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# Biodecolorization of Azo Dye Reactive Orange 4 By Enterobacter Gergoviae, Isolated From Textile Effluent

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**Abstract :** The power of microbes to decolorize and metabolize azo dyes has long been known and they are exploited for their use in treatment of waste water from textile industries .The decolorization of Reactive Orange 4, a very important commercial azo dye in textile industries was investigated. A dye decolorizing bacterium was isolated from effluent collected from GIDC, Pandesara, Surat, India. Optimization of various physicochemical parameters like pH, temperature, various carbon sources and nitrogen sources were carried out for maximum dye decolorization. For color removal, the most suitable pH and temperature were pH 7.0-10.0 and 25$^0$C -39 $^0$C, respectively. The dye was decolorized more than 90% in aerobic culture conditions. The dye can be used as sole source carbon and energy source for cell growth. The addition of glucose increased decolorization rate. These results suggest that isolated bacterium Enterobacter gergoviae is suitable for the biological processing of dye-containing wastewater.

**Keywords** : Decolorization, Waste water, Reactive azo dye, *Enterobacter gergoviae*, Textile effluent

## INTRODUCTION

Synthetic dyes have wide application in the food, pharmaceutical, textile, leather, cosmetics and paper industries due to their ease of production, fastness, and variety in color compared to natural dyes. [1] Textile industry is one of the largest consumers of synthetic dyes. The environmental problems associated with textile activities mainly arise from the extensive use of organic azo dyes. Azo dyes are characterized by the presence of one or more azo groups (-N=N-). They are the largest and most versatile class of dyes, and more than half of the annually produced amount of dyes (estimated for 1994 worldwide as 1 milliontons) are azo dyes.[2] It is estimated that 10%-15% of the dyes are released in the environment during the dyeing process. [3,4] The two major sources of release of dyes in to the environment are the effluents from textile processing units and dyestuff manufacturing industries.[5] Excess dyestuff in process water is highly undesirable because of environmental concerns, [6,7] health hazards and aesthetical aspects.[8] Color is the first contaminant in the wastewater, which should be recognized and has to be removed before it discharged in to the environment.[8] Much research has been focused on chemical and physical removal of dyes from the wastewater. However, many of these technologies are cost prohibitive and therefore are not viable preference for treating large waste streams. [9] It is known that 90% of reactive textile dyes entering activated sludge sewage treatment plants will pass through unchanged and will be discharged to rivers.[10] Biological processes represent eco-friendly and cost competitive alternatives to abiotic treatments.[11] Number of laboratories have investigated the ability of bacteria, fungi, and algae in removing the color of azo dyes. However, it is difficult to keep them in functional form in the activated sludge systems, due to their special nutritional requirements and environmental conditions. Moreover, bacterial degradation is much faster than fungal degradation of textile dyeing effluents. [4] The ability of microorganisms to carryout dye decolorization has recently received much attention. Microbial decolorization of dyes is a cost effective method for removing them from the wastewater. [5] Textile industry is among the most important industrial sector where Reactive dyes are frequently used for dyeing of Cellulosic fabrics. The goals of present study were to isolate efficient dye decolorizing bacteria and optimize various parameters for dye decolorization.

## MATERIAL AND METHODS

### Reactive dye and chemicals :

An azo dye, Reactive Orange 4 was procured from Cell India Textile, Surat, India. The various chemicals used in this study were of analytical grade and procured from Hi-Media Pvt. Ltd., Mumbai.

**Isolation and identification of microbial culture:**

The dye decolorizing bacterial strain was isolated from highly colored effluent from dyeing unit in the GIDC, Pandesara, Surat, India. The pH of effluent was 7.5. The effluent was collected in airtight sterile plastic container and filtered through ordinary filter paper to remove large suspended particles. The effluent sample was inoculated with 50 mg $l^{-1}$ of Reactive Orange 4 and incubated on rotary shaker (100 rpm) at 30°C. After 24 h 5% of inoculum was transferred to fresh effluent along with Reactive Orange 4. Three such transfers were made. After third transfer cell suspension from last enriched flask was plated on the Bushnell Hass agar medium for screening of dye decolorizing microorganism. Composition of BH agar medium $gl^{-1}$ ; $MgSO_4$,0.2; $CaCl_2$,0.02; $KH_2PO_4$,1.0; $(NH_4)NO_3$,1.0; $FeCl_3$,0.05 supplemented with Reactive Orange 4, 200 mg $l^{-1}$; pH 7.4. From that five bacterial colonies were selected on the basis of formation of decolorization zone surrounding the colonies. Out of those five colonies most promising bacterial colony was selected on its capacity to produce largest decolorization zone on BH agar plate containing dye. The isolated bacteria were characterized by various morphological and biochemical test according to Bergey's Manual of Systematic Bacteriology. [12]

