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Original Research Article

Decolorization potential of *Bacillus* sp. for removal of synthetic textile dyes

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A B S T R A C T

During the last few years it has been demonstrated that several bacterial strains decolorize the synthetic textile dyes. In the present study, the decolorization potential of two textile dyes (malachite green and indigo carmine) by the *Bacillus* sp. isolated from dye contaminated soil of the local dying houses in Nagpur-India was determined. Decolorization rate was monitored by spectrophotometry. Different parameters such as pH, time and temperature were optimized for the present study. It was found that *Bacillus* sp. decolorized 95.12% of malachite green (100mg/L) at pH 9.5 and 66.6% of indigo carmine (100mg/L) at pH 6 at 37^0C within 96 hours under shaking condition. According to the ability of *Bacillus* sp. to decolorize the textile dyes, this bacterial strain could be used to decolorize and degrade different dyes of textile industry.

Introduction

The control of water pollution is becoming increasingly important these days. Release of dyes into environment constitutes only a small portion of water pollution. Government legislation is forcing textile industries to treat their waste effluent. Currently, removal of dyes from effluents is by the physic-chemical means. Such methods are often very costly and though the dyes are removed but accumulation of concentrated sludge creates a disposal problem. There is a need to find alternative methods of treatment that are effective in removing dyes from large volumes of effluents and are low cost such as biological or a combination systems.

For biological treatment of the wastewater containing dyes, the microbial decolorization and degradation of dyes has been of considerable interest. Aerobic degradation of triphenylmethane dyes has been demonstrated repeatedly; however these dyes resist degradation in activated sludge systems (Sarnaik and Kanekar, 1999). Malachite green (MG) is used extensively for dyeing silk, wool, jute, leather, ceramics, and cotton and used to treat fungal and protozoal infection (Srivastava *et al.*, 2000). The members of triphenylmethane family are animal carcinogens. The Food and Drug

Administration nominated MG as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993. MG and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time. Therefore, there are both environmental and human health concerns about bioaccumulation of MG and leucomalachite green in terrestrial and aquatic ecosystems. The initial reduction of MG using an intestinal bacterium (Jones and Falkinham, 2003) has been already reported; recently our laboratory has shown 85% MG decolorized within 7 h by yeast (Kalme *et al.*, 2006).

Vat dyes are extensively used for dyeing cotton fabrics. Amongst the vat dyes, indigo dyes are commonly used for the manufacture of denim (Harazono and Nakamura, 2005). The world consumption of dyes for cellulosic fibers is about 60,000 ton per year, 5% of which is indigo dye (Paradise, 1999 and Spadaro *et al.*, 1994).

The annual production of synthetic indigo dye was estimated at 22,000 tons in 2001 (Schrott, 2001). Therefore, very large amounts of indigo dye-containing wastewater, especially textile effluent, must be treated before being discharged into the environment. Indigo dye is water-insoluble and is considered a recalcitrant substance that causes environmental concern (Balan and Monteiro, 2001).

Among the various bioremediation technologies, decolorization using microbial cells has been widely used. A newer approach on the use of microbial enzymes holds promises for effective decolorization of industrial wastewater from dyeing industries as well as degradation of ecosystems contaminated with dyes. During the last years, several bacterial strains have been described that aerobically decolorize azo dyes by reductive mechanisms (Banat *et al.*, 1996).

The objectives of the present study were: 1) To screen for the bacteria capable of decolorizing malachite green and indigo carmine. 2) To study the effects of physicochemical parameters on decolorization capacity of the bacterial isolates.

Materials and Methods

Chemicals

The textile dyes, malachite green and indigo carmine, and all the microbiological media and medium ingredients used in the present study were purchased from Himedia (Mumbai, M.S. India).

