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DECOLORIZATION POTENTIAL OF BACILLUS SPECIES FOR REMOVAL OF SYNTHETIC DYES SUCH AS MALACHITE GREEN AND METHYLENE BLUE

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ABSTRACT

Bacteria have potential ability to decolorize synthetic commercial dyes used for textile dyeing. Effluents from textile and dyeing industries cause serious pollution of soil, water and environment. Therefore, this study was aimed to screen for the bacteria capable of decolorizing Malachite green and Methylene blue. The decolorization potential for two textile dyes by the Bacillus species isolated from dye contaminated soil of a local dying industry near Bangalore was determined. Different parameters such as pH, time and temperature were optimized for the present study. The optimum pH, temperature and incubation time were found to be pH 7, 45°C and 72 hours respectively. It was found that Bacillus species decolorize 92% of Malachite green (100mg/L) and 69% of Methylene blue (100mg/L) at 45°C after 72 hours under shaking conditions. As these Bacillus species have the ability to decolorize the textile dyes, these bacterial strains could be used to degrade different dyes of textile industry.

KEYWORDS: Dye decolorization, Malachite green, Methylene blue, and Bacillus species.

INTRODUCTION

Textile industry generates waste water which is a complex mixture of various chemicals such as dyes, chlorinated compounds, heavy metals, etc.[1]

Water pollution control is currently one of the major areas of scientific activity. Release of dyes into environment constitutes only a small portion of water pollution.[2] Environmental regulation in many countries have made it mandatory to decolorize dye waste water prior to

discharge into waste treatment systems and neighboring water bodies.[3] Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tons of synthetic dyes are produced annually, worldwide. Most of the dyes are reported as carcinogenic to humans.[4]

Removal of dyes from effluents done by the physico-chemical means have drawbacks due to the high cost involved and though the dyes are removed but the accumulation of concentrated sludge which are hazardous secondary pollutants create a disposal problem. Disposal of synthetic dyes improperly in wastewater depicts acute toxic effects on aquatic flora and fauna, causing severe environmental problems.[5] For biological treatment of the wastewater containing dyes, the microbial decolorization and degradation of dyes has been considerable interest.

Several studies have been reported that different microorganisms such as Bacteria[6], Yeast[7], and Fungi[8] are involved in the subject of color removal, in the mechanism of bioadsorption, biotransformation or degradation. The members of triphenylmethane family are animal carcinogens. The Food and Drug Administration nominated Malachite Green as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993.[2] Methylene blue has been described as the first fully synthetic drug used in medicine. Methylene blue is a thiazine (cationic) dye exploited in the coloring paper, hair, cotton and wools.[9] It is also commonly used by biologist as a dye that assists in the identification of bacteria because bacteria are practically colorless. Notably, about 10-15% of the dyes are lost in the effluents of textile units making them highly colored, it was reported that 280,000 tons of textile dyes are inflow in such industrial effluents every year worldwide.[10] Once they are released into receiving water bodies, they cause environmental problems where they affect water transparency, gas solubility, blocking the permeation of oxygen and sunlight necessary for the survival of various aquatic forms.[11]

Among the various bioremediation technologies, decolorization using microbial cells has been widely used. A newer approach on the use of microbial enzyme holds promise for effective decolorization of industrial wastewater from dyeing industries as well as degradation of ecosystems contaminated with dyes. During the last year several bacterial strains have been described that aerobically they decolorize azo dyes by reductive mechanisms.[12] The objectives of the present study were to screen the bacteria capable of

decolorizing malachite green and methylene blue and to study the effects of physicochemical parameters on decolorization potential of these isolated bacterial strains.

MATERIALS AND METHODS

Chemicals

The textile dyes, malachite green, methylene blue, all the microbiological media and medium ingredients used in the study were purchased from Himedia.

