Dye Decolourization and identification of isolated bacterial strains from collected water sample

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Abstract

The contamination of water bodies with synthetic dyes from textile and industrial effluents poses serious environmental and health challenges. This study investigates the decolourization potential of bacterial strains isolated from dye-contaminated water samples. Bacteria were isolated using nutrient agar and screened for their ability to decolourize various dyes under controlled conditions. Morphological and biochemical analyses were conducted to identify the most effective strains. The results revealed that certain isolated bacteria exhibited significant decolourization activity, with potential applications in bioremediation of dye-laden wastewater. These findings highlight the promise of using indigenous bacterial isolates as eco-friendly and cost-effective agents in wastewater treatment technologies.

Keywords- Decolourization, textile effluents, waste water, dye-contaminated, bacterial isolates etc.

1. Introduction

Wastewater discharge and remaining dyes, which are mutagenic and carcinogenic, are the main issues (Kumar et al., 2016). There are many different kinds of chemicals and dyes that are used in the textile industry. Because they are long-lasting, colorfast, and available in a variety of shades, synthetic dyes are frequently used (Chaurasia and Kumar, 2022). Seventy percent of the more than 100,000 dyes that are commercially available are azo dyes. More than 8,000 chemicals Compounds used in the dying industry's operations include metals, surfactants, formaldehyde, salts, and sulphides (Bhatiya and Devraj, 2017). Over 10–25% of the exploited dyes have been lost during this wet process, and about 2–20% is released into the environment

directly as effluents (Sharma and Yadav, 2021). Aside from dyes, other additives have also been used in different stages as substrates or as aqueous systems with large volumes of water and nearly the same volume released into the environment with variable parameters and characteristics. These additives include antifoaming agents, solvents, whitening agents, pH conditioners, and finishing agents (Hassan and Carr, 2018). Metal ions, inorganic compounds, dissolved solids; biological oxygen demand, suspended particles, chemical oxygen demand, and numerous other soluble compounds are among the other important contaminants found in textile wastewater (Sarkar et al., 2017).

Water bodies, sunlight penetration, photosynthetic activity, aquatic organisms, and ecosystem integrity are all negatively impacted by the disposal and dumping of toxic wastewater from a variety of industries (Sarkar et al., 2017; Carvalho and Santos, 2015). As a result, before being discharged into freshwater streams, this wastewater must be treated. Physical and chemical Techniques for decolorizing and deteriorating dyes are frequently employed. Nevertheless, the majority of these methods have disadvantages, such as high operating expenses and the need to dispose of a large amount of sludge produced during these processes (Kumar et al., 2016). As a result, the majority of research has focused on using the most useful and eco-friendly methods, like biological processes. Among the many advantages of biological treatments are their affordability, simplicity, reduced production of surplus sludge, and versatility due to their ability to be applied to a variety of effluents.

Bio-decolorization and deterioration of textile azo dyes have gained considerable recognition due to their low capital requirement, environmental friendliness, and decreased sludge production (Carvalho and Santos, 2015; Lade, 2015). A key element in the success of microbial decolorization is the ability of specific microbial communities such as bacteria, fungi, yeasts, algae, actinomycetes, and others—that may break down textile azo dyes to adapt and become active (Kumar et al., 2016). Despite the fact that plants carry out many important ecological functions, such as supplying habitat for wildlife, halting soil erosion, and contributing a substantial amount of organic materials that are essential for soil fertility, numerous studies demonstrate that textile dyes and industrial effluents have a devastating impact on the biomass as well as on the rates at which seeds of different crops germinate.

Anaerobic microbial degradation of textile dyes, particularly azo, involves the reductive cleavage of azo (-N=N-) bonds with the production of several enzymes, including laccase, lignin peroxidase, and azoreductase. It consequently creates a colorless solution. The ensuing intermediate metabolites undergo multi-step transformation using either anaerobic or aerobic techniques (Lopes et al., 2022; Koller, and Mukherjee, 2022).

The majority of azo dye biodegradation research focuses on bacteria and fungi (Parshetti et al 2010; Mani and Hameed, 2019). Bacterial decolorization is typically quicker and more effective than that of fungi (Chundawat et al., 2022). For effective dye removal in textile dye effluents, bacteria are an affordable and environmentally friendly substitute. Furthermore, compared to other remediation decolorization methods, the biological treatment process has lower operating costs (Abiri et al., 2017). Many microbes were used as bioremediation agents to treat textile industry dye effluents. The first a dye-degrading bacteria to be isolated were *Bacillus subtilis* in 1977, *Aeromonas hydrophila* in 1978, and *Bacillus cereus* in 1980. Numerous additional aerobic bacteria, Numerous researchers have reported that certain bacteria, including *Bacillus sp., Bacillus cereus, Bacillus subtilis SPR42, Corynebacteria, Exiguobaterium sp. RD3 for reactive blue 172, Georgenia sp. CC-NMPT-T3, Pseudomonasputida, and Pseudomonassp.,* are strong dye degraders. Furthermore, for the azodyed degradation, bacterial consortia comprising *Bacillus sp., Bacillus cereus, Bacillus myocoides, Bacillus subtilis*, and *Micrococcus sp.* with *Pseudomonas sp*. have also been used (Mahmood et al., 2015).

Water pollution caused by textile effluent may be remedied by physical, chemical, and biological methods. Because physical and chemical treatments are more expensive and require more attention, local industries cannot afford them. Therefore, an economic approach is necessary to overcome these kinds of issues. Despite the use of a multidisciplinary treatment approach, Indian environmental policy still does not strictly regulate zero discharge. In order to recycle, collect, and ensure zero wastewater discharge to the environment, the National Green Tribunal in India has established an environmental law of Zero Liquid Discharge (ZLD) from textile industrial units. ZLD system employs a number of contemporary wastewater treatment technologies. India's highly fragmented wastewater treatment industry makes it challenging to achieve economies of scale (Brydges, 2021). Because the dyeing industry cluster is widely distributed, it becomes difficult to collect and treat effluents. Nonetheless, certain technologies are now

available, which makes ZLD systems efficient and profitable. The disposal of untreated wastewater in nearby ponds or rivers instead of the Common Effluent Treatment Plant (CETP) represents a major research gap. Research on the significance of decolorization by the individual bacteria and the consortium formed is discussed. Examining how well the isolated native bacteria decolorize the dye in the effluent is the primary goal of this research paper.

