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ORIGINAL ARTICLE

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A sustainable approach for remediation of Reactive dye contaminated water

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ABSTRACT

The treatment of effluent from textile industries that contains synthetic dyes poses a significant environmental issue due to its toxic effects. Addressing the effluent containing reactive dyes is crucial because these dyes are mainly used for dyeing cotton, which is the most commonly used fiber. They also exhibit a low degree of fixation to fibers and resist conventional treatment methods. Reactive dyeing demands substantial water during processing, contributing to water scarcity, and if left untreated, results in the build-up of persistent organic pollutants in the soil. The primary challenges associated with physical and chemical dye treatment methods include high costs and the need for strict reaction conditions. In contrast, biological treatment methods are more affordable and environmentally safe. Microbial and enzymatic systems can be employed to biodegrade dyes. However, the microbial cultures capable of degrading reactive dyes must be transformed into a format that can be easily and safely handled at effluent treatment plants (ETP), even by individuals with minimal training. Consequently, this research developed a formulation that includes a microbial consortium of *Enterobacter hormaechei, Neobacillus niacini,* and *Enterobacter ludwigii* for the treatment of Reactive Brown HER and Navy Blue HER dyes. Three treatment approaches were studied: T1, which used the microbial formulation; T2, which involved ozonation; and T3, a combination of the microbial formulation and ozonation. The percentage decolorization tests showed that using the microbial formulation for two days, followed by ozonation, achieved a decolorization of 98.54% for Reactive Brown HER and 97.92% for Navy Blue HER dyes.

Key words:
Microbial formulation
Navy Blue HER
Ozonation
Reactive Blue HER

1 | INTRODUCTION

Dyes are coloring agents used in industries to add color to materials, enhancing their attractiveness. Dyes can be categorized into natural and synthetic types. Natural dyes are environmentally friendly but have limited applications in the textile industry due to their weak adhesion to textile fibers and low light fastness properties. Synthetic dyes typically have complex chemical structures, superior binding properties, and resistance to washing, rubbing, and light; however, most of them pose environmental hazards. When selecting dyes in the textile industry, factors such as dyeing costs, color persistence after harsh processes like washing and rubbing, fastness to light, and durability during fabric use are considered. Consequently, synthetic dyes supplanted natural dyes in 1856 after H. Perkin synthesized Mauveine (Gupta, 2020). Annually, around seventy million tons of synthetic dyes are produced globally for use in the textile industry. Approximately 10% of these dyes are released into the environment as effluents. During the dyeing process, as much as 50% of the dyes used are not covalently bonded to the fibers, leading to ecological issues (Yuan *et al.,* 2020). The color of dye effluents diminishes sunlight penetration into water, adversely affecting the whole aquatic ecosystem. Fish and other organisms absorb synthetic dyes in water, and the consumption of these contaminated fish by humans can lead to various diseases, including mental illness, hypertension, cramps, and severe toxic effects on the human bladder. Synthetic dyes are highly soluble in water and can penetrate the skin, causing allergic reactions, cancer, and eye irritation (Soumi *et al.,* 2021). Untreated textile effluents can also pollute soil, diminishing its quality and fertility while potentially entering the food chain. Treating synthetic dyes from textile effluents before they are discharged into water resources is crucial (Savita *et al.,* 2024).

Pali city in the state of Rajasthan, known for its textile dyeing and printing work, discharges its effluents into the Bandi River. This river is highly polluted with textile effluent, which has rendered the fields of Jaitpur and

Gadhwara villages infertile. Farmers in these villages have been unable to grow crops for 40 years due to the pollution of the Bandi River (TOI, 2023). Case studies have examined the pollution of the Vena River located in Hinganghat, Wardha, Maharashtra, the Noyyal River and its tributaries in Tamil Nadu due to the textile industries of Tirupur, and groundwater contamination resulting from textile industries in the Kadodara region, Surat, Gujarat. Due to increasing environmental and health concerns, 23 different research projects were supported by DBT for the development of treatment technology for dye and textile effluents. *Fig. 1* provides an overview of DBT-sanctioned research projects. It was observed that the majority of these research projects were sanctioned to states with a high concentration of textile industries and have led to the development of strategies and technologies for the remediation of textile and dye-containing effluents.

Several Indian scientists have been awarded patents for developing novel technologies in textile effluent management, including 'Processes for Decolorization of Coloured Effluents' in 2011; 'Process for Decolorizing Dye-Containing Wastewater Using Dye-Degrading Bacteria' in 2016; and a patent on 'Decolorization and Degradation of Textile Industry Wastewater Using Laboratory-Isolated Consortia Containing *Pseudomonas aeruginosa, Alcaligenes sp.* and *Bacillus latrosphorus'* in 2011 (Anonymous, 2019). Based on chemical structure, dyes can be categorized as anthraquinone, azo, sulfur, nitro, and nitroso. Based on their implementation, they can be grouped as reactive, basic, acidic, dispersed, direct, and vat dyes (Benkhaya *et al.,* 2020). Reactive dyes are used for cotton dyeing and printing due to their availability, the wide array of colors they provide, good fastness properties, and ease of application. Reactive dyes covalently bind to the fiber, forming a permanent attachment

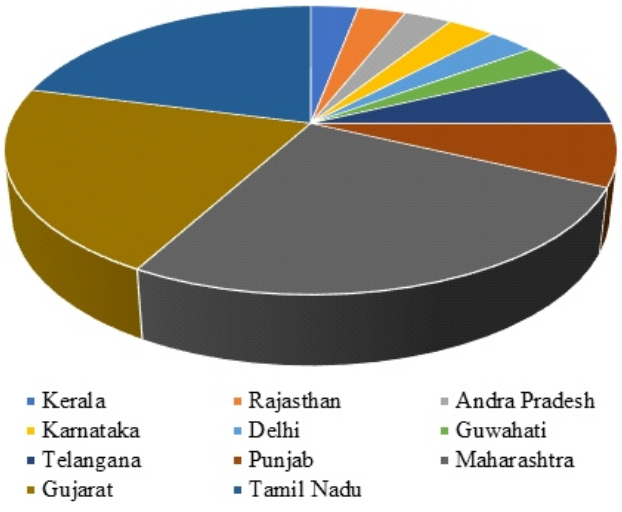


FIGURE 1 Distribution of the sanctioned projects amongst the state of the country

HIGHLIGHTS

- Synthetic dye pollution of textile effluent can effectively treated by Efficient Microbial Decolorization.
- Ozonation can achieve 97–98% decolorization; microbial pre-treatment reduced time and cost.
- Ozonation neutralized microbes; GC-MS confirmed toxic dye breakdown, enabling water reuse.

