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A Sustainable Approach to Water Pollution Control: Microbial Decolorization and Detoxification of Basic Fuchsine-Contaminated Textile Effluent

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Abstract
The discharge of dye-containing textile effluents poses a significant environmental challenge owing to their high color intensity, toxicity, and resistance to natural degradation. Among synthetic dyes, basic fuchsin is a recalcitrant triphenylmethane dye widely used in the textile industry and is known for its adverse effects on aquatic ecosystems and human health. In the present study, we isolated and evaluated potential bacterial strains capable of biodecolorizing textile wastewater amended with basic fuchsin dye. Textile effluents and contaminated soil samples were collected from a textile industrial site, and bacterial isolates were enriched and screened for decolorization efficiency. Eight bacterial isolates (TP1-TP8) were obtained, among which isolate TP7 exhibited the highest decolorization efficiency of 70.25 ± 1.0% under initial conditions. Morphological and microscopic characterization suggested that TP7 belonged to the genus *Bacillus*. Various physicochemical parameters affecting biodecolorization were optimized, including medium concentration, carbon source, nitrogen source, inoculum size, pH, and temperature. The optimum conditions were found to be 1× nutrient medium, 0.25% sucrose, 0.2% peptone, 3% inoculum size, pH 7, and 37°C, under which TP7 achieved a maximum decolorization efficiency of 99.99 ± 0.01% within 24 h. Enzymatic studies revealed significant manganese peroxidase (MnP) activity with a peak value of 7.9 U mL^{-1} h^{-1}, indicating its involvement in dye degradation. Furthermore, phytotoxicity assessment using *Vigna radiata* demonstrated improved seed germination and growth in treated effluents compared to untreated wastewater, confirming substantial detoxification. The findings indicate that *Bacillus* sp. TP7 possesses remarkable potential for the efficient biodecolorization and detoxification of Basic Fuchsin-containing textile effluents, highlighting its suitability for sustainable bioremediation applications in industrial wastewater treatment.

Keywords: Basic Fuchsin, Textile wastewater, Biodecolorization, Manganese peroxidase, Bioremediation, Phytotoxicity

1. Introduction

Textile industries are among the largest consumers of water and major contributors to industrial wastewater generation. The extensive use of synthetic dyes during textile processing results in the discharge of large volumes of colored effluents containing complex organic compounds that are often resistant to natural degradation processes. It is estimated that a considerable fraction of the dyes used during textile manufacturing is lost during dyeing operations and released into wastewater streams, causing severe environmental pollution. Dye-contaminated wastewater reduces light penetration in aquatic ecosystems, affects photosynthetic activity, decreases dissolved oxygen levels, and adversely affects aquatic flora and fauna. These pollutants also contribute significantly to the biochemical oxygen demand (BOD) and chemical oxygen demand (COD), leading to the deterioration of water quality. (Dutta, 2022; Sudarshan et al., 2022)

Among the various synthetic dyes, Basic Fuchsin (pararosaniline hydrochloride) is a cationic triphenylmethane dye widely used in textile dyeing, microbiological staining, histological analysis, and analytical chemistry because of its intense magenta color and high chemical stability. The dye possesses a highly conjugated aromatic structure and amino functional groups that impart remarkable color strength and affinity toward textile fibers. However, the same structural characteristics make Basic Fuchsin highly resistant to biodegradation and environmental decomposition. Its persistence in wastewater systems has raised significant environmental concerns because of its toxic, mutagenic,

genotoxic, and potentially carcinogenic properties. Continuous release of untreated Basic Fuchsin-containing effluents into natural water bodies can cause long-term ecological damage and health risks. (Aouni et al., 2024)

Conventional methods employed for treating dye-bearing wastewater include adsorption, coagulation-flocculation, membrane filtration, advanced oxidation processes, ion exchange, and chemical precipitation. Although these techniques can effectively remove color from wastewater, they often suffer from limitations such as high operational costs, excessive chemical consumption, sludge generation, and incomplete detoxification. In recent years, biological treatment approaches have emerged as promising alternatives because they are environmentally friendly, cost-effective, and capable of transforming complex dye molecules into less-toxic end products. Biological processes utilize the metabolic activities of microorganisms and their enzymes to achieve the decolorization and degradation of dye pollutants under mild environmental conditions. (Bharathi et al., 2022)

Bacteria have received considerable attention for textile wastewater treatment because of their rapid growth, adaptability, and ability to produce various oxidative and reductive enzymes involved in dye degradation. Several bacterial genera, including *Bacillus*, *Pseudomonas*, *Aeromonas*, and *Alcaligenes*, have been reported to effectively decolorize and biodegrade synthetic dyes through enzymatic mechanisms. Enzymes such as laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), azoreductase, and dye-decolorizing peroxidases (DyPs) play important roles in the breakdown of chromophoric structures responsible for dye coloration. These enzymatic reactions facilitate decolorization while simultaneously reducing the toxicity of the effluent. (Harish et al., 2023)

