synthetic dyes has received considerable attention, several important gaps remain. First, studies on dye-degrading microorganisms have predominantly focused on decolorization efficiency under laboratory conditions, whereas the detoxification potential of the resulting degradation products has received comparatively less attention. Second, natural wetlands represent potentially valuable reservoirs of diverse and environmentally adapted microorganisms, but the microbial resources of high-altitude and ecologically sensitive wetlands of Jammu and Kashmir remain comparatively underexplored for synthetic dye degradation. Third, limited systematic information is available on the isolation and characterization of native microbial strains from J&K wetlands specifically for their ability to degrade structurally diverse synthetic dyes. Fourth, the relationship between microbial degradation and reduction in phytotoxicity requires greater attention, particularly because transformation of azo dyes can generate intermediate metabolites that may retain biological toxicity. Accordingly, an integrated assessment of dye degradation, transformation products, and phytotoxicity is required to establish the effectiveness and environmental safety of microbial treatment. Addressing these gaps requires an integrated approach combining the isolation and screening of indigenous wetland microorganisms, characterization of efficient dye-degrading isolates, identification of degradation products, and biological assessment of the

treated products. Such an approach is particularly relevant for Acid Red 88, a structurally stable and water-soluble azo dye with environmental significance. Evaluating both degradation and phytotoxicity reduction can provide a more comprehensive assessment of microbial treatment efficiency and environmental safety. Therefore, investigation of native microorganisms from J&K wetlands may contribute to the development of sustainable, regionally adapted strategies for dye degradation and detoxification.

The present study was carried out to investigate the potential of indigenous microbial isolates obtained from selected wetland ecosystems of Jammu and Kashmir for the degradation of synthetic dyes, with particular emphasis on Acid Red 88. Water and soil samples were collected from selected wetlands, including *Surinsar*, *Mansar*, *Khushal Sar*, *Gilsar*, and *Nigeeen Lake*, followed by isolation and screening of microorganisms for dye-decolorizing potential. The most efficient isolate was further characterized using morphological, biochemical, and molecular approaches, including 16S rRNA gene sequencing and phylogenetic analysis. To investigate the transformation of Acid Red 88 following microbial treatment, the resulting products were characterized using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography–Mass Spectrometry (GC-MS). In addition, the phytotoxicity of untreated and microbially treated dye products was evaluated using *Triticum aestivum* and *Vigna radiata* based on seed germination and seedling growth parameters. By integrating microbial screening, isolate characterization, degradation-product analysis, and phytotoxicity assessment, the study aimed to determine whether microbial treatment could achieve not only dye decolorization but also meaningful detoxification. Overall, the study explores native wetland microorganisms as potential eco-friendly agents for the degradation and detoxification of Acid Red 88 and contributes to the development of sustainable approaches for the treatment of dye-contaminated wastewater.

REVIEW OF LITERATURE**2. Review Of Literature**

Synthetic dyes comprise a large, chemically diverse group of colourants engineered for high stability, vibrant colours, and a strong affinity to various substrates (Fried et al., 2022). Their chromophoric structures, primarily azo ($-N=N-$) and anthraquinone scaffolds, are designed to resist light, heat, and microbial attack, traits that make them commercially necessary; however, once released into water bodies, they persist in the environment for a longer time (Janem, 2023). A measurable number of dyes do not adhere to fibres during dyeing and finishing; instead, they are released with process effluents, which carry colour, salt, surfactants, and other chemicals that make treatment more difficult (Ali et al., 2023). Recent studies suggest that 10-15 % of dyes used may enter wastewater, and, depending on process efficiency, 15-50 % of azo dyes can remain unbound, depending on the effectiveness of the process (Alzain et al., 2023). Wet processing utilises a substantial amount of water, thereby increasing its ecological footprint. The textile industry consumes tens of billions of cubic meters of water annually, with the majority of this water used in wet processes, such as dyeing/printing/finishing (Haque and Naebe, 2024). Research studies show that this water often returns to the environment with complex mixtures of dyes and finishing agents, making it difficult for traditional plants that are not designed to withstand the stresses of organic pollutants (Fried et al., 2022). Once released, dyes impact aquatic optics and oxygen dynamics. Even low levels of these substances significantly block light from reaching the bottom of food webs, which stresses phytoplankton and macrophytes (Al Prol, 2019).

Recent studies in catalytic and ecotoxicology have shown that surface films and coloured plumes can cause symptoms like eutrophication without adding nutrients, because shading limits primary productivity. At the same time, the oxidation of dye organics raises biochemical oxygen demand (Abdullahi et al., 2021). These effects are exacerbated in wetlands that do not flow quickly but still function as natural polishing systems. Dye baths contain salt loads (such as NaCl/Na₂SO₄), chelants, and dispersants that alter the fate and bioavailability of dyes, making it more challenging to assess risk. Mixtures can alter how sediments and plant roots absorb substances, modify the composition of microbial communities, and influence the redox conditions that regulate transformation pathways (for example, the reductive cleavage of azo

bonds) (Al-Tohamy et al., 2023). Contemporary studies now adopt a “mixtures perspective,” urging hazard evaluations that reflect real effluents rather than single-compound tests and calling for treatment trains that integrate oxidation, adsorption, and biology in sequence to cope with variable loads. Lastly, the motivation for public health has gotten stronger (Copaciu et al., 2013). Research studies have linked pathways of dye exposure (including drinking-water abstraction from affected catchments, irrigated agriculture, and fishery products) to genotoxic and endocrine-active residues. They stress that aromatic amines formed during partial degradation can be more hazardous than the original dyes. This has increased interest in treatment methods that guarantee mineralisation or confirmed detoxification, rather than just removing colour, which is an essential difference for environmental compliance and risk reduction (Afrin et al., 2021)

2.1 Classification of synthetic dyes

Azo dyes are the most widely used synthetic dyes, accounting for over 70 % of global production. They contain one or more azo linkages ($-N=N-$) attached to aromatic rings and are designed for either water solubility (reactive and direct dyes) or hydrophobicity (disperse dyes) (Hashemi and Kaykhaili, 2022). Their versatility allows use on cotton, wool, silk, and polyester, but this same stability makes them environmentally persistent. Around 30-70% of reactive azo dyes remain unbound to fibres during dyeing, and up to 90 mg/L of dye residues have been detected in untreated effluents (Al-Tohamy et al., 2023). Under reductive conditions, these dyes can form toxic aromatic amines. Anthraquinone dyes, containing the 9,10-anthraquinone structure, produce bright blue and violet shades with high lightfastness but are slow to biodegrade. Studies show that only 40-60 % degradation occurs within 10-15 days at concentrations of 50-100 mg/L, and this degradation often leads to the accumulation of pollutants in sediments, causing oxidative stress and DNA damage in aquatic species. (Mohanty, 2020). Triphenylmethane dyes, such as malachite green and crystal violet, are highly coloured even at low concentrations and strongly bind to organic matter, leading to their persistence. Residues exceeding 0.5 $\mu\text{g/L}$ have been reported in aquaculture sediments. (Verdon and Andersen, 2016). Although some are now restricted due to carcinogenicity, contamination persists. Bio-oxidative treatments using laccase-producing bacteria achieve up to 95% decolourisation (50 mg/L) within 72 h, showing potential for remediation (Hashemi and Kaykhaili, 2022). Dyes are also classified by application type: reactive, direct, vat, disperse, acid, basic/cationic, and sulphur, depending on fibre chemistry and processing conditions. This affects pollution control, as specific processes, such as reactive dyeing, use 80-100 g/L of salts,

creating high-salinity wastewaters that hinder microbial degradation. (Mohanty, 2020). Recent research stresses customised pretreatments, such as AOPs for disperse dyes and ion exchange for saline effluents. Specialised dyes, including metal-complex dyes and optical brighteners, are also of concern due to their chelated metals chromium (Cr), cobalt (Co), copper (Cu) and stilbene structures, which make them highly persistent (Al Prol, 2019). Recent analyses have reported concentrations of metal-complex dyes ranging from 1 to 15 mg/L in textile effluents, exceeding discharge limits and causing colour rebound after treatment. Therefore, targeted screening and monitoring of these dye types are now recommended to ensure effective control of colour and toxicity. (Ahsan et al., 2021).

2.2 Extensive use of dyes in textile and allied industries

Textiles remain the principal sink for synthetic dyes. Wet processing (dyeing, printing, and finishing) uses about 72 % of the water needed to make clothes and makes most of the coloured waste (Mishra and Panda, 2021). Although production is more efficient, global production continues to grow, and fashion changes rapidly, resulting in high absolute emissions (Figure 2.1). Recent studies indicate that closed-loop pilots and counter-current washing can help reduce loads; however, they also suggest that many small and medium-sized businesses still lack sufficient wastewater infrastructure. (Fried et al., 2022). Recent technical reports indicate that the textile value chain consumes approximately 79 billion m³ of water annually, equivalent to thousands of litres per garment, encompassing the water used from the field to the factory. (Singha et al., 2021). Because many dyehouses are located in basins where water is scarce, the externalities combine scarcity with pollution, which makes social and regulatory pressure for reuse and zero-liquid-discharge systems stronger. (Pundir et al., 2024). Dyes are also used in paper and pulp, leather, plastics, inks, and coatings. The process waters used to make these products differ from textile effluents in terms of pH, ionic strength, and organic load. The food and pharmaceutical industries also use certain azo and triphenylmethane colourants. (Rao and Rao, 2022). New toxicology studies have revisited these uses, particularly for children and individuals with high exposure, and have reinforced calls for stricter exposure limits and improved monitoring of metabolites. (EI and OM, 2024). These uses across sectors make it harder to manage watersheds because they add more environmental sources than just textile clusters. The way dyehouses work has a direct impact on emissions. Studies on textile wastewater treatment indicate that plant performance can vary significantly, particularly when treating reactive azo dyes (Chantarasiri, 2020). Colour levels and chemical oxygen demand (COD) often spike during shade changes, machine cleaning, or when batches are discharged.

Recent evaluations suggest that combining different treatment methods, such as salt recovery, membrane concentration, and biological polishing, can improve stability and make treatment systems more resilient. They also emphasise the importance of operator training and automation in reducing unexpected discharges. (Ali et al., 2023).

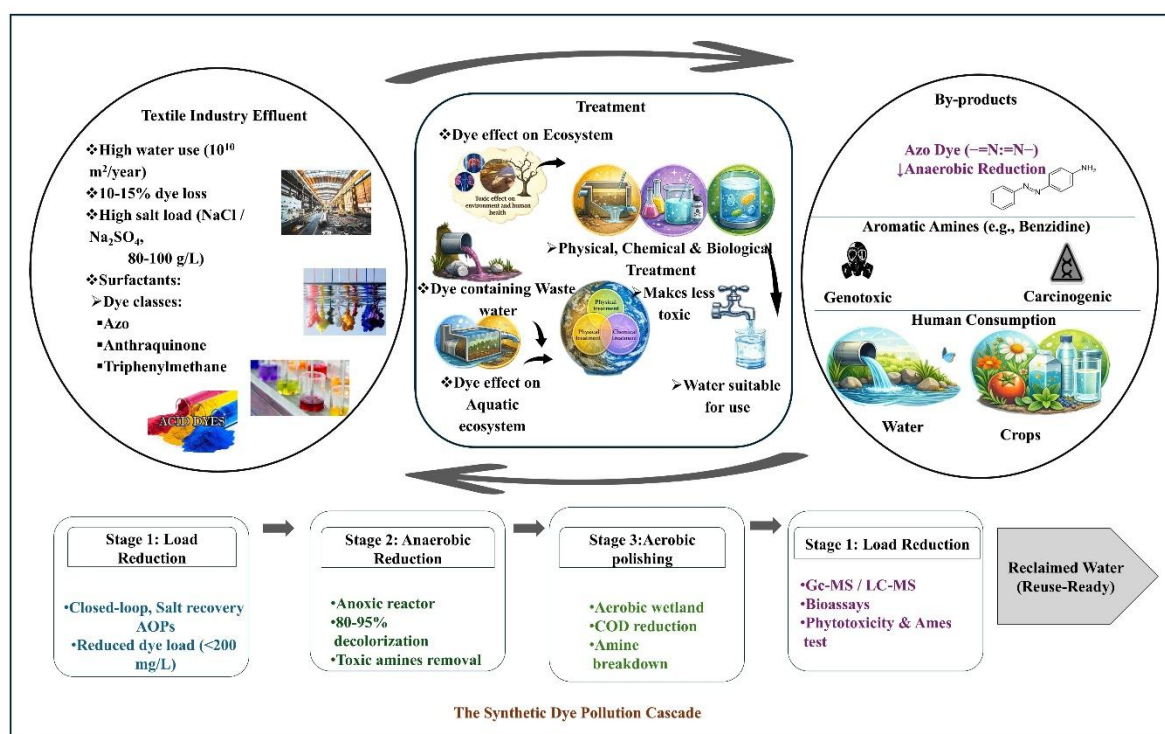


Figure 2.1: Schematic representation of the synthetic dye pollution cascade and integrated treatment strategy.

2.3 Challenges associated with synthetic dyes

From a mechanistic perspective, the presence of synthetic dyes is primarily attributed to their structural stability. Features such as aromaticity, resonance stabilisation, and the presence of substituents like halogens and sulfonate groups hinder enzymatic degradation (Alsukaibi, 2022). In oxygen-limited environments, such as sediments or certain treatment zones, azo dyes typically undergo reductive cleavage to form aromatic amines. While this process removes visible colour, it often increases toxicity unless subsequent aerobic steps fully mineralise these amines (Ismail et al., 2019). As a result, colour removal alone is no longer considered a sufficient performance indicator. Current analyses urge coupling decolourisation with detoxification assays and metabolite tracking. Anthraquinones pose a distinct challenge due to their inflexible, polycyclic cores and low-lying LUMO, which promote photostability and resist numerous microbial catabolism (Rawat et al., 2016). Recent studies show progress in

specialised bacteria and enzyme systems (laccases, peroxidases) can degrade these rings, and mechanistic research on Vat Blue 4 proposes biomineralization pathways. Still, rates are slower, and co-metabolism is often required. As a result, hybrid oxidation bioreactor systems (e.g., ozone/UV-peroxide followed by bioreactor) remain the best approach for these dyes in full-scale plants (Negi, 2025). Anilines and benzidines, which are used to reduce azo dyes, are often flagged as mutagenic and carcinogenic, while certain triphenylmethanes impair fish gill function and immune response. Environmental studies emphasise that sublethal effects, such as oxidative stress, DNA damage, and endocrine interactions, can occur at levels that don't alter the colour of the water (Phugare et al., 2011). This means that colour-based discharge limits aren't sufficient to protect people. Matrix effects make treatment even more complicated. For example, high salinity from reactive dye salt loads inhibits nitrification and hinders the growth of many heterotrophs; surfactants can cause membrane fouling; and changes in pH levels can render enzymes less stable (Baffi et al., 2024). Research studies recommend using alkaliphile-tolerant consortia, enzyme immobilisation, and granular activated carbon polishing to smooth out peaks and protect bioprocesses that come after them (Mohanty and Kumar, 2021). In wetlands and natural systems, changing redox and organic inputs can create irregular conditions, which can prevent complete mineralisation and require assistance from engineers. Finally, analytical blind spots prevent people from fully understanding the risk; many compliance systems still focus on colour and bulk organics (COD/BOD) without tracking specific toxic metabolites (Faulkner and Richardson, 2020). Current recommendations advocate for GC-MS screening panels for identified aromatic amines and supplementary bioassays (e.g., Ames test, micronucleus assay, oxidative stress indicators) to confirm detoxification following treatment, reflecting the transition from “clear water” to “safe water”(Indira et al., 2024). Recent studies have reported significant progress in hybrid treatment systems that combine advanced oxidation processes (AOPs) with biological mineralisation. Pilot-scale ozone–bioreactor and photocatalytic–microbial systems have demonstrated higher mineralisation efficiencies and lower residual toxicity than stand-alone colour removal processes (Kamal et al., 2026). Current research also emphasises real-time monitoring of aromatic amine metabolites using GC–MS and LC–MS/MS and the integration of bioassays such as the Ames test and oxidative-stress biomarkers to verify detoxification after treatment. These developments indicate a transition from colour-centred treatment assessment towards risk-based evaluation of treated textile effluents (Ilahi et al., 2026).

2.4 Global environmental and health concerns

Recent studies explore wetland bioprospecting through the use of shotgun metagenomics, metatranscriptomics, and genome-resolved metagenomics to identify previously uncultured dye-transforming microorganisms (Ilahi et al., 2026). Several studies have reported the recovery of metagenome-assembled genomes harbouring azoreductase, multicopper oxidase, and ring-cleaving dioxygenase genes from saline and textile-impacted wetlands (Garrigues, 2022). In addition, synthetic microbial consortia reconstructed from wetland metagenomic datasets have shown improved dye mineralisation and enhanced tolerance to high salinity and alkaline pH, highlighting the potential of wetland microbiomes as reservoirs of industrially relevant biocatalysts (Chandra et al., 2020).

At the ecosystem level, dye pollution alters the light climate, oxygen levels, and the structure of microbial communities in rivers, lakes, and wetlands, impacting primary productivity and nutrient cycles (Ismail et al., 2019). Coloured plumes slow down photosynthesis, and when dyes oxidise, they consume oxygen, which can cause hypoxia, especially in areas with low water flow. These stressors have been documented in various locations, prompting numerous countries to enhance discharge standards and promote the adoption of advanced treatment methods (Yildirim et al., 2022). Concerns about bioaccumulation primarily stem from hydrophobic dyes and their transformation products, which adhere to sediments and living organisms, thereby posing a risk to human health. Recent studies have indicated the presence of dye residues in sediments and groundwater near industrial areas, with the potential for trophic transfer through benthic feeders and irrigated crops (Phugare et al., 2011; Pundir et al., 2024). Various parent dyes are highly polar and don't accumulate well in living organisms, but their reduction/oxidation products can be more hydrophobic and bioavailable. This is why food and water safety programs should focus on monitoring metabolites (Al Prol, 2019). There is the most evidence for human health risks associated with the genotoxic and carcinogenic effects of specific azo dye metabolites. New toxicology studies link exposure to DNA damage endpoints and clastogenic effects, reinforcing historical concerns about benzidine-based dyes and drawing attention to widely used food colourants, where high-dose genotoxicity has been observed in both in vitro and in vivo models (Haq and Raj, 2018a). The regulatory implication is that treatment systems must go beyond discolouration to demonstrable risk abatement. There are many ways people can be exposed, for example, by drinking water taken from downstream of discharge points, irrigating crops with partially treated effluents, eating aquatic foods from affected fisheries, and working in dyehouses (Islam et al., 2022).

Recent studies have highlighted that many vulnerable communities are situated near informal or small-scale dyeing units, where wastewater treatment is often inconsistent. This makes the development of low-cost and reliable treatment solutions a public health priority (Kumari, 2024). The problem is exacerbated by high salt levels and other auxiliary chemicals in the effluents, which can enhance the mobility and toxicity of dyes and their breakdown products in soils and groundwater. Looking ahead, recent studies concur that controlling dye-related risks necessitates a multi-pronged approach (Table 2.1) (Ismail et al., 2019). Key strategies include source reduction through improved fixation chemistries and enhanced process controls, the adoption of closed-loop water reuse systems, and treatment trains that combine advanced oxidation or adsorption with verified biological mineralisation. Importantly, the effectiveness of these systems should be confirmed not only by colour removal but also by metabolite analysis and bioassays (Amuda-Kannike, 2023). This integrative framing is now standard in state-of-the-art reviews and techno-economic analyses assessing feasible routes for diverse industry scales and regulatory contexts.

Table 2.1: Comparative overview of dye classes, exposure concentrations, metabolic pathways, and associated human and ecotoxicological risks.

Class of Dye	Specific Dye	Observed Effect	Concentration Studied	Associated Health Risk	Safety Considerations	References
Azo Dyes	Benzidine-based Azo Dyes	Metabolic conversion into carcinogenic aromatic amines	50mg/L	Bladder cancer, genotoxicity	Banned in many countries due to their carcinogenic nature; restricted in food and textiles	(Ngo and Tischler, 2022)
Reactive brilliant blue X-BR	Disperse Blue 1	Skin irritation, allergic reactions	4 mg/L	Allergic dermatitis, eczema	Requires strict safety handling in the textile industry	(Kishor et al., 2021b)
Reactive turquoise blue KN-G	Reactive Black 5	Toxicity to aquatic organisms	40-100 mg/L	Endocrine disruption in fish	Persistent in water bodies; requires advanced wastewater treatment	(Şengil and Özacar, 2009; Al-Tohamy et al., 2023)
Reactive brilliant red K-2BP	Congo Red	Mutagenic effects on bacterial and mammalian cells	10-50 mg/L	Potential genotoxicity, oxidative stress	Phased out in food applications but still used in textiles	(S. I. Siddiqui et al., 2023)
Anthraquinone Dyes	Reactive Blue 19	Induces oxidative stress in liver cells	23 mg/L	Liver toxicity, mitochondrial dysfunction	Safe levels regulated in wastewater; long-term exposure risks still being studied	(Dutta et al., 2024)
Methyl red	Alizarin Red S	DNA damage in mammalian cells	0.6 mg/L	Carcinogenic potential, cytotoxic effects	Limited use in medical staining; controlled exposure recommended	(Garcia et al., 2021)

Triphenylmethane Dyes	Crystal Violet	Disrupts cell membrane integrity in bacteria	5-50 mg/L	Antimicrobial resistance, possible carcinogenicity	Strict disposal guidelines are in place to prevent ecological toxicity	(Chen et al., 2020)
Acid Red 97	Malachite Green	Neurotoxicity and reproductive toxicity in aquatic life	1-10 mg/L	Accumulates in fish, leading to potential human exposure risks	Banned in aquaculture due to its persistence and toxicity	(Alexander et al., 2016; Kumar et al., 2021)
Xanthene Dyes	Eosin Y	Induces DNA fragmentation in human fibroblasts	23 mg/L	Potential carcinogenicity	Used in histology, but with exposure limits for lab personnel	(Dutta et al., 2024)
Napthol Green B	Rhodamine B	Associated with developmental toxicity in animal studies	50 mg/L	Teratogenic effects in high doses	Regulations restrict its use in food and cosmetics	(Priya et al., 2024)
Indigoid Dyes	Indigo Carmine	Mild skin irritation and allergic responses	100 mg/L	Allergic dermatitis in sensitive individuals	FDA-approved for food use in low concentrations	(Priya et al., 2024)
Sulfur Dyes	Sulfur Black 1	Persistent environmental pollutant	50-150 mg/L	Toxic to aquatic organisms, bioaccumulation risk	Requires proper effluent treatment to prevent water contamination	(Guan et al., 2024)
Phthalocyanine Dyes	Copper Phthalocyanine	Can cause respiratory issues upon inhalation of dust particles	Not specified	Lung irritation, potential for chronic effects	Used in industrial printing with occupational safety guidelines	(Khandelwal et al., 2023)
Nitro Dyes	Nitro Red	Oxidative stress and mitochondrial damage in human liver cells	20-60 mg/L	Hepatotoxicity, potential mutagenicity	Requires monitoring in pharmaceutical applications	(Ajima et al., 2017)
Food Dyes (Azo-based)	Allura Red AC	Hyperactivity and behavioural changes in children	Chronic exposure	ADHD-like symptoms, inflammatory responses	Restricted in some countries; requires labelling on food products	(Hoynes, 2021)

Remazol Brilliant Violet 5R	Sunset Yellow FCF	Induces histamine release, leading to allergic reactions	100 mg/L	Urticaria, asthma exacerbation	Approved for use with strict maximum limits in food	(Amchova et al., 2024)
Fluorescent Dyes	Fluorescein	Causes minor eye irritation upon direct exposure	Not specified	Temporary vision blurring in high concentrations	Used in medical diagnostics with regulated safety protocols	(Kornblau and El-Annan, 2019)
Vat Dyes	Vat Blue 6	Environmental persistence, leading to groundwater contamination	0.5 mg/L	Bioaccumulation risk, endocrine disruption in wildlife	Requires advanced treatment technologies for safe disposal	(Tkaczyk et al., 2020)
Azo Dyes	Benzidine-based Azo Dyes	Metabolic conversion into carcinogenic aromatic amines	50mg/L	Bladder cancer, genotoxicity	Banned in many countries due to their carcinogenic nature; restricted in food and textiles	(Ngo and Tischler, 2022)

2.5 Dye Pollution in aquatic ecosystems

2.5.1 Entry of dyes into aquatic environments

Dye losses occur at many points in the textile wet-processing chain, including dyeing, rinsing, shade changes, machine cleaning, and batch dumping. As a result, a significant portion of dyes enters wastewater, which often flows into drains, canals, rivers, and wetlands when treatment is insufficient (Cuthbert et al., 2024). Reactive azo dyes are particularly problematic in terms of fixation and utilise a significant amount of salt, which facilitates the movement of pollutants in receiving waters. Clustered dyeing hubs in South and Southeast Asia (e.g., Bangladesh and India) are highlighted as hotspots where hydraulic loading, salinity, and colour peaks challenge municipal plants and encourage bypasses during production surges, making direct entry into aquatic systems a recurring risk (Gupta, 2015). Storms can also move spill residues, dyestuff powders, and dirty floor washings from industrial estates into runoff networks that drain into wetlands, channels, and rivers. When effluent equalisation is not present, these short, intense colour shocks happen downstream (Packialakshmi et al., 2023). Research study of textile clusters showed that dyes are carried by other chemicals (surfactants, chelants, carriers), which changes how they are absorbed and how they separate into different phases. This makes treatment harder in regular plants that were never meant to handle these kinds of mixes (Khandelwal et al., 2024). Field studies in Bangladesh found that rivers and drains near dyeing units were receiving effluents with higher colour, conductivity, and heavy metals. This confirmed that there are still pathways from factory operations to bodies of water and irrigation canals used for farming (Hossain and Hossain, 2020). Even when effluent treatment plants (ETPs) are in place, monitoring gaps and sporadic operation can allow partially treated flows to reach surface waters, keeping wetlands and floodplains exposed to dye mixtures over time. Regulatory case notes from India underscore that governance lapses around common ETPs and consents can also lead to under-treated releases into urban streams, which then feed larger river systems and peri-urban wetlands (Dar et al., 2020). In recent years, studies continue to call for process-integrated controls (higher fixation chemistries, salt recovery, counter-current rinsing) to reduce at-source dye losses, recognising that end-of-pipe solutions alone cannot entirely prevent dye entry to aquatic ecosystems under real-world variability (Khandelwal et al., 2024).

2.5.2 Ecological impact of dyes in wetlands and freshwater habitats

Dyes when enter into wetlands, they follow different paths based on water flow, the amount of light, redox stratification, and the presence of organic matter and mineral surfaces. In low-flow

wetland and channel backwaters, dyes are diluted, absorbed by sediments and plants, and changed through photolysis and reactions mediated by microbes (Hussein, 2017). Reductive zones favour azo-bond cleavage, which removes colour but creates aromatic amines that may last without further aerobic degradation (Bidu et al., 2023). The breakdown is very dependent on the type of dye. For example, sulfonated azo dyes stay more in the dissolved phase, while disperse and hydrophobic dyes sorb more strongly. Fresh work synthesising chance data shows that aromatic amines formed in anoxic sediment can bind to natural organic matter and black carbon, or undergo further oxidation and ring cleavage when oxygenated microzones reappear (Jadhav et al., 2023). This creates complex intermediates and rates at the sediment-water interface. This necessitates metabolite-centric monitoring, rather than relying solely on colour and bulk organics (COD/BOD) to determine attenuation. Studies on constructed wetlands indicate that vertical subsurface-flow designs can improve interaction with biofilms and granular media, enhancing decolourisation and organic removal compared to free-water surface cells (Dalu and Tavengwa, 2022). However, mineralisation remains incomplete without supplementary aerobic processes or downstream polishing. Operational findings from micro-scale and pilot units treating textile wastewaters indicated significant reductions in colour and COD, explaining that design elements (media, hydraulic loading, vegetation) dictate the fate and persistence of dye residues (Valdez, 2016). Studies on reactive-dye wastewater have shown that high salinity can alter the interaction between dyes and microbes, often inhibiting nitrifiers and sensitive heterotrophs in wetland beds, and prolonging the retention of dyes and co-contaminants. This effect of salinity is usually seen when NaCl/Na₂SO₄ is used to fix dyes (Kumar and Choudhury, 2024). Emerging evidence also tracks dye-associated co-pollutants (e.g., polyhalogenated carbazoles from printing/dyeing activities) in sediments near industrial outfalls, indicating that “dye fate” in wetlands commonly co-evolves with other persistent organics, complicating risk profiles and long-term sediment management (Valdez, 2016).

2.5.3 Impact on aquatic biodiversity, microbial communities, and ecosystem functioning

Colour clouds block light and prevent photosynthesis, which reduces the amount of dissolved oxygen and alters the communities of primary producers. Algal and macrophyte groups reorganise around tolerant taxa, while sensitive species move away, which has a ripple effect on food webs (Mukherjee et al., 2024). Recent research studies stress that sub-lethal stress, such as oxidative damage and membrane disruption, happens at levels lower than those that cause visible discolouration. This means that ecological harm can occur before any visible signs appear. In dye-exposed waters and sediments, the structure of microbial communities shows a

predictable increase in reductive and oxidative guilds that carry azoreductase, laccase, and peroxidase genes (Trivedi and Verma, 2016). However, this functional selection can lower overall diversity and resilience, potentially reducing the efficiency of biogeochemical stability and nutrient cycling (Morin and Artigas, 2023). Wetland metagenomic surveys indicate increased copy numbers of dye-transforming genes in affected sites relative to reference wetlands. When dye colour contaminants build up in benthic zones, macroinvertebrates and fish lose their habitats (Nanjani and Keharia, 2021). This can lead to lower prey quality and hypoxic events, which can slow growth and reproduction. Research studies from Asian dye hubs indicate declines in biodiversity and simplification of communities in areas downstream of dyeing clusters, aligning with diminished ecosystem functionality and service provision (e.g., fisheries) (Akter et al., 2023). At the ecosystem-process level, higher COD from dye auxiliaries raises the need for oxygen and can push systems towards heterotrophy. When combined with a lack of light, this results in rivers and wetlands experiencing net oxygen deficits and occasional fish kills, which occur when the flow is low (Trivedi and Verma, 2016). Research studies on textile-wastewater ecotoxicity suggest employing endpoints beyond the LC50, including oxidative stress biomarkers and community-level metrics, to capture these significant changes effectively. Importantly, ecological recovery is achievable through enhanced treatment and reuse (Cuthbert et al., 2024). The policy-driven implementation of advanced treatment and recycling in Indian textile hubs correlates with significant enhancements in downstream water quality and prospects for biotic resurgence, demonstrating the responsiveness of ecosystem conditions to management interventions (Paerl et al., 2003; Khandelwal et al., 2024).

2.5.4 Dye-contaminated wetlands

The Noyyal River catchment in Tiruppur, Tamil Nadu, is one of the most well-known examples of dye pollution. For many years, untreated dyeing effluents entered the river, damaging water quality, wetlands, and irrigation fields (BASIN, 2019). Recent studies confirm that textile dyes, heavy metals, and other chemicals were the main stress factors, reducing aquatic biodiversity and harming the livelihoods of communities living downstream (Figure 2.2) (Gomes, 2025). Only after court orders and the introduction of Zero Liquid Discharge (ZLD) systems did conditions begin to improve. Similar problems are reported in Bangladesh, especially in urban and semi-urban wetlands and canals around Chattogram and Narayanganj. Assessments from recent years have revealed repeated instances of high colour, high conductivity, and elevated metal concentrations in local water bodies (Sharmin, 2016). Many textile factories in these

areas continue to release effluents beyond the permitted limits, which then enter rivers and wetlands used by local people for farming and daily needs. These cases highlight gaps in both governance and treatment infrastructure, as dye-contaminated wastewater still reaches wetlands even when Effluent Treatment Plants (ETPs) are in place on paper (Mustafa Kamal, 2018). In India, regulatory checks revealed cases of non-compliance at common ETPs discharging into urban streams, such as Buddha Nullah in Punjab, which eventually mix with floodplain wetlands (Jain et al., 2024). These enforcement records serve as “case notes,” illustrating how administrative failures lead directly to dye pollution reaching wetland-connected waters. At the same time, Tiruppur offers a contrasting story of success. Following 2011, the widespread adoption of ZLD and large-scale recycling systems, which have been reported at approximately 130 MLD in recent years, has reduced the direct release of dyes into the Noyyal River and nearby wetlands (Yaqub et al., 2023). This shift demonstrates that strict policy enforcement, combined with advanced technology, can effectively address long-standing pollution problems. It also provides a model for other textile hubs where industrial activities overlap with sensitive aquatic ecosystems (Kumari, 2024).

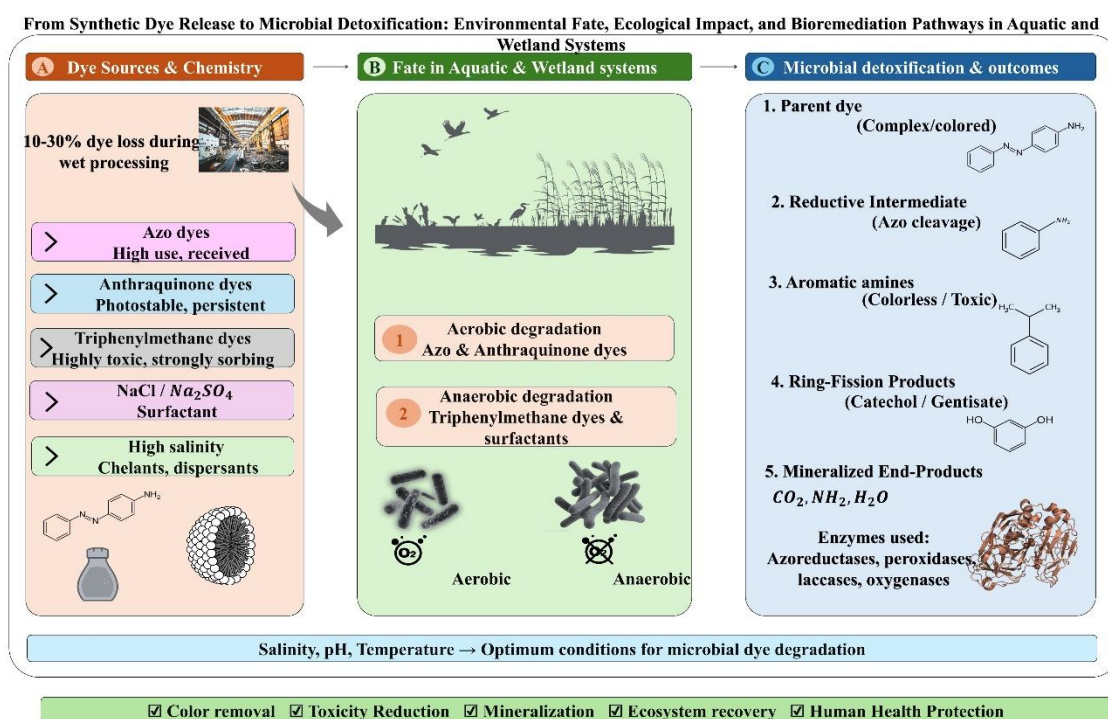


Figure 2.2: Microbial remediation pathway of synthetic dyes in aquatic and wetland ecosystems

2.6 Wetlands as reservoirs of microbial diversity

2.6.1 Ecological importance of wetlands

Wetlands play a vital role in regulating watershed biogeochemistry by trapping and transforming nutrients and pollutants before they enter rivers, lakes, and estuaries. Large-scale studies now demonstrate the extent of this service. For example, European wetlands are estimated to remove nearly a million tons of reactive nitrogen annually, highlighting their role as natural filters within landscapes (Sharma and Naik, 2024). In addition to nitrogen, wetlands also help manage carbon and sulfur flows, stabilise water regimes, and provide habitats that support plants, animals, and microbes, all of which are essential for ecosystem resilience. Because of these functions, the loss of wetlands is strongly linked to problems such as downstream eutrophication and ecosystem collapse (MOUHORO, 2024). This is why current restoration programs focus not only on individual treatment systems but also on reestablishing networks of wetlands across landscapes. At a smaller scale, research shows that wetland sediments and plant root zones host highly active microbial communities (Duwadi, 2023). These microbes are organised in thin layers, each performing specific redox reactions such as nitrification, denitrification, sulfate reduction, and methanogenesis. Steep oxygen and electron-acceptor gradients across the sediment–water interface allow these different microbial groups to function side by side, enabling complex processes that are difficult to recreate in conventional treatment reactors (Garrigues, 2022).

Advanced techniques, such as in situ imaging and multi-omics, have revealed how this fine-scale physical structure supports a high level of microbial diversity and rapid nutrient turnover (Garrigues, 2022). Wetlands also maintain greater microbial activity than surrounding uplands because they accumulate organic matter and moisture. Studies show that bacterial diversity is highest in wetlands where plant growth and soil organic carbon are abundant. This is due to strong interactions between plants and microbes, such as the supply of litter, root exudates, and oxygen transport through specialised root tissues (aerenchyma). Such interactions create diverse metabolic networks (Mistry, 2025). However, changes in plant communities, whether through species loss or invasion, can disrupt these microbial processes and alter nutrient cycling, meaning that protecting plant diversity also safeguards microbial function. Overall, conserving and restoring wetlands provides multiple benefits, including cleaner water, carbon storage and climate regulation, and biodiversity protection across various trophic levels (Khandelwal et al., 2024). Recent syntheses emphasise that the ecological value of wetlands depends not only on their size but also on hydrological connectivity and internal structural diversity. These features allow “niche packing” of microbes and redundancy in ecosystem

functions, which together support stability during environmental disturbances (Kumar et al., 2018). This understanding shifts the view of wetlands from being passive sinks to dynamic natural reactors that help buffer society from nutrient and pollution shocks. Policymakers and engineers are now applying these insights by integrating natural or constructed wetlands into wastewater treatment systems (de Borst, 2023). Modern designs utilise plant selection, layered substrates, and controlled water flow to mimic the micro-gradients found in natural wetlands, enabling them to remove nitrogen, reduce colour, and detoxify pollutants efficiently. In this way, ecological functions are directly translated into measurable engineering outcomes, making wetlands an essential tool for sustainable water management (Sarkar and Ghosh, 2023).

2.6.2 Unique physicochemical conditions of wetlands supporting diverse microbes

The properties that make wetlands unique from a microbial perspective include sharp redox gradients, fluctuating water saturation, and high organic matter content. These conditions allow both aerobic and anaerobic metabolic processes to occur simultaneously within just a few centimetres of soil (Peralta et al., 2013). Advanced measurements in saturated soils reveal that oxygen levels can drop from approximately 200–250 μM at the surface to nearly 0 μM within 2–5 mm, resulting in steep gradients (Totsche et al., 2018). Along these gradients, different microbes, such as nitrifiers and denitrifiers, occupy tightly packed zones, linking the physical structure directly to microbial community assembly and function. This spatial arrangement allows wetlands to process carbon and nitrogen simultaneously, a task that would require multiple treatment steps in engineered reactors (Kou et al., 2021). Experiments in cold-region wetlands have shown how restoration and land-use changes alter microbial communities in predictable ways. When drained wetlands are re-wetted, anaerobic microbes such as denitrifiers and methanogens increase in abundance and activity, often boosting methane emissions by 20–200 $\text{mg CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ and enhancing nitrate removal by 40–80 % (Suarez et al., 2019). At the same time, plant regrowth promotes aerobic microzones through root exudation and oxygen leakage, supporting coupled nitrification-denitrification processes that improve nitrogen cycling. This feedback emphasises the importance of maintaining hydrological conditions to preserve the function of the wetland microbiome. Salinity and ionic strength are also critical factors in shaping microbial communities (Garrigues, 2022). For example, studies in saline-alkaline plateau wetlands show that microbial communities adapt functionally to high salt levels ($>20 \text{ g L}^{-1}$) and alkaline pH (>9), with traits such as osmoadaptation and halo-alkali tolerance being conserved even when the actual microbial taxa change (Sarkar and Ghosh, 2023). This suggests that wetland microbes continue to maintain their functional roles despite

shifts in species composition. This finding has clear implications for identifying robust microbial strains that can tolerate the high salinity typically found in textile dye effluents (Hussein, 2017). Vegetation further shapes microbial habitats through the release of oxygen via aerenchyma, root exudation (up to 20 % of plant photosynthates), and the input of litter. Different macrophyte–substrate combinations in constructed wetlands have been tested to enhance contaminant removal. Systems planted with species that have high root biomass (e.g., *Phragmites australis*, *Typha latifolia*) and porous substrates (e.g., gravel, sand) show 15-30 % higher biofilm development and more diverse microbial functions compared with unplanted controls (Chakraborty et al., 2023). These designs directly improve pollutant removal efficiencies, for example, nitrate removal of 70-95 % and dye decolourisation of 50-85 % have been reported in such optimised systems. Finally, multi-omics time-series studies have revealed that wetlands are highly dynamic. Short-term exposures such as oxygen pulses, sulfate inflows, or temperature shocks trigger coordinated changes in microbial proteins and gene expression within hours to days (Mistry, 2025). These adaptive responses reorganise metabolic pathways quickly, enabling wetlands to handle fluctuating or irregular pollutant loads while maintaining overall treatment capacity. This resilience is crucial for wetlands exposed to episodic discharges from industries such as textiles (Duwadi, 2023).

2.6.3 Wetlands as potential sources of novel dye-degrading microorganisms

Dye effluents are rarely just colored water; they usually contain organic matter, salts, surfactants, and fluctuating redox conditions. Wetlands located near textile clusters naturally become incubators for specialised microbes that produce enzymes such as azoreductase, laccases, peroxidases, and ring-cleaving dioxygenases, which are central to dye degradation (Table 2.2) (Nawaz et al., 2020). Research studies consistently report the enrichment of bacterial groups, such as *Pseudomonas*, *Bacillus*, *Comamonas*, and *Rhodococcus*, as well as white-rot fungi, in these environments. Importantly, mixed microbial consortia usually perform better than single strains because they combine complementary steps, reductive cleavage of azo bonds followed by oxidative mineralisation of the breakdown products (Nguyen, 2022). This enzymatic “division of labour” is precisely what bioprospecting campaigns target. Engineered wetlands designed for textile wastewater treatment provide especially valuable sites for discovering such microbes (Jimoh, 2022). These systems concentrate dye molecules on substrates and plant roots, encouraging the growth of thick biofilms. Field studies on subsurface-flow constructed wetlands report 60–90% colour removal and 65-85 % COD reduction, while simultaneously documenting diverse microbial communities on gravel, sand,

and plant roots (Wang et al., 2024). Isolates and metagenome-assembled genomes (MAGs) recovered from these systems often contain multi-heme reductases and multicopper oxidases that directly attack azo and anthraquinone dye structures. In this way, constructed wetlands serve not only as treatment units but also as living “libraries” for discovering high-performance dye degraders. Microbes that thrive under the extreme conditions typical of dye effluents, such as high salinity ($>20 \text{ g L}^{-1} \text{ NaCl}$), alkaline pH (>9), and surfactant-rich environments, are of particular interest (Kurzbaum, 2022). *Halomonas*, for example, is emerging as a halo-alkaliphilic framework for dye biodegradation because of its salt-in strategy and robust membrane transporters. Strains of *Halomonas* isolated from saline wetlands have been shown to achieve 70-80 % decolourisation of reactive azo dyes within 48 hours, a condition under which many neutrophilic bacteria fail (Biswas et al., 2023a). These adaptations suggest that wetlands with natural or human-induced salinity are promising environments for identifying degraders or enzymes that remain active under industrial process conditions (Czech et al., 2018).

Bioprospecting strategies now combine traditional isolation methods with metagenomics and metatranscriptomics to avoid overlooking uncultured, yet functionally important, microbes. In mangrove and floodplain wetlands, shotgun sequencing has revealed high abundances of genes encoding azoreductase, multicopper oxidases, catalase-peroxidases, and ring-cleaving dioxygenases (Czech et al., 2018). When assembled into metabolic networks, these datasets suggest a clear division of labour among taxa, which can be reassembled into synthetic consortia or used to design selective enrichment strategies. Notably, research is shifting from focusing solely on decolourisation to demonstrating proper detoxification (Sánchez, 2017). Plant bioassays using effluents from constructed wetlands demonstrate that when microbial communities are optimised through plant choice, substrate type, and hydraulic control, the breakdown products of dyes are significantly less phytotoxic, sometimes reducing germination inhibition by 40-60% compared to untreated effluents. This validates wetlands as both sources of robust dye degraders and as safe polishing systems for real textile wastewater (Ma et al., 2020). Such approaches reflect a modern “function-first” perspective in bioprospecting, where the priority is to identify microbes and enzymes that not only remove colour but also lower toxicity under actual effluent conditions. Recent research have studied wetland bioprospecting through the use of shotgun metagenomics, metatranscriptomics, and genome-resolved metagenomics to identify previously uncultured dye-transforming microorganisms (Yeruva, 2023). Several studies have reported the recovery of metagenome-assembled genomes

harbouring azoreductase, multicopper oxidase, and ring-cleaving dioxygenase genes from saline and textile-impacted wetlands (Bhargawa et al., 2025). In addition, synthetic microbial consortia reconstructed from wetland metagenomic datasets have shown improved dye mineralisation and enhanced tolerance to high salinity and alkaline pH, highlighting the potential of wetland microbiomes as reservoirs of industrially relevant biocatalysts (Nawaz et al., 2020).

Table 2.2: Summary of microbial and enzymatic systems involved in dye degradation under saline and thermotolerant conditions.

Strain	Dye tested	Salinity (mg/L)	Temperature ⁰ C)	Time (h)	% Decolorization	References
<i>Halomonas meridiana</i> SAIBP-6	Reactive Red-195	50 mg/L	40	48	87.15	(Saha et al., 2023)
<i>Sterigmatomyces halophilus</i> SSA-1575	Reactive Black - 5	30 mg/L	35	18	80	(Al-Tohamy et al., 2020)
<i>Meyerozyma guilliermondii</i> SSA-1547	Reactive Black-5	50 mg/L	35	18	96.1	(Al-Tohamy et al., 2023)
<i>Pseudomonas aeruginosa</i> RGB11	Reactive black 250	-	37	24	88.91	(Chetry and Lakshmaiah, 2025)
Halophilic Bacillus	Orange II	66.67 mg/L	45.38	9.96	80.9	(Ikram et al., 2022)
<i>Bacillus licheniformis</i> KIBGE IB21	Sewage 1 and 2	30 g/L	35	18	88	(Ansari et al., 2025)
<i>Klebsiella pneumoniae</i> MW815592	Reactive Blue 214 and Reactive Red 195	20 g/L	46	42	80	(Sennaj et al., 2023)

<i>Geobacillus thermodenitrificans</i> A333	Orange II	-	70	24	97	(Marcolongo et al., 2015)
Recombinant enzyme immobilised (salt-stable)	Malachite green	50 g/L	-	24	80–95	(Kovinchuk, 2025)
<i>Escherichia coli</i>	Malachite green	30–80 g/L	-	24–48	70–95	(Xu et al., 2020)
<i>Halomonas xianhensis</i> SUR308	Malachite Green	50 g/L	35	84	95	(Biswas et al., 2023b)
<i>Paenibacillus dendritiformis</i> 17OS	Various	30–100 g/L	35	2–24 h	98	(Dawwam et al., 2023)
Halophilic laccase (recombinant)	Phenolic dyes	30–60 g/L	37	24	75–90	(Ayub et al., 2025)
Marine sediment isolates	Reactive Orange 16	30 g/L	35-37	36	70	(Tan et al., 2023)
Pilot saline wetland inoculation	Reactive dyes	30–50 g/L	45	48–96	55–80	(Riva et al., 2019)

2.6.4 Comparative diversity of microbes in pristine vs. polluted wetlands

Comparative metagenomics across various aquatic ecosystems reveals a consistent pattern: as pollution levels increase, microbial communities change. Taxonomic diversity typically decreases by 15-40 % (declines in the Shannon index have been reported in multiple freshwater studies), while functional genes related to contaminant breakdown, stress tolerance, and antimicrobial resistance often increase by 1.5-3 fold (Navarrete-Euan et al., 2021). In freshwater lakes, side-by-side comparisons of protected versus human-impacted systems reveal altered bacterial community structures and enriched resistomes in areas receiving nutrient and chemical inputs (Xu et al., 2017). These observations provide a valuable framework for studying wetlands exposed to textile dyes and their associated chemicals. Functionally,

selection under pollution pressure favours microbial groups capable of reductive and oxidative processing of xenobiotics (Xu et al., 2017). However, this adaptation may reduce redundancy and resilience in the community. For example, metagenomic surveys of mangrove wetlands have shown that relatively pristine zones are enriched in phototrophs and nitrifiers. In contrast, impacted zones are dominated by heterotrophs with elevated xenobiotic degradation pathways (Jeong and Ham, 2017). In such polluted areas, mobile genetic elements and resistance determinants increase by 30-70 %, reflecting an intensified process of horizontal gene transfer. This represents an ecological trade-off: microbes adapt to contaminants but at the expense of native functional diversity. Large-scale metagenomic studies in iconic wetland systems such as the Sundarbans confirm these trends (García-Estrada et al., 2024). Even in this globally significant biodiversity hotspot, pollution gradients leave clear microbial signatures. While the capacity for carbon cycling and nutrient turnover is retained, the microbial community composition and the relative dominance of metabolic pathways shift in response to exposure (Kumari et al., 2021). These findings have direct implications for bioprospecting: impacted wetlands may be richer in dye-transforming genes (e.g., azoreductase, multicopper oxidase), while pristine wetlands may host unique, underexplored lineages with potentially novel enzymes (Ter Horst et al., 2023). A paired sampling approach, polluted versus reference wetlands, can therefore maximise the discovery of both robust degraders and novel biocatalysts. Experimental wetland restoration studies provide cautious optimism (Payne et al., 2025). As hydrology and vegetation recover, microbial networks tend to regain complexity, and biogeochemical functions (e.g., nitrogen cycling, organic matter turnover) partially rebalance. However, pollution-selected traits, such as antibiotic resistance genes, often persist, with resistome abundances reduced by only 20-40 % compared to pre-restoration levels (Reji et al., 2022). This persistence highlights the importance of conserving pristine wetlands as reservoirs of baseline microbial diversity and genetic resources for future restoration efforts. Finally, recent meta-analyses suggest that under harsh conditions (e.g., high salinity, alkaline pH, chemical stress), microbial communities converge functionally even if their taxonomic composition differs (Kumar et al., 2018). In saline-alkaline wetlands, for example, functional gene inventories for xenobiotic degradation and osmotic protection are strikingly similar across sites despite less than 40% overlap in species-level taxa.

2.7 Microorganism-mediated dye degradation

Utilising microorganisms instead of traditional physico-chemical methods for dye degradation offers significant environmental benefits, providing clean, green, cost-effective, and

sustainable solutions for treating contaminated effluents without generating toxic by-products or substantial sludge (Khandare et al., 2023). Microbial dye degradation can be categorised based on the types of microorganisms involved, including fungi, bacteria, algae, and microbial consortia. Each group exhibits distinct mechanisms and efficiency in dye degradation, offering potential solutions for bioremediation.

2.7.1 Dye degradation by fungi

Fungi play a crucial role in dye degradation, either as bio-sorbents or bio-degraders, benefiting from their high biomass availability, cost-effectiveness, and strong mechanical stability across different pH conditions (Ali et al., 2023). The rigid structure of macrofungi enhances biosorption properties, with adsorption capacity increasing at higher temperatures; however, excessive temperatures may inhibit decolourisation by deactivating the adsorbent surfaces (Sun et al., 2023). Species such as *Cunninghamella elegans* and *Trametes versicolor* have exhibited superior dye removal efficiency compared to activated carbon. In contrast, others, such as *Aspergillus ochraceus*, *Phlebia*, *Pleurotus*, and *Bjerkandera adusta*, demonstrate significant biodegradation capabilities (Chatterjee and Pandey, 2023). The enzymatic systems of fungi, particularly lignin peroxidase, manganese peroxidase, and laccase, play a critical role in breaking down complex dye structures. However, challenges such as long hydraulic retention times and the need to sustain fungal viability in bioreactors for complete degradation need to be addressed to improve their large-scale applicability (Mary et al., 2020) for example, *Saccharomyces cerevisiae* is capable of absorbing high concentrations of dye. A study showed that dyes like Basic Green 4 and Basic Yellow 2 are discoloured by *Saccharomyces cerevisiae* to the extent of 96 % for Basic Green and 93 % for Basic Yellow (Kelewou et al., 2014). Another example, *Candida tropicalis* adsorbed 94 % of Remazol blue and 44% of Reactive Red, while *Trichosporon akiyoshidainum* achieved 63 % adsorption of Reactive Blue and 90 % of Reactive Red (Dhanavade and Patil, 2023). Yeast-mediated dye degradation is linked to their metabolism, which requires glucose as a carbon source. It triggers the production of oxidases, reductases, and NADH-DCIP reductase for the reduction of azo bonds (Al-Tohamy et al., 2023).