**Dye decolorization experiments :**

Dye decolorization by isolated bacterium was tested in 250 ml Erlenmeyer flask with 100 ml BH Medium containing 200 mg $l^{-1}$ of dye. The sterilized medium was inoculated with the isolated bacterial culture of uniform cell density (1.0 optical density [OD] at 550 nm). The medium-to-inoculum ratio (v/v) was 50:1. Inoculated medium was incubated at 30°C on rotary shaker (100 rpm). After 24 h of incubation, 3 ml of medium was withdrawn. Aliquot was centrifuged at 10,000 rpm for 15 min to separate cell mass, clear supernatant was used to measure the decolorization at absorbance maxima of dye (431 nm) using spectrophotometer (UV 2400 series, Shimadzu). Uninoculated medium was incubated as a control to check abiotic decolorization. Experiment was performed in triplicate. Decolorization efficiency was expressed as percentage of decolorization and was calculated using equation,

$$\text{Decolorization (\%)} = A_C - A_T / A_C \times 100$$

Where $A_C$ is the absorbance of the control and

$A_T$ is average absorbance of the test samples.

To ensure that the change in pH of the dye solution had no effect on the decolorization, the visible spectrum was recorded between pH 5.0 to 11.0, in which the pH did not show any effect in spectrum.

**Optimization of condition for maximum decolorization:**

**Effect of different carbon sources on decolorization**

Three different carbon sources i.e. glucose, lactose and sucrose were tested for decolorization at various concentration i.e. 0.2%, 0.5%, 1.0% (w/v). 2 ml of inoculum was inoculated in 100 ml BH medium along with dye and different concentration of carbon source. All flasks were incubated at 30°C on rotary shaker. Aliquot was removed for the determination of decolorizing activity at different time intervals.

**Effect of nitrogen sources on decolorization**

Two nitrogen sources were tested for decolorization of dye. The concentration of organic nitrogen (urea) and inorganic nitrogen source (ammonium chloride) were 0.2%, 0.5%, 1.0% (w/v). 2 ml of inoculum was added to 100 ml of BH medium along with dye, 0.5% glucose and different concentration of nitrogen source. All flasks were incubated at 30°C on rotary shaker. Aliquot was removed for the determination of decolorizing activity at different time intervals.

**Effect of pH and temperature on decolorization**

Effect of pH and temperature decolorization was observed by growing the isolate in the BH medium containing dye having pH range from pH 5.0 to 11.0. in the same way the effect of temperature was examined by growing cultures at 25°C, 27°C, 29°C, 31°C, 33°C, 35°C, 37°C, 39°C, 41°C by keeping the pH of the medium 7.4 for 7 days. Samples were withdrawn at different time intervals and decolorizing activity was determined.

**RESULTS**

From the effluent sample collected from a dyeing unit, we isolated a promising decolorizing bacterial strain. This strain formed a distinct clear zone on BH agar plate containing dye. To identify this bacterium, we investigated its morphological and physiological properties using various biochemical media. On the basis of results the isolate was identified as *Enterobacter gergoviae*. (Table:1)

**Microbial decolorization**

The isolated strain was tested for its capacity to remove dye, Reactive Orange 4. Dye was added to BH medium at concentration of 200 mg $l^{-1}$ as sole source of carbon and nitrogen. The results indicate that the strain is capable of decolorizing the dye up to 90% in 7 days. Decolorization of dye is depicted in Figure 1. The result shows that the strain is effective in decolorization.

**Optimization of culture condition**

For the maximization of decolorization of the dye by the isolated strain, experiments were conducted for the optimization of carbon source, nitrogen source, pH and temperature.

