COMPARATIVE STUDY OF DYE DECOLORIZING FFICIENCY OF MALACHITE GREEN BY BACILLUS MEGATERIUM, PSEUDOMONAS AERUGINOSA, AND ASPERGILLUS NIGER

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ABSTRACT

The triphenylmethane dye, malachite green used widely in various industrial processes possesses severe environmental concern, causing major health problems to human beings. In the present study Comparative study of, decolourization of malachite green using Bacillus megaterium, Pseudomonas aeruginosa and Aspergillus niger were investigated by optimizing various Parameter like pH,temperature,BOD,COD,static and shaking condition.It was found that maximum Decolorization given by Bacillus megaterium 98% at pH-7,and 96 % by P.aeruginesa. During study it was observed that Static and anaerobic condition prove most effective for dye decolorization.The dye decolorization was further confirmed by COD & BOD Analysis.

Key words: Biodegradation, Bushnell & Hass medium (BHM), Malachite green

I. INTRODUCTION

In industries like textile, paper and leather, about 20 variants of dyes *viz* azo dyes, reactive dyes, triphenyl methane dyes, etc. are used for dyeing [1] . These chemical dyes may be organic, polymeric or inorganic in nature and can be visible and viable even at very low concentrations. Mostly, synthetic dyes are recalcitrant in nature and furthermore found to be carcinogenic and mutagenic[2]. Malachite green, one of the basic and widely used dye was chosen as model dye compound for the present investigation.

Various physical and chemical methods like coagulation or adsorption of dyes, ultra-filtration, ion-exchange, chemical oxidation, electrolysis etc have been developed for the elimination of dyes from the wastewater since many years [4] . However these methods are not very much applied because of their high cost, high energy requirements and hazardous by-products[5]. Also these techniques generate a huge volume of sludge and cause secondary pollution due to the formation of sludge and hazardous by-products [6].

Biological methods are generally considered environment friendly as they can lead to complete mineralization of organic pollutants at low cost [7]. Bioremediation may be the most effective method for treating industrial dyes wastewater [8].

Literature review indicates numerous bacteria, fungi were able to decolorize as well as degrade the dye compounds. According to this, biological method must be an alternative method of choice because of its eco-friendly products [9][10][11].so present study conducted to find decolorization efficiency of *Bacillus megaterium, Pseudomonas aeruginosa* and *Aspergillus niger.*

II.MATERIALS AND METHODS

2.1 Dyes and Chemicals

All media component and chemicals are analytical grade and purchased from Hi-media laboratories(Mumbai, India). Malachite green was purchased from Loba chemie Pvt. Ltd. All other reagents used were of analytical grade.

2.2 Medium

The *Bacillus Megaterium, Pseudomonas aeruginosa* cultures were routinely grown at 37°C and *Aspergillus Niger* grown at 27°C in the basal culture medium, Bushnell and Hass medium (BHM) containing the following in g/l, $MgSo_4$ 0.2,$CaCl_2$ 0.02, KH_2 PO 1.0, K $_2HPO_4$ 1.0, NH $_4$No 1.0, FeCl 0.05, Glucose 0.9, Yeast extract 0.9, Malachite Green 10-200 ppm.

2.3 Plate Assay

Plate assay was performed for the detection of decolourizing activity of bacteria. The nutrient agar and Malachite Green dye was autoclaved at 121oC for 15 minutes. Cultures were plated on nutrient agar plates containing Malachite Green (100ppm). The plates were wrapped with parafilm and were incubated at 37oC for 7days. The plates were observed for clearance of the surrounding the colonies.

2.4 Measurement of Dye Concentration

The dye concentrations were measured with a UV/VIS spectrophotometer at regular intervals during the decolorisation process.The concentration of azo dye was detected spectrophotometrically by reading the culture supernatant at its specific max after centrifugation at 10,000 rpm for 10 min. The dye concentrations were determined from the attenuance (O.D) of the culture 610 nm.

2.5 Decolorization Activity was Calculated as Follows:

$$\text{Decolorization}(\%) = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

2.6 Study of Physico-Chemical Parameters

Decolorization was studied using Static and shaking condition as well as different temperature ,27 ◦c37˚C, and 55 ˚C and Different pH [12].

