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Ultraviolet and Ethyl Methanesulfonate-Induced Mutagenesis in *Aspergillus niger* and *Salmonella typhi* for Enhanced Azoreductase Production in Azo Dyes Bioremediation

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ABSTRACT

Azoreductase, an enzyme capable of degrading toxic industrial azo dyes, holds significant potential for environmental remediation. **Objective:** To enhance azoreductase production through innovative approaches, addressing the challenge of azo dye persistence in industrial wastewater. **Method:** Mutagenesis using EMS and UV irradiation was applied to *Aspergillus niger* and *Salmonella typhi*, followed by treatment with L-cysteine HCl to enhance azoreductase production. **Result:** Mutant strains showed significantly higher azoreductase activity and more efficient azo dye degradation than wild types. **Conclusions:** Mutagenesis is a promising strategy to boost azoreductase production for effective industrial dye bioremediation. Chemical mutagenesis using Ethyl Methane Sulfonate (EMS) (1–6 mM) and physical mutagenesis via UV irradiation (254 nm, 10–120 minutes) were applied to *Aspergillus niger* and *Salmonella typhi* to induce mutations. Further enhancement of enzyme production and strain resistance was achieved through treatment with L-cysteine HCl monohydrate. Comparative analysis using spectrophotometry and Fourier Transform Infrared (FTIR) spectroscopy demonstrated increase in azoreductase activity in mutant strains compared to wild strains. Additionally, textile dye degradation tests validated the enzyme's efficacy for bioremediation.

INTRODUCTION

The discharge of azo dyes from industrial sources poses significant environmental and health risks due to their persistence, toxicity, and mutagenic potential [1]. These synthetic dyes, extensively used in textiles, pharmaceuticals, food, and cosmetics, are resistant to natural degradation and can cause genetic mutations, cancer, and organ damage [2]. Traditional treatment methods such as adsorption, ion exchange, and advanced oxidation are often costly and generate toxic by-products [3]. Biological methods using microbial enzymes offer a more sustainable and cost-effective approach to degrading these compounds [4]. Among these, azoreductases play a central role in breaking azo bonds under both aerobic and anaerobic conditions. These enzymes are known for their stability across a wide pH and temperature range and exhibit varied cofactor dependencies and electron donor preferences [5]. Several bacterial genera, including *Pseudomonas, Enterobacter, Bacillus*, and fungal species such as *Aspergillus niger*, are known to secrete azoreductases [6-8]. Enhancing enzyme yield through induced mutagenesis is a promising strategy [9]. Physical mutagens like ultraviolet (UV) radiation and chemical agents such as ethyl methanesulfonate (EMS) are commonly employed to increase microbial enzyme production [10]. UV primarily causes thymine dimers, while EMS induces GC-AT transitions via alkylation [11, 12].

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Aspergillus niger is recognized for its metabolic versatility and capacity to produce enzymes like azoreductase [13]. Likewise, *Salmonella typhi*, despite its pathogenicity, shows potential for enzyme production due to its adaptable metabolism [14]. Mutagenesis in these organisms could enhance their ability to degrade azo dyes more efficiently [15]. Fermentation using low-cost substrates such as soybean meal has been reported to enhance enzyme production due to its rich nutrient content [16]. Additionally, spontaneous and induced mutations contribute to strain improvement and elevated enzyme yield [17]. This study investigated the effect of UV and EMS-induced mutations on *Aspergillus niger* and *Salmonella typhi* for enhanced azoreductase production. A comparative analysis of wild-type and mutant strains was conducted using FTIR and spectrophotometric assays to evaluate enzyme activity in the bioremediation of azo dyes [18]. Azoreductases are a diverse group of enzymes classified based on their cofactor dependency (FMN, FAD, or flavin-independent) and electron donor preference (NADH, NADPH-dependent or independent) [19]. Their ability to function under both aerobic and anaerobic conditions, coupled with their tolerance to a broad pH (5–9) and temperature (25–85°C) range, makes them particularly effective for industrial applications [20]. In addition to their role in azo dye degradation, azoreductases have applications in xenobiotic metabolism, biosensor development, and prodrug activation [21]. Many microorganisms harbor multiple azoreductase isoforms, complicating the understanding of their physiological roles [22]. The variation in their catalytic mechanisms further emphasizes the need to study organism-specific enzymes to identify high-efficiency candidates for industrial use. Moreover, comparative data on bacterial and fungal azoreductases remain limited, particularly regarding their activity levels, substrate specificities, and optimal production conditions under mutagenic enhancement. *Aspergillus niger*, a filamentous fungus widely used in biotechnology, is known for its robust enzyme secretion capabilities and metabolic adaptability. On the other hand, Salmonella typhi, though pathogenic, demonstrates metabolic versatility that could be exploited under controlled, inactivated conditions for biotechnological applications [23]. In recent years, omics-based approaches such as genomics, transcriptomics, and proteomics have emerged as powerful tools to elucidate the genetic and molecular basis of azoreductase expression and activity. These techniques enable the identification of regulatory genes, promoter regions, and metabolic pathways involved in dye degradation, offering potential targets for genetic engineering. Integrating mutagenesis with high-throughput screening and molecular characterization could accelerate the discovery of hyper-producing strains. Additionally, immobilization of microbial cells or enzymes on suitable carriers has shown promise in enhancing enzyme stability and reusability, making the bioremediation process more economically viable. The development of bioreactors tailored for continuous dye degradation under optimized environmental conditions—such as pH, temperature, aeration, and nutrient supplementation—further supports the scalability of microbial azoreductase systems. Future research should focus on designing synthetic microbial consortia and engineering microbial communities capable of synergistic dye breakdown, thereby overcoming the limitations of single-strain systems. Furthermore, comprehensive risk assessments and containment strategies must be implemented when employing genetically modified or pathogenic organisms like *Salmonella typhi*, ensuring biosafety and regulatory compliance. By combining classical microbiological techniques with modern molecular and bioprocess engineering, the field of enzyme-based dye bioremediation can transition from laboratory-scale success to industrial-scale implementation. A systematic comparison between these two organisms under mutagenic stress could provide insight into strain-specific enzyme enhancement strategies. Despite advances in enzyme biotechnology, there remains a gap in optimizing physicochemical parameters and identifying the most productive microbial strains for azoreductase-mediated bioremediation [24]. Addressing this gap could enable more targeted and cost-effective strategies for large-scale treatment of dye-contaminated effluents.

METHODS

Microbial Strains and Culture Conditions

Aspergillus niger and *Salmonella typhi* were obtained from Forman Christian College University, Lahore. Fungi were cultured on Potato Dextrose Agar (PDA) at 30°C and bacteria on nutrient agar at 37°C. Morphological identifications was confirmed via lactophenol cotton blue staining for *A. niger* and Gram staining for *S. typhi.*

Induced Mutagenesis

Mutagenesis was carried out using Ultraviolet (UV) light and Ethyl Methanesulfonate (EMS) to enhance azoreductase production. EMS was applied at concentrations of 1–6 mM for 10–120 minutes, followed by neutralization with 0.05 mM sodium thiosulfate. UV exposure ranged from 10–140 minutes at 260 nm. Post-treatment, cultures were incubated (fungi at 30°C for 3 days; bacteria at 37°C for 24 hours) and the best-growing mutants were selected.

Fermentation and Enzyme Extraction

Selected strains were grown in 50 mL Potato Dextrose Broth (PDB) for *A. niger* and LB broth for *S. typhi.* After incubation (fungi: 3 days at 30°C; bacteria: 24 hours at

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37°C), cultures were centrifuged at 4000 rpm for 25 min. Mycelial pellets (fungi) were ground and suspended in phosphate buffer (pH 7.4), incubated for 2 hours at 30°C, and centrifuged again to collect the supernatant. Bacterial pellets were processed similarly.