Isolation, screening and identification of dye degrading bacteria

The dye decolorizing bacteria were isolated from the soil of local dying houses in Nagpur-India. 10 gm of soil sample was suspended in 100 ml of nutrient broth supplemented with malachite green (100mg/L) and indigo carmine (100mg/L) individually and acclimatized for 5 days at 30^0C at 120 rpm. The dye decolorizing bacteria were isolated from acclimatized soil sample by serial dilution and plating appropriate dilutions on Nutrient agar medium containin, Peptone 5 g/L, Beef extract 3 g/L, Sodium chloride 5 g/L, Agar 15 g/L, Dye (Malachite Green or Indigo Carmine) 100mg, Agar 20 g/L (pH 7.0). All the isolated cultures were studied by inoculating them in nutrient broth containing dye. The inoculated medium was incubated at 30^0C under shaking condition at 120 rpm for 5 days. The decolorization effect was observed visually. The isolates showing significant decolorization of the dyes were selected for further studies. Dye

decolorizing isolates were identified on the basis of morphological and biochemical tests according to Bergey's Manual of Systematic Bacteriology (Sneath *et al.*, 1984).

Dye decolorization assay

Decolorization activity was determined in 100 ml of nutrient broth containing 10 mg of dye (Malachite Green or Indigo Carmine) and 10 % (v/v) inoculums of each isolate used separately. Uninoculated medium containing dye served as control. Inoculated medium and control was incubated at 30^{o} C for 3 days on rotary shaker at 120 rpm. About 5 ml samples were withdrawn aseptically and centrifuged at 10,000 rpm for 15 minutes.

The supernatant was used for measuring absorption at 630 nm for Malachite Green and 610 nm for Indigo Carmine using UV-Vis spectrophotometer (Shimadzu, Japan). The decolorizing activity was expressed in terms of percent decolorization which was determined by using the formula:

% decolorization ={Initial Absorbance – Final absorbance/Initial Absorbance} X100

Dye decolorization optimization

Decolorization of Malachite green and Indigo Carmine by bacterial isolate was optimized with respect to temperature (20^{0}C, 33^{o}Cand 37^{o}C), pH (5–10) and time (24–96 hours). Initial experiments were carried out with 10% (v/v) inoculum of each selected isolate in Nutrient broth medium and Nutrient broth medium without culture was served as control.

All the flasks were incubated at mentioned temperature under shaking conditions (120 rpm) for 1–4 days.

Results and Discussion

Isolation, screening and identification of dye degrading bacteria

All the isolates were screened for dye degrading ability with respect to Malachite green (100mg/L) and Indigo Carmine (100mg/L) in nutrient broth medium. Visual screening revealed that a single bacterial isolate was able to decolorize the dye from moderate to intense. The bacterial isolate was presumably identified by microscopic, biochemical characters (Table 1) and identified as *Bacillus* sp. Percentage of decolorization was calculated with respect to control.

Optimization of decolorization process

The decolorization efficiency of *Bacillus* sp. was compared across a range of pH(5-10). The maximum decolorization of malachite green (95.12%) and indigo carmine (66.66%) was recorded at pH 9.5 and pH6 respectively. At neutral pH the strain exhibited percentage decolorization value of 86.95% for malachite green and 61.29% for indigo carmine, whereas it was 23.07% at pH6 for malachite green and 48.38% for indigo carmine (Fig. 1). The optimum temperature at which effective decolorization observed was found to be at 37^{0}C; *Bacillus* sp. decolorized 90.69% of malachite green and 68.75% of indigo carmine (Fig. 2).

Effective decolorization of malachite green and indigo carmine by *Bacillus* sp. was observed after 96 hours of incubation period (Fig. 3). In the present investigation a bacterium was isolated from soil and identified as *Bacillus* sp. It decolorized 95.12% of malachite green at pH 9.5 and 66.66% of indigo carmine at pH 6 within 96 hours of incubation under shaking conditions.