2.7.2 Dye degradation by algae

Algae are increasingly recognised as cost-effective and scalable solutions for dye effluent treatment. As photosynthetic organisms, they thrive in contaminated environments without being inhibited by dyes, making them promising candidates for bioremediation (Premarathna

et al., 2024). Both live and dead algal cells exhibit dye removal properties through biosorption and enzymatic degradation, with azoreductase facilitating the breakdown of azo dyes into less toxic aromatic amines. Different algal species degrade dyes at varying efficiencies, depending on the dye structure and environmental conditions (Panigrahi et al., 2024). Given their renewable biomass potential and self-sustaining nature, algae present an eco-friendly alternative to conventional dye removal techniques.

2.7.3 Dye degradation by bacteria

Bacteria are widely employed in industrial wastewater treatment due to their rapid degradation rates and adaptability to diverse environmental conditions. Under anaerobic conditions, bacteria can transform azo dyes into aromatic amines, while certain aerobic bacteria utilise azoreductase enzymes to break azo bonds efficiently (Khandelwal et al., 2023). For example, *Micrococcus* spp. Demonstrates rapid decolourisation of reactive dyes under anaerobic conditions, with further enhancement of degradation rates under aerobic conditions. Other bacteria, including *Pseudomonas* and *Geobacillus stearothermophilus*, exhibit high mineralisation efficiency, leading to the complete breakdown of dyes into non-toxic metabolites (El-Sheekh et al., 2023). Additionally, lignolytic bacteria, known for their ability to degrade lignin, have emerged as potent agents for degrading synthetic dyes. Species such as *Pseudomonas*, *Bacillus*, and *Rhodococcus* possess lignin peroxidase, manganese peroxidase, and laccase enzymes, which enable them to oxidise and fragment complex dye molecules. Their ability to operate under diverse environmental conditions and utilise low-cost substrates, such as agricultural and industrial waste, makes them attractive for large-scale wastewater treatment (Mary et al., 2020). However, challenges such as heavy metal interference and optimisation of conditions for maximum degradation efficiency remain significant hurdles. (Yadav and Yadav, 2015).

2.7.4 Microbial consortia and genetic engineering for enhanced dye degradation

Single microbial strains often struggle to completely degrade complex dye molecules; microbial consortia exhibit superior performance due to their combined enzymatic activities. Mixed microbial populations can decolourise dyes more efficiently than pure cultures by targeting different dye structures simultaneously or utilising metabolites produced by other strains (Wen et al., 2023) for example, microbial consortia containing *Yarrowia* sp. SSA1642, *Barnettozyma californica* SSA1518 and *Sterigmatomyces halophilus* SSA1511 decolourised 99 % of Red HE3B within 12 hours, compared to over 60 hours for single strains (Ali et al.,

2021). Fungal-bacterial co-cultures further enhance dye degradation by exploiting oxidoreductive enzymes from both microbial types (Khandelwal et al., 2023). For example, coculturing of *Dichotomomyces cejpai* MRCH 1-2 plus *Phoma tropica* MRCH 1-3 exhibited maximum decolourisation of dyes, such as methyl red and reactive blue, at a concentration of up to 50 mg/L (Krishnamoorthy et al., 2018, pp. 1–3). Genetic modification of microorganisms offers another promising approach to enhance dye degradation stability and efficiency. Engineered strains with enhanced azoreductase activity have demonstrated improved degradation rates, making them highly effective for bioremediation applications (Huang et al., 2024). For example, recombinant *Escherichia coli* expressing azoreductase genes from *Klebsiella pneumoniae* has successfully degraded methyl orange dye. Similarly, genetically modified *E. coli* strains have demonstrated enhanced efficiency in degrading various synthetic dyes (Wang et al., 2023). The potential of genetic engineering to improve microbial resilience against inhibitory substances and optimise dye degradation pathways holds immense promise for the future of sustainable wastewater treatment. Microorganism-mediated dye degradation presents a sustainable and efficient alternative to conventional physicochemical methods. Fungi, yeasts, algae, and bacteria each offer unique advantages in dye degradation (Table 2.3), while microbial consortia enhance decolourisation efficiency through synergistic metabolic interactions (Mary et al., 2020). The integration of genetic engineering techniques further strengthens microbial capabilities, enabling higher degradation rates and stability under industrial conditions. However, challenges such as optimising microbial survival in bioreactors, overcoming inhibitory substances in wastewater, and scaling up these technologies require further research and innovation. The most recent studies have focused on engineering microbial consortia and enzymes for operation under industrial textile-effluent conditions. CRISPR-assisted strain improvement, adaptive laboratory evolution, and AI-guided enzyme design have been employed to enhance azoreductase and laccase activity, salt tolerance, and operational stability (Srivastava and Bandhu, 2022). Immobilised recombinant laccases and azoreductase have demonstrated sustained decolourisation efficiency during repeated treatment cycles, while biofilm-based consortium reactors have shown improved resistance to fluctuations in dye concentration and salinity (Dey and Mandal, 2026). These advances strengthen the prospects for large-scale and continuous bioremediation of textile wastewater. Moving forward, interdisciplinary approaches that combine microbiology, genetic engineering, and environmental engineering will be crucial in advancing microbial bioremediation strategies for effective and sustainable management of dye pollution (Barathi et al., 2022).

Table 2.3. Microbial decolourisation and degradation of different microbial species toward a range of synthetic dyes, including azo, anthraquinone, triphenylmethane, and acid dyes

Dye	Microbe	Incubation time	Decolourisation percentage (%)	Reference
Reactive brilliant red K-2BP	<i>Pseudozyma Rugulosa</i>	24h	99	(Mohammed et al., 2023)
Reactive brilliant blue X-BR	<i>Candida krusei</i>	24h	78	(Shah et al., 2016)
Reactive turquoise blue KN-G	<i>Candida krusei</i>	24h	62	(Mendes et al., 2024)
Reactive brilliant red K-2BP	<i>Pseudozyma rugulosa</i>	24h	62	(Rana et al., 2023)
Remazol Blue	<i>Saccharomyces cerevisiae</i>	432h	51	(Mohammed et al., 2023)
Methyl red	<i>Galactomyces geotrichum</i>	1h	100	(Mendes et al., 2024)
Malachite green	<i>Trichosporon beigeli</i>	24h	90	(Shah et al., 2016)
Acid Red 97	<i>Gloeocapsa pleurocapsoides</i>	26 days	83	(Anandita et al., 2023)
Orange II	<i>Geobacillus stearothermophilus</i> (UCP 986)	24 h	96	(Evangelista-Barreto et al., 2009)
Napthol Green B	<i>Shewanella oneidensis</i> MR-1	24h	95	(Xiao et al., 2012)
Synazol red 6HBN	<i>Alcaligenes aquatilis</i> 3c	96h	82	(Ajaz et al., 2019)
Azure-B	<i>Serratia liquefaciens</i>	48h	90	(Haque et al., 2024)

Acid Orange 7	<i>Aeromonas caviae</i>	16h	90	(Dafale et al., 2008)
Reactive Dark Blue K-R	<i>Penicillium</i>	24h	97	(Al-Tohamy et al., 2023)
Disperse Red 1	<i>Microbacterium</i> sp	72h	80	(Franciscon et al., 2012)
Remazol Brilliant Violet 5R	<i>Bacillus</i> sp	18h	100	(Shah et al., 2016)
Reactive Orange 16	<i>Acinetobacter</i> sp	72h	80	(Meerbergen et al., 2018)
Disperse Red 1	<i>Microbacterium</i> sp	72h	80	(Franciscon et al., 2012)
Coomassie Brilliant Blue G-250	<i>Bacillus aryabhatai</i> DC100	72h	100	(Paz et al., 2017)
Synazol red 6HBN	<i>Alcaligenes aquatilis</i> 3c	96 h	82	(Mishra et al., 2020)

2.7.5 Mechanism of microbial degradation

In in-vivo process, microorganisms typically produce enzymes that degrade azo dyes. However, for higher concentrations of azo dye degradation, isolated enzymes are preferred. Commercially available enzymes, such as azoreductase, laccase, peroxidase, and oxidase, are utilised in industrial applications for this purpose, functioning both intracellularly and extracellularly (Zhong et al., 2023). These enzymes can be substrate-specific and can be immobilised on substrates to enhance degradation efficiency. Because enzymes are self-degradable, they do not pose environmental risks, and the byproducts of enzyme-mediated degradation can serve as media supplements for microbial culture growth (Table 2.4) (Sun et al., 2023). Enzymes follow different degradation mechanisms, with some being substrate-specific and others not (Figure 2.3). The process often involves cofactors and redox-active coenzyme intermediates like FADH, NADPH, and NADH, which transfer electrons from the enzyme to the azo dye, weakening and breaking the azo bonds to produce colourless, odourless,

and sometimes toxic intermediates like aromatic amines (Baffi et al., 2024) These intermediates can further degrade into smaller compounds such as water, carbon dioxide, and ammonia in aerobic conditions. Generally, azo dye degradation starts with the reduction of double bonds under anaerobic conditions, followed by aerobic processes for complete mineralisation. Despite the formation of some auto-oxidised by-products, enzymes such as azoreductase and laccase are commonly used for the efficient degradation of azo dyes (Meng et al., 2024).

Azoreductase enzymes, found in both the cytoplasm and membrane of microbial cells, play a crucial role in the reductive cleavage of azo dyes. These enzymes degrade dyes through redox-active intermediate-dependent pathways, requiring low-molecular-weight reducing equivalents, such as flavin adenine dinucleotide (FADH) or nicotinamide adenine dinucleotide (NADH), as electron donors. (Singh et al., 2024). The electron transfer process leads to the cleavage of the azo bond ($-N=N-$), resulting in the formation of colorless but potentially toxic aromatic amines. Cytoplasmic azoreductase exhibit variable efficiency depending on dye structure; they are less effective for sulfonated azo dyes due to their complex composition and poor diffusion properties, but efficiently degrade water-soluble, non-sulfonated azo dyes under anaerobic conditions. (Baffi et al., 2024) . Conversely, membrane-bound azoreductases catalyze electron transfer directly to azo dyes using redox-active coenzymes, resulting in decolorization. The resulting aromatic amines, which are often cytotoxic and mutagenic, require further aerobic or microaerophilic oxidation to achieve complete mineralisation into non-toxic end products (Khandare et al., 2023).

Laccases, extracellular copper-containing oxidoreductases primarily secreted by fungi and certain plants, follow distinct oxidative degradation pathways. These enzymes catalyse the oxidation of phenolic and non-phenolic aromatic compounds through a nonspecific free radical mechanism, generating phenoxy radicals that undergo secondary oxidative transformations (Mohanty and Kumar, 2021) . Unlike azoreductases, which rely on reductive cleavage, laccase-driven degradation occurs through oxidative polymerisation, resulting in the breakdown of complex dye structures into less toxic derivatives. While anthraquinone dyes can be directly oxidised by laccases, azo and indigo dyes require the presence of redox mediators such as ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and anthraquinone dyes, which facilitate electron transfer between the enzyme and dye molecules (Morsi et al., 2020) . The oxidation of azo dyes by laccases results in the formation of phenolic and quinonoid intermediates, rather than toxic aromatic amines, which subsequently undergo hydrolysis and

further oxidation into simpler, environmentally benign compounds, such as nitrophenols and nitrobenzene derivatives (Unuofin et al., 2019) .

In addition to azoreductases and laccases, other key enzymatic systems involved in dye degradation include peroxidases, such as lignin peroxidase (LiP) and manganese peroxidase (MnP), which are predominantly secreted by white-rot fungi. These enzymes catalyse hydrogen peroxide-mediated oxidative cleavage of dye molecules, breaking down complex aromatic structures into smaller, biodegradable compounds (Gałązka et al., 2023). LiP can cleave non-phenolic aromatic rings via one-electron oxidation, while MnP oxidises phenolic dyes through manganese-dependent radical formation. These oxidative reactions often lead to the formation of carboxylated or hydroxylated intermediates, which can be further mineralised through microbial metabolic pathways (Li et al., 2022). The role of oxidoreductases such as NADH-DCIP reductase has gained increasing attention in microbial dye degradation. This enzyme facilitates the electron transfer necessary for azo bond cleavage under anaerobic conditions, allowing efficient degradation of azo dyes in wastewater treatment systems (Vaithyanathan et al., 2022). The synergistic action of multiple enzymes, often within microbial consortia, enhances the degradation process by providing complementary pathways for dye breakdown, ultimately ensuring the complete mineralization of complex dye molecules into environmentally safe end products (Pandey et al., 2024).

Table 2.4: Key enzymes involved in the biodegradation of synthetic dyes: sources, catalytic properties, and degradation efficiencies under different environmental conditions:

Enzyme	Typical source	Reported optimal pH	Optimum T (°C)	Typical activity (U/mg, reported range)	Substrate example	% Degradation	References
Azoreductase and MnP enzyme	<i>Pseudomonas aeruginosa</i>	7	30	0.2-5.0 U/mg	Methyl orange	99	(Kishor et al., 2021a)
Azoreductase, NADH-quinone reductase (membrane-bound)	<i>Shewanella</i> spp. strain IFN4	8.0	45	0.1-3.5 U/mg	Reactive Black 5, Acid red 88, Direct red 81	80-90	(Imran et al., 2016)

TP-Lac 2	<i>Trametes polyzona</i>	4.0-6.0	50	1-48 U/mg	Malachite green oxalate, Orange G, amido black 10 B and Bromocresol purple	75	(Bucchieri et al., 2024)
Manganese peroxidase (MnP)	<i>Pleurotus pulmonarius</i>	4.0-6.0	50	135.52 U/mg	Remazol brilliant blue R	80-90	(da Silva et al., 2017)
Lignin peroxidase (LiP)	<i>Lentinus squarrosulus</i> MPN12	5.0	25	0.25-15 U/mg	Acid Blue 62	92	(Dinh Giap et al., 2022)
NADH-DCIP, laccase, and azoreductase	<i>Enterobacteriaceae</i> , <i>Staphylococcus</i> , <i>Bacillus</i> , and <i>Kosakonia</i>	6.0-8.0	25-35	0.1-2.0 U/mg	Congo red, Direct Black G (DBG), Amaranth, methyl red, and methyl orange	80-90	(Qiu et al., 2022)

DyP-type peroxidase	<i>Ascomycetous fungus</i>	4.0-5.0	40	0.3-10 U/mg	Reactive dyes	80-90	(Kimani et al., 2021)
Multicopper oxidase, alginate laccase	<i>Streptomyces flavomacrosporus</i>	5.0-8.0	28–50	0.2-8.0 U/mg	Phenolic compounds	75-85	(Salwan et al., 2023)
Catalase-peroxidase	<i>Pseudomonas</i> sp. AN-1	6.0-8.0	25–35	0.5-12 U/mg	Dye intermediates	80-85	(Qiao et al., 2024)
Peroxidase	<i>Nigella sativa</i>	5.0	37	0.2-5.0 U/mg	Yellow, red, Black, and Blue dyes	76.9, 88.7, 91, and 88	(Aziz et al., 2021)
Halotolerant azoreductase	Halophilic bacterium	7.5-9.0	30-45	0.1-3.0 U/mg	Synthetic dyes	70-80%	(Tian et al., 2019)
Azoreductase	<i>Chlorella vulgaris</i>	6.5-7.0	40	0.05–1.0 U/mg	Azo dyes	80-90	(Moradi et al., 2024)
Immobilised laccase (support)	Recombinant	4.0-6.0	25-35	10-100 U/g (immob.)	Malachite green	70-85	(Husain, 2017)
Thermophilic azoreductase	<i>Geobacillus</i>	7.0-8.0	90	0.5–6.0 U/mg	Orange II	85-90	(Matsumoto et al., 2010)

Recombinant enhanced azoreductase	Engineered <i>E. coli</i>	7.0-8.0	28-37	1-20 U/mg	Methyl orange	75-85	(Zhang et al., 2016)
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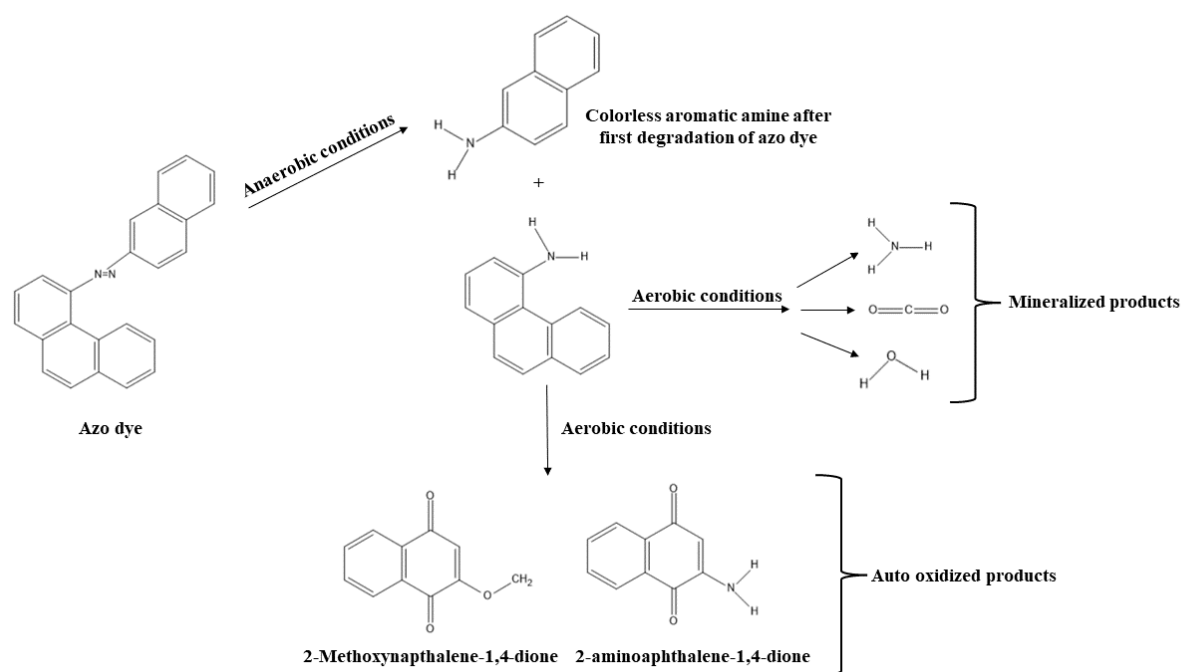


Figure 2.3. Mechanisms of azo dye degradation into less toxic metabolites involve enzymatic or chemical processes that break down the dye molecules, reducing their toxicity (Ayub et al., 2025).

2.7.6 Aerobic vs. anaerobic degradation pathways

A widely validated example in azo dye remediation is that anaerobic reduction cleaves azo bonds, removing visible colour, while aerobic steps are required to mineralise the resulting aromatic amines (Tyagi et al., 2025). Modern integrated treatment designs leverage this complementarity, with sequential or spatially coupled redox zones consistently outperforming single-mode systems. Recent engineering studies have demonstrated that two-stage anaerobic-aerobic reactors achieve 80-95% decolourisation within 24–48 hours and 60-85 % mineralisation within 72-96 h, compared with $\leq 30\%$ mineralisation in purely anaerobic setups (Zhang et al., 2021). Likewise, constructed wetlands engineered with vertical or horizontal subsurface flow show 20-40 % higher COD and aromatic amine removal when designed to sustain microaerophilic niches compared with strictly anaerobic beds. Under strict anaerobic conditions, certain bacteria, such as *Shewanella oneidensis* and *Clostridium* spp., can rapidly reduce azo dyes by coupling the azo bond to their cellular electron transport pathways (Sossalla et al., 2022). Laboratory studies report complete colour removal of 100-200 mg/L Reactive Red 120 in 12-24 h under glucose-fed anaerobic conditions, but with accumulation of aromatic amines that persist unless oxygenases are introduced. Rates of reduction can be accelerated with external electron donors (glucose, lactate, acetate) or redox mediators such as anthraquinone-2,6-disulfonate (AQDS), which can increase decolourisation rates by 2- to 5-fold. Still, without oxidative follow-up, detoxification remains incomplete (Rahman and Thomas, 2021). Aerobic degradation pathways, dominated by mono- and dioxygenases, hydroxylate and cleave aromatic rings, funnelling amines and anthraquinone intermediates into central carbon metabolism via catechol and gentisate pathways. Sequencing-based studies have shown that aerobic degraders, such as *Pseudomonas* and *Rhodococcus*, harbour plasmid-borne dioxygenase clusters, enabling the rapid acquisition and horizontal transfer of oxidative capacity within mixed consortia (Otaiku and Alhaji, 2020). This plasmid-driven flexibility is one reason enrichment from wetlands and textile-impacted sediments often yields robust aerobic strains suitable for “polishing” post-anaerobic effluents (Ismail et al., 2019b). Some engineered and natural systems exploit simultaneous anaerobic and aerobic micro-niches, biofilms, sediment-water interfaces, or vertical subsurface flow beds to host both reductive and oxidative activities in proximity (Choquel, 2021). Microscale coupling reduces transport losses of intermediates and shortens mineralisation times. Pilot-scale studies have reported that integrated wetland reactor systems treating textile wastewater achieve 90-95 % colour removal

and 70-80 % toxicity reduction (as determined by phytotoxicity assays with *Lepidium sativum*), whereas conventional single-mode reactors achieve only 40-55 % toxicity reduction (Bożym et al., 2021). A key caveat illustrated in recent literature is that some reduction products (e.g., benzidine, naphthylamines) are more toxic or mutagenic than their parent dyes if not further degraded. Consequently, performance metrics are shifting away from colour removal alone toward verified detoxification. State-of-the-art protocols now combine targeted metabolite analysis (LC-MS, GC-MS) with bioassays, such as Ames mutagenicity tests and plant germination assays, to demonstrate the efficacy of anaerobic/aerobic treatment systems. This functional emphasis aligns with the modern paradigm of treatment design, which ensures not only decolourisation but also genuine detoxification and ecosystem safety (Lioupi et al., 2021).

2.7.7 Factors influencing microbial dye degradation

pH has a strong influence on both enzyme activity and microbial community balance. Many fungal laccases and peroxidases work best at acidic to near-neutral pH levels (4.0-6.5), whereas bacterial azoreductases generally show optimal activity in neutral to slightly alkaline ranges (pH 7.0-8.5) (Liu et al., 2025). Optimisation studies demonstrate that shifting the pH by even ± 1 unit can alter decolourisation efficiency by 20-40%, underscoring the importance of precise control. For mixed consortia, staged or compromise pH management is often necessary to ensure both reductive and oxidative pathways remain active (Kalra and Gupta, 2023). Temperature dictates enzymatic kinetics and microbial growth. Mesophilic conditions (20-35 °C) are commonly reported as optimal for bacterial and fungal degraders in laboratory studies. However, isolates from more remarkable wetlands can retain significant activity at 10-15 °C, which is essential for applications in seasonal or high-altitude wetlands (Mohanty and Kumar, 2025). Engineering studies emphasise that matching the degrader community to the local temperature regime can enhance long-term stability, with reported differences of up to 30 % in COD and colour removal efficiency between mismatched and adapted strains (Yuan et al., 2020). Nutrients and co-substrates such as glucose, acetate, or yeast extract play a critical role by enabling co-metabolism. Microbes preferentially consume these labile substrates, and in the process, also transform dyes. Studies show that supplementing with glucose or acetate can double or even triple decolourisation rates, for example, boosting removal from 40 % to over 90 % within 24-48 hours (Das and Mishra, 2025). However, excess nutrient dosing risks eutrophication in receiving waters. To manage this, staged nutrient addition or immobilised delivery systems are now recommended in process design. Salinity and ionic strength are

crucial factors for textile effluents, which often contain 30-80 g/L of salts (NaCl, Na₂SO₄) from reactive dyeing processes. Such levels inhibit many freshwater microbes and destabilise enzymes, but they favour halotolerant taxa, such as *Halomonas* and some *Bacillus* spp., as well as salt-stable laccases and azoreductases (Contreras-Salgado et al., 2024). Recent bioprospecting in saline wetlands has identified strains that retain more than 70 % activity at 5-10 % NaCl, making them promising for direct effluent treatment without dilution. Dye concentration and auxiliaries (surfactants, metal-complexing agents) also influence outcomes (Ruginescu et al., 2020). High dye loads can be toxic, while auxiliaries may inhibit enzymes or cause misleading results due to sorption. Many studies recommend either dilution, staged loading, or pre-treatment (e.g., adsorption or advanced oxidation) to reduce concentrations into a biologically manageable window, typically < 200 mg/L for azo dyes (Alsukaibi, 2022). Recent process optimisation using central composite design (CCD) and design of experiments (DoE) approaches demonstrated that adjusting inoculum size, pH, and incubation time together can improve decolourisation yields by 15–35% compared with unoptimized setups (Table 2.5).

Table 2.5: Influence of environmental and operational factors on microbial dye degradation efficiency

Factor	Typical Range Tested	Average Effect on Decolourisation Efficiency (%)	Mechanistic Influence	Relative Influence Level	Experimental Observations	References
pH	5-9	±20-45 %	Optimal near neutral (6.8-7.5); extreme pH reduces enzyme stability and alters redox potential.	High	Sharp decline below pH 6 due to enzyme denaturation and proton imbalance.	(Sonwani et al., 2020, p. 2)
Temperature	20-40°C	±25-35 %	Enzyme-mediated reactions accelerate up to 35°C; thermal stress >40°C causes enzyme denaturation.	High	Thermotolerant consortia maintain an efficiency of greater than 80 % at 35°C.	(Rajesh Kumar et al., 2024)
Salinity (NaCl)	0-100 g/L	10-30 % decrease beyond 50 g/L	Osmotic stress affects cell membranes and enzyme conformation.	Moderate–High	Halotolerant strains retain >70% activity at 50-100 g/L.	(Aziz et al., 2021)
Co-substrate (e.g., glucose, acetate)	0-5 g/L	150-300 % rate increase	Acts as an electron donor in azo bond reduction, enhancing NADH regeneration.	High	Glucose at 2-3 g/L yields 2-3 times faster decolourisation.	(Alam et al., 2021)
Initial dye concentration	25-500 mg/L	20-60 % reduction at high load (>200 mg/L)	Excess dye inhibits enzymes and blocks active sites.	High	The optimum range is 50-200 mg/L for most consortia.	(Tripathi et al., 2023)

Surfactants (SDS, Tween 80)	0-2 g/L	10-40 % inhibition	Disrupts cell membranes and enzyme–substrate affinity.	Moderate	A low concentration (<0.5 g/L) can sometimes enhance the solubility of dyes.	Harish et al., 2023
Heavy metals (Pb, Cd, Cr)	0-10 mg/L	15-60 % inhibition	Competes with enzyme cofactors, inhibits azoreductase and laccase activity.	High	5 mg/L Cd ²⁺ reduces degradation rate by ~50 %.	(Patel and Gupte, 2023)
Hydraulic Retention Time (HRT)	1-7 days	+20-40 % increase with longer HRT	Improves contact time and biofilm stability in CW/bioreactors.	Moderate	A period of 5-7 days is optimal for the stable degradation of reactive dyes.	(Khandare et al., 2023)
Inoculum size	1-10% (v/v)	+15-35 % increase	A higher inoculum accelerates dye utilisation and reduces the lag phase.	Moderate	Optimum is around 5% for balanced growth and oxygen demand.	(Rajesh Kumar et al., 2024)
Immobilisation support (biochar, alginate, PUF)	-	+20-60 % increase	Increases cell density, enzyme stability, and reusability.	High	PUF-immobilised cells show 99 % CR removal vs. 85 % free cells.	(Sonwani et al., 2020)
Redox mediators (AQDS, humic acid)	0-50 µM	200–500 % rate increase	Enhance electron transfer to azo groups; promote anaerobic reduction.	Very High	AQDS addition (25 µM) accelerates the reduction by ~4 times.	(Patel and Gupte, 2023)
Aeration rate (polishing phase)	0-4 L/min	+15-40% improvement in mineralisation	Promotes the cleavage of aromatic rings and the oxidation of intermediates.	Moderate-High	Sequential aerobic polishing enhances the	(Abdullahi et al., 2021)

					reduction of COD and BOD.	
Substrate media size (packed-bed reactors)	1-10 mm	+15–30 %	Smaller particles favour biofilm formation and mass transfer.	Moderate	Media <3 Mm diameter improves degradation by ~25 %.	(Patel and Gupte, 2023)
Temperature control stability	±2°C	10–30 % variability reduction	Ensures reproducible enzyme kinetics and stable reaction rate.	Moderate	Stable temperature conditions improve continuous operation.	(El-Liethy et al., 2024)
Pre-oxidation dose (O ₃ or H ₂ O ₂)	10-30 mg/L	+20–40 % enhancement	Oxidises dye molecules into simpler intermediates, thereby improving biodegradability.	High	Integrated ozonation-bio systems outperform single-step biodegradation.	(Belhalfaoui et al., 2021)

2.8 Overview of dye-induced phytotoxicity in plants

Synthetic dyes mainly reach the environment through wastewater from textile and other industries, as well as through irrigation with contaminated water, and once in soil or surface water, they act as foreign chemicals that plants absorb through roots or leaves (Figure 2.4) (Akter et al., 2023). Both dyes and their breakdown products interfere with vital plant processes such as seed germination, membrane stability, and hormonal signalling, resulting in stress that reduces growth and yield. (Fried et al., 2022). Due to their persistence, dyes have both short-term and long-term effects, depending on their concentration, binding to soil particles, and rate of degradation. For example, azo dyes at concentrations as low as 25 mg/L have been shown to reduce seed germination of red amaranth by 40 % compared with untreated controls, while mung bean (*Vigna radiata*) exposed to 50 mg/L Reactive red 141 Red showed only 46 % germination compared with 92 % in controls, and root and shoot lengths dropped by more than 60 % at 100 mg/L (Sompark et al., 2024). Sensitivity differs among species; crops like mung bean and spinach are highly vulnerable, exhibiting substantial reductions in growth and pigment content. In contrast, more tolerant species, such as wheat or grasses, survive moderate dye levels but still experience a 15-30 % loss of chlorophyll, which reduces photosynthetic efficiency (HanumanthaRao et al., 2016). Soil conditions also influence dye toxicity, as associated salts and surfactants can alter soil texture, microbial balance, and enzyme activity. A recent study reported that untreated dye effluent reduced soil dehydrogenase activity by 50 %, while zinc nanoparticles (Zn NPs) improved enzyme activity by 30-40% and raised mung bean germination from 52 % under dye stress to 80 % when Zn NPs were applied, demonstrating a possible remediation strategy (Mazhar et al., 2024). Modern assessments no longer rely on germination alone but use multiple indicators, including root and shoot length, biomass, vigour index, pigment levels (chlorophyll a, b, and carotenoids), and oxidative stress markers such as malondialdehyde (MDA) and antioxidant enzyme activity (SOD, CAT, POD) (Griffo, 2024). For example, maize exposed to 75 mg/L Reactive Red 120 showed a 35 % drop in chlorophyll content and a two-fold rise in MDA, clearly indicating oxidative damage. Current best practice, therefore, combines these biological markers with physicochemical analysis of effluents (COD, pH, heavy metals, and salts) to provide a comprehensive picture of dye toxicity and to determine whether treated dye wastewater is safe for agricultural reuse (Kaya et al., 2012).

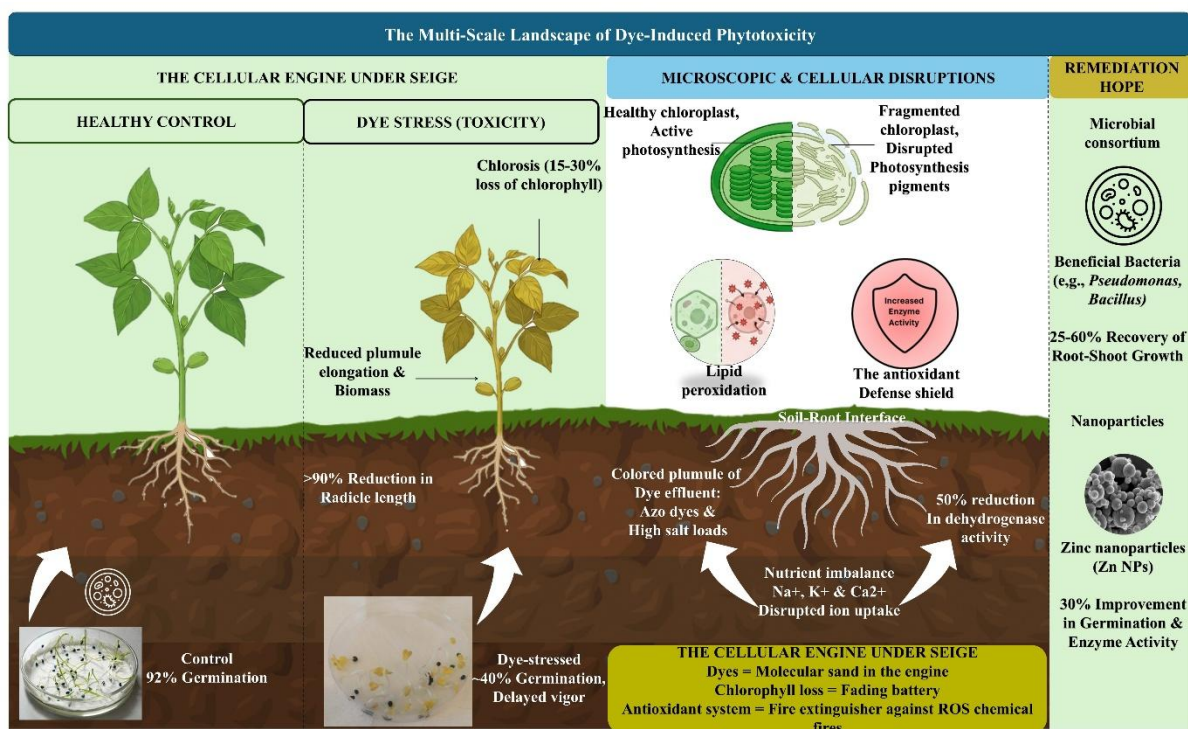


Figure 2.4: Overview of how synthetic dyes affect plants at different levels, from visible growth symptoms to internal cellular and biochemical changes.

2.8.1 Impact on seed germination, root/shoot elongation, and biomass accumulation

Seed germination is one of the earliest and most sensitive indicators of dye toxicity in plants. Studies on red amaranth (*Amaranthus tricolour*) exposed to loom-dye effluents ranging from 0 to 100% concentration demonstrated that germination, capacity, and seedling vigour progressively declined with increasing effluent strength (Rahman et al., 2020). At 100 % effluent, both root and shoot lengths were severely reduced, with relative toxicity ranking black > violet > pink effluents. Similarly, mung bean (*Vigna radiata*) seedlings exposed to Reactive Red 141 showed delayed germination and significantly lower vigor index; plumule (shoot) and radical (root) lengths dropped markedly at 50 and 100 mL/L exposures over both short-term 14 days and long-term (up to 60 days) periods, indicating acute and cumulative phytotoxic effects (Sompark et al., 2024). In general, increasing effluent concentrations (25-100 %) led to a graded decline in germination rates, root and shoot elongation, seedling vigour index, and growth index, with phytotoxicity percentage peaking at full-strength effluent, indicating a direct correlation between dye load and growth inhibition (Rusan et al., 2015). Quantitative biomass measurements further revealed that both fresh and dry weights decreased under dye stress, reflecting impaired photosynthesis, nutrient uptake, and energy allocation. Even moderate dye levels (25-50%) caused measurable reductions in biomass, which can hinder

early growth and reduce competitive ability in agricultural systems (Shi et al., 2025). Recent studies have complemented these morphological observations with physiological and biochemical parameters, such as chlorophyll content, antioxidant enzyme activity (SOD, CAT, POD), and membrane integrity, capturing the cumulative impact of dye exposure on seedling health (Zafar et al., 2024). Collectively, these multifaceted assessments confirm that dyes not only stunt physical growth but also compromise functional viability, especially under prolonged exposure, emphasising the need for careful evaluation of textile effluents before their reuse in agriculture.

2.8.2 Alterations in photosynthetic pigments, antioxidant systems, and metabolic processes

Synthetic dyes primarily interfere with plant metabolism by damaging photosynthetic pigments, inducing oxidative stress, and disrupting the balance of essential nutrients. In the cyanobacterium *Spirulina platensis*, exposure to 5-40 ppm of textile dyes resulted in a reduction of up to 90 % in phycocyanin and approximately 50 % loss of chlorophyll and carotenoids, indicating that dyes directly inhibit pigment synthesis and degrade existing pigments, thereby weakening light-harvesting efficiency (Magalhães et al., 2024). Similar effects are observed in higher plants, where dye stress triggers the accumulation of reactive oxygen species (ROS), increases lipid peroxidation as measured by malondialdehyde (MDA) levels, and enhances antioxidant enzyme activity (Magalhães et al., 2024). For example, studies report that SOD activity can increase by 30–55% and CAT activity by 20-40% under dye exposure, reflecting a defensive response to oxidative damage. These biochemical markers, when studied in conjunction with growth reductions, highlight the extent of cellular stress (Accipe et al., 2023). Dye effluents also alter soil properties, such as pH and salinity, which can disrupt nutrient uptake and absorption. This imbalance often causes deficiencies or toxicities of key ions, such as Na^+ , K^+ , and Ca^{2+} , which slow metabolic processes and reduce crop yield potential (Kaya et al., 2012). Interestingly, remediation experiments using zinc nanoparticles (Zn NPs) in dye-polluted soils showed improved plant performance, including 20-22 % increases in biochemical compounds, 11-45 % rises in soil enzyme activity, and around 4 % higher membrane stability index, all of which indicate restored antioxidant and metabolic functions (Sompark et al., 2024). Modern phytotoxicity studies therefore rely on a combined approach, involving the measurement of chlorophyll and pigment levels, tracking the accumulation of ROS and MDA, quantifying antioxidant enzyme activity, and analysing nutrient uptake. This holistic profiling helps distinguish between visible growth suppression

and more profound biochemical disruptions, providing a clearer understanding of how synthetic dyes compromise plant metabolism and health (Haque et al., 2024).

2.8.3 Long-term effects on soil-plant interactions and crop productivity

Long-term exposure to dye-contaminated wastewater gradually declines both soil quality and plant productivity. Residual dyes accumulate in soil, altering its physical properties, such as porosity and water-holding capacity, as well as its chemical properties, including pH, salinity, and organic load (Ebsa et al., 2024). These changes disrupt root-soil interactions, weaken nutrient cycling, and contribute to a steady decline in yield across successive crop cycles. A significant impact is observed in soil microbial ecology: repeated irrigation with untreated textile effluents shifts microbial communities away from beneficial groups, such as nitrogen-fixing bacteria and mycorrhizal fungi, while favouring pollutant-tolerant species. This shift reduces nutrient mineralisation, enzyme activity, and overall fertility (Schröder et al., 2016). For example, field studies in India have shown that *Amaranthus tricolour* irrigated with 100% textile wastewater accumulated excess salts and displayed a disturbed ion balance, suggesting that impaired microbial facilitation of nutrient uptake directly affects plant health (Sultana et al., 2025). Accumulated salts, surfactants, and dye byproducts also promote secondary salinisation and sodicity, leading to poor soil structure, reduced infiltration, and stressed plant-water relations. Over time, these combined effects translate into measurable agronomic losses: experiments have recorded 15-30 % declines in wheat and rice biomass, 10-25 % yield reductions in legumes and vegetables, and delays in maturity, along with lower grain or fruit quality (Ma et al., 2020). Such chronic exposure scenarios pose a threat to food security in regions where untreated or poorly treated effluents are reused for irrigation. To address these risks, researchers recommend routine monitoring of dye residues, salinity, and microbial enzyme activities in soil, along with adaptive practices such as alternating cropping cycles and leaching to minimise accumulation. Promising remediation approaches, including microbial degradation, phytoremediation, and soil amendments, have shown encouraging results. For instance, the use of zinc nanoparticles has improved soil enzyme activity by 11-45 % and enhanced membrane stability in plants by approximately 4 % under dye stress. Together, these findings highlight the importance of integrating soil rehabilitation strategies with plant assays to sustain agricultural productivity in dye-affected landscapes (Alamu et al., 2024).

2.8.4 Dye toxicity studies using model plants

Mung bean (*Vigna radiata*) has been one of the most widely used model plants to evaluate phytotoxicity, and results consistently show strong sensitivity to dye contamination. Exposure to Reactive Red 141 dye resulted in apparent dose-dependent declines in germination percentage, plumule and radicle lengths, and overall seedling vigour (Sompark et al., 2024). Notably, both short-term 14 days and long-term (60 days) soil exposure experiments revealed sustained inhibition, confirming that the effects are not only acute but also cumulative. Red amaranth (*Amaranthus tricolour*) seedlings irrigated with loom-dye effluent at concentrations ranging from 5 % to 100 % displayed significant reductions in germination energy, capacity, and elongation of roots and shoots, with black dye effluents proving the most toxic, followed by violet and then pink (Rahman et al., 2020). Similar responses were reported in lady's finger (okra, *Abelmoschus esculentus*) and mung bean seedlings exposed to textile dyeing effluent (25-100 %), where progressive reductions in germination percentage, shoot and root length, seedling vigor index, and growth index were recorded, highlighting the consistency of dye toxicity across multiple dicot models (Sadek et al., 2016). Beyond higher plants, primary producers like microalgae are also highly vulnerable. *Spirulina platensis*, exposed to 5-40 ppm textile dyes, experienced a loss of up to 90 % of phycocyanin pigments, a nearly 50% reduction in chlorophyll and carotenoids, along with measurable decreases in protein and elemental composition (Gita et al., 2021). Such pigment and nutrient losses in microalgae can impair photosynthetic efficiency and cascade into higher trophic levels in aquatic systems. Notably, mitigation experiments using zinc nanoparticles (Zn NPs) in mung bean cultivation have demonstrated a partial reversal of dye-induced damage (Lau et al., 2022). Zn NP treatments restored germination by 30-36.5 %, improved biochemical compound levels by 20-22 %, enhanced soil enzyme activity by 11-45 %, and increased membrane stability index by around 4 %. These results provide substantial proof of concept for integrating nanomaterial-assisted remediation with phytotoxicity assays to counteract the harmful impacts of synthetic dyes on plants and soil ecosystems (Lau et al., 2022).

2.8.5 Reduced phytotoxic effects after microbial degradation of dyes

Recent studies strongly confirm that microbial treatment of dye-containing waters can substantially reduce phytotoxicity in plants (Table 2.6). Bacterial, fungal, algal, and mixed consortia are widely reported to not only remove visible colour but also lower COD and TOC, producing treated effluents that support higher seed germination rates, longer root and shoot growth, and greater biomass compared to untreated effluents (Gayathiri et al., 2022). For example, the germination rates of crops like mung bean, tomato, and lettuce irrigated with

biologically treated dye wastewater often increase by 20-40 % compared to raw effluent, while root and shoot lengths improve by 25-60 % (Egbuikwem et al., 2020). Reductions in biochemical stress markers support these physiological gains; plants exposed to treated effluents consistently show lower reactive oxygen species (ROS) accumulation, reduced lipid peroxidation (as measured by malondialdehyde, MDA), and normalised antioxidant enzyme activity, such as superoxide dismutase (SOD) and catalase (CAT) (Hasanuzzaman et al., 2021). Such changes demonstrate that recovery is due to actual detoxification at the cellular level rather than mere dilution or adsorption of dyes. Advanced metabolite profiling (e.g., GC-MS) strengthens this mechanistic link. Studies demonstrate that microbial treatments not only decolourise but also reduce toxic aromatic amines and ring-cleavage intermediates (Franciscon et al., 2012). A direct correlation is often reported between the disappearance of specific metabolites, such as aniline derivatives, and improved plant germination or reduced oxidative stress responses. This highlights that proper detoxification depends on biodegradation pathways progressing beyond azo-bond cleavage toward complete ring fission and mineralisation (Mishra et al., 2020). Pilot-scale systems, including constructed wetlands, microbial fuel cell (MFC)-integrated wetlands, and packed-bed bioreactors, have reproduced these lab-scale benefits under field-relevant conditions. For example, lettuce irrigated with microbially treated textile wastewater showed biomass recovery of up to 50 % compared to untreated controls, provided COD reductions exceeded 70% and aromatic intermediates were substantially removed (Ait-Mouheb et al., 2022). Such findings reinforce that carefully designed microbial systems can yield irrigation-safe water even under realistic industrial loading conditions. However, the literature also cautions that partial degradation can sometimes produce intermediate metabolites with equal or higher phytotoxicity than the parent dye (Singh, 2022). For example, cases where azo-bond cleavage occurs without subsequent oxidative breakdown can generate aromatic amines that retain or enhance toxicity. This makes clear that decolourisation alone cannot serve as a sufficient success metric; a comprehensive assessment must pair colour removal and COD data with phytotoxicity bioassays and metabolite analysis to confirm genuine detoxification (Alamu et al., 2024).

Table 2.6: Summary of immobilised microbial and enzymatic systems in dye degradation, highlighting operational conditions, decolourisation efficiency, underlying enzymatic mechanisms, and the outcomes of detoxification assays.

Strain	Dye	Immobilization	Conditions (pH, Temp)	Reactor System	Decolorization %	COD / BOD / TOC reduction (%)	Enzymes Mechanism	Toxicity Detoxification Assay	References
<i>Bacillus</i> sp. (immobilised)	Congo Red	Polyurethane foam + polypropylene (MBBR carriers)	pH 7, Temp 30 °C	Moving bed biofilm reactor (MBBR)	85	95	Azoreductase-mediated + biofilm adsorption.	Phytotoxicity/bio degradation characterisation	(Sonwani et al., 2020)
<i>Lysinibacillus</i> sp.	Congo Red	Polyurethane foam combined with activated carbon &	pH 7, temp 30 °C	Immobilised carriers; batch/reactor tests	85	85-93	Azo-bond reduction + adsorption.	Toxicity reduction assessed via standard assays	(Sonwani et al., 2020)

		sodium alginate							
Immobilised <i>Aspergillus niger</i> (fungal)	Congo Red	Calcium alginate beads (fungal biomass immobilised)	pH 6–7, temp 30 °C	Batch adsorption/biodegradation experiments	92–98	98→72	Adsorption + azo bond reduction	Phytotoxicity	(Hamad and Saied, 2021)
Crude/purified laccase (from <i>Streptomyces sviveus</i>)	Congo Red	Calcium alginate immobilised enzyme	pH 6, temp 30 °C	Batch enzyme treatment	92	75	Laccase-mediated oxidative cleavage	Toxicity tests + spectral analysis	(Chakravarthi et al., 2021)
Indigenous bacterial consortium (free & immobilised)	Real textile effluent	Immobilised consortium	pH 6-7, temp 30 °C	Semi-pilot scale column/bio reactor	85	85	Oxidoreductases /azoreductase).	Phytotoxicity/acute tests	(El-Liethy et al., 2024)

Autochthonous bacterial consortium	Methyl Orange	Calcium alginate beads (immobilised consortium)	pH 7, temp 30 °C	Batch immobilised bead experiments	90	75	Azoreductase + consortium metabolic pathways.	Toxicity assays	(Ibrahim et al., 2022)
Enriched bacterial consortium	Mixed textile dyes	Consortium (free & immobilised variants tested)	pH 7, 35 °C	Batch & reactor trials	75-85	85	Enzymatic induction of reductases and oxidases.	Toxicity assay	(Patel and Gupte, 2023)
Heterogeneous biocatalytic systems (various microbial + material combos)	Azo dyes	(immobilised cells/enzymes)	Conditions varied	Batch and fixed-bed systems	90.	70-65	Both reductive and oxidative enzymes (azoreductase, laccase, and peroxidase).	Many included toxicity reduction tests (less toxic metabolites).	(Harish et al., 2023)

Natural microbial consortia (activated sludge / enriched consortia)	Simulated textile effluent	Free and immobilised systems (alginate, PUF)	pH 7. temp 37 °C	Anoxic reactors/se quencing reactors	80–95	75-70	Community metabolism + enzymatic azo bond cleavage.	Toxicity assays reported a decrease in phytotoxicity and aquatic toxicity.	(Iqbal et al., 2022)
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2.8.6 Phytotoxic effects of untreated and biodegraded dyes on plants

A growing body of laboratory experiments directly compares plant responses to untreated and microbially treated dye samples using standardised seed germination and seedling growth assays with species such as *Vigna radiata*, *Sorghum spp*, and *Lactuca sativa* (Chaturvedi et al., 2021). These studies consistently report higher germination percentages, longer root and shoot lengths, and greater vigour indices in plants exposed to treated effluents, with improvements proportional to the degree of mineralisation (TOC/COD reduction) achieved, making such side-by-side bioassays routine for validating detoxification claims. (Sari, 2018). For example, a recent study using a bacterial consortium optimised for Reactive Black 5 (RB5) decolourisation demonstrated not only rapid colour removal but also marked reductions in mutagenicity and phytotoxicity in seedling assays compared to untreated RB5, with LC-MS confirming decreased levels of aromatic amines, thereby linking chemical and biological endpoints (Elnabi et al., 2025). Constructed wetlands and algal bacterial bioreactors treating real textile wastewaters often incorporate plant assays with species such as lettuce, tomato, or radish, which are irrigated with treated effluents. Pilot-scale reports indicate improved plant growth and reduced biochemical stress markers relative to untreated wastewater. However, outcomes depend strongly on hydraulic retention time, substrate media, and plant species used in the wetland (Amori, 2022). These real-world studies highlight that scaled biological systems can deliver significant reductions in phytotoxicity under operational conditions. However, comparative experiments also reveal limitations, as partial biodegradation leading to the accumulation of aromatic amines may leave treated effluents with residual genotoxic or phytotoxic potential in sensitive species, underscoring the need for combined germination/growth assays, genotoxicity tests such as Ames, and metabolite profiling to avoid false positives where mere colour removal masks persistent chemical hazards (Sompark et al., 2024). Moreover, time-series analyses demonstrate that phytotoxicity reduction often lags decolourisation, with plants exposed shortly after treatment still exhibiting toxic responses until downstream oxidation or complete mineralisation occurs. This temporal aspect highlights the importance of evaluating treated effluents after a stabilisation period or incorporating polishing steps to ensure their reuse in irrigation. Accordingly, recent protocol papers recommend waiting until key metabolites decline or treated water passes targeted bioassays before classifying it as non-phytotoxic, reinforcing the consensus that only integrated chemical and biological assessments can ensure environmental safety (Haq and Raj, 2018a).

2.8.7 Potential role of microbial consortia in enhancing detoxification and Phyto-protection

Microbial consortia whether naturally assembled biofilms from wetlands or rationally designed mixes of bacteria, fungi, and algae have been shown to outperform single strains in dye remediation because they combine complementary enzymatic activities such as reductive cleavage, oxidative reactions, and aromatic ring degradation, while also exhibiting greater resilience to fluctuating environmental conditions like pH, salinity, and temperature (Rathour et al., 2024). Both experimental and pilot-scale studies consistently demonstrate that consortia achieve deeper mineralisation and greater reductions in phytotoxicity than monocultures when treating complex, real-world effluent matrices (Kuanar et al., 2022). Beyond pollutant breakdown, consortia also confer ecological benefits that indirectly protect plants. Algal-bacterial interactions oxygenate the rhizosphere and supply labile carbon, while fungal partners secrete extracellular oxidases that target recalcitrant dye molecules. Additionally, bacterial members complete aerobic catabolic pathways (Vishwakarma et al., 2020). These synergistic functions collectively reduce the accumulation of phytotoxic intermediates, resulting in effluents that are more compatible with plant physiology. This finding is reinforced by pilot studies using constructed wetlands (CWs) and microbial fuel cells (MFCs), which report improved downstream plant health during irrigation trials (Ijaz et al., 2016). Consortium design can follow two major strategies: empirical assembly of high-performing isolates or omics-informed reconstruction of microbial partnerships (Elnabi et al., 2025). The latter approach, guided by metagenomic and metatranscriptomic profiling, enables researchers to identify keystone taxa and critical pathways, such as azoreductase, dioxygenase, and ring-cleavage enzymes, which may include uncultured organisms. This approach avoids redundancy while ensuring functional coverage (Ali et al., 2021). Translational projects have shown that omics-guided consortia consistently lower phytotoxic endpoints in plant assays by targeting key detoxification steps sequentially. However, bringing these consortia into operational contexts requires careful formulation and ecological stabilisation. Approaches such as immobilisation on carriers, pelletization, or incorporation into bio-support materials help mitigate risks from competition, phage predation, or horizontal gene transfer that might compromise long-term function (Paz et al., 2017). Documented pilot projects highlight successful strategies, including repeated inoculation, protected immobilisation matrices, and the use of physically stabilising media such as granular activated carbon or biochar, which not only adsorb inhibitory compounds but also promote robust biofilm growth (Wang et al., 2022). Integrating microbial

consortia with plants in constructed wetlands provides additional benefits by coupling biotic and abiotic processes. Plant roots regulate hydraulics, supply oxygen, and release exudates that stimulate microbial metabolism, while the consortia simultaneously degrade dyes and detoxify effluents (Sadek et al., 2016). This dual action leads to significant reductions in phytotoxicity. It enhances plant growth, establishing an integrated phytoremediation-bioremediation framework that is increasingly recognised as a best practice for converting textile effluents into water suitable for restricted agricultural reuse, particularly in fodder crops and non-food irrigation systems (Egbuikwem et al., 2020).

