Advances in Bioresearch
Adv. Biores., Vol 16 (3) May 2025: 23-30

Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
DOI: 10.15515/abr.0976-4585.16.3.2330

Advances in Bioresearch

ORIGINAL ARTICLE

Biodegradation of Textile Dye Red Ed3b by Bacteria and Its Impact on Jowar and Mung Seed Germination

Bharati Y. Taware[1], Chhaya D. Sonar[2], Amarja H. Bhosale[3]
[1]Department of Statistics, Vidya Pratishthan's Arts, Science and Commerce College, Baramati
[2]Department of Statistics, Dr. Babasaheb Ambedkar Marathwada University, Chh. Sambhajinagar
[3]Department of Microbiology, Vidya Pratishthan's Arts, Science and Commerce College, Baramati
Corresponding author: Chhaya D. Sonar (Email: chhayajadhav@gmail.com)

ABSTRACT
The textile industry extensively uses synthetic dyes, leading to the discharge of significant amounts of unused dyes in effluents, which pose serious environmental concerns due to the inefficiency of conventional treatment methods. Biological degradation has gained prominence as an eco-friendly and cost-effective alternative. In this study, five novel bacterial strains capable of degrading the widely used textile dye Red ED3B were isolated from acclimatized samples. The isolates were identified through morphological, biochemical, and 16S rRNA gene sequencing techniques. Enzymatic assays revealed that all strains exhibited notable azoreductase activity, along with manganese peroxidase and laccase, indicating their effectiveness in dye degradation. Statistical analysis using multivariate analysis of variance (MANOVA) confirmed the significance of the findings. Phytotoxicity assessments using Jowar (Sorghum) and Mung (Vigna radiata) seeds showed that both the dye and its metabolic byproducts significantly affected radicle length, plumule length, and seed germination percentage. These results underscore the potential of the selected bacterial strains for bioremediation of textile dye pollutants and their environmental impact.

Key words: Biodegradation, Dye degradation, Enzyme activity, MANOVA, Phytotoxicity, Red ED3B

Received: 04. 03.2025 Revised: 26.04.2025 Accepted: 30.05.2025

How to cite this article:
Bharati Y. Taware, Chhaya D. Sonar and Amarja H. Bhosale. Biodegradation Of Textile Dye Red Ed3b By Bacteria And Its Impact On Jowa & Mung Seed Germination. Adv.Biores. Vol 16 [3] May 2025. 23-30

INTRODUCTION

Pollution due to heavy metals and chemicals has grown into a worldwide difficulty now days because they are everlasting and are toxic to living being in the environment [1]. Aromatic Sulfo and Azo groups are artificially synthesized hence Azo dyes are counted in Xenobiotic compounds. As a concern azo dyes are recalcitrant by their structures which are present in large amount in textile waste water[2]. Textile dyes are recalcitrant in nature also they are not modifiable by common treatment process. Massive research work has been done for the removal of these dyes and also shown excellent results but most of this work has been carried with single model compound [3, 4]. Whereas textile waste water is composed of many kinds of mixture of unknown quantities of complex dyes [5]. The use of microbiological agents or bioremediation techniques to cope up with pollution problem is a strategic research area in the environmental sciences. The tool for the biodegradation of recalcitrant dye in the textile effluent is based on biotransformation by microbial enzymes [6,7]. Currently microbial or enzymatic treatments are implemented for the bioremediation of textile dyes because of certain advantages *viz*. i) Cost effective ii) environment friendly iii) needs less water for the process than physical process iv) byproducts formed after treatment are non-toxic or completely mineralized v) produce less sludge [8]. The improvement of compelling cultures and their use in degradation is one of the best biological features of effluent treatment [9]. The bioremediation is natural process which includes bioengineering the abilities of fundamental microbes to clean up the environment and is effective alternative to conventional methods [10]. In our open environment the bacterial community at each micro-niche is surely one of the excellent life forms to utilize huge diversity of compounds as their carbon and energy source [11]. The Textile effluent containing azo dyes are not only recalcitrant but also, they are phytotoxic, toxic, carcinogenic and

mutagenic [12, 13].It is the fact that many of resources and time have been devoted but till today bioremediation in an accessible field is a huge challenge.