Azoreductase Isolation and Activity Assay

Mid-log phase cells were disrupted by sonication (40% power, 6 min). Enzyme was precipitated using 40% ammonium sulfate, incubated at 4°C for 24 hours, and desalted by dialysis. Azoreductase activity was measured spectrophotometrically at 540 nm using the formula:

Enzyme Activity (U/mL) = $\Delta A \times V / (\varepsilon \times l \times v \times t)$

Where, ΔA is the absorbance change, V is total volume, ε is molar absorptivity, l is path length, v is sample volume, and t is time.

Dye Decolorization Assay

Dye solutions (0.2 g/100 mL) of Reactive Black, Congo Red, Methyl Orange, and Brilliant Blue were used. 400 µL dye, 500 µL enzyme supernatant, and 300 µL phosphate buffer (pH 7.0) were mixed and incubated at 37°C for 72 hours. Absorbance was measured pre- and post-incubation to determine decolorization efficiency using: Decolorization (%) = [(Initial Abs - Final Abs) / Initial Abs] × 100

FTIR Analysis

Pellets from the best mutants (wild, UV-treated, and EMS-treated) of each organism were dried at 30°C for 4 hours and submitted for FTIR analysis at Lahore College for Women University (LCWU), Lahore. This analysis was used to evaluate chemical structure differences among strains.

RESULTS

This figure showed the characteristic smooth, moist, beige colonies of *Salmonella typhi* grown on nutrient agar, confirming successful cultivation and typical morphological features.

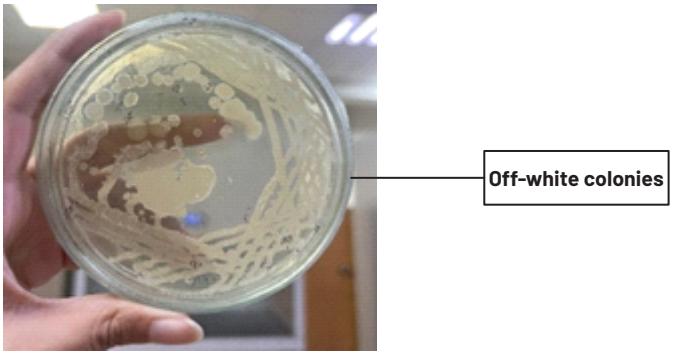

Figure 1: Growth of *Salmonella typhi* on Nutrient Agar

Growth pattern of *Aspergillus niger* on malt extract media showing A) wild type, B) colony morphology after exposure to 3 mM EMS, and C) enhanced growth and sporulation after exposure to 6 mM EMS, indicating mutagenic impact.

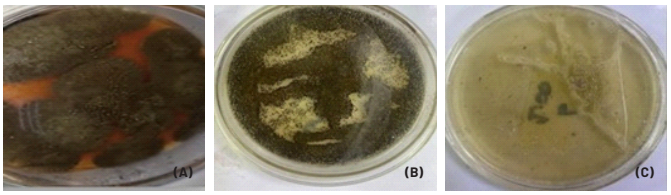

Figure 2: Growth of *Aspergillus niger* on wild and mutated strain on malt extract media. A) Wild type B) exposure to 3mM concentration C) exposure to 6mM concentration of EMS

Growth of *Salmonella typhi* on nutrient agar showing A) wild type, B) colony morphology after exposure to 3 mM EMS, and C) altered growth pattern following 6 mM EMS treatment, indicating successful chemical mutagenesis.

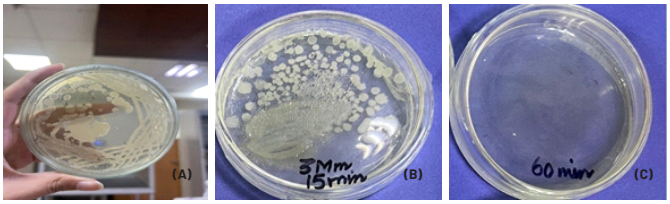

Figure 3: Growth of *Salmonella typhi* on Wild and Mutated Strain on Nutrient Agar. A) Wild Type B) Exposure to 3mM Concentration C) Exposure to 6mM Concentration of EMS

Azoreductase activity (U/mL) of *Aspergillus niger* comparing wild and mutant strains, highlighting enhanced enzyme production following EMS and UV mutagenesis.

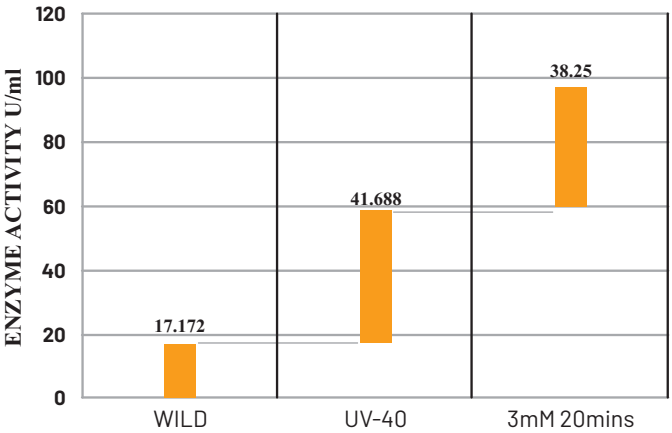

Figure 4: Enzyme Activity of *Aspergillus niger*

Azoreductase activity (U/mL) of *Salmonella typhi* in wild and mutated strains, showing increased enzyme production after EMS and UV-induced mutagenesis.

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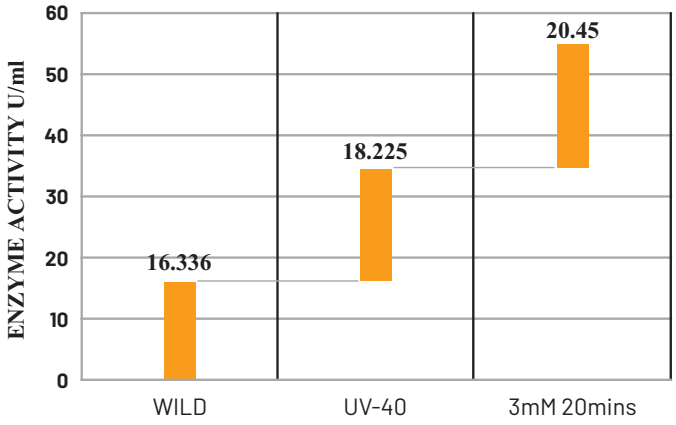

Figure 5: Enzyme Activity of *Salmonella typhi*

FTIR spectrum of UV40-mutated *Aspergillus niger* highlighting key peaks associated with enhanced azoreductase production, including amide I and II regions, polysaccharide, lipid, and hydrogen bonding signatures.

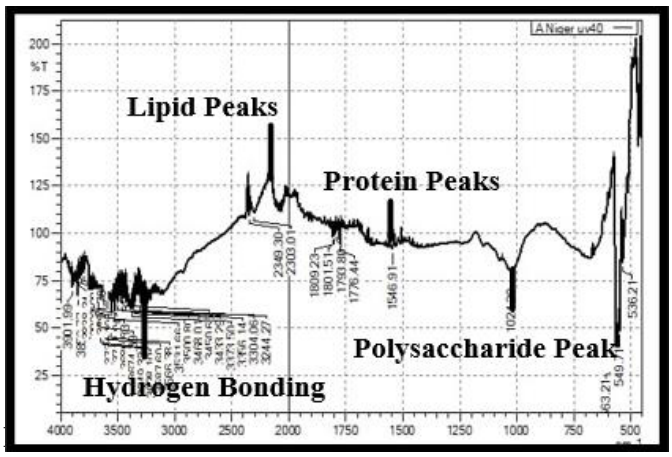

Figure 6: FTIR spectrum showing key peaks for azoreductase production in UV40-mutated *Aspergillus niger*

Based on peaks reported in the literature for azoreductase, the physically mutated strain of *Aspergillus niger* (UV40) exhibited higher intensities in key FTIR regions compared to both the wild strain and the chemically mutated strain (3mM EMS). The UV40 strain showed increased protein-related peaks (~1641 cm^{-1} and ~1549 cm^{-1}), indicating enhanced catalytic efficiency and enzyme stability [22]. Additionally, stronger lipid (~2875–2925 cm^{-1}) and polysaccharide (~1030 cm^{-1}) intensities suggest improved membrane stability and cell wall integrity, facilitating better enzyme secretion. Higher hydrogen bonding (~3317 cm^{-1}) further supports enhanced environmental resistance, making UV40 a more efficient azoreductase producer for azo dye degradation.

