Soil Environ. 30(1): 7-12, 2011
www.se.org.pk
Online ISSN: 2075-1141
Print ISSN: 2074-9546

Isolation and screening of azo dye decolorizing bacterial isolates from dye-contaminated textile wastewater

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Abstract

Azo dyes are released into wastewater streams without any pretreatment and pollute water and soil environments. To prevent contamination of our vulnerable resources, removal of these dye pollutants is of great importance. For this purpose, wastewater samples were collected from dye-contaminated sites of Faisalabad. About 200 bacterial isolates were isolated through enrichment and then tested for their potential to remove Remazol Black-B azo dye in liquid medium. Five bacterial isolates capable of degrading Remazol Black-B azo dye efficiently were screened through experimentation on modified mineral salt medium. Isolate SS1 (collected from wastewater of Supreme Textile Industry) 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) and at temperature 35^{o}C. Similarly, yeast extract proved to be the best carbon source for decolorization purpose. The results imply that the isolate SS1 could be used for the removal of the reactive dyes from textile effluents.

Keywords: *Azo dyes, Remazol Black-B, Wastewater, Yeast extract, Textile effluent*

Introduction

Environmental pollution caused by the release of a wide range of azo dyes through industrial wastewater is a serious problem in present days. The annual world production of azo dyes is estimated to be around one million tons, and more than 2000 structurally different azo dyes are currently in use (Vijaykumar *et al.,* 2007). Azo dyes are used in a number of industries such as textile dyeing, food, cosmetics, paper printing, with the textile industry as the largest consumer. Textile industry is known for using large quantities of water and variety of chemicals (Qin *et al.,* 2007). The high quality water is a crucial factor in many processes such as cleaning, rinsing, dyeing and washing (Fersi *et al.,* 2005). Furthermore, most of areas of Pakistan fall under arid and semi arid climate, where water shortage is a limiting factor for crop production (Ali *et al*., 2009). So, treatment of such high amount of wastewater could help to meet irrigation requirement of the crops.

The discharge of these highly colored industrial effluents can be very dangerous to the receiving water resources, as these dyes in the water absorb sunlight, which in result decreases the intensity of light absorbed by water plants and phytoplankton, ultimately reducing photosynthesis and the oxygenation of water reservoirs. Also, physical appearances of the colored water badly impact its aesthetic value. These dyes are xenobiotic in nature and in some cases are mutagenic and carcinogenic (Daneshvar *et al*., 2007; Dafale *et al*., 2010). Allergic effects of these dyes have also been reported by several scientists (Saunders *et al*., 2004; Sasaki *et al*., 2008).

So, azo dyes-contaminated effluents have emerged as a serious issue because of their negative impact on water ecosystems and human. Due to polluted water, very serious health issues are arising (Jamil *et al*., 2009). Different factors which reduce degradation of dyes include high water solubility, high molecular weight and fused aromatic ring structures, which inhibit permeation through biological cell membranes (Elisangela *et al.,* 2009; Hsueh *et al*., 2009; Mansour *et al*., 2009). Various physiochemical methods can be used for the removal of azo dyes from the wastewater. Some of these methods are effective but are quite expensive because they generate significant amounts of chemical sludge waste whose disposal in a secure landfill increases process cost. Also, there is disposal problem of such waste material to a proper place that also limit the use of these methods (Aguedach *et al*., 2005; Sanghi *et al.,* 2007; Hernandez *et al*., 2008).

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 (Hao *et al.,* 2007; Pandey *et al.,* 2007; Khalid *et al.,* 2008). So, present study was designed to isolate efficient azo dye

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decolorizaing 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.

Materials and Methods

Sampling

Water and sludge samples were collected from Paharang drain near Bawa Chak, Faisalabad 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 (sampling code)	TSS (meq L^{-1})	pH	Notes
Ahsan Habib Processing (AH)	55.2	8.7	effluent
Arif Processing (AP)	76.2	10.2	effluent
Bawa Sam (BS)	80.4	8.0	effluent
Crescent Textile and Processing (C)	52.8	8.0	effluent
Dawood Processing (D)	88.7	10.7	effluent
Jaugar Processing (J)	59.1	7.2	effluent
Mubarak Textile (MT)	83.5	9.9	effluent
Mubarak Processing (MP)	57.8	12.4	effluent
Siddique Processing (S)	60.4	8.2	effluent
Supreme Textile and Processing (Su)	147.6	8.1	sludge
Sludge taken from Baw Sam near Supreme Textile and Processing (SS)	93.7	7.7	sludge
Zafar Fabrics and Processing (Z)	60.13	6.8	effluent

Isolation of azo dye decolorizing bacteria

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 (Khalid *et al*. 2008). Dye was added at a concentration of 100 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 30 °C for 7 days under static conditions. After incubation, cell suspensions from each flask were plated onto MSM agar medium and incubated at 30 °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 200 actively growing colonies were selected for purification.