Table.1 Identification of dye decolorizing bacteria from dye contaminated soil

Test	Isolate
Gram's nature	+
Shape	Rod
Motility	Motile
Glucose fermentation	Acid only
Sucrose fermentation	Acid only
Lactose fermentation	Acid only
Maltose fermentation	Acid only
Mannitol fermentation	Acid only
TSI	A/A, H_2S+
Indole production	-
Methyl red	-
Voges-Prausker	-
Citrate utilization	+
Catalase	+
Oxidase	-
Isolate identification	*Bacillus* sp.

Fig.1 Effect of pH on decolorization of Malachite green and Indigo Carmine by *Bacillus* sp

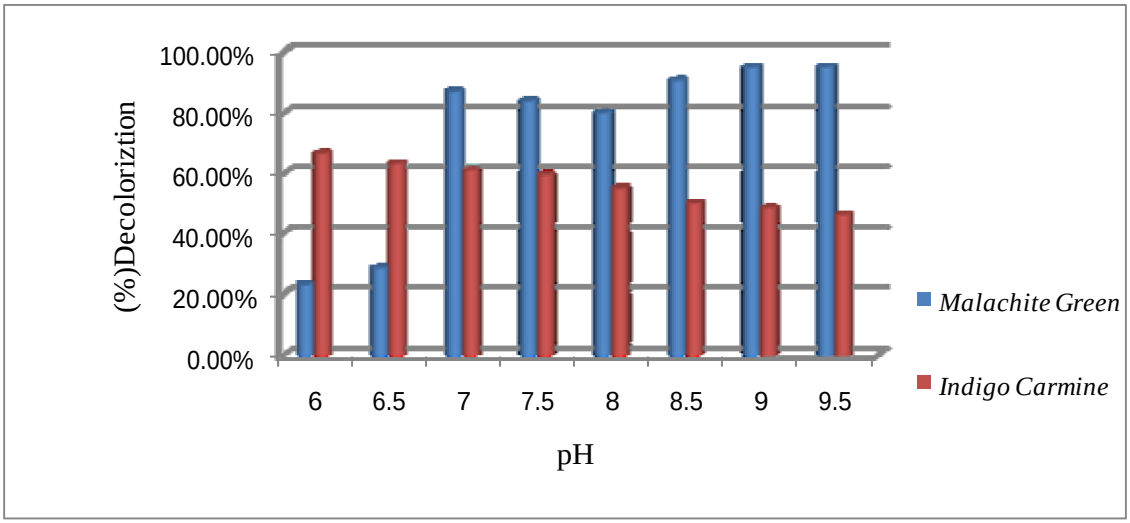


Fig.2 Effect of temperature on decolorization of malachite green and indigo carmine by *Bacillus* sp.