Isolation, screening and identification of dye degrading bacteria

The dye decolorizing bacteria were isolated from the soil of local dyeing house in Doddaballapur, Bangalore. 10 gm of soil sample was suspended in 100 ml of nutrient broth supplemented with malachite green (100mg/L) and methylene blue (100mg/L) individually and acclimatized for 5 days at 30°C at 120 rpm. The dye decolorizing bacteria were isolated from acclimatized soil sample by serial dilution and plating appropriate dilutions on Nutrient agar medium containing, Peptone 5g/L, Beef extract 3 g/L, Sodium chloride 5 g/L, Agar 15 g/L, Dye (Malachite green or Methylene blue) 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°C 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.[13]

Dye decolorization assay

Decolorization activity was determined in 100 ml of nutrient broth containing 10mg of dye (Malachite green or Methylene blue) and 10% (v/v) inoculums of each isolate used separately. Uninoculated medium containing dye served as control. Inoculated medium and control were incubated at 30°C for 3 days on rotary shaker at 120 rpm. About 5ml of 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 670 nm for Methylene Blue using UV-Vis spectrophotometer (Elico, India). The decolorizing activity was expressed in terms of percentage decolorization which was determined by using the formula.

Percentage decolorization = {Initial Absorbance - Final absorbance/Initial Absorbance} X100

Dye decolorization optimization

Decolorization of malachite green and methylene blue by bacterial isolate was optimized with respect to temperature (15°C, 30°C and 45°C), pH (5, 7 and 9) and time (24, 48 and 72 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 methylene blue (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 characteristics (Table.1) and identified as Bacillus species and the percentage of decolorization was calculated with respect to control.

Optimization of decolorization process

The decolorization efficiency of Bacillus species was compared across a range of pH (5-10). The maximum decolorization of malachite green 67.2% and methylene blue 27.2% was recorded at pH 7. At acidic pH 5, the strain exhibited percentage decolorization value of 25.0% for malachite green and 09.0% for methylene blue, whereas it was 35.2% at basic pH 9 for malachite green and 10.0% for methylene blue (Fig.1).

Bacillus species decolorized 92.0% of malachite green and 27.8% of methylene blue at 45°C, At 30°C the decolorization of malachite green and methylene blue was 87.2% and 14.4% respectively, similarly at 15°C the decolorization of malachite green and methylene blue was 46.6% and 12.2% respectively. The optimum temperature at which effective decolorization of both the dyes were found to be 45°C (Fig.2).

The decolorization of malachite green and methylene blue by Bacillus species was observed at different time intervals, after 24 hours of incubation period malachite green and methylene

blue were decolorized at 62.9% and 41.6% respectively, followed by 48 hours of incubation malachite green and methylene blue decolorized at 82.8% and 50% respectively, Similarly after 72 hours of incubation period Bacillus species decolorized 92.6% of malachite green and 69.2% of methylene blue. The optimum incubation period at which effective decolorization of both the dyes were found to be 72 hours (Fig.3).

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

Test	Isolate
Gram's nature	Positive
Shape	Rods
Motility	Motile
Starch Hydrolysis	Positive
Voges-Proskauer	Positive
Citrate utilization	Positive
Isolate identification	*Bacillus* sp.

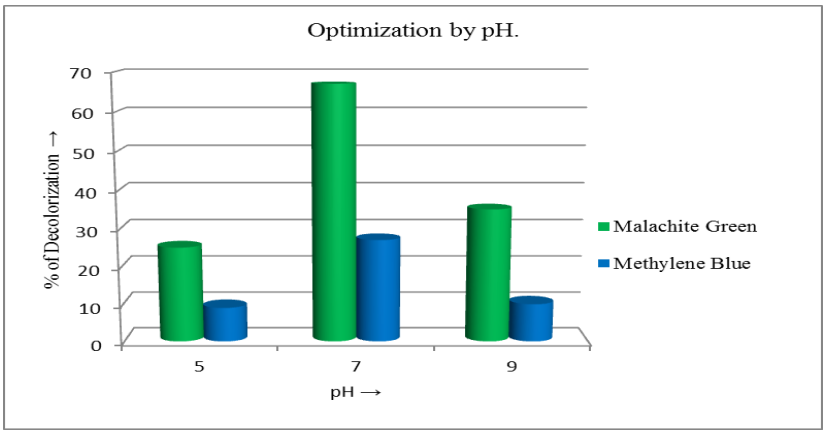

Figure.1: Effect of pH on Decolorization of malachite green and methylene blue by Bacillus species.