(Chapter 20 - Fabric Finishing: Printing Textiles, 2015). Therefore, this research emphasizes the remediation of reactive dye-contaminated water. Different chemical, physical, and biological methods, such as chemical oxidation and reduction, electrochemical precipitation, chemical precipitation, adsorption, photolysis, and membrane processes, along with their combinations, are employed for this purpose. The major limitations in implementing these methods include high costs and strict reaction conditions (Ledakowicz *et al.,* 2021). Biological methods of dye treatment are much cheaper and more environmentally friendly. Microbial and enzymatic systems can be used for the biodegradation of synthetic dyes. In numerous research studies referenced from the Mapping of Research Outcome on Remediation of Dyes, Dye Intermediates, and Textile Industrial Waste (A Research Compendium, Department of Biotechnology Ministry of Science & Technology Government of India, 2019), the presence of tyrosinase, NADH-DCIP reductase, riboflavin reductase, azo reductase, and laccase activity indicated their role in degradation during decolorization. Most cultures identified as dye decolorizers resulted in successful degradation. Therefore, these microbial cultures could be effectively utilized for dye remediation. Although there is abundant literature on microbial dye degradation, for practical purposes, the cultures known to degrade reactive dyes need to be converted into a form that can be safely delivered to the Effluent Treatment Plant and used even by unskilled persons. Further research in this area should facilitate easy transportation, storage, and usage of biological treatments for dye degradation by unskilled communities in remote areas. Achieving complete dye remediation through solely biological methods is challenging. Physical or chemical methods can be combined with biological methods for effective dye remediation. This study compares treatments with microbial formulation and ozonation in isolation and in combination for Reactive Brown HER and Navy Blue HER remediation.

2 | MATERIALS AND METHODS

2.1 | Treatment 1: Microbial formulation

Chemicals and media: Dyes include Reactive Brown HER and Navy-Blue HER. The media utilized are Nutrient agar and Nutrient broth obtained from Hi media. The substrates for formulating are rice flour (RF), wheat flour

(WF), compost (CT), and dried cow dung (CD). Effluent samples from textile industries in Maharashtra, India, were collected as sources of microorganisms for screening those capable of decolourising and degrading textile dyes.

Screening of microorganisms for dye decolourization

Following the appropriate dilution of the effluent sample, the spread plate method was employed to identify organisms that can utilize dyes present in the medium (0.05% Reactive Brown HER and Navy Blue HER dyes in Nutrient agar). The organisms that developed on the Nutrient agar plates containing these dyes were isolated, and their colony characteristics were examined. These isolated organisms were then inoculated into Nutrient Broth with 0.05% of the corresponding dyes. After four days, absorbance was measured with a UV Spectrophotometer to calculate the percentage decolorization of the dyes using the designated formula:

$$\text{Dye decolourization percentage} = \frac{\text{Abs (Control) - Abs (Treated sample)} \times 100}{\text{Abs (Control)}}$$

Three organisms (O3, A1, and A2) were chosen for further studies based on their percentage of dye decolorization. The dye-degrading abilities of these isolated organisms were compared with those of a known dye degrader, *Pseudomonas aeruginosa*.

Microbial Identification using 16S rRNA gene based molecular method: Microbial identification of three organisms (O3, A1, and A2) exhibiting high dye decolorization ability was performed using a 16S rRNA gene-based molecular method at Barcode Biosciences in Bangalore. DNA was isolated from the culture, and its quality was evaluated on a 1.0% agarose gel, where a single band of high-molecular-weight DNA was observed. A fragment of the 16S rRNA gene was amplified using the 16SrRNA-F and 16SrRNA-R primers. A single discrete PCR amplicon band of 1500 bp was noted when resolved on the agarose gel. The PCR amplicon was purified to eliminate contaminants. Forward and reverse DNA sequencing reactions of the PCR amplicon were conducted with the 16SrRNA-F and 16SrRNA-R primers using the BDT v3.1 Cycle Sequencing Kit on the ABI 3730xl Genetic Analyzer. A consensus sequence of the 16S rRNA gene was generated from both forward and reverse sequence data using alignment software. The 16S rRNA gene sequence was used to perform a BLAST search with the 'nr' database of the NCBI GenBank. Based on maximum identity scores, the first ten sequences were selected and aligned using the multiple alignment software program Clustal W. A distance matrix and phylogenetic tree were constructed using MEGA 11.

Effect of temperature, pH, initial dye concentration and mechanical agitation on dye decolourization ability of the isolated organisms: Organisms were inoculated into nutrient broth containing 0.05% dyes and incubated at 0, 27, 37, and 50 ˚C for four days. The percentage of dye decolourization was calculated after incubation under different temperature conditions. Additionally, organisms were inoculated into the nutrient broth with pH levels of 2, 5, 9, and 12, also containing 0.05% dyes, and the percentage of dye decolourization was calculated after four days of incubation at 27˚C. Furthermore, organisms were inoculated in nutrient broth containing 0.25, 0.5, 1, and 2 mg/ml of Reactive Brown HER and Navy-Blue HER dyes, respectively. The percentage of dye decolourization was calculated after four days of incubation at 27˚C. Finally, organisms were inoculated in 100 ml of nutrient broth containing 0.05% Reactive Brown HER and Navy-Blue HER dyes, respectively, and incubated under shaker and static conditions. The percentage of dye decolourization was calculated after four days of incubation at 27˚C.

Optimization of carrier base: Four different substrates - rice flour (RF), wheat flour (WF), compost (CT), and dried cow dung (CT) - were used individually and in combination to prepare a microbial formulation. The individual substrates were weighed in equal ratios (up to 2 g) in a petri dish and autoclaved. Microbial strains (O3, A1, and A2) were inoculated into nutrient broth and incubated for 24 hours. The optical density of each culture was adjusted to 0.8. Under sterile conditions, 2 ml of each culture was added to the Petri dish containing the dried substrate or their combination and mixed well. To reduce the moisture content, the Petri dishes were dried at 40 °C for five days. The powdered formulation was then used for further studies.

2.2 | Treatment 2: Ozonation

For the ozone treatment of Reactive Brown HER and Navy-Blue HER, a commercially available ozone generator from Kent [Product specification: Voltage - Single Phase 220-240 V AC, 50-60 Hz, Rated Power Dissipation - 13 W, Dimensions (mm) - 260 (L) × 145 (W) × 150 (H)] was utilized. Solutions of 100 ml containing 0.025%, 0.05%, 0.1%, and 0.2% of Reactive Brown HER and Navy Blue HER dye were prepared and subjected to ozonation until complete dye decolorization occurred. The percentage of dye decolorization was recorded at 15 min intervals during ozonation.