Two locations from the Raniya textile dyeing industry in Kanpur, U.P, were selected for wastewater collection. Before being used, water samples from the textile industry were gathered in polypropylene bottles that had been previously acid-washed and put in a $40^{O}C$ freezer. At least five red dyes (DR) and eight blue dyes (DB) were gathered. After that, the test wastewater sample was filtered to get rid of solid waste like clothing, threads, etc. The initial samples of Hof wastewater, color, and temperature were confirmed. Prior to storage, these samples were appropriately labeled.

2. Collection of Sample- Two different samples of Waste water from Raniya Industrial area was collected as shown in Fig 1.

Figure 1: Waste water collected in the industrial Area (Raniya)

3. Preparation of Culture Media

Nutrient Agar (NA)- 28 grams of nutrient agar (NA) were combined with 1 liter of purified water and heated until the NA was fully dissolved. After that, it was autoclaved for 15 minutes at 121 degree Celsius. The media was thoroughly mixed and cooled. After that, the media was transferred into sterile autoclaved Petri plates.

Nutrient Broth (NB)- 100 milliliters of ultrapure water were mixed with 2.5 grams of NB, which was then heated until it was fully dissolved. After that, it was autoclaved at standard pressure and temperature (121˚C/15 lbs) for 15 minutes.

4. Samples Physiochemical Analysis- (a) Blue dye (b) Red dye

Temperature, pH, Electric Conductivity (EC), Salinity, Sulfate, Phosphate, and other physio-chemical parameters were also estimated for all collected wastewater samples in accordance with standard protocols. The Central Pollution Control Board's (CPCB) standards were compared to the effluent's obtained physicochemical values.

4.1 Temperature

One crucial factor that affects the rate of chemical reactions is temperature. Temperature affects life. Microorganisms can also be categorized based on their habitat in different temperatures. Wastewater's temperature resulted from the accumulation of various chemical compound constituents that were also directly influenced by a number of other factors.

Method:

The thermometer was dipped into a one-liter sample of wastewater until the reading stabilized. The wastewater sample's temperature was calculated to be °C. A thermometer at the sampling location recorded this.

4.2 pH

The most crucial element on which other physical factors rely is the pH value of wastewater. The pH value, which determines whether water is acidic or alkaline, is the negative log [H+]. A higher value indicates that the solution is acidic (pH<7), while a lower value indicates that it is alkaline (pH>7). Depending on how many contaminants are in the solution, wastewater or contaminated water has different conditions. A specification tool used to ascertain the acidity or alkalinity of an aqueous solution is the pH scale. In essence, it assesses the concentration of hydrogen ions. Variations in pH value could be assigning of toxicity (Ejikeme et al., 2014).

Materials needed:

1. Water that has been distilled.
2. Digital pH Meter.

Method:

The digital pH meter was submerged in a liter of wastewater sample. For an accurate reading, the pH meter's lid was gently and steadily stirred. The test was repeated for accuracy.

4.3 Conductivity of Electrical Energy

The ability of a solution to generate electric current is determined by its electric conductivity. It is a specific evaluation tool to look at the water's purity. The higher range indicates that there are more organic substances in the aqueous solution in the form of ions. It depends on the rate of dissociation, concentration, and velocity of motion in an electric field. As a result, conductivity and dissolved ion concentration are directly correlated (Wani et al., 2017).

Materials needed:

1. Water that has been distilled
2. a digital electrical conductivity meter

Method:

An electrical conductivity meter was used to measure the wastewater sample's electric conductivity. The electrical conductivity meter was dipped into a liter of wastewater sample. After being in this position for two to three minutes, the instrument recorded the wastewater sample's conductivity.

4.4 Salinity

Materials needed:

1. Salinity meter

2. Water that has been distilled

3. The wastewater sample

Method:

A salinity meter was used to determine the wastewater sample's salinity. This salinity meter was immersed in a one-liter sample of wastewater. The instrument recorded the salinity of the wastewater sample after it had been in this position for at least two to three minutes. The soluble nature of dissolved If there are any salts in the wastewater, the oxygen level drops. The DO parameter adjusts for concentration readings in wastewater samples (mg/L).

4.5 Hardness

The amount of calcium and magnesium ions present in an effluent sample is referred to as its hardness. Water hardening is primarily caused by bivalent metallic ions, such as Mg^{+2}, Ca^{+2}, Fe^{+2}, Sr^{+2}, and Mn^{+2}. The Ethylene Di amine Tetra Acetic acid (EDTA) method was used to measure hardness. When EDTA and its sodium salts interacted with metal ions, an ache late-soluble complex was created.

Materials required:

1. Digital salinity meter
2. Distilled water
3. Wastewater sample

Method:

A hardness meter was used to measure the wastewater sample's hardness. The electrode was dipped into a one-liter sample of wastewater. The device recorded the wastewater sample's hardness after it was left in this position for two to three minutes.

4.6 Sulfate

The primary way that sulfur is absorbed by plants is as sulphate. The net downward or upward movement of sulphate in the soil water or the plant is controlled by the texture and composition of the soil (Nair et al., 2023). For sulphate precipitation, barium perchlorate is combined to create barium sulphate. The soluble efficacy of barium sulphate is reduced by the organic solvent

that is utilized. Through a reaction with Thorin, the amount of barium (II) ions is estimated spectrophotometrically at 520 nm.