Optimization of environmental and nutritional parameters is crucial for enhancing microbial dye degradation efficiency. Factors such as pH, temperature, carbon and nitrogen sources, substrate concentration, and inoculum size significantly influence microbial growth and enzymatic activity, thereby affecting the overall decolorization process. Identification of suitable microorganisms and optimization of operational parameters can contribute substantially to the development of efficient bioremediation strategies for textile wastewater treatment. (Ikram et al., 2020)

In the present study, textile effluents amended with basic fuchsin dye were employed to isolate and screen potent dye-decolorizing bacteria. The selected bacterial isolate was evaluated for its decolorization potential under different physicochemical conditions, and the role of manganese peroxidase enzyme in dye degradation was investigated. Furthermore, the detoxification of the treated effluent was assessed using a seed germination assay with *Vigna radiata*. This study aimed to explore the potential application of indigenous bacterial isolates for the sustainable and eco-friendly treatment of textile wastewater contaminated with basic fuchsin dye.(Lazer et al., 2024)

2. Materials and Methods

2.1 Collection and Physicochemical Characterization of Textile Effluent

Textile effluents containing basic fuchsin dye were collected from Parmeshwar Dyeing & Printing Mills P.LTD., Vapi, Gujarat, India. Simultaneously, soil samples were collected from the vicinity of the effluent discharge point for the isolation of indigenous dye-degrading microorganisms. Samples were collected aseptically in sterile containers, transported to the laboratory, and stored at 4°C until further use. (Nivedha et al., 2023)

The collected effluent was analyzed for physicochemical parameters, including pH, temperature, total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total sugars, and total hardness, according to standard APHA procedures. (Gökçekuş et al., 2023)

2.2 Isolation and Enrichment of Dye-Decolorizing Bacteria

Potential dye-degrading bacteria were isolated using enrichment culture techniques. Briefly, 5 g of soil sample was inoculated into 100 mL of nutrient broth supplemented with 2% (v/v) textile effluent and incubated at 37°C under static conditions. Enrichment cultures exhibiting visible decolorization were serially diluted and streaked onto nutrient agar plates supplemented with textile effluent. Distinct colonies were purified by repeated streaking to obtain pure culture. (Bhuiyan et al., 2024)

The nutrient broth consisted of beef extract (3 g L^{-1}), peptone (5 g L^{-1}), and sodium chloride (5 g L^{-1}), while nutrient agar contained the same composition supplemented with 15 g L^{-1} agar. The pH of the media was maintained at 7.2–7.5. (R et al., 2022)

2.3 Morphological and Microscopic Characterization

Purified bacterial isolates were characterized based on colony morphology, including size, shape, margin, elevation, pigmentation, opacity, texture and consistency. Microscopic characterization was performed by Gram staining to determine the cell morphology, cellular arrangement, and Gram reaction. The preliminary identification of potent isolates was based on these phenotypic characteristics. (Elazab et al., 2022)

2.4 Screening of Potential Dye-Decolorizing Isolates

For screening studies, bacterial inocula were prepared by adjusting the optical density to approximately 1.0 at 540 nm, corresponding to approximately 1×10^6 CFU mL^{-1}. Ten percent (v/v) inoculum was transferred into 250 mL Erlenmeyer flasks containing 100 mL nutrient broth supplemented with 100 ppm textile effluent. The cultures were incubated at 37°C under static conditions for 24 h. Samples were collected at 8 h intervals to evaluate the dye decolorization efficiency. (Kachhela et al., 2024)

2.5 Decolorization Assay (Afena et al., 2021)

Aliquots (5 mL) were withdrawn periodically from inoculated cultures and centrifuged at 6000 rpm for 20 min to remove bacterial biomass. The absorbance of the resulting supernatant was measured at 540 nm using a UV-Vis spectrophotometer (Evolution 260 BIO). An uninoculated medium containing textile effluent was used as the control. The decolorization efficiency was calculated using the following equation:

$$\text{Decolorization (\%)} = \frac{A_i - A_f}{A_i} \times 100$$

where Ai and Af represent the initial and final absorbance, respectively.

2.6 Optimization of Biodecolorization Conditions (Mayilsami et al., 2022)

The effects of nutritional and environmental parameters on dye decolorization by the selected isolate were investigated individually while maintaining other factors constant. All optimization experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of medium and 100 ppm textile effluent under static incubation. Study had included Medium concentrations **(**0.5×, 1.0×, 1.5×, 2.0×, and 2.5× w/v); carbon sources (dextrose, galactose, maltose, sucrose, and starch 0.2% w/v); sucrose concentration (0.25, 0.50, 0.75, 1.00, and 1.25% (w/v); nitrogen sources (peptone, yeast extract, beef extract, urea, ammonium sulfate, and sodium nitrate 0.5%, w/v); peptone concentration: 0.1–0.5% (w/v); inoculum size: 1–5% (v/v); textile effluent concentration: 0.1-2.0% (v/v); pH: 6.0-8.0, adjusted using 1 N HCl or 1 N NaOH; Temperature: 25, 30, 35, 40, and 45°C.