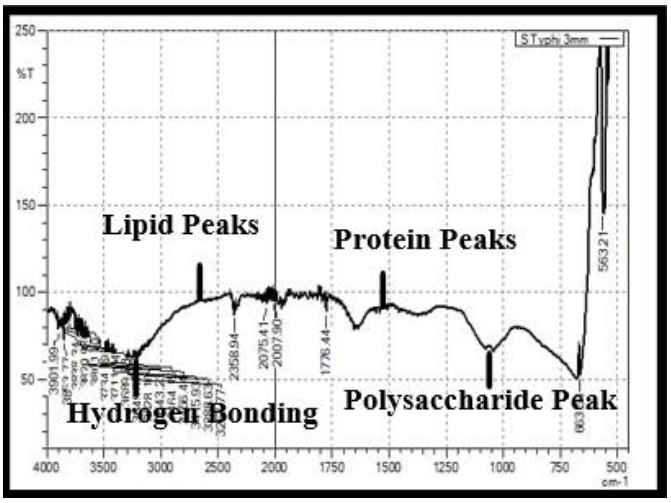

Figure 7: FTIR Spectrum Showing Key Peaks for Azoreductase Production in Chemically Mutated 3mM Strain of *Salmonella typhi*

Based on peaks reported in the literature for azoreductase, the chemically mutated strain of *Salmonella typhi* (3mM EMS) exhibited higher intensities in key FTIR regions compared to the wild strain. The 3mM EMS strain showed increased protein-related peaks (~1641 cm^{-1} and ~1549 cm^{-1}), indicating enhanced catalytic efficiency and enzyme stability. Additionally, stronger lipid (~2875–2925 cm^{-1}) and polysaccharide (~1030 cm^{-1}) intensities suggest improved membrane stability and cell wall integrity, facilitating better enzyme secretion. Higher hydrogen bonding (~3317 cm^{-1}) further supports enhanced environmental resistance, making the 3mM EMS strain a more efficient azoreductase producer for azo dye degradation [23].

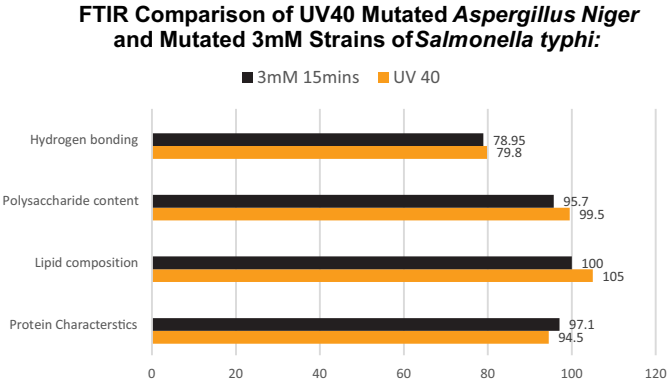

Figure 8: FTIR Comparison of UV40 Mutated *Aspergillus niger* and Chemically Mutated 3mM Strains of *Salmonella typhi*

Aspergillus niger UV40 demonstrated superior results compared to Salmonella typhi 3mM, exhibiting enhanced sustained secretion, higher enzyme stability, and improved long-term functionality, making it a more efficient azoreductase producer [15].

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Biodegradation of Methyl Orange Textile Dye for *Salmonella typhi*

Figure 9: Before Incubation

Figure 10: After Incubation for 72 Hours of Methyl Orange dye for *S. typhi*

Initial absorbance readings of Methyl Orange dye at 464 nm before incubation with wild and mutated *Salmonella typhi* strains, showing uniform baseline across all groups.

Table 1: Absorbance of Methyl Orange Dye at 464 nm before Incubation

Type of Strain	Absorbance at 464 nm
Wild	3.000
20 mins	3.000
40 mins	3.000
2mM 15 mins	3.000
3mM 15 mins	3.000

Absorbance of Methyl Orange dye at 464 nm after 72 hours of incubation with *Salmonella typhi*, showing significant dye degradation by mutated strains compared to the wild type.

Table 2: Absorbance of Methyl Orange Dye at 464 nm after Incubation of 72hrs

Type of Strain	Absorbance at 464 nm
Wild	1.732
20 mins	0.189
40 mins	0.190
2mM 15 mins	0.189
3mM 15 mins	0.191

Wild dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.7332)/3.000
D%=42.2%

Physically mutated strain 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.189)/3.000
D%=93.7%

Physically mutated strain 40 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.190)/3.000
D%=93.6%

Chemically mutated strain 2mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.189)/3.000
D%=93.7%

Chemically mutated strain 3mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.191)/3.000
D%=93.7%

Brilliant Blue Textile Dye for *Samonella typhi*

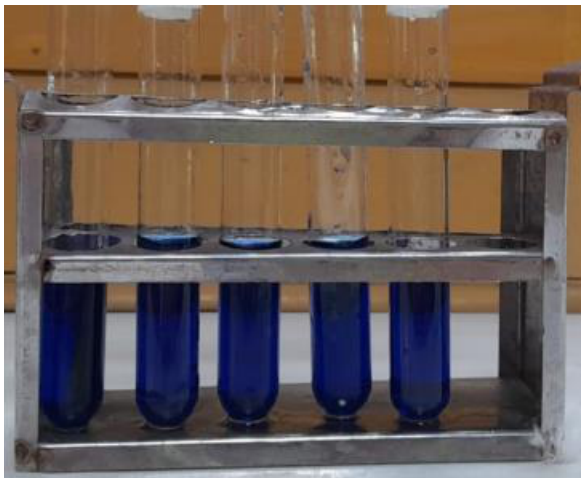

Figure 11: Before Incubation

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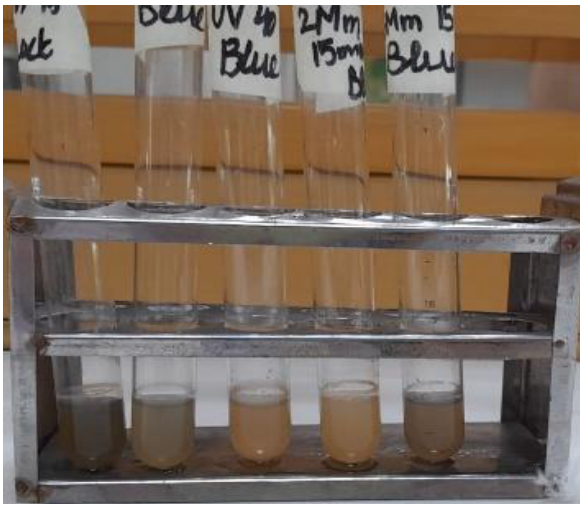

Figure 12: After incubating for 72 hours of brilliant blue dye for *S. typhi*

Baseline absorbance values of Brilliant Blue dye at 628 nm before incubation with wild and mutated *Salmonella typhi* strains, indicating no initial variation among groups.

Table 3: Absorbance of Brilliant Blue Dye at 628 nm before Incubation

Type of Strain	Absorbance at 628 nm
Wild	3.000
20 mins	3.000
40 mins	3.000
2mM 15 mins	3.000
3mM 15 mins	3.000

Absorbance of Brilliant Blue dye at 628 nm after 72 hours of incubation with *Salmonella typhi*, showing enhanced decolorization by EMS and UV-mutated strains compared to the wild type.

