

Decolorization of Remazol Black-B by Three Bacterial Isolates

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Abstract Azo dyes are released into wastewater streams without any pretreatment. To prevent contamination of our vulnerable resources, removal of these dye pollutants is importance. For this purpose, wastewater samples were collected from dye-contaminated sites of Ankleshwar, Gujarat, India. About 48 bacterial isolates were isolated and tested for their potential to remove Remazol Black-B azo dye in liquid medium. Three bacterial isolates capable of degrading Remazol Black-B azo dye efficiently were screened through experimentation on modified mineral salt medium. Isolate ETL-1 was able to completely remove the Remazol Black-B dye from the liquid medium in 18 h. Further, the isolate showed the best performance at the dye concentration of 100 mg L⁻¹ medium (pH 7) & at temperature 35°C. Similarly, yeast extract proved to be the best carbon source for decolorization purpose. The results imply that the isolate ETL-1 could be used for removal of the reactive dyes from textile effluents.

Keywords: azo dye, Remazol Black-B, wastewater, yeast extract

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1. Introduction

Due to rapid industrialization, a lot of chemicals including dyes are manufactured and used in day to day life [1]. Synthetic dyes are extensively used in textile, dyeing, paper printing, colour photography, food, cosmetic and other industries. Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide [2]. Explosion of population coupled with industrial revolution results in pollution of water, air and soil. The discharge of pollutants from various industries poses threat to the biodiversity of the earth. The textile finishing generates a large amount of dyes containing wastewater from dyeing and subsequent steps that forms one of the largest contributions to water pollution [3]. The traditional textile finishing industry consumes about 100 litre of water to process about 1 Kg of textile material. The new closed-loop technologies such as the reuse of microbially or enzymatically treatment of dyeing effluents could help reducing this enormous water consumption [4]. It was already reported that 10-15% of dyes are lost in the effluent during dyeing process [5]. Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic [6]. Azo bonds present in azo dyes are resistant to breakdown, with the potential for the persistence and accumulation of high levels of dye in the environment [7].

These dyes cannot be easily degrade, while some are toxic to higher animals [8]. Azo dyes are very stable in acidic and alkaline conditions and are resistant to temperature and light. However, they can be degraded by bacteria under aerobic and anaerobic conditions [9]. Azo dyes are environmental pollutant [10] and they contribute to mutagenic activity of ground and surface water that are polluted by textile effluents [11,12]. The complex aromatic substituted structures make conjugated system and are responsible for intense color, high water solubility [13]. Their discharge in to surface water also leads to aesthetic problems, obstructing light penetration and oxygen transfer in to water bodies [14,15]. Several physicochemical techniques have been proposed for treatment of colored textile effluents. These include adsorption on different materials, oxidation and precipitation by Fenton's reagent, bleaching with chloride or ozone, photo degradation or membrane filtration [16]. The economic and safe removal of the polluting dyes is still an important issue. Because all these physicochemical methods are very expansive and results in the production of large amount of sludge, which creates the secondary level of land pollution. In this situation bioremediation is becoming important, because it is cost effective and environmentally friendly and produces less sludge [17]. Therefore, in such situations, biological treatment may be a real hope. These methods have the advantages of being environment friendly. Microorganisms have developed enzyme system for the decolorization and mineralization of azo dyes under certain environmental conditions [18,19,20]. So, present study was designed to isolate

efficient azo dye decolorizing bacterial strains from the textile effluents. Since the bacterial isolates were originated from the dye contaminated textile wastewater of local industry, so they can easily adapt to the prevailing local environment. Therefore, such bacteria can be used to develop an effective biological treatment system for the wastewaters contaminated with azo dyes.

2. Materials & Methods

2.1. Sampling

Water and sludge samples were collected from Ankleshwar Industrial Estate, Ankleshwar, Gujarat, India around which many textile processing units are situated. Samples were taken from drain at different locations and sampling sites were selected on the basis of the allocation of outlet from textile units. Electrical conductivity (EC) and pH were determined to assess the presence of total soluble salts (TSS) and acidity or alkalinity of the collected samples (Table 1).

