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Microbial Decolorization of Disperse Textile Dye Brown 21 by E*nterobacter gergoviae* Isolated from Textile Effluent

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Abstract

Decolorization of Disperse textile dye Brown 21, a very important commercial dye in textile industries was investigated. A dye decolorizing bacterium was isolated from effluent collected from a GIDC, Pandesara, Surat, India. Various physicochemical parameters like pH, temperature, carbon sources and nitrogen sources were optimized for maximum decolorization of dye. Dye decolorization was observed in the range of pH 7.0 to 10.0. Temperature ranges from 25˚C - 39˚C was observed as suitable temperature for dye decolorization. The dye was decolorized more than 90% in aerobic culture conditions. The dye can be used as sole source carbon and energy for cell growth. Additional carbon source glucose increases the rate of dye decolorization. Addition of urea also has increased the rate of dye decolorization. These results suggest that isolated bacterium, Enterobacter gergoviae, is suitable for the biological treatment of dye-containing wastewater.

Keywords: Decolorization, wastewater, disperse dye, *enterobacter gergoviae*, textile effluent.

Introduction

Water is considered as a rare and valuable commodity, and only an minuscule part of the earth's water reserves (approximately 0.03%) constitutes the water resource that is available for human activities and considering growth of the world's population, industry and its demand is more than the supply available[1]. The textile industry plays an important role in the world economy as well as in our daily life. Textile industries consumes huge amount of water and at the end produce highly colored effluents[2]. Extensive use of organic dyes in textile activities lead to the environmental problems[3-5]. It is estimated that 10%-15% of the dyes are released in the environment during the dyeing process. The two major sources of release of dyes in to the environment are the effluents from textile processing units and dyestuff manufacturing industries[3]. Excess dyestuff in wastewater is highly objectionable because of ecological concerns[6], health hazards and aesthetical aspects[7]. 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 option for treating large waste streams[3,4]. Biological processes represent eco-friendly and cost competitive alternatives to abiotic treatments[8,9,10]. Many laboratories have investigated the capacity of bacteria, fungi, and algae in removing the color of 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[7]. The ability of microorganisms to carryout dye decolorization has recently received much attention. Microbial decolorization of dyes is a cost effective method from removing them from the wastewater[1, 11]. Textile industry is among the most important industrial sector where disperse dyes are frequently used for dyeing of polyester fabrics[12,13]. The goals of present study were to isolate efficient dye decolorizing bacteria and optimize various parameters for dye decolorization. For this study Disperse Brown 21 dye was used as a model dye.

Material and Methods

Disperse dye and chemicals: Disperse Brown 21 dye was procured from local market in 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: Highly colored textile effluent from a dyeing unit, in the GIDC, Pandesara, Surat, India, was collected for isolation of dye decolorizing bacteria. 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 disperse brown 21 and incubated on rotary shaker (100 rpm) at 30°C. After 24 h 5% of inoculum was transferred to fresh effluent along with disperse brown 21. Three such transfers were made. After third transfer cell suspension from last enriched flask was plated on the Bushnell Hass (BH) 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

disperse brown 21, 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[14].

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 (471 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,

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 and Discussion

From the effluent sample, collected from a dyeing unit, a promising decolorizing bacterial strain was isolated. This strain formed a distinct clear decolorization zone on BH agar plate containing dye. This screening method is also carried out by many author[15,16]. For identification of 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). Some of the similar bacteria were also reported as dye decolorizer like *Enterobacter* sp. EC3[17], *Enterococcus gallinarum*[18], *Enterococcus faecalis*[19], and *Enterobacter agglomerans*[20].

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

Microbial decolorization: The isolated strain was tested for its capacity to remove dye disperse brown 21. Dye was added as sole source of carbon and nitrogen to BH medium at concentration of 200 mg l^{-1}. The results indicate that the strain is capable of decolorizing the dye up to 93% in 7 days. Disperse brown 3 REL was decolorized up to 86% by *Brevibacillus laterosporus,* an aerobic and spore forming bacterium[21] while it was decolorized 100% in optimized anoxic condition[22]. Decolorization of dye is depicted in figure-1. The result shows that the strain is effective in decolorization.