**Effect of different carbon sources**

Three different carbon sources, glucose, lactose and sucrose were tested for maximum decolorization by the isolated strain. Each carbon sources were added at 0.2%, 0.5%, and 1.0% in BH medium containing dye 200 mg $l^{-1}$.The strain is capable of decolorizing the dye in the presence of glucose at various concentrations. Complete decolorization was observed when there was addition of 0.5% of glucose. (Figure 2) There was no increase in the rate of decolorization when lactose and sucrose added. (Figure 3 & 4)

**Effect of different nitrogen sources on decolorization**

Two nitrogen sources urea and ammonium chloride were tested for decolorization of dye by the isolated strain, results of which depicted in figure 5& 6. BH medium containing dye was supplemented with 0.5% and 0.2%., 0.5%, and 1.0% of urea and ammonium chloride. Results suggest that strain showed maximum decolorization at concentration 0.2% of urea and 0.2% of ammonium chloride. The best decolorization was observed at 0.2% of ammonium chloride

**Effect of temperature and pH on dye decolorization**

The effect of temperature and pH on the dye decolorization was tested. It was found that a temperature of 31°C was optimum for maximum decolorization (Figure 7). Decline in decolorization activity at higher temperature more than 39°C can be attributed to the loss of cell viability. Optimum pH for maximum dye decolorization was 7.0 (Figure 8).

**DISCUSSION**

Azo dyes are considered as electron-deficient xenobiotic compounds because they possess the azo (-N=N-) and sulfonic (-$SO^{3-}$) electron-withdrawing groups, generating electron deficiency in the molecule and making the compound less susceptible to oxidative catabolism by bacteria. As a consequence, azo dyes tend to persist under aerobic environmental conditions. [13] They also reported that Reactive orange 4 cannot be utilized by *Enterobacter spp.* as sole carbon source. In present study, when dye is used as carbon source, the degradation is above 90%, suggests that the bacterium is potent candidate which can decolorize the textile effluent containing azo dye. In medium supplemented with 0.5% of additional glucose, 100% decolorization is achieved;

suggest that dye decolorization rate is increased when additional carbon source is added in proper amount, because either less than or greater than 0.5% of additional glucose is not helpful for increase in dye decolorization rate. Additional organic nitrogen source (urea) has no significant effect on dye decolorization but inorganic nitrogen source (ammonium chloride) shows 98% decolorization. Under aerobic conditions, most azo dyes are not degradable by bacteria. However, under anaerobic conditions, the azo linkage in the dye molecule can be reduced to form colorless aromatic amines which are occasionally toxic and carcinogenic. [14] Our results suggest that in shaking condition dye is decolorized. Decolorization capacity of organism at wide range of temperature and pH makes it suitable candidate for bioremediation of textile effluent containing azo dye. The present study has resulted in the isolation of a bacterial strain that has capacity of decolorizing Reactive azo dye in aerobic condition and thus shows the potential to be exploited as possible candidate for bioremediation. Decolorization activity can be enhanced by addition of glucose. The isolated strain can decolorize dye under wide range of pH and temperature, which is the nature of effluent from dyeing industries.

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**Table: 1**. Physiological and biochemical characterization of isolated bacteria

| Sr. No. | Characteristics | Result | Sr. No. | Utilization of | Result |
|---|---|---|---|---|---|
| 1 | Gram Reaction | Negative | 13 | L-Arabinose | Positive |
| 2 | Cell Morphology | Short Rods | 14 | Cellobiose | Positive |
| 3 | Motility | Positive | 15 | Dulcitol | Negative |
| 4 | Pigmentation | Negative | 16 | Glycerol | Positive |
| 5 | Spore Formation | Negative | 17 | Lactose | Positive |
| 6 | Urea hydrolysis test | Positive | 18 | Maltose | Positive |
| 7 | Indole Production test | Negative | 19 | Mannitol | Positive |
| 8 | Methyl Red test | Negative | 20 | Raffinose | Positive |
| 9 | Voges Proskauer test | Positive | 21 | Sucrose | Positive |
| 10 | Gelatin Hydrolysis test | Negative | 22 | Trehalose | Positive |
| 11 | Phenyl alanine deaminase test | Negative | 23 | Xylose | Positive |
| 12 | Glucose Dehydrogenase test | Positive | 24 | D- Sorbitol | Negative |

