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Original Article

Eco-Friendly Bioremediation of Textile Azo Dyes Using Microbial Consortia with Phytotoxicity Assessment

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Abstract

Textile dyeing effluents and in particular, azo dyes are regarded to be among the most recalcitrant environmental pollutants, due to their extremely complex aromatic nature and/or lack of biodegradability. The effluent sample have been taken and identified four bacterial isolates including *Bacillus* sp., *Staphylococcus* sp., *Vibrio cholerae* and *Vibrio parahaemolyticus* characterised morphologically and biochemically. The most effective bacteria in dye removal were Staphylococcus sp. (89%), against direct dyes contaminated wastewater, and *Bacillus* sp. (74%) against azo dye-contaminated wastewater among them. The optimization studies revealed the best carbon source to be lactose and sodium nitrate being the best nitrogen source and that the one-factor-at-a-time optimization study revealed the optimum pH to be 7.5 at 35oC -40oC. FTIR analysis also indicated that certain changes must have occurred in structure of the dye molecules after biodegradation giving indication that detoxification has occurred successfully. The results of the phytotoxicity performed on seven different crop species showed that effluents were subjected to treatment with a bacterial consortium had no significant effects on seed germination or root-shoot growth compared to a control as compared to a marked inhibitory effect of untreated effluents on their seed germination and root-shoot development. This study significantly affirms the elimination of textile dye effluents through an intensive multi-crop phytotoxicity screening, demonstrating restoration of seed germination and the effective growth after bacterial consortium treatment.

Keywords: *Azo dye, Bioremediation, Decolorization, FTIR, Microbial consortia, Phytotoxicity*

Introduction

The textile manufacturing in India with its cultural heritage may be considered one of the most important fields, when it comes to the economic development of this country. It is billed to be the second-largest job-market killer besides agricultural sector, which consumed an estimated 11 percent of the total national exports. With the other reasons that came along with many others natural dyes like that, they had the shelf life, faded very quickly after sun was applied on or even after being washed and the fact that the supply of these dyes was limited in terms of number of hues and colors, more and more people are turning synthetic color (Sigamani *et al*., 2024). These limitations have driven the manufactures to utilize the synthetic fiber, which is stronger, colorfast, and several colors in it provided (Varshan *et al*., 2024). Dyeing is another significant process in production of textile material and involves dyeing of hides the effluent water products of which are in form of large quantity of wastewater (Senthilvelan, 2014). This wastewater holds huge amounts-the dyes, which are

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toxicants and can be harmful to the human health that are already identified to be mutagenic, cytotoxic and carcinogenic (Goralczyk-Binawkowska, 2021). Most likely, these dyes are released in the territory near them equipped with paddies, lakes and rivers and sometimes directly into local landfills. The resulting pollution of the water leads to destabilization of the natural equilibrium in the environment as well as enhancing the chemicals oxygen demand (COD) and biological oxygen demand (BOD) of water. They both alter the pH of water as well as the overall organic and inorganic chemical composition of it (Shankarling *et al*., 2017). This might affect fish, invertebrates, and living organisms negatively because of the toxicity of synthetic colors to aquatic life. They are also able to alter the water chemistry, disrupt nutrient cycling, and persist in the environment long-term, all of which continues to be risks to aquatic ecosystems (Pinheiro *et al*., 2022).

Azo dyes in Textile Industry

Textile, printing, leather, cosmetics, food products, and coloring are some of the industrial processes that use up to 60 and 70 percent of all the synthetic dyes (El-Sayed *et al*., 2024; Solis *et al*., 2012). The dyes have a very high chemical and biological stability, and this is brought about by the presence of one or more azo linkages (-N=N-) in these dyes. The typical backbone of both prepared and naturally occurring azo dyes could be way R -N = N -R' with R and R' aryl or alkyl groups (Thiruppathi *et al*., 2021). This structural stability rendered azo dyes highly worthy against conventional degradation process. However, they often fail to be biodegraded unless the microorganisms have access to bioavailable carbon sources since efficient decolorization is mostly enabled by the coincidence of this combination with the supply of additional nutrients to enter the microbial metabolism (Benkhaya, El Harfi and El Harfi, 2017). Various physical and chemical processes have been involved in removing azo dyes in textile wastewater and these processes include application of coagulation, flocculation, ion exchange, activated carbon adsorption, ozonation, photocatalysis, reverse osmosis and filtration. Some of these chemical reactions could involve forming dangerous compounds in the intermediate (Afrin *et al*., 2021). It is determined that aerobic and anaerobic azo dyes degradation are associated with the generation of toxic aromatic amines including benzidine, p-phenylenediamine, aniline, or toluene which produce carcinogenic and mutagenic effects (Chung, 2016).

Bioremediation of Azo dyes

Among the most recent developments that has captured great concern in regard to dye decolorization are the microbes mainly due to its effectiveness and furthermore due to the fact that it is both ecologically friendly as one of the sustainable methods of removing dyes. Microorganisms utilize genetic and biochemical mechanisms to remediate polluted environments (Singh *et al*., 2024). Xenobiotics degrading microorganisms systems of metabolism. Because of the secretion of specific enzymes (laccase, azoreductase, peroxidase, hydrogenase) that help to destroy the azo bonds, microorganisms are able to break down the dye azo dyes completely (Sharma *et al*., 2024). Microbial strains have gained a lot of interest in degrading dye-containing synthetic wastewater as they have a quick growth period, biomass production and have the ability to degrade the dye. The mixed microbial cultures have increased degradation potential as compared to the single strains of bacteria (Bhatia *et al*., 2017). Conversely, mixed cultures exhibited enhanced activities of detoxification and decolorization and enhanced sensitivity to temperature variations, dye load, and pH.

The present study is focused on the decolorization of liquid untreated effluent from various textile dying units, in Tirupur region (Tamil Nadu, India) and a plant study was conducted using the treated effluent sample. The textile industry in Tirupur, Tamil Nadu, encounters major challenges in effectively managing effluent discharge, especially in terms of decolorization and pollutant removal (Table 1). This study offers a comprehensive evaluation of the decolorization efficiency of four bacterial strains applied to untreated textile effluent. Additionally, it presents findings from phytotoxicity assays using the treated effluent to assess its effects on seed germination, plant growth, and overall development.

Material and Methods

Soil samples, which were collected in the textile industrial units, were processed by the standard serial dilution techniques. Dilutions ranging from 10^{-2} to 10^{-7} were plated in duplicate on Nutrient Agar medium and incubated at 37°C for 24 hours. The plates were incubated, and then individual colonies of bacteria were isolated.

Sample Collection

Untreated wastewater samples of the textile-dying units of Tirupur Tamil Nadu, India were collected in sterile containers, maintained at 4°C, and then processed for examination. The study was carried out in the month of December to June, 2017.