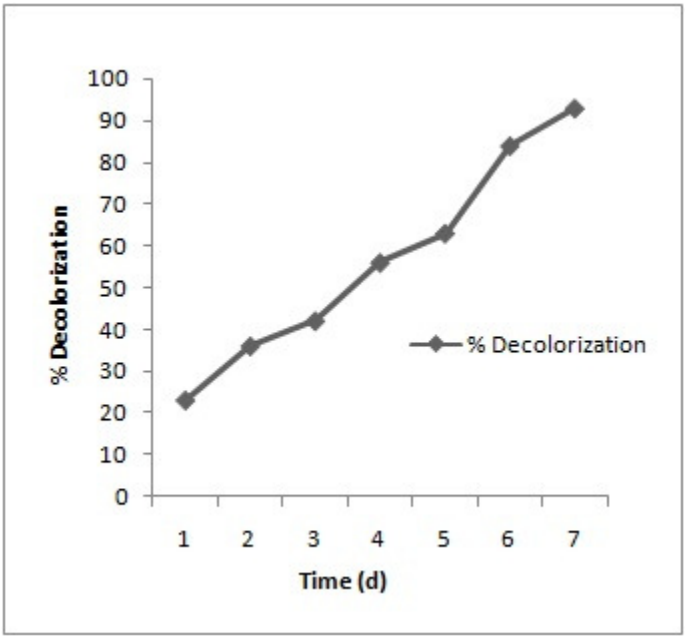

Figure-1
Microbial Decolorization

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. The highest decolorization observed when there was addition of 0.5% of glucose as shown in figure-2. Our findings are supporting the result that the optimum Cibacron Red FN-2BL dye decolorization was achieved when basal media was supplemented with additional glucose[23].

There was no increase in the rate of decolorization when lactose and sucrose added as shown in figure 3 and 4. Maximum percentage decolorization was observed when glucose used as carbon source at 0.5%.

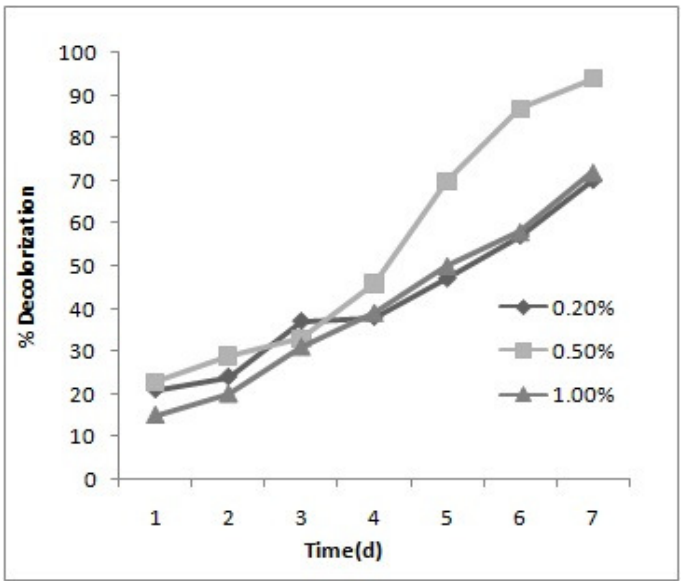

Figure-2
Effect of Glucose on decolorization

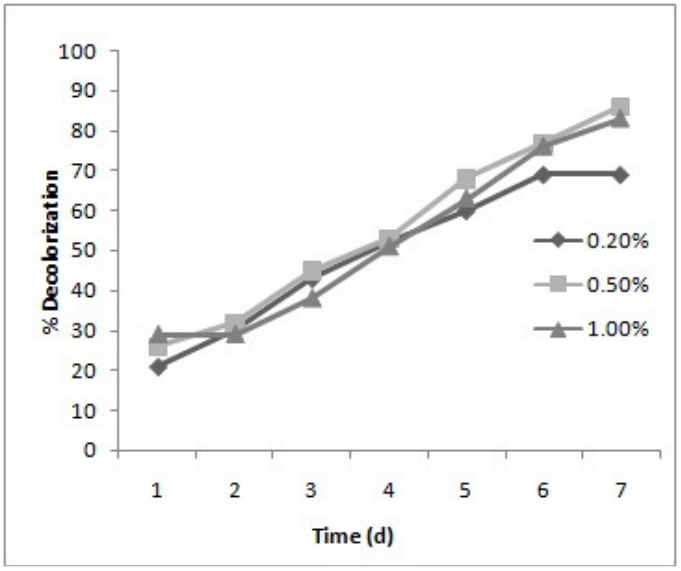

Figure-3
Effect of Lactose on decolorization

Effect of different nitrogen sources: Two nitrogen sources, urea and ammonium chloride, were tested for decolorization of dye by the isolated strain, results of which depicted in figure 5 and 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. Chen *et al*. reported that additional nitrogen source has strong effect on dye decolorization. In their study on Red RBN dye they reported that dye decolorization was enhanced when nitrogen sources were added in basal medium[24]. Jain *et. al*. also reported the same phenomena[25].