2.7 Comparison of Optimized and Unoptimized Conditions

To assess the significance of process optimization, decolorization efficiency under optimized conditions was compared with that obtained under the original unoptimized culture conditions. The maximum color removal achieved under both conditions was used for comparison.

2.8 Enzyme Production and Manganese Peroxidase Assay

To investigate the enzymatic mechanism involved in dye degradation, the selected isolate was cultured in nutrient broth supplemented with 2% textile effluent and incubated at 37°C. Samples were withdrawn at regular intervals and centrifuged at 10,000 rpm for 20 min at 4°C. The resulting supernatant was used as a crude preparation of the extracellular enzyme.

Manganese peroxidase (MnP) activity was determined spectrophotometrically based on the oxidation of phenol red at 610 nm. The reaction mixture consisted of 1.0 mL sodium succinate buffer (50 mM, pH 4.5), 1.0 mL sodium lactate buffer (50 mM, pH 5.0), 0.4 mM manganese sulfate, 0.7 mL phenol red (0.1 mM), 0.4 mL H_2O_2 (50 μM), 1.0 mg mL^{-1} bovine serum albumin or gelatin, and 0.5 mL crude enzyme extract. The reaction was initiated by the addition of H_2O_2 and incubated at 30°C. Enzyme activity was monitored for 4 min, and one unit of MnP activity was defined as an increase of 0.1 absorbance units min^{-1} mL^{-1}.(Chang et al., 2021)

2.9 Phytotoxicity Assessment

The detoxification potential of treated effluents was evaluated using a seed germination assay with *Vigna radiata* seeds. Healthy seeds were surface sterilized using 0.1% mercuric chloride, thoroughly rinsed with sterile distilled water, and soaked for 12–24 h prior to use. Seeds were placed on moist filter paper in sterile Petri dishes and irrigated with untreated, treated, or distilled water (control). Germination was recorded after incubation, and the emergence of the radicle was considered successful germination. (Alessandra Costa Santos et al., 2025)

Germination percentage was calculated as follows:

$$\text{Germination Percentage} = \frac{\text{Number of Germinated Seeds}}{\text{Total Number of Seeds}} \times 100$$

2.10 Statistical Analysis

All experiments were conducted in triplicate, and the results are expressed as the mean ± standard deviation (SD). Decolorization efficiency and enzyme activity were calculated as the average of three independent experiments.

3. Results and Discussion

3.1 Physicochemical Characteristics of Textile Effluent

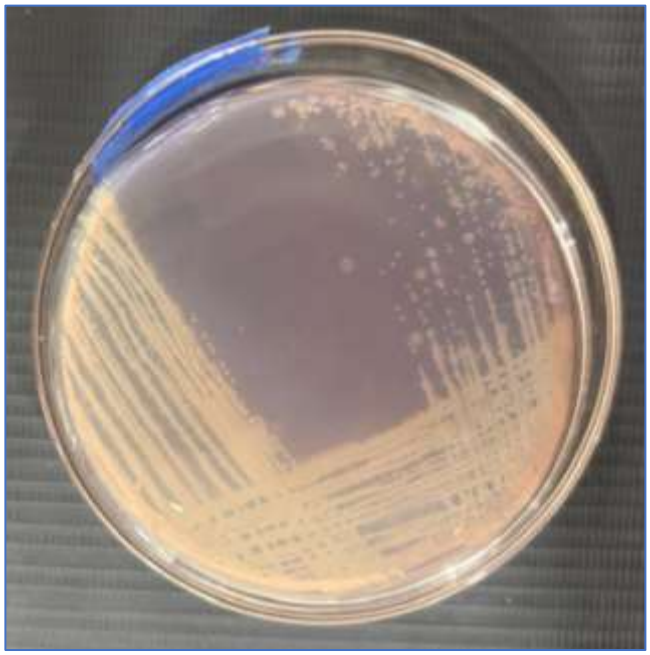
The physicochemical properties of the collected textile effluents revealed a high pollutant load, indicating their potential environmental hazards if discharged without treatment. The raw effluent was dark magenta in color with an acidic pH (6.0 ± 0.2) and elevated levels of total solids, BOD, and COD. The COD and BOD values were 10,724.44 ± 16.5 and 5,412.22 ± 12.3 mg L^{-1}, respectively, indicating the presence of a substantial amount of biodegradable and non-biodegradable organic matter. Following biological treatment, a remarkable reduction in all physicochemical parameters was observed, accompanied by a visible color change from magenta to pale yellow. (Wang et al., 2022)

3.1 Physicochemical Characteristics of Textile Effluent

The substantial decline in BOD and COD suggests the effective degradation of dye molecules and associated organic contaminants by the bacterial treatment process. Similar reductions in organic load during the microbial treatment of textile wastewater have been reported previously, highlighting the potential of bacterial bioremediation as an environmentally sustainable alternative to physicochemical methods. (Sharif, 2020)

3.2 Isolation and Identification of Dye-Decolorizing Bacteria

Eight morphologically distinct bacterial isolates designated TP1 to TP8 were obtained following enrichment and screening procedures. The isolates exhibited considerable variation in colony morphology, pigmentation, and microscopic characteristics. Isolate TP7 demonstrated superior decolorization capability and was selected for further investigation.