Materials needed:

1. A solution of barium perchlorate stock: To create a 100 ml barium perchlorate stock solution, 210 mg of anhydrous barium perchlorate and 0.1 M perchloric acid were combined.
2. Solution of the barium per chlorate reagent: To make 1 L, 10 mL B of barium perchlorate stock was dissolved.
3. Solution of Thorin Reagent: In a volumetric flask, 5 mL 0.01 M perchloric acid was mixed with 125 mg of disodium salt, and the mixture was diluted to 50 mL. It had just been made.
4. Standard solution of sulphate: 1L of 31.25 mL of 0.05 MS sulfuric acid was substituted.
5. Spectrophotometer

Method:

A sample of the pre-treated wastewater (four milliliters) was collected. After dissolving 10 mL of the barium perchlorate reagent solution, 250 µL of Thorin solution was added. A spectrophotometer measured the absorbance of the formed solution at a wavelength of 520 nm after it had been properly dissolved. The calibration curve was created and the standard solutions were created by raising the concentration of the sulfate solution.

Compute:

Milligrams per liter of sulphate were used to measure the sulphate values of wastewater samples, which were assessed using the standard calibration curve.

4.7 The phosphate

Phosphate is insoluble because it is frequently found to be complex with other ions such as calcium, aluminum, etc. Water should contain very little phosphate (µg/mL). Molybdovanadate and ammonium molybdate are widely used in the spectrophotometric method (Kuhad et al., 2012). According to the basic principles, in the solution of potassium antimony tartrate, orthophosphater reacts with ammonium heptamolybdate to form a phosphomolybdic acid

complex, which is then further reduced by ascorbic acid. The amount of orthophosphates in the wastewater sample was ascertained by spectrophotometrically measuring this color shift.

Materials needed:

1. Stock solution for the reagent: The final volume was 1 L after adding 800 ml of 0.5 M HCl, 0.044 g of potassium antimony tartrate trihydrate (in 100 ml of distilled water), and 1.9 g of ammonium heptamolybdate tetra hydrate (in 100 ml of distilled water).

2. Phosphorus calibration sample (10 ml)

3. The wastewater sample

4. Spectrophotometer

Method:

Six milliliters of wastewater sample and six milliliters of reagent solution were added to each test tube. The solution was properly dissolved and given 30 minutes to develop its color. A spectrophotometer was then used to measure the prepared solution's absorbance at a wavelength of 830 nm. The typical solutions were made by preparing the calibration curve by adding more phosphorus to the calibration sample solution.

Compute:

The standard calibration curve was used to estimate the phosphate values of wastewater samples, Which were then expressed in milligrams per liter of phosphate as shown in Fig. 2.

Figure 2: Phosphate Test

5. Isolation of microbes from wastewater

5.1 Isolation of bacteria- Serial dilution was applied to the wastewater sample as shown in Fig. 3. To screen bacterial samples, take 0.1 ml from each tube, spread it onto Nutrient Agar (NA) plates using a glass spreader, and then incubate it for 24 hours at 34 °C. After that single colonies were selected and streaked on nutrient agar media plates to obtain pure culture ads shown in Fig. 4.

Figure 3: Serial dilution of Wastewater Samples

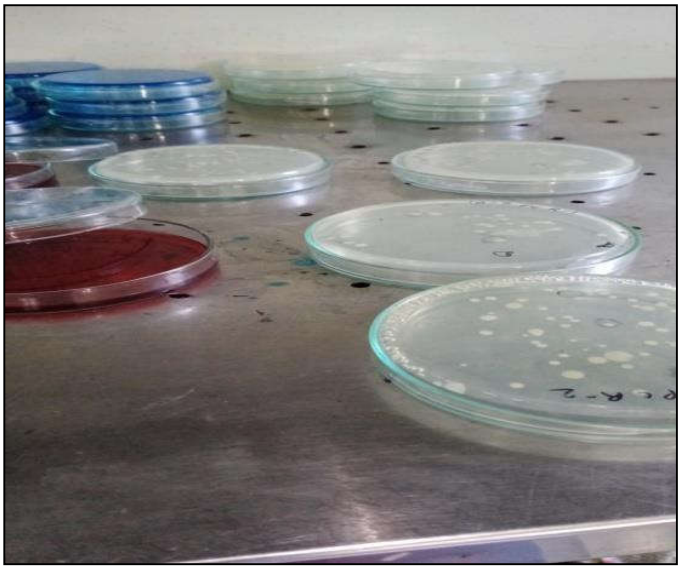
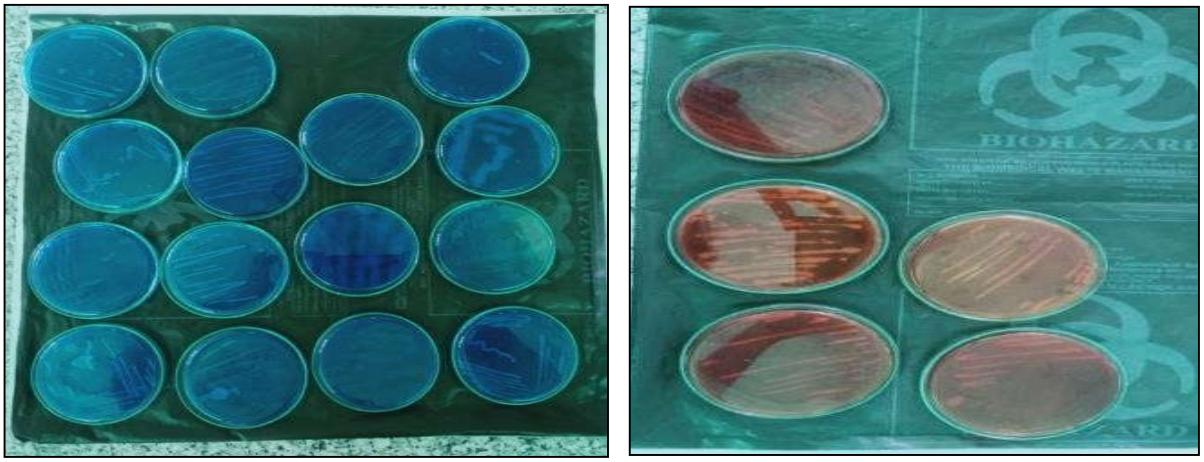