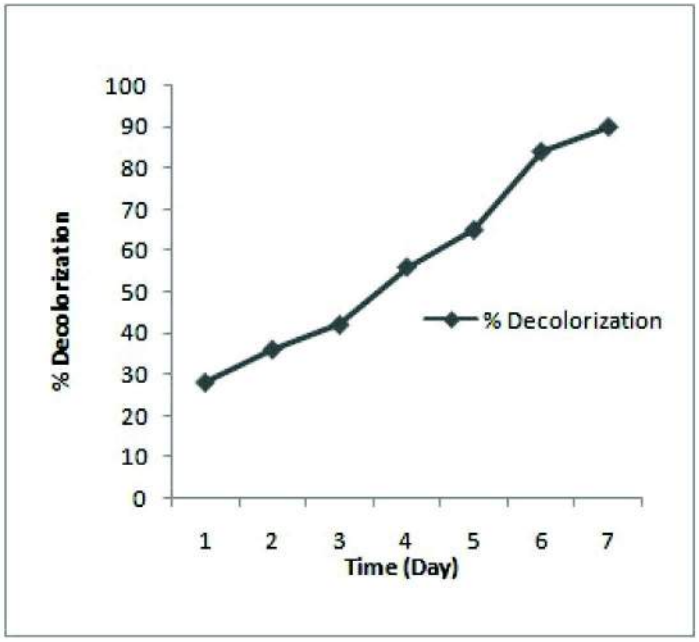


**Figure 1 Microbial Decolorization**

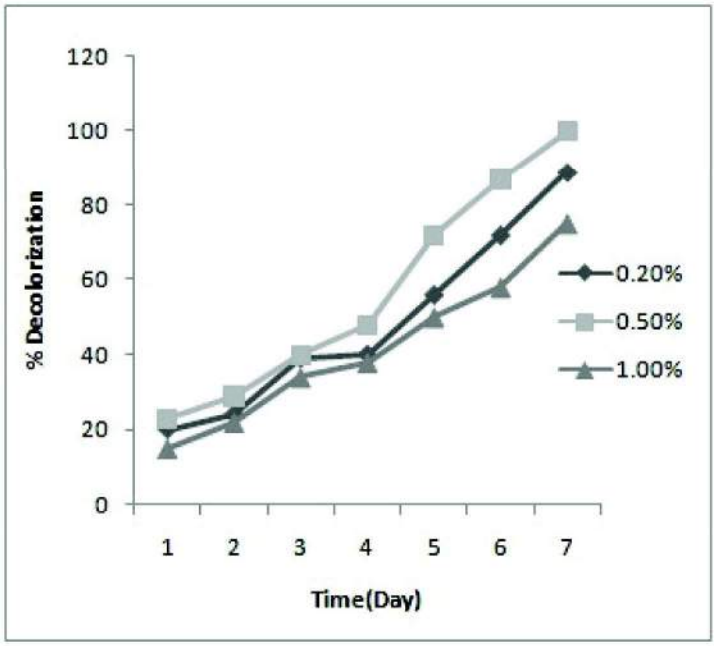


**Figure 2 Effect of Glucose on decolorization**

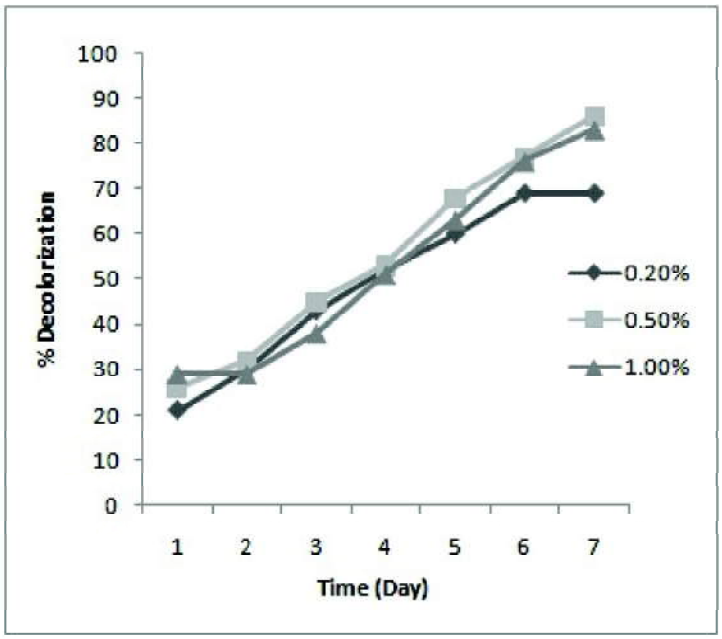


**Figure 3 Effect of Lactose on decolorization**