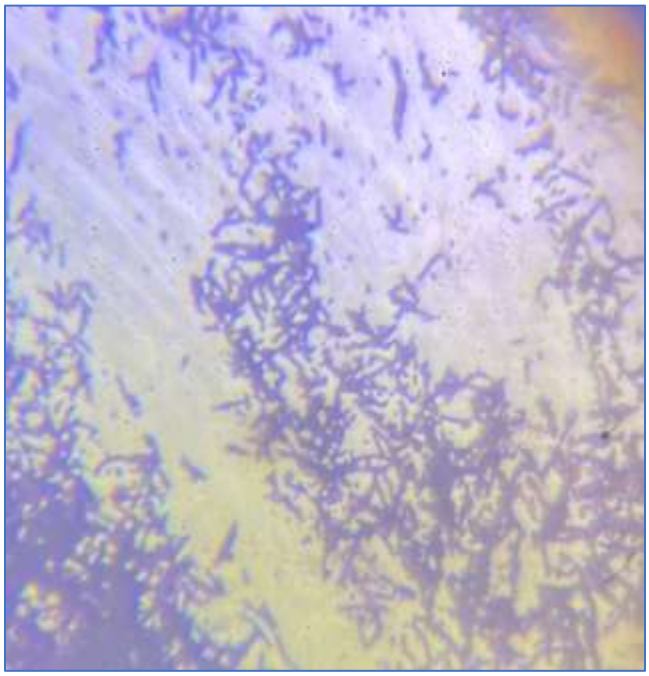


Table 1 Morphological and microscopic characteristics of bacterial isolate TP7

Characteristic	Observation
Colony color	White
Shape	Rod
Gram reaction	Positive
Margin	Undulate
Elevation	Umbilicate
Consistency	Moist
Opacity	Translucent

Morphological examination of TP7 revealed medium-sized, white, smooth, translucent colonies with an undulating margin and umbilicate elevation. Microscopic analysis indicated that the isolate was gram-positive, rod-shaped, and occurred predominantly as single cells. Based on these characteristics, TP7 was tentatively identified as belonging to the genus *Bacillus*.

Species of *Bacillus* have frequently been reported to be efficient degraders of synthetic dyes because of their adaptability and ability to produce oxidative enzymes involved in biodegradation. The predominance of Gram-positive rods among dye-degrading isolates has also been documented in textile wastewater treatment studies. (Akhter et al., 2020)

3.3 Screening of Potent Bacterial Isolates for Decolorization

The decolorization potential of all isolates was evaluated using textile effluents amended with basic fuchsin dye. Among the eight isolates screened, TP7 exhibited the highest decolorization efficiency, achieving 70.25 ± 1.0% color removal within 24 h under static conditions at 37°C. The remaining isolates displayed comparatively lower decolorization efficiencies.