Purification of bacterial isolates

Selected isolates were purified by streaking on MSM medium containing agar at the concentration of 16 g L^{-1}. Streaking was done thrice in zig zag manner. The purified cultures were preserved in a refrigerator for subsequent study.

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, 200 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 ^{o}C for 24 h. After 24 h, the respective cells were harvested by medium centrifugation at 10000 rpm for 10 minutes. Then decolorization was determined with the help of spectrophotometer at 597 nm. Uninoculated blanks were run to determine abiotic decolorization. The five most effective bacterial isolates (SS1, SS2, MT, BS and AP) 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 0.4% 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).

Optimization of environmental factors for efficient decolorization

Factors like substrate concentration, temperature and pH were optimized during the experimentation for

maximizing decolorizing efficiency of the isolates. 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, 100, 150, 200 and 250 mg L^{-1}), pH values (5, 6, 7, 8, 9) and temperatures (25, 30, 35, 40, 45 ^{o}C). All the bacterial isolates SS1, SS2, MT, BS, and AP 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.

Statistical analysis

Data were entered in a Microsoft® Excel 2007 spreadsheet, and means and standard deviations were calculated.

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 (SS1, SS2, MT, AP, and BS) with more than 75% decolorizing efficiency were selected for further experiments (Data not shown).

Biodecolorization of Remazol Black-B by selected bacterial isolates

Biodecolroization of Remazol Black-B by the selective bacterial isolates (SS1, SS2, MT, AP and BS) 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 SS1 with 100% color removal efficiency in 18 h incubation period while remaining isolates displayed maximum decolorization in 24 h. Isolate SS2 was the second most efficient bacterial isolate and it decolorized the Remazol Black-B up to 94% in 24 h. Similarly, MT, AP and BS isolates had decolorization potential of 84, 82 and 76%, respectively.

Factors affecting biodecolorization of Remazol Black-B in liquid medium

Potential of selected isolates (SS1, SS2, MT, AP, and BS) was further investigated for the optimization of various incubation/ environmental conditions for decolorizing the azo dye in liquid medium.

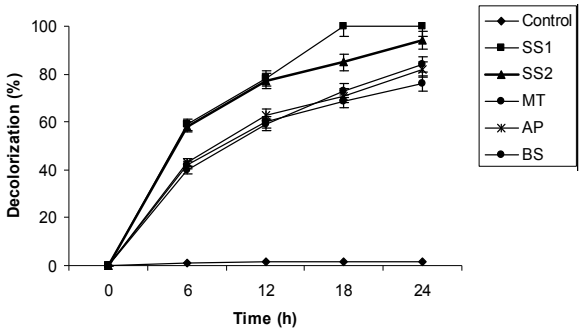

Figure 1: Biodecolorization of Remazol Black-B in liquid medium by selected isolates of bacteria. Standard deviation is presented as bars on each level

Substrate concentration

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 SS1 was the most efficient azo dye decolorizing strain with 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 SS1 showed a decreasing trend. Isolate SS2 was the second at the rank with 93% decolorization at 100 mg L^{-1}. The same trend of decolorization was followed in case of SS2, MT, and BS as observed in the case of SS1. But, AP showed different trend from the other isolates, it indicated enhanced decolorization up to 200 mg L^{-1} (82%).

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 (75 to 100%) that was followed by glucose in which decolorization occurred in the range of 20 to 27%. However, least decolorization was observed in the case of mannitol (15 to 18%). Similarly, maltose application also showed decolorization in the lower range (up to 20%).

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 SS1 (100%) at pH 7 while minimum decolorization occurred at pH 9. Similar trends in remaining isolates SS2, MT, AP, and BS were observed at pH 7. Overall, it was noted that all the bacterial isolates showed optimum decolorization from pH 5 to 7.