Isolation of Dye Degrading Bacteria from textile dye effluent

Soil samples collected from waste disposal areas surrounding textile industries were used to isolate bacteria capable of degrading dyes. Standard microbiological techniques were used to serially dilute 1 mL of effluent-contaminated soil suspension, covering dilutions of 10^{-2} to 10^{-7}. Each dilution was plated on Nutrient Agar using an aliquot of 100 µL using a technique called the spread plate method (Taylor *et al*., 1983). The inoculated plates were left to grow at 37°C overnight promoting the growth of the bacteria. The isolated bacterial colonies that were obtained after incubation were identified. The pure colonies were picked, and maintained on Nutrient agar slants at 4°C.

Identification of Dye-Degrading Bacteria

Standard bacteriological methods, i.e. microscopic examination, staining, motility and biochemical tests were used to describe colony morphology (as in color, shape and size) of the three bacterial isolates. Identification of the isolates was done in accordance to standard procedure of Bergey Manual (1994).

Pre-enrichment condition for bacterial cultivation

Each isolated bacterial strain was individually cultured in 250 mL Erlenmeyer flasks containing 100 mL of nutrient broth. PH of the medium was brought to 7.0 with 0.1 M HCl or NaOH. The medium was autoclaved at 121°C for 20 minutes after which 100 µL of 24-hour-old actively growing bacterial cultures were added separately. The cultures were then used as inocula to carry out further degradation experiments after an enrichment period of 24 h at 30oC ± 0.2°C (Sakpal and Tarfe, 2021).

Dye Decolorization test

Decolorization potential of the isolated bacterial strains was evaluated in 100mL of nutrient broth. All the flasks were inoculated with 1-loop of 24-hour bacterial culture, left under 37°C, and incubated after 48 hours (Afrin *et al*., 2021). After incubation, 10 mL of filtered textile dye effluent was added to each culture flask, which was then placed on a rotary shaker at 37°C for an additional 48 hours. Samples of 10 mL were taken after 24-hour intervals and filtered, and centrifuged at 5000 to 7000 rpm for 20-30 mins. The absorbance of the supernatant was measured by a UV-Visible spectrophotometer in order to determine the extent of decolorization (Ansari *et al*., 2025). The following procedure was used to treat three different textile dye effluent samples, which were collected separately, represented as Sample A, Sample B and Sample C.

Evaluation of bacterial dye decolorization

Using a spectrophotometer, the optical density (OD) was determined at wavelengths specific to effluents, which ranged from 420 to 460 nm, in accordance with the methodology outlined by Ullah Khan *et al* (2023). After that, the percentage of decolorization was computed using the formula below:

$$\%\ \text{Decolorization} = (\text{Initial OD}-\text{Final OD})/(\text{Initial OD}) \times 100$$

Biodegradation of textile effluent by bacterial consortium

Textile dye wastewater was biodegraded by bacteria in 100 mL Erlenmeyer flasks that held 80 mL of dye effluent. Three samples of such effluent of textile dyes (A, B and C) were treated separately. Sample C produced the greatest percentage of decolorization and hence used in carrying out the successive optimization work. The samples to be analyzed were also removed every 24 hours. The 10-milliliter sample volume of the wastewater was filtrated and centrifuged at the speed of 5000-7000 rpm during 20 to 30 minutes. The absorbance of the clear supernatant was also taken in a spectrophotometer at certain wavelength range of 420-460 nm depending on the dye to determine the efficiency of degradation.

Impact of different physicochemical factors on Azo Dye Degradation

The influence of pH on Dye Degradation

The study used sterile nutritional broth containing 20 ppm of dye to determine the dye degrading effect of pH. The pH of broth was adjusted to 6.0, 6.5, 7.0, 7.5, and 8.0 using 1 N NaOH or H2 SO4. Bacterial cultures both individual strains and consortia were added to each pH-adjusted sample and incubated at 37°C to determine the degradation. The cultures were incubated and centrifuged at 5000 rpm for 20 minutes. Based on the aforementioned methodology, the percentage of the dye degradation was estimated. Comparison was done with Abiotic controls implying that no bacterial inoculation in the broth.

Effect of temperature with Dye Degradation

One of the aspects that microbial consortia influence in the degradation process is that of temperature. The 10% (v/v) of each strain were inoculated on the cultures and allowed to incubate in a sterile nutrient broth (pH 7.5) and filter-sterilized dye added to a final concentration of 100mg/L was added after autoclaving. After 24 hours, the cultures were incubated under various temperatures (20ºC, 25ºC, 30ºC, 35ºC, and 40ºC). Abiotic controls were kept under these same conditions. As earlier described, a percentage on the decolorization of the dye was determined.

Effect of Carbon and Nitrogen Source on Dye Decolorization

According to Saratale *et al*. (2011), a semi-synthetic medium was supplemented with different carbon (lactose, mannitol, maltose, dextrose, and sucrose) and nitrogen (protease peptone, ammonium sulfate, ammonium chloride, sodium nitrate, and potassium nitrate) sources at 1% concentrations in order to assess the impact of these sources on the decolorization of Azo dye. After sterilizing each medium, 10% (v/v) bacterial inoculum was added, and filter-sterilized dye was added at a concentration of 100 mg/L. Additionally, abiotic controls devoid of microbial inoculation were added. As explained earlier, the percentage of dye decolorization was calculated.

Effect of agricultural supplements on dye degradation:

Agricultural wastes such rice husk, rice straw, and wood shavings (1 g each) were combined with 100 mL of distilled water and autoclaved at 121°C for 20 minutes in order to increase the process's economic viability. Ten milliliters of each extract were then added to the semi-synthetic medium in order to evaluate their impact on consortium on Azo dye degradation

FTIR Analysis of Spectroscopy

Following decolorization, the supernatant was collected for analysis after the bacterial biomass was eliminated by centrifugation at 1000 rpm for 15 minutes. By contrasting the FTIR spectra of the decolorized samples and the untreated (control) dye, dye degradation was evaluated. The organic layer was dried over anhydrous sodium sulfate to remove any remaining moisture after the supernatant was extracted using an equivalent volume of ethyl acetate. To get the dried metabolites, the solvent was then evaporated for an hour at 50°C in a hot air oven. For spectrum analysis, these were added in the sample holder after being finely pulverized in a 1:300 ratio with spectroscopic-grade potassium bromide (KBr). To assess structural alterations of the dye, FTIR spectra were developed in the mid-infrared range (400–4000 cm^{-1}). Changes in distinctive absorption bands were observed (Pokharia and Ahluwalia, 2016).

Physiochemical analysis of the wastewater from textiles

Textile effluent's pollutant load and environmental impact were estimated using a thorough physicochemical examination. Total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demands (COD), hardness, turbidity, colour, and turbidity were among the physicochemical parameters that were examined in the collected samples. Standard procedures recommended by APHA (1926) were used for the analysis and interpretation of these parameters (Rima *et al.*, 2022).

Phytotoxicity Analysis of Untreated and Bacterial Consortium Treated Textile Dye Effluent

Phytotoxicity tests were conducted to evaluate the toxic effects of untreated and bacterial consortium treated textile dye effluent. The study was carried out at room temperature using seeds of seven commonly cultivated Indian crops. These included Paddy (*Oryza sativa*), Chickpea (*Cicer arietinum*), Mustard (*Brassica nigra*), Fenugreek (*Trigonella foenum-graecum*), Green gram (*Vigna radiata*), Lima bean (*Phaseolus lunatus*), and Horse gram (*Macrotyloma uniflorum*). For each type of seed, ten seeds were treated daily with 10 ml of either untreated or treated textile dye effluent. A control group was maintained by using water instead of effluent. After seven days, the percentage of seed germination, along with the lengths of the plumule and radicle, were measured and recorded (Karthikeyan and Kanchana, 2014).

