ISOLATION AND OPTIMIZATION OF AZO DYE DECOLORIZATION ACTIVITY OF *BACILLUS* SPP

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Abstract: Toxic effluents containing azo dyes are discharged from various textile industries and they adversely affect water resources, soil fertility, aquatic organisms and ecosystem integrity. Over the last two decades, considerable work has been done with the goal of using microorganisms as bioremediation agents in the treatment of dye containing waste waters. In current research work, dye decolorizing microorganisms were isolated from textile effluent samples collected from Kalyan-MIDC area and parameters such as various carbon sources, nitrogen sources, pH and temperature were optimized for decolorization of Congo red and Malachite green using most potent isolates. Six different bacterial spp. were isolated and subjected to dye decolorization assays. Among the six bacterial isolates, B2 strain exhibited the highest potential in decolorizing Congo red and Malachite green up to 79% and 82%, respectively. The isolate was identified as a *Bacillus spp.* and it was more effective at pH 8, temperature 37^{0}C, with peptone as nitrogen source and glucose as carbon source. This decolorization potential suggests the applicability of this isolate for dye removal in effluent from textile industries.

Keywords: Congo red, Decolorization, Malachite green, Textile effluent sample.

Introduction: The textile processing industry adds severe burden on the environment through the release of heavily polluted wastewaters. Azo dyes are a large group of dyes extensively used in textile industry and are common industrial pollutants[1]. Azo dyes are characterized by the presence of one or more azo groups (-N=N-) which are responsible for their coloration. As these dyes are most versatile, have structural properties that are not easily degradable under natural conditions and are not typically removed from water by conventional waste water treatment systems. Azo dyes are designed to resist chemical and microbial attacks and to be stable in light and during washing [2]. Many azo dyes are carcinogenic and may trigger allergic reactions in humans and significantly affect photosynthetic activity in aquatic life due to reduce light potential [3].

The treatment of textile effluents contaminated with dyes is necessary prior to their final discharge to the environment. Various kinds of physicochemical methods are in use for the treatment of wastewater contaminated with dyes. These include filtration, coagulation, use of activated carbon and chemical flocculation. While these methods are effective, they are expensive and involve the formation of a concentrated sludge that creates a secondary disposal problem [4]. Compared to these conventional methods, microbial decolorization is a cost effective method and would be of great importance because it's inexpensive, ecofriendly and has less sludge producing properties.

Many microorganisms belonging to different taxonomic groups of bacteria, fungi, actinomycetes and algae have been reported for their ability to decolorize synthetic dyes [5]. The microorganisms such as *Aeromonas, Pseudomonas, Bacillus, Rhodococcus, Shigella, Klebsiella, Rhizopusoryzae, Penicillium oxalicum* and *Phanerochaete chrysosporium* are used in dye decolorization [6]. Bacteria cleave the azo bond using an azoreductase enzyme which results in decolorization of dye [7].

The present study is focused on the decolorization of Congo red and Malachite green dyes by bacteria isolated from local industrial waste. This approach may be useful in providing an alternative method to accomplish dye

degradation of a wide range of dyes in an ecofriendly manner. **Materials and methods:**

Sample collection:

The textile effluent samples were collected from small dyeing industries located in Kalyan-Dombivali MIDC area, Kalyan, Dist. Thane. The samples were transported to the laboratory in sterile container and stored at 4^0C.

Chemicals and Media:

Textile dye Congo red (CR) and Malachite green (MG), microbiological media and individual medium ingredients were purchased from Himedia laboratories (Mumbai, Maharashtra, India).

Isolation and screening of dye decolorizing bacteria:

5ml of sample was inoculated in 100 ml of sterile Nutrient broth. The flasks were incubated at ambient temperature under shaking conditions with 150 rpm. After 48 hrs of incubation, 1 ml of the culture broth was appropriately diluted and plated on Nutrient agar containing 0.02 gm/L Malachite green and Congo red dye separately. Plates were incubated at 37°C for 24 hrs. After incubation, morphologically distinct bacterial isolate showing clear zone around their colonies due to decolorization of dyes were selected for further studies [8].

Dye decolorization assay:

The selected six isolates were screened for their ability to decolorize Congo red and Malachite green in Nutrient broth.

Decolorization activity was performed in 100ml Nutrient broth containing 0.02 gm/L Congo red and Malachite green individually. The pH was adjusted to 7 using 1*N* HCL and 1*N* NaOH. The flasks were inoculated with 5 ml of 24 hrs. old bacterial culture and incubated at 37^0C on a shaker (Make : Remi) with 150 rpm for maximum 1 week. Medium without dye were used as blank. Uninoculated dye medium served as control. After incubation 10 ml medium was centrifuged at 5000 rpm for 15 min. and supernatant was removed.