Figure 4: Isolation of bacterial cells from collected dye samples

5.2 Screening of isolated bacteria

0.1% red and blue dye was added to the screening medium to inoculate all of the chosen bacterial isolates. The screening medium showed all of the isolated strains, and stored for 24 hours at 37ºC as shown in Fig. 5. A maximum clearance zone in the screening plate was used to choose the potential strain. They were selected based on the isolated bacterial strains' morphological



characteristics. Bacterial strains were regularly maintained on nutrient agar slants. Along with the validation of a number of morphological parameters, the ideal growth criteria of various parameters (such as pH, temperature, etc.) were estimated in accordance with standard procedure (Cappuccino and Sherman, 2008).

Isolates on Blue dye Red dye samples

Figure 5: Morphological Identification

5.3 Mechanistic studies and dye decolorization strategy Assay for Dye Decolorization

Using the chosen bacterial isolates, the laboratory-scale investigations were designed to optimize dye decolorization processes. In order to start the screening and determine an appropriate range of decolorization criteria, the bioremediation process was started. A systematic survey of the textile dye industries in Raniya, Kanpur, UP, was used to choose the particular red and blue dyes. Four red dyes (DR) and seven blue dyes (DB) were gathered from those.

Materials needed:

1. Isolates of bacteria
2. The incubator
3. Centrifuge
4. Spectrophotometer

Method:

Every bacterial isolate was maintained in nutrient broth containing different concentration of DB and DR dyes (25, 50, 100, and 200 mg/L). Following an incubation period of 37ºC and frequent observations of dye degradation at various intervals, including 0 hours, 24 hours, 48 hours, 72 hours, and the fourth, fifth, and sixth days, the culture broths were centrifuged for 30 minutes at 2000 rpm. At a wavelength of 490 nm, the OD value of the supernatant solution was measured spectrophotometrically (Kushvaha et al., 2022). The following was used to estimate the percentage of dye decolorization:

Initial Absorbance − Final Absorbance = $\boldsymbol{Decolourizatio}$n (%) A first absorbance of 100

Finding the best range of decolorization parameters

To maximize the percentage of dye degradation through remediation, all the requirements for dye decolorization using bacterial isolates, either alone or in combination, were verified. **The screening experiments were conducted using the following procedures**:

1. To create the necessary concentration, the chosen dyes (red and blue dye) were dissolved in double-distilled water.

2. Additionally, bacterial isolates were dissolved in double-distilled water to the necessary concentration.

3. Selected bacterial isolates DB1, DB2, DB3, DB4, DB5, DB6, and DB7 isolated from blue textile effluent formed the Bacterial Consortium. Consortium 1 was the name given to it.

4. Selected bacterial isolates DR1, DR2, DR3, and DR4 from red textile effluent were used to create the Bacterial Consortium. It was regarded as consortium 2.

5. To optimize one parameter, the others were held constant.

6. After the pure culture of each isolate reached the exponential phase, it was aseptically transferred into the corresponding flask containing fresh nutrient broth. To create the 2% (v/v) bacterial consortium, a 1:1 ratio was employed.

7. The breakdown of dyes was carried out following the formation of the bacterial consortium.

8. Using NaOH or HCl, the dye decolorization reaction was maintained.

9. A UV-visible spectrophotometer was used to determine the samples' absorbance.

10. To ascertain the decolorization, changes in absorbance at the wavelength that corresponded to maximum absorbance were noted.

11. The following formula was used to calculate the percentage of dye decolorization: Decolorization percentage (%) = (Ai -Af) ×100/Ai

[where Af is the final absorbance (mg/L) and Ai is the initial absorbance (mg/L)]

12. An untreated dye sample in a negative control setup was also examined.

5.4 Spectral Analysis of UV Light

Analysis was performed at λ max peak and observed at each dye's maximum absorbance. To remove the bacterial culture from the supernatant, 2 ml of the inoculated broth culture was taken out of the conical flask and centrifuged for 15 minutes at 2000 rpm. At 490 nm, absorbance was measured.

5.5 Identification of Bacterial Strains Morphologically

The primary method of identifying bacterial isolates morphologically was by identifying their colonies and cells.

Colony attributes: Based on the bacterial colonies' size, shape, elevation, surface, margin, pigmentation, and opacity, the first step is to identify them. Cellular characteristics: Under a microscope, pure colonies of isolated bacteria were obtained in order to characterize the bacterial isolates based on the Gram reaction, cell shape, and motility test.

(i) Gram staining for Bacteria Identification

The most important and widely used microbiological staining technique was the Gram staining method. Gram-positive and/or Gram-negative bacteria were grouped based on the characteristics of their cell wall peptidoglycan layer. Gram-positive or gram-negative characteristics of a bacterial cell are primarily determined by its anatomical features and the composition of its cell wall. Following the application of violet color All of the bacterial strains were deep violet. To fix the bacteria's violet color, iodine solution was utilized. When the violet stain was not properly fixed by iodine, 95% ethanol decolorized some bacteria; when the violet stain was firmly fixed by iodine, it retained the original color of the other bacteria.