Table 4: Absorbance of Brilliant Blue Dye at 628 nm after Incubation of 72hrs

Type of Strain	Absorbance at 628 nm
Wild	0.523
20 mins	0.1926
40 mins	0.1926
2mM 15 mins	0.1927
3mM 15 mins	0.1927

Wild dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.523)/3.000
D%=82.56%

Physically mutated strain 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.1926)/3.000
D%=93.58%

Physically mutated strain 40 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.1926)/3.000
D%=93.58%

Chemically mutated strain 2mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.1927)/3.000
D%=93.57%

Chemically mutated strain 3mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.1927)/3.000
D%=93.57%

The degradation of Congo Red dye by *Salmonella typhi* strains was assessed over a 72-hour incubation period. As shown in figure 13, all dye samples exhibited a uniform deep red color prior to incubation, confirming the consistency of initial dye concentration across all experimental groups. After 72 hours, notable differences in dye intensity were observed (Figure 14), where mutated strains, particularly those treated with EMS and UV exposure, demonstrated significant decolorization compared to the wild type. This visual observation supports the spectrophotometric data and highlights the enhanced azoreductase activity of the mutated strains in degrading complex dye structures.

Congo Red Textile Dye for Salmonella typhi

Figure 13: Before Incubation

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Figure 14: After incubating for 72 hours of Congo Red Dye for *S. typhi*

Baseline absorbance values of Congo Red dye at 497 nm before incubation with wild and mutated *Salmonella typhi* strains, confirming equal initial dye concentration.

Table 5: Absorbance of Congo red Dye at 497 nm before Incubation

Type of Strain	Absorbance at 497 nm
Wild	3.000
20 mins	3.000
40 mins	3.000
2mM 15 mins	3.000
3mM 15 mins	3.000

Absorbance values of Congo Red dye at 497 nm after 72 hours of incubation with *Salmonella typhi*, indicating greater dye degradation by mutated strains compared to the wild type.

Table 6: Absorbance of Congo red dye at 497 nm after incubation of 72hrs

Type of Strain	Absorbance at 497 nm
Wild	0.545
20 mins	0.512
40 mins	0.522
2mM 15 mins	0.525
3mM 15 mins	0.528

Wild dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.545)/3.000
D%=81.8%
Physically mutated strain 20 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.512)/3.000
D%=82.9%
Physically mutated strain 40 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.522)/3.000
D%=82.6%
Chemically mutated strain 2mM 15 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.525)/3.000
D%=82.5%
Chemically mutated strain 3mM 15 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.528)/3.000
D%=82.4%

The biodegradation potential of *Salmonella typhi* against Reactive Black dye was visually assessed before and after incubation. As shown in figure 15, all samples displayed uniform dark coloration prior to treatment, confirming consistent initial dye concentration. After 72 hours of incubation (Figure 16), notable fading of color was observed in the samples exposed to EMS and UV-mutated strains, indicating enhanced decolorization compared to the wild type. These visual findings align with spectrophotometric results, supporting the improved azoreductase activity in mutated strains for dye degradation.

Reactive Black Textile Dye for *Salmonella typhi*

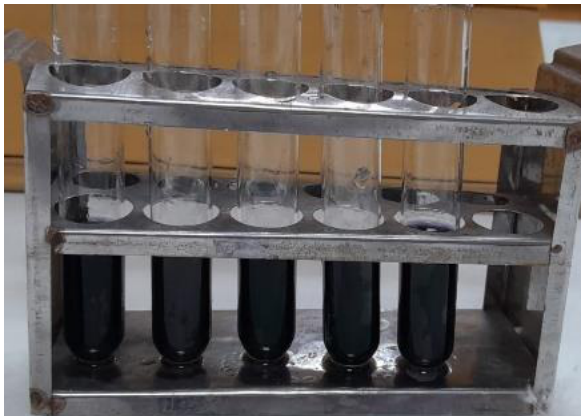

Figure 15: Before Incubation

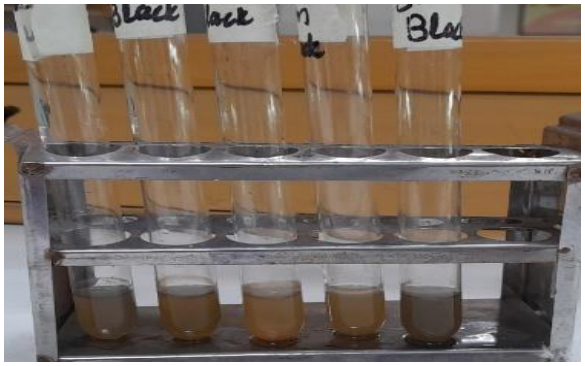

Figure 16: After incubation for 72 hours of reactive black dye for *S. typhi*

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Initial absorbance readings of Reactive Black dye at 597 nm before incubation with wild and mutated *Salmonella typhi* strains, showing identical starting concentrations across all groups.

Table 7: Absorbance of Reactive black dye at 597 nm before incubation

Type of Strain	Absorbance at 597 nm
Wild	3.000
20 mins	3.000
40 mins	3.000
2mM 15 mins	3.000
3mM 15 mins	3.000

Absorbance of Reactive Black dye at 597 nm after 72 hours of incubation with *Salmonella typhi*, showing slightly enhanced dye degradation by EMS and UV-mutated strains compared to the wild type.

Absorbance values of Reactive Black dye at 597 nm after 72 hours of incubation with *Salmonella typhi*, indicating effective dye degradation across all mutated strains compared to the wild type.

Table 8: Absorbance of Reactive black dye at 597 nm before incubation

Type of Strain	Absorbance at 597 nm
Wild	0.539
20 mins	0.535
40 mins	0.537
2mM 15 mins	0.537
3mM 15 mins	0.536

Wild dye degradation percentage

D%=100×(Ci-Co)/Ci

D%=100×(3.000-0.539)/3.000

D%=82.03%

Physically mutated strain 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci

D%=100×(3.000-0.535)/3.000

D%=82.16%

Physically mutated strain 40 mins dye degradation percentage

D%=100×(Ci-Co)/Ci

D%=100×(3.000-0.537)/3.000

D%=82.1%

Chemically mutated strain 2mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci

D%=100×(3.000-0.537)/3.000

D%=82.1%

Chemically mutated strain 3mM 15 mins dye degradation percentage

D%=100×(Ci-Co)/Ci

D%=100×(3.000-0.537)/3.000

D%=82.1%

The decolorization of Congo Red dye by *Aspergillus niger* strains was visually monitored before and after incubation. As shown in figure 17, all dye solutions appeared uniformly red before treatment, indicating consistent initial dye concentrations across wild and mutated strains. After 72 hours of incubation (Figure 18), significant color reduction was observed in samples treated with EMS and UV-mutated strains, highlighting the enhanced azoreductase activity of *A. niger* mutants in dye degradation.