2.9. Future Perspectives

2.9.1 Application of advanced molecular tools to study microbial dye degradation

New sequencing technologies, including short-read and long-read next-generation sequencing (NGS) and metagenomics, enable the rapid identification of a wide range of functional genes, such as azoreductases, multicopper oxidases, DyPs, and dioxygenases, in wetland environments (Figure 2.5) (Mishra et al., 2021). This helps researchers focus on sites where the abundance and diversity of dye-degrading genes are naturally high. Recent studies have shown that metagenome-guided sampling is not only scalable but also a cost-effective method for discovering beneficial microbes. Beyond identifying genes, metatranscriptomics and metaproteomics provide information on which genes and enzymes are actually active when microbes are exposed to dyes (Renganathan et al., 2025). When these tools are combined with metabolomics, scientists can directly link gene activity with the formation of detoxification products. This integrative approach is already proving effective in early pilot projects. CRISPR-based tools further expand the possibilities. They can be used in two main ways: (i) directly editing microbial genomes to improve dye-degradation pathways or stress tolerance, and (ii) performing functional screens in environmental microbial consortia to identify key genes responsible for degradation (Maqsood et al., 2024). While still emerging, protocols for applying CRISPR in environmental bacteria are now being developed, though their use requires strict biosafety measures and regulatory compliance (Piergentili et al., 2021). Additionally, synthetic biology can enhance the efficiency of enzymes. Techniques such as directed evolution and domain shuffling enable the development of laccases, azoreductases, or DyPs with improved stability under challenging conditions, including high salinity or extreme pH (Jaiswal and Jaiswal, 2024). These engineered enzymes can be immobilised and used as stable catalysts in wastewater treatment. Translational research channels that begin with metagenomic

discovery and end with recombinant enzyme testing are already described in recent studies, and their use is expected to grow (Robinson et al., 2021). However, while these molecular tools provide powerful discovery and engineering capabilities, they should not replace ecological and toxicity testing. Advanced sequencing and editing can show what is possible. However, the real-world safety and efficiency of detoxification must still be confirmed through metabolite tracking and bioassays before any field application. The way forward lies in integration, utilising molecular discovery in conjunction with ecological validation to develop reliable, safe, and effective dye bioremediation strategies (Chaturvedi et al., 2021).

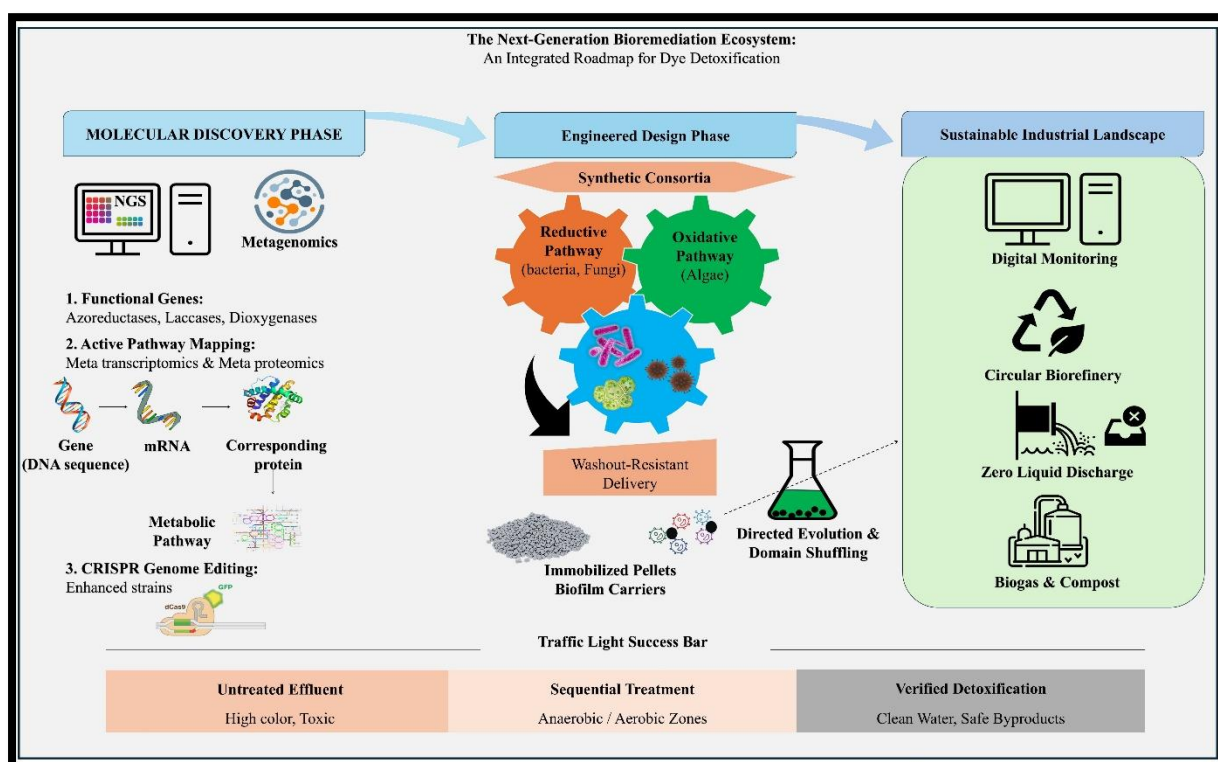


Figure 2.5: Schematic overview of an integrated, next-generation approach for synthetic dye bioremediation.

2.9.2 Development of microbial consortia or engineered strains with enhanced dye-degrading potential

Developing microbial consortia that combine different functions, such as reductive, oxidative, and ring-cleavage pathways, is proving to be a promising strategy for reliable dye detoxification. These consortia can be created by either bringing together complementary wild isolates or by engineering strains to express missing pathway steps (Mawad et al., 2020a). Recent studies show that consortia designed using metagenomic and metatranscriptomic data

are more stable and effective than randomly assembled mixes. In some cases, engineered strains can fill specific gaps, such as adding a stable dioxygenase gene into a salt-tolerant host (Thiruppathi et al., 2021). However, due to safety and regulatory concerns, most experts recommend using engineered enzymes in an immobilised form or in contained bioreactors, rather than releasing genetically modified microbes directly into the environment. Current pilot frameworks follow a precautionary approach, starting with the use of locally adapted, native strains whenever possible, and only considering engineering solutions when the benefits clearly outweigh the risks and containment is feasible (Mani and Hameed, 2019). Delivery formats for consortia are also improving. Options such as immobilised pellets, biofilm carriers, and seed inocula for constructed wetland media have been demonstrated to reduce washout, resist toxic shocks, and simplify large-scale inoculation (Mani and Hameed, 2019). Studies report that materials such as biochar, activated carbon, and polymer beads not only provide attachment sites for microbes but also adsorb toxic inhibitors, improving both stability and performance (Manikandan et al., 2023). Another promising idea is adaptive consortia microbial communities that can self-adjust under process conditions through natural ecological selection. Instead of adding foreign genes, engineers can design treatment processes that steer microbial evolution in desired directions, for example, by adjusting feed composition or using redox cycling (Thiruppathi et al., 2021; Manikandan et al., 2023). This offers a practical balance between engineered strains and entirely natural inocula. Importantly, development channels should include life-cycle analysis, risk assessment, and cost-benefit evaluation from the beginning. This involves combining techno-economic modelling, ecotoxicological testing, and regulatory mapping to ensure that promising microbial consortia are not only effective in the lab but also safe, scalable, and acceptable for field applications (Sadek et al., 2016).

2.9.3 Coupling phytoremediation and microbial remediation for synergistic effects

Plant-microbe partnerships in constructed wetlands (CWs) are increasingly recognised as powerful levers for textile dye remediation, as plants supply oxygen and root exudates that stimulate aerobic mineralisation. At the same time, microbes degrade and detoxify complex organic pollutants (Thakur et al., 2023). Recent pilots and reviews have consistently demonstrated that combining complementary macrophyte species with supportive rhizosphere consortia achieves superior colour removal (often >80-90 %) and toxicity reduction (70-85 % decline in phytotoxic markers) compared to using plants or microbes in isolation (Chaudhary et al., 2023). This synergy not only enhances pollutant removal but also improves biomass outcomes and increases system resilience against fluctuating loads. Rhizosphere engineering,

achieved by manipulating root exudation through careful plant selection or mild fertilisation, provides an additional means to steer microbial community composition toward detoxifying taxa (Kumar and Singh, 2025). For example, studies show that deep-rooted species such as *Phragmites australis* create extended oxygen microzones that support oxidative enzyme pathways, while surface-rooted species like *Eichhornia crassipes* favour reductive microbial metabolisms, thereby influencing the enzymatic dominance within CWs (Thakur et al., 2023). Such differences offer a practical operational handle for optimising treatment pathways. Furthermore, coupled plant–microbe systems enable cascading benefits. Phytoremediation lowers suspended solids and nutrient loads, thereby improving microbial enzyme accessibility to dissolved dyes. Meanwhile, microbial degradation eliminates toxic intermediates that could otherwise suppress plant growth, establishing a mutually supportive loop (Gayathiri et al., 2022). Pilot-scale CWs utilising this reciprocity have reported treatment efficiencies of up to 90 % for colour removal and 75 % for COD reduction in complex textile effluents. Integration with valorisation strategies, such as harvesting macrophyte biomass for biogas or composting (after confirming absence of toxic dye accumulation), can further improve economic feasibility and community acceptance. However, monitoring for dye or metabolite bioaccumulation in plant tissues remains essential to prevent unintended transfer into food chains, and this monitoring is now part of standard pilot protocols. (Ijoma et al., 2024).

2.9.4 Sustainable wastewater treatment solutions tailored for dye-intensive industries

The most promising near-term solutions for textile dye remediation are increasingly hybrid, combining source-control strategies, advanced treatment technologies, and nature-based systems to achieve both cost-effectiveness and regulatory compliance (Bhagwatwar and Wazalwar, 2025). Source control and process improvements, such as higher fixation chemistries, salt recovery, and closed-loop rinsing, significantly reduce the pollutant load entering treatment systems. Meanwhile, advanced oxidation processes (AOPs) provide effective pretreatment for highly recalcitrant dye fractions (Zadra Filho, 2024). These are followed by wetland-based biological polishing, which harnesses the capacity of locally adapted microbes and macrophytes to remove residual colour and toxicity. Recent studies converge on these multi-barrier approaches as the most realistic pathway for industries to meet tightening regulatory standards and water reuse goals, particularly in water-scarce textile hubs. Digital monitoring tools and simple automation, such as sensor-based flow regulation and automated adjustments of hydraulic retention times (HRTs), enhance the robustness of constructed wetlands (CWs) under fluctuating industrial loads (Holloway, 2023). Recent case

studies report that modest automation reduces bypass events by 30-40 % and supports healthier biological activity, directly improving treatment stability (Mani and Hameed, 2019). Circularity measures, including the reuse of treated water, recovery of dye-laden solids for safe disposal or valorisation, and biomass harvesting for bioenergy, further improve economic feasibility while lowering freshwater withdrawals, a factor of critical importance in water-stressed basins (Dadebo et al., 2025). Life-cycle assessments in the latest literature consistently emphasise that integrating circular pathways with biological treatment significantly improves sustainability metrics such as carbon footprint and energy efficiency (Ritesh Kumar et al., 2024). At the policy and finance level, innovations such as incentives for zero-liquid discharge (ZLD) retrofits, subsidies for pilot CW deployments, and public–private partnerships for shared effluent treatment plants (ETPs) have proven effective, with successful regional models demonstrating that regulatory pressure paired with cost-sharing mechanisms accelerates adoption (Ritesh Kumar et al., 2024). Embedding rigorous scientific monitoring, transparent reporting, and community benefit-sharing into these frameworks enhances legitimacy and uptake among industries and stakeholders. At the same time, significant progress has been achieved in microbial bioremediation of synthetic dyes, particularly through the isolation of diverse bacterial and fungal strains capable of degrading a broad spectrum of dyes, including azo, anthraquinone, and triphenylmethane classes (Thiruppathi et al., 2021).

Omics-based approaches have revealed novel catabolic genes and enzymatic pathways. At the same time, experimental trials consistently demonstrate that microbial treatments not only reduce dye concentrations but also mitigate dye-induced phytotoxicity, thereby improving germination rates and biomass recovery in model plants by 25-40 % (Sompark et al., 2024). Despite these advances, significant knowledge gaps remain. Research has disproportionately focused on industrial effluents and a limited set of ecosystems, leaving the microbial diversity of ecologically sensitive and unique wetlands, such as those of Jammu and Kashmir, largely uncharacterized (EI and OM, 2024). Moreover, integration of microbial degradation studies with systematic phytotoxicity evaluations under controlled conditions remains limited, restricting the ecological and translational relevance of existing findings (Gayathiri et al., 2022). Addressing these gaps is crucial to developing sustainable and ecosystem-specific strategies. High-altitude wetlands, with their microbial richness and environmental heterogeneity, represent a largely untapped reservoir of novel dye-degrading microorganisms that may possess unique metabolic and stress-resilience traits. A comprehensive approach combining microbial bioprospecting, molecular and phylogenetic characterisation, and

phytotoxicity assessment will provide new insights into both the ecological roles and potential applications of these microbial communities (Kumari et al., 2021). Against this backdrop, the present study aims to isolate and identify efficient dye-degrading microorganisms from wetlands in Jammu and Kashmir, characterise them using molecular and phylogenetic tools, and assess their role in mitigating dye-induced phytotoxic effects on plants. By linking microbial diversity studies with ecological function, this research aims not only to expand the understanding of high-altitude microbial ecosystems but also to establish their application in eco-friendly dye remediation, bridging fundamental science with real-world sustainability challenges (Mawad et al., 2020a).

2.10 Hypothesis of the study

In view of the research gaps identified in Section 1.8, particularly the limited information on native dye-degrading microorganisms from wetlands of Jammu and Kashmir, the insufficient characterization of degradation products, and the inadequate assessment of phytotoxicity following microbial treatment, the present study was undertaken to test whether indigenous wetland microorganisms possess significant dye-degrading and detoxification potential. The wetlands of Jammu and Kashmir are unique ecosystems characterized by high-altitude conditions, fluctuating physicochemical parameters, and increasing anthropogenic pressure, including contamination by synthetic dyes. These challenging environmental conditions may exert selective pressure on microbial communities, promoting the development of specialized metabolic capabilities that enable survival under stress. Such conditions provide a suitable habitat for the occurrence of microorganisms capable of degrading toxic dyes. Given the abundance of organic matter, diverse microbial populations, and ecological resilience of these wetlands, it was hypothesized that they harbour efficient dye-degrading microorganisms with robust enzymatic and metabolic potential. Furthermore, it was predicted that microbial activity would not only facilitate the breakdown of synthetic dyes but also reduce their phytotoxic effects on plants, thereby providing a sustainable and eco-friendly approach for the bioremediation of contaminated aquatic environments.

AIMS AND OBJECTIVES

An interrogative approach that combines microbial bioprospecting, molecular characterization, and phytotoxicity evaluation can provide new insights into effective bioremediation strategies. Therefore, the present study aims to isolate and identify efficient dye-degrading microorganisms from the wetlands of J&K, characterize them using molecular and phylogenetic tools, and evaluate their role in mitigating dye-induced phytotoxic effects on plants. This research will not only contribute to microbial diversity studies in high-altitude wetlands but also establish their potential application in eco-friendly dye remediation. The Objectives are as follows:

1. Isolation of dye degrading microorganisms from wetlands of J&K.
2. Identification and characterization of dye-degrading microorganisms.
3. Analysis of dye degradation and its study on vegetation.

MATERIAL AND METHODS

4. Materials and Methods

4.1 Chemicals and reagents used in the study

All chemicals used in this study were of molecular grade and of the highest purity. The synthetic azo dyes selected for experimentation were Methylene Blue, Congo Red, Allura Red, Orange II, Acid Red 88, and Methyl Red Sodium Salt, representing different functional groups of industrial dyes. These dyes were procured from HiMedia Pvt. Ltd., India, ensuring reliable quality for experimental use. Additional chemicals and reagents required for media preparation and analytical procedures were purchased from HiMedia Pvt. Ltd. and SRL Pvt. Ltd., both of which are recognized suppliers of high-grade laboratory materials.

4.2 Laboratory instruments and analytical equipment used in the study

All experiments were carried out using standard laboratory equipment. Dye decolorization was monitored using a UV-Visible spectrophotometer (Shimadzu UV-1800). Functional group changes in untreated and degraded dye samples were analyzed using a FTIR spectrometer (Perkin-Elmer spectrum). Degradation metabolites were identified using a GC-MS system (Shimadzu-GC-MS-FID). Bacterial cultures were incubated in an orbital incubator shaker (YSI-573 platelet incubator) at controlled temperature and agitation speed. Centrifugation of culture samples was performed using a refrigerated centrifuge (REMI). The pH of culture media and dye solutions was measured using a digital pH meter. Sterilization of media and glassware was carried out using a vertical autoclave (NSW-India). All microbial handling was conducted under aseptic conditions in a laminar airflow cabinet (LALCO scientific instruments). Accurate weighing of dyes and chemicals was performed using an analytical balance (Shimadzu AUX220, Shimadzu corporation, Japan). Phytotoxicity assays were conducted using *Triticum aestivum* and *Vigna radiata* seeds maintained under controlled conditions in a plant growth chamber (BINDER plant growth chamber).



**Perkin-Elmer
spectrum**



**Shimadzu-GC-MS-
FID**



**Remi-Cold
Centrifuge**



NSW-India



**YSI-573 platelet
incubator**



**Labfit
Incubator**



VeritiPro



**Shimadzu UV-Vis
1800**

Figure 4.1. Major laboratory instruments used in the present study: (A) FTIR spectrometer, (B) GC–MS system, (C) Cold Centrifuge, (D) Laminar, (E) Autoclave, (G) vertical autoclave, (H) Shaking Incubator, (I) Incubator, (J) PCR, (K) UV-Vis spectrophotometer.

4.3 Preparation of microbiological culture media

For strain isolation, Nutrient agar (NA) and Nutrient broth (NB) were used as the basal media to support the growth of mixed bacterial cultures. To screen for dye-degrading microbial strains, NB was prepared and supplemented with varying concentrations of the selected dyes. NB was chosen as it provides sufficient nutrients to support microbial growth while allowing evaluation of dye tolerance and degradation potential. The inclusion of different dye concentrations in the medium led to the assessment of bacterial adaptability and capacity for dye degradation. NA was used for routine cultivation and maintenance of pure isolates.

4.4 Description of wetland sampling sites

The sampling sites selected for this study included five ecologically significant wetlands of Jammu and Kashmir, each facing varying degrees of anthropogenic pressure. Surinsar Wetland, a freshwater lake located in the Jammu region at 32°46'N and 75°23'E, is surrounded by hills and natural vegetation and holds both ecological and cultural importance as part of the Surinsar–Mansar Lake complex, but it also receives surface runoff and untreated discharges from nearby settlements. Close to it lies Mansar Wetland at 32°46'N and 75°22'E, a

waterbody of religious and ecological value that serves as a freshwater resource for local communities but is increasingly threatened by wastewater inputs and human activities. Moving to the Kashmir Valley, Khushalsar Wetland at 34°107195'N and 74°803468237'E has become a shallow, ecologically degraded lake due to continuous urban effluents and anthropogenic encroachments, making it an important site for microbial and phytotoxicity assessments. In its proximity lies Gilsar Wetland at 34°120127'N and 74°803655'E, which was once historically interconnected with other lakes but now suffers from heavy siltation, sewage inflows, and high pollution loads, further justifying its inclusion in the sampling scheme. Finally, Nigeen Lake, located at 34°120508'N and 74°833269'E and often referred to as the “jewel of Srinagar,” remains one of the most picturesque lakes in the Kashmir Valley, surrounded by willow trees and houseboats, yet faces ecological sensitivity due to untreated urban discharges, making it equally valuable for sampling and analysis (Huang et al., 2022).

4.5 Sterilization of culture media and laboratory materials

All culture media and glassware used in microbiological experiments were sterilized by autoclaving at 121 °C under 15 psi pressure and 20 minutes to hold ensure complete elimination of microbial contaminants. Exceptions were made for heat-labile solutions such as urea stock solution, which was sterilized separately using a 0.22 µm membrane filter assembly under aseptic conditions to maintain its stability and sterility. The filtrate was stored at 4°C and protected from light until required for experimental use (Chantarasiri, 2020).

4.6 Collection and transportation of wetland samples

During the collection of soil and water samples from the five selected wetlands of Jammu and Kashmir, strict attention was given to safety protocols and a standard methodology to obtain representative samples (American Public Health Association, 1998; Chantarasiri, 2020). Before beginning sampling, each wetland was carefully surveyed to identify suitable and safe points for collection, as these sites receive untreated effluents and may pose health risks. Soil samples were collected using a sterile trowel and transferred into sterilized Ziplock bags, while water samples were collected in pre-sterilized one-litre screw-capped bottles. At each site, samples were taken from different locations to capture the spatial variation within the wetland ecosystem. Soil was collected from the deeper layers, as surface sediments are more likely to be contaminated by recent anthropogenic inputs, while deeper layers provide a more stable representation of microbial populations. Similarly, water samples were collected after rinsing the bottles with the site water to reduce contamination risks. Immediately after collection, the samples were labelled with the location and coordinates of the site and placed in insulated

containers to maintain a storage temperature of 4°C until further analysis. All collected samples were transported to the laboratory and processed within 24 hours of collection to ensure microbial viability and accuracy of subsequent analyses (Adil, 2020).

4.7 Physicochemical characterization of wetland water samples

The portable Beckmann thermometer was used to measure the temperature of the wetland water at the time of sampling. Measurements were taken at multiple points within each wetland to obtain a representative temperature profile (Barani et al., 2021). The pH of the samples was determined using a Labnox® digital pH meter, supplemented by pH strips for cross-verification. Electrical conductivity was measured using a Mettler toledo conductivity meter, while total dissolved solids (TDS) were determined gravimetrically as per standard procedures. For oxygen-related parameters, Dissolved Oxygen (DO) was measured using the Winkler titration method, while Biological Oxygen Demand (BOD) was determined by incubating the water samples for five days at 25 °C, followed by titration, in accordance with APHA protocols (American Public Health Association, 1998). Similarly, Chemical Oxygen Demand (COD) was estimated using the dichromate reflux method with potassium dichromate as the oxidizing agent (Abdullahi et al., 2021). All physicochemical analyses, including conductivity, oxygen demand, were performed following established protocols outlined by APHA and validated by previous studies (Bharti et al., 2024). These standardized procedures ensured the accuracy and reproducibility of results across all wetland samples.

4.7.1 Biological oxygen demand (BOD)

The biological oxygen demand (BOD) test was conducted to estimate the amount of dissolved oxygen consumed by microorganisms during aerobic degradation of organic matter. The standard BOD assay was performed at 25°C in the dark to minimize photosynthetic interference according to the method outlined in (Gallo and Aulitto, 2024). Effluent samples were first aerated for 24-48 hours to ensure proper oxygen saturation, and then two sets of 50 mL samples were transferred into sterile BOD bottles. The pH of the samples was adjusted using a phosphate buffer solution before analysis. One set was analyzed immediately (Day 0, D0) for dissolved oxygen (DO) content, while the other set was incubated at 25 °C for five days (Day 5, D5). DO was determined using the iodometric method, involving the sequential addition of 2 mL manganous sulphate (MnSO_4) solution and 2 mL alkali-iodide azide reagent, followed by thorough mixing. Formation of a brown precipitate indicated manganese oxide production. After settling, 2 mL concentrated H_2SO_4 was added to dissolve the precipitate, and the liberated iodine was titrated against 0.025 N sodium thiosulphate ($\text{Na}_2\text{S}_2\text{O}_3$) using starch as an indicator.

The endpoint was recorded when the solution changed from blue to colourless. The BOD value was calculated from the decrease in DO concentration between Day 0 and Day 5, using distilled water as a blank for control measurements (Cui et al., 2007; Abdullahi et al., 2021). The BOD of the water samples was calculated by using the formula:

$$DO \text{ (mg/L)} = (\text{Titration value} \times 0.025) \div (\text{Volume of sample}) \times 100$$

Here, 0.025 = Normality of sodium thiosulphate solution

$$BOD \text{ mg/L} = (D0 - D5 - BD) \times (\text{Volume of diluted sample} \div \text{Volume of sample})$$

Here, D0 = dissolved oxygen level on day 0

D5 = dissolved oxygen level on day 5

BD = Difference in dissolved oxygen levels between the zero and fifth days of a blank sample

Volume of diluted sample volume = 50 mL

Volume of the sample = 5 mL

3.7.2 Chemical oxygen demand (COD)

Chemical oxygen demand (COD) was determined to estimate the total oxygen requirement for the oxidation of organic and inorganic matter in the effluent according to the previously published (Gallo and Aulitto, 2024). A 0.1 N potassium dichromate ($K_2Cr_2O_7$) solution was prepared by dissolving 3.676 g in 1000 mL distilled water, while a 2 M sulfuric acid (H_2SO_4) solution was prepared by carefully adding 10.8 mL concentrated H_2SO_4 to 100 mL distilled water. Additional reagents included 10 % potassium iodide (KI), 1% starch indicator, and 0.1 M sodium thiosulphate ($Na_2S_2O_3$) solution prepared by dissolving 15.811 g in 1000 mL distilled water. For the COD test, 10 mL of effluent sample was mixed with 5 mL of 0.1 N potassium dichromate solution in an Erlenmeyer flask and incubated in a water bath at 100 ° for 1 hour. After cooling, 5 mL KI solution and 10 mL H_2SO_4 were added, followed by 1 mL starch solution just before titration. The liberated iodine was titrated against 0.1 M sodium thiosulphate until the blue color disappeared (Abdullahi et al., 2021). COD values were calculated from the difference in sodium thiosulphate volumes consumed between the effluent sample (VS) and blank control (VB), using the formula:

$$COD \text{ (mg/L)} = (VS - VB \times 0.1 \times 8 \div \text{Volume of the sample})$$

Here, VS = volume of sodium thiosulfate utilized for effluent sample.

VB = volume of sodium thiosulfate used in the blank sample

0.1M = Molarity of sodium thiosulfate solution

Volume of sample taken = 10 ml

4.8 Isolation and purification of microorganisms from wetland water and soil samples

The isolation of microorganisms from the wetland samples was carried out using the spread plate technique. For soil samples, 1 g of soil was suspended in 1 mL of sterile distilled water to prepare the initial suspension. Serial dilutions were then made by subsequently transferring 1 mL of the suspension into 9 mL of sterile distilled water, and this process was repeated stepwise until a 10^{-6} dilution was made. From each dilution, 100 μ L aliquots were placed on nutrient agar plates, spread evenly using a sterile glass spreader to allow microbial colonies to grow (de Vasconcelos et al., 2024). For water samples, wetland water was first filtered through a sterile membrane filter to concentrate the microbial population. The retained microorganisms on the filter were then resuspended in a sterile solution, and 100 μ L of this suspension was directly inoculated onto nutrient agar plates (prepared at 1.3 % agar concentration) and spread uniformly to ensure proper distribution of microbial cells. The inoculated plates were incubated at 37 °C for overnight, after which distinct colonies became visible. Each morphologically distinct bacterial colony was carefully picked and further purified through repeated streaking on fresh nutrient agar plates until pure, single-colony cultures were obtained. The purified plates were again incubated at 37 °C for overnight, and the growth pattern of colonies was examined to confirm the isolation of discrete strains. Following purification, representative colonies were transferred into nutrient broth and incubated under the same conditions to establish actively growing cultures for further experimental analysis and characterization (Khan and Malik, 2018).

4.9 Screening and selection of potent dye-degrading isolates

The screening of bacterial isolates for dye decolorization potential was carried out in two stages: primary screening using plate assay and secondary screening using broth assay.

4.9.1 Dye decolorization using plate assay

Primary screening for dye decolorization was carried out using the plate assay method (Anamica et al., 2014). Nutrient agar medium was prepared and sterilized at 121 °C for 15 minutes. After cooling to 45-50 °C, sterile dye was aseptically added to the molten medium to achieve a final concentration of 100 ppm for each dye tested. The dye-supplemented medium was thoroughly mixed to ensure uniform distribution and then poured into sterile petri plates under aseptic conditions. Once solidified, previously isolated bacterial colonies were streaked

individually onto the dye-containing plates. The inoculated plates were incubated at 37 °C for 24 h to allow bacterial growth and interaction with the dye (de Vasconcelos et al., 2024). Following incubation, plates were examined for zones of decolorization surrounding the colonies. Colonies capable of decolorizing the dye produced distinct white or clear zones against the colored agar background, whereas non-decolorizing isolates showed no such effect.

4.9.2 Dye decolorization using broth assay

Secondary screening of the dye-decolorizing potential of bacterial isolates was carried out using liquid culture assay (Anamica et al., 2014). For this, nutrient broth (NB) medium was prepared, autoclaved, and supplemented with the respective dyes to maintain a final concentration of 50 ppm. Each dye, Methylene Blue (MB), Congo Red (CR), Orange II (O-II), Allura Red (AR), Methyl Red Sodium Salt (MRSS), and Acid Red 88 (AR88) was tested individually (Al-Limoun et al., 2019).

4.9.3 Quantitative evaluation of dye decolorization efficiency by isolates

Quantitative dye decolorization was calculated using a liquid broth assay with all positive bacterial isolates. For the assay, nutrient broth was prepared, sterilized, and supplemented with the respective dyes to a final concentration of 5 mg per 100 mL. A 10 % (v/v) inoculum was prepared from overnight cultures of each isolate, and the optical density was adjusted to $OD_{600} = 0.88$ to ensure equal bacterial cell numbers in all experimental flasks. The cultures were then incubated at 37 °C with continuous static and shaking for 14 h. This incubation temperature was chosen based on preliminary screening results and previous studies reporting optimal growth and enzymatic activity of isolates under mesophilic conditions (Al-Limoun et al., 2019). Uninoculated flasks containing dye were maintained alongside the experimental sets to account for any abiotic changes. After incubation, the cultures were centrifuged and the supernatants were analyzed using a UV-Vis spectrophotometer to measure residual dye concentration at MB (665 nm), CR (498 nm), O-II (486 nm), AR (504 nm), MRSS (515 nm), and Acid Red 88 (505 nm) (de Vasconcelos et al., 2024). All experiments were carried out in triplicate to ensure reproducibility. Dye-containing broth without bacterial inoculation served as the negative control, while inoculated dye-free broth was used to correct for blank turbidity. Dye decolorization efficiency was calculated as a percentage using the following equation:

$$\%D = [A_i - A_t \div A_i] \times 100$$

where %D represents the percentage of decolorization, A_i is the initial absorbance, and A_t denotes the final absorbance of the dye solution.

4.9.4 Selection criteria for efficient dye-degrading isolates and dyes

Selection of efficient dye-degrading bacterial isolates was carried out based on quantitative decolorization data obtained from UV–Vis spectrophotometric analysis. The percentage decolorization for each isolate against different dyes under static and shaking conditions was calculated using the standard formula (section 4.10.3). A cut-off criterion of $\geq 80\%$ decolorization efficiency was applied to identify potential high-performing isolates. Isolates meeting or exceeding this threshold were considered efficient and selected for further experimental studies. Similarly, dyes for detailed degradation studies were selected based on their decolorization efficiency. A higher threshold of $\geq 90\%$ decolorization was used to identify dyes showing consistent and significant removal by the selected isolates. Only those dyes that satisfied this criterion were chosen for subsequent biodegradation and analysis experiments (Chakravarthi et al., 2021; Anjali et al., 2022).

4.10 Morphological, biochemical, and molecular characterization of isolates

The bacterial isolates obtained from soil and water samples of wetlands were subjected to a series of morphological, staining, and microscopic analyses to aid in their preliminary identification. These characteristics provided the first level of classification before biochemical and molecular assays were performed.

4.10.1 Morphological characteristics

Morphological features are an important criterion for differentiating bacterial species. The isolates were examined both at the colony level (macroscopic features) and at the cellular level (microscopic features).

4.10.2 Colony morphology

Colony morphology was studied on nutrient agar plates after 24–48 hours of incubation at 37°C. Each distinct isolate was carefully observed for the following parameters:

- Size (small, medium, large)
- Shape (circular, irregular, filamentous, rhizoid)
- Surface (smooth, rough, wrinkled, mucoid)
- Pigmentation (color of colony or diffusible pigments in agar)

These characteristics were systematically recorded to generate a colony profile for each isolate.

4.10.3 Cell morphology

The microscopic characteristics of bacterial isolates were studied using staining techniques to determine the size, shape, and cellular arrangement. Cell morphology included classification as cocci, bacilli, coccobacilli, or spirilla, with further observations on whether the cells occurred singly, in chains, clusters, or pairs.

4.10.4 Gram staining

Gram staining was performed according to the previously reported protocol (Kumar et al., 2019). A thin smear of bacterial culture was first prepared on a clean glass slide, air dried, and heat-fixed to ensure that the cells adhered firmly to the slide surface. The smear was then stained with crystal violet for one minute and gently rinsed with distilled water to remove excess dye. Following this, the slide was flooded with gram's iodine, which acts as a mordant, for one minute and again washed with water. Decolorization was carried out using 95 % ethanol for 20-30 seconds, a crucial step that differentiates bacteria based on the structural composition of their cell walls. After decolorization, the slide was counterstained with safranin for one minute, rinsed thoroughly with water, and left to air dry. The prepared slide was finally examined under a bright-field microscope using an oil immersion lens (100 X objective) for clear visualization of cell morphology and gram reaction. Bacteria that retained the crystal violet-iodine complex due to the presence of a thick peptidoglycan layer in their cell walls appeared purple and were classified as gram-positive. In contrast, bacteria that lost the primary stain during decolorization and subsequently absorbed the counterstain safranin appeared pink or red and were identified as gram-negative. This distinction provided a fundamental basis for the preliminary classification of the isolated dye-decolorizing bacteria (Adil, 2020).

4.10.5 Biochemical characterization

Biochemical tests were performed to characterize the bacterial isolates based on their metabolic properties and enzymatic activities. These tests provided a rapid and cost-effective approach to differentiate the isolates by evaluating their ability to utilize specific substrates and produce characteristic metabolic byproducts. For this purpose, the Hi Assorted™ biochemical test kits KB002 and KB001 (HiMedia, India) were employed, and the assays were carried out strictly according to the manufacturer's instructions. The panel of biochemical tests included citrate utilization, lysine utilization, urease activity, nitrate reduction, and various sugar assimilation assays to determine the metabolic potential of the isolates. Carbohydrate fermentation tests were also performed to assess the ability of isolates to metabolize different sugars with or without the production of gas and acid. Enzyme activity tests, such as urease and nitrate

reductase assays, provided additional insights into their physiological and ecological roles (Gałązka et al., 2023).

4.11 Genomic DNA extraction and molecular characterization of selected isolates

4.11.1 DNA extraction protocol

The genomic DNA was extracted from the overnight grow cultures of the strains according to the previously reported protocol (Adil, 2020). Briefly, 1.5 mL overnight-grown culture was taken and 30 µL of 10 % SDS was added and mixed well. Next, 3 µL of proteinase K (20 mg/mL), 3 µL RNase (20 µg/mL), 100 µL of 5M NaCl, 80 µL of CTAB/NaCl were added and incubated at 37 °C for 60 minutes. After incubation an equal volume of phenol/chloroform: iso-amyl alcohol (25:24:1), was added, mixed and centrifuged for 10 minutes at 10,000 rpm. Pipetted supernatant into a fresh tube. Added equal volume of chloroform: iso-amyl alcohol and thoroughly, centrifuged at 10000 rpm for 10 minutes. Precipitated the DNA by adding 1/10 vols of 5M NaCl and 2 vols of 100 % chilled ethanol. The tubes were incubated at -20°C for 60 minutes. Centrifuged at 12,000 rpm for 5 minutes and the supernatant was removed. The pellet was washed by adding 100 µL of 70 % ethanol, followed by centrifugation as 12,000 rpm for 5 minutes. The pellet was air dried and suspended in 100 µL of TE and stored at -20 °C for further use (Adil, 2020).

4.11.2 Agarose gel electrophoresis of genomic DNA

Agarose gel electrophoresis was performed to analyze and confirm the integrity of DNA samples obtained from bacterial isolates. A 1 % agarose gel was prepared by dissolving 1 g of agarose powder in 100 mL of 1X Tris-Acetate-EDTA (TAE) buffer. The mixture was heated in a microwave oven until the agarose was completely melted and a clear solution was obtained. After cooling the solution to approximately 45-50 °C, ethidium bromide (EtBr) at a final concentration of 0.5 µg/mL was added as an intercalating dye to allow visualization of DNA under UV light. The molten agarose solution was poured into a casting tray fitted with a comb to form wells and was allowed to solidify at room temperature. Once solidified, the comb was carefully removed, and the gel was placed in an electrophoresis tank filled with 1X TAE buffer until the gel was completely submerged. DNA samples were mixed with 6X loading dye to increase sample density and facilitate visualization of migration during electrophoresis. The prepared samples, along with a 100 bp DNA ladder (HiMedia, India) as a molecular size marker, were carefully loaded into the wells using a micropipette. Electrophoresis was carried out at a constant voltage of 80-100 V for 45-60 minutes, depending on the size of the DNA fragments. The progress of electrophoresis was monitored by the migration of the tracking dye

towards the positive electrode. After completion, the gel was placed on a UV transilluminator, and DNA bands were visualized and documented using a gel documentation system (NuGenus SYNGENE). The presence of sharp, distinct bands indicated intact DNA, whereas smearing suggested degradation (Adil, 2020).

4.11.3 Quantification of extracted DNA

The isolated high molecular weight DNA was checked for yield and purity based on the A_{260} value and absorbance ratio $A_{260/280}$ (DNA/Protein) spectrophotometrically (Adil, 2020). The purity of extracted DNA with respect to v high ratio was an indicative of the purity with respect to the protein contamination. The DNA concentration was calculated using the formula:

$$ds\ DNA\ concentration = 50\mu g/mL \times OD260 \times Dilution\ factor$$

4.11.4 PCR amplification

For the amplification of DNA isolated from the isolates is done by using the universal pair of primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') for the sequencing. Each PCR reaction mixture, with a total volume of 20 μ L per reaction, comprised 2 μ L of DNA sample, 2 μ L of Forward primer (0.05 μ M), 2 μ L of Reverse primer (0.05 μ M), 4 μ L master mix, and 10 μ L of distilled water. PCR amplification began with the initial denaturation at 94°C for 5 minutes then denaturation at 94°C for 45 seconds followed by annealing temperature at 57°C for 45 seconds and elongation at 72°C for 1 minutes and final extension at 72 °C for 5 minutes (Kumar et al., 2019).

4.11.5 Electrophoresis and determination of PCR product

The reaction mixtures were stored at 4°C for future use. After PCR, 5 μ L of the product was loaded onto a 1.8 % agarose gel to confirm amplification. The amplified sample was then sent to Gene explore -Diagnostics & Research Centre Pvt.Ltd, Ahmedabad for sequencing (Kumar et al., 2019).

4.12 Phylogenetic analysis and sequence identification of selected isolates

The purified PCR products were sequenced bidirectionally at Gene explore -Diagnostics & Research Centre Pvt.Ltd, Ahmedabad, using a 3500 Genetic Analyzer (Applied Biosystems, USA). The obtained sequences were processed and analyzed using the BLASTn algorithm

available at the NCBI GenBank database to determine the closest phylogenetic affiliations. Multiple sequence alignments and phylogenetic tree construction were performed using MEGA 11 software, enabling evaluation of evolutionary relationships among the isolates (Adesioye et al., 2016; Rima et al., 2022). The molecular data provided species-level resolution and validated the morphological and biochemical findings, ensuring accurate identification of the bacterial isolates capable of dye degradation.

4.12.1 Submission of the sequence to the GenBank

The nucleotide sequences obtained from the 16S rRNA gene amplification of the selected bacterial isolates were submitted to the National Center for Biotechnology Information (NCBI) GenBank database for accession number assignment.

4.13 Antibiotic susceptibility profiling of selected bacterial isolates

To evaluate the antibiotic resistance profile of bacterial isolates SC4, MC2, KC2 and NC2, antibiotic susceptibility testing was performed using the standard disc diffusion method on nutrient agar plates (Salam et al., 2023). Freshly cultured bacterial colonies were adjusted to 0.5 McFarland turbidity standard and evenly spread on the agar surface. Commercially available (Himedia) antibiotic discs representing commonly used antibiotics (e.g., ampicillin, ciprofloxacin, tetracycline, gentamicin) were placed on the inoculated plates, followed by incubation at 37°C for 24 h. After incubation, zones of inhibition around the discs were measured in centimetres and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The results revealed varying degrees of susceptibility and resistance among the isolates, indicating the presence of both sensitive and multidrug-resistant strains. These findings provide valuable insights into the antimicrobial resistance patterns of environmental bacterial isolates and help in assessing potential public health risks (Salam et al., 2023).

4.14 Preliminary compatibility testing of bacterial isolates for consortium development

Before developing the microbial consortium, it was essential to assess the compatibility of the selected bacterial isolates. To determine whether the isolates could grow in proximity without inhibiting each other, cross-streaking was performed on a single nutrient agar plate in accordance with the previously reported protocol (Mani and Hameed, 2019). In this method, each isolate was streaked in separate sectors on the same plate, ensuring overlapping zones where interactions could be observed. The plates were incubated under optimal conditions

temperature 37°C and incubation time 24 h, and growth patterns were carefully monitored (Sari, 2018).

4.15 Effect of initial dye concentration on bacterial decolorization efficiency

The effect of initial dye concentration on the decolorization efficiency of bacterial isolates SC4, MC2, KC2, and NC2 was evaluated in triplicates using AR88 under optimized conditions (pH 7.5, temp 37 °C, and incubation time of 14 h). Different concentrations of AR88 viz, 50, 100, 200, and 300 ppm were prepared in sterile nutrient broth. Each flask containing 100 mL of dye solution was inoculated with actively growing cultures of the respective bacterial isolates. Uninoculated flasks containing dye served as controls. All experimental flasks were incubated at 37 °C under static conditions for 14 h. After incubation, samples were collected and centrifuged at 10,000 rpm for 3 minutes to separate the bacterial biomass. The clear supernatant was analysed by UV-Vis spectrophotometric analysis. The residual dye concentration was determined by measuring the absorbance at the maximum wavelength λ_{max} of AR88 at 505 nm. The percentage decolorization was calculated using the following formula in section 4.10.3.

4.16 Optimization of culture conditions for Acid Red 88 degradation

4.16.1 Statistical optimization and experimental design

To optimize the dye degradation performance of the bacterial isolate, RSM was applied using the box-behnken design (BBD). All experiments were conducted under aseptic conditions in 250 mL Erlenmeyer flasks, with continuous incubation at controlled temperatures. The selection of parameters for BBD was based on their preliminary influence on dye degradation efficiency. In this study, three independent variables pH, temperature, and incubation time were evaluated to determine their individual and interactive effects on the degradation of azo dyes. Each parameter was assessed at three coded levels (-1, 0, +1), as presented in (Table 1). The BBD experimental matrix consisted of 17 experimental runs, including 12 factorial points and 5 center point replicates, to ensure adequate estimation of experimental error and reproducibility of the model. Dye degradation percentage served as the primary response variable and was quantified spectrophotometrically after incubation under the specified conditions. The experimental design enabled systematic evaluation of the combined effects of the variables and facilitated the development of an empirical model for predicting optimal degradation conditions (Manjarrez-Paba et al., 2025).

4.16.2 Statistical modelling and analysis

Based on preliminary concentration-dependent decolorization findings, a dye concentration of 100 ppm was selected as the working concentration for RSM optimization, as it exhibited efficient, stable, and reproducible decolorization while remaining representative of typical textile effluent conditions. The experimental data generated using the BBD were then fitted to a second-order polynomial equation to describe the relationship between the selected independent variables and the dye degradation response. The mathematical model is expressed as:

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \beta_{ij} x_i x_j$$

where Y represents the predicted dye degradation percentage, β_0 denotes the constant term, β_i the linear coefficients, β_{ii} the quadratic coefficients, and β_{ij} the interaction coefficients, while X_i and X_j represent the coded independent variables.

Model construction, regression analysis, and generation of response surface and contour plots were performed using Design-Expert® Version XII. Model adequacy was evaluated by ANOVA, considering the coefficient of determination (R^2), F-values, p-values, and lack-of-fit. Terms with $p < 0.05$ were considered statistically significant. The optimized conditions for pH, temperature, and incubation time were determined based on the maximum predicted degradation response, and model validity was confirmed through diagnostic plots and close agreement between experimental and predicted values.

Table 4.1: Variable level affecting degradation of azo dyes AR88

Parameters	-1 (low value)	+1 (high value)
pH	5	10
Temperature °C	25	45
Incubation Time	1	24

4.17 Analysis and characterization of dye degradation products

4.17.1 FT-IR analysis

Structural modification in dye molecules before and after microbial treatment was analysed through FTIR spectroscopy using the previously reported protocol (Bankole et al., 2018). The dye solution, prepared at a uniform concentration, was incubated with the selected bacterial isolates to facilitate the decolorization process. After treatment, the samples were centrifuged

at 4500 rpm for 15 min to remove biomass, and the resulting supernatant containing degradation metabolites was collected. The supernatant was then dried overnight in a hot air oven at 50 °C, forming a thick, gum-like residue. This residue was carefully placed onto a thin film for direct FT-IR spectral acquisition. The FT-IR spectra were recorded using a PerkinElmer Spectrum FT-IR spectrophotometer, scanning the spectral region of 400 - 4000 cm⁻¹ with 16 scans per sample (Bankole et al., 2018; Rima et al., 2022). Comparative analysis of the FT-IR spectra before and after bacterial treatment revealed the functional group alterations, thereby confirming the breakdown of dye chromophoric structures and the formation of new degradation intermediates. FT-IR spectra were recorded for untreated dye samples (controls) and bacterially treated samples, and each analysis was performed in triplicate to ensure spectral reproducibility (Bankole et al., 2018).

4.17.2 GC-MS analysis

Analysis of the degradation of AR88 dye involved several critical steps, including sample preparation, GC-MS analysis, and data interpretation (Bankole et al., 2018). The degraded dye solution, obtained after microbial treatment, was centrifuged at 10,000 rpm for 10 minutes to separate the bacterial biomass. The resulting supernatant was filtered using a 0.22 µm membrane filter, and the metabolites were extracted with ethyl acetate (1:1 v/v). The organic phase was dried over anhydrous sodium sulfate, evaporated under reduced pressure, and reconstituted in methanol prior to GC-MS analysis. GC-MS analysis was performed using a Shimadzu GCMS-TQ8040 NX system equipped with a DB-5MS column (30 m × 0.25 mm × 0.25 µm). The injection was carried out in split less mode at an injection temperature of 280°C, with helium as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature program was set as follows: an initial temperature of 50°C held for 4 minutes, then ramped at 10 °C /min to 280°C (0 min hold), and further increased at 10 °C/min to 290 °C with a final hold of 7 mints. The total flow was maintained at 14.0 mL/min with a purge flow of 3.0 mL/min and a split ratio of 10:1. The ion source temperature was maintained at 240 °C, and the interface temperature at 300 °C. A solvent cut time of 4.00 minutes was applied to prevent solvent interference. Mass spectrometric data were acquired in Q3 scan mode with a scan speed of 3333, covering an m/z range starting from 45. The data acquisition started at 4.00 minutes and ended at 35.00 minutes. The acquired spectra were analyzed using the NIST and Wiley spectral libraries for the identification of degradation products, based on retention times, molecular masses, and characteristic fragmentation patterns (Anil Kumar et al., 2015).

4.18 Phytotoxicity assessment of untreated and degraded dye samples

The phytotoxicity assay was performed to evaluate the possible toxic effects of the degraded dye metabolites on seed germination and early seedling growth parameters of selected crop plants wheat (*Triticum aestivum* L.), and mung bean (*Vigna radiata* L.)(Nandi et al., 2019). The study aimed to assess whether the biodegradation process reduced the toxicity of the dye compared to the untreated control. Healthy and uniform seeds of each plant species were carefully selected and pooled surface sterilized using 1% sodium hypochlorite solution for 2-3 minutes, followed by thorough rinsing with sterile distilled water to remove any residual disinfectant. Three treatments were prepared for each plant species: control (sterile distilled water), untreated dye solution, and degraded dye solution obtained after microbial degradation and subsequent filtration. For the germination test, twelve surface-sterilized seeds were placed in sterile petri plates lined with Whatman No. 1 filter paper moistened with 0.5 mL of the respective test solution (Haq and Raj, 2018b). The plates were incubated in a growth chamber maintained at 25 ± 2 °C under dark conditions for the first 48 hours to initiate germination, followed by a 12-hour light/dark photoperiod for seven days. The filter papers were moistened daily with the corresponding test solution to maintain adequate moisture levels. Each treatment was performed in triplicate to ensure accuracy and reproducibility. After three days of incubation, germination percentage, root length, shoot length, and seedling vigor index were determined. Germination percentage was calculated as:

$$G_P(\%) = [TN_s \div TG_s] \times 100$$

G_P = Germination percentage

TN_S = Total number of seeds in control (without treatment)

TG_S = Total number of seeds germinate after treatment.

Phytotoxicity percentage was calculated using the formula:

$$PT(\%) = [(C - T) \div C] \times 100$$

where C and T represent the mean lengths of control and treated seedlings, respectively. All data were expressed as mean $\pm$ standard deviation (SD) of three replicates. Statistical analysis was carried out using one-way ANOVA followed by duncan's multiple range test (DMRT) to determine significant differences between treatments ($p < 0.05$). The comparative analysis of

seed germination and seedling growth among control, untreated, and degraded dye treatments was used to assess the reduction in phytotoxic effects after microbial degradation of Acid Red 88. Enhanced germination rate and seedling growth in the degraded dye treatments compared to untreated dye indicated the detoxification and reduced toxicity of the degradation products.

RESULTS AND DISCUSSION**Results and discussion****5.1 Sample site**

The sampling sites selected for this study were located across important wetlands of Jammu and Kashmir, India (Figure 5.1). These wetlands were chosen because they receive untreated dye effluents and other pollutants from surrounding areas. The selected sites included Surinsar and Mansar wetlands in the Jammu region and Khushal Sar, Gil Sar, and Nigeen Lake in the Kashmir region (Benny and Chakraborty, 2024). These wetlands are valuable natural ecosystems that support aquatic life but have become polluted due to increasing human activities such as waste dumping, sewage inflow, and tourism (Dar et al., 2021). Surinsar Wetland, located at 32°46'1"N and 75°23'7"E, lies in the foothills of the Shivalik range surrounded by green forests and agricultural fields. The temperature recorded at the time of sample collection was 24.3°C at 9:30 AM. Mansar Wetland, situated at 32°46'2"N and 75°22'0"E, is one of the largest freshwater bodies in the Jammu region. It is surrounded by hilly terrain and lush vegetation and serves as a popular tourist and religious site (Dar et al., 2021). However, it faces pollution from nearby human settlements. The temperature during sampling was 23.4°C at 10:00 AM. In the Kashmir region, Khushal Sar and Gil Sar are two interconnected urban wetlands located in Srinagar city. Khushal Sar is located at 34°10'7195"N and 74°80'3468"E, while Gil Sar is at 34°12'0127"N and 74°80'3655"E. Both wetlands are heavily affected by untreated sewage, solid waste, and encroachments from surrounding residential areas. The temperature recorded during sampling ranged between 25.6°C and 25.7°C. Nigeen Lake, located at 34°12'0508"N and 74°83'3269"E, is another major freshwater lake in Srinagar connected to Dal Lake through a narrow channel. Although famous for its scenic beauty, it also receives domestic effluents from nearby households, leading to gradual water quality deterioration. The temperature at this site was 25.8°C at 12:00 PM during sampling.

