

## Biodegradation of Azo Dye by Bacterial Species Isolated from Dye Contaminated Area of Jetpur, Gujarat

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### ABSTRACT

The varieties of synthetic azo dyes are widely used in textile industries to generate range of color tones in the textile and paper industries. Such textile dyes are toxic for the animals, birds and human along with environment. It is needful to remove those dyes from the effluent. The degradation of such dyes is accompanied by the involvement of dye degrading microorganisms. The purpose of this research is to isolate, identify, and screen bacterial species capable of decolorizing reactive pink, congo red, and malachite green. Soil and water samples were collected from the dye contaminated area of Jetpur, Gujarat, India. Dye degrading bacteria were isolated and selected through primary and secondary screening. The effect of yeast extract amount on the dye degradation properties of bacteria was examined using a visible spectrophotometer.

After secondary screening MD2, MD8, MD20, MD31, MD33 and MD34 were selected for further analysis. All isolated are able to degrade reactive pink, congo red, and malachite green after 120hrs of incubation at a 4mg<sup>l</sup><sup>-1</sup> concentration of yeast extract. Dye degradation remains constant for 4 mg<sup>l</sup><sup>-1</sup> and 2 mg<sup>l</sup><sup>-1</sup> but gets reduced in 1 mg<sup>l</sup><sup>-1</sup> of yeast extract concentration. Wheat germination rate in control was 65% compared to 96%, 88%, and 85% in MD2, MD8, MD20 respectively. Sorghum germination rate in control was 62% while in isolates no MD31, MD33, MD34 were 96%, 87%, 83% and 65%.

**Keywords-** Reactive pink, Malachite green, Congo red, Dye degradation, Toxicity assay, Yeast extract, Spectrophotometer.

utilized in excess of 1000 tons per year [5][6]. Textile dye comprises colors, causing artistic harm as well as preventing light diffusion in the water, resulting in a drop in dissolved oxygen levels and has an impact on the rate of photosynthesis in aquatic life. The presence of colors in aquatic bodies causes an aesthetic problem and can be harmful to public health [7].

Majority of these dyes are water soluble and are absorbed through inhalation. Skin contact with these dyes can cause allergic reactions as well as cancer [8][9][10]. Because of their extensive applicability and uses, azo dyes are the most common constituents of such pollution, and they are found in large quantities in textile industrial effluents. Microorganisms are used in industrial biological wastewater treatment systems to eliminate toxins from the environment. The majority of azo dyes are carcinogenic, poisonous and mutagenic [11] and may be harmful to aquatic life [12].

Biological treatments provide various advantages, including low cost, ease of use, lesser volumes of excess sludge produced, and high flexibility, as they may be applied to a wide range of effluents. Textile industry effluents are hazardous, containing a high level of color (from reactive dyes and chemical residues), acidic and alkaline pollutants, and high amounts of organic compounds [13]. It is critical to develop effective and environmentally friendly bioremediation systems [14]. Chemical precipitation, Filtration, coagulation, adsorption, chemical oxidation, photolysis, reduction, chemical flocculation, usage of activated carbon, and other physicochemical techniques can be used to treat industrial effluents containing a range of colors [15].

The survival and adaptation of microorganisms during the treatment procedures are critical to the success of these treatment systems [16][17]. The goal of this study is to isolate, identify, and screen bacterial species that can decolorize a range of colors. The breakdown of organic matter is carried out by the microorganisms utilized. The general goal of bioremediation is to increase native organisms natural biodegradation potential by providing adequate environmental conditions for degradation to reduce dye

### I. INTRODUCTION

Dyeing is a well-established method of altering the color properties of various substrates. Azo dyes are used to color a variety of items, including textile, leather, plastic, food and pharmaceuticals, as well as to make paints and for printing [1][2][3]. India produces approximately 80,000 tons of dyestuff and pigments [4]. According to estimates, there are 10,000 different textile dyes commercially accessible worldwide, with annual output of 7 × 10<sup>5</sup> metric tons; 30% of these colors are

content in wastewater to an appropriate level at a reasonable cost.