Two-way MANOVA is an extension of two-way ANOVA that evaluates the effect of two independent variables on multiple dependent variables simultaneously [14]. It is helpful in various fields such as Textile industries, Medicine, Psychology, Microbiology etc.

The present research work deals with the use of wide variety of bacterial cultures which not only cleaved the complex structure of dyes but also detoxify it accompanied by continuous growth without requirement of much nutrients.

MATERIAL AND METHODS

The effect of five bacteria viz. RED-1, RED-2, RED-3, RED-4 and RED-5 at the two time points (24 hrs, 48 hrs) on the Red ED3B dye was observed. The textile dye Red ED3B was procured from Sigma-Aldrich (USA).The experiment is designed statistically. In the present study the degradation efficiency of textile dye Red ED3B by five potent bacterial isolates viz. RED-1, RED-2, RED-3, RED-4 and RED-5 were considered. The textile dye Red ED3B was treated by using these bacteria in simple nutrient medium. Activity of various Oxidoreductase enzymes for dye degradation was also determined. Manganese Peroxidase and Azoreductase enzymes were responsible for cleavage of dye structure. Mainly Azoreductase initiates the metabolic cleavage of dye Red ED3B by cleavage of azo bridge [15]. Phytotoxicity studies revealed that the bacterial isolates degraded toxic and harmful dye into non-toxic and safe metabolites.

Sample collection

Soil and water samples were collected from area near waste discharge sites at various textile industries located at Pune, Maharashtra (India). The collected samples were stored in sterile plastic container for further use.

Acclimatization of samples

To get dynamic microbial flora for efficient removal of dyes from textile effluent, the collected soil and water samples were mixed properly, homogenized and acclimatized by adding increasing concentration of Red ED3B dye for the period of one month daily. These acclimatized samples were used for isolation of prominent dye degrading bacteria.

Isolation and identification of promising bacterial isolates

Nutrient agar medium containing 100 ppm of Red ED3B dye was used for isolation and screening of dye degrading bacteria. Total 15 bacterial isolates were isolated among all 5 isolates were showing highest decolorization and primarily designated as RED-1, RED-2, RED-3, RED-4, RED-5. Identification of selected bacteria was done by routine morphological, biochemical and 16S rRNA sequencing.

Statistical Analysis for determination of enzyme activity

Some Oxidoreductive enzymes such as Azoreductase, Manganese Peroxidase and Laccase are mainly involved in bioremediation of textile dyes [16, 17, 18, 19, 20]. Quantitatively Azoreductase activity was determined by 0.1 mM NADH [21, 22], Manganese Peroxidase enzyme activity assay was performed by using Guaiacol as a substrate [23] whereas activity of Laccase enzyme was determined by using 0.5 mM ABTS [2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] in 1 mM (pH 5.0) Sodium Acetate Buffer. The reaction kinetics was monitored by spectrophotometer at 420 nm (Shimadzu-UV-3600 Plus) at 30^0C for 5 minutes. All the assays were performed in triplicate. One unit of enzyme activity was expressed as 1 µmol of ABTS oxidized per minute ($\varepsilon 420 = 3.6 \times 104\ M^{-1}\ Cm^{-1}$) [24].

RESULTS

We have conducted the experiment to find the effect of five bacteria viz. RED-1, RED-2, RED-3, RED-4 and RED-5 at the two time points (24 hrs, 48 hrs) on the Red ED3B dye. We have measured the three enzyme activities such as Azoreductase, Manganese peroxide and Laccase. Here the two independent variables are bacteria and Time. Three dependent variables are enzyme activities. There are different multivariate test statistics that are used to test the significance of effects of independent variables such as Wilk's Lambda, Pillai's trace test, Roy's Largest Root and Hotelling's Trace test. The output of Pillai's trace test from R software is given in Table 1.

Table1: Two-way MANOVA for Enzyme Activity of various bacteria and time

Source	Df	Pillai	approx F	numDf	den Df	P-value
Bacteria	4	0.843	3.262	4	5	0.029
Time	1	0.732	8.542	1	5	0.023
Bacteria: Time	4	0.615	2.089	4	5	0.082

From table 1, it is observed that p value for Bacterial activity is 0.039 < 0.05 which indicates that there is significant difference between at least two activities of Azoreductase, Manganese peroxide and Laccase. Also, p value for time is 0.023<0.05 indicating significant difference in enzyme activity with respect to time. However, the interaction effect of various bacteria and time is not statistically significant (p value = 0.082 >0.05); hence the bacteria effects and time effect can be clearly interpreted. It is of interest to find out exactly which bacteria differ significantly out of Azoreductase, Lacasse and Manganese peroxide enzymes. Hence multiple comparison tests are conducted using R software. The results are enlisted in Table 2.