Congo Red Textile Dye for *Aspergillus niger*

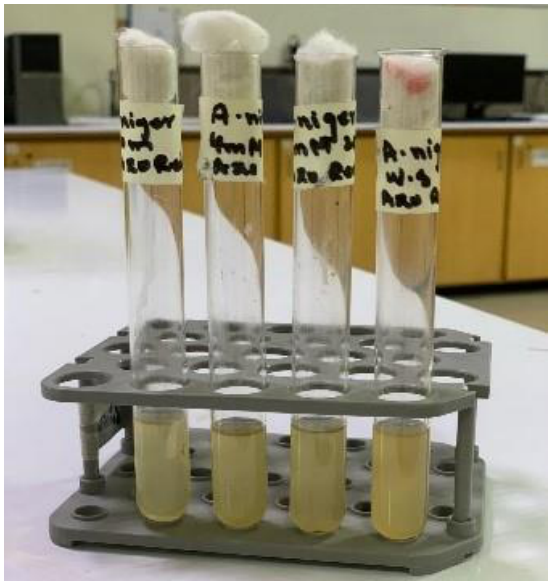

Figure 17: Before Incubation

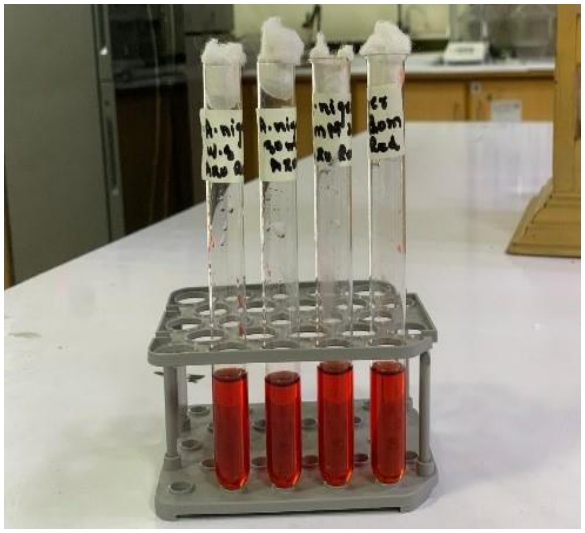

Figure 18: After incubation for 72 hours of Congo red dye for A.

Baseline absorbance of Congo Red dye at 497 nm before incubation with wild and mutated *Aspergillus niger* strains,

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showing equal starting concentrations.

Table 9: Absorbance of Azo orange dye at 497 nm before incubation

Type of Strain	Absorbance at 497 nm
Wild	3.000
30 mins	3.000
3mM 20 mins	3.000
4mM 20 mins	3.000

Absorbance of Azo Orange dye at 520 nm after 72 hours of incubation with Aspergillus niger, showing enhanced dye degradation by EMS and UV-mutated strains compared to the wild type.

Table 10: Absorbance of Congo red textile dye at 520 nm after incubation of 72hrs

Type of Strain	Absorbance at 520 nm
Wild	0.195
30 mins	0.193
3mM 20 mins	0.190
4mM 20 mins	0.189

Wild dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.195)/3.000
D%=93.5%
Physically mutated strain 30 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.193)/3.000
D%=93.5%
Chemically mutated strain 3mM 20 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.190)/3.000
D%=93.66%
Chemically mutated strain 4mM 20 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.189)/3.000
D%=93.7%

The decolorization of Reactive Black dye by *Aspergillus niger* was visually evaluated before and after treatment. As illustrated in Figure 19, all samples exhibited uniform dark coloration prior to incubation, confirming consistent initial dye levels across the strains. Following 72 hours of incubation (Figure 20), noticeable fading of the dye was observed in cultures exposed to EMS and UV-induced mutations, indicating improved azoreductase activity in the mutated *A. niger* strains.

Reactive Black Textile Dye for *Aspergillus niger*

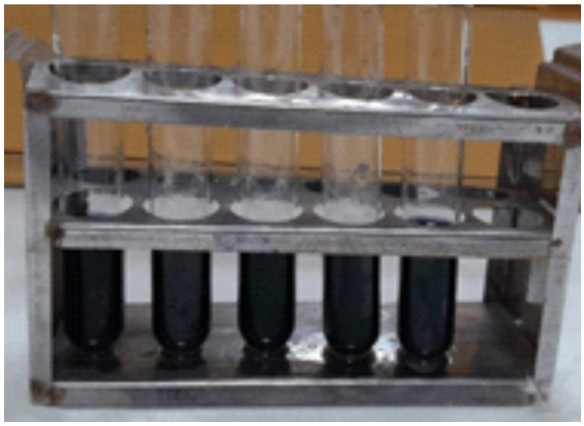

Figure 19: Before Incubation

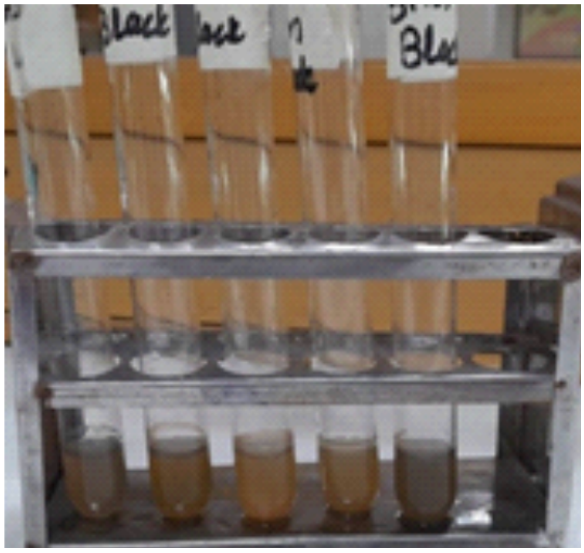

Figure 20: After incubation for 72 hours of reactive black dye for *A. Niger*

Initial absorbance values of Reactive Black dye at 597 nm before incubation with wild and mutated *Aspergillus niger* strains, confirming uniform starting concentrations.

Table 11: Absorbance of Reactive black dye at 597 nm before incubation

Type of Strain	Absorbance at 597 nm
Wild	3.000
30 mins	3.000
3mM 20 mins	3.000
4mM 20 mins	3.000

Absorbance of Reactive Black dye at 597 nm after 72 hours of incubation with *Aspergillus niger*, showing slightly enhanced dye degradation by mutated strains compared to the wild type.

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Table 12: Absorbance of Reactive black dye at 597 nm before incubation

Type of Strain	Absorbance at 597 nm
Wild	0.537
30 mins	0.536
3mM 20 mins	0.536
4mM 20 mins	0.535

Wild dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.537)/3.000
D%=82.1
Physically mutated strain 30 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.536)/3.000
D%=82.1%
Chemically mutated strain 3mM 20 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.536)/3.000
D%=82.1%
Chemically mutated strain 4mM 20 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-0.535)/3.000
D%=82.2%

The degradation of Methyl Orange dye by *Aspergillus niger* was visually assessed before and after incubation. As shown in figure 21, all samples exhibited the same deep orange hue prior to treatment, confirming equal initial dye concentrations. After 72 hours of incubation (Figure 22), minimal visual change was observed across all strains, indicating limited azoreductase activity and poor degradation efficiency of Methyl Orange by both wild and mutated *A. niger* strains.

Methyl Orange dye for *Aspergillus niger*

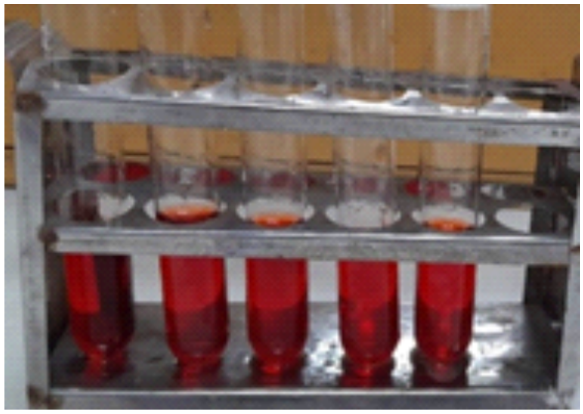

Figure 21: Before Incubation

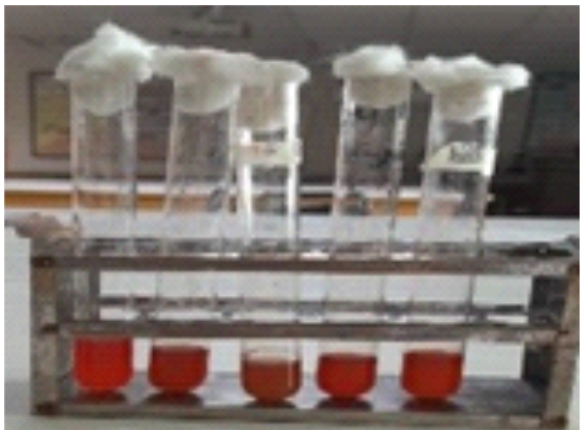

Figure 22: After incubation for 72 hours of methyl orange dye for *A. Niger*

Baseline absorbance values of Methyl Orange dye at 464 nm before incubation with wild and mutated *Aspergillus niger* strains, showing uniform initial concentrations across all samples.