Materials needed:

1. Bacterial sample
2. Glass slide
3. A microscope
4. The inoculating loop

5. The Bunsen burner
6. Reagents for Gram staining

Method:

It was placed on a glass slide and allowed to dry. Before being stained, it was first fixed by heating. The slide was now filled with crystal violet, completely covered with a coverslip, and left for two minutes. After that, rinse with water. Iodine solution was then added to the slidegram and allowed to stand for a minute. Rinsed with water once more. After adding the 95% ethanol solution, it was left to stand for a minute. After that, water was used to rinse it. If there were still violet patches on the smear, it was treated with ethanol once more for 15 seconds and then washed with water. Safranine solution was then added, allowed to sit for ten seconds, and then rinsed with water. The slide was dried and then examined under a microscope.

ii) The shape of cells

Microscopic identification of cells is possible. They can be seen under a microscope as Vibrio, Coccus, and Bacillus because of their rigid bacterial cell walls. The shape of cells was additionally noted under a microscope during the gram staining observation, which allowed the bacterial isolates to be distinguished by their cell shapes.

6. Result and Discussion

Dyes are unsaturated, highly complex organic macromolecules that can absorb light and then emit reflected color. The majority of synthetic dyes are categorized primarily based on their physical and chemical characteristics, chromophore structure, and particle charge (Patel et al., 2013; Mathur et al., 2023). Due to their structural peculiarities and the presence of chromatophores, which enable them to absorb visible spectrum light at any wavelength with regulation through the presence of one or more double bonds, dyes can impart any color (Benkhaya et al., 2020). However, because of their stoichiometric arrangements and structural resistance, the majority is not biodegradable (Singh, D., & Gupta, N. (2020). Very few of the leftover components of synthetic dyes dissolve in water, creating extremely toxic material that can readily enter a living organism through the food chain (Bilal et al., 2021). However, due to inadequate waste disposal practices and carelessness, there are a number of conventional methods and techniques available for the treatment of the waste delivered from the domestic,

industrial, and domestic sectors. Additionally, the majorities of harmful contaminants either goes untreated or produce intermediate products. These resistant dye residues raise a number of physiochemical parameters, including pH, BOD, and COD, when they are discharged into bodies of water (Katheresan et al., 2018). In this case, the dye degradation through remediation approach is commonly used and widely accepted only when the remediation process is shorter, the effluents are partially or completely degraded, a low-cost approach is used and less or no secondary sludge is produced (Prasad et al., 2014).

Wastewater effluents from textile dyeing industries contain a number of stubborn dye traces along with other toxic pollutants that can have a dangerous effect. In Raniya, Kanpur (UP), India, the textile dyeing industry's waste disposal sites provided the effluent samples. At the first stage, these samples were examined to confirm physicochemical parameters as summarized in Table 1 which lists the CPCB-permitted data as well as the physiochemical parameters of the blue and red dye effluent.

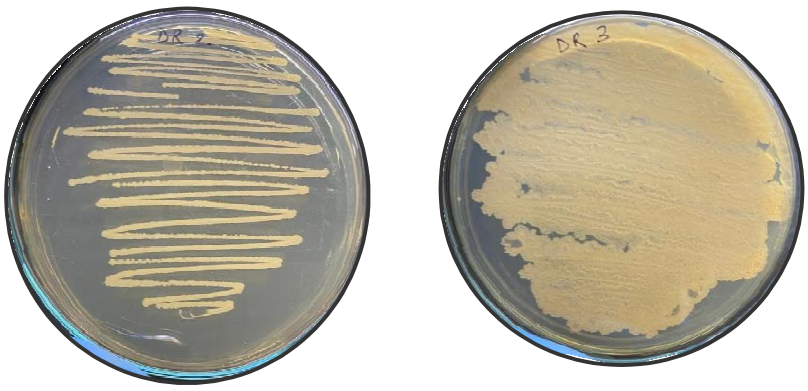
Table 1: Physical-Chemical Characterization of Raniya, Kanpur Wastewater Effluent Sample Before and After Treatment

Physiochemical Parameters	Effluent1 (Blue)	After Treatment by Consortium1	Effluent 2 (Red)	After Treatment by Consortium 2	Permissible limits for Textile Effluents CPCB
pH	8.1	3.02	8.4	2.77	6.5-6.8
Temp (^{0}C)	18.1	-	18.2	-	40
Electrical Conductivity (mS/Cm)	3.65	3.9	3.51	6.2	Nil
Salinity (% saturation)	5.9	4	5.48	3.7	Nil

Total Dissolved Solids (PPM)	1422	1100	1220	1140	1321
Dissolved Oxygen (mg/L)	12.2	7.6	13.5	8	4 to 6
Biological Oxygen Demand (mg/L)	197.4	134	181.6	94	20 to 30
Chemical Oxygen Demand	501.65	319.8	589.2	171.54	250
Nitrate (220nm)	18.73	13.63	20.34	12.23	10
Sulphate	392.1	-	299.421	-	1000
Phosphate	10.18	-	8.4	-	5

6.1 List of Bacterial Isolates

Thirteen sample isolates were independently obtained from the two sample sites in the current study, including blue and red-colored effluents, and cultivated in a nutrient agar medium with optimal temperature and culture conditions (Cappuccino and Sherman, 2008). The plates were checked for colonies that were well-isolated. The colonies underwent initial screening after being chosen based on various morphological characteristics as shown in Fig 6 and 7.