Table 2: Multiple Comparisons of action of Azoreductase, Manganese peroxide and Laccase enzymes from 5 bacterial isolates

Azoreductase enzyme			Manganese peroxide enzyme			Laccase enzyme		
Bacteria	diff	p adj	Bacteria	diff	p adj	Bacteria	diff	p adj
RED-2 - RED-1	-0.78	0.1162	RED-2 - RED-1	-0.25	0.3377	RED-2 - RED-1	-0.11	0.8989
RED-3 - RED-1	-1.05	0.0463	RED-3 - RED-1	0.14	0.7427	RED-3 - RED-1	-0.30	0.5462
RED-4 - RED-1	-0.23	0.8456	RED-4 - RED-1	0.24	0.3656	RED-4 - RED-1	-0.28	0.5984
RED-5 - RED-1	-0.995	0.0551	RED-5 - RED-1	1.34	0.0014	RED-5 - RED-1	-0.08	0.9993
RED-3 - RED-2	-0.27	0.7684	RED-3 - RED-2	0.39	0.1132	RED-3 - RED-2	-0.19	0.9434
RED-4 - RED-2	0.55	0.2821	RED-4 - RED-2	0.49	0.0562	RED-4 - RED-2	-0.17	0.9969
RED-5 - RED-2	-0.215	0.8717	RED-5 - RED-2	1.59	0.0007	RED-5 - RED-2	-0.03	0.9999
RED-4 - RED-3	0.82	0.1004	RED-4 - RED-3	0.1	0.8929	RED-4 - RED-3	0.02	0.9998
RED-5 - RED-3	0.055	0.9990	RED-5 - RED-3	1.2	0.0022	RED-5 - RED-3	0.17	0.9995
RED-5 - RED-4	-0.765	0.1228	RED-5 - RED-4	1.1	0.0031	RED-5 - RED-4	0.15	0.9997

From Table-2, RED-1 bacteria differs significantly from the RED-3 bacteria with respect to the Azoreductase Enzyme activity. Also, levels of the time are significant for this enzyme activity, at 24 hours it has maximum activity. Bacteria RED-5 is significantly different from all other bacteria moreover there is highly significant difference between RED-5 and RED-2 with respect to the Manganese peroxide enzyme activity.

Table 3: Multiple Comparisons of action of Azoreductase, Manganese peroxide and Laccase enzymes from two time periods

Time: A: 24 Hrs B: 48 Hrs

Enzyme Activity	Time	diff	p adj
Azoreductase	B-A	-0.201	0.094
Manganese peroxide	B-A	-0.251	0.452
Laccase	B-A	-0.222	0.003

From Table 3, Time factor is showing significant difference for the Laccase enzyme activity and it is seen to be more active than enzyme activity in Azoreductase and Manganese peroxide.

Phytotoxicity studies of degraded products:

The phytotoxicity study was performed by observing seed germination with two types of seeds. Jowar (*Sorghum vulgare*) and Mung (*Vignaradiata*)are used for phytotoxicity study of treated dye products because they are common agricultural plants. These seeds were watered with 1 ml of original dye solution and treated dye solution separately in respective Petri plates. The distilled water was used as control for this test. These plates were kept at room temperature. All the samples were added for consecutive 7 days. The percent germination, length of root and shoot was recorded and compared with control distilled water.