Results

Physiochemical characteristics of the sample of the textile dye effluent

The table 1 and 2 shows initial estimated values of parameters in the pooled untreated sample of effluent released by tannery and the limit beyond which safe release of effluent is reasonable into the natural water bodies. The physico-chemical properties of each bacterial strain and each of the four microbial consortia were recorded after samples were taken every 24 hours

***Table 1 :** Physiochemical Characteristics of Textile dye effluent*

Parameters	Standards for pollution control	Raw Effluent Initial values
Hardness (mg/L)	Unobjectionable	452.75
COD (mg/L)	400-450 mg/L	2120.23
TS (g/L)	50 mg/L	82.01
TSS (g/L)	3500 mg/L	351.52
TDS	3500 mg/L	325.12
pH	5-9	10.2
Color	Nil	Slightly brown
Turbidity	Unobjectionable	1.87

(COD: Chemical Oxygen Demand; TS:Total Solids; TSS:Total Suspended Solids;TDS:Total Dissolved Solids)

COD was reduced the most by the consortia (85%), *Bacillus* sp. (70%), *Vibrio cholerae* (61%), *Staphylococcus* sp. (80%), and *Vibrio parahaemolyticus* (50 %). The microbial consortia also reduced TDS by the highest percentage (82%) with *Staphylococcus* sp. (72%), *Bacillus* sp. (65%), *Vibrio cholerae* (60%), and *Vibrio parahaemolyticus* (52%). Based on these results the mixtures of microbes were more effective than the individual strains when it comes to reducing TDS. Furthermore, a considerable drop in suspended particulates was suggested by the consortium treatment's about 80% reduction in turbidity.

Table 2: *Physiochemical Characteristics of Textile dye effluent by microbial strain*

Parameters	**Initial Values**	***Bacillus* sp.**	***Staphylococcus* sp.**	***Vibrio cholerae***	***Vibrio parahaemolyticus***	**Consortial strain**
Hardness (mg/L)	452.75	178.12	250.75	375.23	425.98	112.98
COD (mg/L)	2120.2	1123.87	812.11	1435.12	1652.98	720.09
TS (g/L)	82.01	62.23	57.19	70.87	77.12	50.12
TSS (g/L)	351.52	204.12	157.12	341.06	349.98	108.12
TDS	325.12	259.89	168.21	317.85	285.08	118.98
pH	10.2	9.8	8.5	9.9	10.1	8.2
Color	Slightly brown	Colorless	Colorless	Light brown	Light brown	Colorless
Turbidity	1.87	0.67	0.4	0.57	0.97	0.31

(COD: Chemical Oxygen Demand; TS:Total Solids; TSS:Total Suspended Solids;TDS:Total Dissolved Solids)

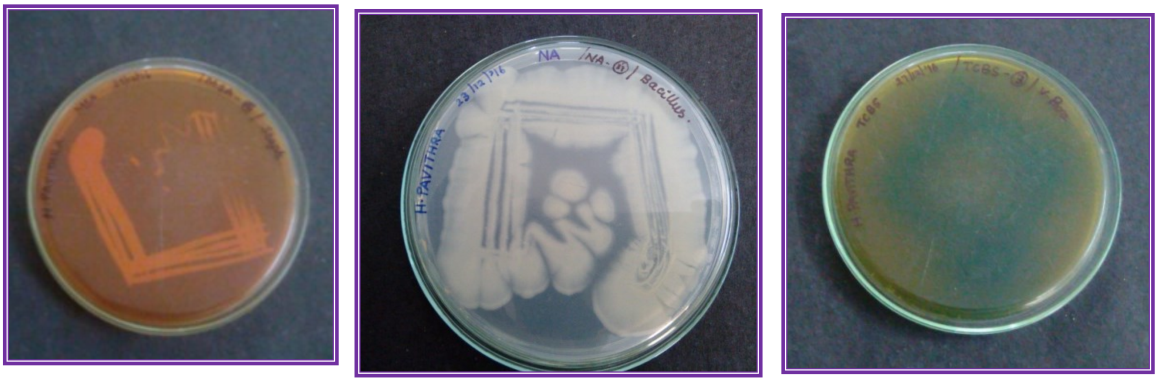
Isolation and Screening of Textile Effluent-Adapted Indigenous Microbial Consortia

Soil samples contaminated with textile dyes effluent were screened for bacterial strains capable of degrading azo dyes. Four morphologically distinct colonies were isolated on nutrient agar, each demonstrating the ability to decolorize azo dyes present in textile effluent (Paul *et al.*, 2020).

Table 3: *Morphological Characteristics*

ORGANISM	**GRAM STAINING**	**MOTILITY**	**CATALASE TEST**	**OXIDASE TEST**
Bacillus species	Gram positive Rods	Actively motile	Positive	Positive
Staphylococcus species	Gram positive Cocci arranged in clusters	Non – motile	Positive	Positive
Vibrio cholera	Gram negative Rods, comma shaped	Actively motile	Positive	Positive
Vibrio parahaemolyticus	Gram negative Rods, comma shaped	Actively motile	Positive	Positive

Table 3 reveals that more contaminants were reduced overall in the microbial consortia than in individual strains at the point of the end of the incubation period. Based on morphological (Table 3), cultural (Table 4), and biochemical characteristics (Table 5), the isolates were identified as *Bacillus* spp., *Staphylococcus* spp., *Vibrio cholerae*, and *Vibrio parahaemolyticus* shown in Figure 1. Among them, two were Gram-positive and two were Gram-negative bacteria.



Staphylococcus* spp** ***Bacillus* sp** ***Vibrio cholerae

Figure 1 : *Identification of bacterial strain from effluent soil sample*

Table 4: *Cultural Examination*

Organism	Nutrient Agar	Mac Conkey Agar	Selective Media	
			Media	**Colonies**
Bacillus species	Irregular, large, raised dull, opaque and grayish white colonies	Lactose fermenting colonies	-----	-----
Staphylococcus species	Large, circular, convex, smooth, shiny and opaque colonies	Lactose fermenting colonies	Mannitol Salt Agar (MSA)	Yellow colored colonies
Vibrio cholera	Moist, translucent, round disc with bluish tinge in transmitting light	Lactose fermenting colonies	Thiosulphate Citrate Bile Salt agar (TCBS)	Yellow colored flat colonies, become green on continued incubation
Vibrio parahaemolyticus	Moist, translucent, round disc with bluish tinge in transmitting light	Non-lactose fermenting colonies	TCBS	Green colored, opaque, flat colonies

Table 5: *Biochemical test*

ORGANISM	I	MR	VP	C	U	TSI
Bacillus species	-----	-----	-----	-----	-----	——————
Staphylococcus species	-	+	+	+	+	A/A, Gas-,H_2S-
Vibrio cholera	+	+	+	+	-	A/A, Gas-,H_2S-
Vibrio parahaemolyticus	+	+	-	+	-	A/A, Gas-,H_2S-

(I- Indole test; MR- Methyl red test; VP- Voges praskauer test; C- Citrate utilisation test;U- Urease test; TSI-Triple sugar iron agar)

Dye decolorization experiment

Using three distinct effluent types, the ability of four bacterial isolates—*Bacillus species*, *Staphylococcus species, Vibrio cholerae,* and *Vibrio parahaemolyticus* to decolorize textile effluent containing azo dyes was studied both separately and in combination. The decolorization efficiency was measured and documented using *Bacillus sp*, *Staphylococcus sp* and *Vibrio sp*, and shown in the relevant figure as well as in Figure 2. The four bacterial strains and its consortia showed different degradation potentials which was illustrated in Figure 3, 4 and 5.