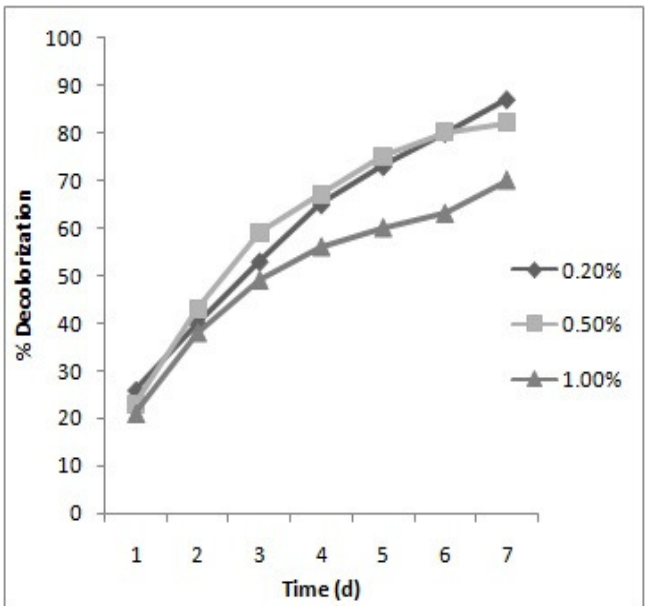

Figure-4
Effect of Sucrose on decolorization

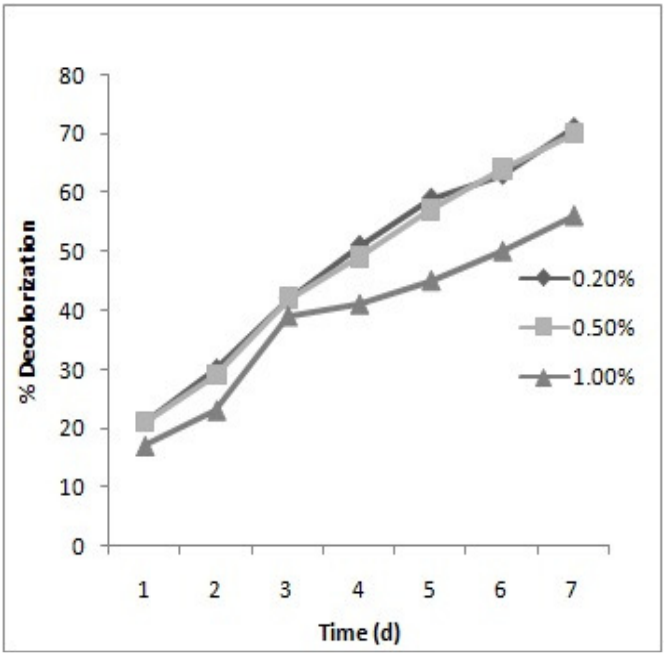

Figure-5
Effect of Urea on decolorization

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 as shown in figure-7. Similar results were observed that *Bacillus megaterium* can decolorize dye at 37°C[26]. Decline in decolorization activity at higher temperature more than 39°C can be due to the loss of cell viability. Optimum pH for maximum dye decolorization was observed 7.0 as depicted in figure-8. pH and temperature play very important role in decolorization of Red 3BN by various fungi[27].