## II. MATERIAL AND METHODS

### *Azo dyes*

Reactive pink dye, Malachite green dye and Congo red dye were collected from dyeing industries.

### *Collection of water sample and soil sample*

The dye contaminated soil and water collected from the effluent sites of Jetpur, Rajkot, Gujarat. Collected polluted samples were transfer to the laboratories and store at refrigerated condition for further analysis.

### *Culture media*

Nutrient agar and mineral salt medium broth were used for the isolation of dye-degrading bacterial species. The mineral salt medium composition is 2.0g of sodium chloride, 0.5g of calcium chloride, 0.4g of magnesium sulphate, 0.7g of magnesium chloride, 0.5g of ammonium sulfate, 0.3g of dipotassium hydrogen phosphate, 0.3g of potassium dihydrogen phosphate, 1.0 g of yeast extract, 0.1 g of nutrient broth, 20.0 g of agar, distilled water 1000ml.

### *Isolation and Identification of Dye Decolorizing Bacteria*

In the case of soil, 1 g of soil was added in 100 ml of distilled water and agitated on shaker for 30 min. Suspension of soil was allowed to soil particles to settle down. While dye contaminated water was serial diluted from  $10^{-1}$  to  $10^{-6}$  using serial dilution method. All the dilutions were used for the isolation and identification of dye degradation bacteria. 100  $\mu$ l of each dilution was spread on the nutrient agar plate. The plates were incubated at 37°C for the 24 hours to develop colony. Morphologically distinct colonies were isolated and stored at 4°C for further analysis [18].

### *Primary screening was carried out*

The primary screening of dye degradation was carried out by using titer plate and scree cap tubes. In 96 well micro titer plate method, Mineral Salt medium broth were prepared for decolorization investigation. 0.1%(v/v) aliquots of each isolated bacterium in nutritional broth were inoculated into 96 well micro titer plates, each containing 200 $\mu$ l (20mg $l^{-1}$  concentration) unique dye solutions for initial screening. After 72hours of incubation, the dye solution was visually checked for decolorization. Isolates with a strong decolorizing potential were chosen for further testing.

In screw cap tube method, MSM-containing screw cap tubes were combined with 20mg  $ml^{-1}$  (2 ml per test tube) dye solutions. These dye-supplemented broths were autoclaved after being poured into screw cap test tubes. These modified broths were individually inoculated with isolated test organisms and cultured at 35°C in a static environment for 3 days for dye degradation analysis.

### *Physical and biochemical characterization*

The morphological and staining features of the chosen isolates were assessed. The isolate was stained using gram staining. The isolate was subjected to biochemical analysis for biochemical characterization for Citrate utilization test, Methyl red test, Voges-proskauer test, Gelatinase production test, Amylase production test, Zinc solubilisation test, Test for fermentation of carbohydrates, Indole test.

### *Secondary screening*

Secondary screening was carried out in MS medium broth with different dyes. In nutrient broth, each strain was grown for 24 hours. The inoculum was then transferred to a sugar tube containing 20 ml of MS media at a rate of 5% (v/v). Each sugar tube received a final concentration of 100mg $l^{-1}$  dye, and absorbance was measured at their absorbance maxima (540nm of reactive pink; 617nm of malachite green; 497nm of congo red after 24 hours (t24) and/or up to 72 hours (t72) at  $30\pm 2^\circ C$  under shaking (aerobic) conditions (110 rpm). Reduction in absorbance of dye at particular O.D. represent the degradation was estimated. On the basis of dye degradation potentiality few isolates were chosen for further analysis.

### *Toxicity Analysis*

Several studies have been carried out on the degradation metabolism of toxic dye. Several metabolites generated through the degradation of dye molecules [19][20]. Toxicity response of dye degradation product and parent dye must be measured because it can be extremely harmful for the aquatic as well to the terrestrial life. The effect of metabolites and dye on the germination of *Sorghum bicolor*, *Triticum aestivum* were examined by inoculating dye degraded culture media. The sorghum seeds were sowed in paper cup (12seeds per cup) in the presence of dye and metabolites. Root length, shoot length and germination percentage were measures after 10 days of germination. Simultaneously, control with only tape water and dye solution(100mg $l^{-1}$ ) was carried out under ambient conditions [21].