2.7 Estimation of Chemical Oxygen Demand (COD)

Chemical oxygen demand was measured by the standard Potassium dichromate method. 1ml of initial medium containing dye solution, decolorized medium, distilled water was added to COD Tube sample 1, sample 2, Blank respectively. Then 1.5ml of distilled water & reducing agent potassium dichromate and 3.5ml COD acid were added to each tube. Duplicates were put up for all the tubes. All the tubes were kept in the COD incubator at 148oC for 2 hrs. After incubation the entire content were transferred to a conical flask. A drop of ferroin indicator was added to it and was titrated against FAS in the burette.(

The readings were noted

$$\text{COD mg/l} = \frac{A-B \times N \times \text{Equi weight of } O_2 \times 1000}{\text{Volu of sample}}$$

A-volume of Ferrous Ammonium Sulphate used for blank

B-volume of ferrous Ammonium Sulphate used for sample

Equivalent weight of oxygen - 8

N-Normality of FAS - 0.1

COD values were compared between the initial medium containing dye solution and decolorized medium.

2.8 Estimation of Biological Oxygen Demand (BOD)

1ml of initial medium containing dye solution, decolorized medium, distilled water was added to airtight BOD bottles sample 1, sample 2, Blank respectively. Place desired volume of water in a suitable bottle and add 1ml of each of Phosphate buffer, MgSO , 4FeCl and seeding/L of water. Before use 3 bring dilution water temperature to 200C. Dilution water was aerated with organic free filtered air. All the bottles are kept in BOD incubator at 200C for 5 days. After incubation 1ml of MnSO , Alkali iodide solution and sulphuric acid was added to form brown color solution. After color formationthey were titrated against their Na SO for their BOD values.

The readings were noted.

$$BOD \ \ Mg/l \ \ = \frac{B - T(v) \times 250}{S(v)}$$

B-volume of Na SO used for blank ,

T (v)-volume of Na So used for sample 2

S (v)-volume of sample

III. RESULT

3.1 Effect of Culture Conditions on Dye Decolorization

(Table-1 &2) The static and shaking conditions showed a profound effect on the dye decolourization efficiency [13] Both the bacterial species and fugie were exhibit dye decolorizing activity only when incubated under the stationary condition (36 hr) .out of them *B.megaterium* was give 100% decolorization at static and 91 % at static condition at 36 hr. Anaerobic or static conditions were necessary for bacterial decolorization through the cell growth was poorer than that under aerobic conditions [14].Under aerobic conditions azo dyes are generally resistant to attack by bacteria[15]. Azo dye decolorization by bacterial species if often initiated by enzymatic reduction of azo bonds, the presence of oxygen normally inhibits the azo bond reduction activity since aerobic respiration may dominate utilization of NADH; thus impeding the electron transfer from NADH to azo bonds..

Table-1 Effect of static condition on Decolorization

Sr No.	Static condition	% of Decolorization		
		Bacillus Megaterium	*Pseudomonas Aeruginosa*	*Aspergillus Niger*
1	12	80	65	18
2	24	97	82	31
3	36	100	98	60

Table-2-Effect of shaking condition on Decolorization

Sr No.	Shaking condition	% of Decolorization		
		Bacillus Megaterium	*Pseudomonas Aeruginosa*	*Aspergillus Niger*
1	12	69.5	60	13
2	24	80	72	25
3	36	91	88	50
		–		

3.2 Effect of Temperature on Dye Decolorization

The dye decolorization activity of our culture was found to increase with increase in incubation temperature from 27c◦ to 37°C with maximum activity attained at 37°C(Table-3)and (graph-1). Further increase in temperature resulted in maginal reduction in decolorization activity of isolates may due to sensitivity at higher temperature.

3.2 Table-3 Effect of Temprature on Decolorization

Sr No.	Temprature	% of Decolorization		
		Bacillus Megaterium	*Pseudomonas Aeruginosa*	*Aspergillus Niger*
1	27◦c	89	65	43
2	37◦c	98	96	21
3	55◦c	70	20	12

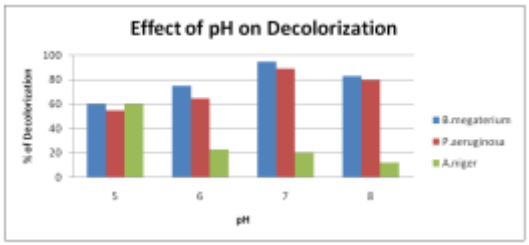
3.3 Effect of Ph on Dye Decolorization

Bacterial culture generally exhibited maximum decolorization rate at pH values near 7 and fungi near pH 5.Table-4 and Grah -2 shows that decolorization rate increase as pH shift from 5-7 .Further decreased decolorization rate obtain at pH 8.Fungi Give maximum decolorization near pH 5 indicate acidic environment needed for growth.