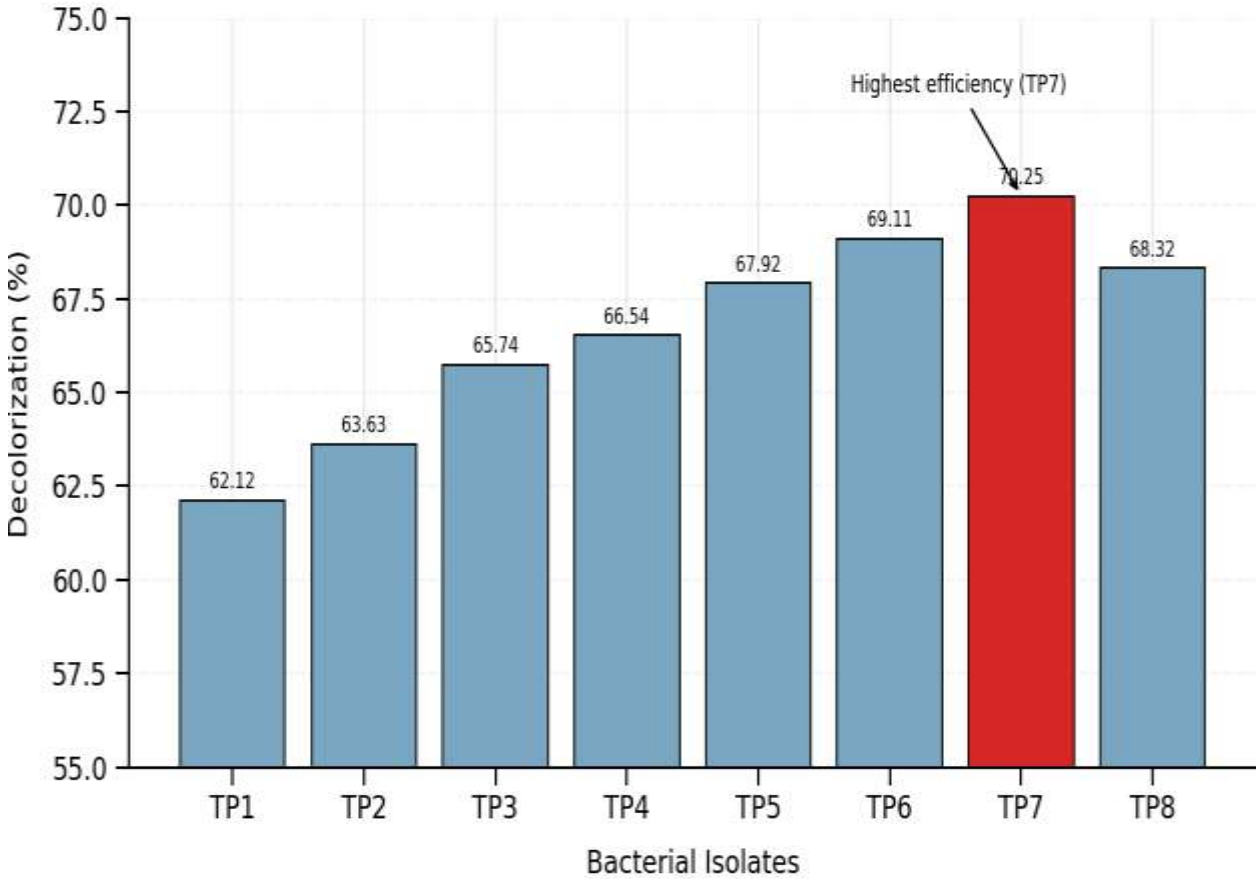


Figure 2. Decolorization efficiency of bacterial isolates (TP1–TP8) isolated from textile effluent-contaminated soil. Isolate TP7 exhibited the highest decolorization efficiency and was therefore selected for further study.

The superior performance of TP7 may be attributed to its enhanced metabolic adaptability and enzymatic capability to attack and degrade the chromophoric structure of the Basic Fuchsin dye. Similar observations have been reported for *Bacillus* spp., which possess efficient extracellular oxidoreductases that are involved in dye degradation. (Diksha Bhapkar et al., 2026)

3.4 Optimization of Biodecolorization Conditions

3.4.1 Effect of Medium Concentration

A medium concentration plays a crucial role in supplying nutrients required for bacterial growth and enzyme production. Among the tested concentrations, 1× nutrient medium resulted in maximum decolorization (99.99 ± 0.1%) after 24 h. Both lower and higher medium concentrations showed comparatively reduced performance.

This observation suggests that balanced nutritional conditions are essential for optimal bacterial metabolism and dye degradation. (Patel et al., 2016)