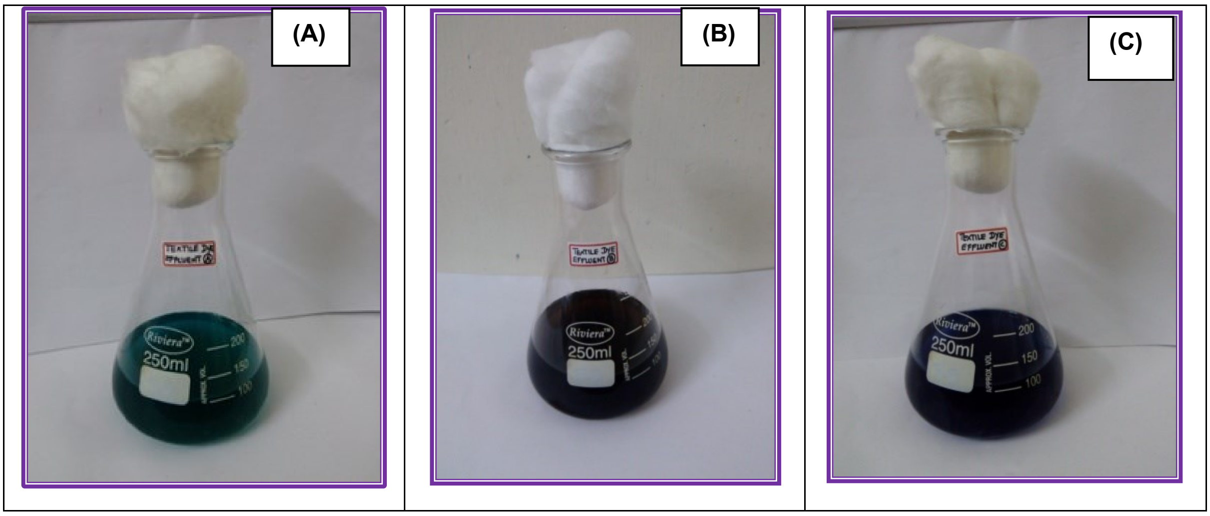


Figure 2: *Dye decolorization experiment using (A) Staphylococcus sp, (B) Bacillus sp, & Vibrio sp(C)*