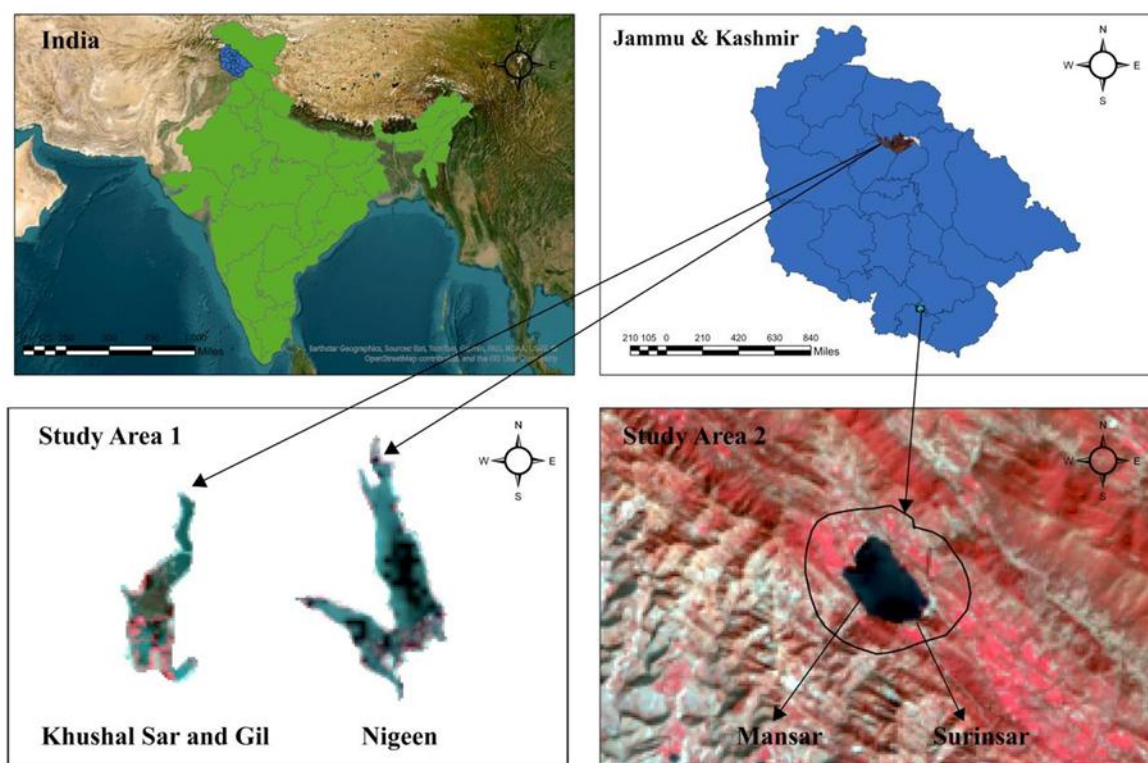


Figure 5.1: Maps showing the geographical location of the wetlands of J&K (Surinsar, Mansar, Khushal Sar, Gilsar & Nigeen Lake)

5.2 Sample collection

Soil and water samples were collected from all selected wetlands following proper safety measures and standard collection procedures to avoid contamination (Figure 5.2 & 5.3). Sampling was carried out mainly from areas where the presence of dye effluents or waste materials was visible, as these sites were expected to contain microorganisms capable of tolerating or degrading pollutants. Before starting the collection, all tools and containers were properly sterilized according to the methods described by the (American Public Health Association, 1998). Soil samples were collected with sterile spatulas from the upper 5-10 cm layer of soil at different points around each wetland. Approximately 100 grams of soil from each site was placed in sterile ziplock bags, which were labelled with the site name, date, and time of collection. Water samples were collected in one-litre sterilized plastic bottles with tight-fitting caps from areas where effluent discharge was observed. The water was collected from the middle depth to avoid floating debris and to obtain representative samples. After collection, all the samples were immediately placed in insulated iceboxes and transported to the laboratory. The samples were stored at 4°C to prevent any change in microbial composition or activity

before analysis (Anamica et al., 2014). Observations such as water colour, odour, and the presence of waste were also recorded at each site. The processing of samples was started within 24 hours of collection to ensure that microbial viability and activity were maintained, following the guidelines of (DeBritto et al., 2020).

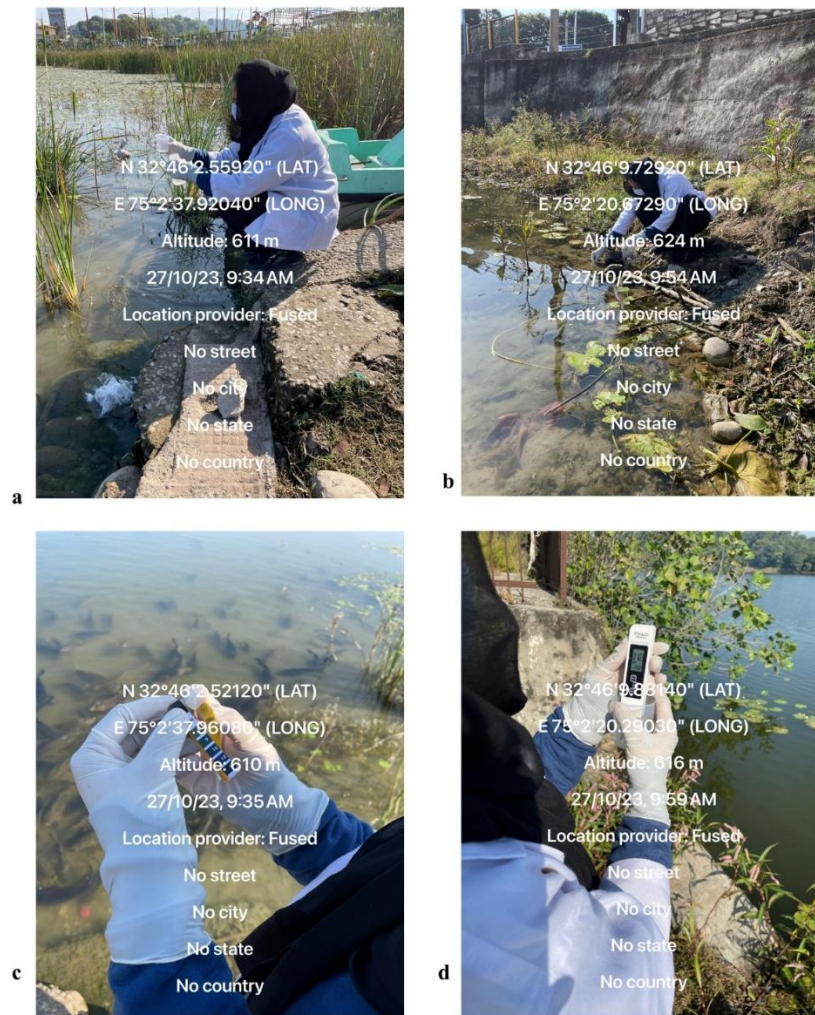


Figure 5.2: Sampling sites and sample collection at wetlands of Jammu: (a, b) Collection of water and soil samples from Surinsar Lake and Mansar Lake, respectively, showing representative sampling sites along the wetland margins. (c, d) On-site measurement of pH and temperature of water samples using a portable digital meter at (c) Surinsar wetland and (d) Mansar wetland during field sampling.



Figure 5.3: Sampling sites and sample collection at wetlands of Kashmir:(a-c) Representative views of (a) Khushal Sar, (b) Gilsar, and (c)Nigeen Lake showing the general condition of the wetlands at the time of sampling. (d-f) Collection of water and soil samples from (d)Khushal Sar, (e)Gilsar, and (f)Nigeen Lake carried out under aseptic conditions during field sampling.

5.3 Physicochemical analysis of water samples

During the sampling process, pH and temperature of the water at each site were measured on-site, while BOD, COD, DO, EC, and TDS were analyzed in the laboratory following standard

protocols recommended by (American Public Health Association, 1998) . The physicochemical characteristics of water samples collected from different wetlands of Jammu and Kashmir showed noticeable altitudinal variation (Table 5.1). The pH values across the studied wetlands ranged from 6.53 to 9.50, indicating that most water bodies were slightly to moderately alkaline in nature. Among all sites, Khushal Sar exhibited the highest pH values 8.80-9.50, suggesting elevated biological activity and higher organic load, whereas Nigeen Lake recorded the lowest pH 6.53-6.80, indicating slightly acidic to near-neutral conditions. Surinsar and Mansar lakes showed moderately alkaline pH values ranging between 7.33-8.50, while Gilsar maintained a consistently alkaline pH 8.23-8.80, reflecting stable carbonate buffering systems typical of freshwater ecosystems (Thakur et al., 2023). Water temperature showed relatively minor variation across all wetlands, ranging from 22.57°C to 25.90°C. The lowest temperatures were recorded at Mansar 22.57-24.57°C and Surinsar 23.50-25.73°C, whereas slightly higher temperatures were observed in Khushal Sar and Gilsar. Such narrow variation indicates relatively stable climatic conditions during sampling, which are favourable for sustaining microbial metabolic activity (Stanić et al., 2025). BOD values ranged from 3.15-5.50 mg/L, indicating varying levels of biodegradable organic matter. The highest BOD values were observed in Khushal Sar 5.20-5.50 mg/L, suggesting higher organic pollution, possibly due to sewage inflow and anthropogenic activities (Wang et al., 2025). In contrast, Nigeen Lake showed the lowest BOD values 3.15-3.67 mg/L, followed by Surinsar and Gilsar, indicating comparatively lower organic load. COD values varied between 55.17-68.29 mg/L, demonstrating the presence of both biodegradable and non-biodegradable oxidizable substances (Abdullahi et al., 2021). Maximum COD was recorded at Surinsar up to 68.29 mg/L and Khushal Sar 66.10-67.00 mg/L, reflecting the presence of both biodegradable and non-biodegradable oxidizable substances (Zhu et al., 2022). Lower COD values were observed in Nigeen Lake 54.33-57.33 mg/L, suggesting relatively better water quality compared to other wetlands. DO concentration ranged from 4.47 -7.60 mg/L. Khushal Sar exhibited the highest DO values 7.40-7.60 mg/L, possibly due to enhanced photosynthetic activity, while Nigeen Lake showed the lowest DO levels 4.47-4.57 mg/L, which may be attributed to oxygen depletion caused by organic matter decomposition (Chantarasiri, 2020). Surinsar, Mansar, and Gilsar showed moderate DO levels, indicating balanced oxygen conditions (Edori and Edori, 2021). The EC values varied widely from 198.03-396.00 $\mu\text{S}/\text{cm}$, indicating differences in ionic composition among wetlands. Khushal Sar recorded the highest EC values 393.17-396.00 $\mu\text{S}/\text{cm}$, followed by Gilsar 363.33-365.00 $\mu\text{S}/\text{cm}$, reflecting higher concentrations of dissolved ions. In contrast, Surinsar and Mansar showed comparatively lower EC values 198-216 $\mu\text{S}/\text{cm}$,

suggesting lower ionic strength (Yadav et al., 2025). Similarly, TDS ranged from 95.33-334.00 ppm. The highest TDS values were recorded in Khushal Sar 332-334 ppm, followed by Gilsar 184-187 ppm, indicating higher levels of dissolved salts and minerals. Surinsar, Mansar, and Nigeen Lake exhibited lower TDS values 95-108 ppm, reflecting comparatively lesser mineral and contaminant load. Overall, the results indicate that Khushal Sar and Gilsar wetlands exhibited higher BOD, COD, EC, and TDS values, suggesting greater pollution stress and anthropogenic influence (Abdullahi et al., 2021). In contrast, Surinsar, Mansar, and Nigeen Lake showed relatively lower pollution levels. These variations highlight the impact of sewage discharge, domestic waste input, and human activities on the water quality of the wetlands across Jammu and Kashmir. Recent investigations have shown that urban wetlands receiving domestic sewage generally exhibit increased organic load and ionic enrichment, but the simultaneous occurrence of high organic pollution and alkaline pH observed in the present study suggests intensified eutrophic conditions (Dar et al., 2022). Unlike many temperate wetlands where high organic loading is associated with reduced dissolved oxygen, Khushal Sar maintained relatively high DO values, indicating that photosynthetic oxygen production may temporarily offset oxygen depletion during daylight sampling. This combination of eutrophication and sustained microbial activity provides a favourable ecological niche for the enrichment of pollutant-tolerant microorganisms and supports the rationale for exploring these wetlands as reservoirs of dye-degrading bacteria (Zhang et al., 2026).

Table 5.1: Physicochemical characteristics of water samples collected from different wetlands of Jammu and Kashmir.

Wetland	Sample Code	pH	Temperature (°C)	BOD (mg L ⁻¹)	COD (mg L ⁻¹)	DO (mg L ⁻¹),	EC (μS cm ⁻¹)	TDS (ppm)
Surinsar	SW1	7.73 ± 0.07	25.73 ± 0.10	3.60 ± 0.2	55.17 ± 0.94	5.59 ± 0.12	198.10 ± 0.72	98.03 ± 0.72
	SW2	7.33 ± 0.14	24.27 ± 0.24	3.51 ± 0.10	64.07 ± 0.19	5.40 ± 0.07	198.03 ± 0.47	96.00 ± 0.47
	SW3	7.96 ± 0.12	23.73 ± 0.10	4.21 ± 0.10	68.29 ± 0.72	6.40 ± 0.14	216 ± 0.89	108.03 ± 0.54
	SW4	7.40 ± 0.19	23.50 ± 0.14	3.80 ± 0.8	60.10 ± 0.54	5.86 ± 0.14	214.03 ± 0.72	102.97 ± 0.54
Mansar	MW1	8.40 ± 0.07	24.57 ± 0.07	4.30 ± 0.00	65.00 ± 0.03	6.30 ± 0.00	210.00 ± 0.05	103.00 ± 0.03
	MW2	8.30 ± 0.07	22.63 ± 0.12	4.40 ± 0.00	65.00 ± 0.03	6.40 ± 0.00	209.33 ± 0.07	104.00 ± 0.05
	MW3	8.50 ± 0.06	25.37 ± 0.07	4.20 ± 0.01	65.17 ± 0.00	6.30 ± 0.00	207.29 ± 0.00	102.00 ± 0.03
	MW4	8.20 ± 0.12	22.57 ± 0.09	4.40 ± 0.01	65.17 ± 0.05	6.20 ± 0.11	209.33 ± 0.03	103.00 ± 0.03
Khushal Sar	KW1	8.80 ± 0.05	25.50 ± 0.19	5.30 ± 0.05	66.10 ± 0.47	7.50 ± 0.05	394.00 ± 0.47	334.00 ± 0.47
	KW2	9.20 ± 0.05	25.30 ± 0.26	5.20 ± 0.05	67.00 ± 0.47	7.40 ± 0.05	393.17 ± 0.54	332.00 ± 0.47
	KW3	9.10 ± 0.05	25.90 ± 0.27	5.40 ± 0.05	66.10 ± 0.49	7.50 ± 0.05	396.00 ± 0.69	334.00 ± 0.47
	KW4	9.50 ± 0.05	25.80 ± 0.23	5.50 ± 0.05	66.53 ± 0.49	7.60 ± 0.05	395.00 ± 0.54	333.83 ± 0.47
Gilsar	GW1	8.40 ± 0.05	25.47 ± 0.09	3.64 ± 0.05	63.67 ± 0.05	5.51 ± 0.05	363.33 ± 0.47	184.33 ± 0.47
	GW2	8.80 ± 0.05	25.47 ± 0.12	3.67 ± 0.05	65.00 ± 0.47	5.57 ± 0.05	365.00 ± 0.14	186.67 ± 0.47

	GW3	8.23 ± 0.05	25.40 ± 0.05	3.50 ± 0.05	62.67 ± 0.29	5.40 ± 0.05	364.33 ± 0.47	184.67 ± 0.47
	GW4	8.47 ± 0.05	25.67 ± 0.12	3.37 ± 0.05	61.33 ± 0.26	5.33 ± 0.05	364.00 ± 0.47	184.00 ± 0.36
Nageen Lake	NW1	6.53 ± 0.22	25.57 ± 0.12	3.15 ± 0.11	56.63 ± 0.72	4.47 ± 0.10	327.67 ± 0.19	97.67 ± 0.72
	NW2	6.60 ± 0.12	25.50 ± 0.07	3.33 ± 0.07	55.33 ± 0.94	4.47 ± 0.14	325.00 ± 0.94	96.00 ± 0.72
	NW3	6.80 ± 0.14	25.67 ± 0.14	3.67 ± 0.12	57.33 ± 0.98	4.53 ± 0.09	324.00 ± 0.98	95.33 ± 0.78
	NW4	6.53 ± 0.14	25.63 ± 0.15	3.50 ± 0.10	54.33 ± 0.27	4.57 ± 0.10	325.67 ± 0.47	97.67 ± 0.25

Values represent mean ± standard deviation (SE) (n = 3).

5.4 Isolation of microorganisms from water and soil samples

Microorganisms were isolated from both water and soil samples collected from different wetlands of J&K, including Surinsar, Mansar, Khushal Sar, Gil Sar, and Nigee Lake. Isolation was carried out using the spread-plate method on nutrient agar medium. A total of 20 morphologically distinct colonies were isolated from Surinsar and designated as SC1-SC20, 18 colonies from Mansar as MC1-MC18, 15 colonies from Khushal Sar as KC1-KC15, 10 colonies from Gil Sar as GC1-GC10, and 8 colonies from Nigee Lake as NC1-NC8. For soil samples, 1 g of soil was aseptically suspended in sterile distilled water and subjected to serial dilution up to 10^{-6} to reduce microbial load and facilitate the isolation of discrete colonies. Subsequently, 100 μ L aliquots from appropriate dilutions were spread uniformly onto nutrient agar plates. In the case of water samples, aliquots 100 μ L were filtered and then spread onto nutrient agar plates containing 1.3% agar, following standard microbiological protocols (Jasińska et al., 2019). All inoculated plates were incubated at 37 °C for 24 h under aerobic conditions. Post-incubation, well-defined bacterial colonies exhibiting diverse morphological characteristics, including variations in size, shape, pigmentation, and texture, were observed (Figure 5.4). This morphological diversity reflects the heterogeneity of microbial communities present in wetland ecosystems subjected to varying environmental and pollution gradients. Individual colonies displaying distinct morphological features were carefully selected and purified through repeated streaking on fresh nutrient agar plates to obtain axenic cultures (Figure 5.5). The purified isolates were subsequently inoculated into nutrient broth and maintained under controlled conditions for further experimental analysis, including dye degradation assays (Figure 5.6). The successful isolation of morphologically diverse bacterial populations from these wetlands suggests the presence of metabolically versatile microorganisms. Wetlands exposed to organic pollutants and dye effluents are known to serve as reservoirs of adaptive microbial communities capable of utilizing complex xenobiotic compounds, thereby highlighting their potential for application in bioremediation processes (Zhuang et al., 2020).

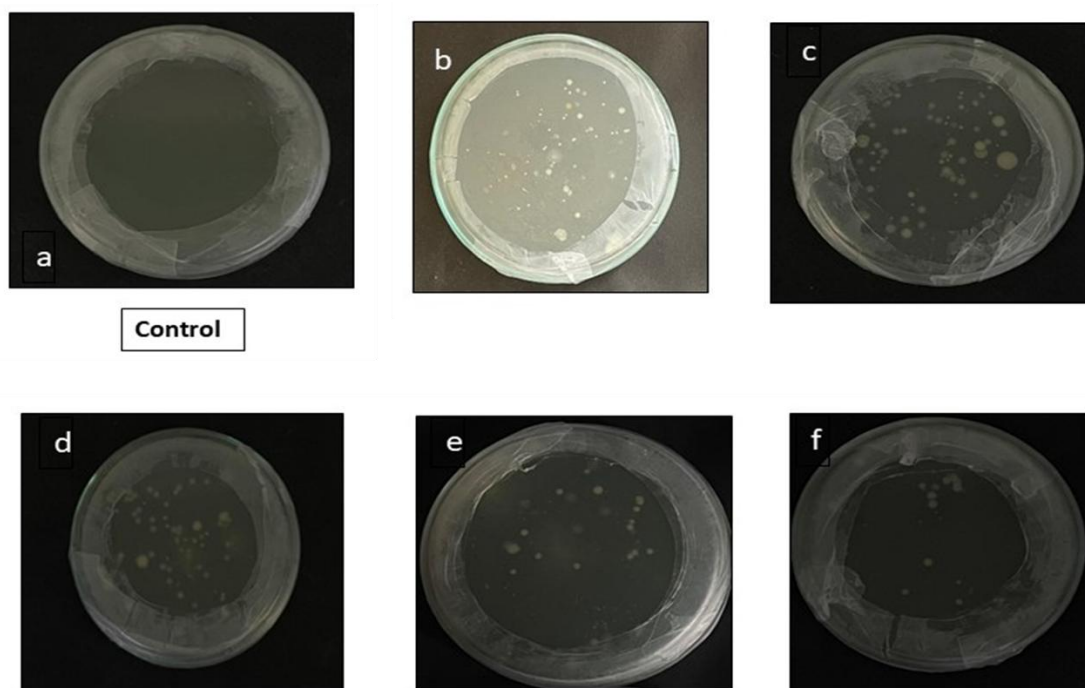


Figure 5.4: Nutrient agar plates showing colonies obtained from different wetlands of J&K (a) Control, (b) Surinsar wetland, (c) Mansar wetland, (d) Khushal Sar, (e) Gilsar, and (f) Nigeen Lake.

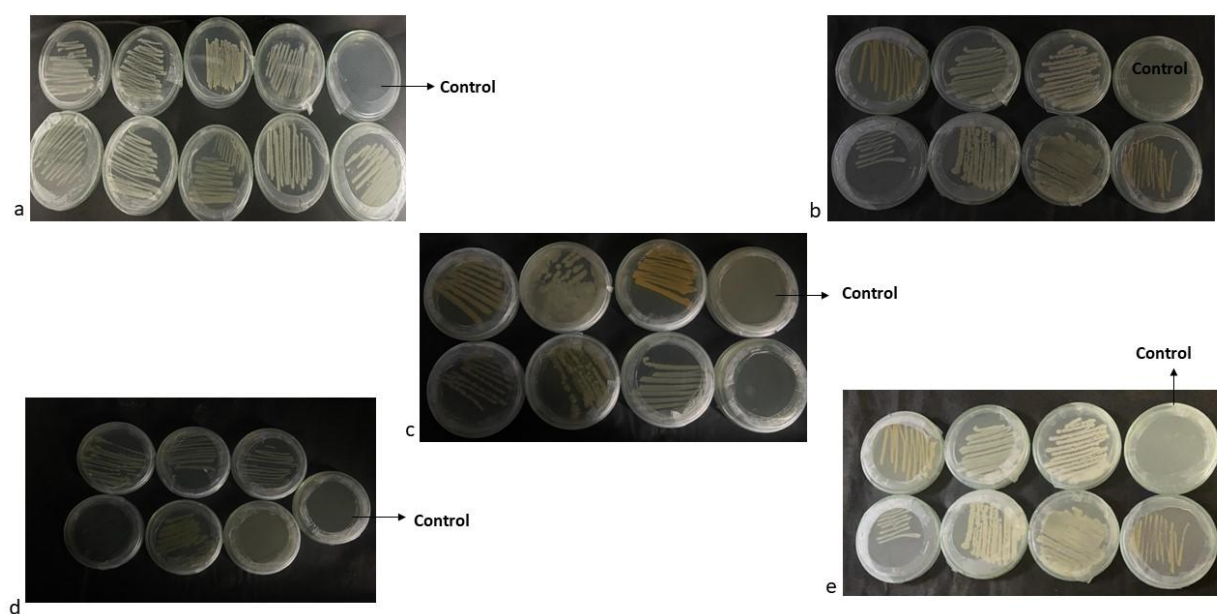


Figure 5.5: Nutrient agar plates showing streaking of colonies obtained from (a) Surinsar wetland, (b) Mansar wetland, (c) Khushal Sar, (d) Gil Sar and (e) Nigeen Lake.

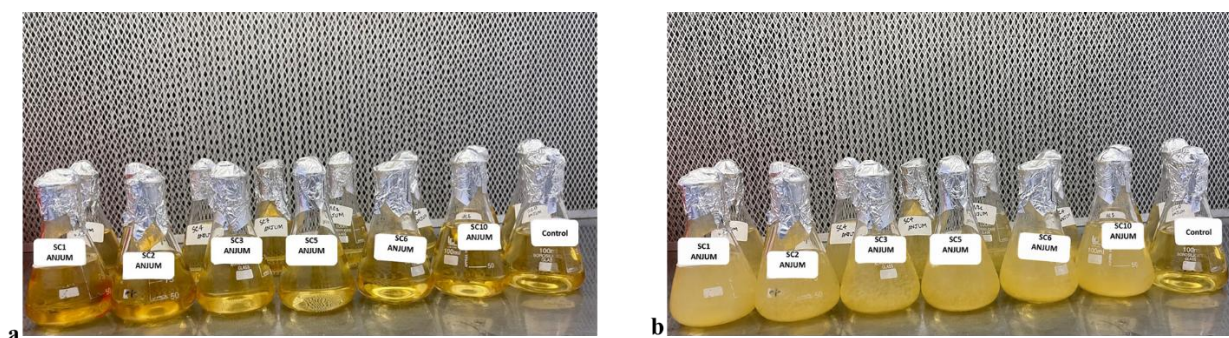


Figure 5.6: Nutrient broth flasks containing cultured isolates obtained from different wetlands of Jammu and Kashmir: (a) before incubation and (b) after incubation showing bacterial growth in the inoculated flasks, while the control remained unchanged.

5.5 Screening of isolates for dye decolorization potential

A total of seventy-one bacterial isolates were obtained from soil and water samples collected from wetlands of Jammu and Kashmir, including Surinsar Wetland, Mansar Wetland, Khushal Sar, Gil Sar, and Nigeeen Lake. The isolates were initially screened on nutrient agar plates supplemented with synthetic dyes, including MB, CR, O-II, MRSS, AR, and AR88, to evaluate their dye-decolorizing potential. Several isolates exhibited visible discoloration of the dyes along the streaked growth lines, indicating their ability to interact with and potentially degrade dye molecules under solid medium conditions (Figure 5.7). The formation of clear or decolorized zones around bacterial colonies is widely recognized as a preliminary indicator of microbial dye degradation, as microorganisms capable of producing oxidative and reductive enzymes can disrupt the chromophoric structure of dyes, leading to loss of colour (Jasińska et al., 2019). Isolates that showed positive discoloration on agar plates were subsequently subjected to secondary screening in liquid nutrient broth containing the respective dyes under controlled conditions. In the broth assays, dye degradation was observed as a reduction in colour intensity from dark to light within 24 h of incubation at 37 °C under both static and shaking conditions (Figure 5.8). The discoloration in liquid culture reflects active microbial metabolism and enzymatic transformation of dye molecules. Microorganisms isolated from contaminated aquatic environments often develop adaptive metabolic pathways that enable them to utilize dye compounds as alternative carbon or nitrogen sources, thereby enhancing their degradation efficiency (Amalanathan et al., 2022). A comparative evaluation of the primary screening of isolates from different wetlands revealed variation in dye-decolorizing

potential. From Surinsar wetland, twenty isolates (SC1-SC20) were obtained. Out of these, nine isolates (SC1, SC2, SC4, SC5, SC6, SC7, SC8, SC9, and SC10) showed strong positive decolorization for all five dyes, while the remaining isolates did not show any color removal. Similarly, Mansar wetland yielded eighteen isolates (MC1–MC18), among which seven isolates (MC1, MC2, MC3, MC4, MC5, MC6, and MC8) showed significant decolorization of all dyes tested. From the Khushal Sar wetland, fifteen isolates (KC1-KC15) were screened. Among these, five isolates (KC1, KC2, KC5, KC6, and KC7) exhibited high decolorization potential for all five dyes. In Gilsar wetland, ten isolates (GC1-GC10) were tested, and three isolates (GC1, GC5, and GC6) showed strong dye-degrading activity. Similarly, Nigeeen Lake yielded eight isolates (NC1-NC8), out of which five (NC1, NC2, NC3, NC4, and NC5) showed efficient decolorization of all dyes tested (Table 5.2). Overall, the screening results revealed variations in dye-decolorizing potential among isolates from different wetlands. The highest percentage of positive isolates was recorded from Nigeeen Lake and Surinsar wetland, followed by Mansar and Khushal Sar, whereas Gilsar Wetland had comparatively fewer dye-degrading isolates. These differences could be attributed to variations in nutrient composition, organic load, and pollutant exposure in the wetlands, which influence the diversity and metabolic potential of the microbial populations (Amalanathan et al., 2022). The results clearly demonstrate that the wetlands of Jammu and Kashmir harbour diverse microbial communities with the ability to decolorize a wide range of synthetic dyes. These indigenous isolates hold significant promise for application in eco-friendly bioremediation of dye-contaminated aquatic environments (Seyedi et al., 2020). Recent studies generally reported that 20-45% of isolates recovered from contaminated aquatic environments exhibited measurable dye-decolorizing activity. In the present investigation, a comparatively higher proportion of active isolates was obtained from Surinsar and Nigeeen wetlands, suggesting that these ecosystems harbour a substantial population of metabolically adaptable bacteria. Furthermore, most recent studies have evaluated only one or two model dyes, whereas the present work screened isolates against six structurally different synthetic dyes, including Acid Red 88. The ability of several isolates to decolorize all six dyes indicates broad substrate specificity and suggests the presence of multiple oxidative and reductive enzyme systems, representing a notable advancement over studies limited to single-dye screening (Amalanathan et al., 2022).

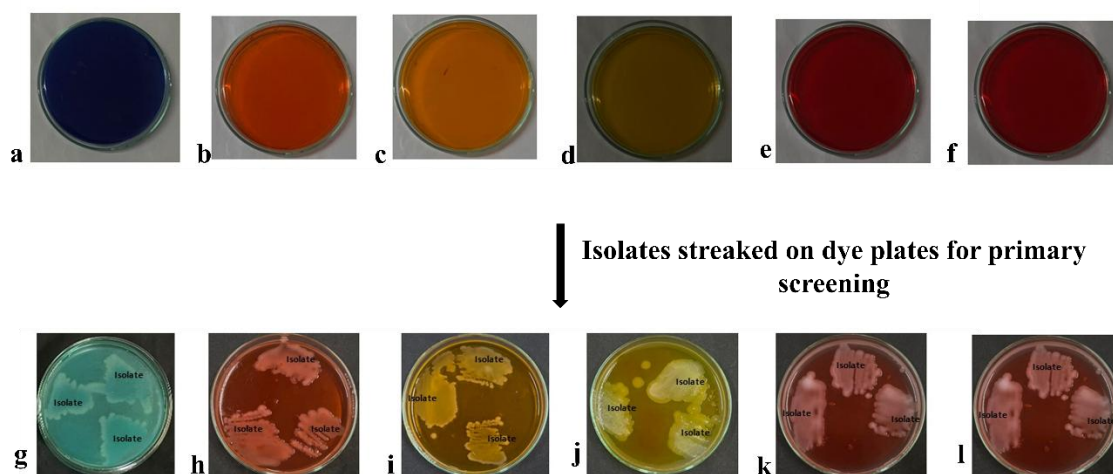


Figure 5.7: Primary screening of J&K wetland isolates showing(a-f) control (a) MB, (b) CR, (c) O-II, (d) MRSS, (e) AR, (f) AR88 and (g-i) shows decolorization of dyes by isolates in plates.

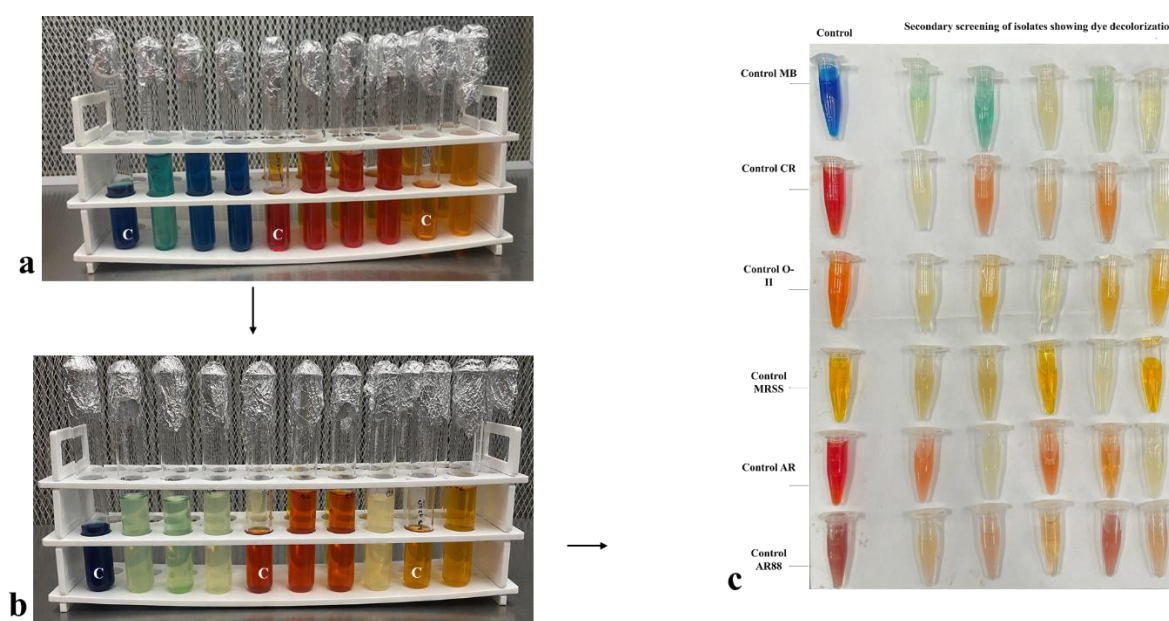


Figure 5.8: Secondary screening of J&K wetland isolates showing decolorization of dyes MB, CR, O-II, MRSS, AR and AR88. (a) test tubes before incubation, (b) test tubes after incubation and (c) visualisation of dye degradation.

Table 5.2: Dye decolorization summary of isolates from wetlands of J&K (Surinsar, Mansar, Khusal Sar, Gil Sar and Nigeen lake):

Wetland	Isolates	Methylene blue	Congo Red	Orange II	Methyl Red	Allura Red	Acid Red 88

					Sodium Salt		
Surinsar	SC1	+ve	+ve	+ve	+ve	+ve	+ve
	SC2	+ve	+ve	+ve	+ve	+ve	+ve
	SC3	-ve	-ve	-ve	-ve	-ve	-ve
	SC4	+ve	+ve	+ve	+ve	+ve	+ve
	SC5	+ve	+ve	+ve	+ve	+ve	+ve
	SC6	+ve	+ve	+ve	+ve	+ve	+ve
	SC7	+ve	+ve	+ve	+ve	+ve	+ve
	SC8	+ve	+ve	+ve	+ve	+ve	+ve
	SC9	+ve	+ve	+ve	+ve	+ve	+ve
	SC10	+ve	+ve	+ve	+ve	+ve	+ve
	SC11	-ve	-ve	-ve	-ve	-ve	-ve
	SC12	-ve	-ve	-ve	-ve	-ve	-ve
	SC13	-ve	-ve	-ve	-ve	-ve	-ve
	SC14	-ve	-ve	-ve	-ve	-ve	-ve
	SC15	-ve	-ve	-ve	-ve	-ve	-ve
	SC16	-ve	-ve	-ve	-ve	-ve	-ve
	SC17	-ve	-ve	-ve	-ve	-ve	-ve
	SC18	-ve	-ve	-ve	-ve	-ve	-ve
	SC19	-ve	-ve	-ve	-ve	-ve	-ve
	SC20	-ve	-ve	-ve	-ve	-ve	-ve
Mansar	MC1	+ve	+ve	+ve	+ve	+ve	+ve
	MC2	+ve	+ve	+ve	+ve	+ve	+ve
	MC3	+ve	+ve	+ve	+ve	+ve	+ve
	MC4	+ve	+ve	+ve	+ve	+ve	+ve
	MC5	+ve	+ve	+ve	+ve	+ve	+ve
	MC6	+ve	+ve	+ve	+ve	+ve	+ve
	MC7	-ve	-ve	-ve	-ve	-ve	-ve

	MC8	+ve	+ve	+ve	+ve	+ve	+ve
	MC9	-ve	-ve	-ve	-ve	-ve	-ve
	MC10	-ve	-ve	-ve	-ve	-ve	-ve
	MC11	-ve	-ve	-ve	-ve	-ve	-ve
	MC12	-ve	-ve	-ve	-ve	-ve	-ve
	MC13	-ve	-ve	-ve	-ve	-ve	-ve
	MC14	-ve	-ve	-ve	-ve	-ve	-ve
	MC15	-ve	-ve	-ve	-ve	-ve	-ve
	MC16	-ve	-ve	-ve	-ve	-ve	-ve
	MC17	-ve	-ve	-ve	-ve	-ve	-ve
	MC18	-ve	-ve	-ve	-ve	-ve	-ve
Khushal sar	KC1	+ve	+ve	+ve	+ve	+ve	+ve
	KC2	+ve	+ve	+ve	+ve	+ve	+ve
	KC3	-ve	-ve	-ve	-ve	-ve	-ve
	KC4	-ve	-ve	-ve	-ve	-ve	-ve
	KC5	+ve	+ve	+ve	+ve	+ve	+ve
	KC6	+ve	+ve	+ve	+ve	+ve	+ve
	KC7	+ve	+ve	+ve	+ve	+ve	+ve
	KC8	-ve	-ve	-ve	-ve	-ve	-ve
	KC9	-ve	-ve	-ve	-ve	-ve	-ve
	KC10	-ve	-ve	-ve	-ve	-ve	-ve
	KC11	-ve	-ve	-ve	-ve	-ve	-ve
	KC12	-ve	-ve	-ve	-ve	-ve	-ve
	KC13	-ve	-ve	-ve	-ve	-ve	-ve
	KC14	-ve	-ve	-ve	-ve	-ve	-ve
	KC15	-ve	-ve	-ve	-ve	-ve	-ve
Gilsar	GC1	+ve	+ve	+ve	+ve	+ve	+ve
	GC2	-ve	-ve	-ve	-ve	-ve	-ve
	GC3	-ve	-ve	-ve	-ve	-ve	-ve

	GC4	-ve	-ve	-ve	-ve	-ve	-ve
	GC5	+ve	+ve	+ve	+ve	+ve	+ve
	GC6	+ve	+ve	+ve	+ve	+ve	+ve
	GC7	-ve	-ve	-ve	-ve	-ve	-ve
	GC8	-ve	-ve	-ve	-ve	-ve	-ve
	GC9	-ve	-ve	-ve	-ve	-ve	-ve
	GC10	-ve	-ve	-ve	-ve	-ve	-ve
Nigeen Lake	NC1	+ve	+ve	+ve	+ve	+ve	+ve
	NC2	+ve	+ve	+ve	+ve	+ve	+ve
	NC3	+ve	+ve	+ve	+ve	+ve	+ve
	NC4	+ve	+ve	+ve	+ve	+ve	+ve
	NC5	+ve	+ve	+ve	+ve	+ve	+ve
	NC6	-ve	-ve	-ve	-ve	-ve	-ve
	NC7	-ve	-ve	-ve	-ve	-ve	-ve
	NC8	-ve	-ve	-ve	-ve	-ve	-ve

5.6 UV–Vis spectrophotometric screening of dye-decolorizing isolates isolated from wetland of J&K

The isolates showing positive dye decolorization in primary and screening were quantitatively evaluated using UV-Vis spectrophotometric analysis to determine their decolorization efficiency. Each isolate was inoculated in nutrient broth containing 100 ppm of different dyes MB, CR, O-II, MRSS, AR, and AR88 and incubated at 37 °C and pH 7 for 14 h under both static and shaking conditions. The reduction in absorbance at respective λ_{max} values was used to calculate the percentage of decolorization. The results showed statically significant variation in dye decolorization efficiency among the isolates. Some isolates exhibited high decolorization, while others showed moderate to low activity, indicating differences in their metabolic capacity and enzyme systems involved in dye degradation (Seyedi et al., 2020). The percentage of dye decolorization varied widely among the isolates and across the six dyes tested. Under static conditions, decolorization efficiency ranged from 0.08 % to 97.23 %, whereas under shaking conditions, it ranged from 1.58 % to 99.54 % (Table 5.3 and 5.4). This wide variation highlights significant differences in the metabolic capabilities and enzymatic

systems of the isolates, with certain strains exhibiting high degradation efficiency while others showed minimal activity. A consistently higher decolorization efficiency was observed under shaking conditions compared to static conditions. This enhancement can be attributed to improved aeration, uniform nutrient distribution, and increased microbial activity, which collectively enhance the production and activity of dye-degrading enzymes. Aerobic conditions are known to promote oxidative enzymes such as laccases and peroxidases, facilitating more effective breakdown of dye molecules (Amalanathan et al., 2022). Overall, the spectrophotometric analysis confirmed the differential dye-degrading potential of the isolates and highlighted the importance of environmental conditions in influencing microbial decolorization efficiency. Among all the isolates tested, SC4 from Surinsar, MC2 from Mansar, KC2 from Khushal Sar, and NC2 from Nigeen Lake consistently exhibited superior decolorization efficiency across multiple dyes under both incubation conditions. Their ability to effectively decolorize a broad spectrum of dyes indicates the presence of versatile enzymatic systems capable of targeting different chromophoric groups. Similar findings have been reported in previous studies, where bacterial strains isolated from contaminated aquatic environments demonstrated high and stable dye degradation efficiency due to their adaptive metabolic capabilities (Zhuang et al., 2020). The high performance of these isolates highlights their potential application in the bioremediation of dye-contaminated wastewater. Indigenous microorganisms from wetland ecosystems are particularly valuable for such applications, as they are naturally adapted to fluctuating environmental conditions and pollutant exposure, making them more efficient and sustainable candidates for large-scale biotreatment processes (Seyedi et al., 2020). Overall, the UV–Vis spectrophotometric analysis confirmed the superior dye-degrading potential of selected isolates and provided a reliable basis for selecting efficient strains for further optimization and molecular characterization. The present study identified SC4, MC2, KC2, and NC2 as consistently efficient degraders across multiple dyes under both static and shaking conditions. Recent research commonly describes efficient decolorization of individual azo dyes under optimized laboratory conditions; however, comparatively few studies have demonstrated stable activity against multiple dye classes under identical experimental conditions. The superior performance of indigenous wetland isolates indicates that natural exposure to fluctuating environmental stresses may have selected for metabolically versatile strains (Gayathri et al., 2026). Such versatility is particularly important for textile wastewater treatment because industrial effluents usually contain mixtures of dyes rather than a single compound. Therefore, the selected isolates have potential for development as

regionally adapted biocatalysts for integrated treatment systems, reducing dependence on costly physicochemical treatment methods (Amalanathan et al., 2022).

Table 5.3: Comparative UV-Vis assessment of dye decolorization by isolates against MB, CR, O-II, MRSS, AR and AR88 under standardized conditions. Values represent mean $\pm$ SE (n = 3). SC4 and MC2 exhibited the highest decolorization efficiency and was selected for further studies.

			Static					Shaking				
Sample	Bacterial Colony	WL	I	II	III	% Decolorization Mean Static	p Value	I	II	III	% Decolorization Mean Shaking	p Value
Methylene Blue with colony SC1	SC1	665.00	1.59	1.55	1.50	49.56 ± 0.72	0.00	1.39	1.35	1.30	56.35 ± 0.72	0.00
Methylene Blue with Colony SC2	SC2	665.00	1.45	1.50	1.51	48.58 ± 0.55	0.00	1.25	1.30	1.21	56.95 ± 0.75	0.00
Methylene Blue with Colony SC4	SC4	665.00	0.73	0.72	0.73	75.70 ± 0.05	0.00	0.53	0.52	0.53	82.87 ± 0.05	0.00
Methylene Blue with Colony SC5	SC5	665.00	2.53	2.59	2.49	8.54 ± 0.88	0.02	2.33	2.39	2.39	14.65 ± 0.60	0.00
Methylene Blue with Colony SC6	SC6	665.00	2.54	2.50	2.59	8.33 ± 0.78	0.01	2.34	2.30	2.39	15.66 ± 0.78	0.00
Methylene Blue with Colony SC7	SC7	665.00	0.89	0.90	0.90	73.75 ± 0.07	0.00	0.69	0.70	0.70	82.62 ± 0.07	0.00
Methylene Blue with Colony SC8	SC8	665.00	0.77	0.67	0.75	72.40 ± 0.93	0.00	0.57	0.57	0.55	79.00 ± 0.25	0.00

Methylene Blue with Colony SC9	SC9	665.00	1.52	1.63	1.55	48.23 ± 0.93	0.00	1.32	1.33	1.35	56.22 ± 0.27	0.00
Methylene Blue with Colony SC10	SC10	665.00	1.21	1.25	1.29	64.51 ± 0.70	0.00	1.01	1.05	1.09	71.99 ± 0.70	0.00
Methylene Blue with Colony MC1	MC1	665.00	1.63	1.67	1.70	44.83 ± 0.60	0.00	1.43	1.47	1.50	51.66 ± 0.60	0.00
Methylene Blue with Colony MC2	MC2	665.00	0.81	0.81	0.81	74.15 ± 0.03	0.00	0.61	0.61	0.61	80.98 ± 0.03	0.00
Methylene Blue with Colony MC3	MC3	665.00	1.22	1.29	1.25	58.93 ± 0.58	0.00	1.02	1.09	1.05	65.76 ± 0.58	0.00
Methylene Blue with Colony MC4	MC4	665.00	2.79	2.87	2.83	5.93 ± 0.66	0.02	2.59	2.57	2.53	14.99 ± 0.48	0.00
Methylene Blue with Colony MC5	MC5	665.00	2.65	2.60	2.69	12.17 ± 0.72	0.01	2.45	2.40	2.49	18.97 ± 0.72	0.00
Methylene Blue with Colony MC6	MC6	665.00	2.58	2.67	2.65	12.58 ± 0.75	0.01	2.38	2.37	2.35	21.64 ± 0.25	0.00
Methylene Blue with Colony MC8	MC8	665.00	2.95	2.93	3.00	1.48 ± 0.56	0.16	2.75	2.73	2.80	8.28 ± 0.56	0.01
Congo Red with Colony SC1	SC1	498.00	0.64	0.62	0.67	72.47 ± 0.56	0.00	0.44	0.42	0.47	82.69 ± 0.56	0.00

Congo Red with Colony SC2	SC2	498.00	0.80	0.90	0.85	77.46 ± 0.84	0.00	0.60	0.70	0.65	84.93 ± 0.84	0.00
Congo Red with Colony SC4	SC4	498.00	0.52	0.59	0.56	83.63 ± 0.60	0.00	0.32	0.39	0.36	90.59 ± 0.60	0.00
Congo Red with Colony SC5	SC5	498.00	1.94	1.98	1.95	5.21 ± 0.46	0.01	1.84	1.88	1.85	10.32 ± 0.46	0.00
Congo Red with Colony SC6	SC6	498.00	2.86	2.80	2.87	1.54 ± 0.67	0.20	2.66	2.70	2.67	7.75 ± 0.36	0.00
Congo Red with Colony SC7	SC7	498.00	0.74	0.75	0.75	75.01 ± 0.21	0.00	0.64	0.65	0.65	84.78 ± 0.21	0.00
Congo Red with Colony SC8	SC8	498.00	0.76	0.77	0.77	72.99 ± 0.14	0.00	0.66	0.67	0.67	82.76 ± 0.14	0.00
Congo Red with Colony SC9	SC9	498.00	1.37	1.36	1.39	57.19 ± 0.29	0.00	1.17	1.16	1.19	63.92 ± 0.29	0.00
Congo Red with Colony SC10	SC10	498.00	1.11	1.15	1.20	64.60 ± 0.72	0.00	0.91	0.95	0.99	71.44 ± 0.64	0.00
Congo Red with Colony MC1	MC1	498.00	1.47	1.46	1.49	53.88 ± 0.27	0.00	1.27	1.26	1.29	60.61 ± 0.27	0.00
Congo Red with Colony MC2	MC2	498.00	0.75	0.76	0.75	78.03 ± 0.07	0.00	0.54	0.55	0.54	85.10 ± 0.07	0.00

Congo Red with Colony MC3	MC3	498.00	1.51	1.56	1.59	51.12 ± 0.66	0.00	1.11	1.16	1.19	64.58 ± 0.66	0.00
Congo Red with Colony MC4	MC4	498.00	1.18	1.17	1.20	44.95 ± 0.40	0.00	0.78	0.77	0.80	65.39 ± 0.40	0.00
Congo Red with Colony MC5	MC5	498.00	0.58	0.61	0.66	73.84 ± 0.94	0.00	0.48	0.41	0.46	82.35 ± 0.89	0.00
Congo Red with Colony MC6	MC6	498.00	0.90	0.98	0.95	57.03 ± 0.99	0.00	0.90	0.98	0.95	57.03 ± 0.99	0.00
Congo Red with Colony MC8	MC8	498.00	0.88	0.90	0.86	60.42 ± 0.47	0.00	0.68	0.70	0.66	70.64 ± 0.47	0.00
Orange II with colony SC1	SC1	486.00	1.39	1.40	1.39	61.02 ± 0.07	0.00	1.19	1.20	1.19	67.12 ± 0.07	0.00
Orange II with colony SC2	SC2	486.00	0.65	0.58	0.61	73.52 ± 0.83	0.00	0.45	0.48	0.41	82.05 ± 0.90	0.00
Orange II with colony SC4	SC4	486.00	0.55	0.56	0.56	76.46 ± 0.06	0.00	0.35	0.36	0.36	86.70 ± 0.06	0.00
Orange II with colony SC5	SC5	486.00	1.99	2.00	1.99	3.00 ± 0.16	0.00	1.79	1.80	1.79	13.24 ± 0.16	0.00
Orange II with colony SC6	SC6	486.00	1.99	1.99	1.98	3.26 ± 0.09	0.00	1.79	1.79	1.78	13.50 ± 0.09	0.00

Orange II with colony SC7	SC7	486.00	1.62	1.60	1.59	51.16 ± 0.37	0.00	1.42	1.40	1.49	58.64 ± 0.94	0.00
Orange II with colony SC8	SC8	486.00	2.00	1.98	1.92	34.90 ± 0.83	0.00	1.80	1.78	1.72	43.89 ± 0.83	0.00
Orange II with colony SC9	SC9	486.00	1.65	1.72	1.64	50.87 ± 0.66	0.00	1.45	1.42	1.44	58.24 ± 0.23	0.00
Orange II with colony SC10	SC10	486.00	1.92	1.96	2.00	41.85 ± 0.60	0.00	1.72	1.76	1.80	48.17 ± 0.60	0.00
Orange II with colony MC1	MC1	486.00	1.84	1.90	1.83	45.03 ± 0.50	0.00	1.64	1.70	1.63	51.35 ± 0.50	0.00
Orange II with colony MC2	MC2	486.00	0.84	0.84	0.84	77.21 ± 0.02	0.00	0.64	0.64	0.64	83.52 ± 0.02	0.00
Orange II with colony MC3	MC3	486.00	1.66	1.74	1.63	50.61 ± 0.85	0.00	1.46	1.44	1.43	57.98 ± 0.24	0.00
Orange II with colony MC4	MC4	486.00	2.05	1.93	1.99	42.77 ± 0.84	0.00	2.00	1.99	1.99	42.72 ± 0.05	0.00
Orange II with colony MC5	MC5	486.00	0.99	1.00	1.00	72.25 ± 0.05	0.00	0.79	0.80	0.80	78.56 ± 0.05	0.00
Orange II with colony MC6	MC6	486.00	1.04	1.06	1.08	70.21 ± 0.26	0.00	1.01	1.06	1.06	70.74 ± 0.38	0.00

