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Assessment of The Bioremediation Potential of Selected Bacterial Species Isolated from the Textile Printing Wastewater Inoculated with Cow Dung

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Abstract

This study was conducted to isolate and identify bacteria from the wastewater of the textile printing industry inoculated with a mixture of cow dung, jaggery, and urea to assess the bioremediation potential of isolated bacterial species for reducing color and other polluting parameters of the wastewater. Based on colony characteristics, we were able to isolate nine types of bacteria (Pri 1 to 9), capable of thriving in textile printing wastewater. Out of the nine isolates examined, four (Pri 3, Pri 4, Pri 8, and Pri 9) significantly reduced the color and values of other physicochemical parameters of the wastewater. Isolated bacterial cultures were identified using various biochemical tests, antibiotic sensitivity tests, and rRNA sequence analysis. At the end of a 24-h incubation period at room temperature under shaking conditions at 100 rpm on an orbital shaker, isolate Pri 3 was identified as *Alcaligenes aquatilis* LMG 22996 (T), capable of reducing color by 86.13%, biological oxygen demand (BOD) by 70.44%, chemical oxygen demand (COD) by 80.65%, total dissolved solids (TDS) by 47.31%, total suspended solids (TSS) by 56.56%, and ammoniacal nitrogen by 75.95%; isolate Pri 4 as *Priestia aryabhattai* B8W22(T), capable of reducing color by 78.35%, BOD by 66.35%, COD by 74.92%, TDS by 34.94%, TSS by 31.66%, and ammoniacal nitrogen by 71.14%; isolate Pri 8 as *Citrobacter werkmanii* NBRC 105721(T), capable of reducing color by 90.37%, BOD by 82.13%, COD by 85.06%, TDS by 54.83%, TSS by 61.97%, and ammoniacal nitrogen by 80.76%; and isolate Pri 9 as *Shewanella chilikensis* JC5(T), capable of reducing color by 90.17%, BOD by 84.68%, COD by 83.46%, TDS by 59.13%, TSS by 62.45%, and ammoniacal nitrogen by 90.37%.

Keywords: Textile Printing Wastewater, Cow Dung, Bacterial Isolates, Bioremediation, Physicochemical Parameters, Antibiotic Sensitivity, 16S rRNA Sequencing

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INTRODUCTION

Since the existence of mankind, human beings have used colors in their routine lifestyles, which were traditionally derived from natural materials.[1] However, with the increase in population and industrial development, these natural materials are being replaced by synthetic colors and dyes. Currently, more than 100,000 types of synthetic dyes are being used across various industrial sectors.[2] Among these sectors, the textile industry stands out as the largest consumer of synthetic dyes and chemicals in its manufacturing process. Textile manufacturing depends on the freshwater requirements, and approximately 20–25% of the used dyes and chemicals return as wastewater from these industries.[3] Many of the textile dyes inhibit oxygen transfer in the generated wastewater, thereby increasing both biological and chemical oxygen demands.[4] This may lead to toxicity for aquatic life, and continuous exposure to such dyes might pose health risks to humans.[5,6] Consequently, proper treatment of industrial wastewater before discharge into the natural environment becomes crucial. Previously, treatment processes relied on chemical and physical methods,[7] which helped in color removal of the dyes to some extent. However, these methods often generate undesirable by-products, such as aromatic amines, which are challenging to maintain,[8] Instead, the biological procedure for the removal of such compounds from the wastewater is cheaper due to the availability of the vast array of microbes in the natural environment and their diverse metabolic pathways that remove or convert complex forms of pollutants to simpler forms.[9,10] This can be achieved through biosorption, biodegradation, bioaccumulation, and biostimulation.[11] As the bacterial system has diverse metabolic pathways, numerous innovations have been conducted in textile wastewater treatment. Various bacterial genera, including *Bacillus sp.* ADR, ETL-2012, SF, VUS, UN2,[12–22] *Caulobacter subvibrioides*[23]; *Enterobacter aerogenes* and its spp. SXCR, CV–S1[24-26]; *Klebsiella* (ST16.16/034), (facultative) strain VN-31, strain Y3, DA26 [11,27,28]; *Moraxella osloensis*[29,30]; *Paenibacillus alvei* MTCC 10625 and *P. azoreducens*[7,31]; *Pseudomonas spp.* SU-EBT, LBC1, SUK1[32-34]; *Pseudomonas luteola*;[35-37] *Rhodobacter erythropholis* MTCC 4688[38]; and *Stenotrophomonas spp.* BHUSSp X2,[12,39] have been utilized in the treatment process.

The success of microbial wastewater treatment highly depends on the isolation of microorganisms from the polluted sample, such as textile wastewater[6,26,40-44] textile effluent-contaminated soil,[19,43,45] and activated sludge.[46] In this study, we isolated bacteria from textile printing wastewater inoculated with a mixture of cow dung, jaggery, and urea. The efficiency of different types of bacteria naturally present in manmade polluted samples such as textile printing wastewater, and those present in natural samples such as cow dung that survived in textile printing wastewater was evaluated for their capability to reduce color and values of other physicochemical parameters of the wastewater.

MATERIALS AND METHODS

Collection of textile printing wastewater

Wastewater was collected from the effluent treatment plant of the textile printing industry, Western Overseas, situated at 21°43’25.20" N and longitude 70°34’10.27" E, in the village of Jetalsar, Jetpur, Rajkot, Gujarat, India. The sample was collected in a 10-L sterile plastic container and preserved at 4°C in the refrigerator until examination.

Enrichment and isolation of bacteria

Bacteria are present in all ecosystems and are part of all living systems on the Earth.[47] Typically, bacteria capable of dye decolorization and degradation are isolated from textile wastewater, textile effluent-contaminated soil, and activated sludge. In this study, we prepared a cocktail of textile printing wastewater, cow dung, jaggery, and urea for isolating bacteria suitable for the bioremediation of pollution caused by the colored effluent.

To obtain a diverse range of bacterial systems, enrichment of bacteria is essential before the isolation process. In 500 L of fresh water, a mixture of 2 kg cow dung, 250 g jaggery, and 100 g urea was prepared and aerated for 48 h using a Nanobubble pump (Century Pump, Udhyog Nagar, Rajkot, India), producing bubbles that can survive in water for up to 60 min or

more.[48] Subsequently, 250 L of this mixture was combined with 250 L of textile printing wastewater, supplemented with 2 kg cow dung, 250 g jaggery, and 100 g urea. Aeration with the Nanobubble pump was continued for 24 h, and this cycle was repeated for 7 days. This method enriched only those bacteria capable of surviving in textile printing wastewater. The microbially enriched textile printing wastewater was diluted 100 times in sterile distilled water, streaked aseptically onto nutrient agar plates (HiMedia, Mumbai, India) prepared in sterile textile printing wastewater, and incubated at 37°C for 24 h. After incubation, numerous morphologically distinct bacterial colonies were observed on the nutrient agar plates. Nine morphologically different colonies were selected, maintained by subculturing onto nutrient agar slants, and stored in a refrigerator at 4°C.

Study of bacterial growth in textile printing wastewater

For the examination of bacterial growth, nutrient broth (HiMedia, Mumbai, India) was prepared directly in sterile textile printing wastewater. In a 250 mL Erlenmeyer flask, 100 mL of this nutrient broth was added and sterilized in an autoclave. A loopful of the pure culture of all nine selected bacterial isolates was individually inoculated into separate flasks and agitated at 100 rpm in an orbital shaker (Remi, Bharat) at room temperature for 10 h. Aseptically, 10 mL samples were harvested from each flask and collected in 12 mL sterile centrifuge tubes. The tubes containing isolates were centrifuged at 5000 rpm for 10 min using a laboratory centrifuge (Remi, India). The cell-free supernatants were discarded, and pellets of bacterial cells were resuspended in 10 mL distilled water. The absorbance was measured at λ660 nm (Systronics 106, India) to estimate the growth.