## III. RESULT AND DISCUSSION

The pH of collected effluent was near to 7.4 that is near to the permissible range [22]. Total 20 morphologically distinct bacterial colonies were selected for the primary screening of dye degradation properties.

The biodegradation of commercially available textile dyes, reactive pink dye, malachite green dye and Congo red dye was investigated in the presence of bacterial isolates recovered from the dye effluent sample using the MS media. Six of the ten isolates examined were further screened for dye degradation. The morphological, physiological, and biochemical characteristics of the selected bacterial isolates were studied (Table 1).

Primary screening and secondary screening of bacteria isolated from dye contaminated

area. Each isolated strains were inoculated into 96 well micro titer plates, After 48 hours of incubation at  $30\pm 2^{\circ}\text{C}$ , six isolates namely MD2, MD8, MD20, MD31, MD33, MD34 had completely degrade the reactive pink and reactive yellow. Bacterial isolates were initially screened with different dyes using micro titer plate technique and screening of bacterial isolates showing positive response to decolorization of different dyes [23].

Dye degradation properties of primary screened isolates were estimated rotary shaker method at the concentration of  $20\text{mg l}^{-1}$ . In the secondary screening

reactive pink, malachite green, congo red is completely degraded after 120hrs of incubation. Reactive pink dye was completely degraded by MD8 within 24hrs, Malachite green after 48hrs by MD8 and MD33, malachite green after 72hrs by MD20, reactive pink after 72hrs by MD20, MD33, MD34(Figure 1&2). Strain ETL-1942 exhibited 44-49% decolorization of Orange G, Reactive Red HE7B, Reactive Golden HR, Remazol Brown GR and Evan's Blue within 24h [24]. The azo dye MR (750 ppm) was decolorized by *S. paucimobilis* within 10h at shaking condition in MSM [25].

**Table 1: Biochemical characteristics of isolates selected through primary screening**

Sr no	Test name	MD2	MD8	MD20	MD31	M33	MD34
1	Citrate utilization test	+	+	+	+	+	+
2	MR test	+	+	+	+	+	+
3	VP test	-	-	-	-	-	-
4	Gelatinase production test	+	-	+	-	+	-
5	Amylase production test	+	+	+	+	+	+
6	Zinc solubilisation test	+	-	+	-	-	-
7	Indole test	+	+	+	+	+	+
8	Carbohydrate fermentation test						
	Dextrose	+	+	+	+	+	+
	Fructose	+	+	+	+	+	+
	Sucrose	+	+	+	+	+	+

#### **Effect of yeast extract on dye degradation under lab condition**

Effect of carbon source on the degradation of various dyes was estimated using visible spectrophotometer at different concentrations of yeast extract (0.1g/250ml, 0.5g/250ml, 1g/250ml). According to this experiment dye degradation can be greatly affected by the carbon and nitrogen source.

Maximum degradation of congo red dye was 64.75%, 61.71%, 63.42% and malachite green was 81.37%, 79.91%, 79.74% in various concentration of yeast extract ( $0.4\text{g ml}^{-1}$ ,  $2\text{g ml}^{-1}$ ,  $4\text{g ml}^{-1}$ ) by the isolates MD2 after 120hrs of incubation. while in the case of reactive pink was 94.87%, 71.11% hydrolyzed by MD8 at the yeast extract concentration of  $4\text{g ml}^{-1}$  and  $2\text{g ml}^{-1}$  and 16% by MD34 at the concentration of  $0.4\text{mg l}^{-1}$  [26]. Observed that after 90 h of incubation a maximum dye degradation value (92 %) was achieved for RY 145 dye.

#### **Effect of yeast extracts source in the bio-degradation of dye**

Yeast extract is used as a co-substrate to improve the decolorization azo dye. The best MSM in maintaining decolorization of Reactive pink, Malachite

green, Congo red as the sole carbon supply was yeast extract concentrations of 0.1g/250ml, 0.5g/250ml, and 1.0g/250ml. The concentration not show any change when Yeast extract added more than 1 g/l Yeast Extract concentration along with 1 g/l glucose and decolorized 200 mg/l of RV5 within 18 h under static condition [27].