Orange II with colony MC8	MC8	486.00	1.11	1.11	1.02	69.54 ± 0.78	0.00	1.11	1.11	1.02	69.54 ± 0.78	0.00
Methyl Red Sodium Salt with Colony SC1	SC1	515.00	0.34	0.34	0.34	57.01 ± 0.17	0.00	0.32	0.32	0.32	60.46 ± 0.17	0.00
Methyl Red Sodium Salt with Colony SC2	SC2	515.00	0.23	0.23	0.23	43.37 ± 0.27	0.00	0.21	0.21	0.21	50.81 ± 0.27	0.00
Methyl Red Sodium Salt with Colony SC4	SC4	515.00	0.12	0.13	0.13	79.68 ± 0.97	0.00	0.10	0.10	0.11	88.35 ± 0.56	0.00
Methyl Red Sodium Salt with Colony SC5	SC5	515.00	0.34	0.34	0.34	1.49 ± 0.18	0.02	0.32	0.33	0.33	6.44 ± 0.86	0.03
Methyl Red Sodium Salt with Colony SC6	SC6	515.00	0.34	0.33	0.34	2.73 ± 0.54	0.05	0.33	0.32	0.33	6.44 ± 0.54	0.01
Methyl Red Sodium Salt with Colony SC7	SC7	515.00	0.24	0.24	0.25	47.04 ± 0.44	0.00	0.22	0.22	0.23	53.27 ± 0.44	0.00

Methyl Red Sodium Salt with Colony SC8	SC8	515.00	0.22	0.22	0.22	55.14 ± 0.14	0.00	0.21	0.21	0.21	58.26 ± 0.15	0.00
Methyl Red Sodium Salt with Colony SC9	SC9	515.00	0.24	0.25	0.25	68.00 ± 0.33	0.00	0.22	0.23	0.23	71.93 ± 0.33	0.00
Methyl Red Sodium Salt with Colony SC10	SC10	515.00	0.23	0.23	0.23	71.47 ± 0.22	0.00	0.21	0.21	0.21	75.47 ± 0.22	0.00
Methyl Red Sodium Salt with Colony MC1	MC1	515.00	0.29	0.29	0.30	58.60 ± 0.25	0.00	0.27	0.27	0.28	62.60 ± 0.25	0.00
Methyl Red Sodium Salt with Colony MC2	MC2	515.00	0.12	0.12	0.12	93.60 ± 0.19	0.00	0.11	0.11	0.11	95.60 ± 0.19	0.00
Methyl Red Sodium Salt with Colony MC3	MC3	515.00	0.21	0.22	0.22	73.80 ± 0.25	0.00	0.20	0.21	0.21	75.80 ± 0.25	0.00
Methyl Red Sodium Salt with Colony MC4	MC4	515.00	0.29	0.30	0.30	64.20 ± 0.29	0.00	0.27	0.28	0.28	67.64 ± 0.29	0.00

Methyl Red Sodium Salt with Colony MC5	MC5	515.00	0.22	0.22	0.22	77.76 ± 0.16	0.00	0.21	0.21	0.21	79.48 ± 0.16	0.00
Methyl Red Sodium Salt with Colony MC6	MC6	515.00	0.29	0.30	0.30	64.25 ± 0.29	0.00	0.27	0.28	0.28	67.70 ± 0.29	0.00
Methyl Red Sodium Salt with Colony MC8	MC8	515.00	0.54	0.53	0.54	22.87 ± 0.29	0.00	0.52	0.51	0.52	26.32 ± 0.29	0.00
Allura Red with colony SC1	SC1	507.00	0.97	0.93	0.90	69.99 ± 0.62	0.00	0.95	0.96	0.95	69.29 ± 0.06	0.00
Allura Red with colony SC2	SC2	507.00	2.40	2.32	2.35	22.15 ± 0.70	0.00	2.39	2.40	2.35	21.36 ± 0.43	0.00
Allura Red with colony SC4	SC4	507.00	0.94	0.95	0.95	70.36 ± 0.04	0.00	0.74	0.75	0.75	77.19 ± 0.04	0.00
Allura Red with colony SC5	SC5	507.00	2.89	2.93	2.89	1.98 ± 0.38	0.05	2.69	2.63	2.69	10.11 ± 0.57	0.00
Allura Red with colony SC6	SC6	507.00	2.94	2.94	2.84	2.02 ± 0.96	0.23	2.74	2.74	2.74	7.80 ± 0.02	0.00
Allura Red with colony SC7	SC7	507.00	1.87	1.89	1.89	40.13 ± 0.16	0.00	1.67	1.69	1.69	46.65 ± 0.16	0.00

Allura Red with colony SC8	SC8	507.00	1.90	1.90	1.90	39.61 ± 0.03	0.00	1.70	1.70	1.70	46.13 ± 0.03	0.00
Allura Red with colony SC9	SC9	507.00	2.20	2.29	2.30	39.18 ± 0.75	0.00	2.10	2.19	2.20	41.98 ± 0.75	0.00
Allura Red with colony SC10	SC10	507.00	2.22	2.23	2.23	40.15 ± 0.11	0.00	2.12	2.13	2.13	42.95 ± 0.11	0.00
Allura Red with colony MC1	MC1	507.00	2.68	2.69	2.79	26.35 ± 0.80	0.00	2.48	2.49	2.49	32.89 ± 0.06	0.00
Allura Red with colony MC2	MC2	507.00	0.87	0.83	0.80	79.18 ± 0.49	0.00	0.67	0.63	0.60	84.79 ± 0.49	0.00
Allura Red with colony MC3	MC3	507.00	2.48	2.49	2.57	32.16 ± 0.70	0.00	2.28	2.29	2.27	38.69 ± 0.12	0.00
Allura Red with colony MC4	MC4	507.00	1.62	1.65	1.69	38.72 ± 0.66	0.00	1.42	1.45	1.49	46.57 ± 0.66	0.00
Allura Red with colony MC5	MC5	507.00	1.82	1.86	1.84	37.66 ± 0.34	0.00	1.62	1.66	1.64	44.80 ± 0.34	0.00
Allura Red with colony MC6	MC6	507.00	1.71	1.75	1.81	40.56 ± 0.86	0.00	1.51	1.55	1.51	48.90 ± 0.36	0.00
Allura Red with colony MC8	MC8	507.00	1.60	1.56	1.56	47.22 ± 0.36	0.00	1.20	1.16	1.16	54.36 ± 0.36	0.00

AR88 with colony SC1	SC1	505.00	0.54	0.54	0.54	53.43 ±0.10	0.00	0.34	0.34	0.34	70.63 ± 0.10	0.00
AR88 with colony SC2	SC2	505.00	0.34	0.34	0.35	70.61 ±0.20	0.00	0.14	0.14	0.15	87.81 ± 0.20	0.00
AR88 with colony SC4	SC4	505.00	0.15	0.16	0.16	86.55 ± 0.10	0.00	0.11	0.12	0.12	89.99 ± 0.10	0.00
AR88 with colony SC5	SC5	505.00	1.15	1.16	1.16	0.32 ±0.16	0.26	1.14	1.15	1.14	1.58 ± 0.19	0.02
AR88 with colony SC6	SC6	505.00	1.14	1.16	1.15	0.8 ± 0.37	0.21	1.11	1.11	1.11	4.24 ± 0.05	0.00
AR88 with colony SC7	SC7	505.00	0.59	0.59	0.59	49.30 ± 0.07	0.00	0.49	0.49	0.49	57.90 ± 0.06	0.00
AR88 with colony SC8	SC8	505.00	0.53	0.53	0.52	54.55 ± 0.21	0.00	0.43	0.43	0.42	63.15 ± 0.21	0.00
AR88 with colony SC9	SC9	505.00	1.16	1.16	1.16	0.32 ±0.20	0.33	1.13	1.12	1.13	3.18 ± 0.04	0.00
AR88 with colony SC10	SC10	505.00	0.76	0.76	0.76	34.56 ±0.14	0.00	0.66	0.66	0.66	43.16 ± 0.14	0.00
AR88 with colony MC1	MC1	505.00	0.47	0.47	0.46	60.05 ±0.20	0.00	0.44	0.44	0.44	62.35 ± 0.06	0.00

AR88 with colony MC2	MC2	505.00	0.11	0.11	0.11	90.71 ± 0.04	0.00	0.10	0.10	0.10	91.58 ± 0.04	0.00
AR88 with colony MC3	MC3	505.00	0.22	0.22	0.22	81.13 ± 0.19	0.00	0.21	0.22	0.22	81.42 ± 0.11	0.00
AR88 with colony MC4	MC4	505.00	1.16	1.16	1.15	0.57 ± 0.28	0.24	1.13	1.12	1.13	3.15 ± 0.12	0.00
AR88 with colony MC5	MC5	505.00	0.38	0.38	0.38	67.31 ± 0.03	0.00	0.36	0.36	0.35	69.37 ± 0.18	0.00
AR88 with colony MC6	MC6	505.00	0.41	0.41	0.42	64.75 ± 0.18	0.00	0.40	0.40	0.40	65.90 ± 0.07	0.00
AR88 with colony MC8	MC8	505.00	0.34	0.34	0.34	70.83 ± 0.15	0.00	0.31	0.32	0.32	72.84 ± 0.10	0.00

Initial and final absorbance values were blank-corrected. Percentage decolorization was calculated as $(A_i - A_f)/A_i \times 100$, and mean $\pm$ SE was obtained from three replicates.

Table 5.4: Comparative UV-Vis assessment of dye decolorization by isolates against MB, CR, O-II, MRSS, AR and AR88 under standardized conditions. Values represent mean $\pm$ SE (n = 3). KC2 and NC2 exhibited the highest decolorization efficiency and were selected for further studies.

	Static	Shaking
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Sample	Bacterial Colony	WL	I	II	III	% Decolorization Mean	p Value	I	II	III	% Decolorization Mean	p Value
Methylene blue with colony KC1	KC1	665.00	2.66	2.68	2.63	8.30 ± 0.43	0.00	2.46	2.48	2.43	15.54 ± 0.43	0.00
Methylene blue with colony KC2	KC2	665.00	1.97	1.97	1.96	34.09 ± 0.06	0.00	1.77	1.77	1.76	41.15 ± 0.06	0.00
Methylene blue with colony KC5	KC5	665.00	2.82	2.83	2.82	3.80 ± 0,08	0.00	2.62	2.63	2.62	10.86 ± 0.08	0.00
Methylene blue with colony KC6	KC6	665.00	2.79	2.80	2.80	3.34 ± 0.05	0.00	2.59	2.60	2.60	10.51 ± 0.05	0.00
Methylene blue with colony KC7	KC7	665.00	2.80	2.80	2.82	2.97 ± 0.19	0.01	2.60	2.50	2.52	12.53 ± 0.89	0.01
Methylene blue with colony GC1	GC1	665.00	2.90	2.89	2.90	0.08 ± 0.07	0.46	2.70	2.69	2.70	7.12 ± 0.07	0.00
Methylene blue with colony GC5	GC5	665.00	2.91	2.81	2.91	1.97 ± 0.94	0.23	2.71	2.71	2.71	7.77 ± 0.03	0.00
Methylene blue with colony GC6	GC6	665.00	2.89	2.88	2.90	1.47 ± 0.20	0.03	2.69	2.68	2.60	9.71 ± 0.80	0.01
Methylene blue with colony NC1	NC1	665.00	1.97	1.97	1.96	33.60 ± 0.07	0.00	1.77	1.77	1.76	40.55 ± 0.07	0.00

Methylene blue with colony NC2	NC2	665.00	1.77	1.77	1.79	39.25 ± 0.19	0.00	1.57	1.57	1.59	46.3 ± 0.19	0.00
Methylene blue with colony NC3	NC3	665.00	2.43	2.44	2.43	16.04 ± 0.07	0.00	2.23	2.24	2.23	23.09 ± 0.07	0.00
Methylene blue with colony NC4	NC4	665.00	1.98	1.98	1.97	32.48 ± 0.08	0.00	1.78	1.78	1.77	39.51 ± 0.08	0.00
Methylene blue with colony NC5	NC5	665.00	2.89	2.90	2.90	1.28 ± 0.08	0.01	2.69	2.70	2.70	8.34 ± 0.08	0.00
Congo red with colony KC1	KC1	498.00	2.73	2.73	2.72	5.73 ± 0.08	0.00	2.53	2.53	2.52	12.91 ± 0.08	0.00
Congo red with colony KC2	KC2	498.00	0.99	0.93	1.00	68.73 ± 0.64	0.00	0.79	0.73	0.80	75.91 ± 0.64	0.00
Congo red with colony KC5	KC5	498.00	2.70	2.70	2.69	6.82 ± 0.07	0.00	2.50	2.50	2.49	14.00 ± 0.07	0.00
Congo red with colony KC6	KC6	498.00	2.68	2.68	2.67	7.51 ± 0.07	0.00	2.48	2.48	2.47	14.70 ± 0.07	0.00
Congo red with colony KC7	KC7	498.00	2.57	2.57	2.59	11.17 ± 0.18	0.00	2.37	2.37	2.39	18.36 ± 0.18	0.00
Congo red with colony GC1	GC1	498.00	2.76	2.79	2.83	3.31 ± 0.67	0.06	2.56	2.59	2.53	11.69 ± 0.44	0.06

Congo red with colony GC5	GC5	498.00	1.91	1.92	1.92	34.73 ± 0.10	0.00	1.71	1.72	1.72	41.92 ± 0.10	0.00
Congo red with colony GC6	GC6	498.00	2.59	2.55	2.50	12.12 ± 0.76	0.01	2.39	2.35	2.30	19.31 ± 0.76	0.01
Congo red with colony NC1	NC1	498.00	2.73	2.73	2.70	5.90 ± 0.33	0.00	2.53	2.53	2.60	11.86 ± 0.65	0.00
Congo red with colony NC2	NC2	498.00	0.85	0.85	0.84	74.62 ± 0.06	0.00	0.65	0.65	0.64	81.41 ± 0.06	0.00
Congo red with colony NC3	NC3	498.00	1.85	1.85	1.84	40.67 ± 0.06	0.00	1.65	1.65	1.64	47.46 ± 0.06	0.00
Congo red with colony NC4	NC4	498.00	1.79	1.73	1.80	43.18 ± 0.61	0.00	1.59	1.53	1.60	49.97 ± 0.61	0.00
Congo red with colony NC5	NC5	498.00	2.99	2.99	2.98	2.01 ± 0.11	0.00	2.79	2.79	2.78	8.81 ± 0.11	0.00
Orange II with colony KC1	KC1	486.00	2.34	2.34	2.25	8.04 ± 0.96	0.02	2.14	2.14	2.15	15.02 ± 0.18	0.02
Orange II with colony KC2	KC2	486.00	0.69	0.65	0.69	76.45 ± 0.47	0.00	0.49	0.45	0.49	84.83 ± 0.47	0.00
Orange II with colony KC5	KC5	486.00	2.44	2.40	2.47	2.83 ± 0.68	0.08	2.24	2.30	2.27	9.81 ± 0.59	0.08

Orange II with colony KC6	KC6	486.00	2.43	2.44	2.44	2.72 ± 0.09	0.00	2.13	2.14	2.14	15.28 ± 0.09	0.00
Orange II with colony KC7	KC7	486.00	2.23	2.24	2.25	10.90 ± 0.15	0.00	2.03	2.04	2.05	19.28 ± 0.15	0.00
Orange II with colony GC1	GC1	486.00	2.40	2.43	2.41	3.71 ± 0.24	0.01	2.20	2.23	2.21	12.09 ± 0.24	0.01
Orange II with colony GC5	GC5	486.00	1.98	1.99	1.98	21.68 ± 0.09	0.00	1.78	1.79	1.78	30.05 ± 0.09	0.00
Orange II with colony GC6	GC6	486.00	2.37	2.37	2.39	5.35 ± 0.25	0.00	2.17	2.17	2.19	13.72 ± 0.25	0.00
Orange II with colony NC1	NC1	486.00	0.79	0.75	0.79	72.20 ± 0.47	0.00	0.59	0.55	0.59	80.57 ± 0.47	0.00
Orange II with colony NC2	NC2	486.00	0.39	0.35	0.39	86.54 ± 0.57	0.00	0.19	0.15	0.19	96.72 ± 0.57	0.00
Orange II with colony NC3	NC3	486.00	1.05	1.09	1.08	51.07 ± 0.53	0.00	1.01	1.01	1.00	54.46 ± 0.09	0.00
Orange II with colony NC4	NC4	486.00	1.01	1.07	1.05	52.51 ± 0.78	0.00	1.01	1.07	1.05	52.51 ± 0.78	0.00
Orange II with colony NC5	NC5	486.00	1.89	1.88	1.87	10.05 ± 0.16	0.00	1.79	1.78	1.77	15.14 ± 0.16	0.00

Methyl red sodium salt with colony KC1	KC1	515.00	0.49	0.48	0.48	21.77 ± 0.69	0.00	0.39	0.38	0.38	41.94 ± 0.69	0.00
Methyl red sodium salt with colony KC2	KC2	515.00	0.18	0.19	0.19	82.59 ± 0.60	0.00	0.15	0.15	0.15	89.99 ± 0.05	0.00
Methyl red sodium salt with colony KC5	KC5	515.00	0.48	0.49	0.47	22.72 ± 0.67	0.00	0.38	0.39	0.37	42.88 ± 0.67	0.00
Methyl red sodium salt with colony K6	KC6	515.00	0.43	0.44	0.44	31.52 ± 0.33	0.00	0.33	0.34	0.34	51.68 ± 0.33	0.00
Methyl red sodium salt with colony KC7	KC7	515.00	0.44	0.45	0.45	29.57 ± 0.67	0.00	0.34	0.35	0.35	49.73 ± 0.67	0.00
Methyl red sodium salt with colony GC1	GC1	515.00	0.42	0.43	0.44	32.53 ± 0.86	0.00	0.32	0.33	0.34	52.69 ± 0.86	0.00
Methyl red sodium salt with colony GC5	GC5	515.00	0.30	0.30	0.30	59.48 ± 0.16	0.00	0.20	0.20	0.20	79.64 ± 0.16	0.00

Methyl red sodium salt with colony GC6	GC6	515.00	0.37	0.36	0.35	47.04 ± 0.79	0.00	0.27	0.26	0.25	67.20 ± 0.79	0.00
Methyl red sodium salt with colony NC1	NC1	515.00	0.45	0.46	0.47	26.61 ± 0.95	0.00	0.35	0.36	0.37	46.77 ± 0.95	0.00
Methyl red sodium salt with colony NC2	NC2	515.00	0.10	0.10	0.10	97.23 ± 0.19	0.00	0.09	0.09	0.09	99.54 ± 0.19	0.00
Methyl red sodium salt with colony NC3	NC3	515.00	0.37	0.37	0.36	34.95 ± 0.74	0.00	0.27	0.27	0.26	58.04 ± 0.74	0.00
Methyl red sodium salt with colony NC4	NC4	515.00	0.32	0.33	0.33	44.27 ± 0.60	0.00	0.22	0.23	0.23	67.36 ± 0.60	0.00
Methyl red sodium salt with colony NC5	NC5	515.00	0.42	0.43	0.43	21.64 ± 0.87	0.00	0.32	0.33	0.33	44.73 ± 0.87	0.00
Allura red with colony KC1	KC1	507.00	2.79	2.78	2.79	3.55 ± 0.09	0.00	2.69	2.68	2.69	7.11 ± 0.09	0.00
Allura red with colony KC2	KC2	507.00	2.27	2.27	2.23	22.48 ± 0.35	0.00	2.17	2.17	2.13	26.07 ± 0.35	0.00
Allura red with colony KC5	KC5	507.00	2.64	2.67	2.65	8.22 ± 0.19	0.00	2.44	2.47	2.45	15.39 ± 0.19	0.00
Allura red with colony KC6	KC6	507.00	2.76	2.74	2.75	4.67 ± 0.19	0.00	2.66	2.64	2.65	8.26 ± 0.19	0.00

Allura red with colony KC7	KC7	507.00	2.46	2.48	2.45	15.18 ± 0.28	0.00	2.36	2.38	2.35	18.76 ± 0.28	0.00
Allura red with colony GC1	GC1	507.00	2.79	2.77	2.70	4.76 ± 0.78	0.04	2.69	2.67	2.70	7.15 ± 0.26	0.04
Allura red with colony GC5	GC5	507.00	2.45	2.44	2.43	15.78 ± 0.16	0.00	2.35	2.34	2.33	19.36 ± 0.16	0.00
Allura red with colony GC6	GC6	507.00	2.66	2.60	2.65	8.95 ± 0.53	0.01	2.46	2.50	2.45	14.93 ± 0.47	0.01
Allura red with colony NC1	NC1	507.00	2.56	2.52	2.46	13.24 ± 0.92	0.01	2.46	2.42	2.46	15.63 ± 0.39	0.01
Allura red with colony NC2	NC2	507.00	2.31	2.32	2.35	25.54 ± 0.34	0.00	2.11	2.12	2.15	32.06 ± 0.34	0.00
Allura red with colony NC3	NC3	507.00	2.78	2.75	2.77	11.40 ± 0.23	0.00	2.68	2.65	2.67	14.67 ± 0.23	0.00
Allura red with colony NC4	NC4	507.00	2.44	2.43	2.54	20.98 ± 0.92	0.00	2.34	2.33	2.34	25.33 ± 0.14	0.00
Allura red with colony NC5	NC5	507.00	2.97	2.98	2.99	4.39 ± 0.17	0.00	2.77	2.78	2.79	10.91 ± 0.17	0.00
Acid Red 88 with KC1	KC1	505.00	0.53	0.52	0.53	3.30 ± 0.38	0.02	0.43	0.42	0.43	21.69 ± 0.38	0.02

Acid Red 88 with KC2	KC2	505.00	0.03	0.02	0.03	93.38 ± 0.17	0.00	0.03	0.02	0.03	95.22 ± 0.17	0.00
Acid Red 88 with KC5	KC5	505.00	0.50	0.49	0.50	9.01 ± 0.46	0.00	0.40	0.39	0.40	27.39 ± 0.46	0.00
Acid Red 88 with KC6	KC6	505.00	0.52	0.52	0.52	4.60 ± 0.00	#DIV/0!	0.42	0.42	0.42	22.98 ± 0.00	#DIV/0!
Acid Red 88 with KC7	KC7	505.00	0.54	0.54	0.53	1.72 ± 0.30	0.04	0.44	0.44	0.43	20.10 ± 0.30	0.04
Acid Red 88 with GC1	GC1	505.00	0.51	0.51	0.51	6.74 ± 0.13	0.00	0.31	0.31	0.31	43.50 ± 0.13	0.00
Acid Red 88 with GC5	GC5	505.00	0.16	0.17	0.16	70.22 ± 0.52	0.00	0.14	0.15	0.15	73.28 ± 0.26	0.00
Acid Red 88 with GC6	GC6	505.00	0.49	0.50	0.50	8.95 ± 0.25	0.00	0.39	0.40	0.40	27.33 ± 0.25	0.00
Acid Red 88 with NC1	NC1	505.00	0.44	0.45	0.44	18.26 ± 0.26	0.00	0.34	0.35	0.34	36.64 ± 0.26	0.00

Acid Red 88 with NC2	NC2	505.00	0.05	0.05	0.05	94.38 ± 0.18	0.00	0.02	0.02	0.02	96.81 ± 0.18	0.00
Acid Red 88 with NC3	NC3	505.00	0.40	0.39	0.39	27.70 ± 0.44	0.00	0.30	0.29	0.29	46.80 ± 0.44	0.00
Acid Red 88 with NC4	NC4	505.00	0.08	0.08	0.09	84.80 ± 0.30	0.00	0.07	0.08	0.08	86.03 ± 0.30	0.00
Acid Red 88 with NC5	NC5	505.00	0.39	0.40	0.39	28.00 ± 0.39	0.00	0.29	0.30	0.29	46.38 ± 0.39	0.00

Initial and final absorbance values were blank-corrected. Percentage decolorization was calculated as $(A_i - A_f)/A_i \times 100$, and mean $\pm$ SE was obtained from three replicates.

Graphical representation of comparative decolorization efficiency of Jammu isolates:

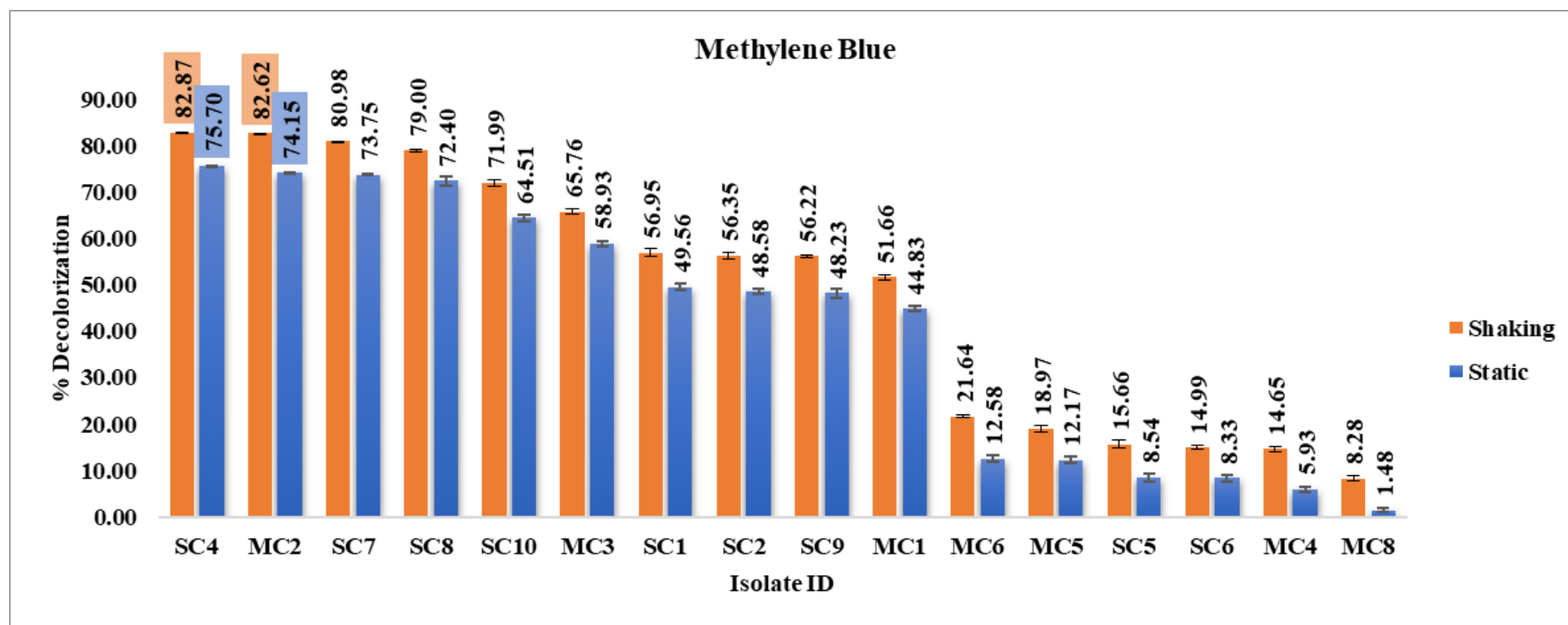


Figure 5.9: Comparative percentage decolorization of MB by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

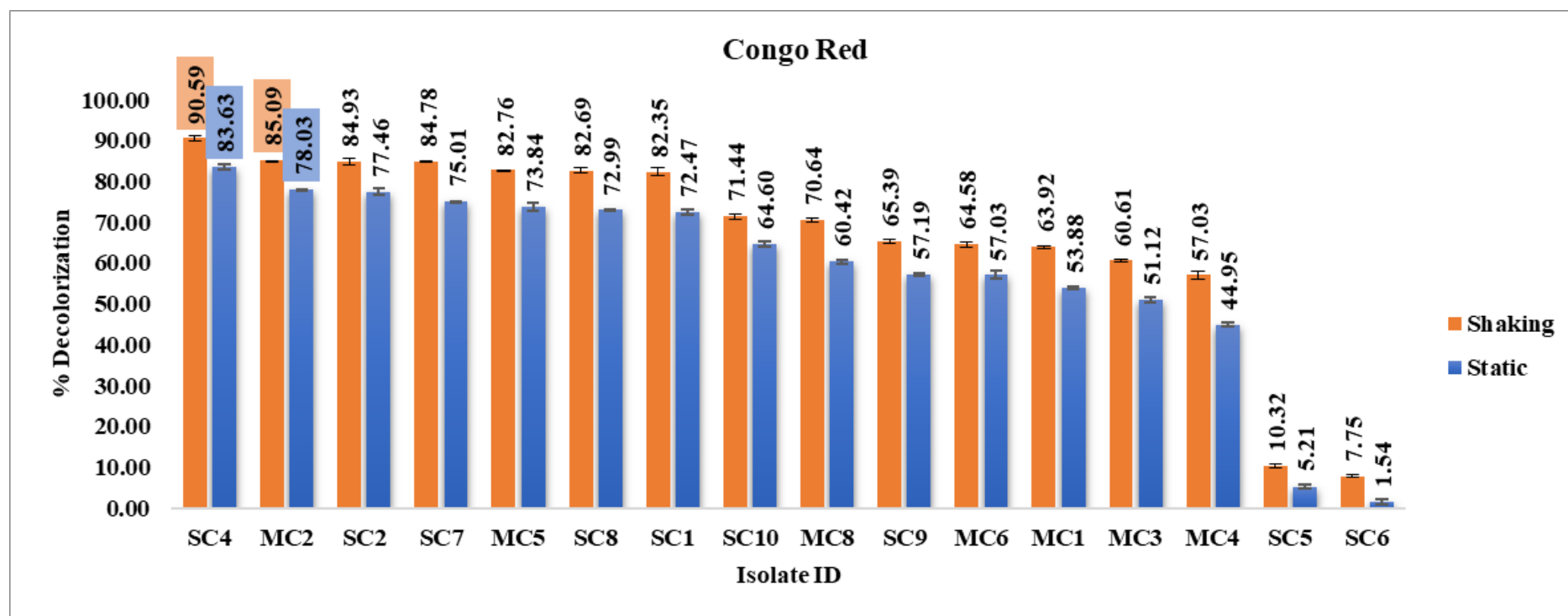


Figure 5.10: Comparative percentage decolorization of CR by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

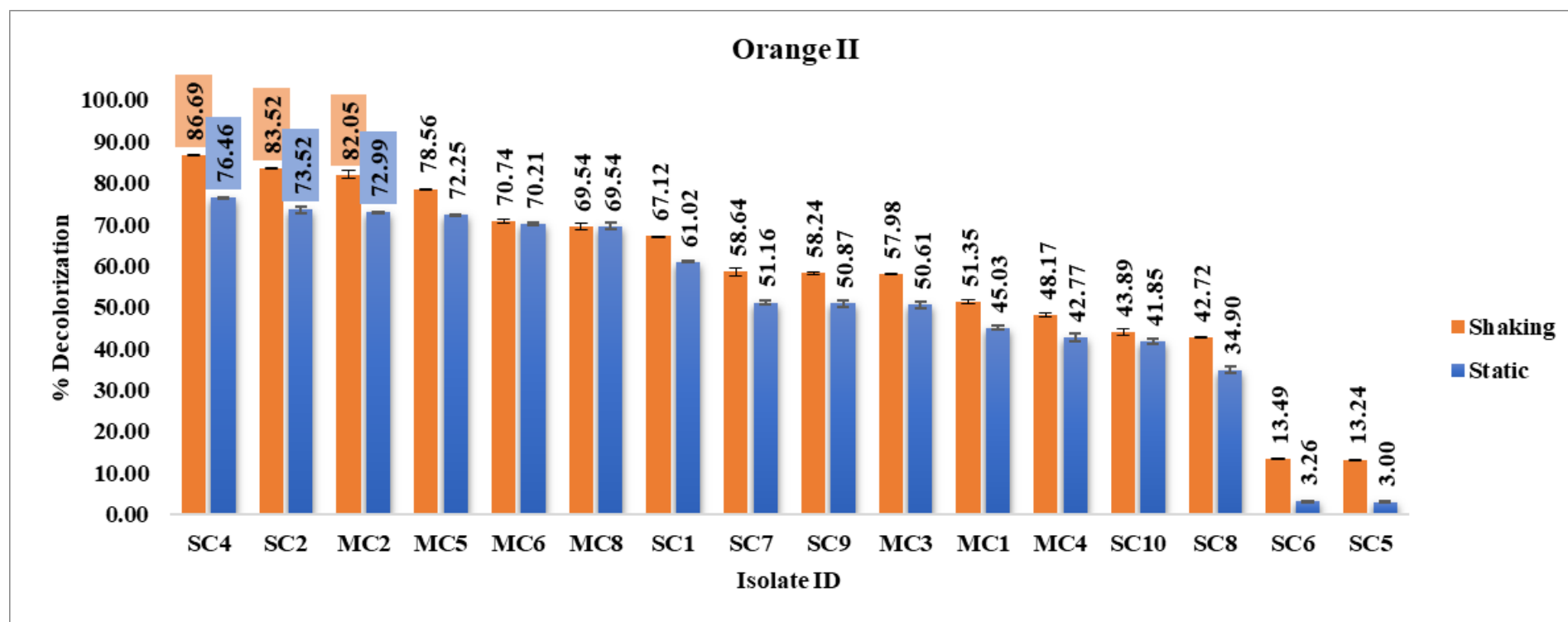


Figure 5.11: Comparative percentage decolorization of O-II by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

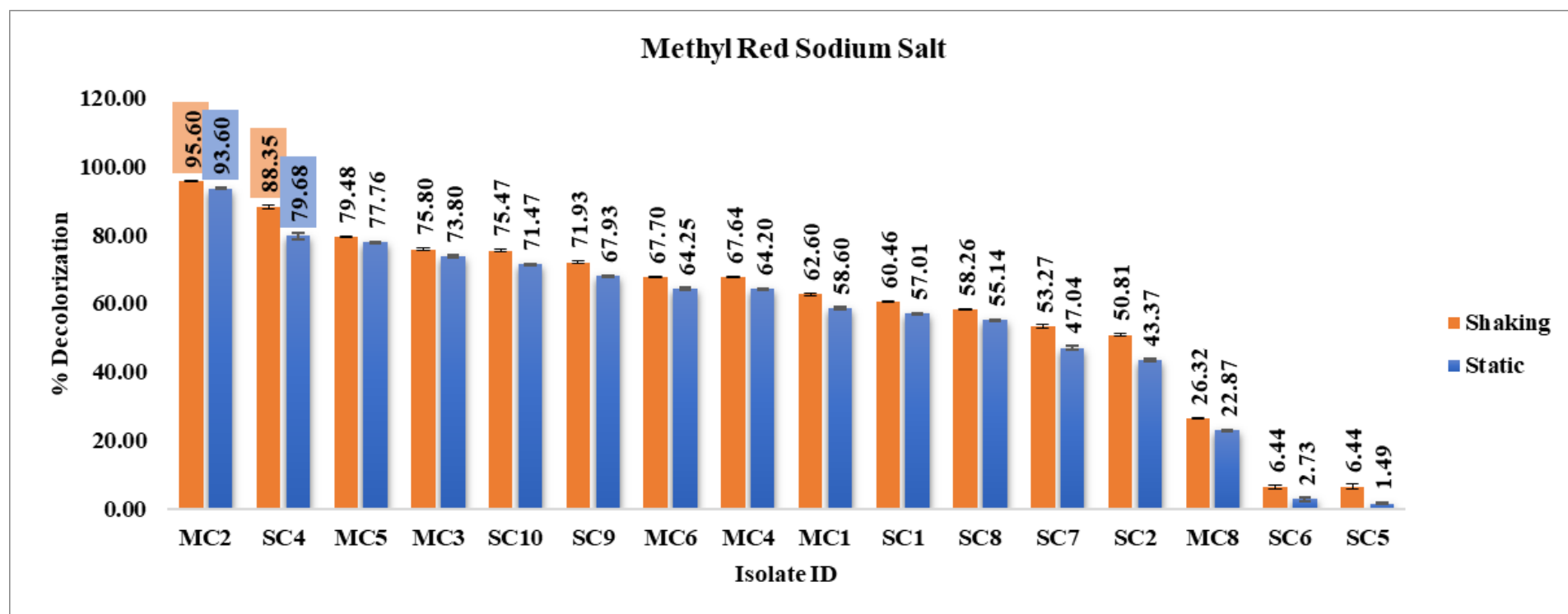


Figure 5.12: Comparative percentage decolorization of MRSS by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

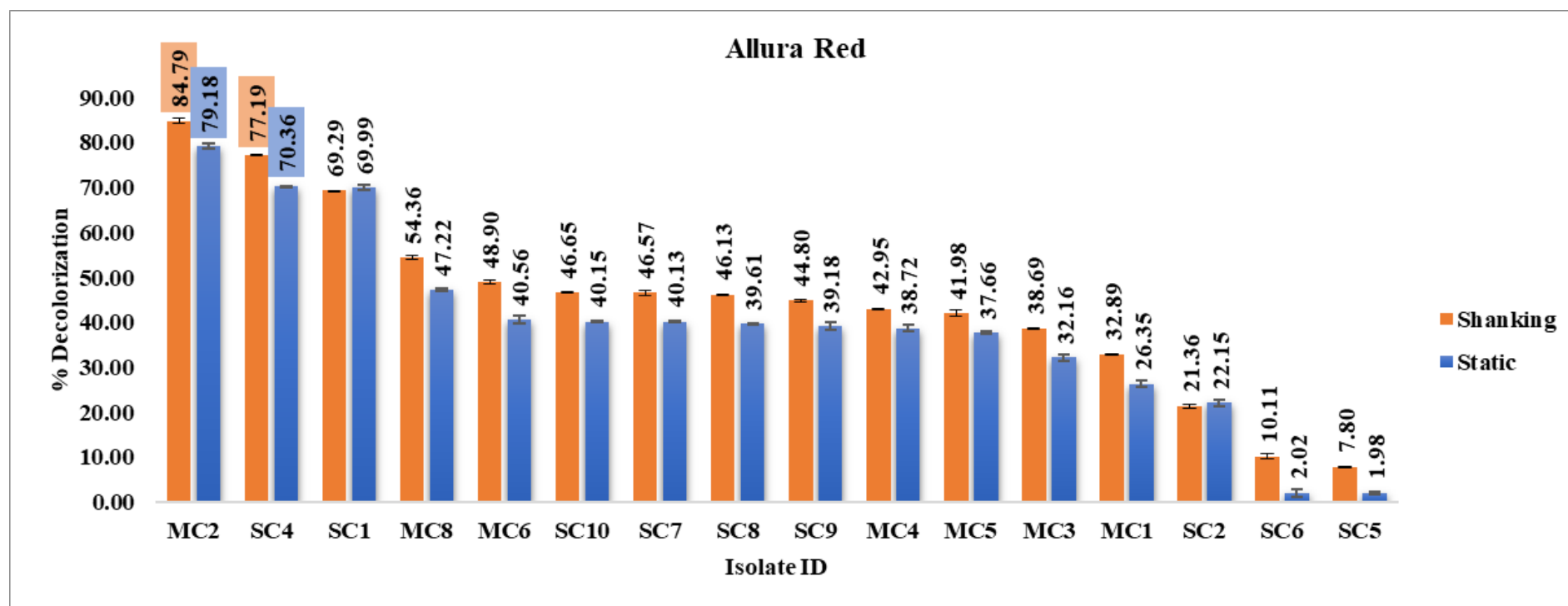


Figure 5.13: Comparative percentage decolorization of AR by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

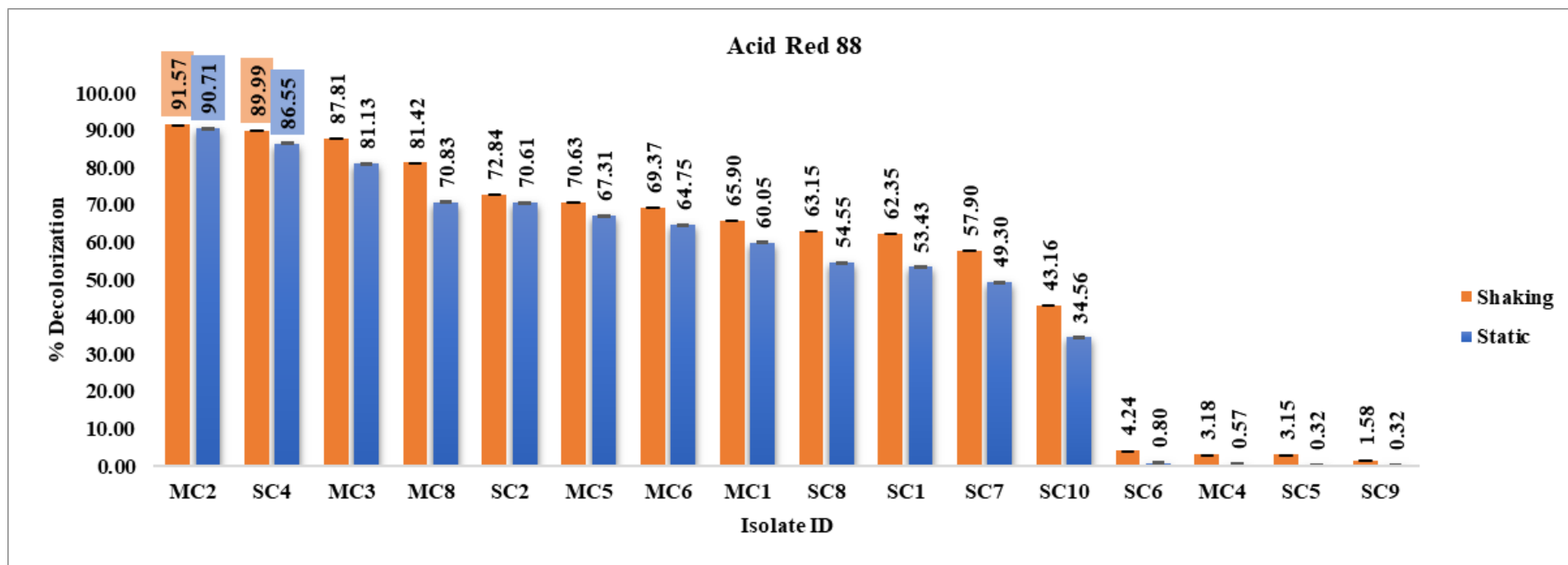


Figure 5.14: Comparative percentage decolorization of AR88 by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

Graphical representation of comparative decolorization efficiency of Kashmir isolates:

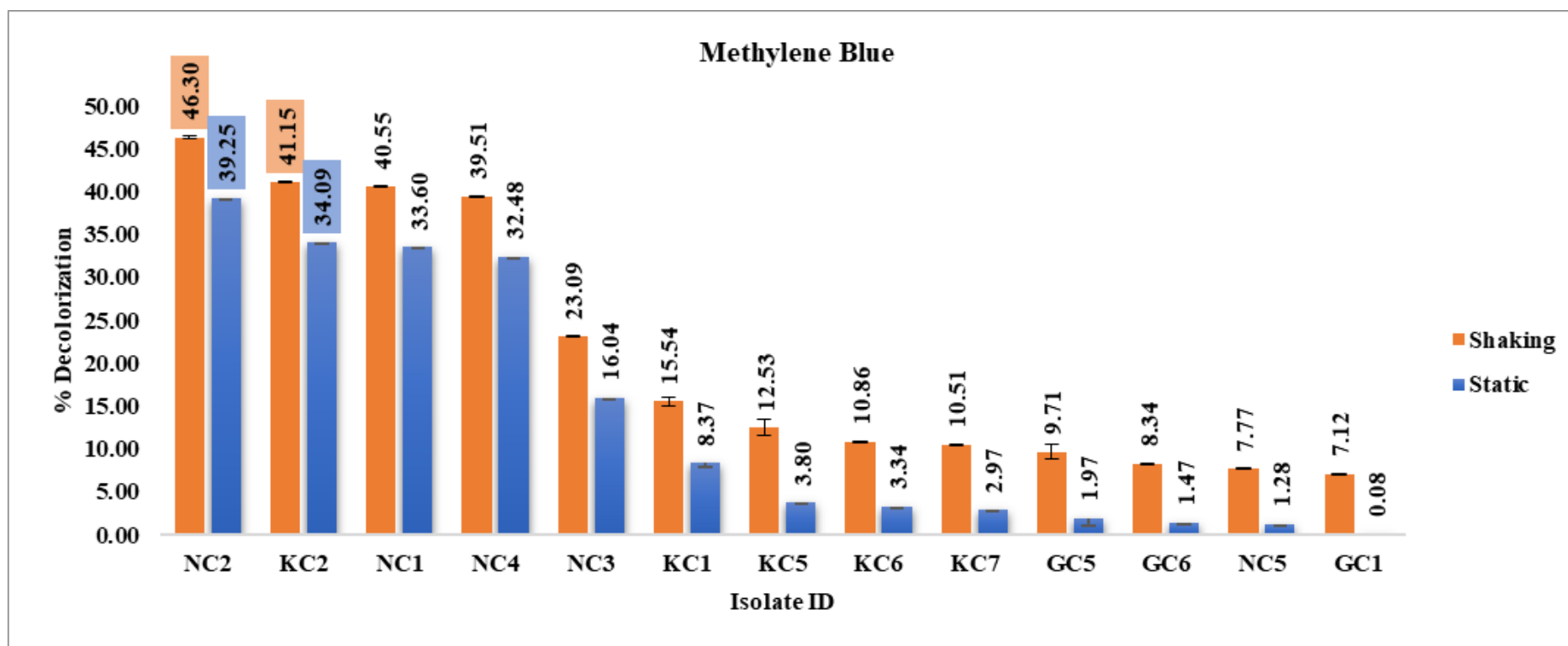


Figure 5.15: Comparative percentage decolorization of MB by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

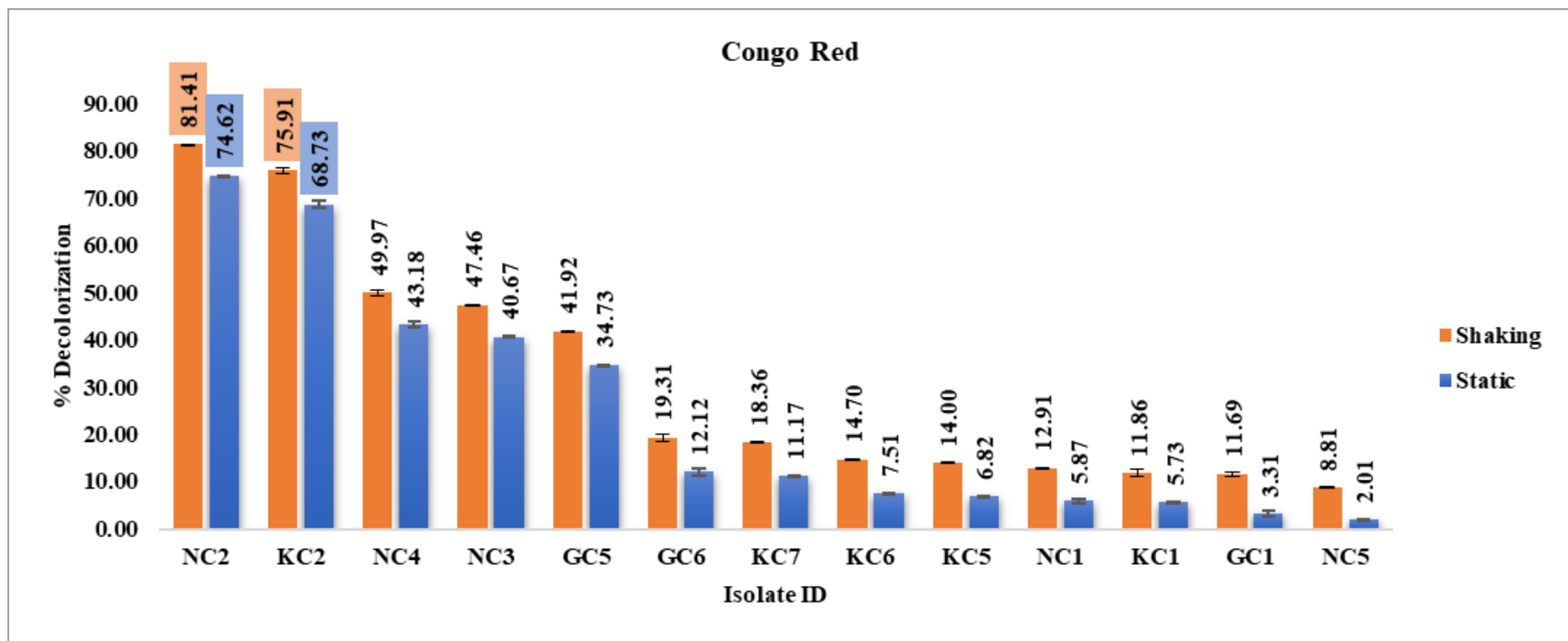


Figure 5.16: Comparative percentage decolorization of CR by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

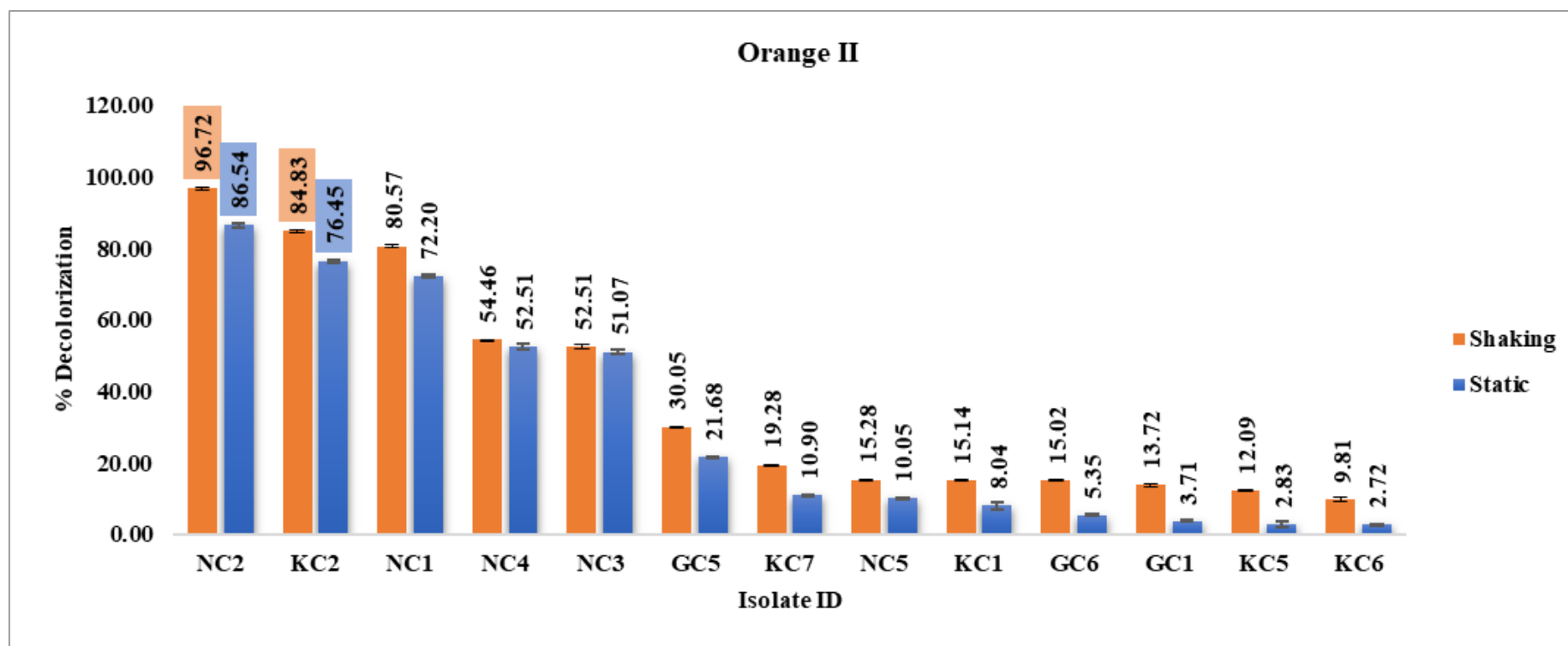


Figure 5.17: Comparative percentage decolorization of O-II by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