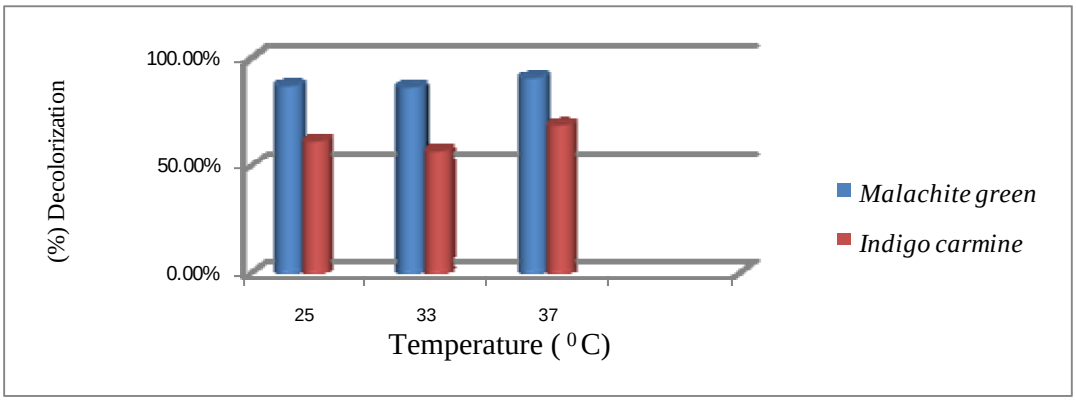
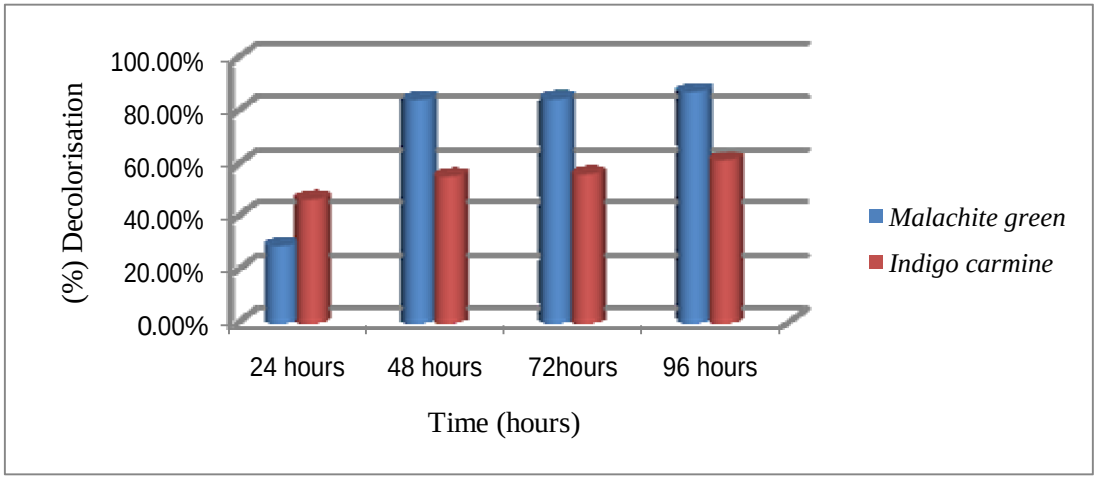


Fig.3 Effect of time on decolorization of Malachite green and indigo carmine by *Bacillus* sp.



Lal and Srivastava in 2011 studied the similar experiment and they bioremediate the malachite green. Result showed that decolorization was 100% by isolated bacterium sp. *Bacillus Strain* MTCC - 3330 comparatively more than that of *Bacillus subtilis.*

Initha Lebanon Ebency *et al.* (2013) isolated a dye decolorizing *Bacillus* sp which was found to have better ability to decolorize indigo blue dye. Effects of physiochemical parameters (time, pH, temperature) on the indigo blue decolorization by the selected bacterium were studied. *Bacillus* sp efficiently decolorize 0.02 gm of Indigo blue dye at pH 8.0 and temperature 45^0C with 144 hour of incubation.

The same trend was also reported by scientist Tom *et al.* (2011) and made an attempt to decolorize the Malachite green by aerobic mixed culture. The effect of pH, temperature, inoculums and initial concentration of dye was studied with an aim to determine the optimal conditions required for maximum decolorization and degradation. Results showed that decolorization of Malachite green were

98% in 24 hour. The culture exhibited maximum decolorization ability at pH between 7 and 8 at 30°C.

The study of Ramya *et al.* (2008) reported the decolorization and biodegradation of indigo carmine by a textile soil isolate *Paenibacillus larvae*. The effects of operational parameters (temperature, pH, dye concentration, shaking/non shaking) were tested. Maximum extent of decolorization was observed when the medium was incorporated with 10 g/l of yeast extract and peptone. Decolorization was strongly inhibited at non-shaken conditions as well as incorporation of inorganic sources (sodium nitrite and ammonium chloride) in the medium. Maximum decolorization was observed at 30 degrees C (100%) and 40 degrees C (92%) at 8 h of incubation. The LC-MS and NMR analysis confirmed the oxidation of Indigo carmine.

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