Screening of bacterial isolates for their decolorization potential

The ability of all bacterial isolates to remove color from the effluent was assessed. To prepare an inoculum, a loopful of the pure culture of bacteria was transferred from a nutrient agar slant to a 250 mL sterile Erlenmeyer flask containing 100 mL nutrient broth (pH 7.0) prepared in sterile distilled water. These flasks were placed on an orbital shaker (Remi, Ahmedabad, India) at 100 rpm and room temperature for 8 h. Then, 1.0 mL of actively growing inoculum was transferred to another 250 mL sterile Erlenmeyer flask containing 100 mL nutrient broth prepared in the textile printing wastewater itself (pH 7.0). Similar flasks for all nine bacterial isolates were prepared and kept on an orbital shaker at 100 rpm and room temperature. Visual observations of reduction or change in color were recorded at 24 h and 48 h. At the end of this process, four out of nine isolates were identified as the most effective decolorizers of textile printing wastewater and were selected for further testing.

Reduction in color and values of other polluting parameters of textile printing wastewater by selected bacterial isolates

Four bacterial isolates were subjected to further testing to assess their efficacy in reducing color and the values of other polluting parameters of wastewater, including BOD, COD, TDS, TSS, and ammoniacal nitrogen. To conduct the experiment, 100 mL of textile printing wastewater was introduced into a 250 mL Erlenmeyer flask, and 0.1 g jaggery was added as a nutrient source. The final pH was adjusted to 7.0, and the flasks were sterilized in an autoclave. A control flask without inoculation served as a reference, while test flasks were inoculated with 1 mL of an actively growing bacterial isolate aseptically. Each bacterial isolate had a dedicated test flask, placed on an orbital shaker at 100 rpm and room temperature. Samples were collected from these flasks at 8 h, 16 h, 24 h, and 48 h and analyzed for color reduction (decolorization), BOD, COD, TDS, TSS, and ammoniacal nitrogen. Decolorization was measured using a UV-visible spectrophotometer (Elico, Model No. SL 159) at λ660 nm, while other parameters were measured following the standard methods for the examination of water and wastewater (APHA).[49]

Decolorization percent was calculated using equation (1)[4]:

1. % Decolorization = [Initial absorbance – Final absorbance) / (Initial absorbance] x100

Reduction in all other parameters, i.e., BOD, COD, TDS, TSS, and ammoniacal nitrogen, was calculated using the following equation (2)[50]:

2. % Reduction in the parameters = [Initial result-Final result)/(Initial result] x100

Identification of selected bacteria

Morphological examinations

Morphological examination of bacteria involves observing colony characteristics and conducting microscopic examinations of cells. The colony characteristics of bacterial isolates were visually recorded, while cellular characteristics were documented through Gram and spore staining. Additionally, motility was observed through a hanging drop preparation of pure culture suspension.

Biochemical tests

By performing various biochemical tests, bacterial systems can be identified up to the genus level, and sometimes even to the species level.[44] These tests were performed following the procedures outlined in Bergey's Manual of Systematic Bacteriology. The identification process was conducted in VITEK-2 (BioMerieux USA), a fully automated microbial identification system.

Antibiotic sensitivity test

All four selected bacterial isolates underwent testing for their sensitivity to various antibiotics using the disk diffusion method.[51] Mueller–Hinton agar plates (HiMedia, Mumbai, India) served as a nutrient medium, with 47 different antibiotic discs, each with a suggested dose for the tests. Antibiotics included amikacin, tobramycin, gentamicin, netilmicin, aztreonam, cefazolin, cefuroxime, ceftizoxime, cefpodoxime, cefixime, ceftazidime, cefepime, piperacillin + tazobactam, cefoperazone + tazobactam, cefoperazone + sulbactam, amoxicillin + clavulanic acid, trimethoprim/sulfamethoxazole, colistin, imipenem, meropenem, doripenem, tetracycline, doxycycline, minocycline, ciprofloxacin, levofloxacin, moxifloxacin, penicillin G, erythromycin, azithromycin, rifampicin, linezolid, vancomycin, and clindamycin. All plates were incubated at 37°C for 24 h to measure sensitivity.

16S rRNA sequencing

For the identification of bacterial species, 16S rRNA sequencing of the selected bacterial isolates was conducted at the National Center for Microbial Resources (NCMR), Pune, India.

RESULTS AND DISCUSSION

Colony morphology of bacterial isolates

Colony morphology refers to the visible characteristics and appearance of bacterial colonies grown on solidified agar media. This information is valuable for understanding the bacterial species present and their growth patterns.[52] The Detailed colony characteristics are mentioned in Table 1. The colonies of isolates Pri 1 to 8 exhibited a circular form, whereas those of isolate Pri 9 displayed an irregular form. Colony

Table 1. Colony morphology of bacterial Isolates

Isolates	Form	Size (mm)	Elevation	Margin	Surface	Opacity	Color
Pri 1	Circular	>0.5	Convex	Entire	Smooth	Opaque	White
Pri 2	Circular	<2.0	Umbonate	Entire	Smooth	Opaque	White
Pri 3	Circular	1.0–1.5	Convex	Entire	Smoot	Slightly opaque	White
Pri 4	Circular	1.5–1.8	Convex	Entire	Smooth and shiny	Opaque	Yellow
Pri 5	Irregular	<2.0	Flat	Filamentous	Rough	Opaque	Grey
Pri 6	Circular	1.5–1.7	Umbonate	Entire	Rough	Opaque	Bluish white
Pri 7	Circular	1.0–1.5	Convex	Entire	Smooth	Slightly opaque	White
Pri 8	Circular	2.0–3.0	Convex	Filamentous	Rough and shiny	Transparent	Grey
Pri 9	Irregular	1.2–1.8	Convex	Entire	Smooth and shiny	Opaque	Milky white

sizes ranged from 0.5 mm to 3.0 mm among all isolates. The elevation of colonies varied: isolates Pri 1, Pri 3, Pri 4, Pri 7, Pri 8, and Pri 9 had convex elevations; isolates Pri 2 and Pri 6 were umbonate; and isolate Pri 5 had a flat elevation. The margin of colonies showed filamentous characteristics for isolates Pri 5 and Pri 8, whereas that of other isolates had entire margins. The color of colonies differed: isolates Pri 1, Pri 2, Pri 3, and Pri 7 were white; Pri 9 was milky white; Pri 5 and Pri 8 were grey; Pri 4 was yellow; and Pri 6 was bluish white. The surface of the colonies also varied: Pri 1, Pri 2, Pri 3, and Pri 7 had smooth surfaces; Pri 4 and Pri 9 had smooth and shiny surfaces; Pri 5 and Pri 6 had rough and white surfaces; and Pri 8 had rough and shiny surfaces. Most colonies appeared opaque or slightly opaque, with the exception of isolate Pri 8, which produced transparent colonies. Based on these observed colony characteristics, all nine microbial isolates were primarily identified as bacteria.