The sampled water is the water which contains degraded metabolites of dye by the bacteria after the enzyme activity (treated dye sample) and original dye sample. Here we have taken distilled water as a control group and checked the effect of treated dye sample from five bacteria RED-1, RED-2, RED-3, RED-4 and RED-5 on the plant of Jowar (*Sorghum vulgare*) and Mung beans (*Vignaradiata*). The plant parameters considered for present study were radicle, plumule and percentage of seed germination. The length of plumule and radical were measured in millimeters manually by ruler [25]. In the presence of dye Red ED3B, the reduced germination of *Sorghum vulgare*, and *Vigna radiata* seeds was observed with only 40% - 50% of seeds germination, respectively. After decolorization/degradation of dye, the germination extended further to more than 80% - 90% in presence of dye metabolites. We also measured plant's root length and shoot length to validate our results and it was observed that in the presence of the original dye, root and shoot growth was stunted and significantly reduced. However, when exposed to the treated dye metabolites both root and shoot length showed considerable improvement. In comparison to dye, better growth of experimental plants was observed when grown under dye degraded products and results are nearly similar to plant parameters in distilled water. The results are shown in Figure 1 and 2. We have conducted two way MANOVA for testing the effect of bacteria and sampled water on the plant parameters like radicle, plumule and percentage of seed germination. Here the two independent variables are bacteria and Time. Three dependent variables are length of radicle, plumule and percentage of seed germination. The output for present study in Table 4.

Table 4: Two Way MANOVA for Jowar

Source	Df	Pillai	Approx F	numDf	den Df	P- value
Bacteria	4	0.232	1.98	4	16	0.15
Sampled water	2	0.174	2.13	6	16	0.04
Bacteria: Sampled Water	8	0.343	1.56	8	16	0.23

From Table 4, p value for bacteria is 0.15 >0.05; hence effect of bacteria is not significant. Also the interaction effect of different bacteria and sampled water (p value = 0.23 > 0.05) is not statistically significant hence the bacteria effects and sampled water effect can be clearly interpreted**.** However**,** effect of sampled water is statistically significant on the parameters of Jowar viz. radicle, plumule and percentage of seed germination (p value = 0.04 < 0.05); hence it is of interest to find out exactly which parameters differ significantly which can be evaluated applying multiple comparison tests. The multiple comparison tests for Distilled water, treated dye sample, original dye was conducted in R; output of it is listed in Table 5.

Table 5: Multiple Comparisons of different sampled water on the parameters of Jowar plant

A: Distilled water B: Treated dye sample C: Original dye

Length of Radicle			Plumule of Jowar			Germination of seed		
	diff	p adj		diff	p adj		diff	p adj
B-A	-0.728	0.0095	B-A	-0.742	0.00588	B-A	-0.06	0.07323
C-A	-1.146	0.0005	C-A	-2.156	< 0.001	C-A	-0.42	< 0.0001
C-B	-0.418	0.1118	C-B	-1.414	< 0.001	C-B	-0.36	< 0.0001

From Table 5, p-value for germination of seed is 0.07323 > 0.05 which indicates that there is no significant difference between distilled water and treated dye sample. p value for length of radicle is 0.1118 > 0.05, hence there is no significant effect of treated dye sample and original dye sample on the length of radicle. For the comparison of distilled water and original dye sample (p value is < 0.05); indicates that there is significant difference with respect to length of radicle, length of plumule and percentage of seed germination of the Jowar. Also, there is significant difference for the comparison of treated dye sample and original dye sample (p value is <0.05) with respect to length of plumule and percentage of seed germination for the plant of Jowar.