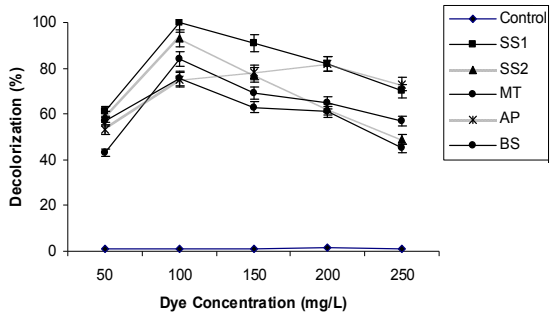

Figure 2: Effect of substrate (Remazol Black-B) concentration on bacterial decolorization. Standard deviation is presented as bars on each level

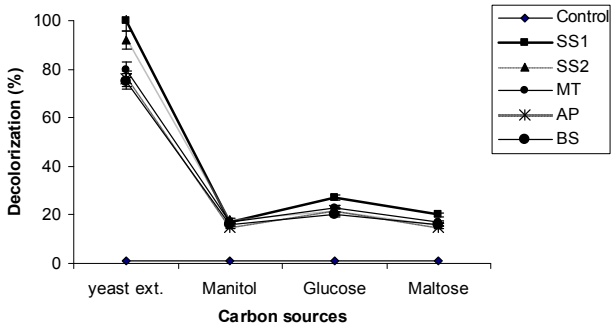

Figure 3: Effect of different sources of carbon on biodecolorization of Remazol Black-B by bacterial isolates. Standard deviation is presented as bars on each level

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 inconsistant trend in decolorization by different isolates. The SS1, SS2, and BS isolates showed gradual increase in decolorization while one isolate MT displayed maximum decolorization at 25 °C and isolate AP displayed maximum value at 30 °C. Remaining three bacterial isolates (SS1, SS2 and BS) 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 SS1 (100%) at 35 °C and it is followed by SS2 (92%) at the same temperature. Least decolorization was observed at 45 °C in all the selected isolates.

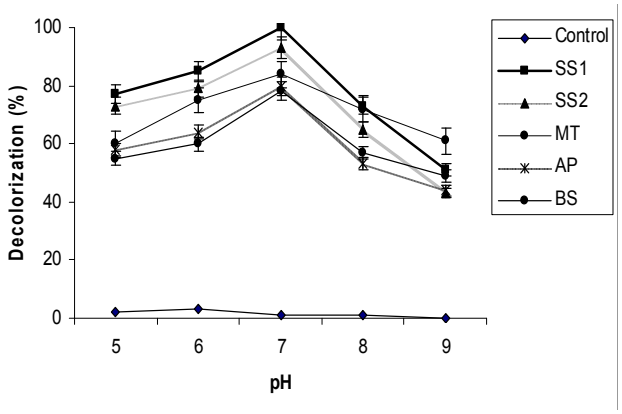

Figure 4: Effect of different levels of pH on biodecolorization of Remazol Black-B by bacterial isolates. Standard deviation is presented as bars on each level

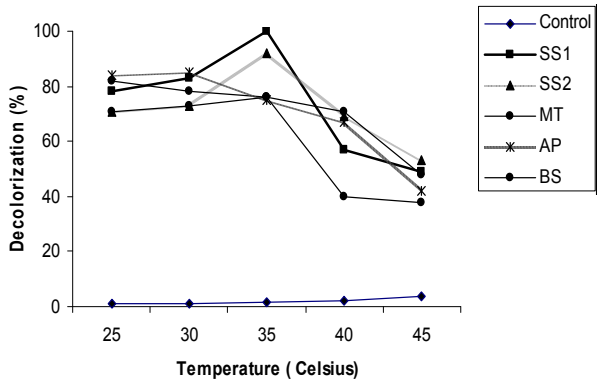

Figure 5: Effect of incubation temperature on biodecolorization of Remazol Black-B by bacterial isolates. Standard deviation is presented as bars on each level

Discussion

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 (Nachiyar and Rajkumar, 2005; Khalid *et al*., 2008). Azoreductase activity had been identified in several species of bacteria recently, such as *Staphylococcus aureus*, *Shewanella putrefaciens, Shewanella* strain J18 143 and *Pseudomonas* sp (Nachiyar and Rajkumar, 2005; Khalid *et al*., 2008; Li and Guthrie, 2010**;** Lin *et al*., 2010)

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 (Cruz and Buitron, 2001; Kapdan and Oztekin, 2003; Sponza and Isik, 2005). Decrease in decolorization ability at high substrate concentration might be due to the toxicity of the dye (and co contaminants) (Chen *et al*., 2003). Azo dyes generally contain one or more sulphonic-acid groups on aromatic rings, which might act as detergents to inhibit the growth of microorganisms (Chen *et al*., 2003). 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 non-hydrolyzed reactive groups which may retard the bacterial growth (reactive dyes) (Sponza and Isik, 2005). 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 (Hu, 1998; Moosvi *et al.,* 2005).

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 (Mali *et al.,* 1999; Chang *et al.,* 2000). 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 (Varel *et al*., 1980) 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 (SS1) 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.

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