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Original Research Article

The application and assessment of bacterial isolates for textile dye decolorization

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ABSTRACT

Background: In the present study has been carried out to find the suitable bio degradation measures of textile dye by using biological agents. The wastewater containing dye was obtained from a textile factory situated in Thiruvananthapuram.
Materials and Methods: The study focused on three specific types of dyes: direct black, direct blue, and direct orange. Based on Microscopic, Macroscopic, Biochemical, Hydrolysis tests and selective plating upon comparing with Bergy's Manual (9^{th} edition) the selected organism were identified as, *Micrococcus* sp, *Bacillus* sp and *Pseudomonas* sp. The isolates were then tested for their ability to degrade the dyes.
Result: In this study it was identified that *Micrococcus* sp possessed the higher dye degrading ability. This specific isolate is then carried out for 16SrRNA sequencing analysis and it was confirmed that the highest degraded organism (AZ2) was *Micrococcus lutes* strain JW-22. The toxicity of the textile dyes determined using seed germination.
Conclusion: The isolated bacteria demonstrate the capability to remove color from commonly used textile dyes. In the future, these bacterial strains could serve as effective tools for bioremediation in treating various textile effluents, transforming toxic dyes into harmless, colorless products. The treated effluents could then be recycled for use within the textile industry.

1. Introduction

The global textile industry is expansive, contributing significantly to environmental pollution due to its heavy reliance on synthetic dyes for coloring.[1] Annually, around 30 million tonnes of textiles are produced worldwide, requiring about 700,000 tonnes of various dyes.[2] These dyes, containing chromophore groups, bind to materials to impart color. Among them, azo dyes are particularly notable for their durability and resistance to degradation,[3] finding applications in textiles, rubber products, color photography, paper printing, pharmaceuticals, cosmetics, and food processing.

Textile industry effluents present significant treatment challenges due to their high chemical oxygen demand (COD), biological oxygen demand (BOD), elevated temperatures, intense coloration, variable pH levels, and the presence of metal ions.[4] Approximately 10-15% of dyes are lost during dyeing processes, and traditional textile finishing consumes about 100 liters of water per

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kilogram of textile material processed.[5] Despite being cost-effective and easy to synthesize,[6] azo dyes pose considerable health and environmental risks due to their toxicity, carcinogenicity, and mutagenicity.[7] Their resistant azo bonds hinder breakdown, leading to persistence and potential accumulation in the environment.[8]

The increasing need for potable water and its decreasing availability underscore the importance of treating and reusing industrial effluents.[9] Colored effluents, especially those from industrial sources, reduce light penetration and gas solubility in water bodies, impairing photosynthesis in phytoplankton and posing aesthetic concerns.[10,11] Azo dyes and their breakdown products, such as aromatic amines, are known carcinogens and mutagens.[12,13], contributing to health risks like bladder cancer and hepatocarcinoma.[14] In soil, high dye concentrations inhibit seed germination, stunt plant growth, and suppress the elongation of shoots and roots.

Physicochemical methods for treating colored textile effluents are often expensive and generate substantial sludge, leading to further pollution.[15] As a result, the economical and safe disposal of pollutant dyes remains a critical issue.[16,17] Microorganisms, including bacteria, fungi, and yeast, have shown diverse capabilities in decolorizing various dyes, although the degradation of dye molecules can be slow, allowing for persistence and accumulation. Bioremediation, using microorganisms,[18,19] offers a financially viable and environmentally friendly alternative for managing textile effluents by biologically breaking down or converting hazardous chemicals into less harmful forms.[20,21] Compared to physicochemical methods, biological processes are favored for their cost-effectiveness, minimal sludge formation, and environmental compatibility.[22,23]

2. Materials and Methods

The wastewater sample containing textile dyes was obtained from a dyeing industry in Nemam, Thiruvananthapuram, Kerala. Three types of dyes—direct black, blue, and orange—were selected for this study.

2.1. Physical analysis of samples

The samples were assessed for color and odor characteristics upon collection. Dilutions were prepared and spread onto nutrient agar plates, then incubated at 37°C for 24 hours to enumerate bacterial counts.

2.2. Characterization of bacterial strains

Characterization involved macroscopic and microscopic observations, biochemical tests, and molecular methods.

2.3. Decolorization activity assay

Commercial dyes (direct orange, direct blue, and direct black) were used in the decolorization study. Nutrient broth containing 0.1 g of dye per 100 ml was inoculated with 1 ml of each isolated test organism and incubated at 37°C for 1-3 days. Decolorization percentages were determined using a colorimeter before and after incubation, referencing control samples.