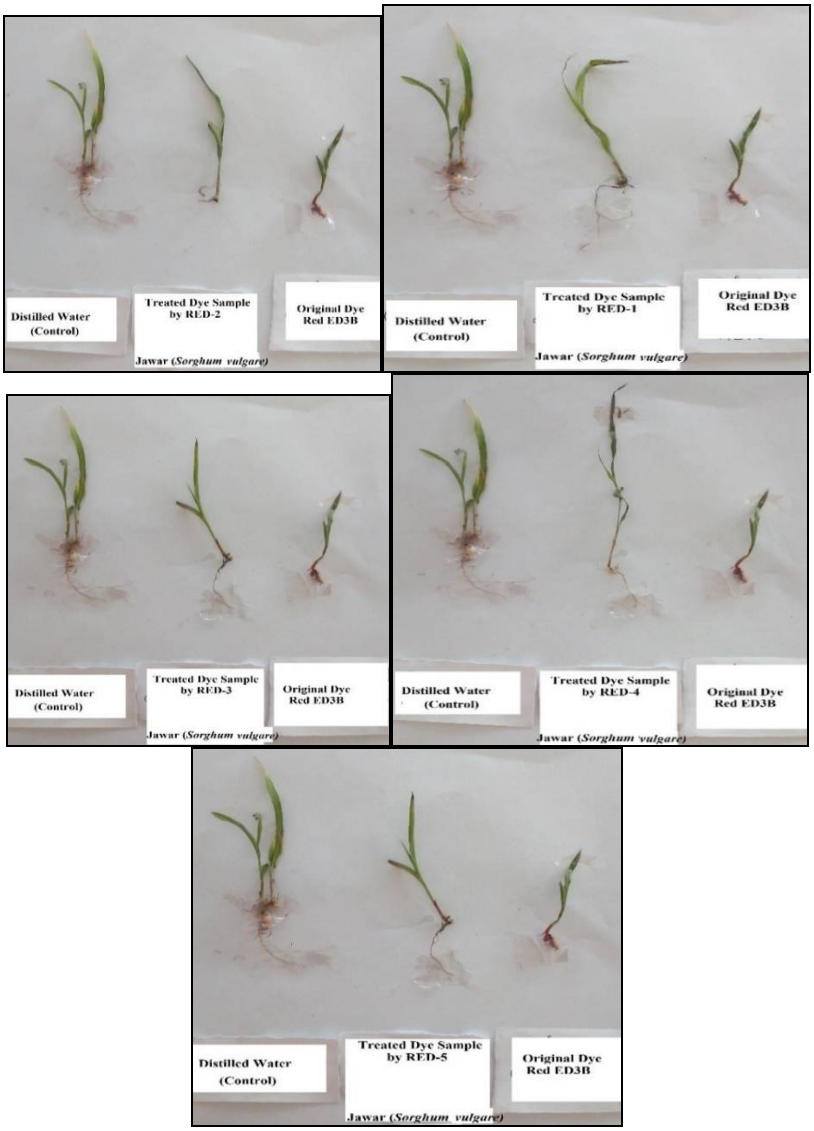

Figure 1 :Phytotoxicity studies of treated and untreated Red ED3B dye on Jowar (Sorghum vulgare)

Table 6: Two way MANOVA for the plant parameter of Mung beans.

Source	Df	Pillai	Approx. F	num Df	den Df	P-value
Bacteria	4	0.934	4.118	4	16	0.094
Sampled water	2	0.975	3.249	6	16	0.035
Bacteria: sampled water	8	0.950	1.903	8	16	0.087

From Table 6,p value for bacteria = 0.094 > 0.05; hence effect of bacteria is not significant. Also the interaction effect of different bacteria and sampled water (p value = 0.087 > 0.05) is not statistically significant hence the bacteria effects and sampled water effect can be clearly interpreted**.** However**,** effect of sampled water is statistically significant on the parameters of Mung beans like radicle, plumule and percentage of seed germination (p value = 0.035 < 0.05); hence it is of interest to find out exactly which parameters differ significantly which can be evaluated applying multiple comparison tests. The multiple comparison test for Distilled water, Treated dye sample and Original dye are conducted in R; output of it is listed in Table 7.

Table 7: Multiple Comparisons of different sampled water on the plant parameters of Mung beans.

A: Distilled water B: Treated dye sample C: Original dye

Radicle			Plumule			Germination of seed		
	diff	p adj		diff	p adj		diff	p adj
B-A	-1.004	0.0003	B-A	-0.4	0.2865	B-A	-0.1	0.1643
C-A	-3.258	0.00001	C-A	-4.18	0.0004	C-A	-0.46	0.0003
C-B	-2.254	0.0009	C-B	-3.78	0.0008	C-B	-0.36	0.0002

From the table 7, there is no significant difference between the treated dye water and distilled water with respect to the plant parameter like length of plumule (p value = 0.2865 > 0.05) and percentage of seed germination (p value = 0.1643> 0.05), but it is significant for length of radicle (p value = 0.0003 <0.05. For the comparison of treated dye sampled water and original dye (p value is < 0.05) hence there is significant difference with respect to length of radicle, length of plumule and percentage of seed germination. Also there is significant difference (p value is <0.05) for the comparison of distilled water and original dye sample with respect to length of radicle, length of plumule and percentage of seed germination for the plant of Mung beans.