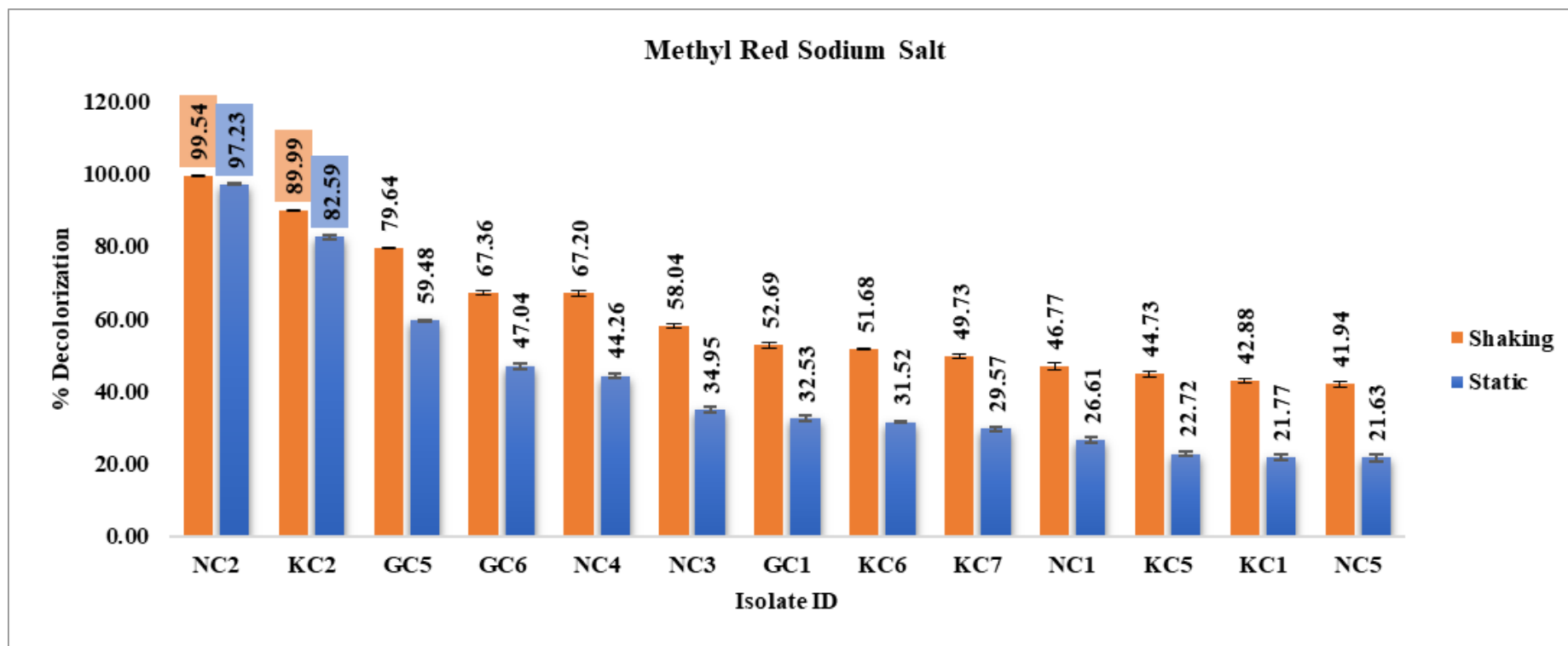


Figure 5.18: Comparative percentage decolorization of MRSS by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

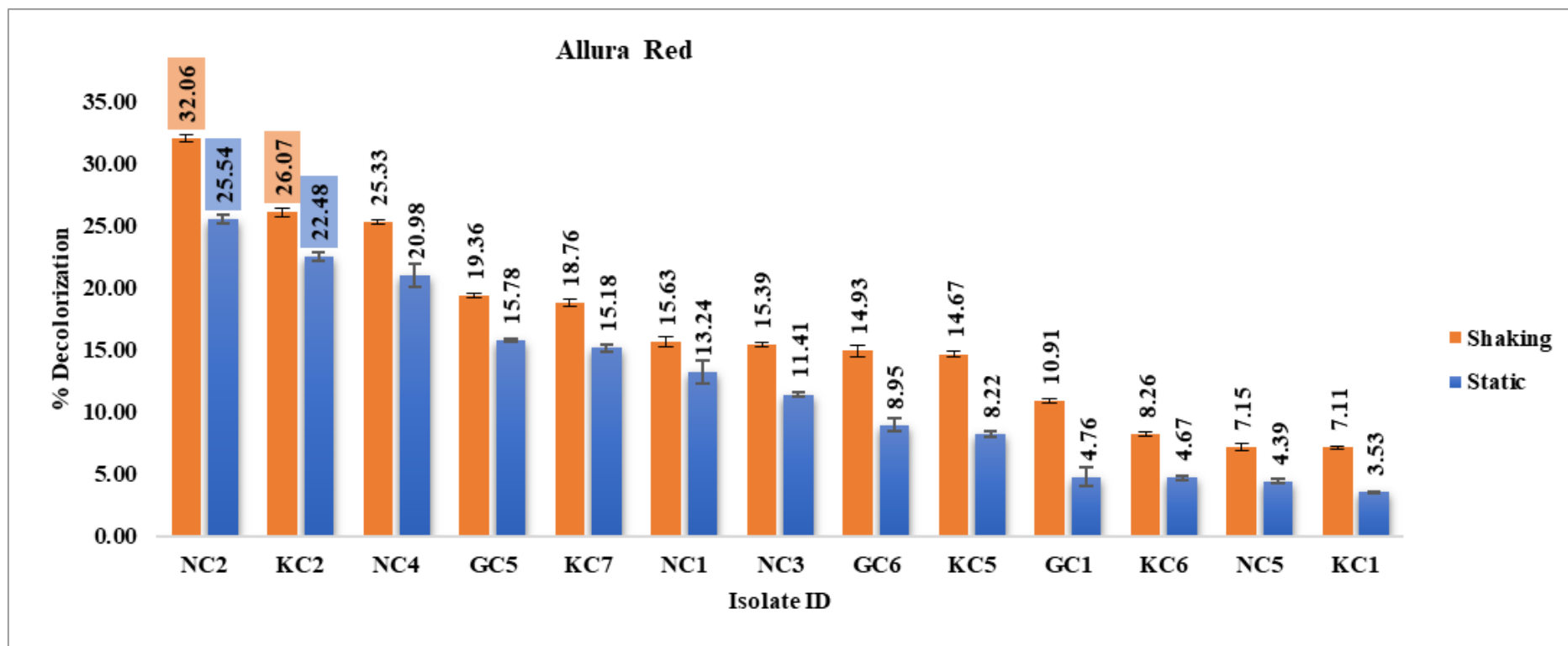


Figure 5.19: Comparative percentage decolorization of AR by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

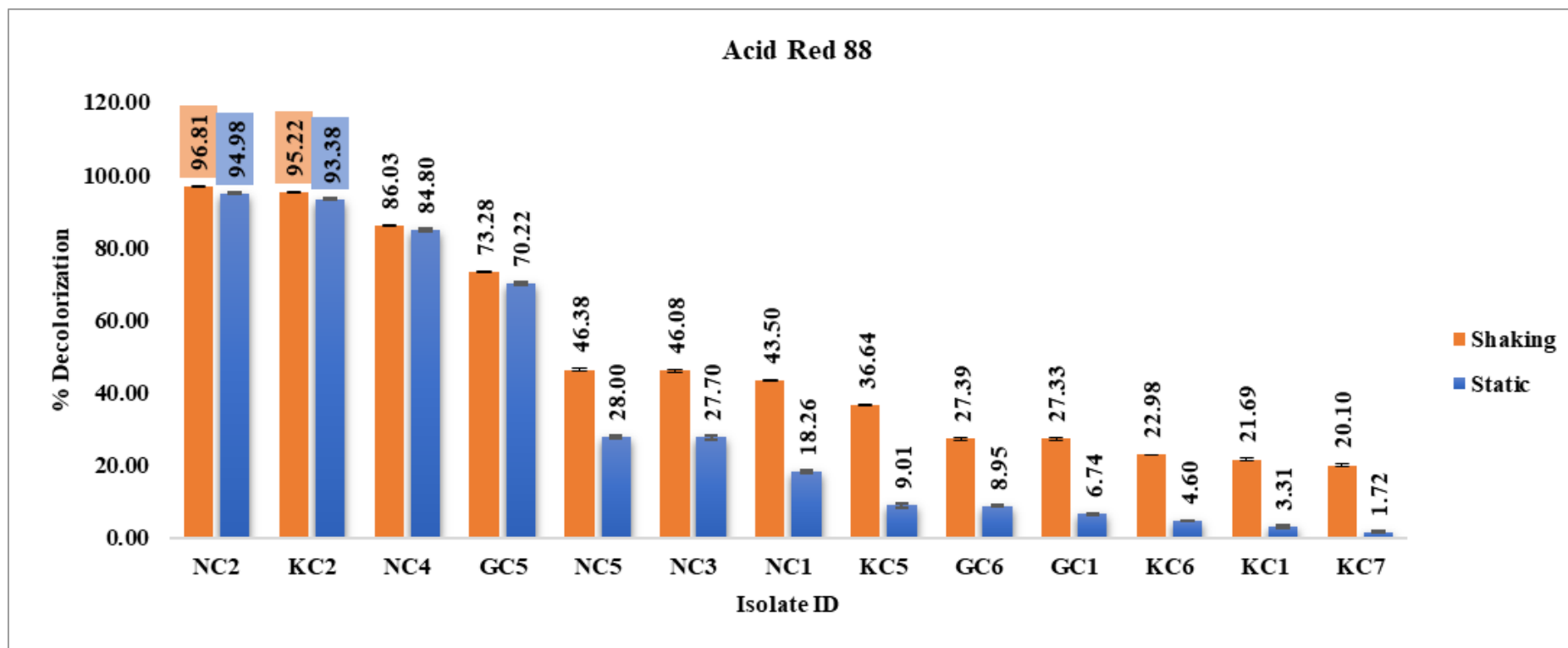


Figure 5.20: Comparative percentage decolorization of AR88 by different isolates under shaking and static incubation conditions, showing variation in dye degradation efficiency among isolates.

To select efficient strains for further analysis, a cut-off criterion of 80 % decolorization efficiency was applied. Only isolates meeting this threshold were considered significant and retained for subsequent studies. The application of such selection criteria is widely practiced in biodegradation research to ensure the selection of metabolically active and high-performing strains suitable for practical applications (Bozkurt et al., 2026). Based on the results obtained from UV–Vis spectrophotometric analysis, four bacterial isolates SC4, MC2, KC2, and NC2 showed consistently high decolorization efficiency across all the tested dyes under both static and shaking conditions. These isolates were therefore identified as the most potent dye-degrading isolates among the screened microbial isolates.

5.7 Morphological characteristics

5.7.1 Colony morphology, cell morphology, and gram staining

Among all the isolates screened, four isolates SC4, MC2, KC2, and NC2 showed the highest dye-decolorization efficiency and were selected for further identification. The morphological and staining observations revealed that these isolates belonged to different bacterial genera, each with unique structural and pigmentation traits. The isolate SC4, obtained from Surinsar wetland, formed creamy-white, smooth, opaque colonies with a sticky texture, indicating capsule formation (Figure 5.21c). Microscopic examination showed rod-shaped cells, and gram staining confirmed it as gram-negative (Figure 5.22a). Similarly, the isolate MC2, obtained from Mansar wetland, showed moist, smooth, yellowish colonies that were slightly convex and opaque (Figure 5.21b). Microscopic and gram staining results also identified MC2 as a gram-negative rod (Figure 5.22b). The isolate KC2, obtained from Khushal Sar, showed off-white to light gray colonies with a dry, rough surface (Figure 5.21d). Microscopic observation revealed that the cells were rod-shaped and gram-negative (Figure 5.22c). The isolate NC2, obtained from Nigeen Lake, showed orange to yellowish-orange colonies with smooth surfaces and entire margins (Figure 5.21e). Microscopic observation showed rod-shaped cells, and gram staining indicated gram-positive nature (Figure 5.22d). The observed variations in colony morphology and gram characteristics reflect the physiological diversity among the isolates and their possible adaptation to different wetland environments. The presence of both gram-positive and gram-negative bacteria indicates that the wetlands support a diverse microbial population capable of utilizing different metabolic pathways for dye degradation (Zhao et al., 2023).

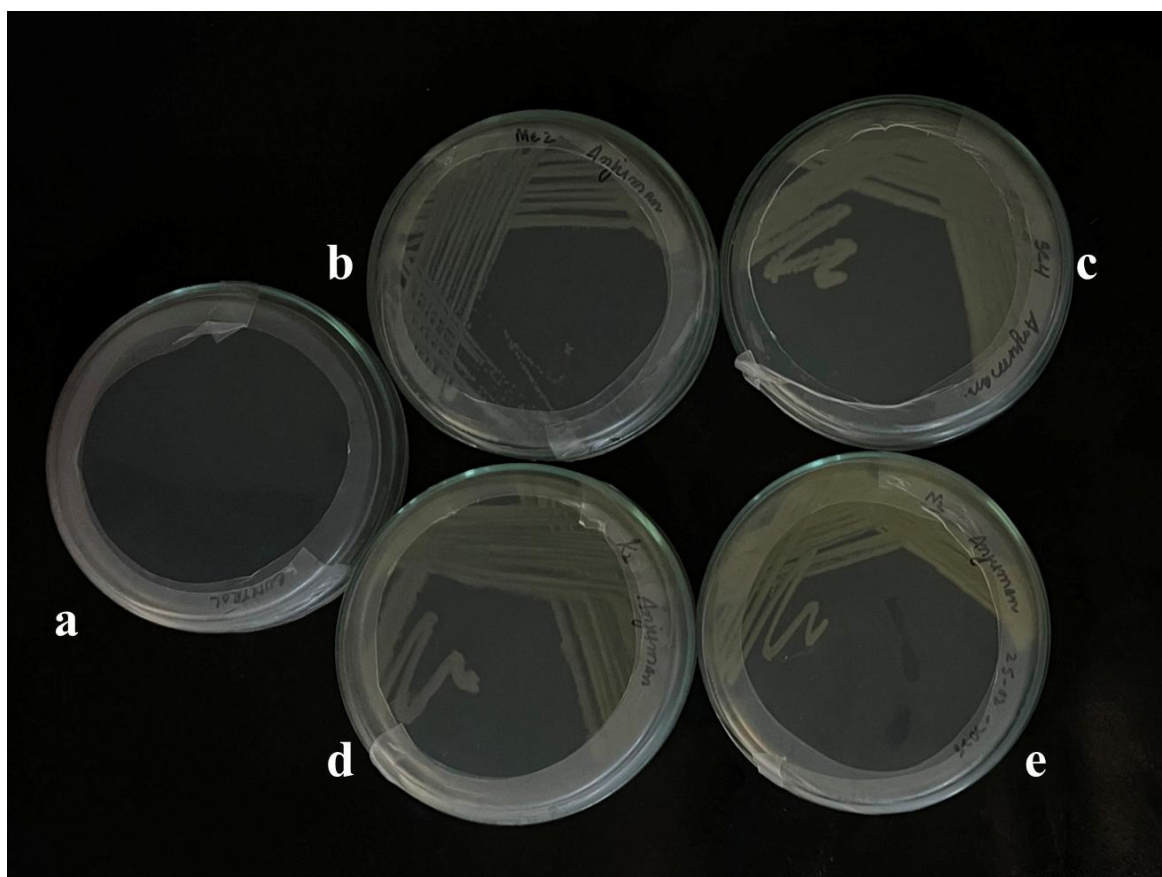


Figure 5.21: Isolates exhibited distinct physical characteristics after streaking and incubation on nutrient agar plates, (a: Control), (b:MC2), (c:SC4), (d: KC2) and (e:NC2).

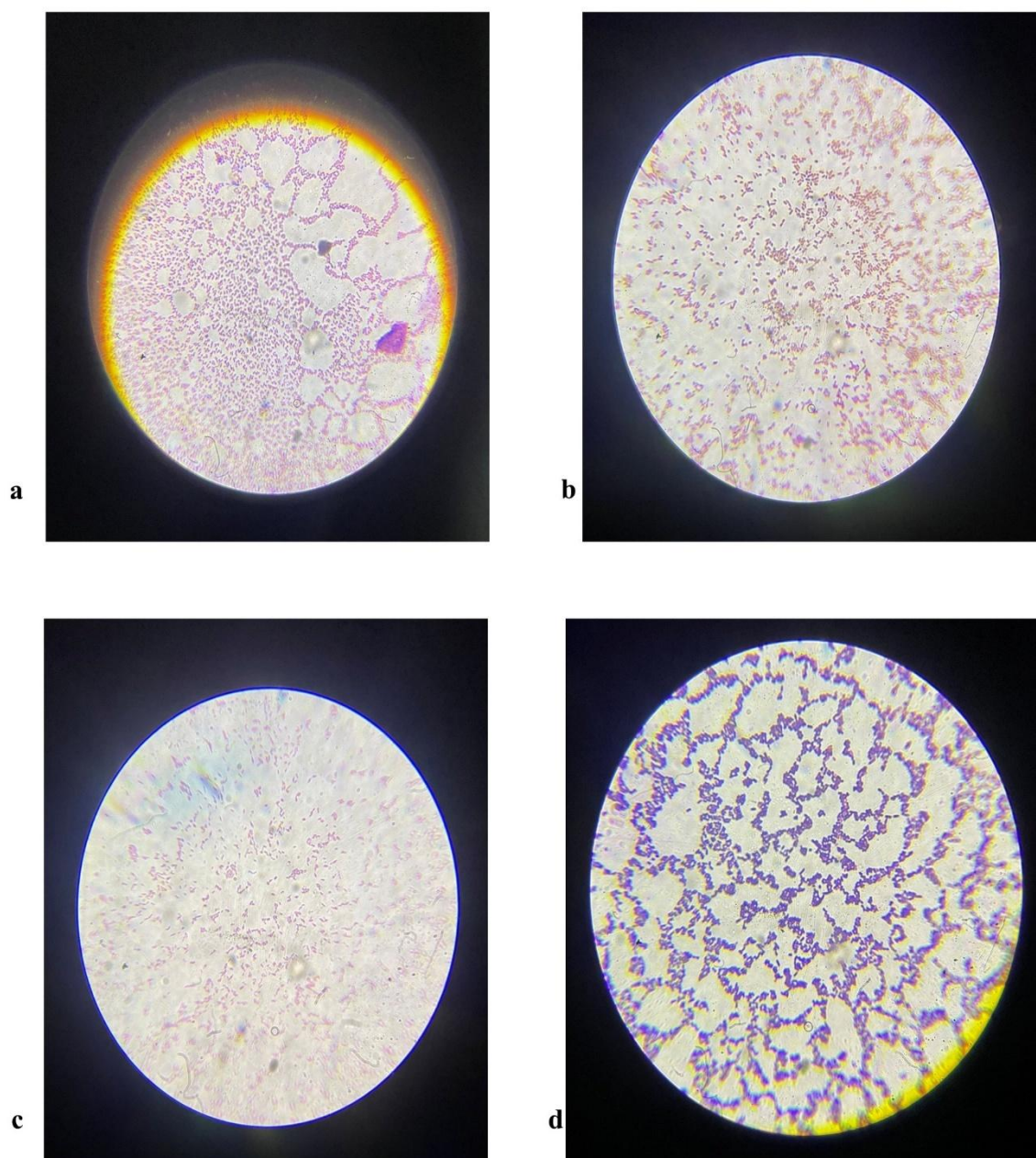


Figure 5.22: Gram staining and microscopic morphology of selected dye-decolorizing isolates: (a) SC4, (b) MC2, (c) KC2, and (d) NC2, observed under oil immersion.

5.7.2 Biochemical characterization of isolates SC4, MC2, KC2 and NC2

Biochemical tests provide a quick and cost-effective way to identify and classify bacteria based on their metabolic abilities. These tests evaluate how bacteria use specific nutrients or produce certain enzymes and byproducts, helping to group isolates according to their metabolic characteristics. In this study, all bacterial isolates were subjected to a series of biochemical tests to determine their metabolic properties and phenotypic traits. The tests were performed using

the Hi Assorted™ Biochemical Test Kits (KB001 and KB002) according to the manufacturer’s instructions. The assays examined activities such as citrate and lysine utilization, urease activity, nitrate reduction, and carbohydrate fermentation. The biochemical results showed a wide range of metabolic capabilities among the bacterial isolates, indicating high metabolic diversity (Zhao et al., 2023). The carbohydrate utilization patterns helped in grouping the isolates based on their ability to ferment different sugars. Overall, these results suggest that the isolates have developed diverse adaptive mechanisms to survive in their specific wetland environments. The detailed biochemical profiles of the isolates are summarised in (Table5.5) and the visual representation of biochemical reactions is provided in (Figure 5.23).

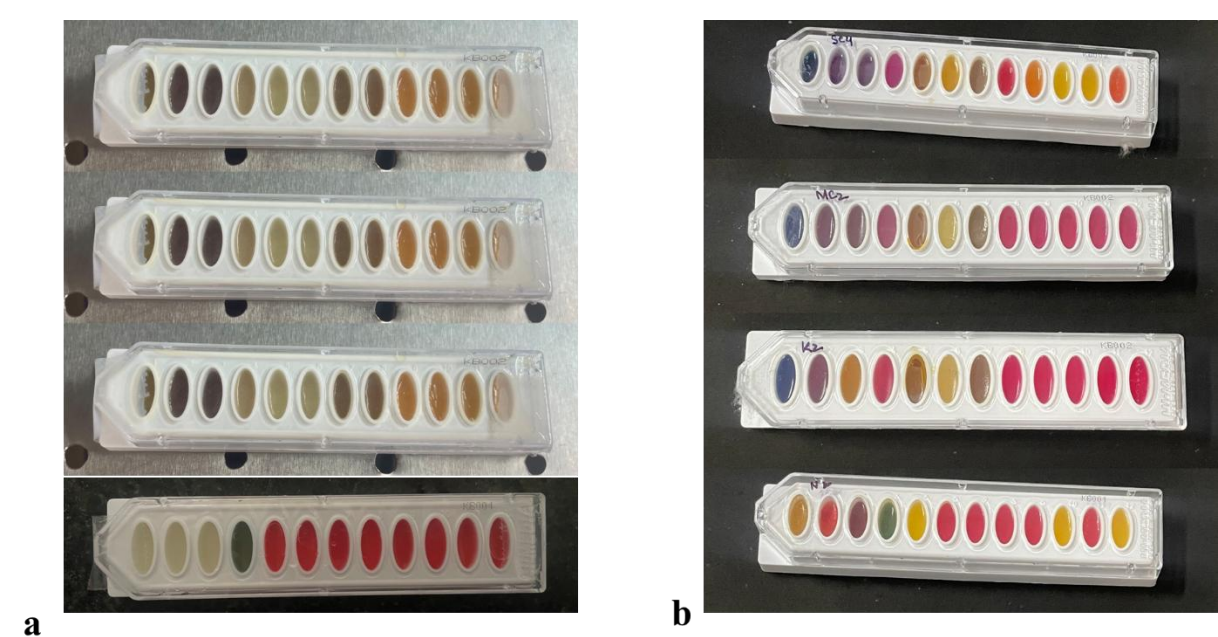


Figure 5.23: Biochemical characterization of selected high-performing dye-decolorizing isolates using Hi Assorted™ Biochemical Test Kits (KB001 and KB002). The (a) left panel shows the biochemical test kits before incubation, while the (b) right panel shows the corresponding color changes after incubation for SC4, MC2, KC2, and NC2, indicating different biochemical reactions of the isolates.

Table 5.5: Biochemical test results of selected dye-decolorizing isolates SC4, MC2, KC2, and NC2.

Test	Gram negative			Test	Gram positive
	Isolate SC4	Isolate MC2	Isolate KC2		Isolate NC2

Citrate utilization	+ve	+ve	+ve	Indole	-ve
Lysine utilization	+ve	+ve	+ve	Methyl red	+ve
Ornithine utilization	+ve	-ve	-ve	Voges Proskauer's	+ve
Urease	+ve	+ve	+ve	Citrate utilization	-ve
Phenylalanine Deamination	-ve	-ve	-ve	Glucose	+ve
Nitrate reduction	-ve	-ve	-ve	Adonitol	-ve
H₂S production	+ve	+ve	-ve	Arabinose	-ve
Glucose	-ve	-ve	-ve	Lactose	-ve
Adonitol	+ve	-ve	-ve	Sorbitol	-ve
Lactose	+ve	-ve	-ve	Maintol	+ve
Arabinose	+ve	-ve	-ve	Rhamnose	-ve
Sorbitol	-ve	-ve	-ve	Sucrose	+ve

5.8 Molecular characterization of SC4, MC2, KC2, and NC2

5.8.1 Genomic DNA extraction and quality assessment

High-quality genomic DNA was successfully extracted from all four isolates SC4, MC2, KC2, and NC2. The extracted DNA appeared as clear, intact high-molecular-weight (HMW) bands when resolved on a 0.8% agarose gel (Figure 5.24), indicating minimal degradation. The presence of sharp and prominent bands confirmed that the DNA was suitable for downstream applications such as PCR amplification and sequencing.

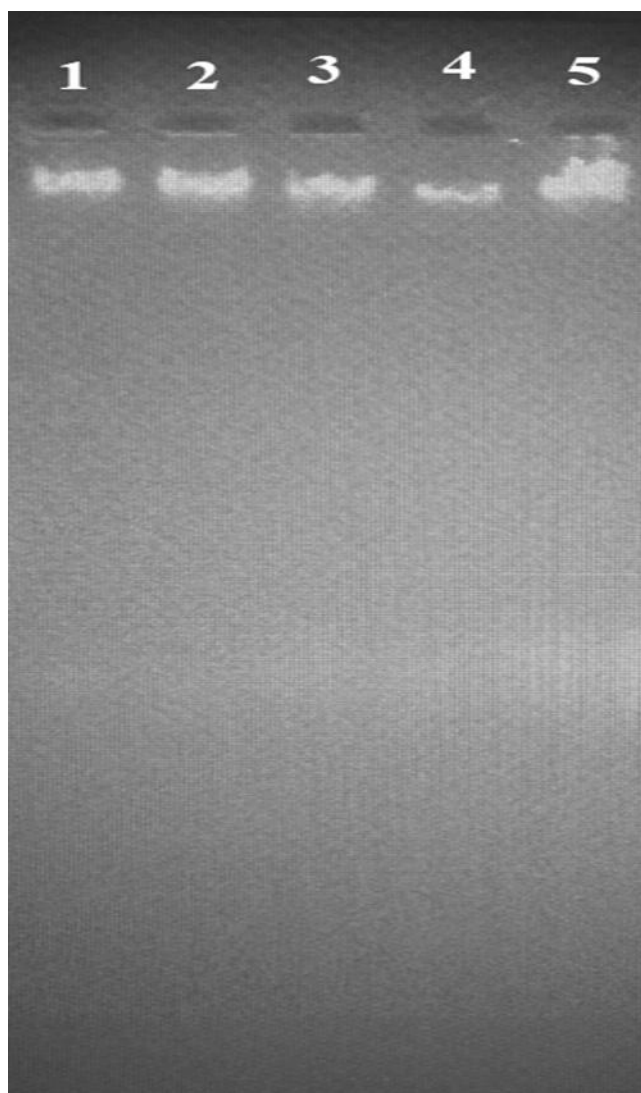


Figure 5.24: 0.8% Agarose gel electrophoresis of isolated DNA from Bacteria SC4, MC2, KC2 and NC2 with uncut lambda isolated from Wetlands J & k. Well 1: Uncut Lambda DNA. Well 2: DNA extract (SC4); Well 3: DNA extract (MC2), Well 4: DNA extract (KC2) and Well 5: DNA extract (NC2)

5.8.2 Spectrophotometric analysis of DNA purity and concentration

Spectrophotometric analysis further confirmed the good quality of the extracted DNA. The A₂₆₀/280 ratios for all isolates ranged from 1.79 to 1.93, which falls within the acceptable range for pure DNA, indicating low protein contamination. The highest concentration was observed in isolate KC2 (916 ng/μl), followed by NC2, MC2, and SC4 (Table 5.6).

Table 5.6: Spectrophotometric analysis of Isolated DNA from SC4, MC2, KC2 and NC2:

Sample	A ₂₆₀ /280	Concentration (ng/μl)	Quantity used(ng)

SC4	1.80	810	1620
MC2	1.93	871.650	1743
KC2	1.832	916	1832
NC2	1.792	896	1792

5.8.3 PCR amplification and phylogenetic characterization of 16s rRNA gene

Amplification of the 16S rRNA gene was carried out for all bacterial isolates using universal primers 27F and 1492R. The PCR reactions resulted in successful amplification, which was confirmed by the appearance of clear and distinct bands corresponding to the expected amplicon size on a 1.8 % agarose gel (Figure 5.25). The consistent amplification across all isolates indicated the reliability of the PCR conditions and the quality of the extracted genomic DNA. The amplified PCR products were subsequently purified and sent for sequencing. The obtained 16S rRNA gene sequences were analyzed using the BLASTn tool available at the NCBI GenBank database to determine the closest phylogenetic affiliations of the isolates (Table 5.7). Sequence similarity analysis revealed a high degree of homology with known bacterial species, enabling accurate taxonomic identification. Further phylogenetic analysis was performed using MEGA 11 software, where multiple sequence alignment and phylogenetic tree construction were used to assess evolutionary relationships. The resulting phylogenetic trees placed all four isolates within their respective evolutionary lineages, thereby confirming their molecular identity and supporting the taxonomic classification of the isolates (Wang et al., 2025).

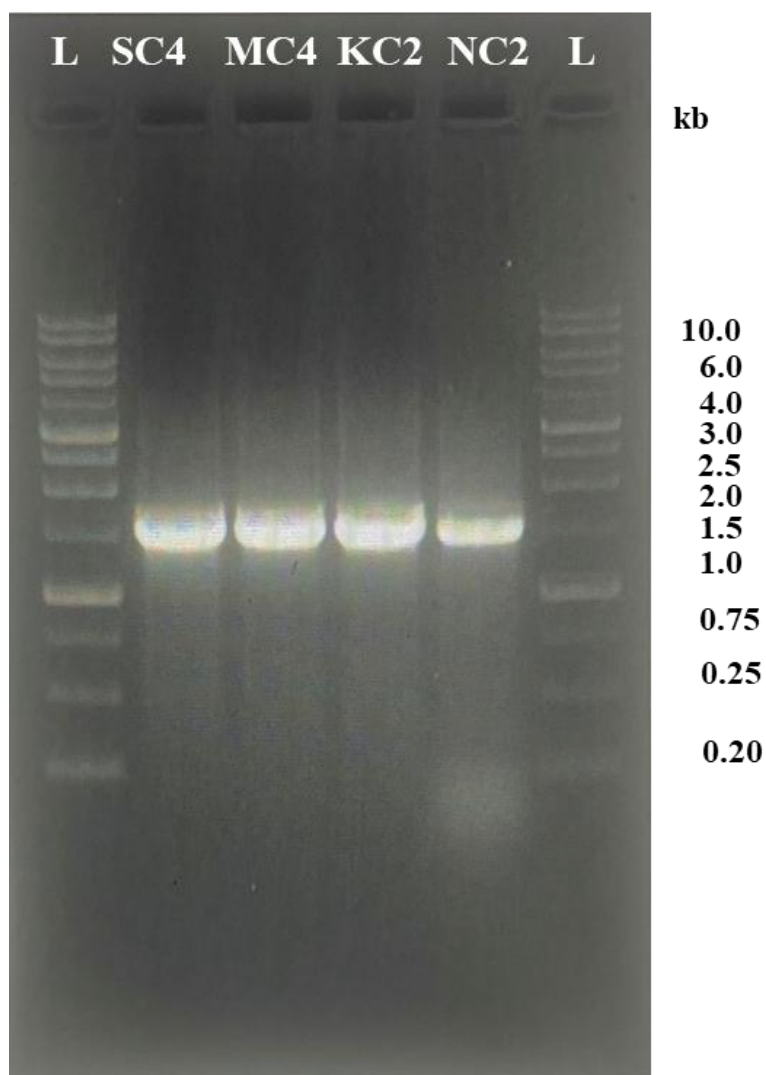


Figure 5.25: 1.8 % Agarose gel electrophoresis of isolated PCR DNA from isolate SC4, MC2, KC2 and NC2 with 10kb DNA ladder isolated from soil and water sample wetlands of J&K. Well 1: DNA Ladder. Well 2: PCR DNA (SC4); Well 3: PCR DNA (MC2), Well 4: PCR DNA (KC2), Well 5: PCR DNA from (NC2) and Well 6: 1: DNA Ladder).

5.8.3.1 Phylogenetic analysis of isolate SC4

The phylogenetic tree (Figure 5.26) based on 16S rRNA gene sequences revealed that the isolate SC4 clustered within the *Klebsiella pneumoniae* species complex. The isolate grouped closely with reference strains of *Klebsiella quasipneumoniae* subsp. *similipneumoniae*, particularly with *K. quasipneumoniae* subsp. *similipneumoniae* strain 07A044, supported by a high bootstrap value 88 %. The clustering clearly separated the isolate from other closely related species such as *Klebsiella pneumoniae* subsp. *rhinoscleromatis*, *Klebsiella africana*, and *Klebsiella quasipneumoniae* subsp. *quasipneumoniae*, indicating distinct phylogenetic

positioning. The strong bootstrap support across the major nodes confirms the robustness of the tree topology and validates the taxonomic placement of isolate SC4 as *Klebsiella* sp.SC4.

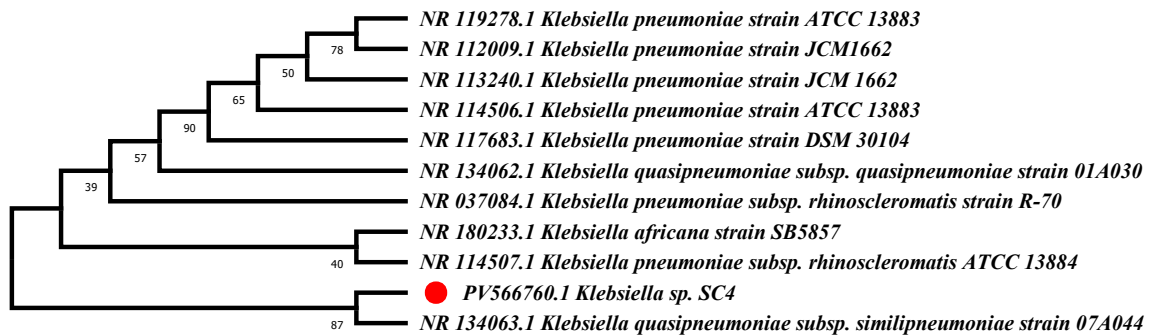


Figure 5.26: Phylogenetic tree based on 16S rRNA gene sequences showing the evolutionary relationship of isolate SC4 with closely related species of the genus *Klebsiella*.

5.8.3.2 Phylogenetic analysis of isolate MC2

Phylogenetic analysis (Figure 5.27) of the 16S rRNA gene sequence showed that isolate MC2 clustered within the genus *Stenotrophomonas*. The isolate formed a well-supported clade with *Stenotrophomonas pavanii* reference strains, particularly *S. pavanii* strain LMG 25348, indicating close evolutionary relatedness. The *S. pavanii* cluster was clearly distinct from other *Stenotrophomonas* species such as *S. maltophilia*, *S. hibiscicola*, *S. beteli*, and *S. geniculata*. Bootstrap values at the internal nodes supported the reliability of the clustering pattern. Based on its phylogenetic position and sequence similarity, isolate MC2 was identified as *Stenotrophomonas* sp.MC2.

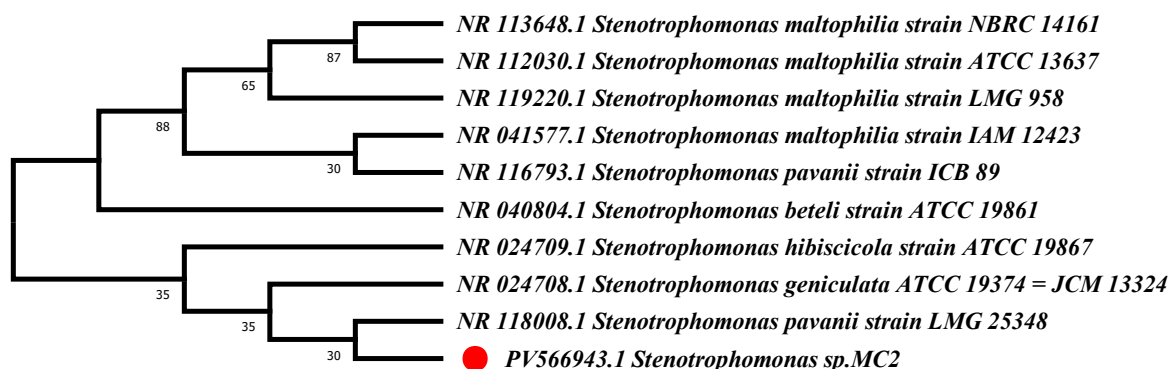


Figure 5.27: Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship of isolate MC2 with representative species of the genus *Stenotrophomonas*.

5.8.3.3 Phylogenetic analysis of isolate KC2

The 16S rRNA gene-based phylogenetic tree (Figure 5.28) demonstrated that isolate KC2 clustered within the genus *Alcaligenes*. The isolate showed the closest phylogenetic affiliation with *Alcaligenes faecalis* strains, forming a coherent clade with *A. faecalis* NBRC 13111 and *A. faecalis* IAM 12369. The clustering was supported by moderate to high bootstrap values, indicating reliable phylogenetic placement. The isolate was clearly separated from other related species such as *Alcaligenes pakistanensis*, *A. aquatilis*, *A. parafaecalis*, and *A. ammonioxydans*. More distantly related genera, including *Paralcaligenes* and *Pollutimonas*, formed separate branches. These results confirm that isolate KC2 belongs to the species *Alcaligenes* sp.KC2.

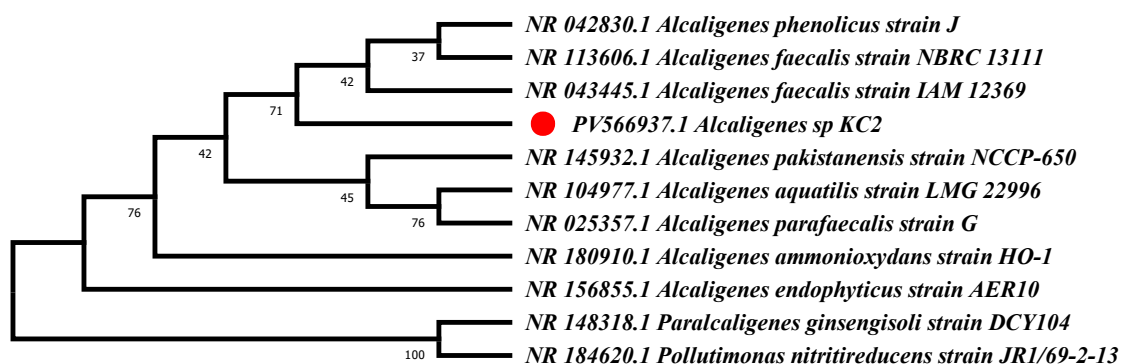


Figure 5.28: Phylogenetic analysis of isolate KC2 based on 16S rRNA gene sequences, showing its evolutionary position among species of the genus *Alcaligenes* and related genera.

5.8.3.4 Phylogenetic analysis of isolate NC2

The phylogenetic analysis (Figure 5.29) based on 16S rRNA gene sequences revealed that isolate NC2 belongs to the genus *Exiguobacterium*. In the neighbor-joining phylogenetic tree, the isolate clustered within the *Exiguobacterium indicum* clade and showed the closest evolutionary relationship with *E. indicum* reference strains HHS 31 and NIO-1109. This clustering was supported by a high bootstrap value (93%), indicating strong confidence in the phylogenetic placement. The *E. indicum* clade was clearly separated from other closely related *Exiguobacterium* species, including *E. acetylicum*, *E. oxidotolerans*, *E. artemiae*, *E. undae*, and *E. antarcticum*. Distinct clustering of these species with high bootstrap support further confirmed the robustness of the tree topology. Based on the phylogenetic positioning and sequence similarity, isolate HHS 31 was conclusively identified as *Exiguobacterium indicum*.

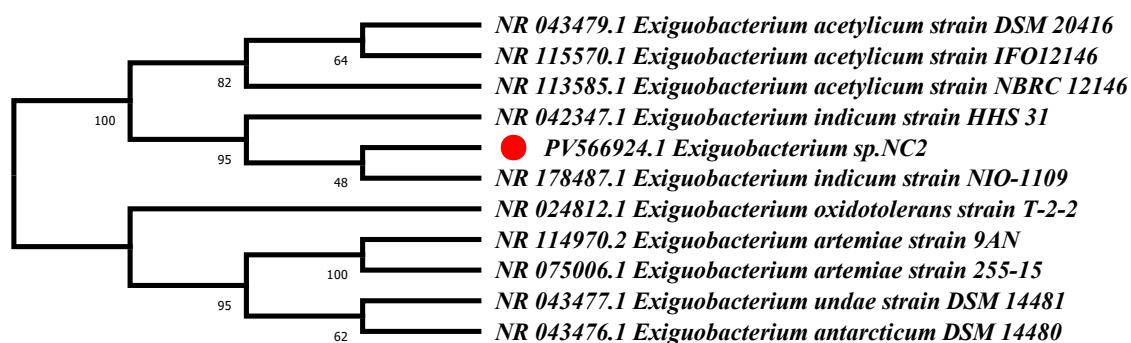


Figure 5.29: Neighbor-joining phylogenetic tree derived from 16S rRNA gene sequences depicting the relationship of NC2 with closely related *Exiguobacterium* species.

Table 5.7: Molecular identification of efficient dye decolorizing bacterial isolates:

Isolate code	High similarity with	Accession No.	Query cover (%)	Percent identification
SC4	<i>Klebsiella quasipneumoniae</i> subsp. <i>similipneumoniae</i> strain 07A044	NR-134063.1	100	99.03
MC2	<i>Stenotrophomonas pavanii</i> strain LMG 25348	NR-118008.1	100	99.35
KC2	<i>Alcaligenes faecalis</i> strain NBRC 13111	NR-113606.1	100	99.35
NC2	<i>Exiguobacterium indicum</i> strain HHS 31	NR-042347.1	100	99.92

5.8.4 GenBank submission of 16S rRNA sequences of SC4, MC2, KC2 and NC2

Table 5.8: Submission ID and the accession no of the isolates:

Isolate code	Submitted as	Submission No.	Accession No
SC4	<i>Klebsiella</i> sp.SC4	SUB15281216	PV566760
MC2	<i>Stenotrophomonas</i> sp.MC2	SUB15281376	PV566943
KC2	<i>Alcaligenes</i> sp.KC2	SUB15281362	PV566937
NC2	<i>Exiguobacterium</i> sp.NC2	SUB15281312	PV566924

5.9 Antibiotic susceptibility testing of bacterial isolates:

The antibiotic resistance patterns of the selected dye-decolorizing bacterial isolates SC4, MC2, KC2, and NC2 were evaluated using the disc diffusion method on nutrient agar plates. After incubation, clear zones of inhibition were observed around several antibiotic discs, indicating varying levels of susceptibility among the isolates (Figure 5.30). The measured diameters of the inhibition zones are presented in Table 5.9. As shown in Table 5.9, the isolate SC4 showed high sensitivity to meropenem (2.50 cm), norfloxacin (2.03 cm), and gentamycin (2.0 cm), while no inhibition zones were observed against vancomycin and penicillin, indicating resistance to these antibiotics. MC2 exhibited maximum sensitivity to meropenem (2.8 cm) and norfloxacin (2.03 cm) but showed resistance to penicillin and tetracycline, with no visible zones of inhibition. The isolate KC2 demonstrated good sensitivity to gentamycin (2.27 cm) and streptomycin (1.7 cm), whereas it showed complete resistance to vancomycin, penicillin, and tetracycline. In contrast, isolate NC2 displayed a broader susceptibility pattern, showing inhibition zones against most tested antibiotics, including meropenem (2.2 cm), gentamycin (2.2 cm), tetracycline (2.1 cm), and penicillin (1.5 cm), indicating comparatively lower resistance. Overall, the results revealed noticeable variation in antibiotic susceptibility among the four isolates. While some isolates exhibited resistance to multiple antibiotics, others showed broad sensitivity, reflecting the diverse antimicrobial resistance profiles of wetland-derived bacteria (Syal et al., 2017). These findings highlight the environmental adaptability of the isolates and provide important information for assessing their biosafety and potential application in bioremediation processes.

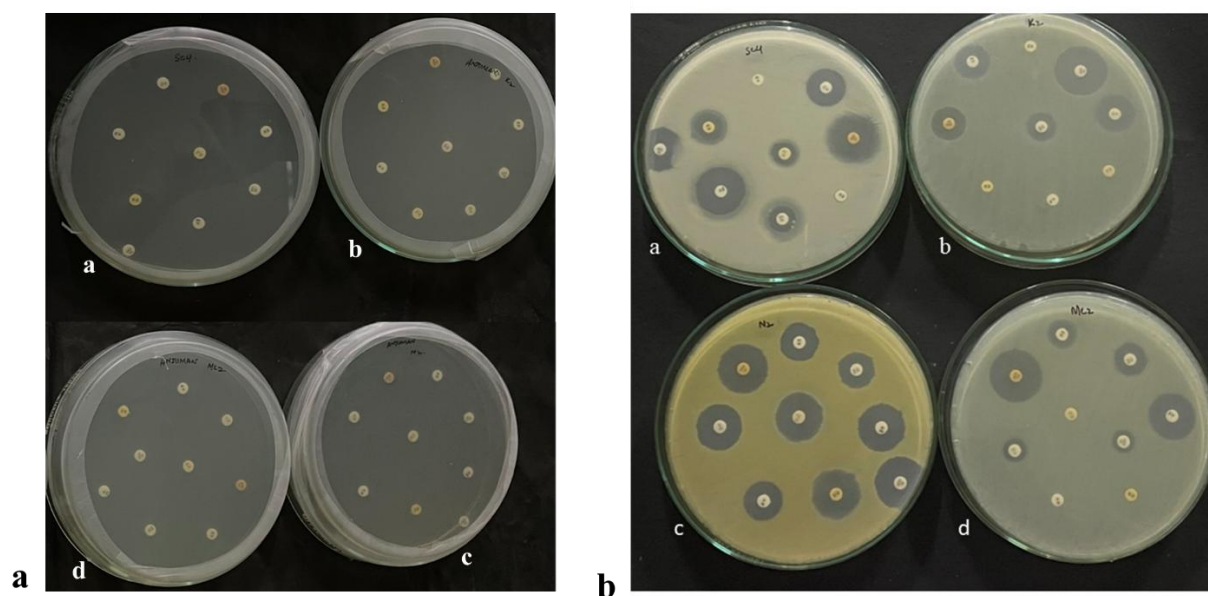


Figure 5.30: Antibiotic susceptibility patterns of selected dye-decolorizing bacterial isolates using the disc diffusion method. The left panel shows the antibiotic discs placed on inoculated plates before incubation, while the right panel shows the zones of inhibition formed after 24 h incubation for (a) SC4, (b) KC2, (c) NC2, and (d) MC2 against different antibiotics.

Table 5.9: Antibiotic susceptibility pattern of selected dye-decolorizing bacterial isolates (SC4, MC2, KC2, and NC2) determined by the disc diffusion method, expressed as diameter of inhibition zones (cm).

Antibiotic (Disc potency)	SC4 (cm)	MC2 (cm)	KC2 (cm)	NC2 (cm)
Streptomycin (10 µg)	1.70 ± 0.05	1.31 ± 0.00	1.70 ± 0.00	1.50 ± 0.00
Meropenem (10 µg)	2.50 ± 0.05	2.80 ± 0.00	1.50 ± 0.00	2.20 ± 0.05
Vancomycin (30 µg)	0.00 ± 0.00	1.70 ± 0.00	0.00 ± 0.00	1.60 ± 0.00
Norfloxacin (10 µg)	2.03 ± 0.05	2.03 ± 0.03	1.51 ± 0.00	1.90 ± 0.05
Nalidixic acid (30 µg)	1.00 ± 0.05	1.30 ± 0.00	1.10 ± 0.05	1.50 ± 0.00
Gentamycin (10 µg)	2.00 ± 0.05	0.70 ± 0.05	2.27 ± 0.11	2.20 ± 0.05
Penicillin (30 µg)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.50 ± 0.00
Tetracycline (10 µg)	1.10 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	2.10 ± 0.05

5.10 Compatibility testing of bacterial isolates for consortium development

Before developing microbial consortium, the compatibility of the selected dye-decolorizing bacterial isolates SC4, MC2, KC2, and NC2 was evaluated using the cross-streaking method on nutrient agar plates. All four isolates were streaked on a single plate in an intersecting pattern to allow direct interaction and to observe any inhibitory effects among the strains. The plates were incubated at 37°C for 24 h, and growth was examined along the intersecting streak lines. The results showed that all four isolates were able to grow well in close proximity without the formation of inhibition zones at the points of intersection (Figure 5.31). Continuous and uninterrupted growth was observed for each isolate, indicating the absence of antagonistic interactions. None of the isolates exhibited growth suppression or clearing zones against the others. These findings confirm that the selected isolates are mutually compatible and can coexist under the same growth conditions. The absence of antagonism suggests that these strains can be safely combined to form a stable microbial consortium for enhanced dye decolorization and bioremediation applications (MOR et al., 2025).

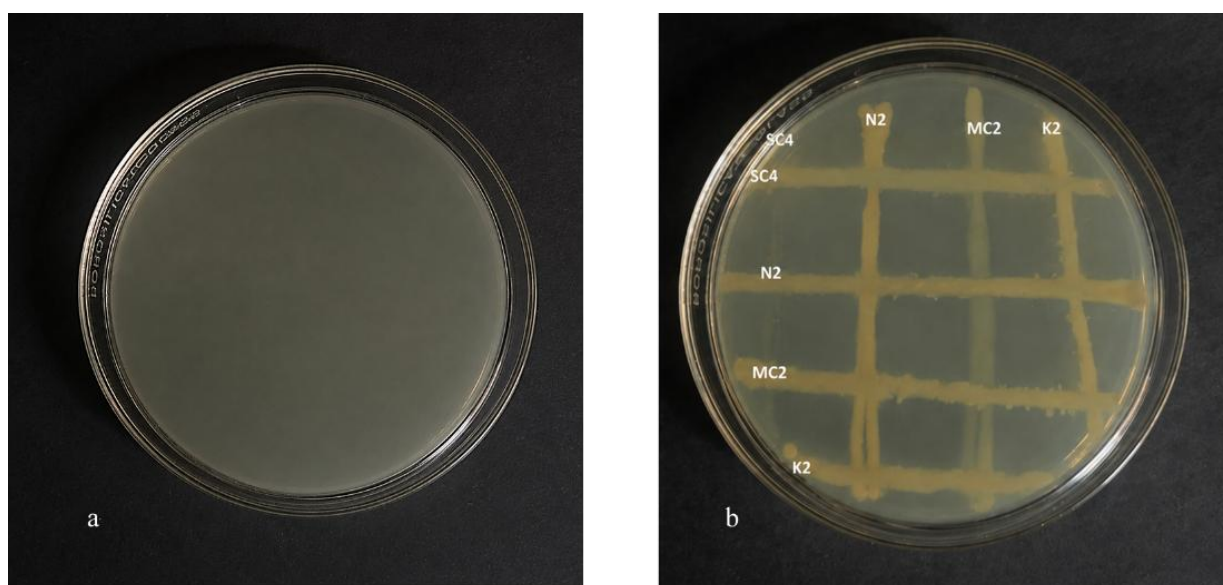


Figure 5.31: Cross-streak compatibility assay of selected dye-decolorizing isolates (SC4, MC2, KC2, and NC2) on nutrient agar: (a) control plate and (b) plate showing absence of inhibitory interactions among the isolates.

Dye selection for detailed degradation studies was carried out based on the established decolorization threshold 90 % obtained from UV-Vis spectrophotometric analysis. Accordingly, CR, MRSS and AR88 met this criterion, as they exhibited high and consistent decolorization efficiency with the selected bacterial isolates. CR and MRSS have been

extensively studied and are widely used as model dyes in biodegradation research. Their inclusion allows for effective comparison with previously reported studies and provides a standardized basis for evaluating microbial degradation efficiency (Chakravarthi et al., 2021; Anjali et al., 2022). In contrast, Acid Red 88 was selected due to its relatively higher toxicity and environmental relevance, making it an important target pollutant for remediation studies. The relative toxicity of the dyes investigated in recent study follows the order: CR > AR 88 > O- II > AR > MRSS (Kaur et al., 2023; Sharf Ilahi Siddiqui et al., 2023). Therefore, the inclusion of Ar88 enhances the significance of the study by addressing a comparatively more hazardous dye, while CR and MRSS serve as reference compounds for assessing biodegradation performance.