Isolate–DR1 **Isolate-DR2**

Isolate–DR3 **Isolate–DR4** **Isolate–DR5**

Figure 6: Isolates from Red Samples

Isolate-DB2 ***Isolate-DB3***

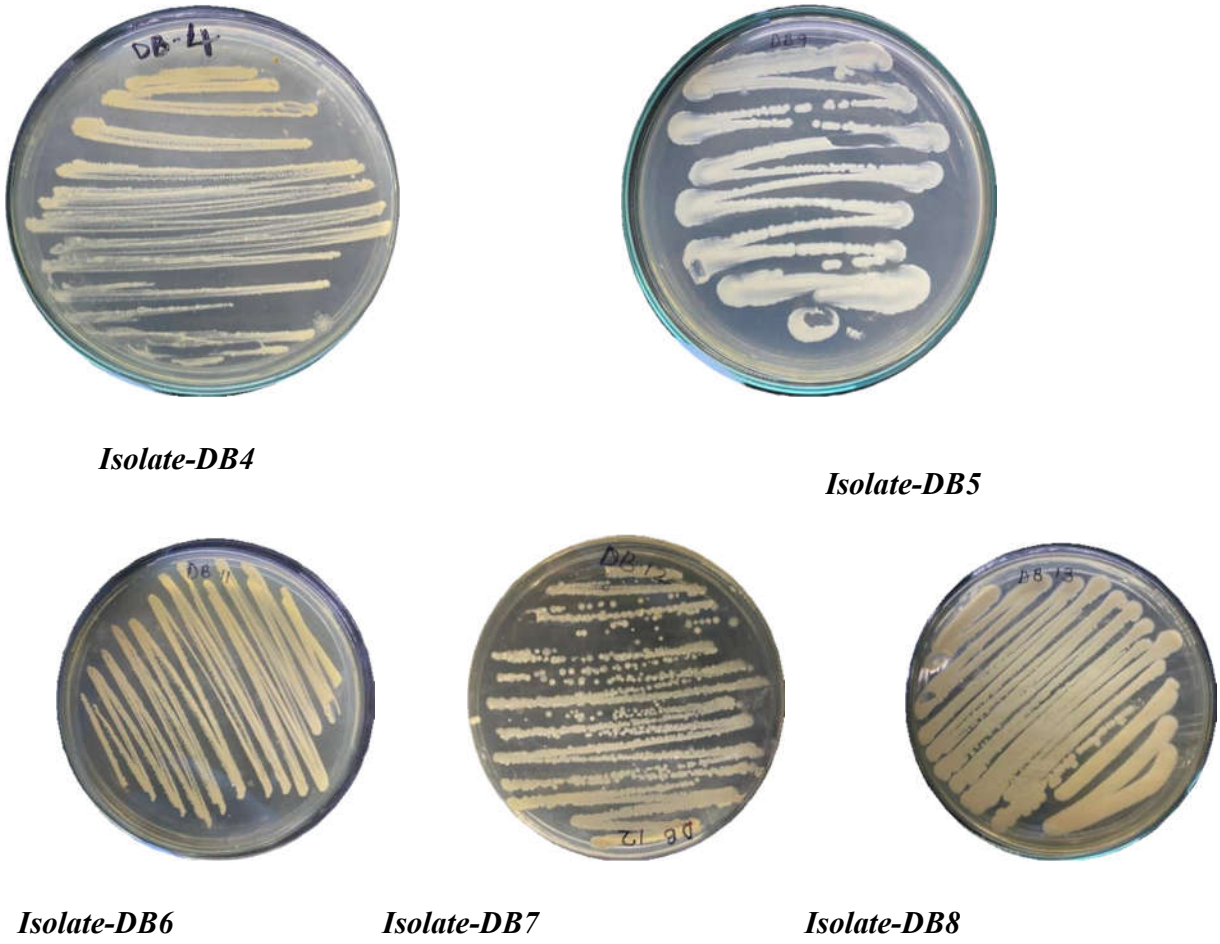


Figure 7: Isolates from Blue dye Samples

6.2 Initial Examination of Dye-Degrading Bacteria

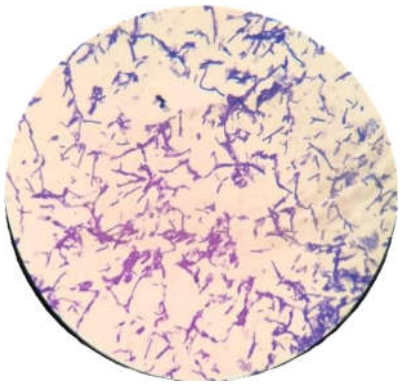
By inoculating them onto nutrient agar plates containing methylene blue and congo red dyes, the obtained isolates were screened and chosen based on the degradation of different textile dyes. The plates were checked for the clearance zone following the incubation period. The colonies were surrounded by a clear zone of bacteria that were positive dye-degrading, and the results were chosen for additional degradation. Eleven of the total isolates demonstrated positive outcomes, including a distinct zone of dye degradation.

6.3 Analysis of Dye-Degrading Bacteria Morphologically

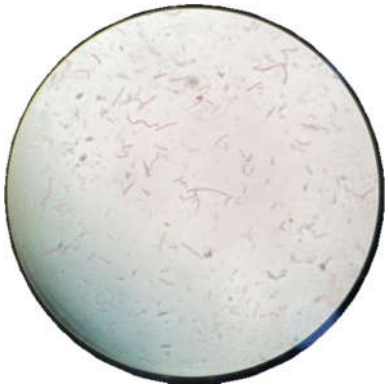
Gram staining analysis was used to validate all of the bacterial isolates for morphological evaluations using microscopical assessment, taking into account a number of parameters,

including size, shape, elevation, texture, margin, odor, and pigment, as shown in Table 2. The majority of the screened bacterial isolates were gram-positive, according to the morphological validations. It was found that the majority of the blue dye bacterial isolates had rounded edges. The red dye isolates, on the other hand, had filamentous, smooth, wavy, or curved edges and were either round or irregular in shape. No particular odor was present in any of the bacterial isolates. Blue dye bacterial isolates displayed smooth, dry, and somber textures, whereas the majority of red dye bacterial isolates had dry textures. Numerous diverse bacterial consortiums in a group or individual bacterial cells have also been used in a number of other studies to degrade different kinds of toxic and resistant dyes (Kumar et al., 2016; Prasad et al., 2014; Sanmuga Priya et al., 2015; Sanmuga Priya et al., 2016; Mustafa et al., 2021). All isolates' morphological and gram stain microscopic views are shown in Figure 8 (a–k).