Microscopic examination of bacterial isolates

Bacterial cells were examined under a compound light microscope to determine their size, shape, organization, motility, and other structural traits. Gram staining was employed to determine the composition of cell walls, and

Table 2. Microscopic examination of bacterial isolates

Isolates	Grams Stain	Spore Staining	Motility
Pri 1	Gram-positive cocci	Non-sporulating	Non-motile
Pri 2	Gram-positive cocci	Non-sporulating	Non-motile
Pri 3	Gram-negative coccobacilli	Non-sporulating	Motile
Pri 4	Gram-positive rods	Sporulating	Motile
Pri 5	Gram-positive rods	Sporulating	Non-motile
Pri 6	Gram-positive rods	Non-sporulating	Non-motile
Pri 7	Gram-negative coccobacilli	Non-sporulating	Non-motile
Pri 8	Gram-negative bacilli	Non-sporulating	Motile
Pri 9	Gram-negative bacilli	Non-sporulating	Motile

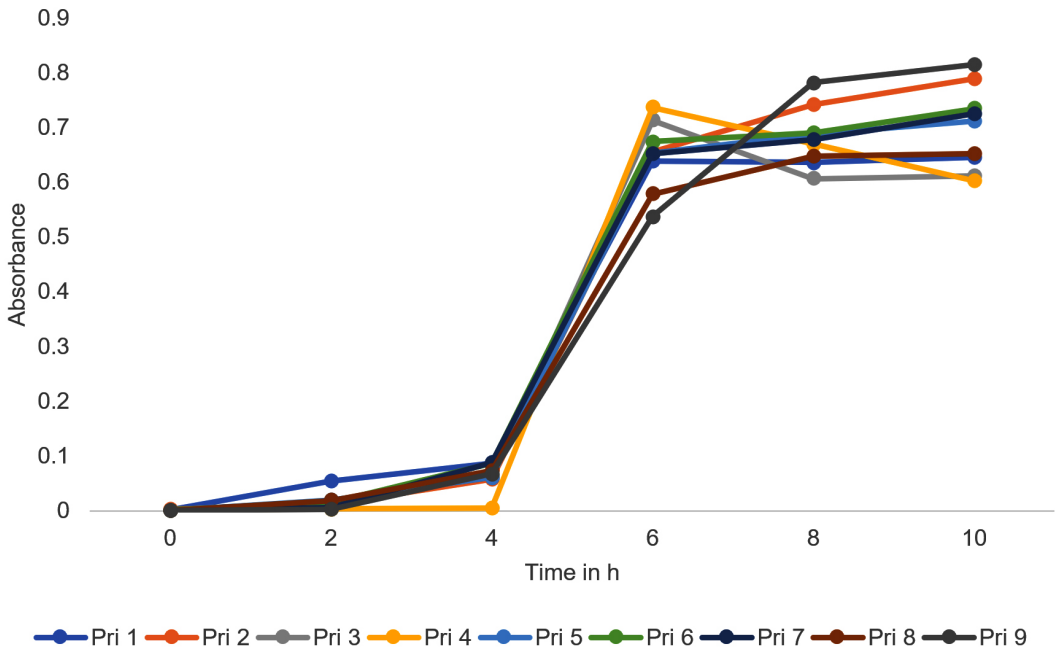

Figure 1. Growth curve of bacterial isolates

spore staining was employed to assess whether the bacteria possessed a survival mechanism under unfavorable environmental conditions. Additionally, motility observation was conducted to determine the motility of bacteria in the given medium. The Details of the staining and Motility test are mentioned in Table 2. In our study, cells of isolates Pri 1 and Pri 2 were observed as gram-positive cocci, Pri 4 as gram-positive bacilli, Pri 5 and Pri 6 as gram-positive rods, Pri 3 and Pri 7 as gram-negative coccobacilli; and Pri 8 and Pri 9 as gram-negative bacilli. Except for isolate Pri 5, cells of all isolates were found to be non-sporulating. Furthermore, cells of isolates Pri 4, Pri 8, and Pri 9 were motile, whereas those of the other isolates were non-motile.

Growth curve of bacterial isolates

Bacterial growth curve patterns (Figure 1) revealed that all bacterial isolates were

Table 3. Screening of bacterial isolates for their decolorization potential

No.	Isolates	Visual observation for color change	Color after 24 h	Color after 48 h
1.	Reference (without bacterial inoculation)	No color change	Dark brown	Dark brown
2.	Pri 1	Minor decolorization was observed compared with a reference	Brown	Light brown
3.	Pri 2	Minor decolorization was observed compared with a reference	Brown	Brown
4.	Pri 3	A light yellowish-colored layer formed in more than half of the flask	Light green	Light yellow
5.	Pri 4	Complete decolorization was observed compared with a reference	Light green	Light yellow
6.	Pri 5	No decolorization was observed compared with a reference	Brown	Brown
7.	Pri 6	No decolorization was observed compared with a reference	Brown	Brown
8.	Pri 7	A light yellowish-colored layer formed in more than half of the flask	Yellowish green	Yellow
9.	Pri 8	Complete decolorization was observed compared with a reference	Light yellow	Light yellow
10.	Pri 9	Complete decolorization was observed compared with a reference	Light yellow	Light yellow

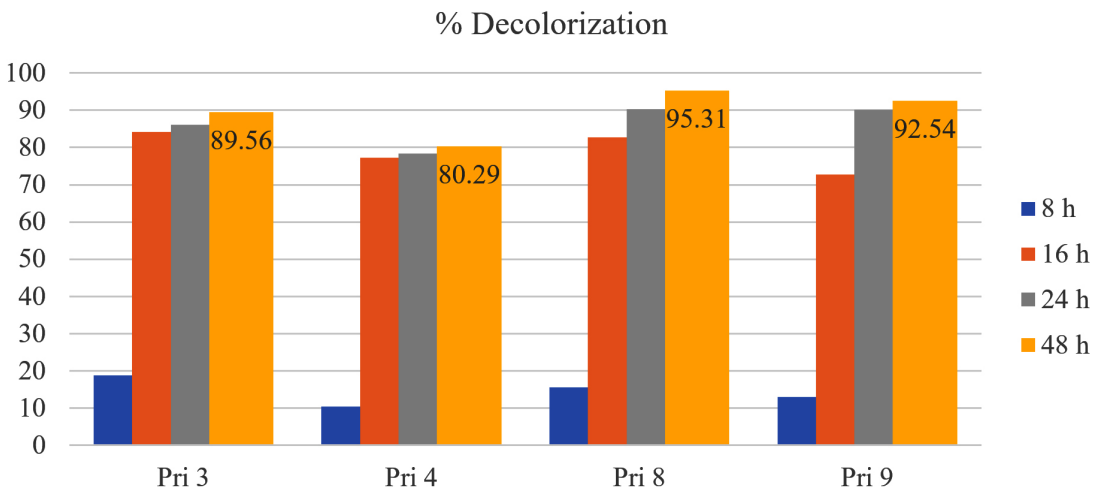

Figure 2. Color reduction by selected isolates

capable of surviving and growing in the challenging environment of textile printing wastewater and exhibited normal growth patterns characterized by lag, log, and stationary phases. Consequently, all bacterial isolates demonstrated their potential as viable candidates for textile printing wastewater treatment. However, to comprehensively analyze the reduction in color and values of other polluting