Table 1. Total soluble salts (TSS) and pH of the dye contaminated textile effluent and sludge

Sampling Site	TSS	pH	Notes
Near K Patel Dye Unit	58	8.2	Effluent
Near Dynamic Dye Unit	78	8.5	Effluent
Near Harpal Dye Unit	152	10.3	Sludge
Near Chemcrux Unit	118	9.4	Effluent
Near Suyog Dye Unit	98	8.7	Sludge

2.2. Isolation of Bacterial Strain by Enrichment Method

Bacterial strains were isolated from water and sludge samples. Isolates from each inoculum source were first enriched using MSM medium amended with an azo dye Remazol Black-B as the sole source of C and N (20). Dye was added at a concentration of 150 mg L⁻¹. The cultures containing 200 mL of MSM broth with dye in 500 mL Erlenmeyer flasks were inoculated with 10 mL volume of wastewater or sludge suspensions. The flasks were incubated at 32°C for 7 days under static conditions. After incubation, cell suspensions from each flask were plated onto MSM agar medium and incubated at 32°C for 24 h. Microbial colonies that appeared on the agar medium were washed gently with sterile water and resuspended into the flasks containing fresh MSM broth spiked with the Remazol Black-B dye. About 50 actively growing colonies were selected for purification.

2.3. Purification of Bacterial of Isolates

Selected isolates were purified by streaking on MSM medium containing agar at the concentration of 20 g L⁻¹. Streaking was done thrice in zig zag manner. The purified cultures were preserved in a refrigerator for subsequent study.

2.4. Screening Efficient Azo Dye Decolorizing Bacterial Isolates

Screening was done to find out the efficient bacterial strains capable of decolorizing the Remazol Black-B azo dye using modified MSM. For this purpose, 48 isolates having the ability to decolorize Remazol Black-B from all samples were selected. After that decolorization, ability of each isolate was tested in the liquid medium. Media inoculated with the respective inocula were incubated at 35°C for 24 h. After 24 h, the respective cells were harvested by medium centrifugation at 10,000 rpm (REMI R-23, India) for 10 minutes. Then decolorization was determined with the help of spectrophotometer (SHIMADZU, Japan) at 597 nm. Uninoculated blanks were run to determine abiotic decolorization. The three most effective bacterial isolates (ETL-1, ETL-2 & ETL-3) from the final screening were further examined for their decolorization potentials in test tubes at different time periods. Ten milliliters of the sterilized MSM broth containing Remazol Black-B at the concentration of 100 mg L⁻¹ was added to autoclaved test tubes supplemented with 0.5% yeast extract as a co-substrate. The medium was inoculated with the respective bacterial strains by adding inocula of uniform cell density (OD: 0.6) at 597 nm. The test tubes were tightly sealed and incubated at 35°C under static conditions. Uninoculated test tubes with MSM containing azo dye plus yeast extract were incubated under similar conditions to check for abiotic decolorization of dye. Decolorization was measured after 6, 12, 18 and 24 h at 597 nm by spectrophotometer as described by Khalid *et al.* (2008).

2.5. Optimization of Environmental Factors for Efficient Decolorization

Factors like substrate concentration, temperature and pH were optimized during the experimentation for Different carbon sources (glucose, yeast extract, Mannitol and maltose) at the concentration of 4 g L⁻¹ were also tested as co-substrate in the decolorization process. Optimization studies included various concentration of dye (50,75,100,125,150,200 and 250 mg L⁻¹), pH values (5,6,7,8,9) and temperatures (25,30,35,40,45°C). All the bacterial isolates ETL-1, ETL-2 & ETL-3 were tested to optimize their decolorization efficiency. While culture conditions were the same as used in decolorization experiment i.e., minimal salt medium was used along with the 100 mg L⁻¹ of Remazol Black-B azo dye. Uninoculated blanks were run to check the abiotic decolorization during the experimentation.

2.6. Statistical Analysis

Data were entered in a Microsoft® Excel 2007 spreadsheet.

3. Results

Efficiency of the bacterial isolates to decolorize Remazol Black-B was examined by measuring color intensity in liquid medium. Based upon the relative decolorization efficiency of different isolates, five the most efficient isolates (ETL-1, ETL-2 & ETL-3) with more than 80% decolorizing efficiency were selected for further experiments (Data not shown).

3.1. Biodecolorization of Remazol Black-B by Selected Bacterial Isolates

Biodecolorization of Remazol Black-B by the selective bacterial isolates (ETL-1, ETL-2 & ETL-3) was confirmed by conducting another experiment in liquid medium at different time periods (Figure 1). It was found that different bacterial isolates had variable potential to

remove Remazol Black-B in the growing cultures. The most efficient bacterial isolate to decolorize the Remazol black-B was ETL-1 with 98% color removal efficiency in 18 h incubation period while remaining isolates displayed maximum decolorization in 24 h. Isolate ETL-2 was the second most efficient bacterial isolate and it decolorized the Remazol Black-B up to 94% in 24 h. Similarly, ETL-3 isolates had decolorization potential of 80.