Table 13: Absorbance of Reactive black dye at 597 nm before incubation

Type of Strain	Absorbance at 597 nm
Wild	3.000
30 mins	3.000
40 mins	3.000
3mM 20 mins	3.000
4mM 20 mins	3.000

Absorbance of Methyl Orange dye at 464 nm after 72 hours of incubation with *Aspergillus niger*, indicating minimal dye degradation by both wild and mutated strains.

Table 14: Absorbance of Methyl orange dye at 464 nm after incubation of 72hrs

Type of Strain	Absorbance at 464 nm
Wild	1.732
30 mins	1.818
40 mins	1.812
3mM 20 mins	1.819
4mM 20 mins	1.824

Wild dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.7332)/3.000
D%=42.9%
Physically mutated strain 30 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.818)/3.000
D%=39.4%
Physically mutated strain 40 mins dye degradation percentage
D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.812)/3.000
D%=39.6%

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Chemically mutated strain 3mM 20 mins dye degradation percentage

D%=100×(Ci-Co)/C
D%=100×(3.000-1.819)/3.000
D%=39.36%

Chemically mutated strain 4mM 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.824)/3.000
D%=39.2%

The results showed that *Aspergillus niger* did not degrade methyl orange dye when incubated for 72 hours. There was no significant reduction in dye concentration, indicating a lack of azoreductase activity against this particular dye.

Brilliant blue dye for *Aspergillus niger*

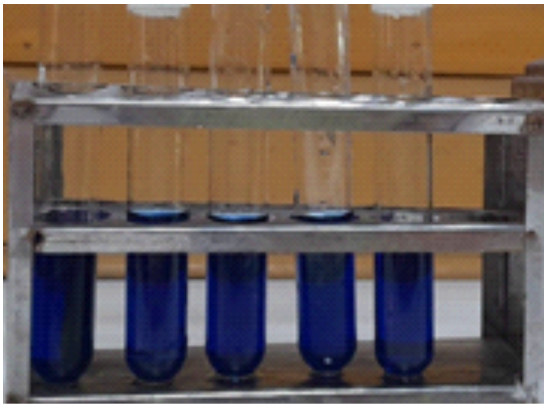

Figure 23: Before Incubation

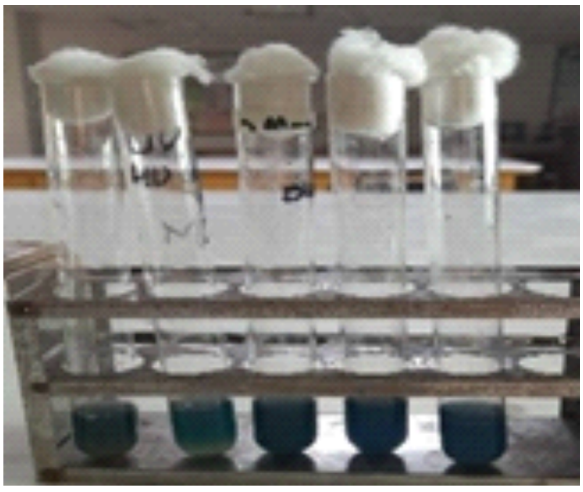

Figure 24: After incubation for 72hours of Brilliant Blue Dye for *A. Niger*

Initial absorbance values of Brilliant Blue dye at 628 nm before incubation with wild and mutated *Aspergillus niger* strains, confirming equal starting concentrations.

Table 15: Absorbance of Brilliant Blue Dye at 628 nm before Incubation

Type of Strain	Absorbance at 628 nm
Wild	3.000
30 mins	3.000
40 mins	3.000
3mM 20 mins	3.000
4mM 20 mins	3.000

Absorbance of Brilliant Blue dye at 628 nm after 72 hours of incubation with *Aspergillus niger*, indicating minimal decolorization and limited azoreductase activity across all strains.

Table 16: Absorbance of Brilliant Blue Dye at 628 nm after incubation of 72hrs

Type of Strain	Absorbance at 628 nm
Wild	1.825
30 mins	1.815
40 mins	1.848
3mM 20 mins	1.845
4mM 20 mins	1.844

Absorbance of Brilliant Blue dye at 628 nm after 72 hours of incubation with *Aspergillus niger*, indicating minimal decolorization and limited azoreductase activity across all strains.

Wild dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.825)/3.000
D%=39.16%

Physically mutated strain 30 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.815)/3.000
D%=39.5%

Physically mutated strain 40 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.848)/3.000
D%=38.4%

Chemically mutated strain 3mM 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.845)/3.000
D%=38.5%

Chemically mutated strain 4mM 20 mins dye degradation percentage

D%=100×(Ci-Co)/Ci
D%=100×(3.000-1.844)/3.000
D%=38.53%

The results showed that *Aspergillus niger* did not degrade brilliant blue dye when incubated for 72 hours. There was no significant reduction in dye concentration, indicating a lack of azoreductase activity against this particular dye.

Aspergillus niger showed less OD value for brilliant blue compared to Congo Red and Reactive Black.