#### **Phytotoxic effect on seed germination (Sorghum and wheat)**

The purpose of the phytotoxicity study was to examine how dyes and their breakdown products influence sorghum and wheat seed germination and seedling growth. The germination percentage, shoot length, and root length of dye-irrigated seedlings, as well as their degradation metabolites, were measured and compared to those of control seedlings.

When raw dye was compared to dye degradation metabolites, the percentage of seeds that germinated was lower. Sorghum seeds germination percentage was high (96) compared to raw dye control (62). When compared to raw dyes, the lengths of shoot, root, and seedling growth were longer in degradation metabolites, showing that degradation metabolites are less harmful than dyes.

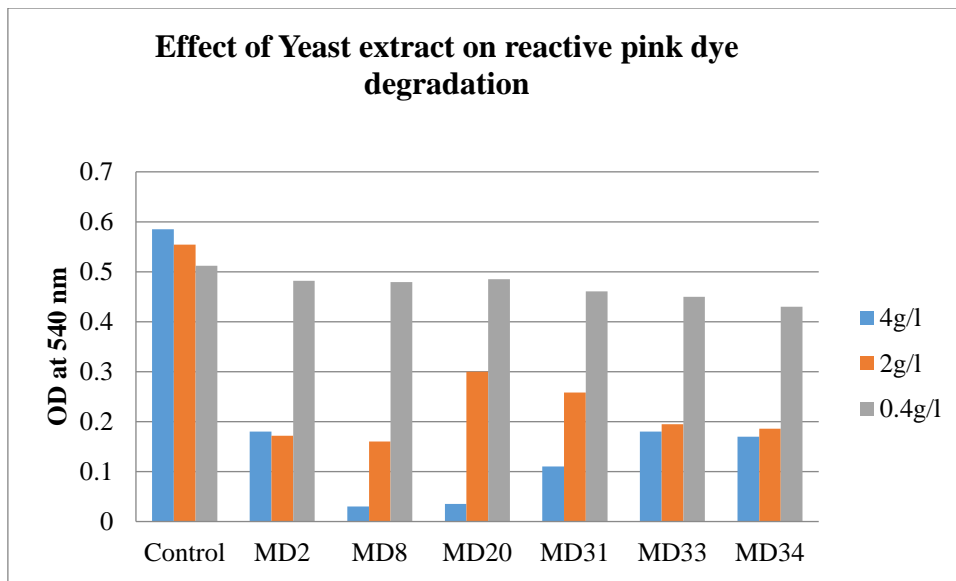


Figure 3. Decolorization of reactive pink dye at different yeast extract concentrations.

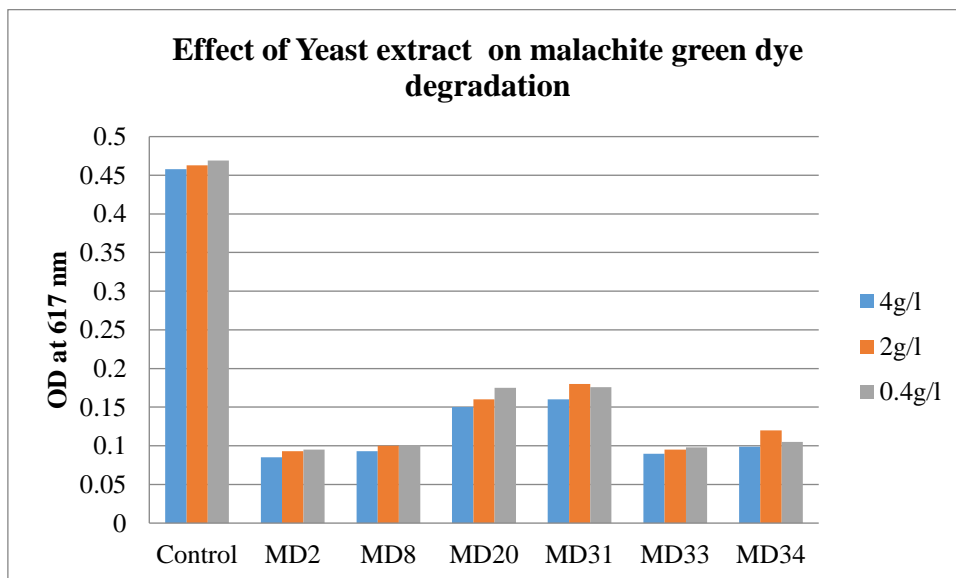


Figure 4. Decolorization of malachite green dye at different yeast extract concentrations.