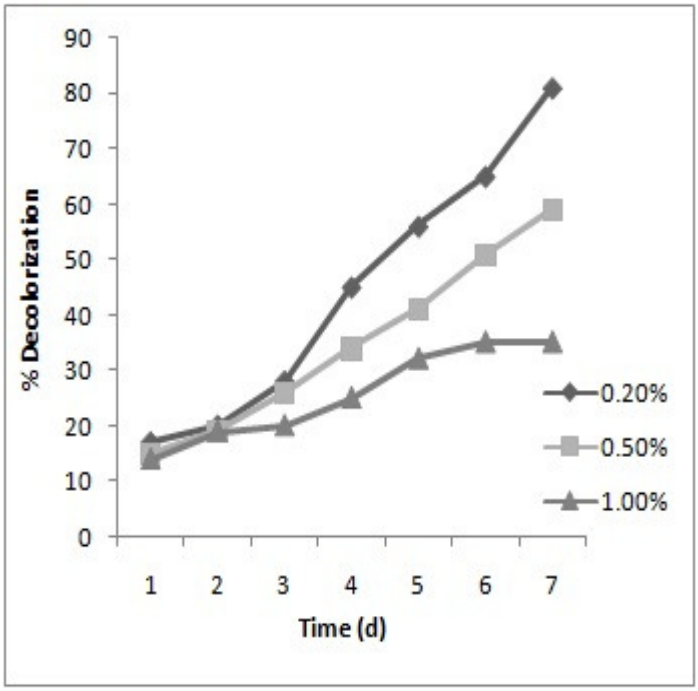

Figure-6
Effect of Ammonium Chloride on decolorization

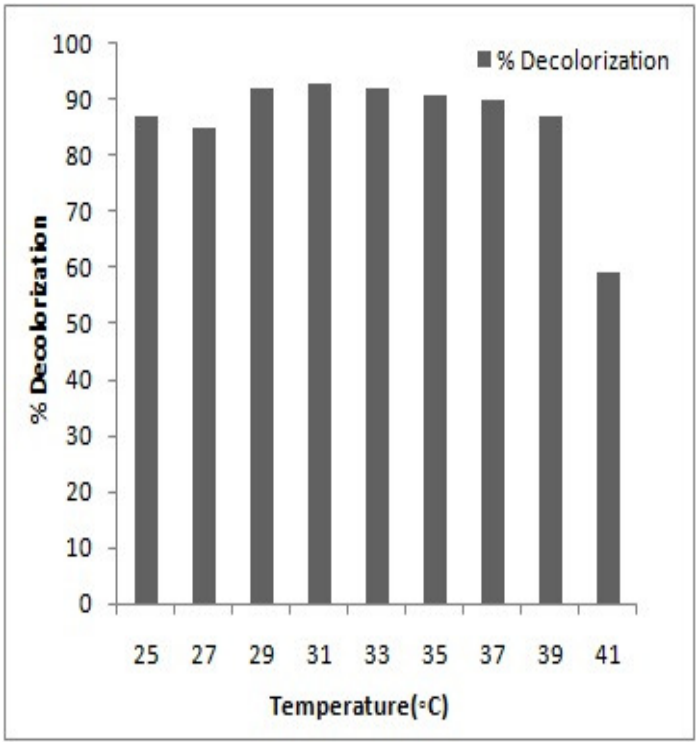

Figure-7
Effect of Temperature on decolorization

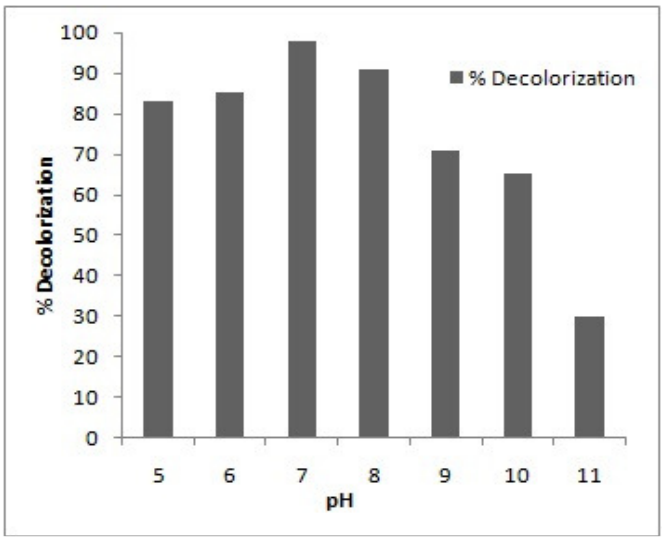

Figure-8
Effect of pH on decolorization

Conclusion

The present study has resulted in the isolation of a bacterial strain that has capacity of decolorizing disperse azo dye and thus show the potential to be exploited as possible candidate for bioremediation. Decolorization activity can be enhanced by addition of glucose. The isolated strain can decolorize disperse dye under wide range of pH and temperature, which is the nature of effluent from dyeing industries.

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