Figure 2 :Phytotoxicity studies of treated and untreated Red ED3B dye on Mung (*Vigna radiata*)

DISCUSSION

The present study concluded that, the five bacterial isolates *viz*. RED-1, RED-2, RED-3, RED-4 and RED-5 are potent strains for the treatment of textile dye Red ED3B as it showed good degradation in all aspects. As it showed complete removal of dye in the medium within 24 hours. The experiments were conducted to determine the effect of above five bacteria at the two time points (24 hrs. and 48 hrs.) on the Red ED3B dye. The activities of three enzymes such as Azoreductase, Manganese peroxide and Laccase were also determined. Manganese Peroxide and Azoreductase enzymes were responsible for cleavage of dye structure. Mainly Azoreductase initiates the metabolic cleavage of dye Red ED3B by cleavage of azo bridge. Phytotoxicity studies revealed that the bacterial isolates degraded toxic and harmful dye into non-toxic and safe metabolites. Enzyme activity is statistically significant on the bacteria. Also, it is significant on the time period. RED-1 bacteria are mostly significant on the Azoreductase and Manganese peroxide enzyme activity. Effect of Time period is statistically significant on the Laccase enzyme activity; it is mostly activated at 24Hrs.

Bacteria and sampled water are statistically significant on the plant parameter like radicle, plumule and germination of seed. In our study there is no significant difference between the treated dye water and

distilled water with respect to the plant parameter like length of plumule and percentage of seed germination, it is significant for length of radicle [26, 27]. There is significant difference between treated dye sampled water and original dye with respect to length of radicle, length of plumule and percentage of seed germination.

CONCLUSION

RED-1 and RED-5 bacteria are significant on the Azoreductase and Manganese peroxide enzyme activity.The results indicate that there is no statistically significant difference in the impact of distilled water and treated dye sample water (after degradation) on the plant parameters. This suggests that the treated dye water, after degradation, is as safe for plants as distilled water under the tested conditions.