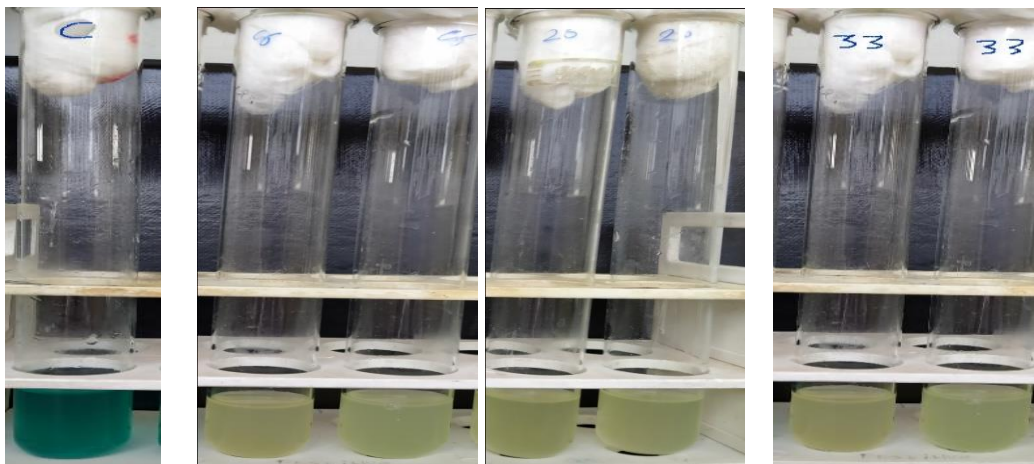


Figure 1. Malachite green dye degradation by MB8, MD20, MD33 after 48hrs of incubation

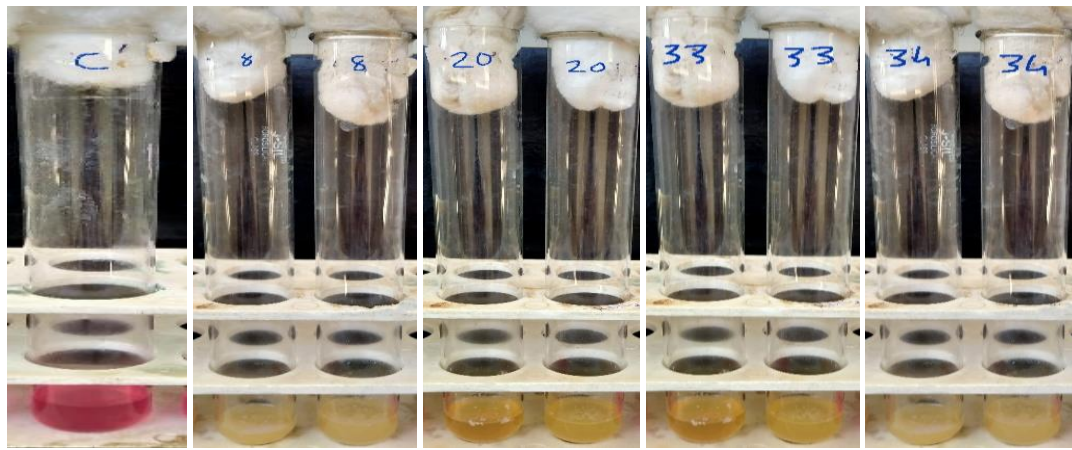


Figure 2. Reactive pink dye degradation by MB8, MD20, MD33, MD34 after 72hrs of incubation

