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Screening of Bacterial Isolates for the Decolourization of Reactive Azo Dyes

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Abstract

The release of textile dye effluents into water bodies poses significant environmental concerns due to their toxicity and resistance to degradation. This study investigates the bioremediation potential of bacterial isolates for the decolourization of reactive azo dyes from textile effluent. The dye-contaminated wastewater was collected from a dyeing unit in Tirupur, Tamil Nadu, India, and bacterial strains were isolated and identified using morphological and biochemical characterization. The identified bacterial species included *Pseudomonas fluorescens*, *Bacillus subtilis*, *Azospirillum brasilense*, *Rhizobium japonicum*, and *Azotobacter chroococcum*. The bacterial isolates were screened for dye decolourization efficiency using a plate assay. The bacterial consortium demonstrated enhanced decolourization efficiency compared to individual isolates, suggesting synergistic interactions. The findings indicate that bacterial isolates, particularly *Pseudomonas fluorescens*, offer an effective, eco-friendly, and cost-efficient alternative for textile wastewater treatment. Compared to conventional chemical treatments, microbial bioremediation presents a sustainable approach to reducing environmental pollution caused by textile dye effluents. Further research on optimizing bacterial consortia and process parameters can enhance the scalability and efficiency of biological dye degradation.

Key words : Textile dye effluent, bacterial decolourization, reactive azo dyes, bioremediation, and wastewater treatment.

Dye degradation has recently become one of the most researched topics due to its outrageous and carcinogenic characteristics. Large amounts of industrial effluents are generated by these industries every year, causing mainly the aquatic pollution, which is quite dangerous for aquatic biota as well as for plants and other animals, like human

beings[5]. As per report, commercial production of these pigments around the globe has about 7 million tons annually[8]. Azo dyes contain at least one nitrogen-nitrogen (N=N) double bond, however, many different structures are possible[6]. Monoazo dyes have only one N=N double bond, while diazo and triazo dyes contain two and three N=N double bonds, respectively. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocycles or enolisable aliphatic groups[3]. These side groups are necessary for imparting the color of the dye, with many different shades and intensities being possible[2]. Furthermore, without any adequate treatment, this dye can cause tremendous damage to the environment and humans[1]. Contemporary research reports that the bacteria can quickly degrade the toxicity of the azo and direct dyes. Under aerobic conditions, azo dyes are not readily metabolised, although the ability of bacteria with specialized reducing enzymes to degrade certain azo dyes aerobically was reported[10]. In contrast, many bacteria reduce azo dyes under anaerobic conditions by the activity of unspecific, soluble, cytoplasmic reductase, known as azo reductases. The anaerobic reduction degrades the azo dyes converted into aromatic amines, which may be toxic, mutagenic, and possibly carcinogenic to mammals[9]. Therefore, to achieve complete degradation of azo dyes, another stage that involves aerobic biodegradation of the produced aromatic amines was necessary[7]. Extensively used coagulation/flocculation techniques produce large amounts of sludge, which requires safe disposal. Adsorption and, to a certain extent, membrane filtration techniques lead to secondary waste streams which need further treatment[4].

Collection of Textile Dye Effluent Sample :

The dye house effluent was collected from a dyeing unit in Tirupur district, Tamil Nadu, India. It was refrigerated at 4°C and used without any preliminary treatment.

Dyes used :

Reactive azo dyes were used in this study. The dye samples were commercially graded and supplied by the dealers of "SIGMA Aldrich, U.S.A". Reactive azo dyes used in this research are, Congo Red, Direct Blue 28, Direct Blue, Direct Green, Direct Red, Direct Orange, Direct Yellow and Direct Black.

Identification of Bacteria isolated from Textile Dye effluent :

The routine bacteriological methods, *i.e.* carried out identification of the bacterial isolates, a) By the Colony morphology (Staining techniques); b) Preliminary tests like Gram staining, Capsule staining, Endospore staining, Motility, Catalase and Oxidase; c) Plating on selective media and d) By performing Biochemical tests.

Screening of Bacterial Isolates for the Decolourization of Reactive Azo Dyes by Plate Assay :

The decolourization of textile Reactive azo dyes by bacterial isolates was determined by the Plate assay technique. The plate assay was performed to detect the decolorizing activity of bacteria isolated and identified from the textile dye effluent. The Nutrient agar and Reactive dyes (500 mg/L) were autoclaved at 121°C for 15 min. The bacterial cultures

Table–1. Identification and characterization of bacteria isolated from textile dye effluent