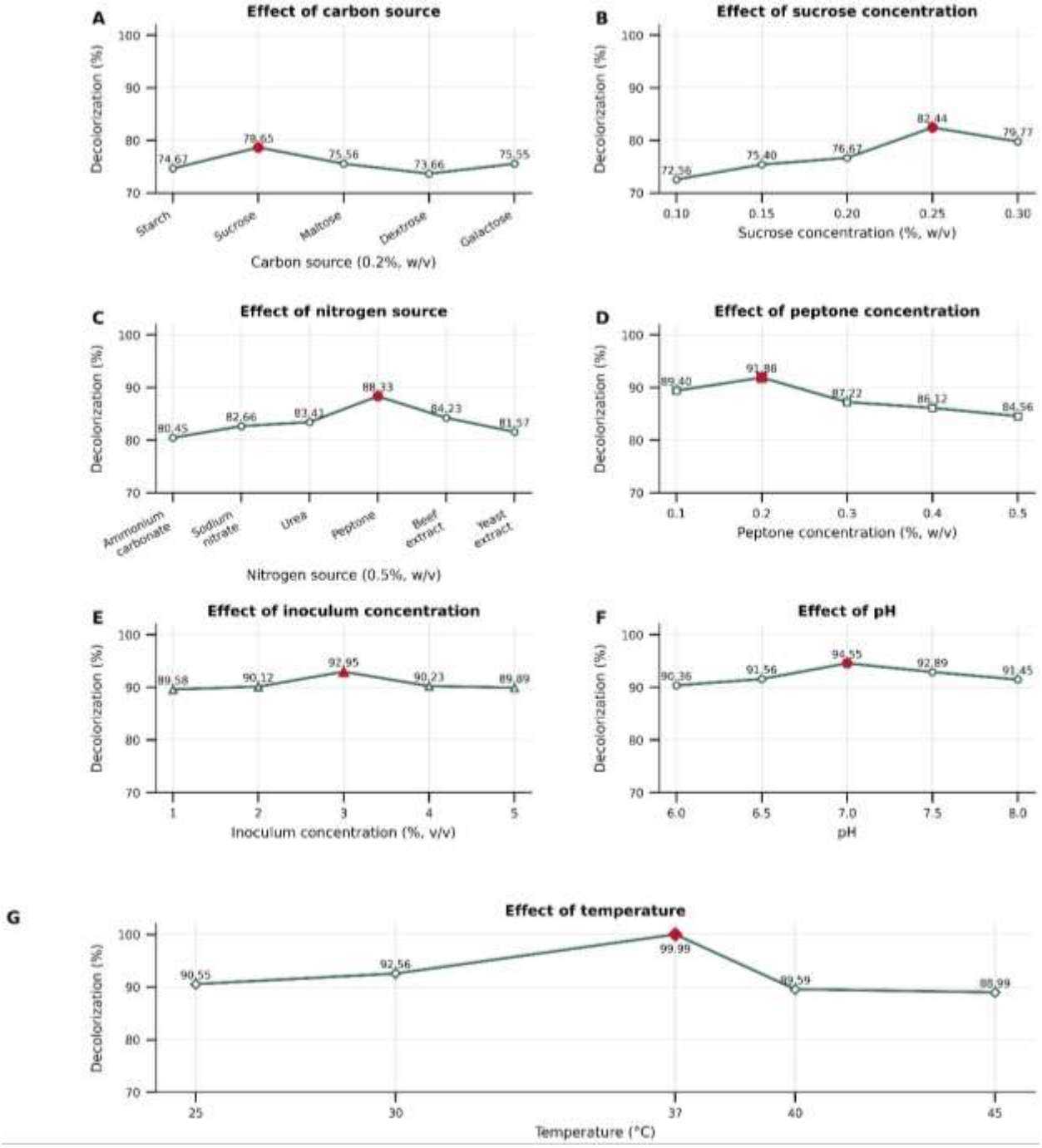


Figure 3 Optimization of nutritional and physicochemical parameters for decolorization of dye by bacterial isolate TP7. The effects of (A) different carbon sources at 0.2% (w/v), (B) sucrose concentration, (C) different nitrogen sources at 0.5% (w/v), (D) peptone concentration, (E) inoculum concentration, (F) pH, and (G) temperature on decolorization efficiency were evaluated. The highest responses within the respective experiments were obtained with sucrose, 0.25% (w/v) sucrose, peptone, 0.2% (w/v) peptone, 3% (v/v) inoculum, pH 7.0, and 37 °C.

3.4.2 Effect of Carbon Source

Different carbon sources significantly affected the decolorization process. Sucrose supported maximum dye removal (78.65 ± 0.1%), followed by other carbon sources, including maltose, galactose, starch and dextrose.

The enhancement of decolorization in the presence of sucrose may be attributed to the increased availability of reducing equivalents and energy required for bacterial growth and enzymatic degradation of dye molecules. (Mishra et al., 2022)

3.4.3 Effect of Sucrose Concentration

Among the various sucrose concentrations evaluated, 0.25% (w/v) was found to be optimal and supported maximum decolorization (82.44 ± 0.1%). Further increases in sucrose concentration did not improve dye removal.

The results indicate that excess carbon supplementation may not necessarily enhance degradation but may alter cellular metabolic priorities. (Mishra et al., 2022)

3.4.4 Effect of Nitrogen Source

Among the nitrogen supplements tested, peptone significantly enhanced dye decolorization, resulting in 78.33 ± 0.2% removal after 24 h. Organic nitrogen sources generally performed better than inorganic ones.

This finding suggests that the amino acids and peptides present in peptone may stimulate enzyme synthesis and support rapid bacterial growth. (Mishra et al., 2022)

3.4.5 Effect of Peptone Concentration

Maximum decolorization (91.88 ± 0.01%) was observed at a peptone concentration of 0.2 % (w/v). Both lower and higher concentrations resulted in reduced efficiencies.

This indicates that a balanced C: N ratio is essential for optimum microbial activity. (Eltarahony et al., 2021)

3.4.6 Effect of Inoculum Size

Among the inoculum levels evaluated, 3% (v/v) produced the maximum decolorization efficiency (92.9 ± 2.0%). Lower inoculum concentrations may have provided insufficient biomass, whereas excessive inoculum sizes may have resulted in nutrient competition. (Buenaño-Vargas et al., 2023)

3.4.7 Effect of pH

The decolorization process was highly dependent on medium pH. Maximum color removal (94.5 ± 0.08%) was achieved at pH 7.0. Decreases or increases in pH beyond neutrality adversely affected decolorization efficiency.