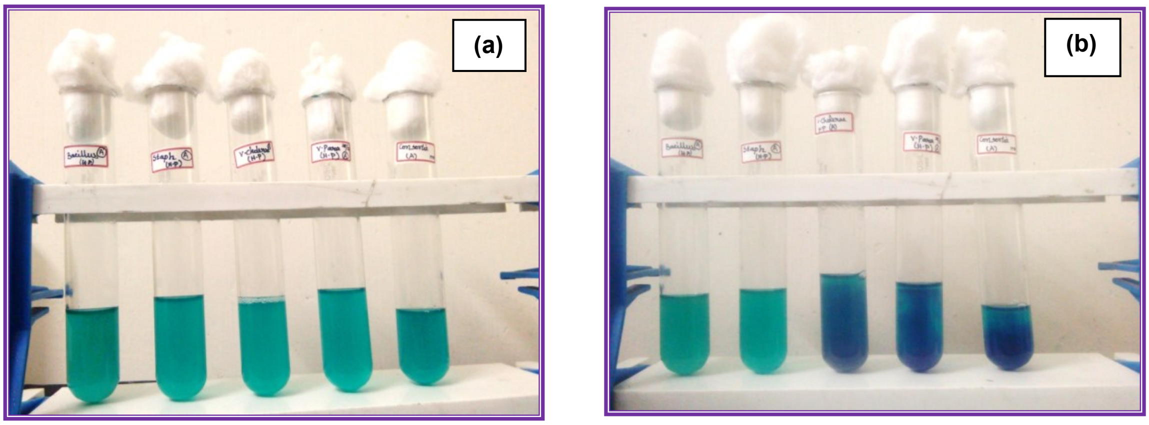


Figure 3: *Sample A (a) Before Dye decolorization (b) After dye decolorization*

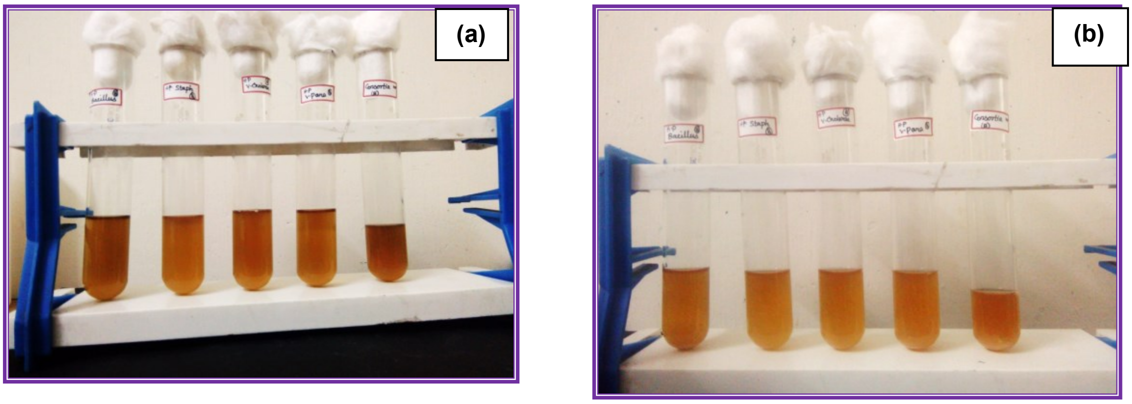


Figure 4: *Sample B (a) Before Dye decolorization (b) After dye decolorization*

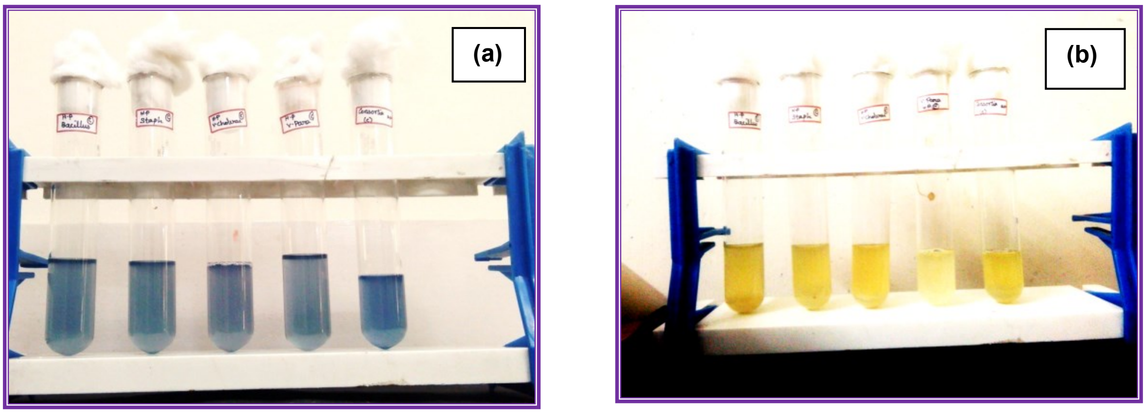
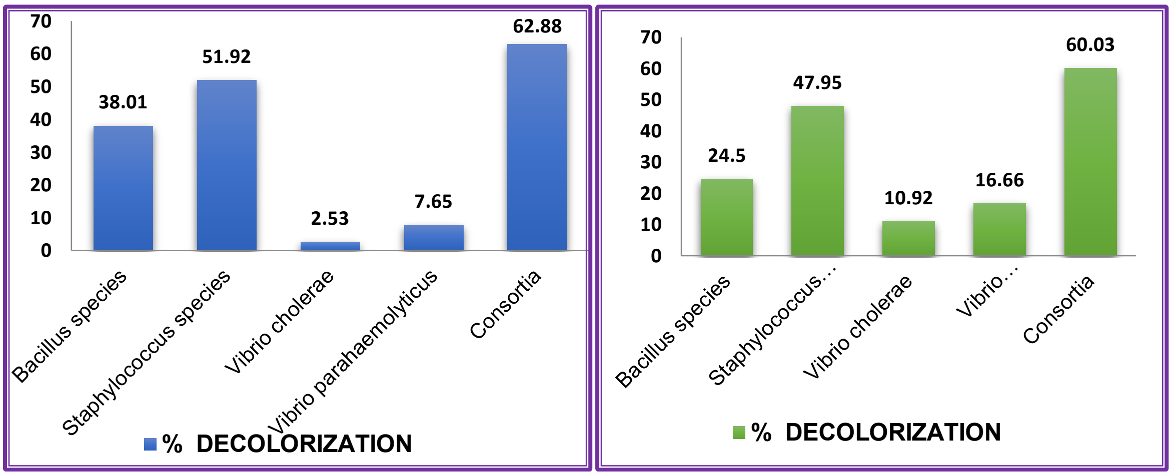
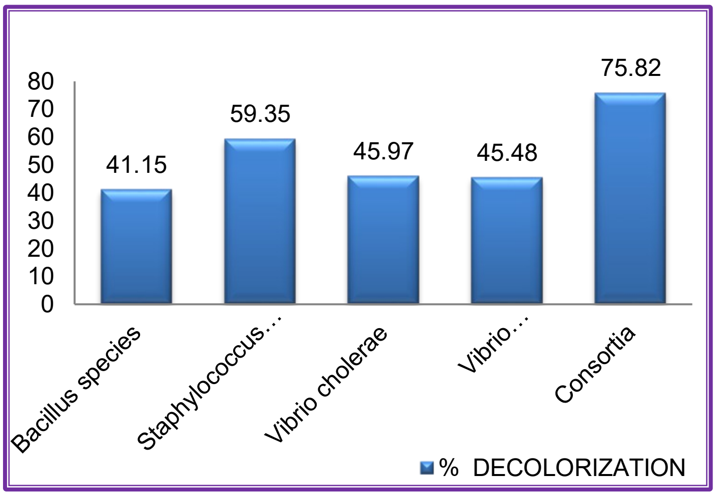


Figure 5: *Sample B (a) Before Dye decolorization (b) After dye decolorization*



Sample -A Sample-B



Sample - C

Figure 6: *Dye decolorization experiment for sample A, B and C using single and microbial consortia*

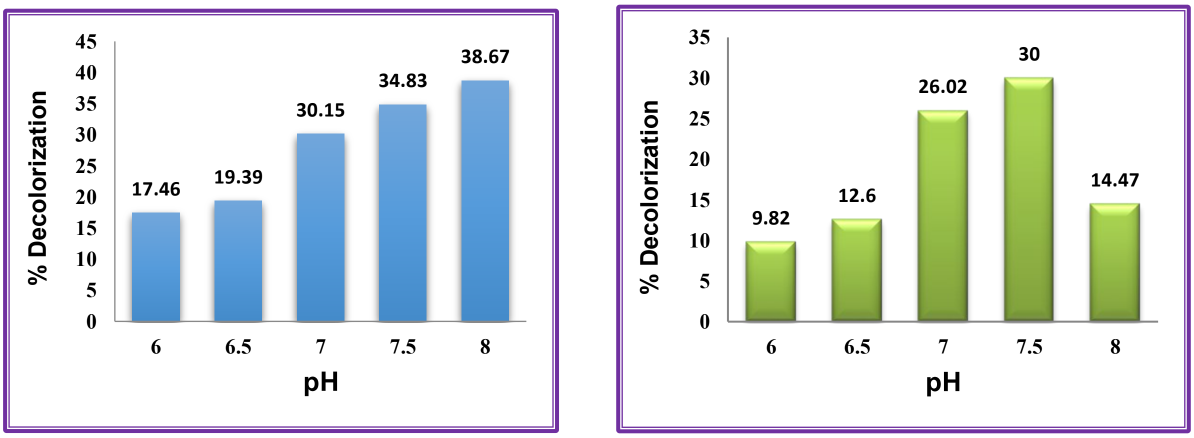
Based on the results, the microbial consortium exhibited the highest decolorization rate (75.82%) for Sample C, compared to Samples A and B. Among the three, Sample C showed the greatest dye removal efficiency. This suggests a synergistic interaction among the microbial strains in the consortium, significantly enhancing the decolorization process (Garg and Tirupathi, 2017). The order of dye degradation efficiency is as follows:

Staphylococcus sp. > *Bacillus* sp. > *Vibrio cholera* > *Vibrio parahaemolyticus*

Among the four isolates, *Staphylococcus species* showed maximum decolorization percentage (76%), followed by *Bacillus* (65%). Hence, it was confirmed that comparatively *Staphylococcus* sp. (Ajaz *et al*., 2024) had high decolorization effectiveness, outperforming both *Bacillus* and *Vibrio* strains was shown in Figure 6.

Effect of pH on dye decolorization

The most important environment variable is pH, since it regulates the growth of microorganisms as well as intracellular enzymes in the degradation of dyes. The influence of pH on the decolorization of azo dyes by *Staphylococcus* spp. and *Bacillus* spp. has been studied in the proposed research in a set of pH values (6.0-8.0) within which the most appropriate pH could be established with the help of bioremediation shown in Figure 7**.** The *Staphylococcus* spp. performed the best with a decolorization efficiency of 38% at pH 8.0.