Character	*Bacillus subtilis*	*Pseudomonas fluorescens*	*Azospirillum brasilense*	*Rhizobium japonicum*	*Azotobacter chroococcum*
Gram staining	Gram positive rods	Gram negative rods	Gram Negative rods	Gram Negative rods	Gram-negative rods.
Endospore	Central spores present	No Endospores	No Endospores	Terminal endospore	No Endospores
Motility Catalase Oxidase	Non-motile Positive Negative	Motile Positive Positive	Motile Positive Positive	Positive Positive Negative	Motile Positive Positive
Nutrient agar	Colonies are large, circular or irregular, grey–yellow, granular and difficult to emulsify.	Smooth colonies, greenish or bluish tint.	Pinkish Colonies are produced.	Colonies are smooth, circular, white-cream, entire, opaque	Large, mucoid and brownish colonies. Due the pigment production. .
MacConkey agar	Non-lactose fermenting colonies	Non-lactose fermenting colonies	Lactose fermenting colonies	No growth	Non-lactose fermenting colonies
Glucose fermentation	Acid produced	No Acid produced	Negative	Acid produced	Negative
Mannitol fermentation	Acid produced	No Acid produced	No Acid produced	No Acid produced	No Acid produced
Sucrose fermentation	Acid produced	No Acid produced	No Acid produced	No Acid produced	Acid produced
Xylose fermentation	Acid produced	No Acid produced	No Acid produced	No Acid produced	Acid produced
Indole	Negative	Negative	Negative	Negative	Negative
Methyl Red Test	Negative	Negative	Negative	Positive	Negative
Voges Proskauer Test	Positive	Negative	Negative	Positive	Negative

Citrate utilization	Positive	Variable	Positive	Negative	Positive
Nitrate reduction	Positive	Positive	Positive	Positive	Variable
Gelatine hydrolysis	Positive	Positive	Negative	Negative	Positive
Starch hydrolysis	Positive	Variable	Negative	Negative	Positive
Urease	Negative	Negative	Negative	Positive	Negative

Table–2. Screening of Bacterial isolates for Dye degradation by Plate assay

S. no	Zone of Formation	Bacterial Isolation				
		Pseudo-monas fluorescens	*Bacillus subtilis*	*Azospi-rillum brasilense*	*Rhizo-bium japonicum*	*Azoto-bacter chroococcum*
1	Congo Red	32 mm	29 mm	25 mm	18 mm	20 mm
2	Direct Blue 28	30 mm	28 mm	24 mm	19 mm	18 mm
3	Direct Blue	32 mm	29 mm	25 mm	20 mm	20 mm
4	Direct Green	31 mm	30 mm	23 mm	19 mm	15 mm
5	Direct Red	29 mm	27 mm	25 mm	16 mm	15 mm
6	Direct Orange	29 mm	27 mm	24 mm	18 mm	16 mm
7	Direct Yellow	30 mm	29 mm	23 mm	17 mm	15 mm
8	Direct Black	31 mm	29 mm	25 mm	18 mm	16 mm

were plated on Nutrient agar plates containing Reactive azo dyes. The plates were wrapped with parafilm and were incubated in an incubator at 37 °C for 4 days. The plates were observed for clearance of the dye surrounding the colonies.

Identification and Characterization of Bacteria Isolated from Textile Dye Effluent:

Six different bacterial isolates were isolated and identified from the textile dye effluent. The characteristics of the identified bacterial isolates were furnished in Table–1. The isolated bacterial isolates were identified and characterized as *Azospirillum brasilense, Rhizobium japonicum, Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens*.

Screening of Bacterial Isolates for the Decolourization of Reactive Dyes by Plate Assay :

The bacterial isolates were screened

for the decolourization of reactive dyes by Plate assay and the results were tabulated in Table-2. The identified bacterial isolates *viz*., *Azospirillum brasilense, Rhizobium japonicum, Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens* were used for Plate decolourization assay. Maximum decolourization was recorded by *Pseudomonas fluorescence* in the plate containing Reactive Orange 16 (32 mm) followed by *Bacillus subtilis* (29 mm), *Azosprillium brasilense* (25mm), *Azotobacter chroococcum* (18 mm), *Rhizobium japonicum* (20 mm). The zone of inhibition in the plates containing the remaining reactive dyes was also recorded by the bacterial isolates in the above given order. Next to Reactive Orange – 16, the bacterial isolates showed maximum zone of inhibition in the plate containing Reactive Black – B followed by Reactive Yellow – MR.

Decolourization of Textile Reactive Azo Dyes by Bacterial Isolates and Consortium:

The decolourization of textile reactive azo dyes by six bacterial isolates *viz*., *Azospirillum brasilense, Rhizobium japonicum, Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens* and bacterial consortium was studied and the results were showed in Table – 3. Maximum decolourization percentage was observed in the medium inoculated with *Azospirillum brasilense, Rhizobium japonicum, Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens*. The decolourization percentage was maximum in the medium containing Reactive Orange – 16. Next to Reactive Orange – 16, maximum decolourization was observed in Reactive Black – B followed by Reactive Yellow – MR.

Application of traditional waste water treatment requires enormous cost and continuous input of chemicals which becomes uneconomical and causes further environmental damage. Hence, economical and ecofriendly techniques using microorganisms like bacteria and fungi can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for colour removal of textile dyes. Thus, by this present study, it was concluded that the bacterial isolates like *Azospirillum brasilense, Rhizobium japonicum, Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens* were used as a good microbial source for the textile Reactive dye decolourization and waste water treatment in textile dye industries. The bacteria *Pseudomonas fluorescens* showed high decolourization percentage against textile Reactive dyes followed by *Bacillus subtilis, Azospirillum brasilense, Rhizobium japonicum* and *Azotobacter chroococcum*. Among the eight dyes tested, the dye Direct Orange was highly decolourized by the bacterial isolates when compared to other reactive dyes. The bacterial isolates showed more effective decolourization of the test textile Reactive dyes when compared to the individual bacterial isolates which was isolated from the textile dye effluent.

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