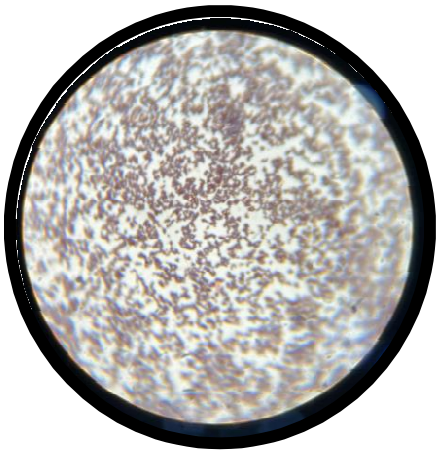
All these bacterial strains were analyzed for dye decolourization effects spectrophotometrically and absorbances were recorded with respect to time then graph were plotted as shown in Fig 9.



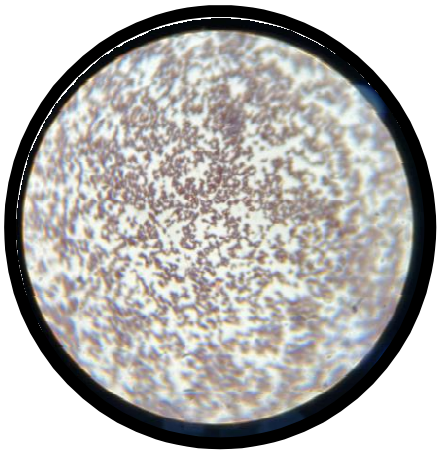
(a) Gram-Positive acilli (DB-1)



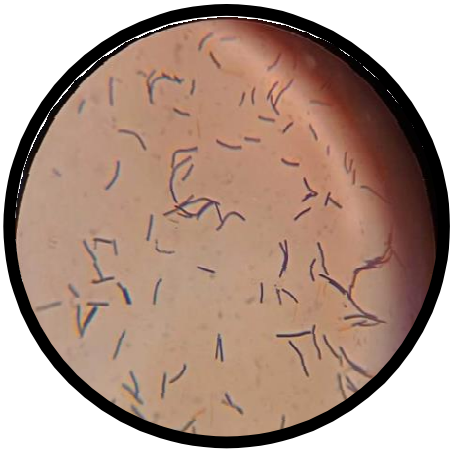
(b) Gram-Positive bacilli (DB-2)



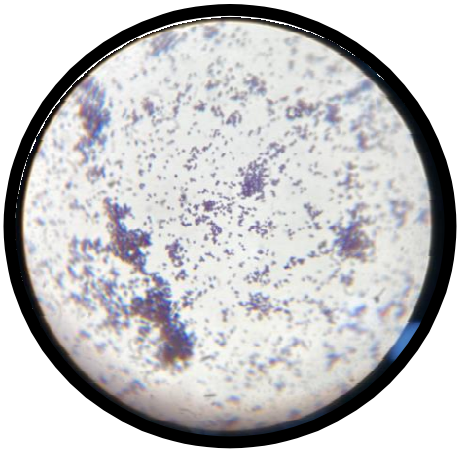
(c) Gram-Positive Cocci (DB-3)



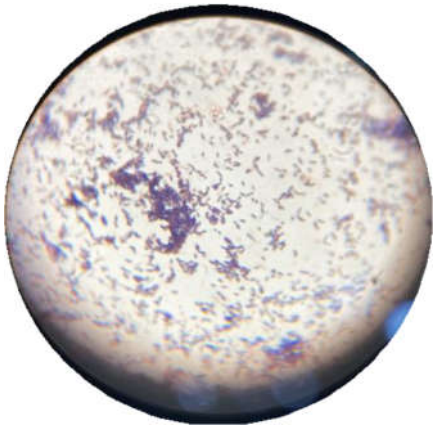
(d) Gram-Positive Cocci (DB-4)

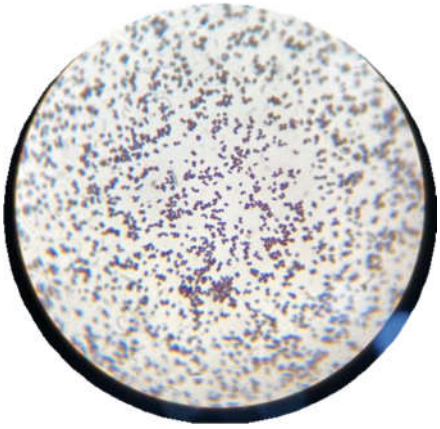


(e) Gram-Positive bacilli (DB-5)

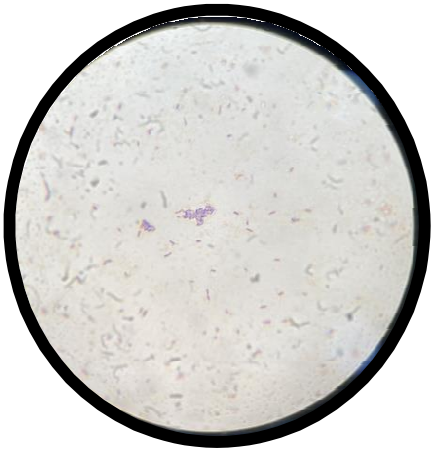


(f) Gram-Positive Cocci (DB-6)

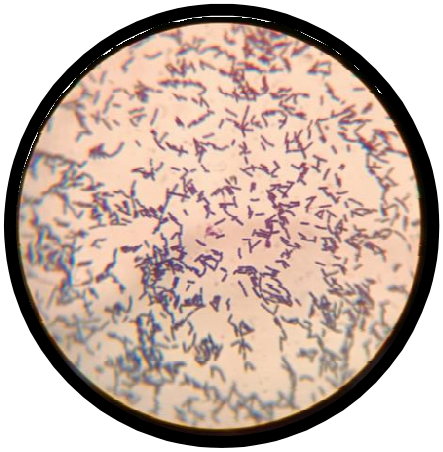




(g) Gram-Positive bacilli (DB-7)