Decolorization was assessed by measuring absorbance of the supernatant at wavelength maxima (λm) of respective dye. (Malachite green = 470 nm and Congo red = 580 nm) [2], [8]. The percentage decolorization was calculated from the following equation,

$$\% \ Decolorization = (Initial\ OD - Final\ OD)\ x\ 100/Initial\ OD$$

Identification of the most potent dye decolorizing bacteria:

The isolate with highest potential of Azo dye decolorization was identified. Morphology characteristics, utilization of sugars sole source carbon source and biochemical tests were studied.

Optimization of decolorization ability for the selected isolate:

Optimization medium:

One gm/L yeast extract was supplemented in Mineral salt medium (MSM) used in optimization experiments to support growth and increase the degradation ability of selected bacterial isolate [6].

All the experiments were conducted in triplicate with standard conditions such as pH-7, incubation temp. 37^0C, 0.02 gm/L dye conc., culture density of 0.6 O.D. at 600nm wavelength. 3 ml of cell suspension was inoculated in 100 ml of MSM broth.

Decolorization was optimized with respect to the effect of 0.5 gm/L carbon sources (glucose, fructose, mannitol, sucrose, lactose), 1 gm/L nitrogen sources (NH_4CL, $NaNO_3$, NH_4SO_4, NH_4 oxalate, peptone), pH (4,6,7,8,10) and temperature (4^0C,15^0C, R.T., 37^0C, 55^0C).

Uninoculated broth served as control. After incubation samples were analyzed for percent decolorization.

Results and discussion:

Isolation and screening of dye decolorizing bacteria:

There is concern for the treatment of industrial effluents from textile and manufacturing units, with attention on this subject throughout India. Several researchers have demonstrated the possibility of utilizing micro-organisms for bio-treatment of textile wastewaters [9].

Six different morphologically distinct bacterial colonies having clear zone were isolated. These were inoculated on Nutrient agar slopes and

after growth, stored at 4^0C.

Dye decolorization assay:

All six bacterial isolates were screened for their efficacy in decolorizing azo dyes from culture medium. All the isolates were able to decolorize Congo red and Malachite green greater than 50% and 60% respectively. Results are shown in **Table 1**. The B2 isolate more efficacious in decolorizing the two dyes both in percentage and time to decolorization. Based on these results, the B2 isolate was chosen for further studies.

The present study indicates that effluent adapted strains may be better candidates for potential bioremediative use.

Identification of the most potent dye decolorizing bacteria:

The isolated organism was found to be Gram positive, rod-shaped bacterium. The isolate was also a spore former and exhibited motility. Biochemical characteristics were found to be negative for indole, methyl red, Voges - Proskauer, citrate utilization, oxidase, nitrate, and urea tests. The strain utilized various sugars D-maltose, D-glucose, D- lactose, mannitol, and sucrose as sole carbon sources with acid production. On comparing the results of morphological and biochemical tests, the isolate was identified as *Bacillus spp.* [10].

The bacterial isolate *Bacillus spp.* originated form the dye contaminated textile wastewater of local industry can easily decolorized the CR and MG.

Optimization of decolorization ability for the selected isolate:

Following are the parameters optimized to achieve effective decolorization rate of Congo red and Malachite green by the selected isolate.

Effect of Ph:

While the selected B2 isolate was able to decolorize the two dyes across a wide range of pH (**Fig. 1**) maximum degradation of 85% and 80% was observed for MG and CR, respectively, at pH 8. The lowest dye degradation of 18% (MG) and 14% (CR) was observed at pH 4.

Effect of temperature:

As seen in **Fig. 2**, it was observed that an increase in temp from 4^0C to 37^0C had positive effect on decolorization of Congo red and malachite green. Optimal temp to decolorize Congo red and Malachite green azo dyes for tested strain was 37^0C showing 85% and 81% decolorization respectively. However decolorization rate dropped sharply as the temp increased from 37^0C to 50^0C.

Table 1: Decolorization activity of selected isolates in percentage

Isolate	% decolorization of CR	Time of decolorization of CR (Hr.)	% decolorization of MG	Time of decolorization of MG (Hr).
A1	52	120	60	48
B1	58	96	72	24
B2	79	72	82	24
C1	70	96	79	48
C2	60	72	64	24
D1	67	96	51	24

Keys: CR: Congo red, MG: Malachite green

Effect of Carbon source:

Textile industrial effluents are deficient in carbon content and biodegradation without any extra carbon source is very difficult to achieve. Therefore, different co-substrates such as sucrose, glucose, mannitol, fructose, lactose were supplemented in the medium and decolorization of the two dyes was studied individually. Results presented graphically in **Fig. 3**.

Among the different carbon sources studied, glucose showed maximum decolorization of 78% and 82% for CR and MG respectively. Maltose and sucrose recorded the lowest decolorization percentage.

Effect of Nitrogen source:

A number of organic and inorganic sources of nitrogen were used in this experiment **(Fig. 4)**. Maximum decolorization with nitrogen source was achieved with peptone (80% for CR and 82% for MG). Ammonium sulphate and ammonium oxalate showed 77% and 68% decolorization of MG respectively. NH_4Cl was the least effective as a nitrogen source for decolorization of the two dyes.