5.11 Effect of initial dye concentration on decolorization efficiency

The effect of initial dye concentration on the decolorization efficiency of the bacterial isolates NC2, KC2, MC2, SC4 and the bacterial consortium was evaluated using UV-Vis spectrophotometric analysis under optimized conditions pH 7.5, 37 °C, and 14 h incubation. Decolorization was measured at dye concentrations of 100, 200, and 300 ppm by monitoring the decrease in absorbance at the characteristic wavelength of AR88. As shown in Figure 5.32 and 5.33, all isolates and the consortium exhibited the highest decolorization efficiency at 100 ppm, followed by a gradual decline with increasing dye concentration. The bacterial consortium showed the highest decolorization efficiency, achieving 97.62% at 100 ppm, which slightly decreased to 95.35 % at 200 ppm and 94.18 % at 300 ppm, indicating strong tolerance to elevated dye concentrations. Among the individual isolates, NC2 and KC2 demonstrated high decolorization efficiency at 100 ppm, recording 95.70 % and 95.38 %, respectively, while MC2 and SC4 showed comparatively lower efficiencies of 93.06 % and 89.02 %, respectively. However, as the dye concentration increased to 200 and 300 ppm, a progressive decline in decolorization efficiency was observed for all isolates, with NC2 decreasing to 84.03% and 80.48 %, KC2 to 78.24 % and 75.17 %, MC2 to 80.38 % and 70.15 %, and SC4 to 78.11 % and 73.46 %, respectively. The high decolorization observed at 100 ppm indicates that bacterial cells were able to efficiently metabolize dye molecules when the substrate concentration remained within a tolerable range. At this concentration, dye molecules are more accessible to bacterial enzymatic systems, enabling effective cleavage of azo bonds and breakdown of chromophoric groups responsible for color formation (Ghosh et al., 2025). Similar trends have been reported in previous studies, where microbial dye degradation was found to be more efficient at moderate dye concentrations due to reduced toxic stress and enhanced enzymatic

activity (Rai et al., 2026). Although the maximum decolorization efficiency was observed at 100 ppm, this concentration also represents a moderately realistic dye load compared to industrial effluents, making it more suitable for practical applications. At higher concentrations (200-300 ppm), the reduction in decolorization efficiency may be attributed to increased dye toxicity, which can inhibit bacterial growth and metabolic activity, as well as enzyme inhibition or saturation affecting key dye-degrading enzymes such as azoreductase. Additionally, high dye concentrations may limit oxygen transfer and interfere with nutrient uptake, thereby reducing overall degradation efficiency. Notably, the bacterial consortium consistently outperformed individual isolates, which may be due to synergistic interactions among different bacterial strains, leading to enhanced metabolic versatility and more efficient degradation of dye molecules and their intermediates (Manjarrez-Paba et al., 2025). Considering both degradation efficiency and environmental relevance, 100 ppm was selected as the optimal dye concentration for subsequent optimization and degradation studies, as it ensures high, stable, and reproducible performance under moderately elevated substrate conditions.

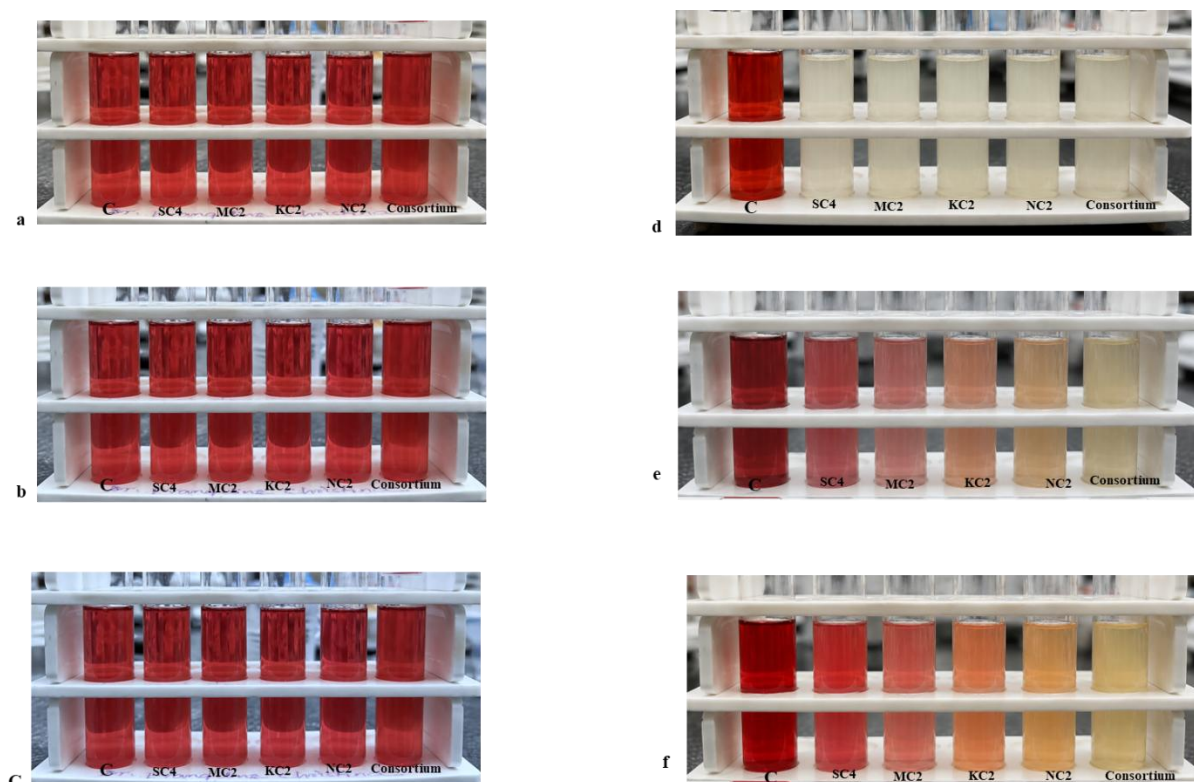


Figure 5.32: Decolorization of dye at different concentrations by selected bacterial isolates and consortium: (a-c) dye-containing media at 100 ppm, 200 ppm, and 300 ppm before incubation, and (d-f) corresponding decolorization after incubation with SC4, MC2, KC2, NC2, and the bacterial consortium.

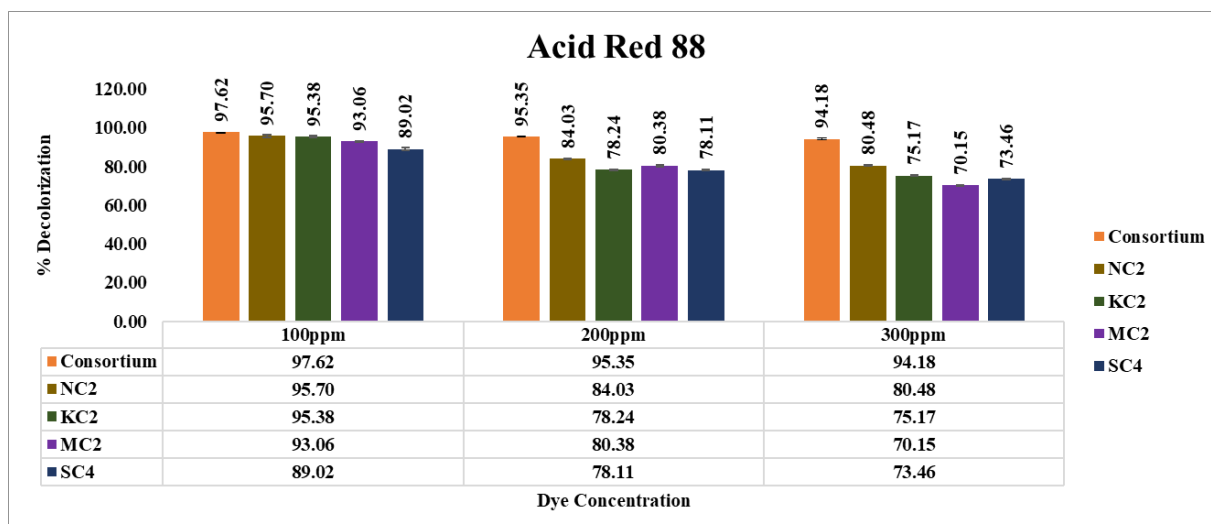


Figure 5.33: Effect of increasing dye concentration (100, 200, and 300 ppm) on the percentage decolorization of Ar88 by individual bacterial isolates (SC4, MC2, KC2, NC2) and the bacterial consortium.

5.12 Analysis of dye degradation product by FTIR and GC-MS:

5.12.1 FTIR analysis of dye AR88 and degraded products by SC4,MC2,KC2 and NC2.

FTIR analysis was performed to identify the functional group changes in Acid Red 88 after biodegradation by the bacterial isolates SC4, MC2, KC2, and NC2, and the corresponding peak positions are summarized in Figure 5.34 and Table 5.10. The comparison of peak shifts between the parent dye and degraded samples clearly indicates structural modifications caused by microbial activity. In the parent dye, a broad absorption band appeared around 3331.10 cm^{-1} , which corresponds to O-H stretching vibrations, commonly associated with hydroxyl or amine groups (Anil Kumar et al., 2015). After degradation, this peak showed noticeable shifts in all bacterial treatments, appearing at 3240.82 cm^{-1} (SC4), 3262.52 cm^{-1} (MC2), 3264.10 cm^{-1} (KC2), and 3251.54 cm^{-1} (NC2). These shifts toward lower wavenumbers suggest changes in hydrogen bonding and indicate microbial modification of hydroxyl or amine-containing functional groups. The characteristic aromatic C=C stretching vibration, which contributes to the stability of the dye structure, was observed at 1614.31 cm^{-1} in the untreated dye. Following biodegradation, this peak shifted to 1557.63 cm^{-1} (SC4), 1567.56 cm^{-1} (MC2), 1563.20 cm^{-1} (KC2), and 1567.51 cm^{-1} (NC2). The consistent shift of this peak across all isolates indicates disruption of the aromatic ring system, suggesting cleavage or modification of conjugated structures within the dye molecule (Madhavan et al., 2010). A strong peak related to S=O stretching of sulfonic groups appeared at 1498.43 cm^{-1} in the parent dye. After bacterial treatment, this peak shifted to 1346.94 to 1349.34 cm^{-1} for SC4, MC2, KC2, and NC2, while a

more pronounced shift to 1122.42 cm^{-1} was observed in one degraded sample. These changes indicate desulfonation or transformation of sulfonic acid groups, confirming enzymatic breakdown of dye-associated sulfonate moieties. The C-N stretching vibration, initially observed at 1227.02 cm^{-1} , showed a clear downward shift after degradation, appearing consistently around 1033 cm^{-1} for all isolates. This shift suggests alteration or cleavage of amine-linked structures, further supporting microbial attack on nitrogen-containing functional groups within AR88. In the lower wavenumber region, peaks associated with C-Br stretching were detected at 680.24 cm^{-1} in the parent dye. After degradation, these peaks shifted slightly to $649\text{-}651\text{ cm}^{-1}$ across all bacterial treatments, indicating changes in substituted aromatic structures. Additionally, peaks corresponding to C-I stretching vibrations, initially observed at 558.71 cm^{-1} , shifted to $530\text{-}535\text{ cm}^{-1}$ after treatment, reflecting significant structural rearrangements in the fingerprint region (Khehra et al., 2006). Overall, the FTIR results clearly demonstrate that biodegradation by SC4, MC2, KC2, and NC2 caused consistent and significant shifts in major functional group peaks of AR88. The differences in peak positions shown in Table 5.10 confirm the breakdown of aromatic rings, modification of sulfonic and amine groups, and overall destabilization of the dye structure, supporting effective microbial degradation rather than simple adsorption.

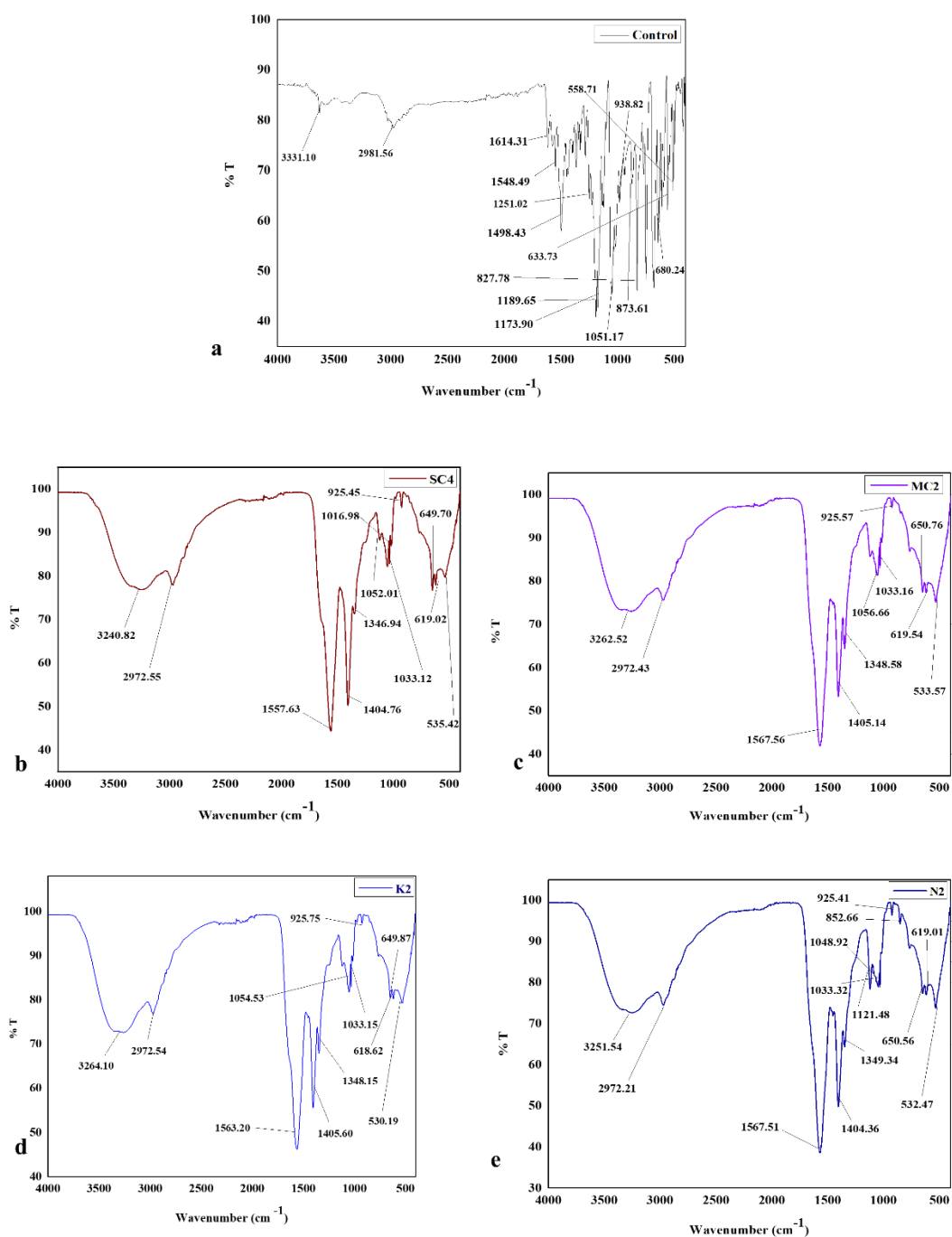


Figure 5.34: Fourier Transform Infrared (FTIR) spectra of the (a)control dye solution and biodegraded samples obtained after treatment with bacterial isolates (b)SC4, (c)MC2, (d)KC2, and (e)NC2.

Table 5.10: FTIR spectral peak comparison of AR88 before and after biodegradation by bacterial isolates SC4, MC2, KC2, and NC2.

S. No.	Parent dye (cm ⁻¹)	SC4 (cm ⁻¹)	MC2 (cm ⁻¹)	KC2 (cm ⁻¹)	NC2 (cm ⁻¹)	Standard range (cm ⁻¹)	Functional group
1	3331.10	3240.82	3262.52	3264.10	3251.54	3200–3550	O–H stretching (hydroxyl / phenolic)
2	1614.31	1557.63	1567.56	1563.20	1567.51	1566–1650	C=C stretching (aromatic ring)
3	1498.43	1346.94	1348.58	1348.15	1349.34	1335–1370	S=O stretching (sulfonate group)
4	1227.02	1033.12	1033.16	1033.15	1033.32	1020–1250	C–N stretching (amine group)
5	680.24	649.70	650.76	649.87	650.56	515–690	C–Br stretching
6	558.71	535.42	533.57	530.19	532.47	500–600	C–I stretching

5.12.2 GC-MS characterization of acid red 88 degradation products

GC-MS analysis was performed to identify the metabolites formed during the biodegradation of AR88 by the selected bacterial isolates SC4, MC2, KC2, and NC2. The chromatographic profiles of the degraded samples revealed distinct peaks corresponding to low-molecular-weight organic acids, indicating the breakdown of the parent dye molecule (Figure 5.34 and Table 5.11). The chromatogram of SC4 showed two prominent peaks at retention times of 4.304 min and 4.937 min, both corresponding to propanoic acid, with relative peak areas of 66.30 % and 10.54%, respectively. Similarly, the isolate MC2 exhibited peaks at 4.704 min and 6.310 min, corresponding to propanoic acid 16.80 % and butanoic acid 15.57 %, respectively. In the case of KC2, a major peak was observed at 4.038 min corresponding to acetic acid 93.51%, along with a minor peak of propanoic acid 4.23% at 4.702 min. The chromatographic profile of NC2 revealed two peaks corresponding to propanoic acid at retention times of 4.478 min and 6.409 min, with relative peak areas of 24.97 % and 60.00 %, respectively. The detection of short-chain organic acids such as acetic acid, propanoic acid, and butanoic acid indicates the formation of low-molecular-weight metabolites following bacterial degradation. The appearance of these simple aliphatic compounds suggests substantial breakdown of the AR88 molecule, which is a complex azo dye containing aromatic rings and azo (-N=N-) linkages (Bankole et al., 2018). Microbial degradation of azo dyes typically proceeds through sequential

enzymatic reactions, beginning with the reductive cleavage of the azo bond mediated by azoreductases, leading to the formation of aromatic amines. These intermediates undergo further biochemical transformations, including oxidation, deamination, and ring cleavage, facilitated by enzymes such as laccases, peroxidases, and dioxygenases (Kishor et al., 2021c). During the degradation process, the aromatic structure of the dye is progressively disrupted, resulting in the formation of intermediate compounds that are further metabolized into simpler molecules. The predominance of acetic acid in KC2 suggests a more advanced stage of degradation and higher mineralization efficiency compared to other isolates. In contrast, the accumulation of propanoic acid in SC4 and NC2, and the presence of both propanoic and butanoic acids in MC2, indicate intermediate stages of degradation where partial breakdown of the dye structure has occurred (Ogunlaja et al., 2020). The final stage of AR88 degradation is reflected in the detection of these low-molecular-weight organic acids as dominant compounds in the chromatograms. The presence of such metabolites suggests extensive cleavage of the aromatic structure and conversion into less complex, potentially less toxic products. Similar formation of low-molecular-weight metabolites during microbial degradation of azo dyes has been reported in previous studies, demonstrating the transformation of complex dye molecules into simpler compounds (Bankole et al., 2018). Overall, the GC-MS results confirm that the selected bacterial isolates are capable of enzymatically transforming Acid Red 88 into simpler metabolites, with KC2 showing the highest degree of mineralization, followed by NC2, SC4, and MC2. These findings highlight the potential application of these isolates in the bioremediation of dye-contaminated wastewater Figure 5.35.

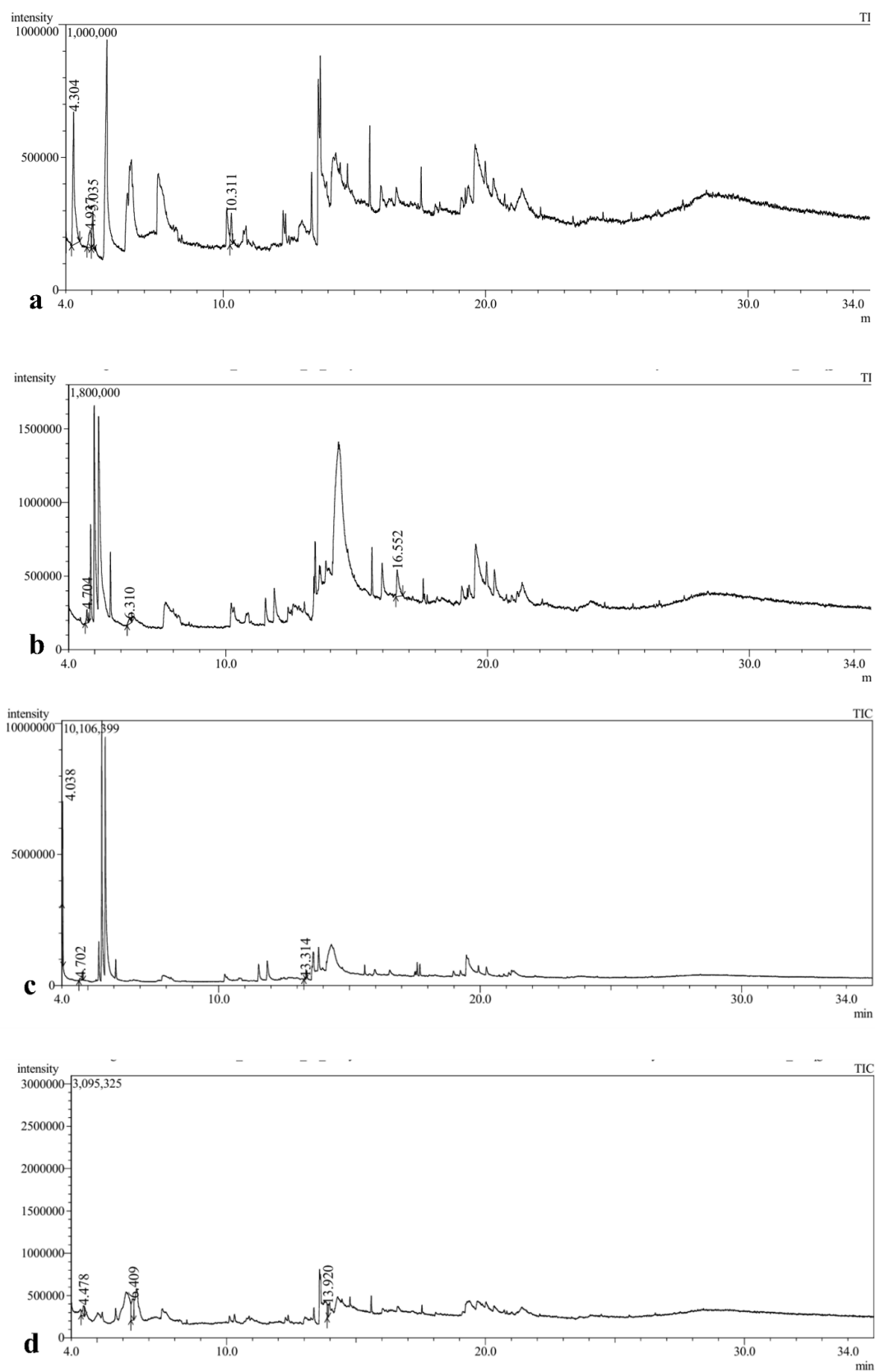


Figure 5.35: GC-MS chromatogram of AR88 degradation products obtained after treatment with the (a) SC4, (b)MC2, (c)KC2 and(d) NC2.

Table 5.11: GC–MS identified degradation metabolites of Acid Red 88 after treatment with the SC4, MC2, KC2 and NC2.

Peak	Name	R.Time (min)	Area (counts)	Area %	Height (Counts)	Height %
SC4	Propanoic acid	4.304	2453828	66.30	499098	60.44
	Propanoic acid	4.937	389973	10.54	68003	8.23
MC2	Propanoic acid	4.704	276205	16.80	91568	30.14
	Butanoic Acid	6.310	255848	15.57	31310	10.31
KC2	Acetic acid	4.038	9107250	93.51	5410110	97.26
	Propanoic acid	4.702	411958	4.23	74538	1.34
NC2	Propanoic acid	4.478	675080	24.97	122990	23.20
	Propanoic acid	6.409	1622247	60.00	254859	48.07

5.12.2.1 Proposed degradation pathway of AR 88 by SC4, MC2, KC2 and NC2

Based on FTIR analysis, GC-MS identification of metabolites, and known microbial enzymatic reactions, a proposed degradation pathway of AR88 by the bacterial isolates SC4, MC2, KC2, and NC2 is illustrated in Figure 5.36. The initial step of degradation involves the reductive cleavage of the azo bond ($-N=N-$), which is the main chromophoric group responsible for the colour of AR88. This step is most likely catalyzed by the enzyme azoreductase, leading to the formation of aromatic amine intermediates such as naphthalene-2-ol and sodium naphthalene-1-sulfonate. Following azo bond cleavage, these aromatic intermediates undergo further transformation by oxidative enzymes such as laccase. The sulfonated aromatic compounds are subjected to desulfonation, while hydroxylated naphthalene derivatives undergo dihydroxylation and oxidation, resulting in the formation of dihydroxy naphthalene. These reactions reduce the aromatic complexity of the dye molecule and destabilize the ring structure.

Subsequently, the aromatic rings are broken down through ring cleavage reactions, producing smaller aliphatic organic acids such as acetic acid and propanoic acid (Bankole et al., 2018). These low-molecular-weight compounds indicate complete detoxification and mineralization of the dye structure rather than mere decolourization. The generated organic acids are then transported into the bacterial cell, where they enter central metabolic pathways. Acetic acid is converted into acetyl-CoA, which feeds directly into the tricarboxylic acid (TCA/Krebs) cycle. Through successive enzymatic steps involving citrate, isocitrate, α -ketoglutarate, succinate, fumarate, malate, and oxaloacetate, the metabolites are fully oxidized to CO₂, producing NADH and FADH₂. These reducing equivalents are further utilized in cellular respiration, leading to ATP generation, which supports bacterial growth and survival. Overall, this pathway demonstrates that SC4, MC2, KC2, and NC2 are capable of not only decolourizing AR88 but also converting it into non-toxic metabolites that are assimilated into normal cellular metabolism. Among the isolates, variations in degradation efficiency may be attributed to differences in enzyme expression levels; however, all isolates follow a similar core degradation mechanism involving azo bond reduction, aromatic ring cleavage, and mineralization via the TCA cycle.

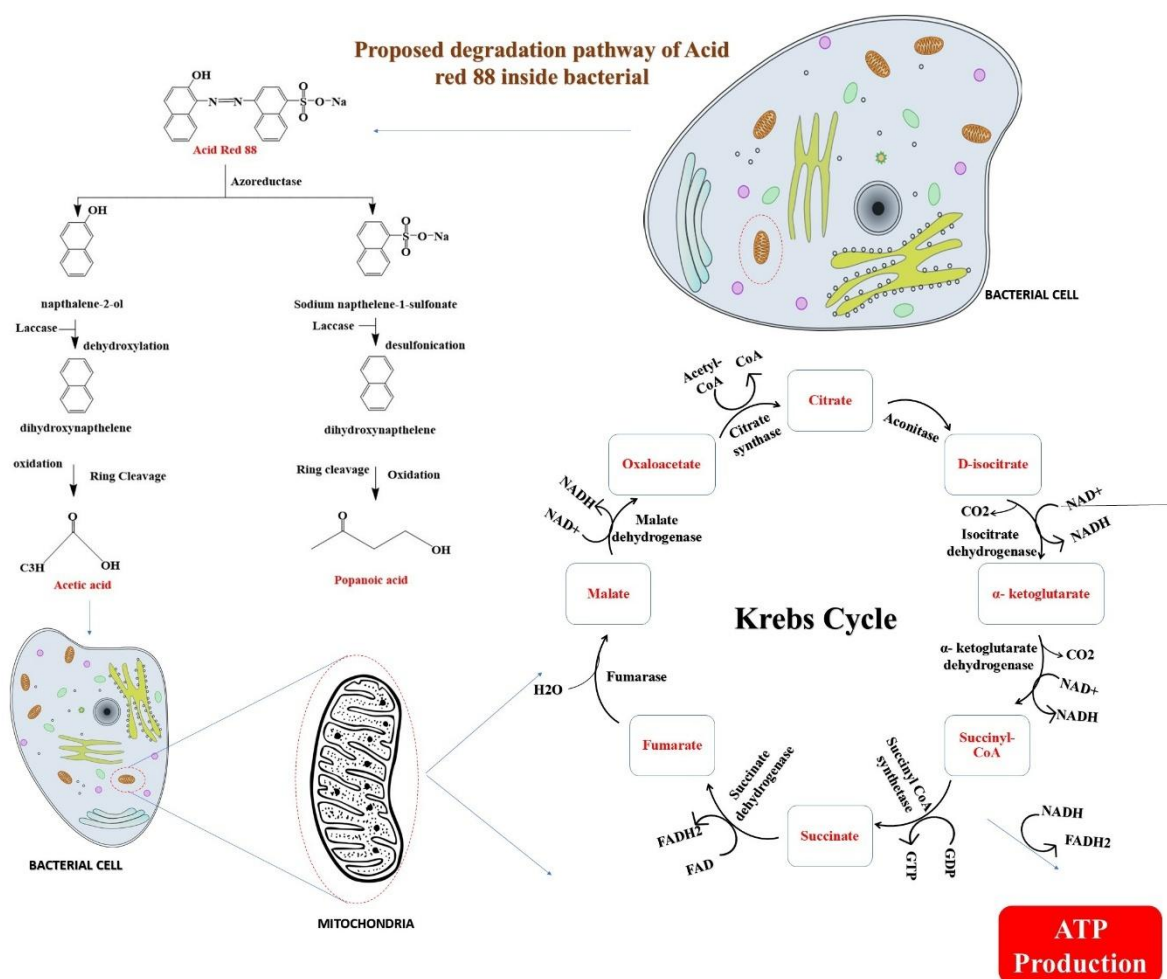


Figure 5.36: Proposed degradation pathway of Acid Red 88 by bacterial isolates SC4, MC2, KC2, and NC2, showing azo bond cleavage by azoreductase, subsequent aromatic ring breakdown, formation of low-molecular-weight organic acids, and their assimilation into the TCA cycle for ATP production.

5.13 Optimisation of culture conditions for dye degradation

5.13.1 Effect of pH, Temperature and Incubation time on dye decolorization

The effect of different physicochemical parameters on the color stability of Acid Red 88 was evaluated under controlled experimental conditions in the absence of bacterial inoculation. The dye solution was adjusted to different pH values (5, 7.5, and 10) and incubated under varying temperature (25, 35, 45°C) and incubation time (1, 12.5, 24 hrs) conditions to determine whether these parameters alone could influence dye decolorization. The decolorization pattern was analyzed visually as well as spectrophotometrically by measuring the absorbance of the dye solution before and after incubation table 5.12. Visual observations revealed no noticeable change in dye color intensity under any of the tested conditions (Figure 5.37). Similarly,

spectrophotometric analysis showed no significant reduction in absorbance values after incubation, indicating the absence of dye decolorization.

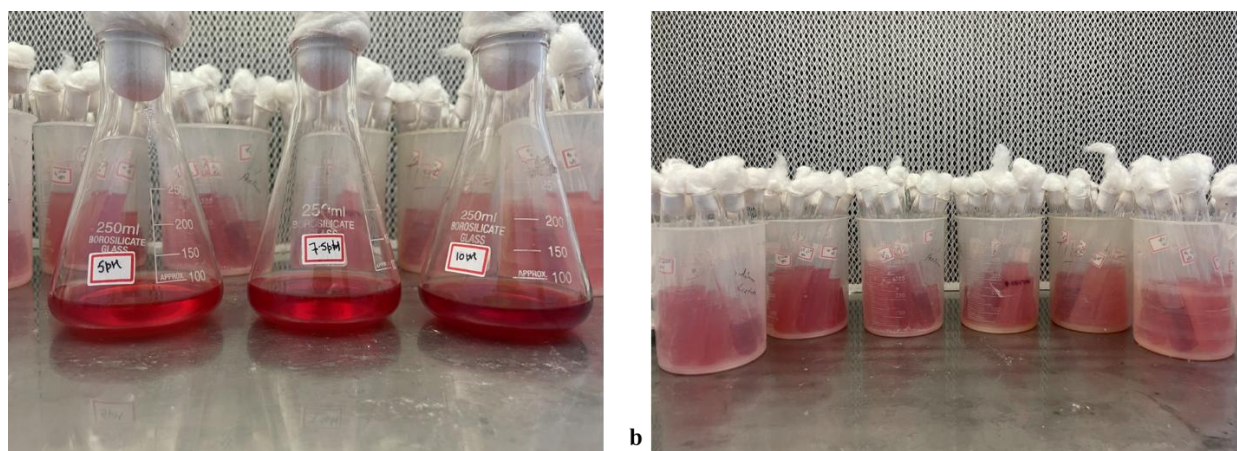


Figure 5.37: Effect of pH on the color stability of Acid Red 88 in the absence of bacterial inoculation. The dye solution adjusted to different pH values (5, 7.5, and 10) showed no visible change in color intensity before and after 24 h of incubation.

Table 5.12: Spectrophotometric analysis of Acid Red 88 at different pH value in absence of bacterial inoculation:

pH	Incubation time(h)	Temp (°C)	Absorbance at 505 nm		% Decolorization	Change in Absorbance
			Before incubation (0 h)	After incubation (24 h)		
5	0-24	25-45	1.291	1.290	0.03	0.001
7.5	0-24	25-45	1.395	1.394	0.03	0.001
10	0-24	25-45	1.354	1.353	0.03	0.001

5.13.2 Response surface analysis and optimisation of AR88 by SC4

To further enhance the dye degradation efficiency of the selected isolate SC4, the effects of key environmental parameters were optimized using RSM based on a BBD. The interactive effects of pH (A), temperature (B) and time (C) on the decolorization of AR88 were evaluated, and the results are represented in Figure 5.38. The response surface plots illustrate the combined influence of these variables on dye degradation, providing insight into the interaction effects and optimal conditions predicted by the model. The interaction between pH and temperature

demonstrated a strong influence on the decolorization efficiency of SC4. The predicted versus actual value plot demonstrated a close agreement between experimental and model-predicted responses, confirming the adequacy and reliability of the developed RSM model for Acid Red 88 biodegradation (Figure 5.38a). The three-dimensional response surface plots further illustrated the significant interactive effects of pH, temperature, and incubation period on dye biodegradation efficiency by the bacterial isolate SC4. The results show that degradation efficiency increased significantly with increasing pH, reaching maximum values under alkaline conditions (pH 10). At pH 10, temp 35 °C and time 24 h, the highest decolorization efficiency 98.00 % was observed. Similarly, high efficiency was maintained at pH 10, temp 45 °C and time 12.5 h (90.88 %) and pH 10, temp 25 °C and time 12.5h (88.23 %), indicating that alkaline pH plays a prominent role in enhancing degradation. At neutral pH 7.5, the decolorization efficiency remained moderately high, with values around 86 – 87 % across different temperatures 25-35 °C, suggesting that SC4 retains substantial activity under near-neutral conditions (Figure 5.38b). However, as the pH shifted towards acidic conditions pH 5, a marked decline in decolorization efficiency was observed. At pH 5 and 45 °C, the efficiency decreased to 70.33 %, and further declined to 63.82 % and 59.65 % at lower temperatures, indicating the inhibitory effect of acidic conditions on microbial activity. The response surface also highlights that extreme combinations of low pH and varying temperatures significantly reduced degradation efficiency. At pH 7.5, temp 45 °C and time 1h, the efficiency dropped sharply to 15.32 %, and similarly low values were observed at other unfavorable combinations, including pH 10 with suboptimal conditions, where efficiencies decreased to approximately 14-15 %, and further to 4% under highly unfavorable conditions (pH 5, temp 35 °C, time 1 h) (Figure 5.38c). This decline may be attributed to enzyme denaturation, reduced microbial growth, and disruption of metabolic pathways under stress conditions (Sharma et al., 2024). Overall, the response surface analysis indicates that maximum decolorization of AR 88 by SC4 occurs under strongly alkaline conditions (pH 10) and moderate temperature (around 35 °C) (Figure 5.38d). The pronounced variation in efficiency across the tested conditions confirms the significant interaction between pH and temperature and supports the applicability of the RSM approach in predicting optimal degradation conditions (Ogunlaja et al., 2020). The observed curvature in the response further validates the importance of quadratic effects in the model and demonstrates that SC4 exhibits optimal enzymatic activity within a narrow range of environmental conditions.

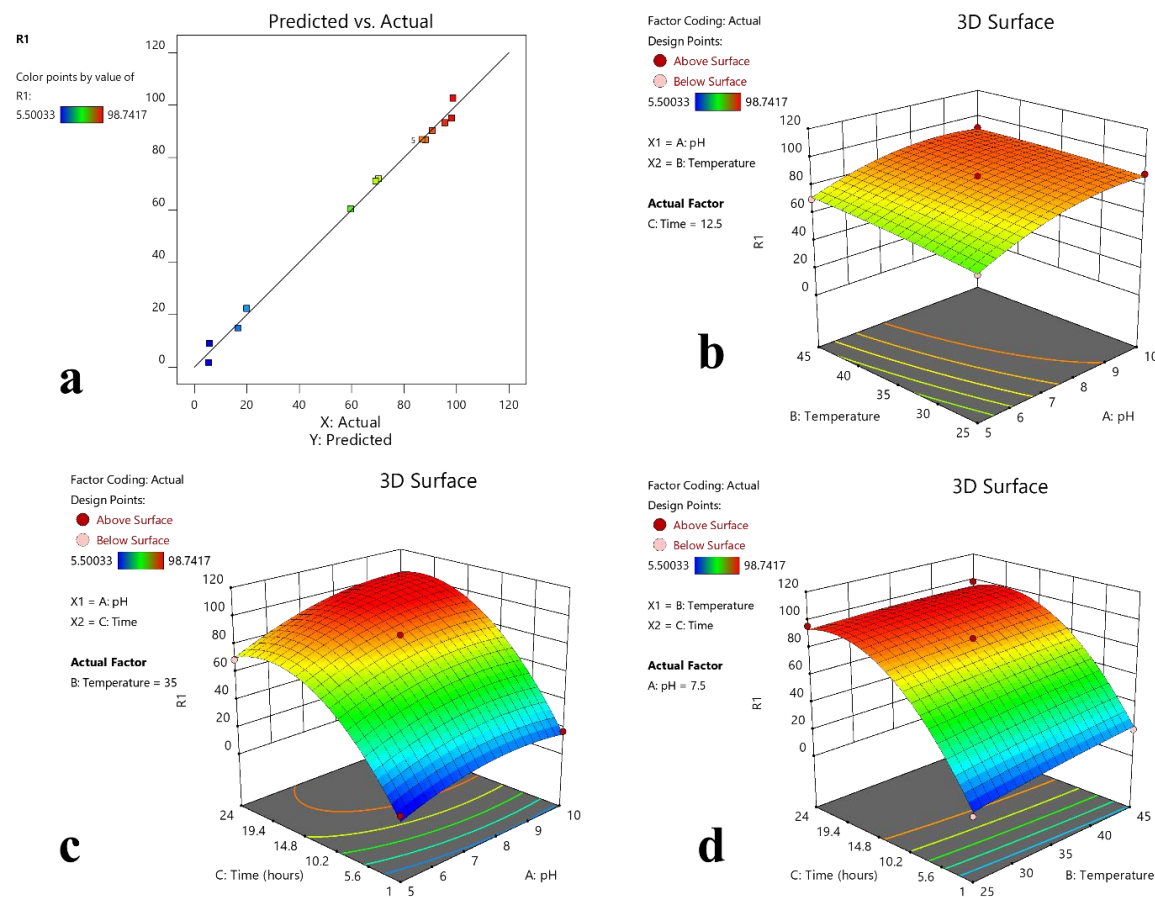


Figure 5.38: RSM plots showing the optimization of Acid Red 88 biodegradation by the bacterial isolate SC4: (a) comparison between predicted and actual values of dye biodegradation, (b) interactive effects of pH and temperature at a fixed incubation period, (c) interactive effects of pH and incubation period at a constant temperature, and (d) interactive effects of temperature and incubation period at a constant pH.

5.13.2.1 Statistical significance (ANOVA analysis)

The analysis of variance (ANOVA) was performed to evaluate the statistical significance and adequacy of the quadratic model developed using the BBD for the SC4 isolate. The ANOVA results are summarized in Table 5.13. The model was found to be highly significant, with a high F-value of 193.52 and a very low p-value ($p = 0.0001$), confirming that the model is statistically valid and reliable at the 95 % confidence level (Venkatesan et al., 2025). This strong significance indicates that the model can accurately predict the percentage degradation of Acid Red 88 by the SC4 isolate. Among the linear factors, incubation period (C) was identified as the most influential parameter, showing an extremely high F-value of 1187.66 and a highly significant p-value ($p = 0.0001$). This clearly demonstrates that incubation time plays a critical role in controlling the degradation efficiency of AR88. The linear effect of pH (A) was also statistically ($p < 0.05$), although its influence was comparatively lower than that of the incubation period, indicating a moderate but important role in regulating degradation efficiency.

Table 5.13: Analysis of variance (ANOVA) for the quadratic response surface model showing the effects of pH, temperature, and incubation period on the biodegradation of AR 88 by the SC4 isolate.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	18061.63	9	2006.85	193.52	0.0001
A-pH	1008.47	1	1008.47	97.25	0.0001
B-Temperature	113.41	1	113.41	10.94	0.0130
C-Time	12316.44	1	12316.44	1187.66	0.0001
AB	16.10	1	16.10	1.55	0.2528
AC	84.89	1	84.89	8.19	0.0243
BC	33.86	1	33.86	3.26	0.1137
A ²	301.94	1	301.94	29.12	0.0010
B ²	5.19	1	5.19	0.5005	0.5021
C ²	4012.34	1	4012.34	386.90	0.0001

5.13.3 Response surface analysis and optimisation of Acid Red 88 by MC2

To the dye degradation efficiency of the selected isolate MC2, the effects of key environmental parameters were optimized using RSM based on a BBD. The interaction between pH (A), temperature (B) and time (C) on AR88 decolorization is illustrated in Figure 5.39. The predicted versus actual value plot demonstrated a close agreement between experimental and model-predicted responses, confirming the adequacy and reliability of the developed RSM model for Acid Red 88 biodegradation (Figure 5.39a). The three-dimensional response surface plots further illustrated the significant interactive effects of pH, temperature, and incubation period on dye biodegradation efficiency by the bacterial isolate MC2. The response surface analysis revealed that maximum decolorization 97.99 % was achieved at pH 7.5, temp 45 °C and time 24 h, indicating that slightly alkaline conditions coupled with elevated temperature favor enzymatic activity in MC2. High degradation efficiency was consistently observed across pH 7.5 at different temperatures, with values ranging between 91-96 %, suggesting that this isolate performs optimally under near-neutral to mildly alkaline conditions (Figure 5.39b). At pH 10, temp 35 °C and time 24 h, the degradation efficiency remained relatively high 89 %, indicating tolerance to alkaline environments, although slightly lower than the optimum. However, a significant decline was observed under acidic conditions pH 5, where efficiency dropped to 73.63 % at 45 °C and further decreased to 49.36 % and 40.02 % at other temperature combinations (Figure 5.39c). The lowest decolorization efficiencies 5-18 % were recorded under unfavourable combinations such as pH 7.5 at suboptimal temperatures and pH 5 at lower temperatures, reflecting strong inhibition of microbial activity and enzyme function (Figure 5.39d). This decline may be attributed to reduced enzyme stability and metabolic suppression under stress conditions (Sharma et al., 2024). Overall, the response surface analysis indicates that MC2 exhibits optimal degradation under pH 7.5, temperature 45°C and time 24h. The observed curvature confirms the significant interaction between pH and temperature, validating the effectiveness of RSM in predicting optimal biodegradation conditions.

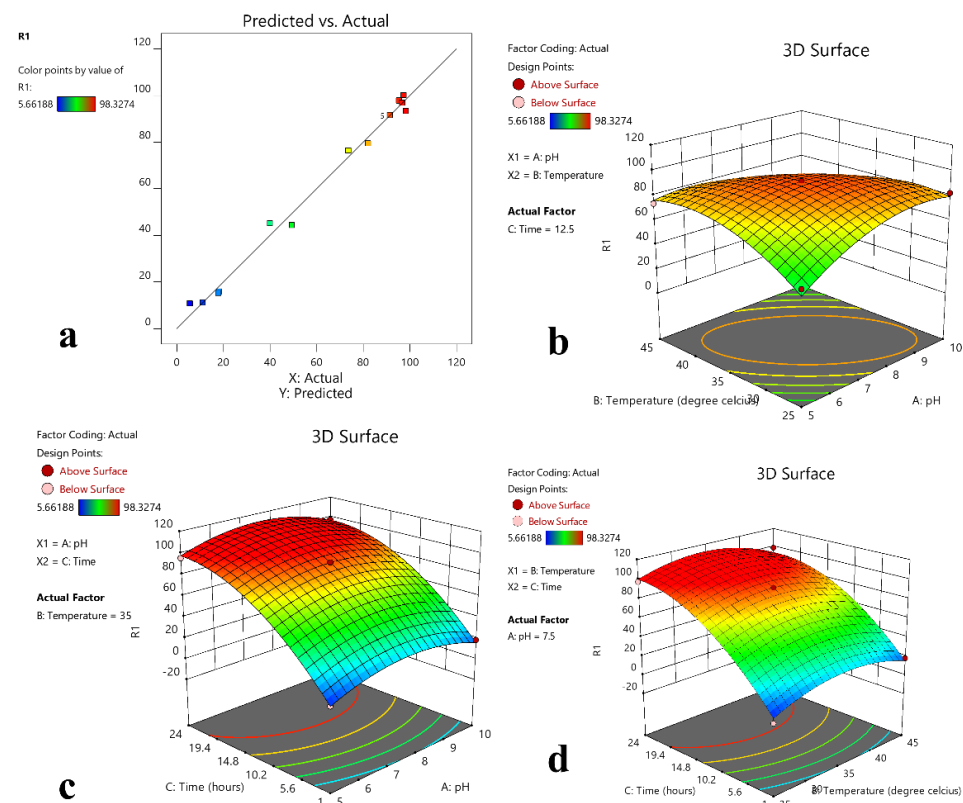


Figure 5.39: RSM plots showing the optimization of Acid Red 88 biodegradation by the bacterial isolate MC2: (a) comparison between predicted and actual values of dye biodegradation, (b) interactive effects of pH and temperature at a fixed incubation period, (c) interactive effects of pH and incubation period at a constant temperature, and (d) interactive effects of temperature and incubation period at a constant pH.

5.13.3.1 Statistical significance (ANOVA analysis)

The analysis of variance (ANOVA) was performed to evaluate the statistical significance and adequacy of the quadratic model developed using the BBD for the MC2 isolate. The ANOVA results are summarized in Table 5.14. The model was found to be highly significant, with a high F-value of 106.53 and a very low p-value ($p = 0.0001$), confirming that the model is statistically valid and reliable at the 95% confidence level. This strong significance indicates that the model can accurately predict the percentage degradation of Acid Red 88 by the MC2 isolate. Among the linear factors, incubation period (C) was identified as the most influential parameter, showing an extremely high F-value of 691.56 and a highly significant p-value ($p = 0.0001$). This clearly demonstrates that incubation time plays a critical role in controlling the degradation efficiency of Acid Red 88. The linear effect of pH (A) was also statistically significant ($p < 0.05$), indicating that pH plays an important role in influencing the degradation efficiency of AR88, although its effect was comparatively lower than that of the incubation period.

Table 5.14: Analysis of variance (ANOVA) for the quadratic response surface model showing the effects of pH, temperature, and incubation period on the biodegradation of AR 88 by the MC2 isolate.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	19439.41	9	2159.93	106.53	0.0001
A-pH	7.27	1	7.27	0.3585	0.5682
B-Temperature	2.61	1	2.61	0.1288	0.7303
C-Time	14021.40	1	14021.40	691.56	0.0001
AB	1099.33	1	1099.33	54.22	0.0002
AC	8.03	1	8.03	0.3963	0.5490
BC	31.50	1	31.50	1.55	0.2527
A ²	925.99	1	925.99	45.67	0.0003
B ²	999.75	1	999.75	49.31	0.0002
C ²	1910.32	1	1910.32	94.22	0.0001

5.13.4 Response surface analysis and optimisation of Acid Red 88 by KC2

The optimization of dye degradation by isolate KC2 was carried out using RSM to evaluate the combined effects of pH (A), temperature (B) and time (C), as presented in Figure 5.40. The predicted versus actual value plot demonstrated a close agreement between experimental and model-predicted responses, confirming the adequacy and reliability of the developed RSM model for Acid Red 88 biodegradation (Figure 5.40a). The three-dimensional response surface plots further illustrated the significant interactive effects of pH, temperature, and incubation period on dye biodegradation efficiency by the bacterial Isolate KC2. The results indicate that maximum decolorization 97.91% was achieved at pH 7.5, temp 45 °C and time 24 h, closely followed by 97.36 % at pH 10 , temp 35 °C and time 24 h, demonstrating that KC2 performs efficiently under both neutral and alkaline conditions. A consistently high degradation range 89-94 % was observed across pH 7.5 at multiple temperatures, highlighting the robustness of this isolate (Figure 5.40b). At pH 10, degradation efficiency remained relatively high 82-89 %, suggesting good adaptability to alkaline environments. However, under acidic conditions pH 5, a notable reduction in efficiency was observed, with values dropping to 82.76 % and further to 67 %, depending on temperature (Figure 5.40c). A sharp decline in decolorization efficiency 11–18 % was recorded under extreme or unfavorable conditions, particularly at low pH combined with suboptimal temperatures, indicating inhibition of microbial growth and enzymatic activity (Figure 5.40d). This behavior is consistent with enzyme sensitivity to environmental stress (M-Ridha et al., 2020). Overall, the response surface analysis suggests that KC2 achieves optimal degradation at pH 7.5-10 and temperatures around 35-45 °C, with maximum performance at pH 7.5, temp 45°C and time 24 h. The significant curvature in the response surface further supports the role of quadratic interactions in determining optimal conditions.

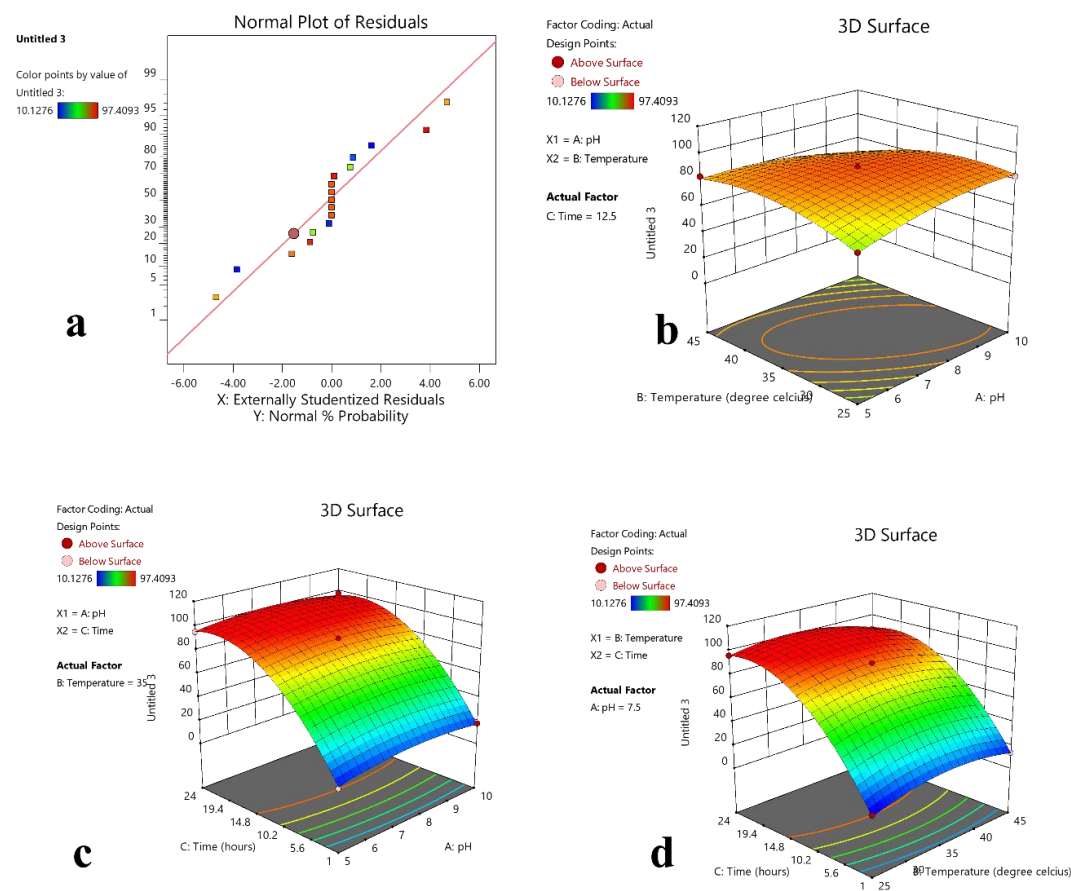


Figure 5.40: RSM plots showing the optimization of Acid Red 88 biodegradation by the bacterial isolate KC2: (a) comparison between predicted and actual values of dye biodegradation, (b) interactive effects of pH and temperature at a fixed incubation period, (c) interactive effects of pH and incubation period at a constant temperature, and (d) interactive effects of temperature and incubation period at a constant pH.