Table-4 Effect of pH on Decolorization

Sr No.	pH	% of Decolorization		
		Bacillus Megaterium	*Pseudomonas Aeruginosa*	*Aspergillus Niger*
1	5	60	55	60
2	6	75	65	23
3	7	95	89	20
4	8	83	80	12

Graph-1 Effect of pH on Decolorization



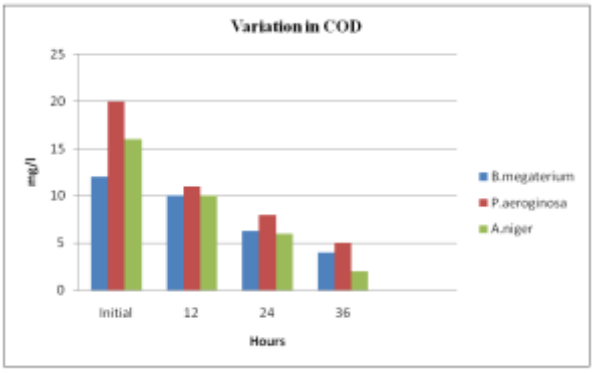
3.4 Cod Determination

The Chemical oxygen demand was measured by calculating the amount of oxidizing agent i.e., K_2 Cr_2 O_4 consumed during oxidation of organic matter (biodegradable and nonbiodegradable) under acidic conditions. Chemical oxygen demand of degraded dye solution gets considerably reduced after degradation. COD of the solutions after degradation shows significant decrease from 20- 2 mg/l (Table-5 and Graph-2).

Table-5 Effect of chemical Oxygen demand on Decolorization

Sr No.	COD(Mg/L)	% of Decolorization		
		Bacillus Megaterium	*Pseudomonas Aeruginosa*	*Aspergillus Niger*
1	Initial	12	20	16
2	12	10	11	10
3	24	6.3	8	6
4	36	4.0	5	2

Graph-2 Effect of Chemical oxygen Demand on decolorization



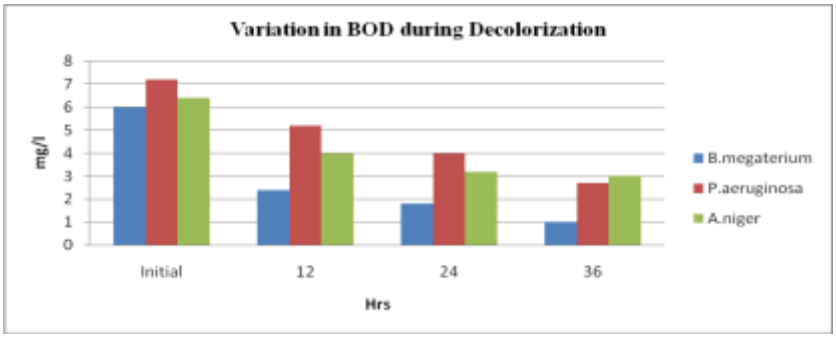
3.5 Bod Determination

The rate of removal (that is Consumption) of Oxygen by microorganism in aerobic degradation of the dissolved or even particulate organic matter in water that is called Biological Oxygen Demand (BOD).The BOD determination was used todetermine the relative oxygen requirements of dye solution.BOD Of all samples dcrease from 7.2-1 mg/l(Table-6 &Graph-3). The test measures the Oxygen utilized during a specified incubation period for the biochemical degradation of organic matter (Carbonaceous demand) and the oxygen used to utilize in organic material such as sulfides and ferrous iron. It also may measure the oxygen used to oxidize reduce forms of Nitrogen (Nitrogenous demand).

Table-6 Effect of Biological oxygen Demand on Decolorization

Sr No.	BOD(Mg/L)	% of Decolorization		
		BacillusMegaterium	Pseudomonas Aeruginosa	Aspergillus Niger
1	Initial	6	7.2	6.4
2	12	2.4	5.2	4.0
3	24	1.8	4	3.2
4	36	1.0	2.7	3

Graph-3 variation in Biological oxygen Demand during Decolorization



IV. CONCLUSION

Bioremediation has proved to be a very effective method in encountering the textile dye pollution in an eco-friendly manner. This approach creates a promising hope for remediation of the environment which is polluted by hazardous dyes.The present study confirms the ability of bacterial culture *Bacillus megaterium* to decolorize the malachite green with decolorization efficiency of 98% and *Peudomonas.aeruginose* with 96% efficiency.

The ability of the strain to tolerate, decolorize and degrade malachite green at high concentration gives it an advantage for the treatment of textile industry wastewater. However the potential of the bacteria needs to be demonstrated for its application in the treatment of dye containing industrial effluents using appropriate bioreactors. This study further recommends the identification and purification of enzymes from isolates and their kinetics involved in the degradation of malachite green. Further research on this bacterial strain could explore new tools and techniques to evolve viable and eco friendly solutions for the treatment of dyes in the industrial effluents.

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