Neutral pH likely provides favorable conditions for bacterial growth and optimum activity of extracellular enzymes involved in dye degradation. (Duan et al., 2024)

3.4.8 Effect of Temperature

Temperature optimization revealed that 37°C was the most favorable temperature for biodecolorization, yielding 99.9 ± 0.02% decolorization within 24 h. Lower or higher temperatures resulted in reduced efficiency.

The results suggest that TP7 is a mesophilic bacterium with maximum metabolic activity at physiological temperatures. (Kachhela et al., 2024b)

3.5 Comparison Between Optimized and Unoptimized Conditions

A comparative study was conducted to evaluate the significance of process optimization. Under unoptimized culture conditions, TP7 achieved only 71.55 ± 3.5% decolorization. In contrast, optimization of nutritional and environmental parameters increased decolorization efficiency to 99.99 ± 0.01% within the same incubation period.

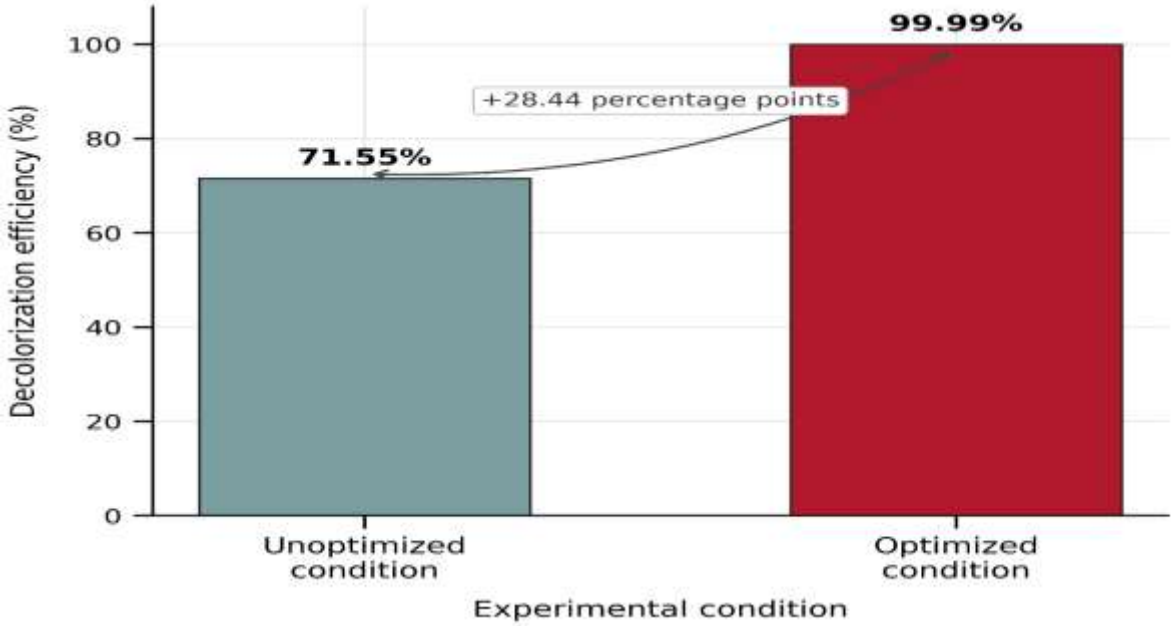
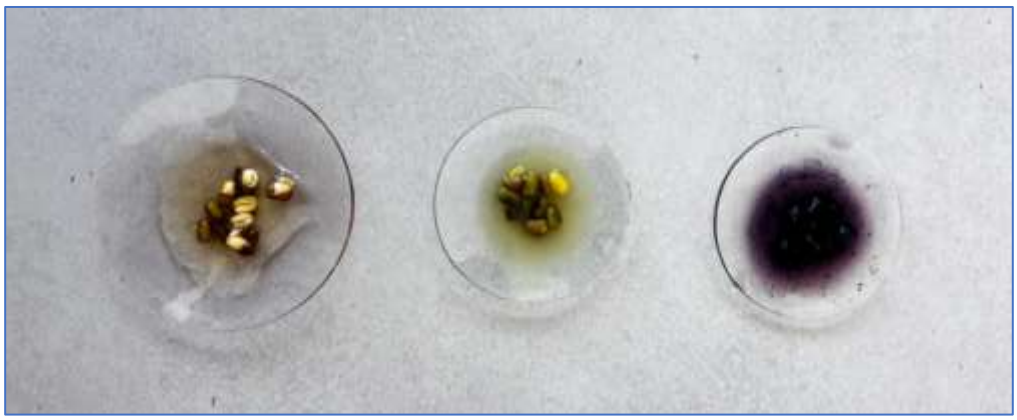


Figure 4. Comparison of decolorization efficiency achieved under optimized and unoptimized culture conditions by isolate TP7

The nearly 28.44% improvement in decolorization efficiency demonstrates the critical importance of process optimization in achieving effective bioremediation of dye-containing wastewater.



