Phenol Red Dye Decolorization by Bacterial Isolates

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Abstract : The textile and dyeing industries produce large amounts of wastewater that are highly colored. This wastewater can be a major environmental pollutant, and it is important to find ways to decolorize it. This study investigated the ability of bacterial isolates to decolorize phenol red dye. Phenol red dye is a common dye that is used in the textile industry. The bacterial isolates were obtained from a previous study, and they were screened for their ability to decolorize phenol red dye in a Bushnell-Hass (BH) medium. The screening revealed that only two of the seven isolates tested positive for dye decolorization. These isolates were named R3 and R7. The degree of decolorization was determined by measuring the percentage of dye loss in relation to the control group. The isolates R3 and R7 decolorized the phenol red dye to a degree of 75 % and 90 %, respectively. The isolates R3 and R7 were identified as Pseudomonas spp. and Staphylococcus spp., respectively. These identifications were based on the morphological, colony, and biochemical characteristics of the isolates. These results suggest that the bacterial isolates R3 and R7 have the potential to be used to decolorize phenol red dye. respectively.
Key Words : Decolorization, Phenol red dye, *Pseudomonas spp*, *Staphylococcus spp*

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1. Introduction

Numerous industrial sectors including the textile, chemicals, leather, pigment, and dyeing industries release a vast amount of colored effluents that contain dyes as a potential source of pollutants (1). The textile industry is one of the largest producers of effluents contaminated with dyes (1). Different organic pollutants are introduced into the natural water resources and the land by the residual dyes that are released from these effluents. (2). About 15% of the dyes that are used extensively in industrial applications end up in effluents. (3). Triarylmethane dyes are synthetic organic compounds containing triphenylmethane backbones. At least two aryl groups in phenol dyes have hydroxyl groups at the para locations.

Phenol red dye is used pH indicator dye widely used as an analytical reagent, diagnostic agent for assessing kidney function (injection), and intestinal absorption of medicines (taken orally). It is beneficial in the pH range of 6.8 (yellow) - 8.4 (red), commonly used as a water-soluble sodium salt or as a the solution in dilute sodium bicarbonate or sodium hydroxide. Unfortunately, traditional wastewater treatment methods are ineffective at getting rid of dyes and are expensive, produce potentially dangerous byproducts, and use a lot of energy (4). One of the biggest issue facing the modern world is the environmental contamination caused by textile dye. For the removal of colors from textile effluent, a variety of physical treatments, including ultrafiltration, reverse osmosis, ion exchange, and dye absorption on different absorbents are available. As an alternative, biological techniques remove dyes from the polluted region using a variety of taxonomic families of microbes, including bacteria, fungi, yeast, and algae (5). Thus, the present study aimed to isolate, screen, and quantify the capability of bacterial isolates for decolorizing dyes.

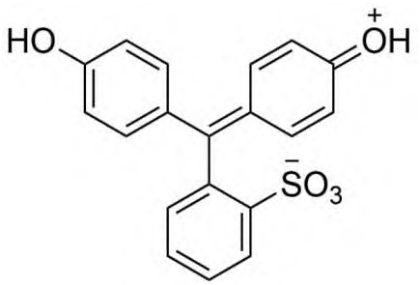

Figure 1 – Chemical structure of Phenol Red dye

2. Material and Methods :

2.1. Sample collection :

In sterile containers, 500 ml of textile effluent samples were taken from the industrially contaminated site at GIDC, Pandesara, Surat, Gujarat, India. Prior to further analysis, every sample that was obtained was kept at 4°C.

2.2. Isolation and Screening of dye-decolorizing bacteria :

1ml of the sample was inoculated in 100ml of sterile modified Bushnell Hass (BH) medium containing 0.4% glucose, 0.% yeast extract, and 100mg/l of phenol red dye were used as the inoculation media. The medium's pH was maintained at 7.0. Flasks were incubated for 24 hours at 37°C and 150 rpm. The broth was serially diluted up to 10 -6 after incubation. A modified Bushnell Hass (BH) medium mixed with 100 mg/l of Phenol red dye was used to plate each dilution. The plates were incubated for 48 hours at 37 °C. Bacterial isolates with a dye decolorization zone were selected, and they were streaked on a nutrient slant until they were used. (5).