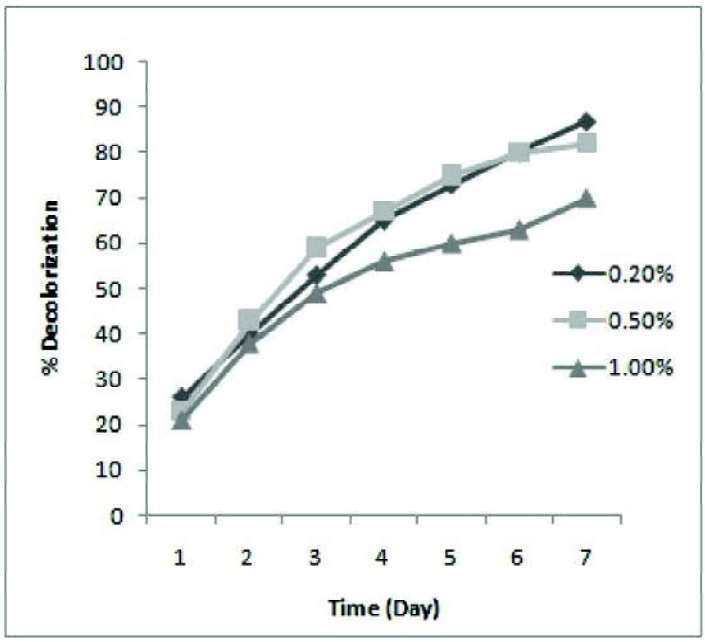


**Figure 4 Effect of Sucrose on decolorization**

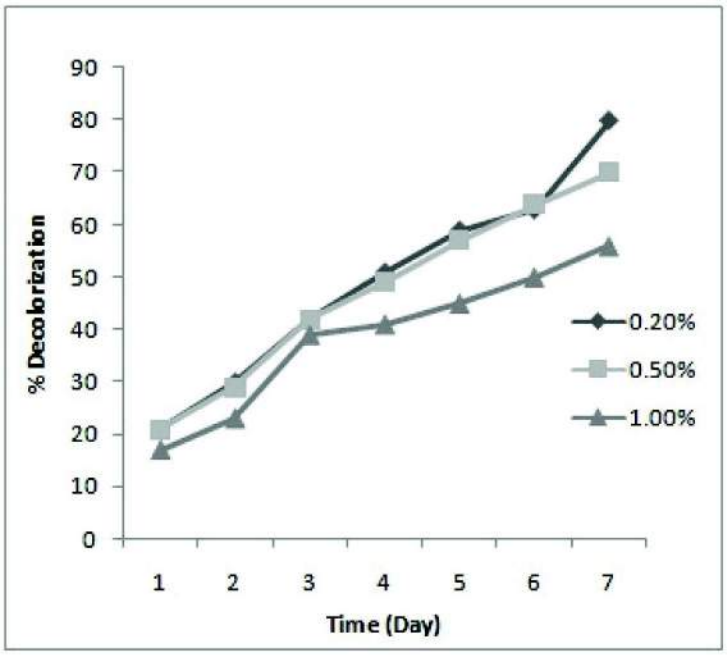


**Figure 5 Effect of Urea on decolorization**

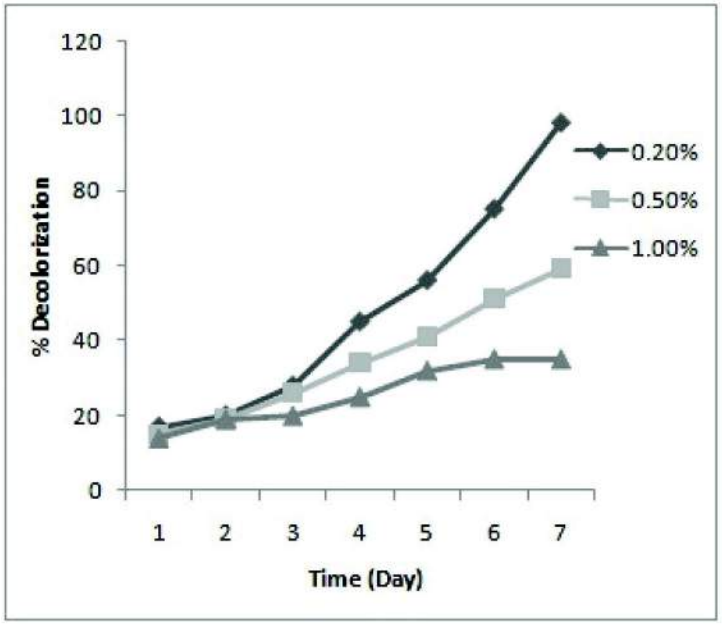