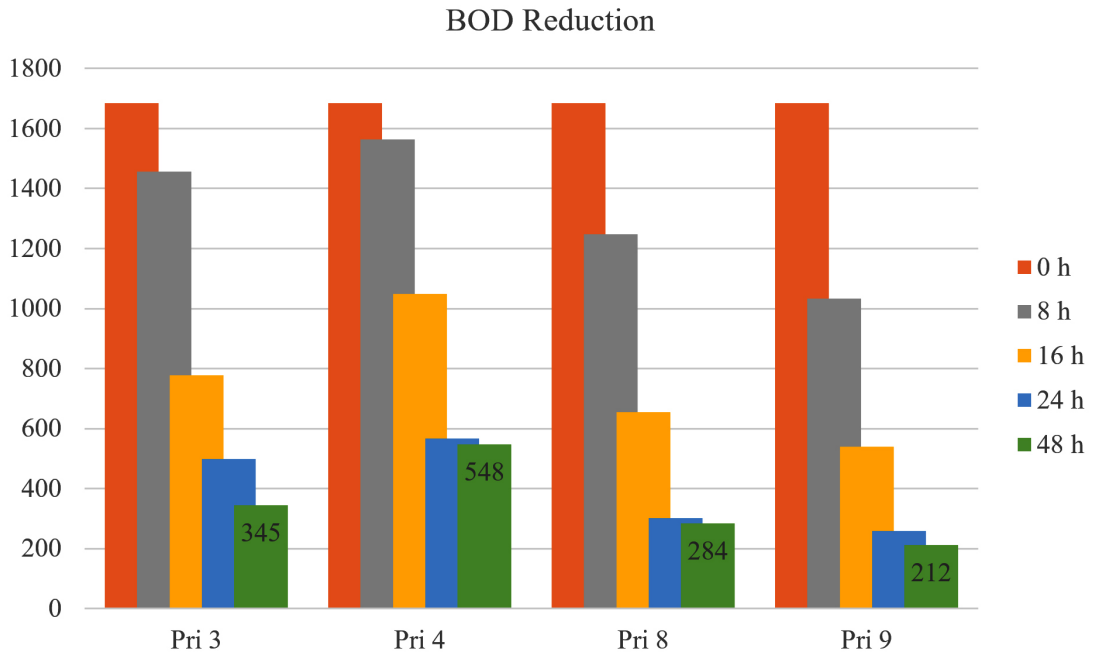

Figure 3. BOD reduction by selected bacterial isolates

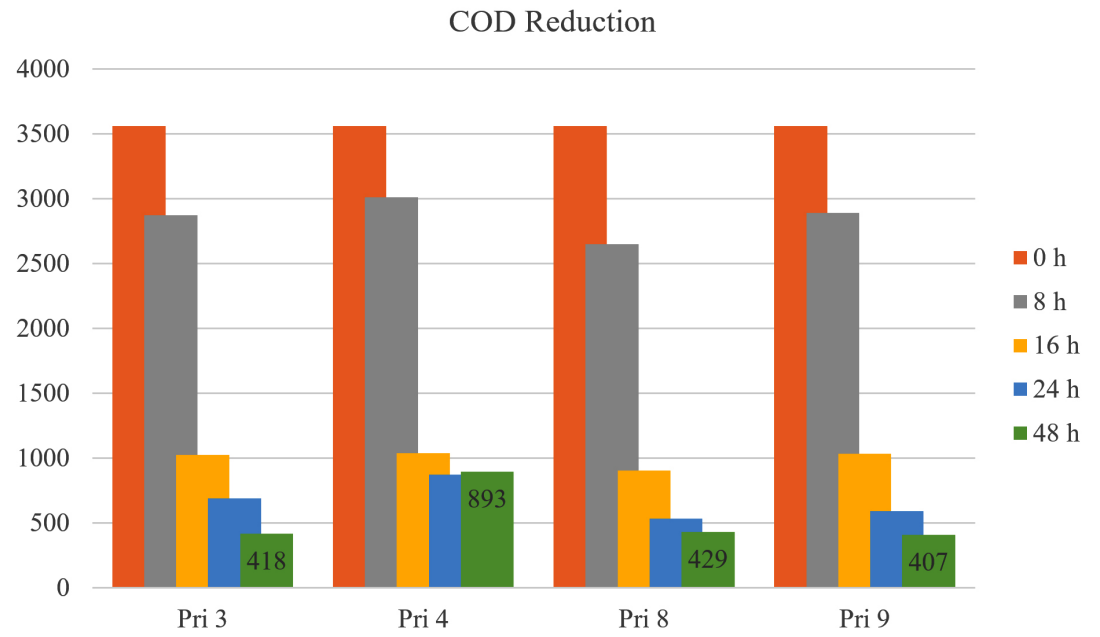

Figure 4. COD reduction by selected bacterial isolates

parameters of textile printing wastewater by bacterial isolates, additional assays are currently being conducted.

Screening of bacterial isolates for their decolorization potential

Through visual observation for color changes after 24 h and 48 h of incubation, four