2.3 | Treatment 3: Ozonation coupled with microbial formulation

One gram of the powdered formulation was inoculated into 100 ml of 0.05% Reactive Brown HER and Navy Blue HER dye solutions, respectively, and incubated for two days under shaking conditions. After two days, the supernatant was subjected to ozonation until complete dye decolourization occurred. The percentage decolourization after two days of microbial formulation treatment and the time taken for complete decolourization via ozonation were recorded. The percentage decolourization results of T1, T2, and T3 were compared.

3 | RESULTS AND DISCUSSION

3.1 | Treatment 1: Microbial formulation

Effluent samples were serially diluted to 10^{-5} and spread on Nutrient Agar containing 0.05 % of Reactive Brown HER (*Fig. 2a*) and Navy-Blue HER (*Fig. 2b*). Reactive Brown HER and Navy Blue HER dye, respectively, and percentage decolourization by individual colonies were studied. Out of 50 and 47 organisms inoculated in Reactive Brown HER and Navy-Blue HER respectively. Nine organisms (O1, O2, O3, A1, A2, A3, B1, B2 and B3) that consistently gave good decolourization for both dyes were screened for further studies and their colony characteristics were recorded. After an incubation period of four days, three organisms (O3, A1 and A2) showed higher dye decolorization of 0.05 % of Reactive Brown HER and Navy-Blue HER compared to others (Fig. 3a and 3b). Therefore O3, A1 and A2 were used for further studies. The dye decolourization capacity of selected isolates was then compared to that of a known dye degrader *Pseudomonas aeruginosa*. 0.05% Reactive Brown HER and Navy Blue HER in Nutrient broth were inoculated with O3, A1, A2 and *Pseudomonas aeruginosa*, respectively. After an incubation period of four days, O3, A1, A2 and *Pseudomonas aeruginosa* gave percentage decolourization of 61.86, 90.03, 91.97 and 57.45 % for Reactive Brown

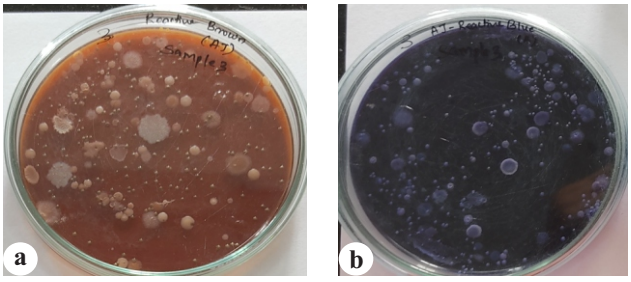


FIGURE 2 Results of spread plate technique (a) Colonies growing on media containing Reactive Brown HER and (b) Navy Blue HER

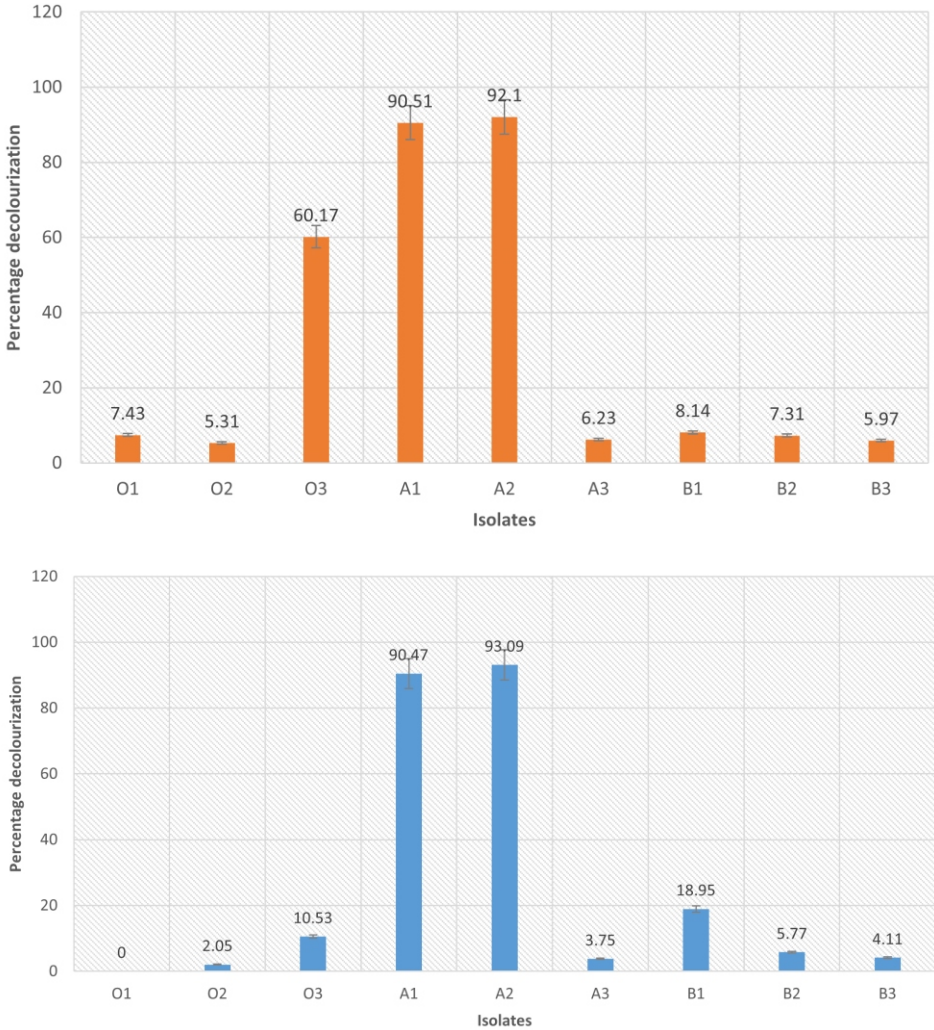


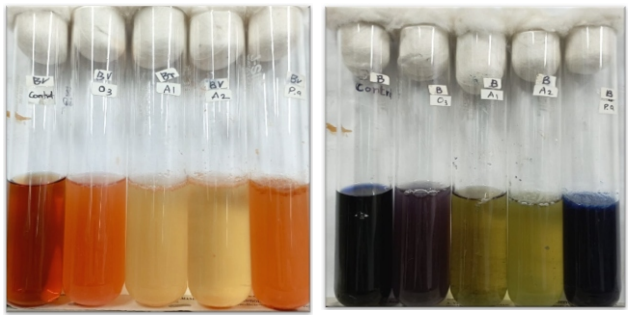
FIGURE 3 Percentage decolourization by isolated organisms (a) Reactive Brown HER and (b) Navy Blue HER

HER and 15.95, 90.53, 91.86 and 7.09 % for Navy Blue HER respectively. Therefore O3, A1 and A2 gave higher percentage decolourization compared to *Pseudomonas aeruginosa* (*Fig. 4a* and *4b*).