2.3. Dye decolorization Assay :

Each isolate screened positive was further assayed for its dye decolorization efficiency. Each isolate was inoculated in Erlenmeyer flasks (250 mL) containing 50 mL of sterilized modified BH medium (pH 7.0) amended with 100 mg/l phenol dye and incubated for 6 days at 37°C with 150 rpm. Control was maintained without inoculation. The culture broth was centrifuged for 15 minutes at 10,000 rpm after 2, 4, and 6 days of incubation to measure the absorbance of the supernatant at 540 nm for each dye solution. The equation following was used to compute the decolorization activity in terms of percentage decolorization.

$$\% \text{ dye decolorization} = \left(\frac{\text{Initial absorbance-final absorbance}}{\text{Initial absorbance}}\right) \times 100$$

2.4. Morphological, Colonial & Biochemical Characterization of bacterial isolates :

Each isolate exhibiting dye decolorization was subjected to morphological, colonial, and biochemical analysis for partial identification. The findings were evaluated utilizing standard descriptions given in Bergey's Manual of Determinative Bacteriology (7).

3. Results and Discussion

3.1. Isolation, screening and identification of dye decolorizing bacterial isolates :

The enrichment medium was used to isolate bacteria that decolorize dyes. 07 isolates selected having distinct colony characteristics were screened by repeated streaking. During the screening process, a zone of decolorization around bacterial colonies was observed. 02 bacterial isolates labeled R3 and R7 were chosen for additional research on the medium.

Figure 2 – Dye decolorization efficiency by isolates R3 and R7

3.2. Analysis of decolorization efficiency

In the current work, phenol red dye at a final concentration of 100 mg/l was examined for its capacity to decolorize several bacterial isolates. According to reports, a typical textile effluent comprises 10 to 50 mg/l of dye material. So, for the duration of the study, a final dye concentration of 100 mg/l was chosen for the decolorization assay. The dye decolorization by chosen bacteria produced good results after 2, 4, and 6 days of incubation. Previous studies (9, 10, 11) revealed that only a few scientists had been successful in identifying bacterial cultures that could use dyes as their entire source of energy. This might be because microorganisms in natural habitats co-metabolize. Certain co-substrates are added during the co-metabolic process, which can cause the biodegradation process and, as a result, shorten the overall process time (12). After the incubation period of six days, decolorization of phenol red by specific bacterial isolates was observed and recorded (Fig. 1). When the decolorization effectiveness of the chosen isolates was further examined, isolates R3 and R7 both demonstrated a significant decolorization of 75% & 90% the phenol red dye respectively (13).

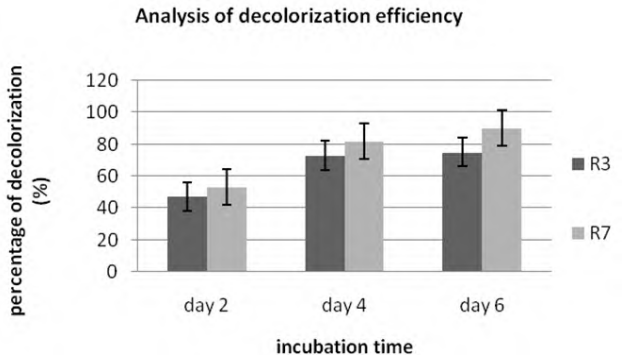

Figure 3 – Analysis of decolorization efficiency of isolates R3 and R7

3.3. Morphological, Biochemical Characterization of bacterial isolates

Based on their cultural, morphological, physiological, and biochemical traits, the chosen bacterial isolates were characterized and the results are shown in Table 1. The conventional Bergey's Manual of Determinative The bacteriology description was then put up against all of these traits. R3 Bacterial isolates were identified as Pseudomonas spp. and isolate R7 as Staphylococcus spp. (7).

4. Conclusions

Based on the results of this experimental work, one can conclude that the bacterial isolates had the ability to decolorize phenol red dye and were of the species Pseudomonas spp. for isolate R3 and Staphylococcus spp. for isolate R7. Both of these bacterial isolates were identified using biochemical characterization. As a result, they can be used to

treat textile effluent. However, additional research is required to validate the isolates as potentially useful bioremediation agents. This includes molecular characterization of the bacteria that were isolated, optimization of the cultural conditions under which they grew, and the detoxification mechanism.

Isolates	R3	R7
Gram reaction	Gram negative	Gram Positive
Morphological characteristic	rods	cocci
Biochemical characteristic		
Indole Production	Negative	Negative
Methyl Red Test	Negative	Positive
Vogas Proskauer Test	Negative	Negative
Citrate utilization Test	Positive	Positive
Nitrate reduction	Positive	Positive
H2S production	Negative	Negative
Urea hydrolysis test	Negative	Positive
Gelatin liquefaction Test	Positive	Negative
Glucose fermentation	Negative	Positive
Lactose Fermentation	Negative	Positive
Mannitol fermentation	Positive	Positive
Maltose fermentation	Negative	Positive
Xylose fermentation	Negative	Negative
Sucrose fermentation	Negative	Positive
Catalase Test	Positive	Positive
Oxidase Test	Positive	Negative

Table 1 – Morphological and Biochemical Characterization

5. Acknowledgement

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