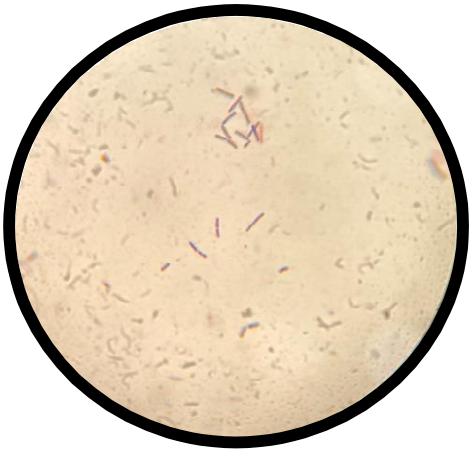


(h) Gram-Positive Cocci (DR-1)



(i) Gram-Positive bacilli (DR-2)

(j) Gram-Positive bacilli (DR-4)



(k) Gram-Positive bacilli (DR-5)

Figure 8 (a-k): Microscopic View after Gram staining of all Eleven Isolates

Table 2: Bacterial Cell Morphological Characteristics

Isolates	**Gram Stain**	**Morphology**							
Red dye		**Shape**	**Size**	**Shape**	**Margin**	**Elevation**	**Texture**	**Odour**	**Pigment**
DR1	Gram+	Cocci	Medium	Round	Filament	Flat	Dry	No	Non pigmented red
DR2	Gram+	Cocci	Large	Irregular	Smooth	Flat	Dry	No	Non pigmented-red
DR3	Gram-	Cocci	Large	Irregular	Wavy	Flat	Dry	No	Non pigmented
DR4	Gram+	Bacilli	Small	Irregular	Curved	Flat	Dry	No	Yellow Pigment
Blue dye		**Shape**	**Size**	**Shape**	**Margin**	**Elevation**	**Texture**	**odour**	**Pigment**
DB1	Gram+	Cocci	Large	Round	Curved	Flat	smooth	No	Off-white Pigmentation - blue
DB2	Gram-	Cocci	Small	Round	Curved	Flat	smooth	No	White pigmentation
DB3	Gram-	Bacilli	Medium	Round	Curved	Flat	Dry	No	White pigmentation
DB4	Gram+	Cocci	Medium	Round	Curved	Flat	Dry	No	White pigmentation - blue
DB5	Gram+	Cocci	Small	Round	Curved	Flat	gloomy	No	Non pigmented

DB6	Gram+	Bacilli	Medium	Round	Curved	Flat	smooth	No	Off White Pigmentation
DB7	Gram-	Cocci	Small	Round	Curved	Flat	Dry	No	Off White Pigmentation

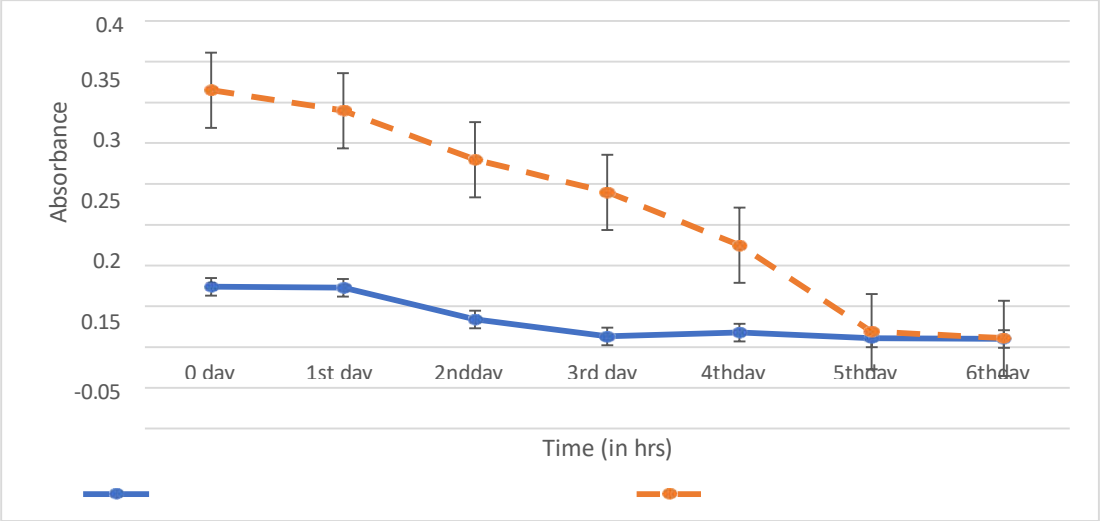


Figure 9: Dye decolorization assay using all bacterial isolates

7. Conclusion

The accumulation of azo dyes and other resistant dyes, which are non-biodegradable and can build up for years, poses major health risks. This is directly caused by the industrial effluents released in the highest concentration from the textile, tannery, and paint industries. On this point, using new microorganisms, particularly bacterial isolates, either alone or in combination can help release a variety of enzymes needed for this kind of dye degradation on a bigger scale. In order to identify possible bacterial isolates and their consortium, wastewater samples were gathered from two dyeing areas in Raniya Kanpur, and validated for the dye decolorization assay. The findings of this study could be used to identify native species in the desired area and break down contaminants more rapidly and effectively without causing harm or danger.

The idea that textile effluent could be a great way to isolate native bacterial species that can break down resistant toxic substances was supported by this study.

The textile effluent was the source of all 11 bacterial isolates, and it was determined that each isolate had a high potential for fully degrading and mineralizing effluent dyes.

The bacterial consortium of strong isolates isolated from two effluent samples totally degraded both of the samples.

Morphological analysis of both samples revealed the presence of gram-positive bacilli and cocci.

The percentage rate of decolorization in the blue dye degradation assay was 75.536% for DB3 and 87.044% for DB4, whereas the percentage rate of degradation in the red dye degradation assay was 59.016% for DR5.

The validation of Raniya dyeing regions and related possible bacterial strains for bioremediation applications is made easier by this study. Additionally, it can help with the thorough investigation of a bacterial consortium for textile effluent bioremediation.

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