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DISCUSSION

This study demonstrates the successful growth of Aspergillus niger and Salmonella typhi, both showing their characteristics features. Aspergillus niger appeared black, velvety with rough conidia and biseriate conidiophores, whereas Salmonella typhi showed smooth, moist, beige colonies under the microscope [24, 25]. The main objective of this study was to compare the azoreductases production capacity of wild and mutated strains of both organisms. Aspergillus niger was grown on PDA media, while Salmonella typhi on nutrient agar media that support effective growth. Both of the organisms proved to be producing azoreductases, the enzyme that has been of considerable interest in textile industries for azo dye degradation and decolorization capability [26, 27]. Azoreductases demonstrates remarkable efficiency in degrading Azo dyes like Methyl Orange, Congo Red, Reactive Black, and Brilliant Blue by converting them into non-toxic amines under aerobic and anaerobic conditions, showing prospects for the bioremediation of industrial wastewaters [2]. Their wide range of pH stability and temperature (25-85°C) stability reinforces their significance in bioremediation [5-9, 28]. Complementary enzymes such as laccase and peroxidases work synergistically with azoreductases to enhance dye degradation, offering a holistic approach for tackling complex pollutants [29]. This combined enzymatic activity underlines the power of bioremediation technologies in mitigating environmental pollution [30]. This study aimed to enhance azoreductases production in Aspergillus niger and Salmonella typhi through physical and chemical mutagenesis. Mutation induction was performed using Ethyl Methane Sulphonate (EMS) and UV radiation with optimal conditions. EMS a known ethylating agent that has been used in previous studies to induce GC- AT and AT-GC transitions as well as insertions and deletions [31]. EMS has been widely used to improve enzyme production in various microorganisms including bacteria and fungi with concentration ranging from 1mM to 6mM, as practiced in this research following previous literature [32]. EMS mutagenesis has been used successfully to enhance protein secretion in Ashbya goossypoi and increase Ethanol production and also been utilized to enhance the characteristics of other microbial strains like Beauveria which is a pest control agent [33, 34]. Similarly, physical mutagenesis methods including UV radiation, X-rays and Gamma rays showed improve enzyme production compared to parental strains [35]. In this research, the results revealed that production of azoreductases has been enhanced in Aspergillus niger (3 mM EMS for 20 min and 40 min UV exposure) and Salmonella typhi (3 mM EMS for 20 min and 40 min UV exposure). In case of Aspergillus niger, chemical mutation resulted in a production of 1.207 mg/ml, while that by physical mutation was 1.116 mg/ml, compared to the wild strain's production of 0.43 mg/ml. For Salmonella typhi, chemical mutation resulted in 0.185 mg/ml, and physical mutation to 0.121 mg/ml, both of which were higher than the wild strain. The use of physical and chemical mutagens holds promise for increasing enzyme production and improving enzyme and strain activity [36]. This mutagenic approach holds promise for applications such as textile dye decolorization and industrial enzyme production [37]. Mutated strains of Aspergillus niger and Salmonella typhi were treated with 1mM L-cysteine HCl for 15 mins, known for its antioxidants and reducing effects during enzyme production. The study revealed that the viability and resistance of the strains improved when treated with 1mM L- cysteine HCl for 15 mins, aligning with the results of [38]. The Quantitative analysis was evaluated through UV- VIS Spectrophotometer and FTIR. The production of azoreductase in both Aspergillus niger and Salmonella typhi was evaluated through Spectrophotometer at 540nm, which revealed that mutated strains had a higher level of enzyme compared to wild control strains. For A. Niger, the absorbance values of the supernatant from fermentation media were 1.158 for 3mM and 1.078 for UV40, which translates to enzyme concentration of 1.207 mg/ml and 1.116 mg/ml respectively, indicated that significant increase of azoreductase production. While in Salmonella typhi mutant strains showed 0.299 for 3mM and 0.235 for UV40, which means 0.185 mg/ml and 0.121 mg/ml of enzyme concentrations, respectively. These results have validated the role of spectrophotometric analysis in measuring azoreductase production and showed the influence of mutations in the increase of enzyme titer, which is also in line with the previous work of [39]. The FTIR results, based on specific intensities and peak positions, provide definitive evidence for azoreductase production, aligning with literature-reported. FTIR analysis revealed the structural changes responsible for strain improvements. Large shifts in amide I (~1650 cm^{-1}) and amide II (~1550 cm^{-1}) regions indicated a change in secondary structure of proteins, probably due to enhance production of azoreductase. Increased in carbohydrate peaks (1000–1200 cm^{-1}) and lipid associated vibrations (2850–2950 cm^{-1}), indicated upregulation of metabolic pathways and membrane restructuring, thus allowing better secretion of enzymes. These results are supported by earlier observation by and in which similar biochemical mechanisms were also exhibited in overexpression strains of modified enzymes [2, 8]. The stronger O-H and N-H stretching's around 3300 cm^{-1} indicated dynamic functional group was improved by FTIR. The result was validated by matching with literature showed UV-induced mutants UV40 showed better growth and enzyme activity than EMS- induced mutants. The Qualitative Analysis was monitored by decolorization of Azo dyes through azoreductase activity, an extensively studied enzymatic process. The decolorization was observed after 72 hours. The bacterial strains were incubated at 37 °C, while fungal strains at 30°C. The readings were taken according to the time interval in the UV- VIS

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spectrophotometer. The % decolorization was measured by standard formula, indicated that mutated strains more efficiently decolorized dye compared to controls. These findings supported by studies that mutagenesis may boost microbial ability for azo dye degradation [40]. In addition, it has been widely reported that azoreductases can break complex dye structures under optimal conditions which supports the observed efficiency of the mutated strains in this study.

CONCLUSIONS

This study demonstrated that chemical and physical mutagenesis significantly enhances azoreductase production in Aspergillus niger and Salmonella typhi. The use of optimized substrates and mutagenic treatments led to increased enzyme activity, showcasing the potential of microbial strain improvement for biotechnological applications. Enhanced degradation of Azo dyes such as Methyl Orange, Brilliant Blue, Reactive Black, and Congo Red by mutated strains highlights a sustainable solution for industrial wastewater treatment. Quantitative analysis through UV-Vis Spectrophotometry and FTIR confirmed higher enzyme levels in mutated strains, while qualitative analysis showed improved dye decolorization compared to wild types. These results support the application of microbial azoreductases in bioremediation, with scalability for industrial use. The findings underscore the versatility of azoreductases in environmental and industrial biotechnology, encouraging future research into strain engineering and enzyme optimization. This work contributes to the development of eco-friendly alternatives to conventional chemical treatments. The authors declare no competing financial or personal interests that could have influenced the outcomes of this study.

Authors Contribution

Conceptualization: FT
Methodology: DB, HR, FT
Formal analysis: HR, DB, FT
Writing, review and editing: MW, HR, FT

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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REFERENCES

[1] Alzain H, Kalimugogo V, Hussein K, Karkadan M. A review of environmental impact of azo dyes. International Journal of Research and Review. 2023 Jun; 10(6): 673-89. doi: 10.52403/ijrr.20230682.

[2] Singh GB, Vinayak A, Mudgal G, Kesari KK. Azo dye bioremediation: An interdisciplinary path to sustainable fashion. Environmental Technology & Innovation. 2024 Sep: 103832. doi: 10.1016/j.eti.2024.103832.

[3] Chaudhary R, Nawaz A, Fouillaud M, Dufossé L, Haq IU, Mukhtar H. Microbial cell factories: biodiversity, pathway construction, robustness, and industrial applicability. Microbiology Research. 2024 Feb; 15(1): 247-72. doi: 10.3390/microbiolres15010018.

[4] Bumpus JA. Biodegradation of azo dyes by fungi. Mycology Series. 2004 Mar; 21: 457-70. doi: 10.1201/9780203913369.ch38.

[5] Bala S, Garg D, Thirumalesh BV, Sharma M, Sridhar K, Inbaraj BS *et al.* Recent strategies for bioremediation of emerging pollutants: a review for a green and sustainable environment. Toxics. 2022 Aug; 10(8): 484. doi: 10.3390/toxics10080484.

[6] Wang R, Li H, Liu Y, Chen J, Peng F, Jiang Z *et al.* Efficient removal of azo dyes by Enterococcus faecalis R1107 and its application in simulated textile effluent treatment. Ecotoxicology and Environmental Safety. 2022 Jun; 238: 113577. doi: 10.1016/j.ecoenv.2022.113577.

[7] Ikram M, Naeem M, Zahoor M, Rahim A, Hanafiah MM, Oyekanmi AA *et al.* Biodegradation of azo dye methyl red by Pseudomonas aeruginosa: optimization of process conditions. International Journal of Environmental Research and Public Health. 2022 Aug; 19(16): 9962. doi: 10.3390/ijerph19169962.

[8] Ayed L, Bekir K, Jabeur C. Modeling and optimization of biodegradation of methylene blue by Staphylococcus aureus through a statistical optimization process: a sustainable approach for waste management. Water Science and Technology. 2022 Jul; 86(2): 380-94. doi: 10.2166/wst.2022.211.

[9] Kumar Shri Guru A and Kumar Singh S. Biodegradation of azo dyes by Bacillus subtilis "RA29" 2018. Available from: https://www.researchgate.net/publication/281959357.

[10] Kumar A, Chopra J, Singh SK, Khan A, Singh RN. Biodegradation of azo dyes by Bacillus subtilis 'RA29'. Der Pharmacia Lettre. 2015 Jan; 7(6): 234-8.

[11] Philip I, Sarojini S, Biswas S, Jayaram S. Exploring the Potential of Bacillus velezensis, an Endophytic Bacteria Isolated from Alternanthera philoxeroides for Plant Growth Promotion and Bioremediation Properties. Journal of Pure & Applied Microbiology. 2023 Sep; 17(3). doi: 10.22207/JPAM.17.3.40.