The dye decolourization ability of single isolates and their combinations was studied. Results indicated that a consortium of O3, A1, and A2 showed decolourization of about 95% in a shorter time duration of two days (*Fig. 5a* and *5b*).

Microbial Identification using 16S rRNA gene-based molecular method

The isolated microbial strains O3, A1, and A2 exhibited high similarity based on nucleotide homology and phylogenetic analysis with *Enterobacter hormaechei* (*Fig. 6a*), *Neobacillus niacini* (*Fig. 6b*), and *Enterobacter ludwigii* (*Fig. 6c*), respectively. The evolutionary history was inferred



(a) (b)

FIGURE 4 Comparative study of percentage dye decolourization by O3, A1 and A2 with *Pseudomonas aeruginosa*. Left to right - Tubes labelled as Control (0.05% dye in Nutrient broth), O3, A1, A2 and *Pseudomonas aeruginosa* for (a) Reactive Brown HER and (b) Navy Blue HER

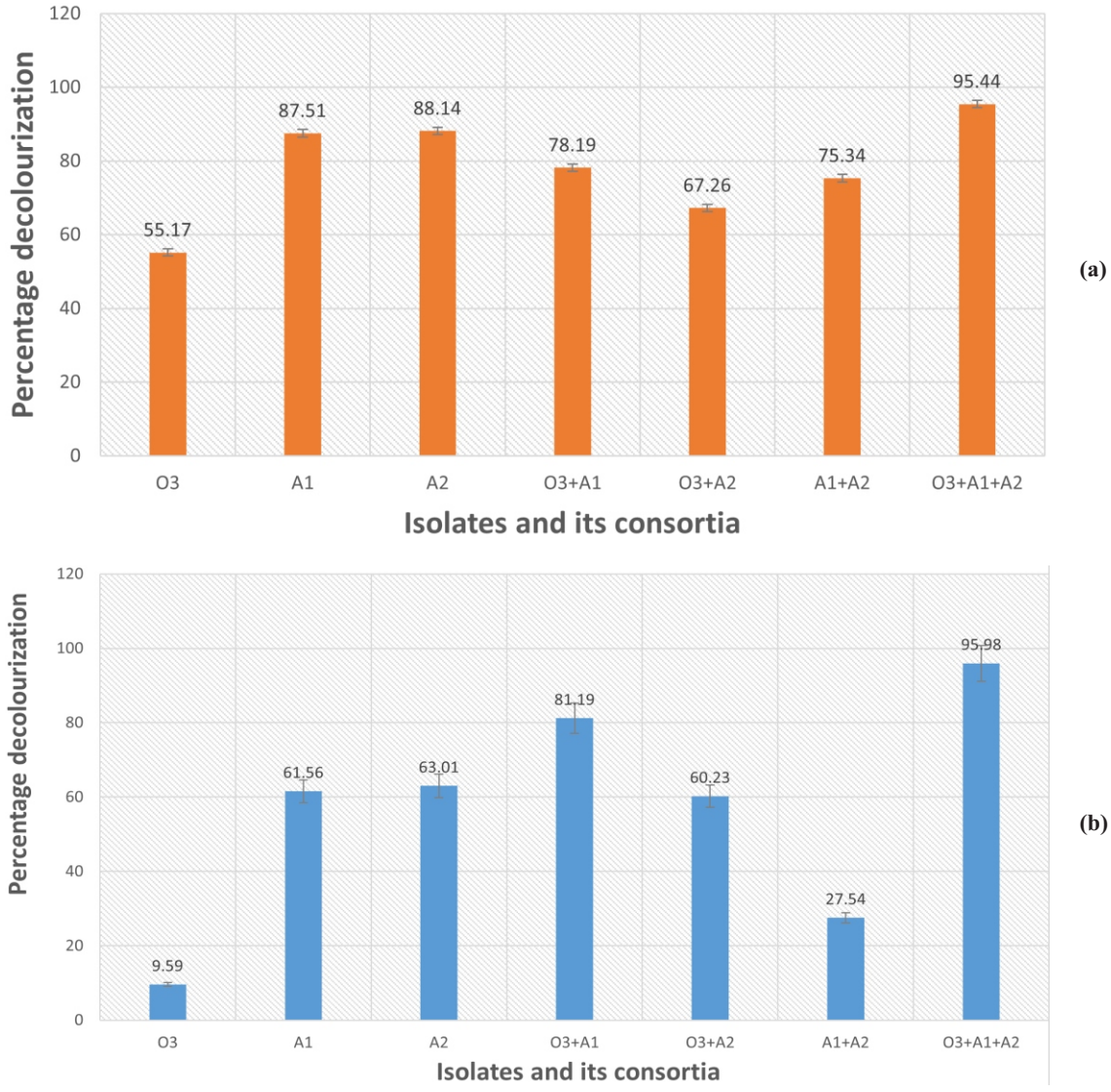
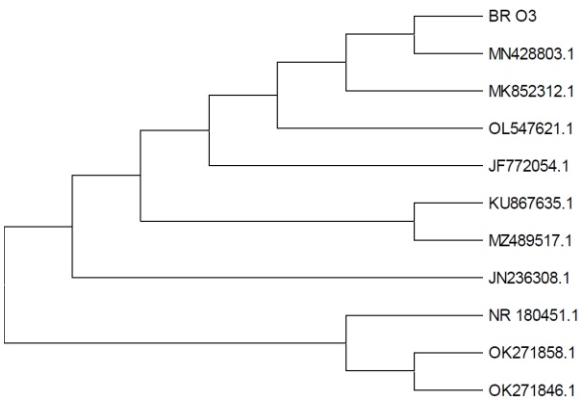


FIGURE 5 Percentage decolourization of (a) Reactive Brown HER and (b)Navy Blue HER by O3, A1 and A2 and their consortia

Phylogenetic Tree

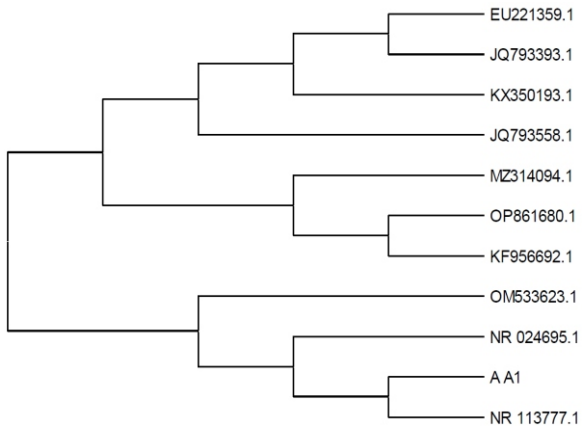


Sequences producing significant alignments

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Enterobacter hormaechei strain IPBCC 19.1426	2638	2638	100%	0.0	100.00%	MN428803.1
Uncultured bacterium clone MT-9	2638	2638	100%	0.0	100.00%	MK852312.1
Enterobacter hormaechei strain PJ062A64	2638	2638	100%	0.0	100.00%	OL547621.1
Enterobacter hormaechei strain LRC5	2638	2638	100%	0.0	100.00%	JF772054.1
Uncultured bacterium clone b1_22	2638	2638	100%	0.0	100.00%	JN236308.1
Enterobacter hormaechei strain CW-3	2636	2636	99%	0.0	100.00%	KU867635.1
Enterobacter quasihormaechei strain WCHEs120003	2632	2632	100%	0.0	99.93%	NR_180451.1
Enterobacter hormaechei strain E1-N-107	2632	2632	100%	0.0	99.93%	OK271858.1
Enterobacter hormaechei strain E1-N-2	2632	2632	100%	0.0	99.93%	OK271846.1
Enterobacter hormaechei strain ABRL036	2632	2632	100%	0.0	99.93%	MZ489517.1