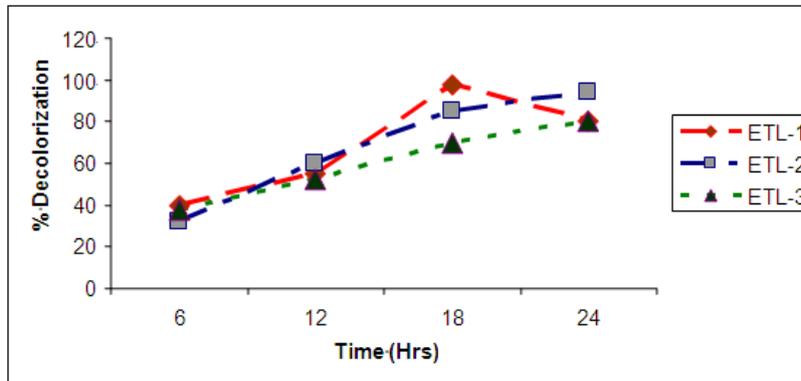


Figure 1. Biodecolorization of Remazol Black -B

3.2. Factors Affecting Biodecolorization of Remazol Black-B in Liquid Medium

Potential of selected isolates (ETL-1, ETL-2, and ETL-3) was further investigated for the optimization of various incubation/ environmental conditions for decolorizing the azo dye in liquid medium. It was evident (Figure 2) that Remazol Black-B azo dye decolorization sharply increased up to 100 mg L-1 of substrate concentration and maximum decolorization was observed at 100 mg L-1 of substrate concentration. Then, there was a gradual

decrease in the azo dye decolorization. Isolate ETL-1 was the most efficient azo dye decolorizing strain with more or less complete removal of the color i.e., 100% decolorization at 100 mg L-1 and minimum decolorization was recorded at 50 mg L-1 while after 100 mg L-1 substrate concentration, again ETL-1 showed a decreasing trend. Isolate ETL-2 was the second at the rank with 90% decolorization at 100 mg L-1. But, ETL-3 showed different trend from the other isolates, it indicated enhanced decolorization up to 200 mg L-1 (82%).

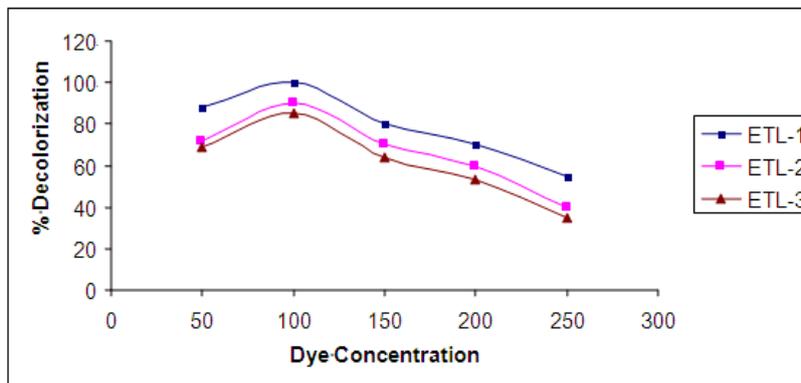


Figure. 2 Effect of Substrate Concentration

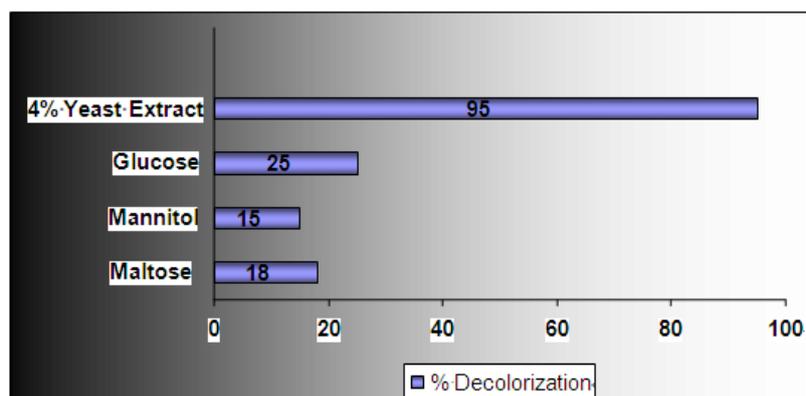


Figure 3. Effect of different Sources of carbon on decolorization of Ramazol Black-B

3.3. Types of Carbon Sources

Effects of different carbon sources such as maltose, mannitol, glucose and yeast extract were evaluated on Remazol Black-B decolorization by bacterial isolates (Figure 3). It was found that maximum decolorization occurred with 4% yeast extract in all selected strains (85 to 95%) that was followed by glucose in which decolorization occurred in the range of 20 to 25%. However, least decolorization was observed in the case of mannitol (10 to 15%). Similarly, maltose application also showed decolorization in the lower range (up to 18%).