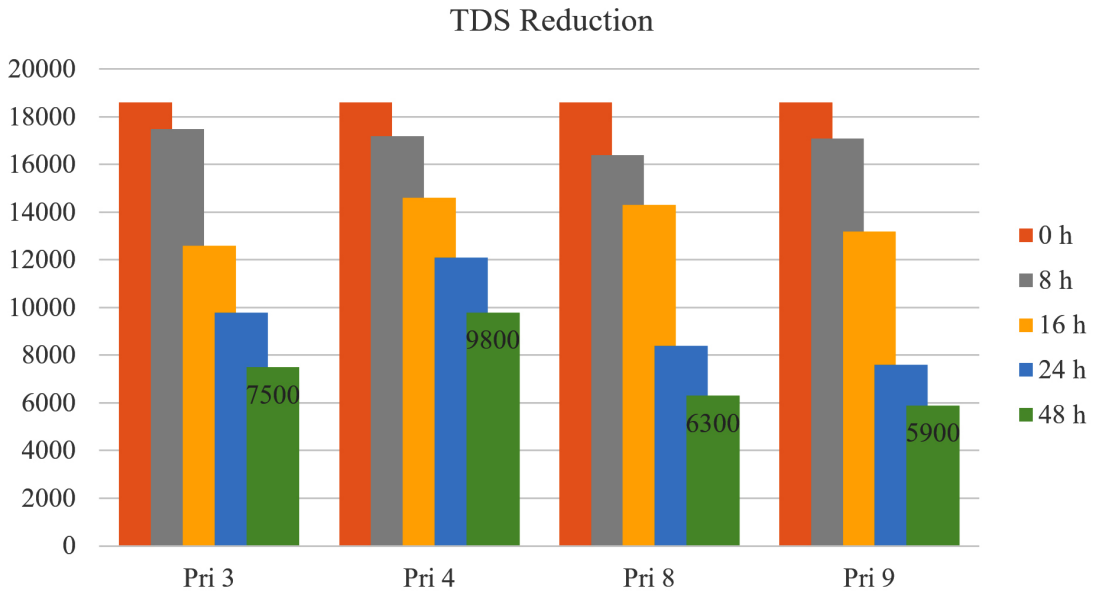

Figure 5. TDS reduction by selected bacterial isolates

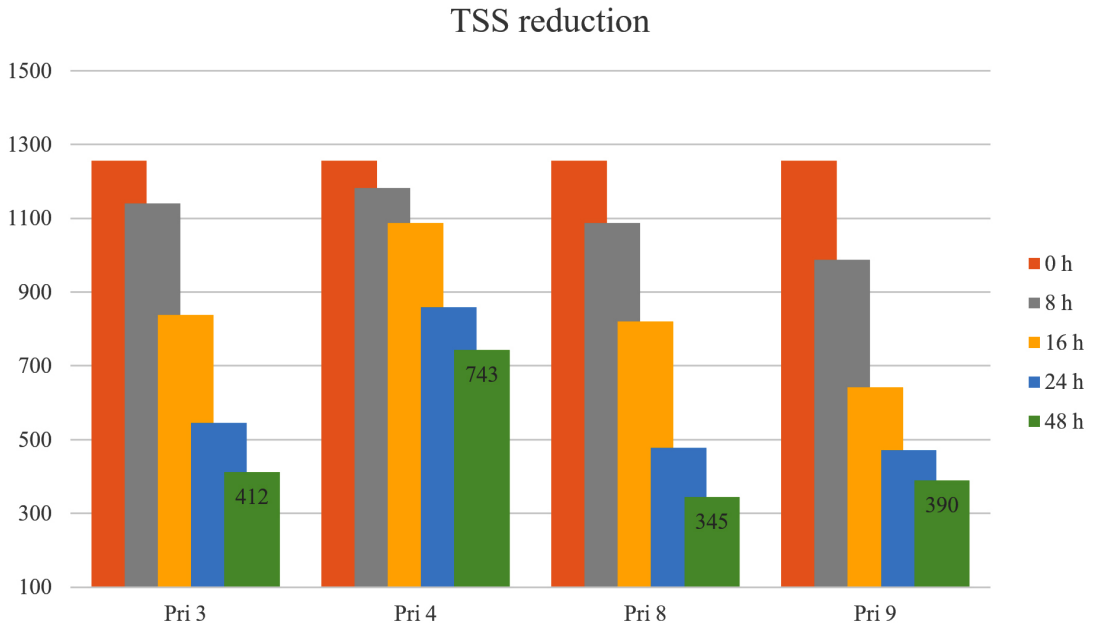

Figure 6. TSS reduction by selected bacterial isolates

Table 4. Bacterial identification using VITEK-2, the microbial identification system

No.	Tests	Mnemonic	Pri-3	Pri-4	Pri-8	Pri-9
1.	Ala-Phe-Pro-Arylamidase	APPA	-	-	-	-
2.	Adonitol	ADO	-	-	-	-
3.	L-pyrrolidonyl-Arylamidase	PyrA	-	+	+	-
4.	L-Arabitol	IARL	-	-	-	-
5.	D-Cellobiose	dCEL	-	+	-	-
6.	Beta-Galactosidase	BGAL	-	+	+	-
7.	H2S Production	H2S	-	-	+	-
8.	Beta-N-Acetyl-Glucosaminidase	BNAG	-	-	-	-
9.	Glutamyl Arylamidase pNA	AGLTp	-	-	-	-
10.	D-Glucose	dGLU	-	+	+	-
11.	Gamma-Glutamyl-Transferase	GGT	-	-	+	-
12.	Fermentation/Glucose	OFF	-	-	+	-
13.	Beta-Glucosidase	BGLU	-	+	-	-
14.	D-Maltose	dMAL	-	+	+	-
15.	D-Mannitol	dMAN	-	+	+	-
16.	D-Mannose	dMNE	-	-	+	-
17.	Beta-Xylosidase	BXYL	-	-	-	-
18.	Beta-Alanine Arylamidase pNA	BAlap	-	-	-	-
19.	L-Proline Arylamidase	ProA	+	-	-	+
20.	Lipase	LIP	-	-	-	-
21.	Palatinose	PLE	-	+	-	-
22.	Tyrosine Arylamidase	TyrA	+	+	-	+
23.	Urease	URE	-	-	+	-
24.	D-Sorbitol	dSOR	-	-	+	-
25.	Saccharose/Sucrose	SAC	-	+	-	-
26.	D-Tagatose	dTAG	-	-	-	-
27.	D-Trehalose	dTRE	-	+	+	-
28.	Citrate	CIT	+	-	-	+
29.	Malonate	MNT	-	-	+	-
30.	5-keto-D-Gluconate	5KG	-	-	+	-
31.	L-Lactate alkalinization	ILATk	+	+	-	+
32.	Alpha-Glucosidase	AGLU	-	+	-	-
33.	Succinate alkalinization	SUCT	+	-	+	+
34.	Beta-N-Acetyl-Galactoaminidase	NAGA	-	-	-	-
35.	Alpha-Galactosidase	AGAL	-	+	-	-
36.	Phosphatase	PHOS	-	-	-	-
37.	Glycine Arylamidase	GlyA	-	-	-	-
38.	Ornithine Decarboxylase	ODC	-	-	-	-
39.	Lysine Decarboxylase	LDC	-	-	-	-
40.	L-Histidine assimilation	IHISa	-	-	-	-
41.	Coumarate	CMT	-	-	+	-
42.	BETA-Glucuronidase	BGUR	-	-	-	-
43.	O/129 Resistance (Comp-Vibrio)	O129R	-	-	-	-
44.	Glu-Gly-Arg-Arylamidase	GGAA	-	-	-	-
45.	L-MALATE assimilation	IMLTa	(-)	-	-	(-)
46.	Ellman	ELLM	+	-	-	+
47.	L-Lactate assimilation	ILATa	+	-	-	+

Table 5. Antibiotic sensitivity tests of the selected bacterial isolates

No.	Antibiotics	Pri 3		Pri 4		Pri 8		Pri 9	
		Zone	Sensitivity	Zone	Sensitivity	Zone	Sensitivity	Zone	Sensitivity
1	Amikacin	23	Sensitive	23	Sensitive	15	Intermediate	21	Sensitive
2	Tobramycin	24	Sensitive	24	Sensitive	14	Intermediate	23	Sensitive
3	Gentamicin	21	Sensitive	22	Sensitive	14	Intermediate	21	Sensitive
4	Netilmicin	22	Sensitive	21	Sensitive	15	Sensitive	22	Sensitive
5	Aztreonam	24	Sensitive	19	Sensitive	22	Sensitive	21	Sensitive
6	Cefazolin	18	Sensitive	21	Sensitive	16	Resistance	26	Sensitive
7	Cefaclor	---	---	---	---	---	---	---	---
8	Cefuroxime	15	Sensitive	21	Sensitive	18	Intermediate	24	Sensitive
9	Ceftizoxime	25	Sensitive	23	Intermediate	18	Intermediate	26	Sensitive
10	Cefpodoxime	22	Sensitive	24	Sensitive			26	Sensitive
11	Cefixime	26	Sensitive	26	Sensitive	---	---	---	---
12	Ceftazidime	25	Sensitive	23	Sensitive	19	Intermediate	23	Sensitive
13	Cefoperazone	---	---	---	---	21	Sensitive	24	Sensitive
14	Cefotaxime	---	---	---	---	26	Sensitive	26	Sensitive
15	Ceftriaxone	---	---	---	---	---	---	23	Sensitive
16	Cefepime	19	Sensitive	26	Sensitive	23	Sensitive	27	Sensitive
17	Piperacillin + tazobactum	31	Sensitive	22	Sensitive	24	Sensitive	23	Sensitive
18	Ticarcillin + clavulanic acid	24	Sensitive	---	---	25	Sensitive	---	---
19	Cefepime + tazobactum	---	---	---	---	23	Sensitive	26	Sensitive
20	Cefoperazone + tazobactum	22	Sensitive	26	Sensitive	---	---	---	---
21	Cefoperazone + sulbactam	24	Sensitive	24	Sensitive	25	Sensitive	24	Sensitive
22	Amoxicillin + clavulanic acid	22	Sensitive	22	Sensitive	18	Sensitive	21	Sensitive
23	Trimethoprim/ sulfamethoxazole	23	Sensitive	24	Sensitive	22	Sensitive	21	Sensitive
24	Colistin	14	Sensitive	13	Sensitive	13	Sensitive	15	Sensitive
25	Imipenem	25	Sensitive	26	Sensitive	27	Sensitive	25	Sensitive
26	Meropenem	26	Sensitive	25	Sensitive	28	Sensitive	25	Sensitive
27	Doripenem	27	Sensitive	27	Sensitive	27	Sensitive	24	Sensitive
28	Tetracycline			21	Sensitive	20	Sensitive	21	Sensitive
29	Doxycycline	18	Sensitive	23	Sensitive	17	Sensitive	22	Sensitive
30	Minocycline	18	Sensitive	19	Sensitive	18	Sensitive	25	Sensitive
31	Tigecycline	18	Sensitive	---	---	19	Sensitive	22	Sensitive
32	Chloramphenicol	21	Sensitive	---	---	20	Sensitive	21	Sensitive
33	Nitrofurantoin	20	Sensitive	---	---	19	Sensitive	24	Sensitive
34	Norfloxacin	19	Sensitive	---	---	21	Sensitive	19	Sensitive
35	Ciprofloxacin	23	Sensitive	26	Sensitive	23	Sensitive	27	Sensitive
36	Levofloxacin	24	Sensitive	23	Sensitive	21	Sensitive	25	Sensitive
37	Fosfomycin	---	---	---	---	19	Sensitive	23	Sensitive
38	Piperacillin	22	Sensitive	---	---	20	Intermediate	22	Sensitive
39	Ticarcillin	21	Sensitive	---	---	24	Sensitive	24	Sensitive
40	Moxifloxacin	---	---	26	Sensitive	---	---	---	---
41	Penicillin G	---	---	28	Sensitive	---	---	---	---
42	Erythromycin	---	---	25	Sensitive	---	---	---	---
43	Azithromycin	---	---	19	Sensitive	---	---	---	---
44	Rifampicin	---	---	23	Sensitive	---	---	---	---
45	Linezolid	---	---	27	Sensitive	---	---	---	---
46	Vancomycin	---	---	19	Sensitive	---	---	---	---
47	Clindamycin	---	---	06	Resistant	---	---	---	---