Conclusion:

We isolated a *Bacillus spp*. from textile waste water sample from Kalyan area which has a potential to decolorize dyes in textile effluents. The study shows that pH, temperature and various carbon and nitrogen sources have a significant influence on dye removal efficiency of the isolate. The use of the microorganisms for the removal of synthetic dyes from industrial effluent offers considerable advantages. The process is relatively inexpensive and the end products of the complete mineralization are not toxic. Further studies on enzymatic and genetic factors are necessary and degradation to enhance decolorization efficacy.

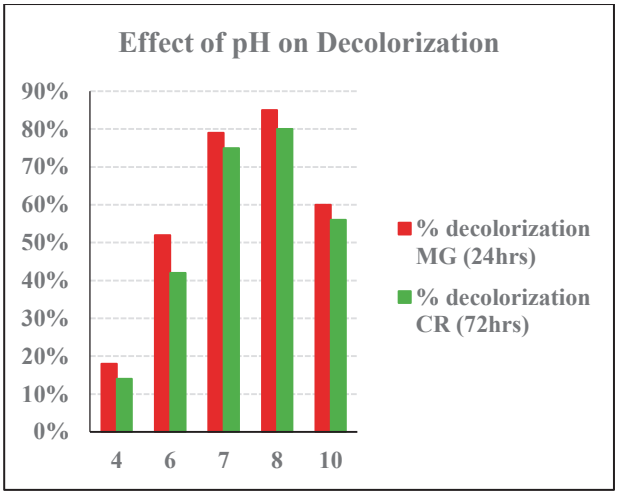

Fig:1 Effect of pH on decolorization

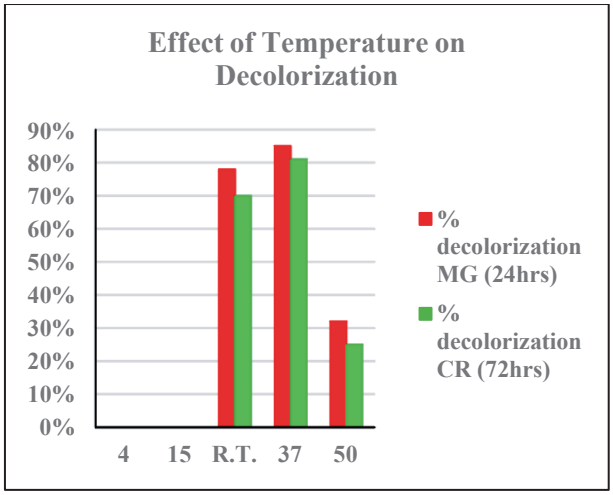

Fig: 2 Effect of temp. on decolorization

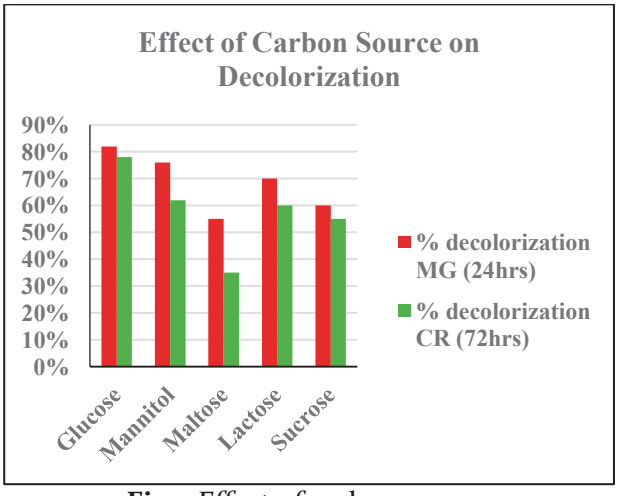

Fig:3 Effect of carbon source

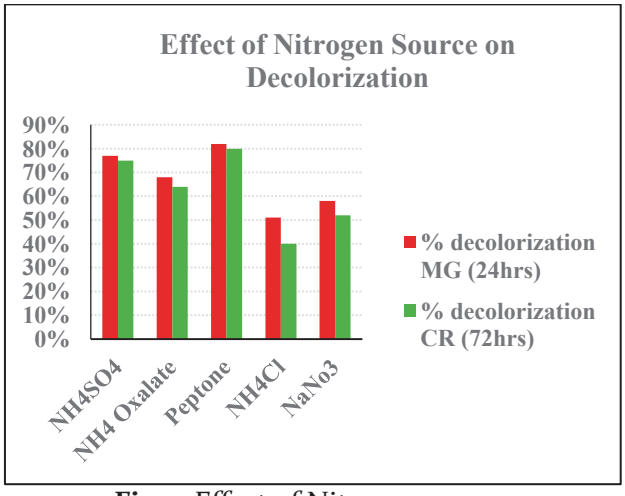

Fig: 4 Effect of Nitrogen source

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