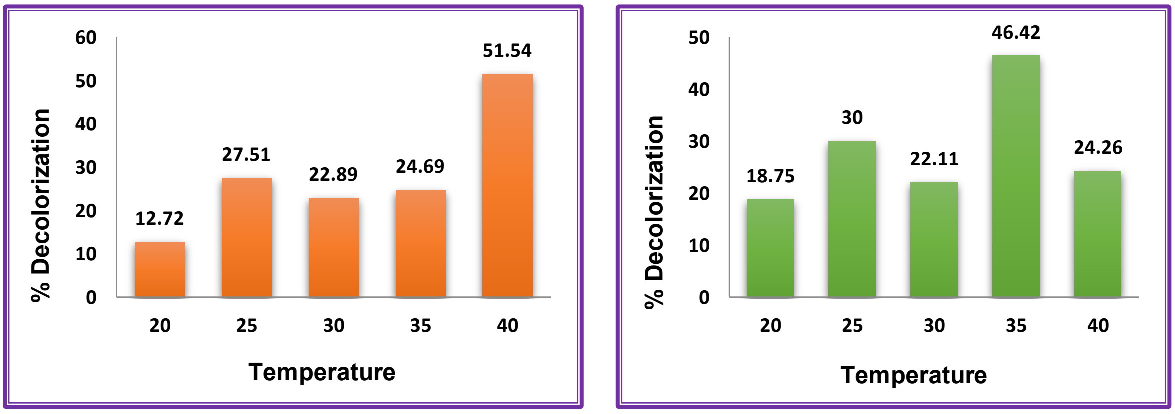
(a) (b)

Figure 7: *Effect of pH on dye decolorization by (a) Staphylococcus sp. (b) Bacillus sp.*

Bacillus spp. on the contrary, was found to have maximum decolorization (30%) at pH 7.5 indicating a medium to slightly alkaline environment (Göktaş, 2024). Decreased decolorization at lower acidic or greater alkaline pH values of both strains probably indicates low efficiency of enzyme and diminished bacterial growth. These findings underline the finding that pH optimization is strain-specific process and is vital to optimizing azo dye biodegradation.

Effect of temperature on Dye Decolorization

The important parameter affecting microbial activity, enzyme activity and in general leads to the efficiency of biodegradation is temperature. The present study has assessed the impact of different incubation temperatures (20°C - 40°C) on azo dye decolorization by *Staphylococcus* sp. and *Bacillus* sp. as shown in Figure 8*. Staphylococcus* sp. was found to exhibit high decolorization efficiency (51 %) at 35°C and this proves that this temperature is favourable to the enzyme metabolism and activity by the cell in the process of degrading the dye. The maximum decolorization rate of *Bacillus* sp., (46%) at 40°C implying greater thermal tolerance and a high capacity of dye degradation at high temperatures.



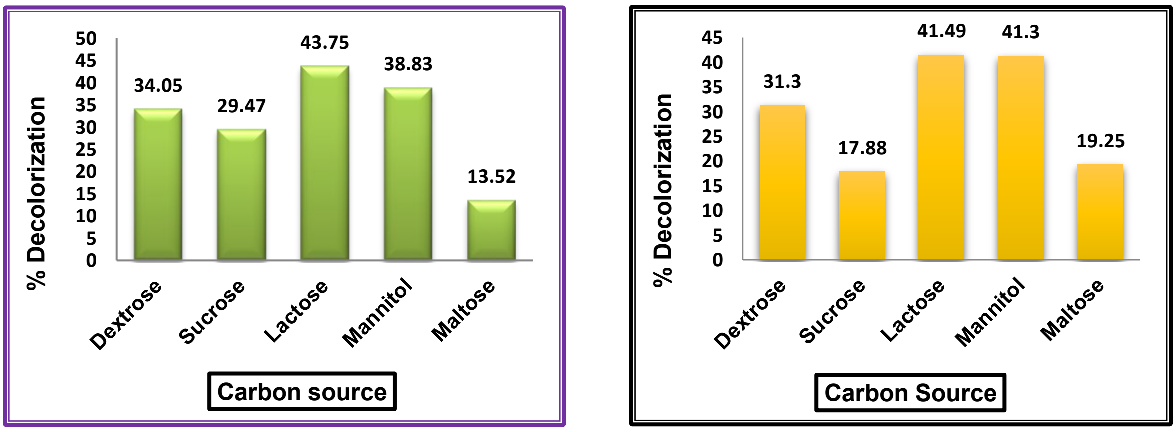
(a) (b)

Figure 8: *Effect of temperature on dye decolorization by (a) Staphylococcus sp. (b) Bacillus sp.*

Effect of Carbon Source on Dye Decolorization

The effect of different carbon sources, including lactose, mannitol, maltose, sucrose, and dextrose, on the decolorization of textile effluent by *Staphylococcus* sp. and *Bacillus* sp. was assessed. The results are presented in the effect of different carbon sources were evaluated on decolorization by bacterial strain Figure 9. The result suggests that the carbon sources serve as effective co-substrates, which supporting bacterial growth and the synthesis of reductive enzymes degrades the azo dye. It was found that the *Staphylococcus species* and *Bacillus* showed maximum decolorization (43%, 41%

respectively) in the presence of lactose, mannitol and moderate activity was shown in presence of dextrose and negligible decolorization in the presence of sucrose and maltose. This results indicates either inadequate use of these sugars or a potential suppression of the enzyme pathways that break down dye. Palanivelan *et al*. (2014), stated that the role of various carbon source in optimizing bioremediation processes. Due to their effective transport and assimilation pathways, lactose and mannitol are preferred by these bacteria, which results in increased microbial activity and dye decolorization



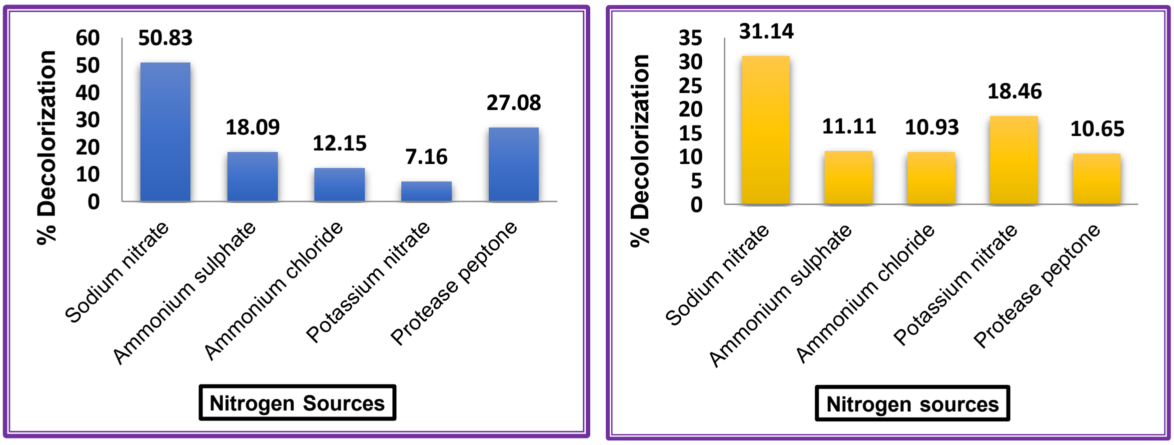
(a) **(b)**

Figure 9: *Effect of carbon source on dye decolorization by (a) Staphylococcus sp. (b) Bacillus sp.*