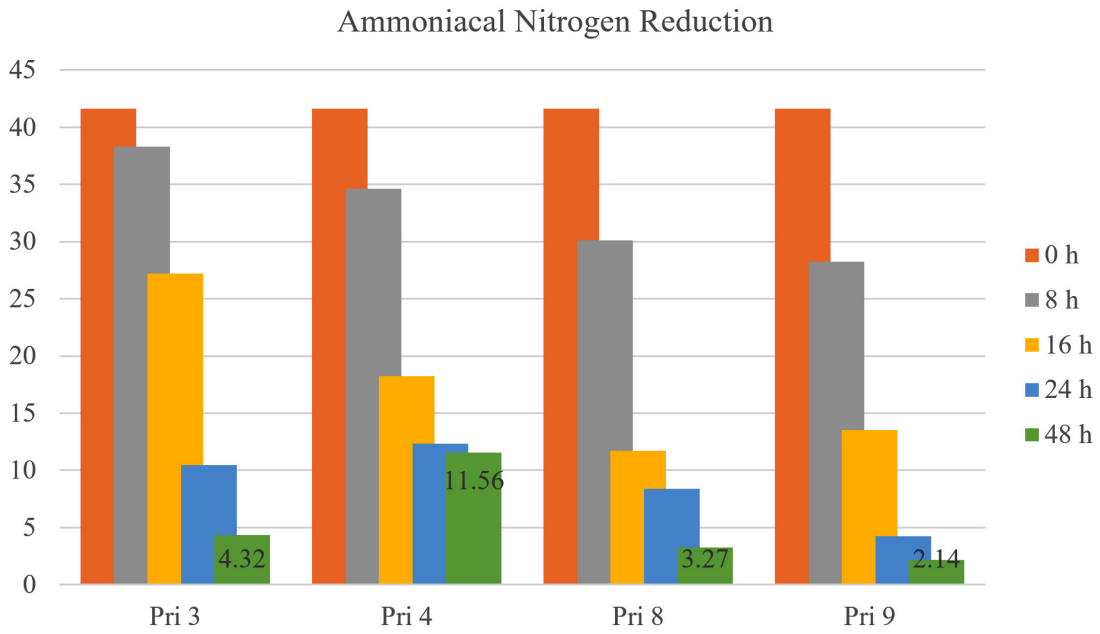

Figure 7. Ammoniacal nitrogen reduction by selected bacterial isolates

isolates, namely, Pri 3, Pri 4, Pri 8, and Pri 9, were identified as the most effective candidates for decolorizing textile printing wastewater and the details of the same are mentioned in Table 3. These four bacterial isolates successfully transformed the color of the textile printing wastewater from dark brown to light yellow after 48 h of incubation. In contrast, the remaining bacterial isolates showed minimal alteration in the color of the wastewater.

Bioremediation of textile printing wastewater by selected bacterial isolates

Reduction in wastewater color, BOD, COD, TDS, TSS, and ammoniacal nitrogen was assessed to evaluate the bioremediation potential of selected bacterial isolates Pri 3, Pri 4, Pri 8, and Pri 9.

Color reduction

All four bacterial isolates achieved >75% decolorization of textile printing wastewater within 16 h. After 24 h, the decolorization further increased, ranging from 85% to 90%, except for isolate Pri 4, which exhibited <80% decolorization. Subsequently, after 48 h, the decolorization percentages reached 89%, 80%, 95%, and 92% for isolates Pri 3, Pri 4, Pri 8, and Pri 9, respectively. Notably, isolate Pri 8 demonstrated the highest color reduction within 48 h. The results of percent decolorization are illustrated in Figure 2.

BOD reduction

All bacterial isolates exhibited >50% reduction in BOD of textile printing wastewater within 16 h, except for isolate Pri 4 (<36%). After 24 h and 48 h, BOD reduction increased to 70.44% and 79.52% for Pri 3, 66.35% and 67.47% for Pri 4, 82.13% and 83.14% for Pri 8, and 84.68% and 87.41% for Pri 9, respectively. Notably, isolate Pri 9 achieved the maximum BOD reduction within 48 h. The data on BOD reduction are presented in Figure 3.

COD reduction

All bacterial isolates demonstrated COD reduction of >70% in textile printing wastewater within 16 h. After 24 h and 48 h, COD reduction increased to 80.65% and 88.26% for Pri 3, 74.92% and 75.46% for Pri 4, 85.06% and 87.95% for Pri 8, and 83.46% and 88.57% for Pri 9, respectively. Notably, isolates Pri 3 and Pri 9 achieved the maximum COD reduction within 48 h. The data on COD reduction are presented in Figure 4.

TDS reduction

All bacterial isolates exhibited approximately 50% reduction in TDS after 24 h, except for Pri 4 (<33%). After 48 h, TDS reduction further increased to 59.67% for Pri 3, 47.31% for Pri 4, 66.12% for Pri 8, and 68.27% for Pri 9. Notably, isolate Pri 9 achieved the maximum reduction in TDS within 48 h. The data on TDS reduction are presented in Figure 5.

TSS reduction

Approximately 60% reduction in TSS was observed after 24 h by all bacterial isolates, except for isolate Pri 4 (<32%). After 48 h, the reduction increased to 67.22% for Pri 3, 40.89% for Pri 4, 72.55% for Pri 8, and 68.97% for Pri 9. Notably, isolate Pri 8 achieved the maximum reduction in TSS within 48 h. The data on TSS reduction are presented in Figure 6.

Ammoniacal nitrogen reduction

All bacterial isolates demonstrated >75% reduction in ammoniacal nitrogen after 24 h. After 48 h, the reduction increased to 90.37% for Pri 3, 73.54% for Pri 4, 92.68% for Pri 8, and 95.18% for Pri 9. Notably, isolate Pri 9 achieved the maximum reduction in ammoniacal nitrogen within 48 h. The data on ammoniacal nitrogen reduction are presented in Figure 7.