2.4. Impact of dye strength on decolorization

Dyes were tested at concentrations of 100 mg/L, 200 mg/L, 300 mg/L, and 400 mg/L in separate test tubes. Each tube was inoculated with 1 ml of isolated test organisms, and decolorization rates were measured colorimetrically.

2.5. Influence of pH on dye decolorization

Dyes were adjusted to pH levels of 5, 6, 7, 8, and 9, and inoculated with 1 ml of each isolated test organism. Decolorization efficiencies were assessed after incubation, with absorbance readings taken using a colorimeter.

2.6. Impact of temperature on dye decolorization

Dyes were exposed to temperatures of 28°C, 37°C, and 40°C in test tubes inoculated with 1 ml of each isolated test organism. Decolorization rates were determined using absorbance measurements.

2.7. Seed germination test

The impact of dyes on soil was evaluated by germinating Setaria italica seeds in sterile plastic dishes filled with fertilized soil. Three dishes were prepared: one with textile dye (10000 ppm), one with test organism AZ2 (10000 ppm), and a control dish with only soil. After six days at room temperature (28±2°C), shoot and root lengths of the seedlings were measured.

2.8. 16S rRNA sequences analysis

DNA from the bacterial isolate AZ2 was prepared and amplified via PCR using specific primers. Sequencing was performed and analyzed for identification and characterization purposes.

3. Results

The wastewater sample from the textile industry exhibited a dark black color and strong odor, with a pH of 8, indicating alkalinity (Tables 1 and 2). A diverse array of microbes was cultured on nutrient agar plates, characterized by distinct colony traits such as shape, size, color, elevation, and transparency(Table 3 &4). Three bacterial isolates—designated AZ1, AZ2, and AZ3—were selected for further analysis.

Bacillus sp., *Micrococcus luteus*, and *Pseudomonas* sp. were identified among the bacterial isolates. The efficiency of these isolates in decolorizing direct blue, black, and orange dyes at concentrations around 1000 ppm was assessed (Table 5). The isolates were incubated under agitation, and color changes were noted after 24 hours. *Bacillus* sp. exhibited the highest average decolorization at 200 mg/L, *Micrococcus luteus* at 400 mg/L, and *Pseudomonas* sp. at 100 mg/L.

The influence of pH on decolorization was tested across pH levels 5, 6, 7, 8, and 9. *Bacillus* sp. showed optimal decolorization at pH 8, while *Micrococcus luteus* and *Pseudomonas* sp. showed highest efficiencies at pH 7. The impact of temperature on decolorization was studied at 28°C, 37°C, and 40°C, revealing that *Bacillus* sp. achieved peak decolorization at 37°C, while *Micrococcus luteus* and *Pseudomonas* sp. exhibited maximum efficiencies at 40°C.

The toxicity of the dyes was assessed by studying their impact on seed germination, plant shoot growth, and root elongation (Table 6). Results indicated that higher dye concentrations were more detrimental to seed germination. The interaction of these dyes with selected bacterial strains correlated with observable changes in plant growth, using Setaria italica seeds as the test plant.

3.1. Identification of Species

The identified organism AZ2 was "*Micrococcus luteus* strain JW-22" (Figure 1). The PCR primers designed for analyzing the 16S rRNA sequence were:

Forward primer: 5'-TGTACACACCGCCCGTC-3'

Reverse primer: 3'-CTCTGTGTGCCTAGGTATCC-5'

Table 1: Showing physical analysis of textile waste water

S.No.	Parameter	Results
I.	pH	8
II.	Colour	Dark black
III.	Colour intensity	0.600
IV.	Odour	Pungent smell

Table 2: Showing microbial volume of textile wastewate

Sample	Dilution	Bacterial count (CFU/ml)
Textile waste water sample	10-2	TLTC
	10-3	TLTC
	10-4	TLTC
	10-5	TLTC
	10-6	TLTC
	10-7	TLTC

4. Discussion

Textile wastewater has significantly contributed to soil pollution. In this study, *Micrococcus luteus* exhibited the highest decolorization efficiency under optimal conditions of pH 7 and a temperature of 40°C. Previous studiesof Saratale et al., (2009) have demonstrated similar results, with optimal decolorization occurring at pH levels ranging from 6 to 8 and temperatures around 37°C.[24] The biological activities of these organisms are influenced by pH, which affects dye molecule movement through cell membranes, a critical step in decolorization kinetics. Temperature is also crucial for microbial growth and enzymatic activity.[6]