Effect of Nitrogen Source on Dye Decolorization

The different nitrogen supplies affected the dye decolorization. *Bacillus* sp. and *Staphylococcus* sp. demonstrated the highest rates of breakdown among the four bacterial strains, at 38% and 50%, respectively. The results showed that sodium nitrate was the most efficient nitrogen supply, allowing for a maximum decolorization of 31% by Bacillus sp. and 50% by Staphylococcus sp. This outcome is in line with research by Bandary *et al*. (2016), which showed that the addition of nitrate-based nitrogen sources increased dye removal efficiency in Figure10.

The enhanced decolorization in the presence of sodium nitrate is caused by nitrate, which in turn stimulates nitrate reductases and other oxidative enzymes that aid in the degradation of azo dye. Other nitrogen sources, such as peptone and ammonium salts, showed substantially reduced decolorization effectiveness, suggesting either inadequate breakdown or potential inhibition of dye-degrading enzymes as a result of organic nitrogen complexity or ammonium accumulation.



(a) **(b)**

Figure 10 : *Effect of nitrogen source on dye decolorization by*
(a) Staphylococcus species (b) Bacillus sp.

FTIR Spectroscopy Analysis

FTIR spectroscopy was used to examine the biodegradation and decolorization of textile dye effluent (Figure 11a and 11b). The bacterial consortium's biodegradation of the dye was validated by a noticeable shift in the untreated dye control's FTIR spectrum (Figure 11a) (Prasad, *et al.*, 2013) . Functional groups like C–H (aliphatic), C≡C (alkyne), C–H (aromatic), C=C (alkene), and N–O (aliphatic ether) are represented by the prominent absorbance bands at 3324.8 cm^{-1}, 2107.3 cm^{-1}, 1992.8 cm^{-1}, 1941.2 cm^{-1}, 1637.2 cm^{-1}, 1542.1 cm^{-1}, and 1094.5 cm^{-1} in the untreated dye's FTIR spectrum. Figure 11 b showed the presence of N–H (amine), N=C=S (isothiocyanate), and C–H (aromatic) groups was confirmed by the FTIR spectra of the decolorized effluent, which showed absorbance bands at 3335.3 cm^{-1}, 2111.1 cm^{-1}, 2088.1 cm^{-1}, 1637.1 cm^{-1}, and 1541.8 cm^{-1} and it confirmed the structural changes and dye degradation (Saranraj , 2025).

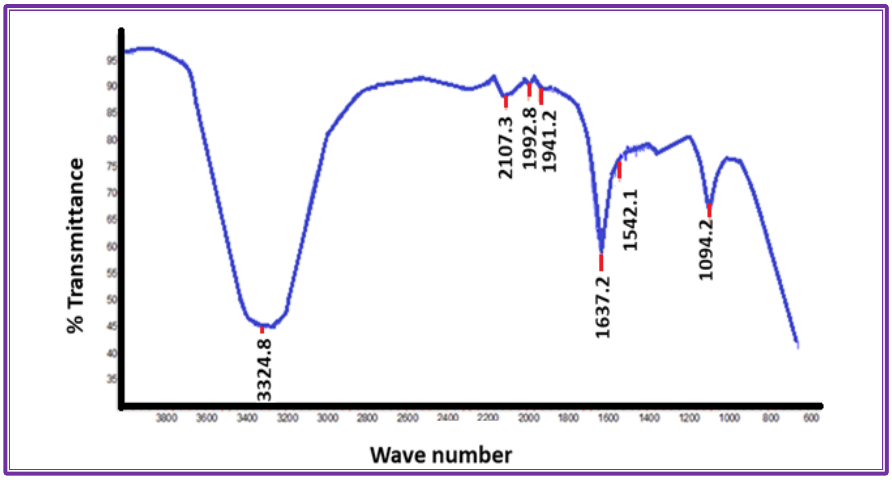


Figure 11a: *FTIR spectrum of control- (a) Untreated dye effluent*

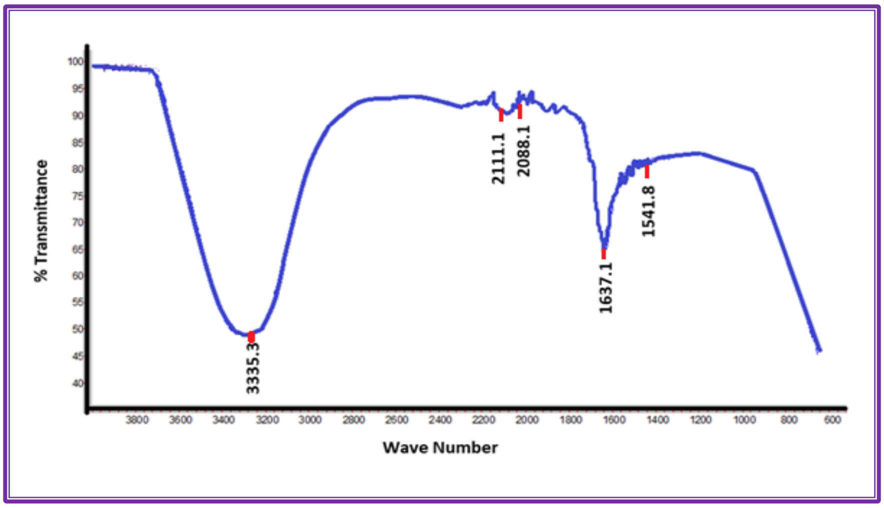


Figure 11b: *FTIR spectrum of control- (b) Treated dye effluent*

Phytotoxicity Analysis

The phytotoxicity bioassay was conducted by evaluating seed germination, shoot (plumule) development, and root (radicle) elongation. Germination percentage, plumule length, and radicle length were recorded for all seven seed types (Table – 6, 7, and 8) (Etminani and Harighi, 2018). The phytotoxic effects of untreated and bacterial consortium-treated textile dye effluent were assessed using seven different agricultural crops: Paddy (*Oryza sativa*), Chickpea (*Cicer arietinum*), Mustard (*Brassica nigra*), Fenugreek (*Trigonella foenum-graecum*), Green gram (*Vigna radiata*), Lima bean (*Phaseolus lunatus*), and Horse gram (*Macrotyloma uniflorum*) (Figure 12).

The findings demonstrated that, in comparison to seeds treated with water and bacterial consortium-treated effluent, seedlings treated with untreated textile dye effluent exhibited significantly shorter plumule and radicle lengths and lower germination percentages as shown in Figure 12. After being treated with bacterial consortium-treated effluent, seedlings treated with water (control) showed the highest germination rates and seedling growth. In terms of radicle length, plumule length, and germination, the untreated textile dye effluent showed the lowest values and the highest degree of phytotoxicity (Shafqat *et al.*, 2017).