3.4. Effect of pH

For studying effect of pH value, different levels of pH ranging from 5 to 9 were used and incubation of all selected isolates was done at these levels (Figure 4). Initially with the increase in pH value from 5 to 7, decolorization increased and maximum occurred at 7 pH. Similarly, further increase in pH from 7 to 9 had negative effect on decolorization capacity of various isolates. The maximum decolorization was observed with the isolate ETL-1 (98%) at pH 7 while minimum decolorization occurred at pH 9. Similar trends in remaining isolates ETL-2, and ETL-3 were observed at pH 7. Overall, it was noted that all the bacterial isolates showed optimum decolorization from pH 5 to 7.

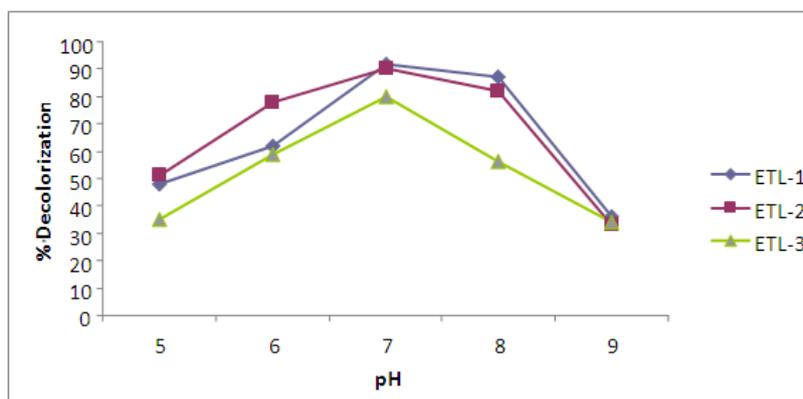


Figure 4. Effect of pH on decolorization of Ramazol Black-B

3.5. Effect of Incubation Temperature

Five levels (25,30,35,40, and 45°C) of temperature were used for assessing optimal biodecolorization of Remazol Black-B by selected bacterial isolates. It is evident (Figure 5) that when the temperature raised from 25 to 35°C there was inconsistent trend in decolorization by different isolates. The ETL-1 and ETL-2 isolates showed gradual increase in decolorization, while one isolate ETL-3 displayed maximum decolorization at 25°C. Remaining two bacterial isolates (ETL-1 and ETL-2) with

a gradual rise from 25 to 35°C showed maximum decolorization at 35°C. As the temperature increased further from 35°C to 45°C, there was sharp decline in decolorization capacity in all the isolates. It was also observed that with rise in temperature, abiotic decolorization also increased. Maximum decolorization was observed with the isolate ETL-1 (98%) at 35°C and it is followed by ETL-2 (94%) at the same temperature. Least decolorization was observed at 45°C in all the selected isolates.

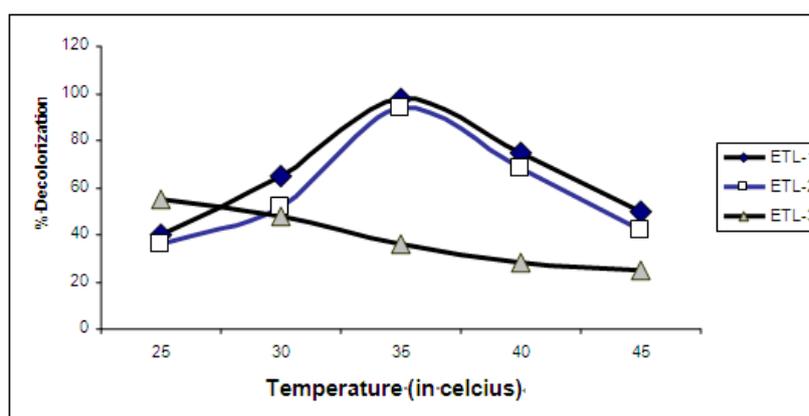


Figure 5. Effect of incubation temperature

4. Discussion

Industrial effluent is not stable and it varies often in a wide range depending upon the process practiced. South Asian countries are experiencing severe environmental problems due to rapid industrialization. This phenomenon is very common where the polluting industries like textile