REFERENCES

1. G. B. MacFarlane, S. O. Ajayi, O. Osibanjo. (1980), "The state of environment in Nig. Pollution studies textile industries in Nigeria", Monogra, 1: 76-86.
2. R. Anliker. (1979), "Ecotoxicology of dyestuffs – a joint effort by industry", Ecotox. Environ. Safe, 3: 59-74.
3. F. J. Cervantes, F. P. Van der Zee, G, Lettinga. (2001). "Enhanced Decolorization of Acid Orange 7 in a continuous UASB reactor with quinones as redox mediators", Water Science and Technology, 44: 123-128.
4. Bhosale Amarja, Khobragade Rahul. (2019). "Biodecolourization and biodegradation of textile azo dye Golden Yellow HER by Pseudomonas sp. GYH-1", Research Journal of Science and Technology,11(4): 246-258. DOI: 10.5958/2349-2988.2019.00035.4.
5. A. E. Bisschops, H. spanjers. (2003). "Literature review on textile wastewater characterization", Environmental Technology, 24: 1399-1411.
6. R. G. Sartale, G. D. Sartale, J. S. Chang, S. P. Govindwar. (2009). "Ecofriendly Degradation of Sulfonated diazoDye CI Reactive Green 19A using Micrococcus glutamicusNCIM-2168", Bioresource Technology, 100: 3897-3905.
7. Ghulam Mustafa, Muhammad Zahid, MayurKurade, SwapnilPatil, Farah Shakoori, ZeeshanShafiq, SirdaIhsan, YongtaeAhn, Azmat Khan, AmelGacem, ByongJeon. (2023), "Molecular characterization of Azoreductase and its potential for the decolorization of Remazol Red R and Acid Blue 29", Environmental Pollution, 335, 12253.
8. H. S. Rai, M. S. Bhattacharyya, J. Singh, T. K. Bansal, P. Vats, U. C. Banerjee. (2005). "Removal of dyes from the effluent of textile and dyestuff manufacturing industry: a review of emerging techniques with reference to biological treatment", Critical Reviews in Environmental Science and Technology, 35: 219-238.
9. C. H. Chen, C. F. Chang, C. H. Ho, T. L. Tsai, S. M. Liu. (2008). "Biodegradation of Crystal violet by a Shewanella sp. NTOU1", Chemosphere, 72: 1712-1720.
10. M. Vidali. (2009). "Bioremediation- an overview", Pure Application Chemistry, 73: 581-587.
11. M. Imran, D. E. Crowley, A. Khalid, S. Hussain, M. W. Mumtaz, M. Arshad. (2015), "Microbial biotechnology for decolorization of textile wastewater", Reviews in Environmental Sciences and Bio/Technology, 14: 73-92.
12. Lekha Nair, Maleeka Begum, R. Ragunathan. (2017). "Biodegradation of Azo dyes using Bacillus megaterium and its phytotoxicity study", IOSR Journal of Environmental Science, Toxicology and Food Technology, 11(7): 12-20.
13. S. Singh, RL. Gautam, DK. Chaudhari, D. Singh. (2024). "Biodecolorization and Biodegradation of Reactive Green 12 textile industry dye and their post-degradation phytotoxicity-genotoxicity assessments", Achievements of Microbiology, 206(6): 1-14.
14. Montgomery, D.C. (2020). Design and Analysis of Experiments, 10thEdn(Wiley).
15. H. G. Kulla, T. Leisinger, A. M. Cook, J. Niiesch, R. Hiitter. (1981), "Microbial degradation of Xenobiotics and Recalcitrant Compounds", Academic Press London, 387-399.
16. R. Campos, A. Kandelbauer, K. H. Robra, A. Cavaco-Paulo, G. M. Gubitz. (2001), "Indigo degradation with Purified Laccases from *Trameteshirsuta* and *Sclerotium rolfsii*", J. Biotechnol., 89: 131.
17. J. Maier, A. Kandelbauer, A. Cavaco-Paulo, G. M. Gubitz. 2004, "A New Alkali thermostable Azoreductase from Bacillus sp. Strain SF", Appl. Environ. Microbiol., 70: 837.
18. S. Ryan, W. Schnitzhofer, T. Tzanov, A. Cavaco-Paulo, G. M. Gubitz. (2003). "An acid-Stable Laccase from Sclerotiumrolfsii with Potential for wool Dye Decolorization", Enzyme Microb. Technol., 33: 766.
19. Y. Suzuki, T. Yoda, A. Ruhul, W. Sugiura. (2001). "Molecular Cloning and Characterization of the Gene Coding for Azoreductase from Bacillus sp. OY1-2 Isolated from soil", J. Biol. Chem., 276: 9059.
20. Heinfling, M. J. Martinez, A. T. Martinez, M. Bergbauer, U. Szewzyk. (1998). "Transformation of Industrial Dyes by Manganese Peroxidases from *Bjerkandera adusta* and *Pleurotus eryngii* in a Manganese-Independent Reaction", Appl. Environ. Microbiol., 64: 2788.
21. S. Blumel, H. J. Knackmuss, A. Stolz. (2002). "Molecular cloning and characterization of the gene coding for the aerobic Azoreductase from *Xenophilus azovorans* KF46F", Appl. Environ. Microbiol., 68: 3948-3955.
22. M. Nakanishi, C. Yatome, N. Ishida, Y. Kitade. (2001), "Putative ACP phosphodiesterase gene (acpD) encodes an Azoreductase", J. Biol. Chem., 276: 46394-46399.
23. J. Putter. (1974), "Peroxidases in Methods of Enzymatic Analysis", 2nd English edn., Edited by H. U. Bermeyer, London: academic press, 685-690.
24. R. Bourbonnais, M. G. Paice. (1990). "Oxidation of non-phenolic substrates: An expanded role for laccase in lignin biodegradation", Fed. Biochem. Soc., 267: 99-102.

25. Siddiqui, M. T., & Khan, M. R. (2017). Plant Growth and Development: Measuring Root and Shoot Lengths Using a Ruler. Journal of Plant Sciences.9(10):90-98
26. Izharul hag, Anhay Raj. (2018). "Biodegradation of Azure-B dye by *Serratia liquefaciens* and its validation by phytotoxicity, genotoxicity and cytotoxicity studies, Chemosphere, 196: 58-68.
27. Ritu Sharma, Poonam Sharma, Naveen Kumar, Neeraj Agrawal. (2025). "Enhanced decolorization of Basic violet 14 dye waste water and its phytotoxicity assessment: A Green approach", Environmental Quality Management, 34(3): e70050