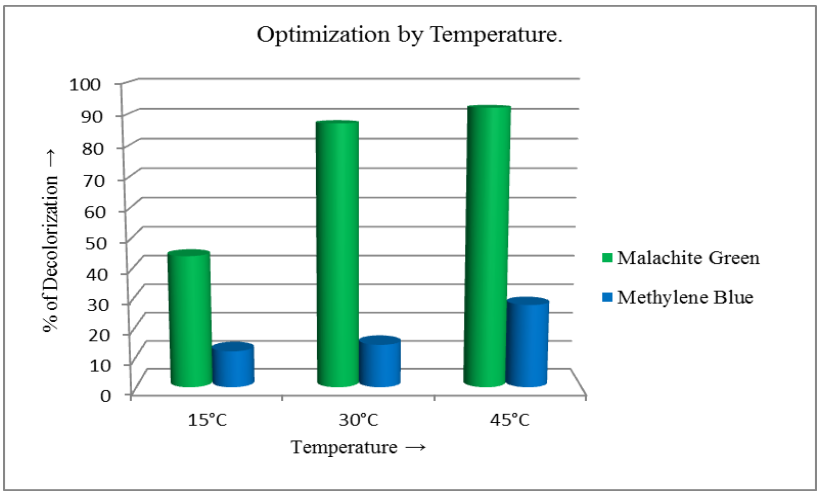

Figure.2: Effect of Temperature on Decolorization of malachite green and methylene blue by Bacillus species.

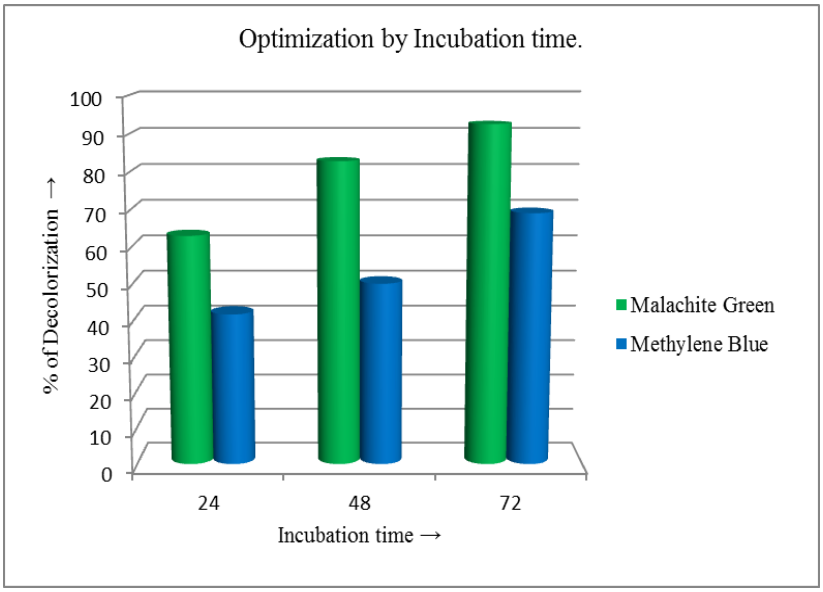

Figure.3: Effect of Incubation Time on Decolorization of malachite green and methylene blue by Bacillus species.

CONCLUSION

After screening the soil sample, *Bacillus subtilis* was obtained which was capable of decolorizing and degrading the textile dyes such as malachite green and methylene blue. Dye decolorization assay showed maximum decolorization for malachite green followed by Methylene blue. Dye decolorization optimization for both the dyes was found with respect to pH, temperature and incubation time. Optimum pH was found to be 7, Optimum temperature was found out to be 45°C and Optimum incubation time was found to be 72 hours. As these bacterial strains have potential ability to decolorize synthetic dyes, these species could be exploited in treating textile dye effluents.

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