5.13.4.1 Statistical significance (ANOVA analysis)

The analysis of variance (ANOVA) was performed to evaluate the statistical significance and adequacy of the quadratic model developed using the BBD for the KC2 isolate. The ANOVA results are summarized in Table 5.15. The model was found to be highly significant, with a very high F-value of 950.22 and a p-value < 0.0001 , confirming that the model is statistically valid and reliable at the 95% confidence level. This strong significance indicates that the model can accurately predict the percentage degradation of Acid Red 88 by the KC2 isolate. Among the linear factors, the incubation period (C) was identified as the most influential parameter, showing an extremely high F-value of 6337.05 and a highly significant p-value < 0.0001 . This clearly demonstrates that incubation time plays a critical role in controlling the degradation efficiency of Acid Red 88. The linear effect of pH (A) was also statistically highly significant ($p < 0.0001$), indicating that pH plays a crucial role in influencing the degradation efficiency of AR88, although its effect was comparatively less pronounced than that of the incubation period.

Table 5.15: Analysis of variance (ANOVA) for the quadratic response surface model showing the effects of pH, temperature, and incubation period on the biodegradation of Acid Red 88 by the KC2 isolate.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	17602.26	9	1955.81	950.22	< 0.0001
A-pH	7.37	1	7.37	3.58	0.1003
B-Temperature	4.85	1	4.85	2.36	0.1686
C-Time	13043.39	1	13043.39	6337.05	< 0.0001
AB	237.56	1	237.56	115.42	< 0.0001
AC	3.17	1	3.17	1.54	0.2549
BC	35.37	1	35.37	17.18	0.0043
A ²	128.34	1	128.34	62.35	< 0.0001
B ²	358.17	1	358.17	174.02	< 0.0001
C ²	3535.37	1	3535.37	1717.64	< 0.0001

5.13.5 Response surface analysis and optimisation of Acid Red 88 by NC2

The effect of environmental parameters on the degradation efficiency of isolate NC2 was optimized using RSM, focusing on the interaction between pH (A), temperature (B) and time (C), as shown in Figure 5.41. The predicted versus actual value plot demonstrated a close agreement between experimental and model-predicted responses, confirming the adequacy and reliability of the developed RSM model for Acid Red 88 biodegradation (Figure 5.41 a). The three-dimensional response surface plots further illustrated the significant interactive effects of pH, temperature, and incubation period on dye biodegradation efficiency by the bacterial isolate NC2. The results demonstrated that maximum decolorization 97.98 % was achieved at pH 10, temp 35 °C and time 24 h, indicating that NC2 performs best under strongly alkaline conditions with moderate temperature. Similarly, high efficiency 97.30 % was observed at pH 7.5, temp 45 °C and time 24 h, suggesting flexibility across a range of favorable conditions (Figure 5.41b). At pH 7.5, the degradation efficiency remained consistently high 8-90 % across different temperatures and time, reflecting stable enzymatic activity under near-neutral conditions (Figure 41c). At pH 10, slightly lower but still substantial degradation 88-90 % was observed. In contrast, under acidic conditions pH 5, the efficiency decreased significantly, with values dropping to 79.22 %, 72.17 %, and 70.38 %, depending on temperature (Figure 5.41d). The lowest efficiencies 11-16 % were recorded under highly unfavorable conditions, indicating strong inhibition of microbial metabolism and enzyme activity (Ram Talib et al., 2019). Overall, the response surface analysis indicates that NC2 achieves optimal degradation under alkaline pH around 10 and moderate temperature 35 °C and time 24 h. The interaction effects and curvature confirm the importance of pH–temperature synergy in controlling dye degradation efficiency.

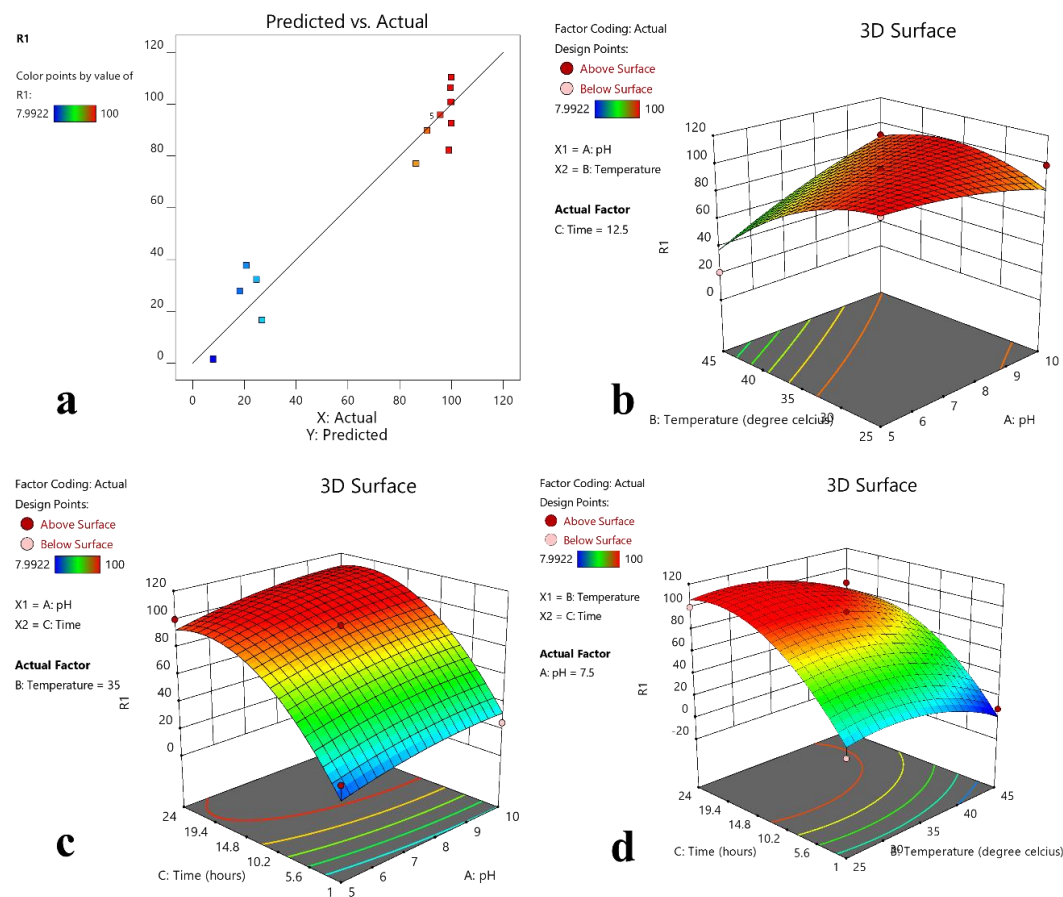


Figure 5.41: RSM plots showing the optimization of Acid Red 88 biodegradation by the isolate NC2: (a) comparison between predicted and actual values of dye biodegradation, (b) interactive effects of pH and temperature at a fixed incubation period, (c) interactive effects of pH and incubation period at a constant temperature, and (d) interactive effects of temperature and incubation period at a constant pH.

5.13.5.1 Statistical significance (ANOVA analysis)

The analysis of variance (ANOVA) was performed to evaluate the statistical significance and adequacy of the quadratic model developed using the BBD for the isolate. The ANOVA results are summarized in Table 5.16. The developed model was found to be statistically significant, with an F-value of 13.37 and a p-value of 0.0012, confirming that the model is reliable and adequate at the 95% confidence level. This significance indicates that the model can satisfactorily predict the percentage degradation of AR88 by the isolate. Among the linear factors, incubation period (C) was identified as the most influential parameter, exhibiting a high F-value of 71.84 and a highly significant p-value ($p = 0.0001$), clearly demonstrating that incubation time plays a crucial role in governing the degradation efficiency of AR88. The linear effect of temperature (B) was also statistically significant ($F = 9.33$, $p = 0.0184$), indicating its important contribution to the biodegradation process. In contrast, the linear effect of pH (A) was not statistically significant ($p > 0.05$), suggesting a comparatively lesser influence within the studied range. Among the interaction terms, the AB (pH–temperature) interaction showed statistical significance ($p = 0.0286$), whereas AC and BC interactions were insignificant. The quadratic term C^2 was also highly significant ($p = 0.0026$), confirming the nonlinear influence of incubation time on dye degradation.

Table 5.16: Analysis of variance (ANOVA) for the quadratic response surface model showing the effects of pH, temperature, and incubation period on the biodegradation of Acid Red 88 by the NC2 isolate.

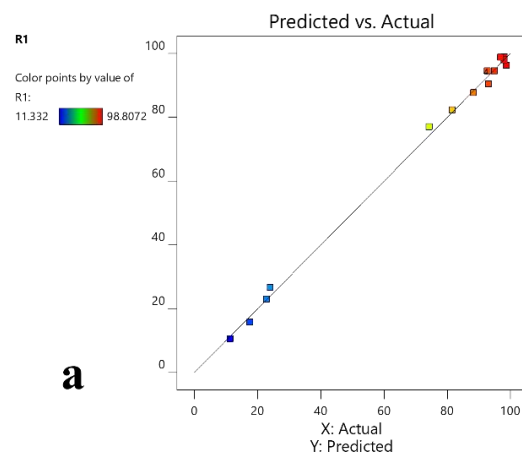
Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	19889.69	9	2209.97	13.37	0.0012
A-pH	558.48	1	558.48	3.38	0.1086
B-Temperature	1542.68	1	1542.68	9.33	0.0184
C-Time	11872.73	1	11872.73	71.84	0.0001
AB	1248.27	1	1248.27	7.55	0.0286
AC	1.12	1	1.12	0.0068	0.9367
BC	2.36	1	2.36	0.0143	0.9083
A ²	74.38	1	74.38	0.4501	0.5238

B ²	827.82	1	827.82	5.01	0.0602
C ²	3462.51	1	3462.51	20.95	0.0026

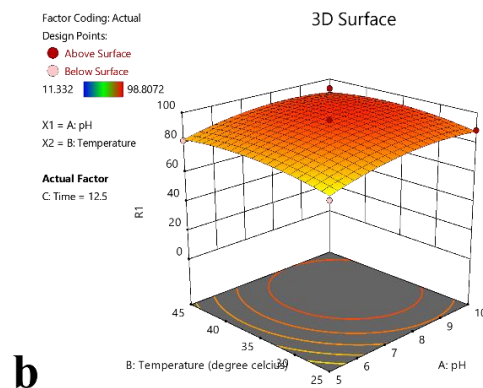
5.13.6 Response surface analysis and optimisation of Acid Red 88 by consortium (SC4,MC2,KC2 and NC2)

The dye degradation efficiency of the bacterial consortium, the effects of key environmental parameters were optimized using RSM based on a BBD. The interaction between pH (A), temperature (B) and time (C) on AR88 decolorization is illustrated in Figure 5.42. The response surface plots provide a clear representation of the combined influence of these variables and help identify the optimal conditions for maximum degradation. The predicted versus actual value plot demonstrated a close agreement between experimental and model-predicted responses, confirming the adequacy and reliability of the developed RSM model for Acid Red 88 biodegradation (Figure 5.42a). The three-dimensional response surface plots further illustrated the significant interactive effects of pH, temperature, and incubation period on dye biodegradation efficiency by the bacterial consortium. The results revealed that the bacterial consortium exhibited the highest decolorization efficiency 98.81% at pH 5, temp 35 °C and time 24 h, indicating that, unlike individual isolates, the consortium performs optimally under slightly acidic conditions (Figure 5.42b). Similarly, high decolorization efficiencies were observed at pH 7.5, temp 25 °C and time 24 h was 97.29 % and pH 7.5, temp 45 °C and time 24 h was 96.94 %, demonstrating the ability of the consortium to maintain strong degradation performance across a broad range of environmental conditions (Ram Talib et al., 2019). At neutral pH 7.5, the consortium showed consistently high decolorization efficiency 87-97 % across different temperature combinations, highlighting its robustness and metabolic versatility. At alkaline pH 10, the degradation efficiency remained relatively high, with values around 87-93 %, indicating good adaptability of the consortium to alkaline environments as well (Figure 5.42c). However, as the environmental conditions deviated from the optimal range, particularly under extreme combinations of pH and temperature, a significant decline in decolorization efficiency was observed. For example, at pH 5, temp 45 °C and time 1 h, the efficiency decreased to 81.62 %, and further dropped to 74.32 % at pH 5 and 25 °C. The lowest efficiencies 7–11 % were recorded under highly unfavorable conditions such as pH 5 at suboptimal temperatures and pH 7.5 at low temperature combinations, indicating strong inhibition of microbial activity (Figure 5.42d). The enhanced performance of the consortium compared to individual isolates can be attributed to synergistic interactions among different

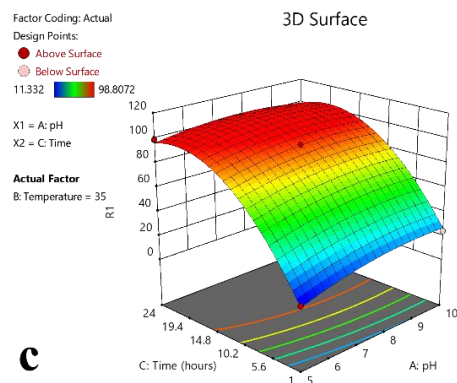
bacterial species, which enable efficient utilization of dye molecules and their intermediates (Ram Talib et al., 2019). The presence of multiple metabolic pathways within the consortium likely facilitates sequential degradation, leading to improved tolerance against environmental stress and higher degradation efficiency (Ram Talib et al., 2019). The response surface analysis also highlights the significant interaction between pH and temperature, as indicated by the pronounced variation and curvature in the degradation profile. These findings confirm that both parameters jointly influence enzymatic activity and microbial metabolism (Ning et al., 2014). Overall, the RSM results indicate that the bacterial consortium achieves optimal degradation across a wider range of conditions, with maximum efficiency observed under mildly acidic to neutral pH and moderate temperature 25-35 °C. The broader adaptability and higher efficiency of the consortium highlight its strong potential for application in the bioremediation of dye-contaminated wastewater under variable environmental conditions.



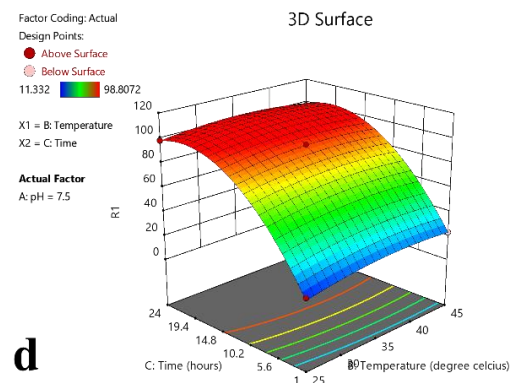
a



b



c



d

Figure 5.42: RSM plots showing the optimization of Acid Red 88 biodegradation by the bacterial consortium: (a) comparison between predicted and actual values of dye biodegradation, (b) interactive effects of pH and temperature at a fixed incubation period, (c) interactive effects of pH and incubation period at a constant temperature, and (d) interactive effects of temperature and incubation period at a constant pH.

5.13.6.1 Statistical significance (ANOVA analysis)

The analysis of variance (ANOVA) was performed to evaluate the statistical significance and adequacy of the quadratic model developed using the BBD for the isolate. The ANOVA results are summarized in Table 5.17. The developed model was found to be highly statistically significant, with a high F-value of 331.46 and a very low p-value ($p = 0.0001$), confirming that the model is reliable and adequate at the 95% confidence level. This strong significance indicates that the model can satisfactorily predict the percentage degradation of Acid Red 88 by the isolate. Among the linear factors, the incubation period (C) was identified as the most influential parameter, exhibiting an extremely high F-value of 2166.97 and a highly significant p-value ($p = 0.0001$), clearly demonstrating that incubation time plays a crucial role in governing the degradation efficiency of AR88. The linear effects of pH (A) and temperature (B) were also statistically significant, with F-values of 30.48 ($p = 0.0009$) and 5.65 ($p = 0.0490$), respectively, indicating their important contribution to the biodegradation process. Among the interaction terms, the AC (pH–time) interaction showed statistical significance ($p = 0.0269$), whereas AB and BC interactions were not significant. Furthermore, the quadratic terms A^2 , B^2 , and C^2 were all statistically significant, with C^2 showing a particularly high F-value (699.95, $p = 0.0001$), confirming the strong nonlinear influence of incubation period on dye degradation.

Table 5.17: Analysis of variance (ANOVA) for the quadratic response surface model showing the effects of pH, temperature, and incubation period on the biodegradation of AR 88 by the consortium.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	17194.67	9	1910.52	331.46	0.0001
A-pH	175.67	1	175.67	30.48	0.0009
B-Temperature	32.59	1	32.59	5.65	0.0490
C-Time	12490.18	1	12490.18	2166.97	0.0001
AB	1.71	1	1.71	0.2962	0.6032
AC	44.90	1	44.90	7.79	0.0269
BC	10.26	1	10.26	1.78	0.2239

A ²	126.58	1	126.58	21.96	0.0022
B ²	92.89	1	92.89	16.12	0.0051
C ²	4034.46	1	4034.46	699.95	0.0001

5.14 Comparative RSM-based evaluation of acid red 88 degradation by individual isolates and bacterial consortium

A comparative evaluation of the selected bacterial isolates (SC4, MC2, KC2, NC2) and the bacterial consortium based on RSM optimization highlights significant differences in their degradation efficiency, environmental adaptability, and tolerance to varying physicochemical conditions (Figure 5.43). Among the individual isolates, SC4 and NC2 exhibited maximum decolorization efficiency under strongly alkaline conditions pH 10, 35 °C, indicating their preference for higher pH environments and suggesting the dominance of alkaline-active enzymes in their metabolic systems. In contrast, MC2 and KC2 showed optimal performance at slightly alkaline pH 7.5, temperature 45 °C and time 24 h, reflecting their ability to function efficiently under comparatively milder pH but higher thermal conditions. This variation among isolates demonstrates the diverse physiological and enzymatic adaptations of bacteria originating from different wetland environments. When comparing overall efficiency, KC2 and NC2 showed relatively higher and more stable decolorization across a wider range of conditions, indicating better tolerance to environmental fluctuations. SC4, although highly efficient under optimal conditions, exhibited a sharper decline under stress conditions, suggesting narrower environmental adaptability. Similarly, MC2 showed moderate efficiency with noticeable sensitivity to acidic pH and lower temperatures. In contrast, the bacterial consortium outperformed all individual isolates in terms of both efficiency and robustness. The consortium maintained consistently high decolorization efficiency across a broader range of pH 5-7.5 and temperature 25-35 °C conditions, demonstrating superior adaptability. Unlike individual isolates, which showed specific optimal ranges, the consortium exhibited functional stability under both neutral and mildly acidic conditions, making it more suitable for real-world wastewater treatment scenarios where environmental conditions may fluctuate. The enhanced performance of the consortium can be attributed to synergistic interactions among different bacterial species, allowing for complementary metabolic pathways, efficient utilization of intermediate degradation products, and improved resistance to environmental stress (Thiruppathi et al., 2021). Such cooperative behavior results in more complete and efficient degradation of complex dye molecules compared to single isolates. Across all systems, acidic

conditions (pH 5) and extreme temperature combinations led to a significant decline in decolorization efficiency, confirming that unfavourable environmental conditions inhibit microbial growth and enzyme activity. However, the consortium showed comparatively better tolerance even under these stress conditions, further emphasizing its advantage. In summary, while individual isolates demonstrate high efficiency under specific optimized conditions, the bacterial consortium offers superior performance, broader environmental tolerance, and greater operational stability. Therefore, the consortium represents a more promising and reliable candidate for large-scale bioremediation of dye-contaminated wastewater, particularly under variable environmental conditions (Mawad et al., 2020b).

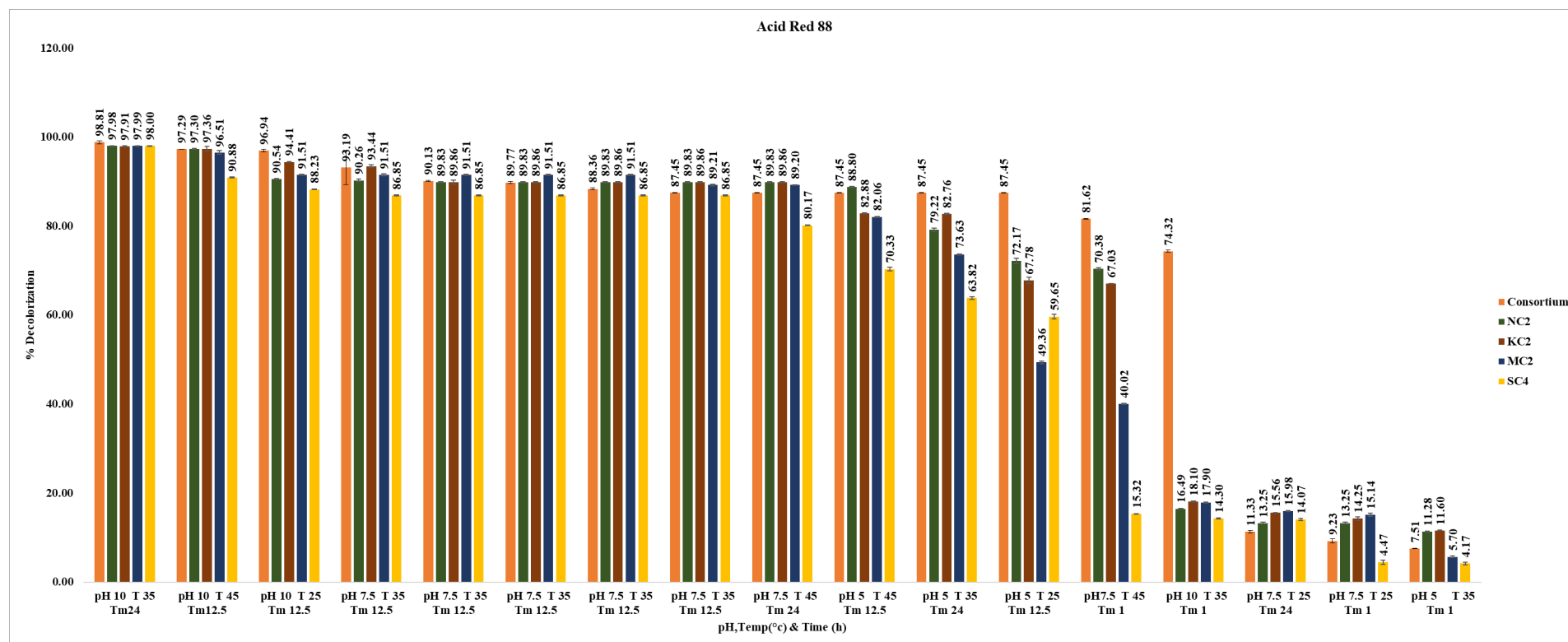


Figure 5.43: RSM based optimization of pH, temperature, and incubation time for the decolorization of AR88 by selected bacterial isolates SC4, MC2, KC2, NC2 and the bacterial consortium. The graph illustrates the comparative effect of different pH (5, 7.5, and 10) temperature (25, 35, and 45 °C) and incubation time (1,12.5 and 24 h) combinations on % decolorization, highlighting the optimal conditions and interaction effects for maximum dye degradation.

5.15 Phytotoxicity Studies

5.15.1 Effect of biodegradation on the phytotoxicity of AR88 on *Triticum aestivum*

The phytotoxic effects of the dye and the toxicity reduction achieved after degradation by the microbial consortium were evaluated using *Triticum aestivum*. Seed germination percentage and seedling growth parameters, including stem length, root length, and leaf length, were recorded after exposure to different concentrations of untreated dye and its degraded products (Table 5.18). Seed germination was highest in the control treatment 92.86 %. Exposure to the untreated dye resulted in a concentration-dependent reduction in germination percentage. Germination decreased to 78.57 % at 100 ppm and further declined to 71.43 % at 200 ppm and 300 ppm, indicating the inhibitory effect of the dye on seed viability and early metabolic processes. In contrast, seeds treated with the consortium-degraded dye products showed relatively higher germination percentages 85.71 %, and 80.00 % at 100, 200, and 300 ppm, respectively, which were closer to the control, demonstrating effective detoxification after microbial degradation. Seedling growth parameters also exhibited a clear dose-dependent toxicity pattern (Figure 5.44). Stem length in the control was 6.17 ± 0.17 cm and remained comparable at 100 ppm untreated dye 6.27 ± 0.15 cm. However, a significant reduction was observed at higher concentrations, with stem length decreasing to 5.43 ± 0.23 cm at 200 ppm and 5.27 ± 0.15 cm at 300 ppm. Root length followed a similar trend, with control seedlings showing 4.17 ± 0.17 cm, while untreated dye exposure resulted in reduced root length of 3.47 ± 0.26 cm at 200 ppm and 3.03 ± 0.09 cm at 300 ppm, indicating higher sensitivity of root tissues to dye toxicity (Figure 5.45). Leaf length was also negatively affected, decreasing from 10.60 ± 0.10 cm in the control to 9.23 ± 0.15 cm at 200 ppm and 8.83 ± 0.17 cm at 300 ppm untreated dye. In contrast, seedlings treated with the degraded dye products showed marked recovery in growth parameters. Stem length 6.00 ± 0.00 cm, 6.00 ± 0.00 cm, and 5.97 ± 0.03 cm, root length 4.03 ± 0.09 cm, 4.00 ± 0.00 cm, and 3.93 ± 0.03 cm, and leaf length 8.87 ± 0.33 cm, 8.50 ± 0.29 cm, and 10.17 ± 0.09 cm at 100, 200, and 300 ppm, respectively, were comparable to or close to control values and significantly higher than those observed in untreated dye treatments at corresponding concentrations. Statistical analysis indicated that most growth parameters in degraded dye treatments showed no significant difference ($p > 0.05$) compared to the control, whereas significant inhibition ($p < 0.05$) was observed at higher concentrations of untreated dye, as indicated by differing superscripts. Overall, the untreated dye exhibited clear concentration-dependent phytotoxic effects on *Triticum aestivum*.

germination and seedling growth. In contrast, the degraded dye products showed substantially reduced toxicity and supported near-normal plant growth, confirming the efficiency of the microbial consortium in detoxifying the dye.

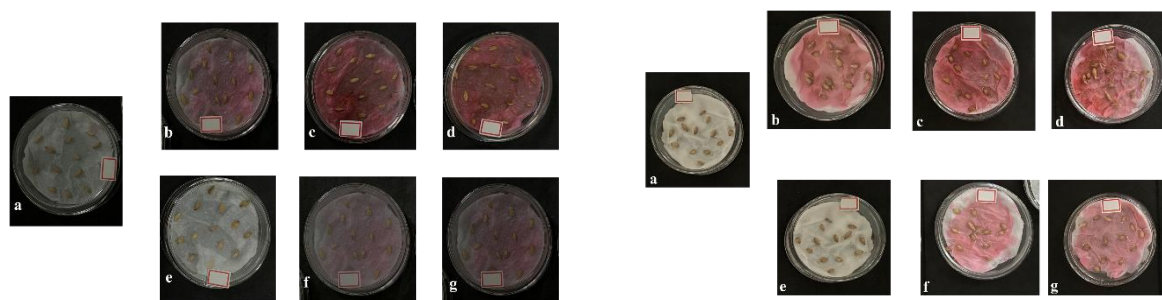


Figure 5.44: Phytotoxicity assessment of Acid Red 88 and its bacterial degradation products using *Triticum aestivum* seeds. The left panel depicts the experimental setup prior to germination, while the right panel shows seedling growth after 3 days of incubation. (a) control (b-d) AR88 at concentrations of 100, 200, and 300 ppm, respectively, and (e-g) corresponding concentrations (100, 200, and 300 ppm) of bacterially degraded dye products.



Figure 45: Effect of Acid Red 88 and its bacterial degradation products on *Triticum aestivum* (a) Control (b) Seedlings exposed to 100 ppm AR88 (c) Seedlings treated with 200 ppm AR88 (d) Seedlings exposed to 300 ppm AR88 (e-g) Seedlings treated with bacterially degraded products of AR88 at 100, 200, and 300 ppm, respectively.

Table 5.18: Effect of AR88 dye and degraded products on the germination and growth parameters of *Triticum aestivum*.

Parameter Studied	Control	100ppm		200ppm		300ppm	
		Undegraded Dye	Degraded Dye	Undegraded Dye	Degraded Dye	Undegraded Dye	Degraded Dye
Germination (%)	92.86 ± 0.05	78.57 ± 0.05	85.71 ± 0.05	71.43 ± 0.05	80.00 ± 0.05	71.43 ± 0.05	80.00 ± 0.05
Stem length (cm)	6.17 ± 0.17a	6.27 ± 0.15a	6.00 ± 0.00a	5.43 ± 0.23b	6.00 ± 0.00a	5.27 ± 0.15b	5.97 ± 0.03a
Root length (cm)	4.17 ± 0.17a	4.47 ± 0.26a	4.03 ± 0.09a	3.47 ± 0.26bc	4.00 ± 0.00a	3.03 ± 0.09c	3.93 ± 0.03ab
Leaf length (cm)	10.60 ± 0.10a	10.67 ± 0.15a	10.00 ± 0.00b	9.23 ± 0.15cd	9.83 ± 0.17b	8.83 ± 0.17d	9.50 ± 0.29bc

Values are expressed as mean ± SE from three replicates. Different superscript letters within the same row represent statistically significant differences among treatments as determined by one-way ANOVA followed by DMRT at $p < 0.05$.

5.15.2 Effect of biodegradation on the phytotoxicity of AR88 on *Vigna radiata*

The phytotoxic effects of the dye and the reduction in toxicity achieved after degradation by the microbial consortium were evaluated using *Vigna radiata*. Seed germination percentage and seedling growth parameters, including stem length, root length, and leaf length, were recorded after exposure to different concentrations of untreated dye and its degraded products (Table 5.19). Seed germination was highest in the control treatment 85.71 %. Exposure to untreated dye resulted in a clear concentration-dependent reduction in germination percentage. Germination decreased to 78.57 % at 100 ppm, further declined to 64.29 % at 200 ppm, and reached 57.14 % at 300 ppm, indicating strong phytotoxic effects at higher concentrations. In contrast, seeds treated with consortium-degraded dye products showed relatively higher germination percentages (79.14 %, 78.71 %, and 78.57 % at 100, 200, and 300 ppm, respectively), which were close to the control, demonstrating effective detoxification following microbial degradation. Seedling growth parameters also exhibited a dose-dependent response

(Figure 5.46). Stem length in the control was 17.27 ± 0.15 cm and showed a slight increase at 100 ppm untreated dye 18.27 ± 0.15 cm, indicating a possible stimulatory effect at lower concentration. However, stem length decreased at higher concentrations, reaching 16.50 ± 0.29 cm at 200 ppm and 16.47 ± 0.12 cm at 300 ppm, with statistically significant differences as indicated by superscripts. Root length showed a gradual decline with increasing dye concentration. The control recorded 4.17 ± 0.17 cm, which decreased to 3.97 ± 0.29 cm at 100 ppm, 3.47 ± 0.26 cm at 200 ppm, and 3.03 ± 0.09 cm at 300 ppm, reflecting the sensitivity of root systems to dye-induced stress (Figure 5.47). Leaf length also exhibited a similar trend. The control value was 2.27 ± 0.15 cm, which increased slightly at 100 ppm 2.63 ± 0.13 cm, followed by a reduction to 2.23 ± 0.15 cm at 200 ppm and 1.87 ± 0.09 cm at 300 ppm, indicating inhibitory effects at higher dye concentrations. In contrast, seedlings treated with degraded dye products showed considerable recovery in growth parameters. Stem length values (17.00 ± 0.00 cm, 16.79 ± 0.03 cm, and 16.80 ± 0.15 cm) were comparable to the control, indicating reduced toxicity. Root length in degraded treatments (4.00 ± 0.00 cm, 3.97 ± 0.03 cm, and 4.00 ± 0.06 cm) closely matched control values across all concentrations, suggesting effective alleviation of toxicity in root development. Leaf length values (2.27 ± 0.15 cm, 2.00 ± 0.00 cm, and 2.00 ± 0.00 cm) were also comparable to the control and higher than untreated dye at elevated concentrations. Statistical analysis revealed that untreated dye at higher concentrations caused significant inhibition ($p < 0.05$) in most growth parameters, as indicated by differing superscripts. In contrast, degraded dye treatments showed no significant difference ($p > 0.05$) from the control in several parameters, particularly root length and stem length. Overall, untreated dye exhibited pronounced, concentration-dependent phytotoxic effects on *Vigna radiata*, affecting germination and seedling growth. In contrast, the degraded dye products demonstrated substantially reduced toxicity and supported near-normal plant growth, confirming the efficiency of the microbial consortium in detoxifying the dye (Kumar and Thakur, 2025).

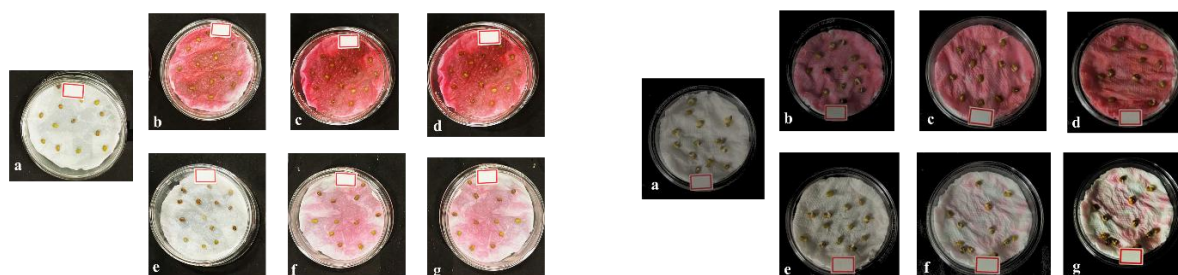


Figure 5.46: Phytotoxicity assessment of Acid Red 88 and its bacterial degradation

products using *Vigna radiata* seeds. The left panel depicts the experimental setup prior to germination, while the right panel shows seedling growth after 3 days of incubation (a)Control, (b-d) AR88 at concentrations of 100, 200, and 300 ppm, respectively and (e-g) corresponding concentrations (100, 200, and 300 ppm) of bacterially degraded dye products.



Figure 5.47: Effect of Acid Red 88 and its bacterial degradation products on *Vigna radiata* seedling growth. (a) Control, (b-f) Seedlings exposed to increasing concentrations of AR88 (100, 200, 300, 400, and 500 ppm), (g) Seedlings treated with bacterially degraded products of AR88.

Table 5.19: Effect of Acid Red 88 and its degraded products on seed germination and seedling growth parameters of *Vigna radiata*.

Parmeter Studied	Control	100ppm		200ppm		300ppm	
		Undegraded Dye	Degraded Dye	Undegraded Dye	Degraded Dye	Undegraded Dye	Degraded Dye
Germination (%)	85.71 ± 0.5	78.57 ± 0.6	79.14 ± 0.0	64.29 ± 0.5	78.71 ± 0.0	57.14 ± 0.0	78.57 ± 0.0

Stem length (cm)	17.27 ± 0.15b	18.27 ± 0.15a	17.00 ± 0.00bc	16.50 ± 0.29cd	16.97 ± 0.03bcd	16.47 ± 0.12d	16.80 ± 0.15bcd
Root length (cm)	4.17 ± 0.17a	3.97 ± 0.29ab	4.00 ± 0.00ab	3.47 ± 0.26bc	3.97 ± 0.03ab	3.03 ± 0.09c	4.00 ± 0.06ab
Leaf length (cm)	2.27 ± 0.15b	2.63 ± 0.13a	2.27 ± 0.15b	2.23 ± 0.15bc	2.00 ± 0.00bc	1.87 ± 0.09c	2.00 ± 0.00bc

Values are expressed as mean ± SE from three replicates. Different superscript letters within the same row represent statistically significant differences among treatments as determined by one-way ANOVA followed by DMRT at $p < 0.05$.

5.16 Novelty and environmental significance of the study

The present investigation provides one of the few integrated assessments of dye-degrading microorganisms from high-altitude wetlands of Jammu and Kashmir. The study combines wetland bioprospecting, multi-dye screening, quantitative UV-Vis evaluation, molecular characterization, degradation-product analysis, and phytotoxicity assessment within a single experimental framework. The identification of indigenous isolates capable of degrading Acid Red 88 and other synthetic dyes under varying incubation conditions demonstrates the ecological adaptation and metabolic versatility of wetland microorganisms. Importantly, the study evaluates not only dye decolorization but also reduction in phytotoxicity, thereby addressing the environmental safety of the treatment process. These findings contribute to the development of sustainable, region-specific bioremediation strategies for textile dye-contaminated wastewater and highlight himalayan wetlands as valuable reservoirs of industrially relevant microbial resources.

SUMMARY AND CONCLUSION

Understanding microbial diversity and its functional potential in environmental remediation has become a key focus in modern microbiology. Industrialization has led to the extensive release of synthetic dyes, particularly azo dyes, into aquatic ecosystems, posing serious ecological and health hazards due to their persistence, toxicity, and recalcitrant nature. In this context, microbial bioremediation has emerged as an eco-friendly, cost-effective, and sustainable approach for the degradation and detoxification of such pollutants. The present study, entitled “Bioprospecting Of Dye Degrading Microorganisms Isolated From Wetlands Of Jammu And Kashmir And The Study Of Dye Phytotoxicity,” was designed to explore the diversity of dye-degrading bacteria from wetland ecosystems and evaluate their efficiency in dye degradation and detoxification. Wetlands of J&K, including Surinsar, Mansar, Nigeen Lake, Khushal Sar, and Gilsar, represent ecologically rich and dynamic environments that harbor diverse microbial communities adapted to fluctuating physicochemical conditions. These habitats provide a promising source for isolating microorganisms with novel metabolic capabilities, including the degradation of complex xenobiotic compounds such as synthetic dyes. The present investigation focused on isolating, screening, and characterizing dye-degrading bacteria, optimizing degradation conditions, analyzing degradation pathways, and evaluating the phytotoxicity of treated and untreated dye samples. A total of seventy-one bacterial isolates were obtained from collected soil and water samples and screened for their ability to decolorize multiple synthetic dyes. Among these, selected isolates (SC4, MC2, KC2, and NC2) exhibited significant decolorization potential and were further investigated. Based on optimization studies, a dye concentration of 100 ppm was selected as optimal for efficient and stable degradation under controlled conditions. The biodegradation of AR88, a representative azo dye, was systematically evaluated. Optimization of environmental parameters using RSM based on BBD revealed that pH, temperature, and incubation period significantly influenced degradation efficiency. Individual isolates exhibited distinct optimal conditions, with SC4 and NC2 favouring alkaline pH (pH 10), moderate temperature (35 °C) and time 24 h, while MC2 and KC2 performed optimally at slightly alkaline pH (7.5), higher temperature (45 °C) and time 24 h. Among all systems, the bacterial consortium demonstrated superior performance, achieving maximum decolorization efficiency (98.81%) under mildly acidic to neutral conditions (pH 5-7.5), moderate temperatures (25-35 °C) and time (24 h), indicating enhanced adaptability and synergistic interactions among microbial members. FTIR

analysis confirmed significant structural alterations in AR88 following microbial treatment. Key functional groups, including azo bonds, aromatic rings, sulfonic groups, and amine linkages, exhibited noticeable shifts, reduction in intensity, or disappearance, indicating enzymatic breakdown rather than mere adsorption. GC-MS analysis further revealed the formation of low-molecular-weight metabolites such as acetic acid, propanoic acid, and butanoic acid. These compounds indicate extensive degradation and mineralization of the dye molecule through sequential enzymatic reactions involving azoreductase, laccase, and related oxidative enzymes. Based on FTIR and GC-MS findings, a plausible degradation pathway was proposed, involving initial azo bond cleavage followed by aromatic ring breakdown, desulfonation, and conversion into simple aliphatic acids. These intermediates are subsequently assimilated into central metabolic pathways such as the tricarboxylic acid (TCA) cycle, leading to complete mineralization into CO₂ and water. The environmental safety of the degraded products was evaluated through phytotoxicity studies using *Triticum aestivum* and *Vigna radiata*. Untreated AR88 exhibited strong concentration-dependent phytotoxic effects, significantly inhibiting seed germination and seedling growth parameters. In contrast, bacterially degraded dye products showed markedly reduced toxicity, with germination rates and growth parameters comparable to control conditions. The recovery of root, stem, and leaf growth in treated samples confirmed effective detoxification and highlighted the eco-friendly nature of microbial degradation. Comparative evaluation demonstrated that while individual isolates showed high degradation efficiency under specific conditions, the bacterial consortium exhibited superior performance, broader environmental tolerance, and enhanced stability. The synergistic interactions within the consortium enabled more efficient utilization of intermediate metabolites and improved resistance to environmental stress, making it a promising candidate for large-scale bioremediation applications.

The major findings of the study are summarized as follows:

- ❖ A total of 71 bacterial isolates were successfully obtained from wetland ecosystems of Jammu and Kashmir and screened for dye-degrading potential.
- ❖ Four potent isolates (SC4, MC2, KC2, and NC2) demonstrated significant decolorization efficiency and were selected for further study.
- ❖ Optimal dye degradation was achieved at 100 ppm concentration under controlled pH and temperature conditions.
- ❖ RSM-based optimization revealed that pH, temperature, and incubation period significantly influence dye degradation efficiency.

- ❖ The bacterial consortium exhibited the highest decolorization efficiency (98.81%) and greater environmental adaptability compared to individual isolates.
- ❖ FTIR analysis confirmed the breakdown of functional groups such as azo bonds, aromatic rings, sulfonic groups, and amine linkages.
- ❖ GC-MS analysis identified low-molecular-weight metabolites (acetic acid, propanoic acid, butanoic acid), indicating advanced degradation and mineralization.
- ❖ A comprehensive degradation pathway was proposed, involving azo bond cleavage, aromatic ring degradation, and assimilation into the TCA cycle.
- ❖ Phytotoxicity studies revealed that untreated dye exhibited significant inhibitory effects on seed germination and growth of *Triticum aestivum* and *Vigna radiata*.
- ❖ Degraded dye products showed significantly reduced toxicity and supported near-normal plant growth, confirming effective detoxification.
- ❖ The bacterial consortium demonstrated superior efficiency, robustness, and potential for practical application in wastewater treatment.

The present study successfully demonstrates the potential of wetland-derived bacterial isolates from Jammu and Kashmir for the biodegradation and detoxification of synthetic azo dyes. A major novelty of this work is the integrated approach combining microbial bioprospecting from Himalayan wetlands with UV-Vis screening, RSM-based optimization, FTIR and GC-MS analyses, and phytotoxicity assessment, which enabled simultaneous evaluation of degradation efficiency, degradation pathways, and environmental safety. Among the investigated systems, the bacterial consortium showed the highest decolorization efficiency (98.81%) and greater adaptability to varying environmental conditions, highlighting the importance of synergistic microbial interactions in dye degradation. The significant reduction in phytotoxicity of treated dye samples confirms the eco-friendly nature of the biodegradation process and supports its practical application in textile wastewater treatment and other dye-contaminated effluents. Overall, this study provides a sustainable and cost-effective bioremediation strategy and establishes Himalayan wetlands as valuable reservoirs of metabolically versatile dye-degrading microorganisms. Future research should focus on pilot-scale validation under real industrial conditions, detailed characterization of dye-degrading enzymes, omics-based analysis of degradation pathways, and genetic improvement of promising microbial strains to enhance degradation efficiency and environmental robustness.

6.1 Future scope

In the field of environmental biotechnology, the bioprospecting of dye-degrading microorganisms from natural ecosystems such as wetlands offers immense opportunities for advancing sustainable wastewater treatment technologies. Moving forward, research efforts should focus on expanding the exploration of diverse and underexplored ecological niches, including high-altitude wetlands, cold deserts, and polluted aquatic systems, to identify novel microbial strains with enhanced dye-degrading capabilities (Al-Mutairi, 2025). Such environments are likely to harbour metabolically versatile microorganisms capable of degrading a wide range of recalcitrant pollutants under extreme conditions. There is a pressing need to integrate advanced molecular and omics-based approaches, including metagenomics, metatranscriptomics, and proteomics, to gain deeper insights into the functional potential and regulatory mechanisms of dye-degrading microbial communities. These approaches will enable the identification of key genes, enzymes, and metabolic pathways involved in azo dye degradation, thereby facilitating the discovery of novel biocatalysts with industrial relevance (Sharma et al., 2024). High-throughput sequencing and bioinformatics tools should be further developed to efficiently analyze large datasets and predict functional annotations with greater accuracy. Future studies should also emphasize the isolation and characterization of specific enzymes such as azoreductase, laccases, and peroxidases, which play crucial roles in dye degradation. Detailed biochemical and structural characterization of these enzymes will provide insights into their catalytic mechanisms, substrate specificity, and stability under varying environmental conditions. Such knowledge can be exploited for the development of enzyme-based treatment systems and immobilized biocatalysts for continuous wastewater treatment processes (Rai et al., 2026). Another important direction involves the scale-up and application of microbial consortia in real industrial settings. Pilot-scale and field-level studies should be conducted to evaluate the efficiency, stability, and economic feasibility of these consortia in treating textile effluents under variable environmental conditions. The synergistic interactions observed in microbial consortia can be further optimized to enhance degradation efficiency, resilience, and adaptability in large-scale bioreactors. The integration of nanotechnology and microbial biotechnology represents another promising avenue for improving dye degradation efficiency. The use of nanomaterials, such as metal nanoparticles or nanocomposites, in combination with microbial systems can enhance catalytic activity, improve pollutant adsorption, and facilitate faster degradation rates. Additionally, hybrid treatment systems combining biological and physicochemical methods could be developed for

more effective remediation of complex industrial effluents. Synthetic biology and genetic engineering approaches offer exciting possibilities for designing genetically modified microorganisms with enhanced degradation capabilities. By manipulating specific genes and metabolic pathways, it is possible to develop strains with improved efficiency, broader substrate range, and tolerance to environmental stress factors (Manjarrez-Paba et al., 2025). Furthermore, genome editing tools such as CRISPR-Cas systems can be utilized to optimize metabolic pathways and regulatory networks involved in dye degradation. Long-term ecological and environmental impact assessments should also be prioritized to ensure the safety and sustainability of microbial bioremediation strategies. Studies focusing on the fate of degradation intermediates, potential toxicity, and impact on soil and aquatic ecosystems are essential for validating the environmental compatibility of these approaches. In this context, advanced toxicity assays, including genotoxicity and ecotoxicological evaluations, can provide comprehensive insights into the safety of treated effluents. Finally, interdisciplinary collaboration between microbiologists, environmental engineers, chemists, and computational biologists will be crucial for translating laboratory findings into practical applications. By integrating innovative technologies, optimizing microbial systems, and adopting sustainable practices, future research can significantly contribute to the development of efficient, eco-friendly, and economically viable solutions for the treatment of dye-contaminated wastewater, thereby addressing one of the major environmental challenges of modern industrialization.

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Dual dye degradation of methylene blue and orange II by a wetland-derived *Acinetobacter* species: RSM optimization, GC–MS pathway elucidation, and phytotoxicity assessment

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ABSTRACT

Synthetic dyes such as Methylene Blue (MB) and Orange II (O-II) cause serious environmental and phytotoxic risks due to their chemical stability and presence in aquatic systems. Although microbial dye degradation has been widely studied, most reports focus on single dyes and lack comprehensive validation of detoxification, particularly using wetland-derived bacterial strains. In this study, *Acinetobacter* sp. SC2 was isolated from the Surinsar wetland (Jammu and Kashmir) and evaluated for its ability to independently degrade two structurally different dyes under optimized conditions. Using response surface methodology (RSM), optimal conditions (pH 7.5–10, 40–45 °C, 24 h) resulted in 90.99% degradation of MB and 95.96% degradation of O-II. Fourier transform infrared spectroscopy (FTIR) analysis confirmed dye degradation through the disappearance and shifting of characteristic azo and thiazine functional group peaks, while gas chromatography–mass spectrophotometry (GC–MS) analysis revealed the formation of low-molecular-weight, less toxic metabolites, indicating structural breakdown of the parent dyes. Phytotoxicity studies using *Vigna radiata* demonstrated severe toxicity of untreated dyes, with significant reductions in seed germination and root growth. In contrast, bacterially degraded dye products showed near-normal germination and growth, confirming effective detoxification. This study provides an integrated evaluation of a wetland derived *Acinetobacter* sp. SC2 for dual-dye degradation by combining RSM optimization, metabolite analysis, and phytotoxicity assessment. Future studies should focus on enzyme level characterization, mixed-dye systems, and scale-up applications for wastewater treatment.

1. Introduction

Industrial growth in recent years has led to the release of large amounts of harmful chemical pollutants into the environment. Among these pollutants, synthetic dyes are one of the most persistent and toxic. The textile industry produces huge volumes of wastewater containing dyes, which negatively affect aquatic life, soil microorganisms, plant growth, and human health. Because textile dyes degrade very slowly and are highly toxic, they accumulate in water bodies, disturb natural ecosystems, and block light penetration, thereby reducing photosynthesis in

aquatic plants (Faouzi et al., 2023). Many textile-processing areas, especially in South Asia and Brazil, discharge large quantities of synthetic dyes into rivers and freshwater sources, leading to pollution of surface water and drinking water. Worldwide, about 2,80,000 metric tons of synthetic dyes enter wastewater every year, while total dye consumption is close to one million metric tons annually (Sudarshan et al., 2023). Among these dyes, MB and O-II are of serious concern because of their strong color, stable chemical structure, wide industrial use, and toxic nature. These dyes are commonly used during textile dyeing, printing, washing, and finishing processes, which results in their

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Review

Advancing Dye Degradation: Integrating Microbial Metabolism, Photocatalysis, and Nanotechnology for Eco-Friendly Solutions

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Highlights

- Microbes degrade dyes through enzymatic reactions, breaking down complex dye molecules into simpler, non-toxic substances.
- Degradation of dyes through exposure to light is often enhanced by catalysts.
- Nanoparticles enhance dye degradation through catalytic activity, often involving advanced oxidation processes (AOPs).

Abstract: Textile dyes pose a major environmental threat due to their toxicity, persistence in water bodies, and resistance to conventional wastewater treatment. To address this, researchers have explored biological and physicochemical degradation methods, focusing on microbial, photolytic, and nanoparticle-mediated approaches, among others. Microbial degradation depends on fungi, bacteria, yeasts, and algae, utilizing enzymatic pathways involving oxidoreductases like laccases, peroxidases, and azoreductases to breakdown or modify complex dye molecules. Photolytic degradation employs hydroxyl radical generation and electron-hole pair formation, while nanoparticle-mediated degradation utilizes titanium dioxide (TiO₂), zinc oxide (ZnO), and silver (Ag) nanoparticles to enhance dye removal. To improve efficiency, microbial consortia have been developed to enhance decolorization and mineralization, offering a cost-effective and eco-friendly alternative to physicochemical methods. Photocatalytic degradation, particularly using TiO₂, harnesses light energy for dye breakdown. Research advancements focus on shifting TiO₂ activation from UV to visible light through doping and composite materials, while optimizing surface area and mesoporosity for better adsorption. Nanoparticle-mediated approaches benefit from a high surface area and rapid adsorption, with ongoing improvements in synthesis, functionalization, and reusability, particularly through magnetic nanoparticle integration. These emerging technologies provide sustainable solutions for dye degradation. The primary aim of this review is to comprehensively evaluate and synthesize current research and advancements in the degradation of azo dyes through microbial methods, photolytic processes, and nanotechnology-based approaches. The review also provides detailed information on salient mechanistic aspects of these methods, efficiencies, advantages, challenges, and potential applications in industrial and environmental contexts.

Keywords: microorganisms; nanoparticles; degradation; dye; contamination; sustainability



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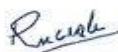
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