Identification of the selected bacterial isolates using biochemical tests and VITEK 2

We have tested all the isolates against 47 different Sugars for the identification of Bacteria with a biochemical test, and the results we got are mentioned in Table 4.

Antibiotic sensitivity tests of the selected bacterial isolates

We have tested all the isolates against 47 different antibiotics for the selected isolates depending on the biochemical identification test, and the results we got after the incubation period are mentioned in Table 5.

Out of 47 antibiotics tested, isolate Pri 3 exhibited sensitivity to 32; Pri 4 showed sensitivity to 33 and intermediate sensitivity to 1; Pri 8 displayed sensitivity to 26, intermediate sensitivity to 7, and resistance against 1; and isolate Pri 9 demonstrated sensitivity to 35 antibiotics. Thus, the selected bacterial isolates were found to be sensitive to the majority of the antibiotics tested, suggesting vulnerability and positive response to antibiotics in case of infections, even after proper disinfection of the bioremediated textile printing wastewater.

Identification of the selected bacterial isolates by 16S rRNA sequencing

According to the report from NCMR, the results of 16S rRNA sequencing for the selected bacterial isolates are as follows:

The Pri 3 isolate matches with the Alcaligenes aquatillis LMG 22996(T) strain with approximately 99.93% similarities, as per the gene-sequencing database.

TGACGGGCGGTGTGTGCAAGACCC
GGGAACGTATTCACCGCGACATTCTGATCC
GCGATTACTAGCGATTCCGACTTCACGCAG
TCGAGTTGCAGACTGCGATCCGGACTACGA
TCGGGTTTCTGAGATTGGCTCCCCCTCGCG
GGTTGGCGACCCTCTGTCCCGACCATTG
TATGACGTGTGAAGCCCTACCCATAAGG
GCCATGAGGACTTGACGTCATCCCCA
CCTTCCTCCGGTTTGTCACCGGCAGTCTCATT
AGAGTGCTCTTGCGTAGCAACTAATGACAA
GGGTTGCGCTCGTTGCGGGACTTAACCCAA
CATCTCACGACACGAGCTGACGACAGCCA
TGCAGCACCTGTGTTCCGGTTCTCTTGCGA
GCACTGCCAAATCTCTTCGGCATTCCAGAC
ATGTCAAGGG TAGGTAAGGTTTTTCGCGTTGCATCG
AATTAATCCACATCATCCACCGCTTGTGCG
GGTCCCCGTCAATTCCTTTGAGTTTTAATCTTGCG
ACCGTACTCCCCAGGCGGTCAACTTCACGCGTTA
GCTGCGCTACTAAGGCCTAACGGCCCCAACAGCT
AGTTGACATCGTTTAGGGCGTGGACTACCAGGGT
ATCTAATCCTGTTTGCTCCCCACGCTTTCGTGTCT
GAGCGTCAGTATTATCCCAGGGGGCTGCCTTCGCC
ATCGGTATTCCTCCACATATCTACGCATTTCACT
GCTACACGTGGAATTCTACCCCCCTCTGACATACTCT
AGCTCGGCAGTTAAAAATGCAGTTCCAAGGTTGAG
CCCTGGGATTTCACATCTTTCTTTCCGAA
CCGCCTACACACGCTTTACGCCCAGTAATTCCGATT
AACGCTTGCACCCTACGTATTACCGCGGCTGCTG
GCACGTAGTTAGCCGGTGCTTATTCTGCAGATACC
GTCAGCAGTATCTCGTATTAGGAGATACCTTTTCTT
CTCTGCCAAAAGTACTTTACAACCCGAAGGC
CTTCATCATACACGCGGGATGGCTGGATCAG
GGTTTCCCCCATTGTCCAAAATTCCCCACTG
CTGCCTCCCGTAGGAGTCTGGGCCGTGTCTC

AGTCCCAGTGTGGCTGGTCGTCCTCTCAAACC
AGCTACGGATCGTTGCCTTGGTGAGCCTTTACC
CCACCAACTAGCTAATCCGATATCGGCCGC
TCCAATAGTGAGAGGTTCCGAAGAATCCCC
CCCTTTCCCCCGTAGGGCGTATGCGGTATT
AGCCACTCTTTCGAGTAGTTATCCCCCGCT
ACTGGGCACGTTCCGATATATTACTCACC
CGTCCGCCACTCGCCACCAAGAGAGCAAG
CTCTCTCGTGCTGCCGTTCGACTTGCATG
TGTAAAGCATCCCCGCTAGCGTTCAAT
CTGAGCCAGGAT

The Pri 4 isolate matches with the Priestia aryabhattai B8W22(T) strain with approximately 99.93% similarities, as per the gene-sequencing database.

TCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGT
TAGCGGCGGACGGGTGAGTAACACGT
GGGCAACCTCCTGTAAGACTGG
GATAACTTCGGGAAACCGAAGCTAA
TACCGGATAGGATCTTCTCCTTCATGGGAGAT
GATTGAAAGATGGTTTCGGCTATCACTTACAGAT
GGGCCCGCGGTGCATTAGCTAGTTGG
TGAGGTAACGGCTCACCAAGGCAACG
ATGCATAGCCGACCTGAGAGGGTGATCG
GCCACACTGGGACTGAGACACGGCCCAGAC
TCCTACGGGAGGCAGCAGTAGGGAATC
TTCCGCAATGGACGAAAGTCTGACGG
AGCAACGCCGCGTGAGTGATGAAGGC
TTTCGGGTCGTAAAACTCTGTTGTT
AGGGAAGAACAAGTACGAGAGTAACTGC
TCGTACCTTGACGGTACCTAACCAGA
AAGCCACGGCTAACTACGTGCCAGCAG
CCGCGGTAATACGTAGGTGGCAAGCGT
TATCCGGAATTATTGGGCGTAAAGCG
CGCGCAGGCGGTTTCTTAAGTCTGATGTG
AAAGCCCACGGCTCAACCGTGGAGGGT
CATTGGAAACTGGGGAACTTGAGTGCA
GAAGAGAAAAGCGGAATTCCACGTGTA
GCGGTGAAATGCGTAGAGATGTGGAGG
AACACCAGTGGCGAAGGCGGCTTTTTG
GTCTGTAACTGACGCTGAGGCGGAAAGC
GTGGGGAGCAAACAGGATTAGATACCC
TGGTAGTCCACGCCGTAAACGATGAGT
GCTAAGTGTTAGAGGGTTTCCGCCCTT
TAGTGCTGCAGCTAACGCATTAAGCACT
CCGCCTGGGGAGTACGGTCGCAAGACTGA
AACTCAAAGGAATTGACGGGGGCCCGCAC
AAGCGGTGGAGCATGTGGTTTAATTCGAA
GCAACGCGAAGAACCTTACCAGGTCTTGA
CATCCTCTGACAACTCTAGAGATAGAGCG
TTCCCCTTCGGGGGACAGAGTGACAGGTG
GTGCATGGTTGTCGTCAGCTCGTGTCGTG
AGATGTTGGGTTAAGTCCCGCAACGAGCGC
AACCCTTGATCTTAGTTGCCAGCATTTAG
TTGGGCACTCTAAGGTGACTGCCGGTGAC
AAACCGGAGGAAGGTGGGGATGACGTCAAA
TCATCATGCCCCTTATGACCTGGGCTA
CACACGTGCTACAATGGATGGTACA
AAGGGCTGCAAGACCGCGAGGTCAAGCCAA

The Pri 8 isolate matches with the Citrobacter werkmanii NBRC 105721(T) strain with approximately 99.78 % similarities, as per the gene-sequencing database.