dyeing, leather tanning, paper and pulp processing, sugar manufacturing, etc. thrive as clusters. Among these the Textile industries are large industrial consumers of waters as well as producers of wastewater. The effluent discharged by this industry leads to serious pollution of groundwater and soils and ultimately affects the livelihood of the poor [21]. During the dyeing process a substantial amount of dyes and other chemicals are lost in waste

water. Estimates put the dye losses between 10 - 15% [22]. Dye is generally not toxic to the environment but the color water bodies may hinder high penetration there by affecting the aquatic life and limiting the utilization [23]. Color removal of industrial effluent has been a major concern in waste water that originates from textile and dye stuff plant with a continuous discharge of great quantity of remaining dyes to the environment. The efficient treatment of the effluent is an eco-friendly method for treatment of textile effluent. The degradation of molecules of dyes in the environment by microorganisms is likely to be slow, which means that it is possible for high levels of dye to persist and potentially accumulate. Due to the low degradability of the dyes, conventional biological treatment process is inefficient in treating dye waste waters. Biological decolorization is employed under either aerobic or anaerobic environment. A number of reports discourage the azo dye decolorization by microorganism under anaerobic conditions as it leads to the formation of corresponding aromatic amines. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms. Over the past decades, many microorganisms are capable of degrading azo dyes, including bacteria, fungi and yeast. The release of colored wastewater into water streams by textile industry represents a serious environmental problem and a public health concern. Major portion of this wastewater contains azo dyes which are increasingly used in industries because of their ease and cost-effectiveness in synthesis compared to natural dyes. Relative effectiveness of the isolated bacteria for the decolorization of Remazol Black-B clearly implies that these can be effectively used for the removal of Remazol Black-B from contaminated industrial wastewater. Azoreductase is reported to be the key enzyme expressed in azo-dye-degrading bacteria and catalyses the reductive cleavage of the azo bond [20,24]. Azoreductase activity had been identified in several species of bacteria recently, such as *Staphylococcus aureus*, *Shewanella putrefaciens*, *Shewanella* strain J18 143 and *Pseudomonas* sp [20,24,25,26]. It was indicated that increase in substrate concentration from its optimum level had negative effect on decolorization capacity of isolates. Investigations with different dye concentrations in other experiments also reported higher net color removal efficiencies at lower dye concentrations [27,28,29]. Decrease in decolorization ability at high substrate concentration might be due to the toxicity of the dye (and co contaminants) [30]. Azo dyes generally contain one or more sulphonic-acid groups on aromatic rings, which might act as detergents to inhibit the growth of microorganisms [30]. Another reason of the toxicity at higher concentration may be due to the presence of heavy metals (metal-complex dyes) and/or the presence of nonhydrolyzed reactive groups which may retard the bacterial growth (reactive dyes) [29]. Similarly, reduction in decolorization at low concentration of the substrate might be due to the decrease in enzyme ability to recognize the substrate efficiently. Whereas in case of different carbon sources tested yeast extract proved to be the best amongst tested carbon source. Our results were in agreement with the research conducted by Guo *et al.* (2008), in which the bacterial strains grew well and completely decolorized K-2BP where either yeast extract or peptone was present in the medium; however, glucose,

glycerol, sucrose, lactose and starch resulted in lower rates of growth and decolorization of these dyes. Other studies also reported the maximum decolorization of azo dyes in the presence of yeast extract by bacteria [32]. In case of pH as a variable, decolorization was on higher side at pH 7. Whereas higher pH values (alkaline conditions) decreased the decolorization efficiency of all the tested isolates. So, from this study, it could be concluded that neutral pH supported bacterial activity to decolorize Remazol Black-B in liquid medium [33,34]. Temperature is another very important parameter for anaerobic treatment of wastewater. Selected isolates were mesophilic bacteria because they all showed better decolorization in the temperature range of 25 to 35°C. Similar results were also reported by Guo *et al.* (2008). The mesophilic range is traditionally used [35] since it is generally thought that maintaining high temperature would be uneconomical, while degradation within the psychrophilic range is too slow. Overall, one of the selected isolate (ETL-1) of bacteria was able to completely remove color of the dye in 18 h. However, these isolates should be tested at large scale treatment system to examine their potential for bioremediation of dye-polluted wastewaters.

5. Conclusion

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 eco-friendly techniques using bacteria can be applied for fine tuning of waste water treatment. Biotreatment offers easy, cheaper and effective alternative for color removal of textile dyes. Thus, by this present study, concluded that the bacterial isolate ETL-1 was used as a good microbial source for waste water treatment.

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