[12] Carruthers DN and Lee TS. Translating advances in microbial bioproduction to sustainable biotechnology. Frontiers in Bioengineering and

DOI: https://doi.org/10.54393/fbt.v4i03.164

Biotechnology. 2022 Aug; 10: 968437. doi: 10.3389/fbioe.2022.968437.

[13] Basu S, Bose C, Ojha N, Das N, Das J, Pal M *et al.* Evolution of bacterial and fungal growth media. Bioinformation. 2015 Apr; 11(4): 182. doi: 10.6026/97320630011182.

[14] Schroeder JW, Yeesin P, Simmons LA, Wang JD. Sources of spontaneous mutagenesis in bacteria. Critical Reviews in Biochemistry and Molecular Biology. 2018 Jan; 53(1): 29-48. doi: 10.1080/10409238.2017.1394262.

[15] Wei W, Ho WC, Behringer MG, Miller SF, Bcharah G, Lynch M. Rapid evolution of mutation rate and spectrum in response to environmental and population-genetic challenges. Nature Communications. 2022 Aug; 13(1): 4752. doi: 10.1038/s41467-022-32353-6.

[16] Tamano K. Enhancing microbial metabolite and enzyme production: current strategies and challenges. Frontiers in Microbiology. 2014 Dec; 5: 718. doi: 10.3389/fmicb.2014.00718.

[17] Yuan L, Chang J, Yin Q, Lu M, Di Y, Wang P *et al.* Fermented soybean meal improves the growth performance, nutrient digestibility, and microbial flora in piglets. Animal Nutrition. 2017 Mar; 3(1): 19-24. doi: 10.1016/j.aninu.2016.11.003.

[18] Brüschweiler BJ and Merlot C. Azo dyes in clothing textiles can be cleaved into a series of mutagenic aromatic amines which are not regulated yet. Regulatory Toxicology and Pharmacology. 2017 Aug; 88: 214-26. doi: 10.1016/j.yrtph.2017.06.012.

[19] Leonard CA, Brown SD, Hayman JR. Random mutagenesis of the Aspergillus oryzae genome results in fungal antibacterial activity. International Journal of Microbiology. 2013; 2013(1): 901697. doi: 10.1155/2013/901697.

[20] Chen H, Hopper SL, Cerniglia CE. Biochemical and molecular characterization of an azoreductase from Staphylococcus aureus, a tetrameric NADPH-dependent flavoprotein. Microbiology. 2005 May; 151(5): 1433-41. doi: 10.1099/mic.0.27805-0.

[21] Saranraj P, Stella D, Sivasakthivelan P. Separation, purification and characterization of dye degrading enzyme azoreductase from bacterial isolates. Central European Journal of Experimental Biology. 2014; 3(2): 19-25.

[22] Cong J, Xie X, Liu Y, Qin Y, Fan J, Fang Y *et al.* Biochemical characterization of a novel azo reductase named BVU5 from the bacterial flora DDMZ1: application for decolorization of azo dyes. RSC advances. 2022 Jan; 12(4): 1968-81. doi: 10.1039/D1RA08090C.

[23] Liu S, Xu X, Kang Y, Xiao Y, Liu H. Degradation and detoxification of azo dyes with recombinant ligninolytic enzymes from Aspergillus sp. with secretory overexpression in Pichia pastoris. Royal Society Open Science. 2020 Sep; 7(9): 200688. doi: 10.1098/rsos.200688.

[24] Diba K, Kordbacheh P, Mirhendi SH, Rezaie S, Mahmoudi M. Identification of Aspergillus species using morphological characteristics. 2007 Oct – Dec; 23(6): 867-872.

[25] Afzal H, Shazad S, Qamar S, Nisa U. Morphological identification of Aspergillus species from the soil of Larkana District (Sindh, Pakistan). Asian Journal of Agriculture and Biology. 2013 Jan; 1(3): 105-17.

[26] Baweja M, Nain L, Kawarabayasi Y, Shukla P. Current technological improvements in enzymes toward their biotechnological applications. Frontiers in Microbiology. 2016 Jun; 7: 965. doi: 10.3389/fmicb.2016.00965.

[27] Pinheiro LR, Gradíssimo DG, Xavier LP, Santos AV. Degradation of azo dyes: bacterial potential for bioremediation. Sustainability. 2022 Jan; 14(3): 1510. doi: 10.3390/su14031510.

[28] Alabdraba W and Bayati M. Biodegradation of Azo dyes a review. International Journal of Environmental Sciences & Natural Resources. 2014 Oct; 1(4): 179-89.

[29] Sudha M, Saranya A, Selvakumar G, Sivakumar N. Microbial degradation of azo dyes: a review. International Journal of Current Microbiology and Applied Sciences. 2014 Mar; 3(2): 670-90.

[30] Pramanik S and Chaudhuri S. Laccase activity and azo dye decolorization potential of Podoscypha elegans. Mycobiology. 2018 Jan; 46(1): 79-83. doi: 10.1080/12298093.2018.1454006.

[31] Sega GA. A review of the genetic effects of ethyl methanesulfonate. Mutation Research/Reviews in Genetic Toxicology. 1984 Sep; 134(2-3): 113-42. doi: 10.1016/0165-1110(84)90007-1.

[32] Chen L, Duan L, Sun M, Yang Z, Li H, Hu K et al. Current trends and insights on EMS mutagenesis application to studies on plant abiotic stress tolerance and development. Frontiers in Plant Science. 2023 Jan; 13: 1052569. doi: 10.3389/fpls.2022.1052569.

[33] Ribeiro O, Magalhães F, Aguiar TQ, Wiebe MG, Penttilä M, Domingues L. Random and direct mutagenesis to enhance protein secretion in Ashbya gossypii. Bioengineered. 2013 Sep; 4(5): 322-31. doi: 10.4161/bioe.24653.

[34] Majumdar A. Molecular techniques for the improvement of microbial biocontrol agents against plant pathogens. Egyptian Journal of Biological Pest Control. 2023 Oct; 33(1): 103. doi: 10.1186/s41938-023-00746-4.

DOI: https://doi.org/10.54393/fbt.v4i03.164

[35] Ghazi S, Sepahy AA, Azin M, Khaje K, Khavarinejad R. UV mutagenesis for the overproduction of xylanase from Bacillus mojavensis PTCC 1723 and optimization of the production condition. Iranian Journal of Basic Medical Sciences. 2014 Nov; 17(11): 844–853.

[36] Kaul P and Asano Y. Strategies for discovery and improvement of enzyme function: state of the art and opportunities. Microbial Biotechnology. 2012 Jan; 5(1): 18-33. doi: 10.1111/j.1751-7915.2011.00280.x.

[37] Tripathi M, Singh S, Pathak S, Kasaudhan J, Mishra A, Bala S *et al.* Recent strategies for the remediation of textile dyes from wastewater: a systematic review. Toxics. 2023 Nov; 11(11): 940. doi: 10.3390/toxics 11110940.

[38] Hussein HA and Alshammari SO. Cysteine mitigates the effect of NaCl salt toxicity in flax (Linum usitatissimum L) plants by modulating antioxidant systems. Scientific Reports. 2022 Jul; 12(1): 11359. doi: 10.1038/s41598-022-14689-7.

[39] Adeniyi AO, Boyro DE, Chindo IY, Mahmoud AA. Spectrophotometric and infra-red analyses of azo reactive dyes derived from 2-methyl-3-(2'-methylphenyl)-6-arylazo-4-oxoquinazoline. Science World Journal. 2023 Oct; 18(2): 231-9. doi: 10.4314/swj .v18i2.10.

[40] El Awady ME, El-Shall FN, Mohamed GE, Abd-Elaziz AM, Abdel-Monem MO, Hassan MG. Exploring the decolorization efficiency and biodegradation mechanisms of different functional textile azo dyes by Streptomyces albidoflavus 3MGH. BioMed Central Microbiology. 2024 Jun; 24(1): 210. doi: 10.1186/s1286 6-024-03347-9.