FIGURE 6a Results of 16S rRNA sequencing for O3. Phylogenetic tree and Sequences producing significant alignments

Phylogenetic Tree

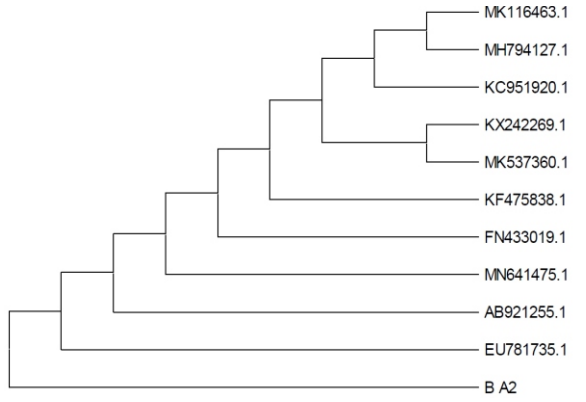


Sequences producing significant alignments

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Neobacillus niacini strain 4B	2652	2791	100%	0.0	99.25%	MZ314094.1
Neobacillus niacini strain HJ01-02-qm	2625	2690	99%	0.0	99.72%	OM533623.1
Neobacillus niacini strain IFO15566	2625	2690	99%	0.0	99.72%	NR_024695.1
Bacillus niacini strain J2S5	2615	2681	100%	0.0	99.58%	EU221359.1
Neobacillus sp. strain Np2.2	2612	2678	99%	0.0	99.72%	OP861680.1
Bacillus sp. S22224	2608	2674	99%	0.0	99.51%	KF956692.1
Uncultured Bacillus sp. clone SGR224	2608	2674	99%	0.0	99.51%	JQ793558.1
Neobacillus niacini strain NBRC 15566	2606	2665	98%	0.0	99.86%	NR_113777.1
Bacillus sp. BAB-5803	2603	2668	99%	0.0	99.72%	KX350193.1
Uncultured Bacillus sp. clone SGR17	2603	2668	99%	0.0	99.58%	JQ793393.1

FIGURE 6b Results of 16S rRNA sequencing for A1. Phylogenetic tree and Sequences producing significant alignments

Phylogenetic Tree



Sequences producing significant alignments

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Enterobacter ludwigii strain OS5.4	2375	2375	100%	0.0	99.02%	KX242269.1
Enterobacter ludwigii strain LHC8	2375	2375	100%	0.0	99.02%	KC951920.1
Enterobacter ludwigii strain IHB B 1518	2375	2375	100%	0.0	99.02%	KF475838.1
Enterobacter sp. CCM6B isolate CCM6B	2375	2375	100%	0.0	99.02%	FN433019.1
Enterobacter sp. VET-7	2375	2375	100%	0.0	99.02%	EU781735.1
Enterobacter sp. NA10140	2370	2370	100%	0.0	98.94%	AB921255.1
Enterobacter ludwigii strain SC1	2370	2370	100%	0.0	98.94%	MN641475.1
Enterobacter sp. strain DB044	2370	2370	100%	0.0	98.94%	MK537360.1
Enterobacter cloacae strain TMX6	2370	2370	100%	0.0	98.94%	MK116463.1
Enterobacter tabaci strain UPM18	2370	2370	100%	0.0	98.94%	MH794127.1

FIGURE 6c Results of 16S rRNA sequencing for A2. Phylogenetic tree and Sequences producing significant alignments

using the Maximum Likelihood method and the Tamura-Nei model (Phylogenetic tree from Figs. 6a, 6b, and 6c). The tree showing the highest log likelihood is presented. Initial tree(s) for the heuristic search were automatically obtained by applying the Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura-Nei model, and then selecting the topology with the superior log likelihood value. This analysis included 11 nucleotide sequences. The codon positions included were 1st, 2nd, 3rd, and noncoding. The final dataset comprised a total of 1429 positions for O3, 1471 positions for A1, and 1326 positions for A2. Evolutionary analyses were performed in MEGA11.

Effect of temperature, pH, Initial dye concentration and mechanical agitation

Enterobacter hormaechei, Neobacillus niacini, and *Enterobacter ludwigii* demonstrated decolorization of Reactive Brown HER and Navy-blue HER at 27 and 37°C after an incubation period of four days (*Fig. 7a*). The organisms regained their decolorization ability after being incubated at 4 and 50°C for four days, followed by two days at 27°C, indicating their thermotolerance. *Enterobacter hormaechei* and *Enterobacter ludwigii* exhibited decolorization within a pH range of 5, 9, and 12, while *Neobacillus niacini* demonstrated decolorization at pH 9 and 12 (*Fig. 7b*). Regarding the effect of initial dye