Table 6: *Germination % of treated and untreated textile dye effluent against various seeds*

Sl.No.	**Common Name**	**Botanical Name**	**No. Of Seeds Sown**	**Control (%)**	**Untreated Effluent (%)**	**Treated Effluent (%)**
1	Paddy	*Oryza sativa*	10	100	40	70
2	Chick pea	*Cicer arietinum*	10	100	30	80
3	Mustard	*Brassica nigra*	10	90	30	70
4	Fenugreek	*Trigonella foeum-graecum*	10	90	40	70
5	Green gram	*Vigna radiata*	10	10	40	80
6	Lima bean	*Phaseolus lunaetus*	10	90	40	60
7	Horse gram	*Macrotyloma uniflorum*	10	80	30	60

Table 7: *Radical length of treated and untreated textile dye effluent against various seeds*

Sl.No.	**Common Name**	**Botanical Name**	**Length of Radical (Cm)**		
			Control	**Untreated Effluent**	**Treated Effluent**
1	Paddy	*Oryza sativa*	7.3	5.3	7
2	Chick pea	*Cicer arietinum*	5.4	3.1	5.2
3	Mustard	*Brassica nigra*	9.2	2.5	7.2
4	Fenugreek	*Trigonella foeum-graecum*	6.7	4	5
5	Green gram	*Vigna radiata*	8	3.1	6.8
6	Lima bean	*Phaseolus lunaetus*	9	4.8	8
7	Horse gram	*Macrotyloma uniflorum*	8.8	4.4	6.9

Table – 8: *Plumule length of treated and untreated textile dye effluent against various seeds*

S.No	**Common Name**	**Botanical Name**	**Length of Plumule (Cm)**		
			Control	**Untreated Effluent**	**Treated Effluent**
1	Paddy	*Oryza sativa*	10.6	5.7	10
2	Chick pea	*Cicer arietinum*	9	5	8.4
3	Mustard	*Brassica nigra*	10	5.5	8.7
4	Fenugreek	*Trigonella foeum-graecum*	9.6	5.7	9
5	Green gram	*Vigna radiata*	21	6.5	20
6	Lima bean	*Phaseolus lunaetus*	16.5	7	13
7	Horse gram	*Macrotyloma uniflorum*	12	3.5	12

Paddy **Chick pea**

Mustard **Green Gram**

Lima Bean **Horse Gram**

Figure 12: *Phytotoxicity analysis of control, untreated and treated effluent sample*

Discussion

Among the three different bacterial strains, the degradation was effective in *Staphylococcus sp* and *Bacillus sp*. Dye Physicochemical property analysis showed a black hue, turbidity, high biological oxygen demand (BOD), total dissolved solids (TDS), and total solids (TS) of the effluent (Rekha, *et al*., 2025; Azanaw *et al*., 2022). In these metrics, the effluent could not have been released directly to the environment since they were much above the permitted thresholds. Thus, in the present study, it is proposed to treat the wastewater by using a microbial consortium-based cheap method of adsorption (Revathi *et al*., 2023). The pH of the consortia-treated samples was found to have slightly decreased, falling below the acceptable discharge limits (Chockalingam *et al*., 2019). The effluent that had been treated by the microbial consortium had reduced hardness and could hence be reused in other purposes. The respective results show that the microbial consortia performed better than the individual bacterial strains as regards to the improvement of the textile effluent quality. Individual strains demonstrated significant elimination of pollutants, but their efficiency was very modest, suggesting limited metabolic flexibility (Ingale and Thorat, 2024). The effect of pH showed various changes on dye degradation. The generation of organic acid during microbial metabolism may be the cause of the little pH drop below discharge limits. Overall, the results show that using mixed microbial consortia for wastewater bioremediation is a more effective and long-term solution. This increased activity could be attributed to better enzyme stability and membrane permeability at alkaline pH condition, which enables effective intake of dye and degradation (Sun *et al*., 2017). These observations are harmonious with other observations that have shown the presence of alkaline pH as beneficial in maximizing azoreductase activity of Gram-positive bacterium. Effect of temperature correlates with other research findings indicating that improvement on the azoreductase activity and the growth of the microorganisms in mesophilic conditions (Nabi *et al*., 2020). Several strains of the bacteria especially Bacillus, which have been observed to exhibit metabolic activity and enzyme stability at moderately high temperatures. At sub-optimal temperatures, the two strains had a slower metabolism and less enzyme kinetics. Likewise, there can also be high/inappropriate temperatures that caused denaturation or stress-mediated inhibition of enzyme activities in microbes (Armalina *et al*., 2020). FTIR analysis and physicochemical parameter optimization verified the dyes' biodegradation. Improved seed germination from phytotoxicity testing further supported the effluent's detoxification. Notably, the effluent became non-toxic due to the breakdown of dangerous color intermediates such p-phenylenediamine. Slow degradation rates, sensitivity to environmental factors (pH, temperature, hazardous intermediates), and challenges in sustaining stable consortia on a wide scale can all be obstacles to dye degradation employing microbial consortia. The features of such outcomes reveal the effectiveness of the indigenous microbial communities in the remediation of the dye effluents due to efficiency and sustainability and therefore the reuse of textile dyes in the agriculture sector. Future studies could concentrate on the usage of biofilm/immobilized systems, integration with cutting-edge treatment technologies, and genetic and metabolic optimization of consortia (Ayub *et al*., 2025).

Conclusion

Environmental pollution is a major global challenge, with watercourse coloration being a significant problem. Effluents discharged by the textile industry cause serious groundwater and soil pollution, ultimately impacting the livelihoods of vulnerable populations. Biodecolorization is recognized as a highly promising method for decolorizing and degrading these dyes. Bacteria capable of degrading textile effluent azo dyes were isolated from soil samples. The current investigation showed that Bacillus species, Staphylococcus species, Vibrio cholerae, and Vibrio parahaemolyticus are the most effective microbiological isolates from textile effluent soil contaminated with azo colors. Bacterial dye decolorization was assessed using spectroscopic analysis. Bacterial inoculums, both individual strains and a consortium, were introduced into flasks containing three different textile dye effluents (Samples A, B, and C) and incubated at 37°C for two days. Sample C was chosen for additional improvement because it had the highest decolorization performance among the examined samples. Hence, Sample C exhibited the highest decolorization percentage and was therefore selected for further optimization. These results imply that the safe repurposing of treated textile effluents for agricultural irrigation is made possible by the application of bacterial consortia, which offer a cost-effective and environmentally beneficial substitute for traditional wastewater treatment techniques. This study significantly affirms the elimination of textile dye effluents through an intensive multi-crop phytotoxicity screening, demonstrating restoration of seed germination and the effective growth after bacterial consortium treatment. The results support that biologically treated dye effluents are offering several properites like degradation, suitable for agricultural use, promotes the reuse for irrigation purposes.

Conflict of Interest

The authors declare they have no conflict of interest.

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