**Figure 6 Effect of Ammonium Chloride on decolorization**

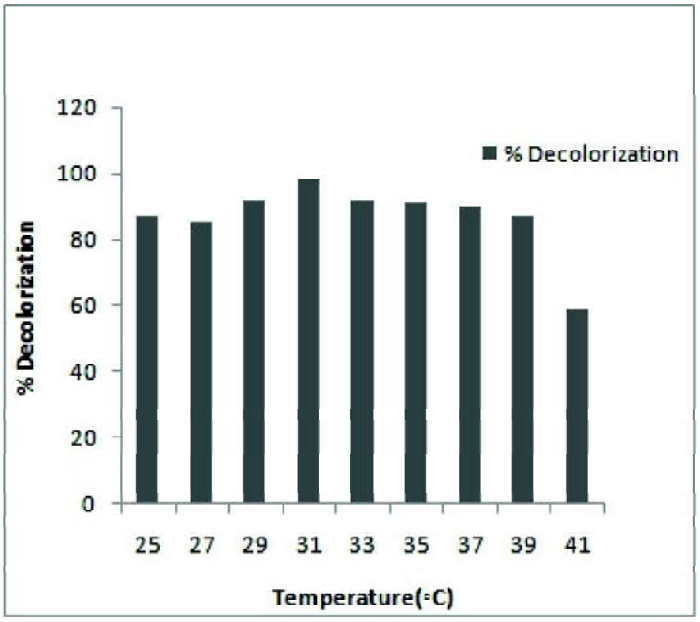


**Figure 7 Effect of Temperature on decolorization**

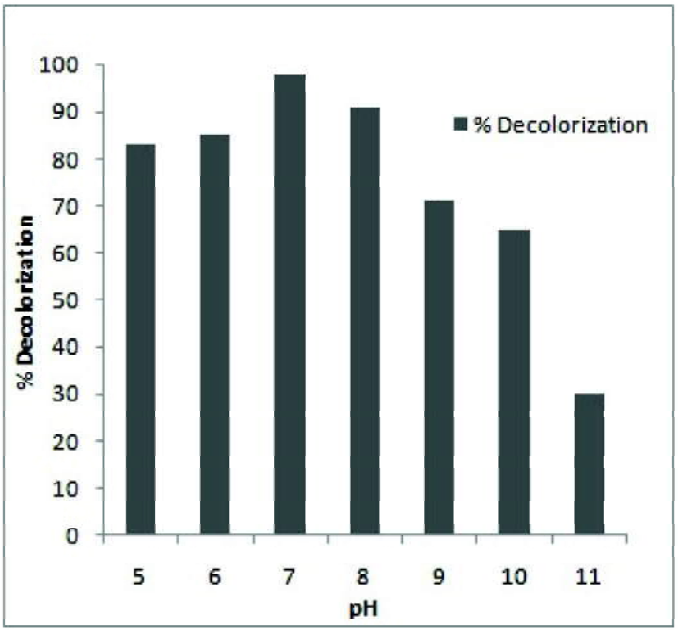


**Figure 8 Effect of pH on decolorization**

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