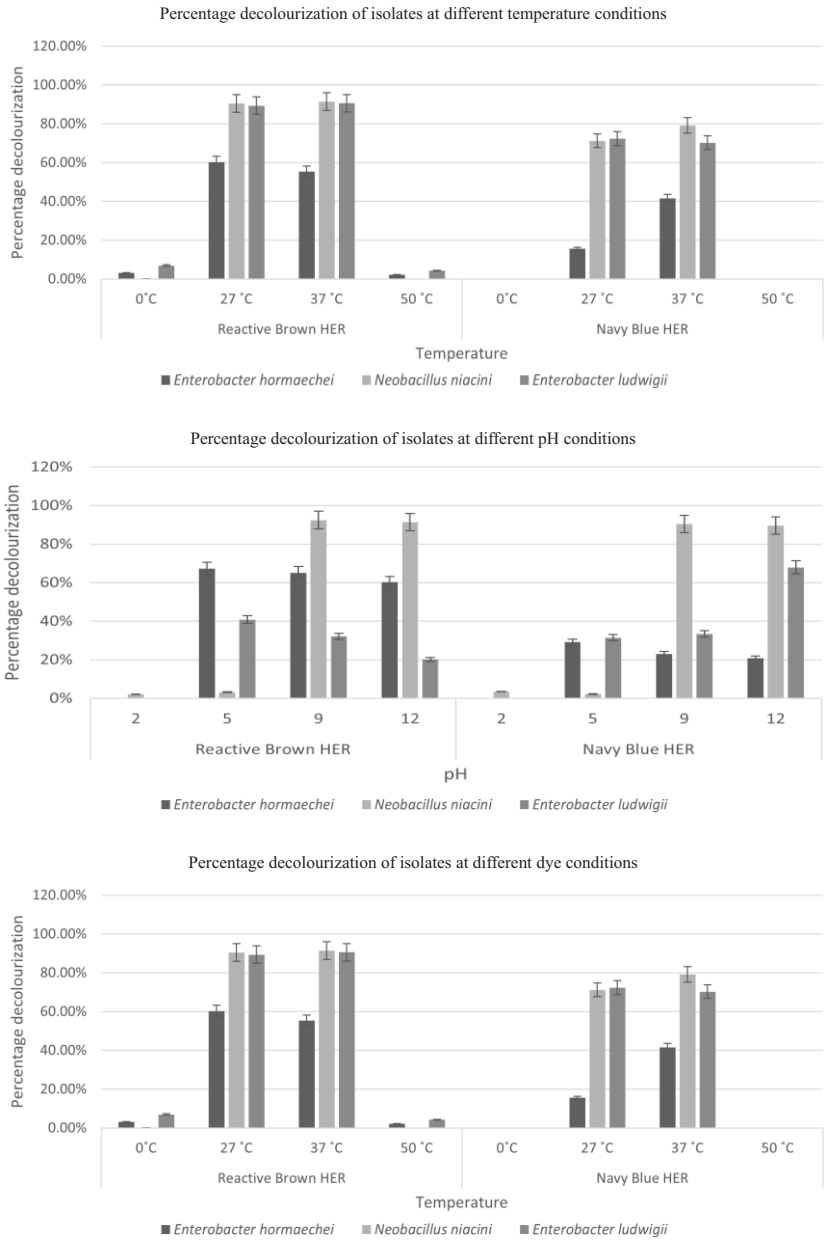


FIGURE 7 Percentage decolourization of Reactive Brown HER and Navy Blue HER by *Enterobacter hormaechei, Neobacillus niacini* and *Enterobacter ludwigii* at different (a) temperature conditions, (b) pH conditions and (c) initial dye concentrations

concentration, *Enterobacter hormaechei* and *Enterobacter ludwigii* could decolorize dyes at initial concentrations up to 1 mg/ml, whereas *Neobacillus niacini* could decolorize at concentrations up to 2 mg/ml (*Fig. 7c*). A higher percentage of decolorization was observed for *Enterobacter hormaechei* under static conditions, while *Enterobacter ludwigii* and *Neobacillus niacini* achieved a higher percentage of dye decolorization under shaker conditions, which facilitated better mixing and aeration of the culture.

Preparation of formulation for microbial growth

Four different substrates - rice flour (RF), wheat flour (WF), compost (CT) and cow dung (CD)(dried) were used individually and in combinations for the preparation of microbial formulation. A high percentage of decolourization was achieved for Reactive Brown HER and Navy-Blue HER, respectively, when using RF+CT, RF+CD+CT, and RF+WF+CD+CT as substrates for the consortia. However, considering the cost and simplicity of production, RF+CT (1:1), which resulted in 81.25% decolourization of Reactive Brown HER (*Fig. 8a*) and 79.7% decolourization of Navy-Blue HER (*Fig. 8b*), was selected for further processing. The consortia of *Enterobacter hormaechei, Neobacillus niacini,* and *Enterobacter ludwigii* inoculated in RF+CT was dried at 40 ˚C for five days to create a powdered formulation (*Fig. 9*), which was inoculated into 100 ml of a 0.05% solution of Reactive Brown HER and Navy Blue HER dye under shaker conditions, and the percentage dye decolourization was measured. The results confirmed that approximately 80% decolourization of Reactive Brown HER and Navy Blue HER could be achieved through this biological treatment method.

3.2 | Treatment 2: Ozonation

The time required for complete dye decolourization of Reactive Brown HER and Navy-Blue HER was directly

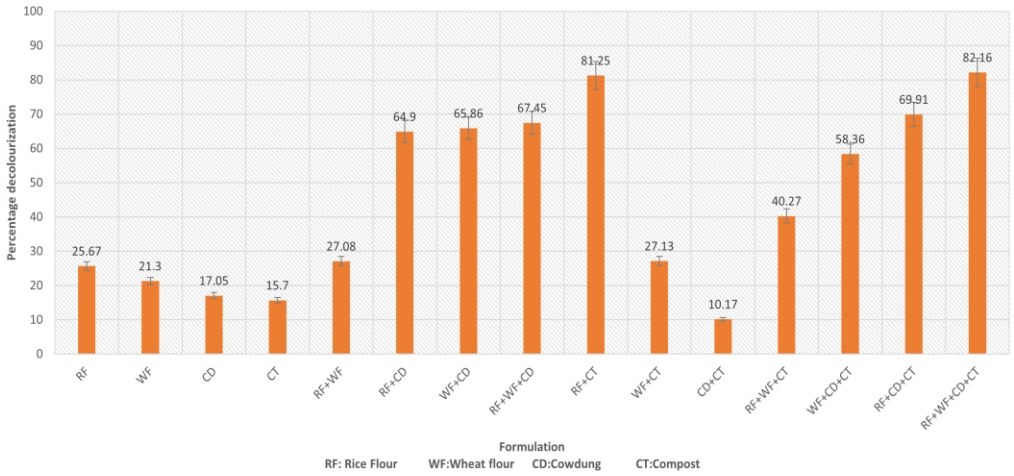


FIGURE 8a Percentage decolourization of Reactive Brown HER using RF, WF, CD, CT and their combinations as substrates for consortia of *Enterobacter hormaechei, Neobacillus niacini* and *Enterobacter ludwigii*