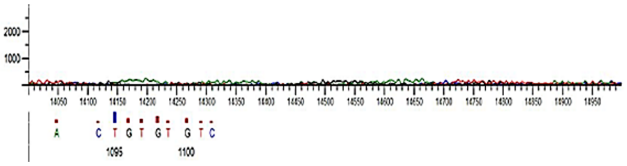

Figure 1: Showing Electrophotogram of the sample *Micrococcus luteu*

The selected organisms in this study showed rapid decolorization rates, achieving nearly 100% color removal within one day of incubation. No phytotoxic effects were observed at the tested dye concentrations, suggesting the potential of effluent-derived isolates in efficient dye degradation. Textile dye pollution poses significant threats to soil and water quality, necessitating the conversion of toxic dyes into non-toxic forms before discharge into the environment.[25,26] The isolates examined in this study show promise in decolorizing commonly used textile dyes and could serve as effective tools for bioremediation strategies, enabling the safe reuse of effluents within the textile industry.

5. Conclusion

This study demonstrates the significant potential of bacterial isolates, particularly *Micrococcus luteus*, in bioremediating textile dye pollution. *Micrococcus luteus* achieved maximum decolorization within one day at an optimum pH of 7 and temperature of 40°C, aligning with previous research on the efficacy of similar conditions. The study underscores the critical role of pH and temperature in enhancing microbial growth and enzyme activity, essential for effective dye decolorization. The bacterial isolates from textile wastewater showed rapid decolorization, achieving almost 100% color removal without causing phytotoxicity, as evidenced by healthy plant germination and growth in both dye-exposed and control groups. These findings suggest that the tested isolates could be valuable tools for bioremediating textile effluents, converting toxic dyes into non-toxic, colorless products, and facilitating the reuse of treated effluents in the textile industry, thus offering a sustainable solution for managing textile wastewater pollution.

Table 3: Showing the structural features of the bacterial isolates

S. No	Bacterial isolates	Growth on plates				
		Colour	Elevation	Transparency	Shape	Size
1.	AZ1	White	Flat	Opaque	Irregular	Large
2.	AZ2	Cream	Convex	transparent	Circular	Small
3.	AZ3	Dissusible green	Umbonate	Transparent	Oval	medium

Table 4: Showing microscopic characterization of bacterial isolates

S. No.	Biochemical, physiological and selective plating	Bacterial isolates		
		AZ1	AZ2	AZ3
1.	Gram's staining	G positive rod	G negative, cocci	G negative rod
2.	Motility	Positive	Negative	Positive
3.	Spore staining	Positive	Negative	Negative
4.	Indole	Positive	Negative	Negative
5.	Methyl red	Negative	Negative	Negative
6.	Voges proskaur	Negative	Negative	Negative
7.	Citrate utilization	Positive	Positive	Positive
8.	TSI test	A/AL	A/AL, gas	A/AL
9.	Catalase test	Positive	Positive	Positive
10.	Oxidase test	Positive	Positive	Positive
11.	Urease	Positive	Positive	Positive
12.	Nitrate	Positive	Negative	Positive
13.	Casein	Positive	Positive	Positive
14.	Gelatine	Positive	Negative	Positive
15.	starch	Positive	Negative	Negative
16.	Lipid	Positive	Negative	Positive
17.	Carbohydrate	Positive	Positive	Positive
18.	Bacterial growth on selective medium	*Bacilluscereus* agar	Tripticasesoy agar	Cetrimide agar
19.	Isolates obtained	*Bacillus*	Micrococcus	Pseudomanas

Table 5: Showing impact of dye strength on decolorization

Isolate	Colour of dye	Decolourization Percentage (%)				
		100 milligrams per liter	200 milligrams per liter	300 milligrams per liter	400 milligrams per liter	500 milligrams per liter
AZ1	Blue	82	86	51	39	36
	Black	11	14	21	28	28
	Orange	82	84	85	85	87
AZ2	Blue	33	37	57	63	60
	Black	96	95	88	85	71
	Orange	88	88	89	93	97
AZ3	Blue	16	17	25	16	19
	Black	70	80	53	64	58
	Orange	66	49	57	22	20

Table 6: Bioassay for dye toxicity (Seed Germination Test)

S. No	Soil type	Length of shoot(cm)	Length of root (cm)
1.	Fertile soil	3.8	1.5
2.	Soil+dye	1	0.3
3.	Soil+dye+organism	3	0.5

6. Source of Funding

None

7. Conflict of Interest

None.

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