ACGGGCGGGTGTGTGCAAGGCCCGGGAACGTAT
TCACCGTGGCATTCTGATCCACGATTACTAG
CGATTCCGACTTCATGGAGTCGAGTTGCAG
ACTCCAATCCGGACTACGACATACTTTATG
AGGTCCGCTTGCTCTCGCGAGGTCGCTTCT
CTTTGTATATGCCATTGTAGCACGTGTGTA
GCCCTACTCGTAAGGGCCATGATGACTTG
ACGTCATCCCCACCTTCCTCCAGTTTATC
ACTGGCAGTCTCCTTTGAGTTCCCGGCCTAAC
CGCTGGCAACAAAGGATAAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATTTCACA
ACACGAGCTGACGACAGCCATGCAGCACC
TGTCTCAGAGTTCCCGAAGGCACCAAAGCAT
CTCTGCTAAGTTCTCTGGATGTCAAGAGTA
GGTAAGGTTCTTCGCGTTGCATCGAATTAA
ACCACATGCTCCACCGCTTGTGCGGGCCCCC
GTCAATTCATTTGAGTTTTAACCTTGC
GGCCGTACTCCCCAGGCGGTCGA
CTTAACGCGTTAGCTCCGGAAGCC
ACGCCTCAAGGGCACAACCTCCAA
GTCGACATCGTTTACGGCGTGGACTACC
AGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGCACCTGAGCGTCAGTCTT
TGTCCAGGGGGCCGCCTTCGCCACCGG
TATTCCTCCAGATCTCTACGCATTTCA
CCGCTACACCTGGAATTCTACCCCCCTC
TACAAGACTCTAGCCTGCCAGTTTCGGAT
GCAGTTCCCAGGTTGA GCCCGGGGATTT
CACATCCGACTTGACAGACCGCCTGCGTGC
GCTTTACGCCCAGTAATTCCGATTAACGCT
TGCACCCTCCGTATTACCGCGGCTGCTGGC
ACGGAGTTAGCCGGTGCTTCTTCTGCGAGT
AACGTCAATTGCTGCGGTTATTAACCACAA
CACCTTCCTCCTCGCTGAAAGTACTTTACAA
CCCGAAGGCCTTCTTCATACACGCGGCATGG
CTGCATCAGGCTTGCGCCCATTGTGCAATAT

TCCCCACTGCTGCCTCCCGTAGGAGTCTGGAC
CGTGTCTCAGTTCCAGTGTGGCTGGTCA
TCCTCTCAGACCAGCTAGGGATCGTCG
CCTAGGTGAGCCGTTACCCCACCT
ACCAGCTAATCCCATCTGGGCACATCCGA
TGGCAAGAGGCCCGAAGGTCCCCCTC
TTTGGTCTTGCGACATTATGCGGTATTAGCT
ACCGTTTCCAGTAGTTATCCCCCTCCATCG
GGCAGTTTCCCAGACATTACTCACCCGTCCG
CCACTCGTCACCCGAGAGCAAGCTCTCTGTGCT
ACCGTTCGACTTGCATGTGTTAGGCCTGCCGCC
AGCGTTCAATCTGAGCCA

The Pri 9 isolate has 100% similarities with the Shewanella chilikensis JC5(T) strain, as per the gene-sequencing database.

CAAGGCCCGGGAACGTATTCACCGTG
GCATTCTGATCCACGATTACTAGCGATTCCGAC
TTCATGGAGTCGAGTTGCAGACTCCAATCCGGAC
TACGACCGGCTTTATGAGATTAGCTCCACCTCGC
GGCTTCGCAACCCTCTGTACCGACCATTGTAGCA
CGTGTGTAGCCCTACTCGTAAGGGCCATGATGA
CTT GACGTCGTCCCCACCTTCCTCCGGTTTATCA
CCGGCAGTCTCCCTAAAGTTCCCGGCATTA
CCCGCTGGCAAGTAAGGATAGGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATTTCACAA
CACGAGCTGACGACAGCCATGCAGCACCTGT
CTCTCAGTTCCCGAAGGCACACCTGCGTCTC
CGCTGGCTTCTGAGGATGTCAAGAGTAGGTA
AGGTTCTTCGCGTTGCATCGAATTAAACCAC
ATGCTCCACCGCTTGTGCGGGCCCCCGTCAA
TTCATTTGAGTTTTAACCTTGCGGCCGTACT
CCCCAGGCGGTCTACTTAATGCGTTAGCTTG
AGAGCCCAGTGTTCAAGACACCAAACTCCGA
GTAGACATCGTTTACGGCGTGGACTACCAGG
GTATCTAATCCTGTTTGCTCCCCACGCTTTC
GTGCCTGAGCGTCAGTCTTTGTCCAGGGGGC
CGCCTTCGCCACCGGTATTCCTCCAGATCTC
TACGCATTTCACCGCTACACCTGGAATTCTA
CCCCCCTCTACAAGACTCTAGTTTGCCAGTT
CGAAATGCGGTTCCCAGGTTGAGCCCGGGGC
TTTCACATCTCGCTTAACAAACCGCCTGCGC
ACGCTTTACGCCCAGTAATTCCGATTAACGC
TCGCACCCTCCGTATTACCGCGGCTGCTGGC
ACGGAGTTAGCCGGTGCTTCTTCTGCGAGTA
ACGTCACAGCTAGCAGGTATTAACTACTAAC
CTTTCCTCCTCGCTGAAAGTGCTTTACAACC
CGAAGGCCTTCTTCACACACGCGGCATGGCT
GCATCAGGGTTTCCCCCATTGTGCAATATTCC
CCACTGCTGCCTCCCGTAGGAGTCTGGGCCG
TGTCTCAGTCCCAGTGTGGCTGATCATCCTCT
CAGACCAGCTAGGGATCGTTGCCTAGGTGAGC
CATTACCTCACCTACTAGCTAATCCCACCTGG
GCTTATCCATCAGCGCAAGGACCGAAGTTCCC
CTGCTTTCCCCCGTAGGGCGTATGCGGTATTA
GCAGTCGTTTCCAACTGTTATCCCCCACAAAT
GGGCAAATTCCCAGGCATTACTCACCCGTCCG
CCGCTCGTCATCTTCAAAAGCAAGCTTTTGAAAT
GTTACCGCTCGACTTGCATGTGTTAGGCCTG
CCGCCAGCGTTCAATCTGAGCCA

CONCLUSION

Addressing environmental restoration amidst the current state of pollution poses a significant challenge. Despite decades of research in colored wastewater treatment, finding an effective approach remains elusive. In light of this, the present study explored the utilization of textile printing wastewater inoculated with cow dung to isolate potential bacterial species for the efficient biological treatment of textile printing wastewater. This bioremediation strategy demonstrated the effectiveness of four enriched bacterial species, namely, *A. aquatilis* LMG 22996(T), *P. aryabhattai* B8W22(T), *C. werkmanii* NBRC 105721(T), and *S. chilikensis* JC5(T), in substantially reducing color, BOD, COD, and other polluting parameters of textile printing wastewater. However, considering their superior capacity to decolorize a diverse range of dyes in natural settings and achieve the complete mineralization of wastewater, *C. werkmanii* NBRC 105721(T) and *S. chilikensis* JC5(T) emerged as the most effective biological candidates among the four isolates. To further understand the depth of dye decolorization by these isolates, we are currently studying the biochemical mechanisms involved. Additionally, we are attempting to isolate the genes responsible for dye degradation. We hope that, in the future, cow dung will be recognized as a reservoir of microbial species with high potential for the bioremediation of pollution.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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None.

DATA AVAILABILITY

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

Not applicable.

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