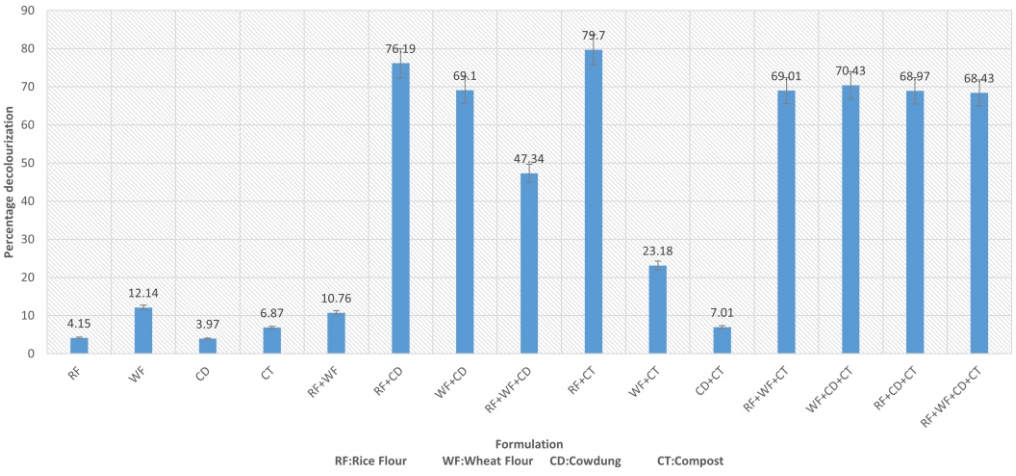


FIGURE 8b Percentage decolourization of Navy-Blue HER using RF, WF, CD, CT and their combinations as substrates for consortia of *Enterobacter hormaechei, Neobacillus niacini* and *Enterobacter ludwigii*

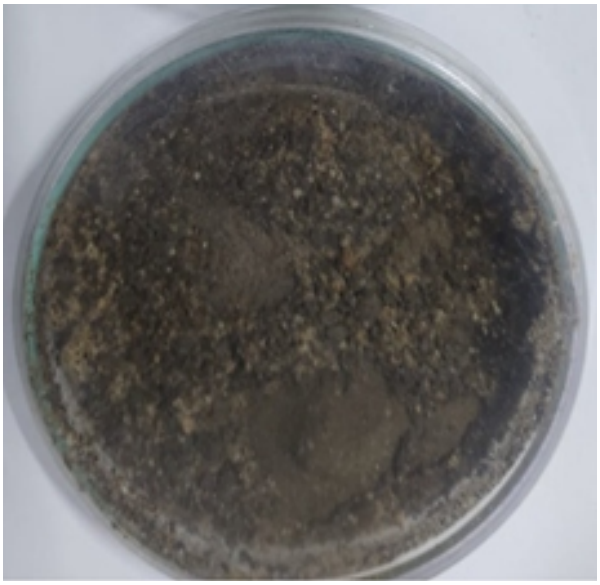


FIGURE 9 Powdered Formulation - Consortia of *Enterobacter hormaechei, Neobacillus niacini* and *Enterobacter ludwigii* in RF+CT

proportional to the initial concentration of respective dyes. Time for complete decolourization of 0.025, 0.05 ,0.1 and 0.2 % Reactive Brown HER was 75, 105, 165 and 225 min respectively (*Fig. 10a*). Time required for complete decolourization of 0.025, 0.05, 0.1 and 0.2 % Navy Blue HER was 30, 45, 105 and 195 minutes respectively (*Fig. 10b*).

3.3 | Treatment 3: Ozonation coupled with microbial formulation

Results from T2 indicate that it takes 105 and 45 minutes, respectively, to achieve complete decolorization of 0.05% Reactive Brown HER and Navy Blue HER dye (*Fig. 10a* and *10b*). However, in T3, where T1 preceded T2, only 15 and 25 minutes of ozonation were needed for complete decolorization of Reactive Brown HER and Navy Blue HER dye, respectively (*Fig. 11a* and *11b*). A significant reduction in the time required for dye decolorization was observed using ozonation in T3. A graphical representation of T3 with respect to percentage decolourization v/s time is given in *Fig. 12*.