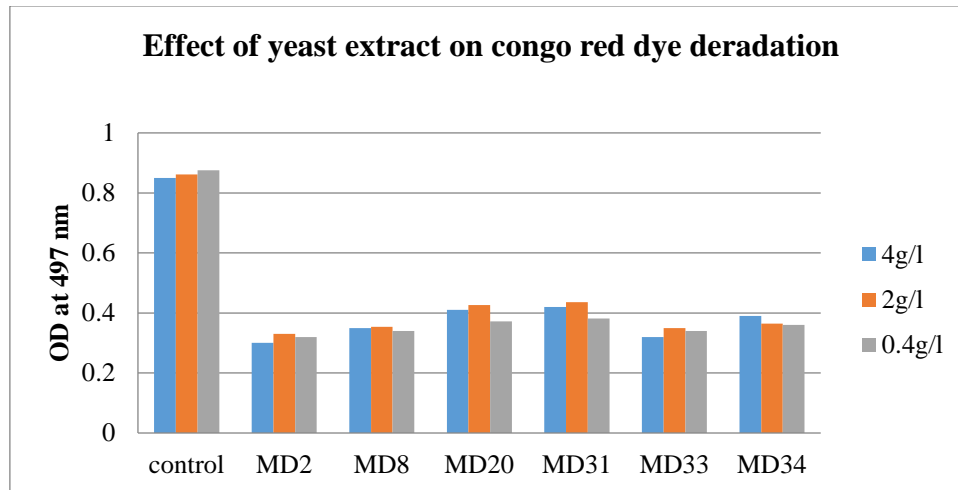


Figure 5. Decolorization of congo red dye at different yeast extract concentrations.

Untreated malachite green had a higher hazardous effect on seed germination and seedling growth than degradation metabolites. The germination percentage of both seeds was high in degradation metabolites treatment than that of raw dye. The findings imply that dumping dye wastewater on land could have a deleterious influence on soil fertility and plant growth.

When wheat seeds treated with malachite green the percent (%) germination of wheat seeds in control was 65 while in isolates no MD2, MD8, MD20 were 96, 88 and 85. Same way sorghum seeds germination percent (%) in control was 62 compared to 96, 87, and 83 in MD31, MD33, MD34 respectively.

Shoot and root length in Wheat were  $59.8 \pm 7.4$  and  $53 \pm 7.58$  while  $53 \pm 4.18$  and  $48 \pm 2.73$  in Sorghum. Shoot length of Wheat plants were  $75 \pm 8.66$ ,  $79 \pm 6.51$ ,  $79 \pm 7.41$  when it's treated by isolates MD2, MD8, MD20 respectively. Root length were  $81 \pm 9.02$ ,  $85.8 \pm 6.30$ ,  $85.4 \pm 7.503$  in MD31, MD33, MD34 respectively. Shoot length of wheat plants were increased by  $73.2 \pm 4.20$ ,  $82.80 \pm 11.23$ ,  $81 \pm 9.30$  and root length  $67 \pm 8.36$ ,  $77 \pm 11.51$ ,  $74 \pm 9.61$  in dye degradation metabolites treatment of isolates MD2, MD8, MD20 respectively.

#### IV. CONCLUSION

Due to the fast changes in consumer expectations, the textile, dyeing, and finishing business uses a wide range of dyestuffs. Dyes are used in textile, tanning, leather, printing and many more industries. For the treatment of industrial wastes containing various colors and dye stuffs, many physicochemical approaches have been used; however, these processes are very expensive and result in resistant wastes. After physical and chemical treatment, the toxic effluent is contained and released into the natural environment. Many industries, on the other side, dispose of their effluent without treatment or with only minor physical and chemical treatment. The degradation of the three dyes is used, congo red and malachite green and reactive pink dye was investigated in this study. The decolorization was due to dye biodegradation into non-colored metabolites, according to the UV visible spectrophotometer. The goal of this phytotoxicity study was to determine how dyes and their degradation products affected seed germination and seedling growth. Degradation metabolites had a higher germination rate

and seedling growth than untreated dyes, indicating that degradation metabolites are less hazardous.

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