3.6Manganese Peroxidase (MnP) Activity

To investigate the mechanism of dye degradation, manganese peroxidase activity was measured during decolorization. MnP activity increased progressively with incubation time and reached a maximum value of 7.9 U mL^{-1} h^{-1} after 24 h.

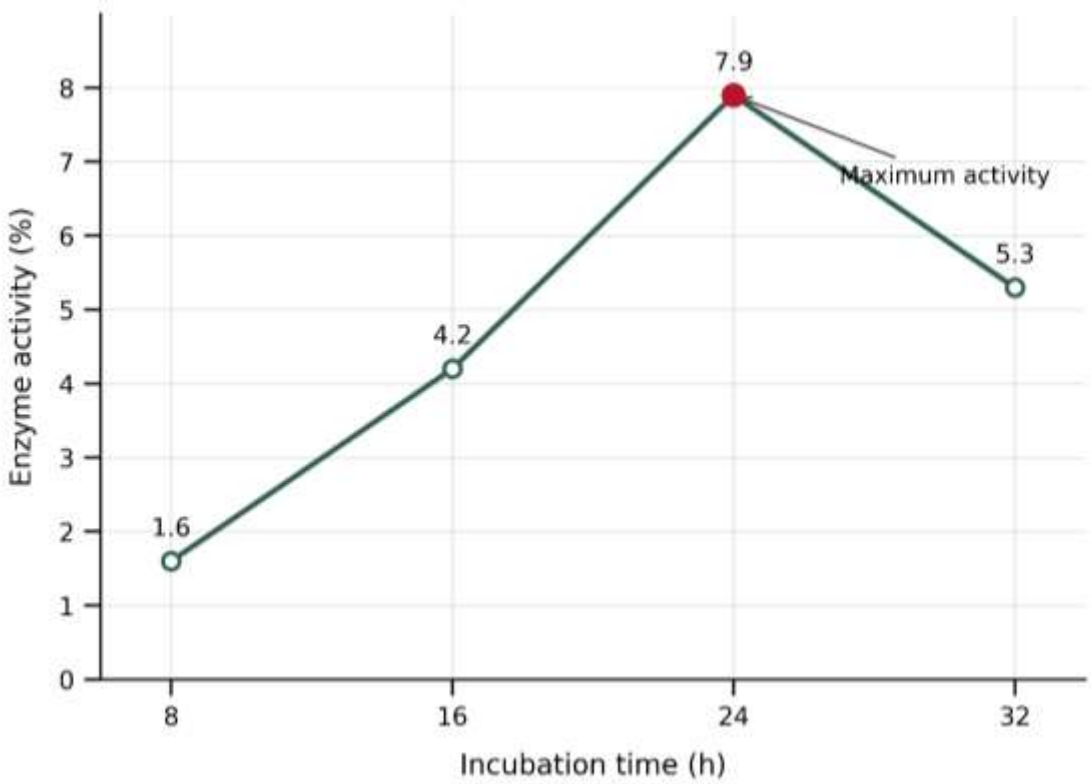


Figure 5. Extracellular manganese peroxidase activity produced by isolate TP7 during the biodecolorization process

The production of MnP suggests that oxidative enzymatic pathways are actively involved in the degradation of Basic Fuchsin dye. MnP is known to oxidize complex aromatic compounds and disrupt chromophoric structures, resulting in dye decolorization and detoxification. The relatively high enzyme activity observed in this study supports the exceptional biodegradation capacity of TP7. (Chang et al., 2021)

3.7 Phytotoxicity Reduction Assessed by Seed Germination Assay

The detoxification efficiency of bacterial treatment was evaluated using *Vigna radiata* seed germination. Seeds exposed to untreated textile effluent showed poor germination and growth, indicating the toxic nature of the dye-containing wastewater. In contrast, seeds treated with biologically treated effluent exhibited normal swelling and seedling development. A germination rate of approximately 66.66% was observed in the treated samples, whereas untreated effluent severely inhibited germination.

The improved germination performance demonstrated that bacterial treatment not only removed color but also substantially reduced the toxicity of the wastewater. These findings indicate that the biodegradation products formed after treatment were considerably less harmful than the parent dye molecules. (Kothari et al., 2022)

4. Conclusion

The present study demonstrated that the bacterial isolate TP7, tentatively identified as *Bacillus* sp., efficiently biodecolorized Basic Fuchsin-amended textile effluents, achieving up to 99.99% decolorization under optimized conditions. Enhanced dye removal was associated with significant manganese peroxidase (MnP) activity, indicating enzymatic degradation of the dye molecules. Furthermore, the treated effluent exhibited reduced phytotoxicity and supported *Vigna radiata* seed germination, confirming effective detoxification. These findings suggest that TP7 has strong potential for sustainable and eco-friendly treatment of textile wastewater contaminated with Basic Fuchsine.

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