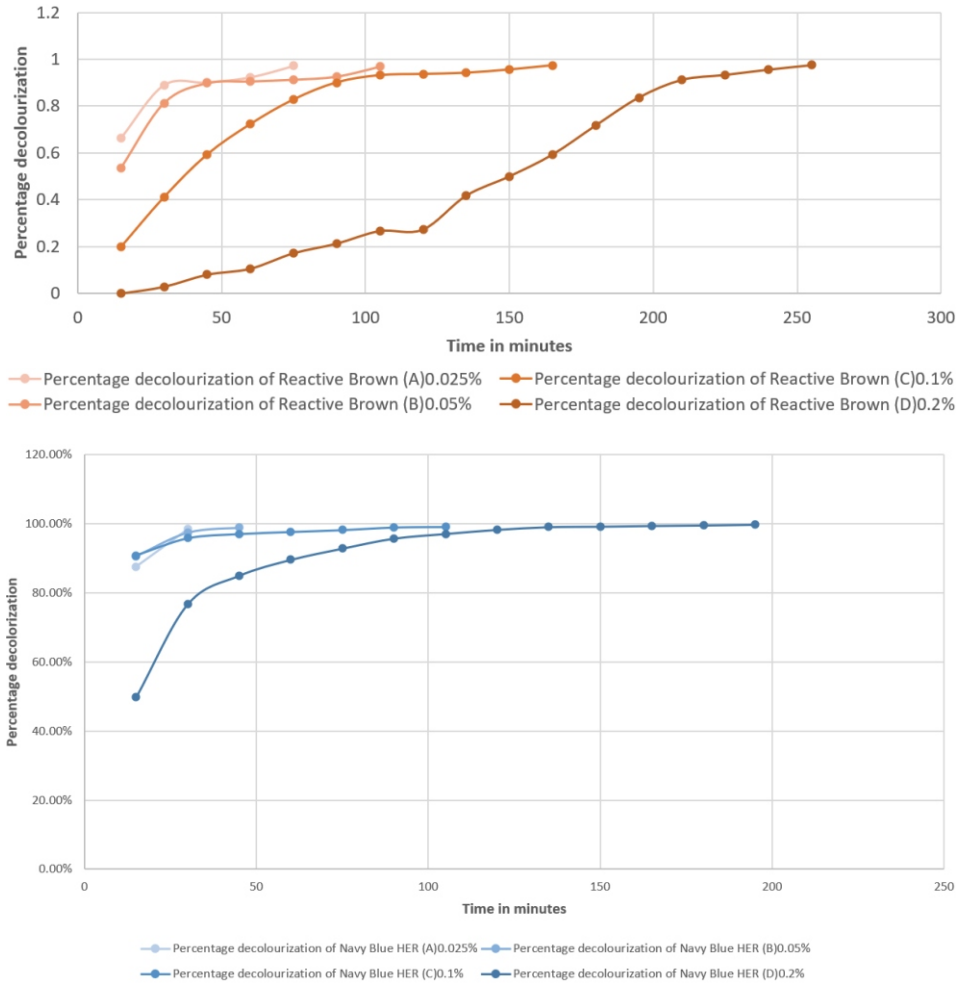
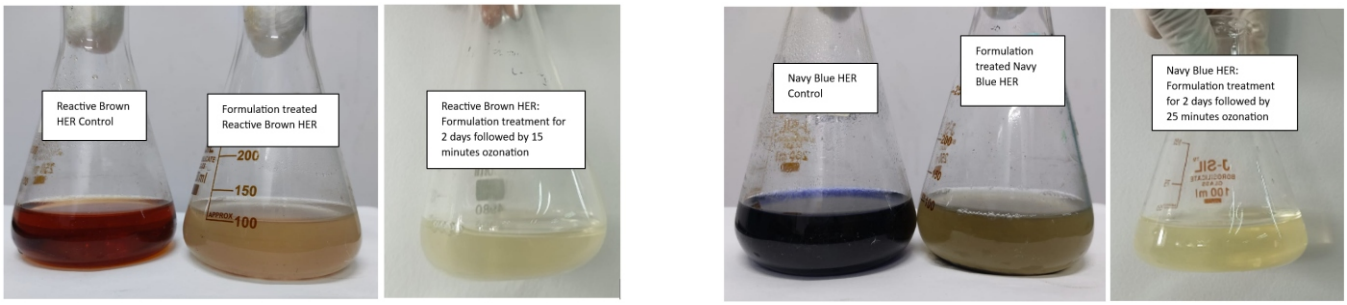
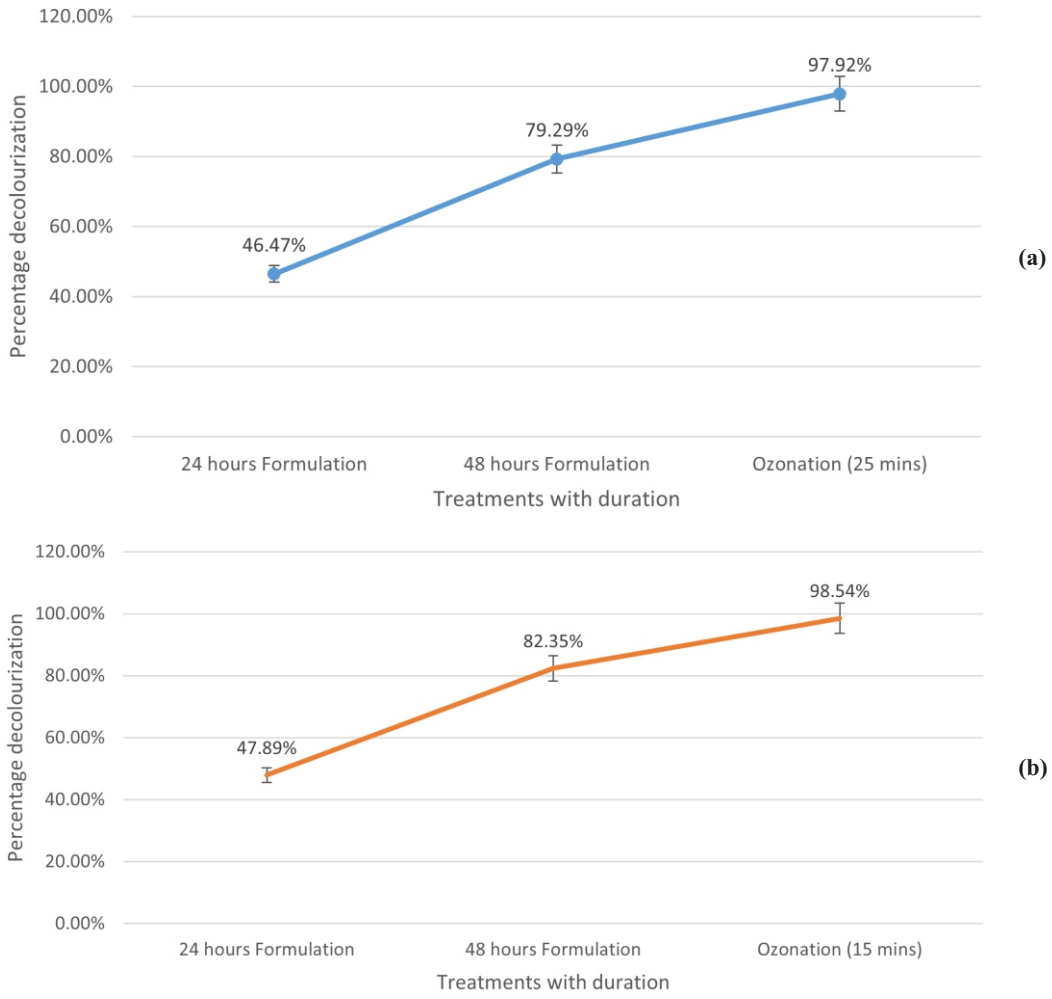


FIGURE 10 Graph indicating time required for complete decolourization at different dye concentrations - 0.025, 0.05, 0.1 and 0.2% of (a) Reactive Brown HER and (b) Navy Blue HER



(a) **(b)**

FIGURE 11 Results of T3. From left to right - Control (0.05% dye solution), formulation treated sample, 2 days formulation treatment followed by ozonation for (a) Reactive Brown HER and (b) Navy Blue HER



(a)

(b)

FIGURE 12 Graph of percentage decolourization vs time for (a) Reactive Brown HER and (b) Navy Blue HER

4 | CONCLUSIONS

The analysis of microorganisms in textile wastewater showed various organisms can survive, flourish, and decolorize harmful dyes. Research indicated *Enterobacter hormaechei, Neobacillus niacini,* and *Enterobacter ludwigii* displayed significant dye decolorization capabilities. Combined with rice flour and compost as substrates, they achieved approximately 82.35% and 79.29% decolorization of Reactive Brown HER and Navy Blue HER in two days. Ozonation resulted in 97% and 98% decolorization of 0.05% Reactive Brown HER and Navy Blue HER in 105 and 45 min. In treatment T3, dye solutions required less ozonation time after two days of microbial application, suggesting T3 reduces both

cost and duration of dye treatment. However, *Enterobacter hormaechei* and *Enterobacter ludwigii,* commonly found in soil, water, and humans, may pose risks of nosocomial infections (Davin-Regli *et al.,* 2019). Ozonation after microbial treatment can help mitigate this. Ozone disrupts cell membranes and oxidizes vital cellular components, effectively killing bacterial cells (Giuliani *et al.,* 2018). GCMS analysis can confirm the breakdown of toxic dye molecules into non-toxic substances. The effectiveness of T3 may also be assessed for remediating other reactive dyes and wastewater samples. After evaluations, treated samples might be reused for washing or cleaning purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting our findings are available within the article.

AUTHOR'S CONTRIBUTION

AJ - Designed and performed experiments, analyzed data, wrote the manuscript. TM - Supervised the project, analyzed data and made corrections in manuscript.

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