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Optimization of malachite green decolourization by *Pseudomonas aeruginosa* MTCC 424

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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, decolourization of malachite green using *Pseudomonas aeruginosa* (MTCC 424) was investigated by screening and optimizing various parameters to determine the optimal conditions required for maximum decolourization. Based on the studies, *Pseudomonas aeruginosa* showed maximum decolourization upon incubation for 18 hrs at 37 °C at pH 7. The decolourization was also enhanced by amending glucose and peptone as carbon and nitrogen sources in the culture medium. The present investigation reveals that the strain, *Pseudomonas aeruginosa* MTCC 424 showed great potential in the decolourization of malachite green dye up to 86 % in the aqueous environment at optimal conditions.

Keywords: Decolourization, Malachite green, *Pseudomonas aeruginosa*.

1. Introduction

Environmental pollution is one of the major concern for various environmentalists and researchers around the world. Water, the elixir of life, occupies the major area of the planet, of which less than three percent of it can be used as drinking water^[1]. As a consequence of global industrialization, the usage of water in industries has increased and processed effluent water is discharged into neighboring water bodies. 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^[2]. 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^[3]. Malachite green, one of the basic and widely used dye was chosen as model dye compound for the present investigation. In spite of various regulations regarding treatment and disposal of effluents, most of the industries pollute the environment through discharge of untreated/ improperly treated dye effluents into water bodies. As a consequence, there arises or evolves the alterations in the water quality parameters via BOD, COD etc threatening the survival of the organisms that lives in such ecosystem^[4]. The treatment of effluents or polluted water bodies has become the serious concern of researchers which can be achieved through physical, chemical or biological methods.

In case of physical methods, such as sedimentation, segregation, equalization and filtration, the suspended solids and organic load cannot be removed efficiently but, it can be achieved through chemical methods including coagulation/ flocculation^[5], ion exchange, adsorption, etc.^[6]. Thus chemical methods were proven to be efficient in the removal of solids, COD and BOD from effluents^[7]. The water polluted with effluents can be treated by biological methods^[8] which involve the use of bacteria, fungi, actinomycetes and yeast for the reduction of biodegradable organics (BOD) and chemicals (COD). The biological treatment is also found to be effective in the oxidation of ammonium to nitrate through denitrification reaction. The present investigation has been carried out in an attempt to analyze the efficacy of the reactive dye (malachite green) using *Pseudomonas aeruginosa* MTCC 424.

2. Materials and Methods

2.1 Dye and Chemicals

The analytical grade malachite green, a triphenylmethane dye {4-[(4-dimethylaminophenyl)-phenylmethylidene]- dimethyl-ammonium chloride} was procured from Himedia, India and all the other analytical chemicals were procured from SRL, Chennai.

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2.2 Bacterial strains

Bacillus subtilis MTCC 121, *Bacillus cereus* MTCC 430 and *Pseudomonas aeruginosa* MTCC 424 cultures were procured from IMTECH, Chandigarh, India for the present study and maintained in nutrient agar slants until further investigation.

2.3 Screening for malachite green decolourization

The efficacy of the bacterial strains *Bacillus subtilis* MTCC 121, *Bacillus cereus* MTCC 430 and *Pseudomonas aeruginosa* MTCC 424 on the decolourization of textile dyes was screened by plate assay technique. The nutrient agar amended with malachite green (0.1 mg/ml) was prepared, inoculated with selected bacteria and incubated for 24 hrs at 37 °C. After incubation, the plates were observed for appearance of clear zones around the colonies ^[9].

2.4 Decolourization studies ^[10]

For the batch decolourization studies, 100 ml of sterile nutrient broth was prepared in 250 ml Erlenmeyer flask and malachite green was amended at a concentration of 0.1 mg/ml. The flask was then inoculated with 1% of *Pseudomonas aeruginosa* culture suspension and incubated at 37 °C for 24 hours. After incubation, the culture media was withdrawn and centrifuged to collect the cell free supernatant. The decolourization activity was expressed in terms of percentage decolourization and was determined by monitoring the decrease in absorbance at the maximum wavelength of the dye (617 nm). A UV-Vis spectrophotometer was used for absorbance measurement and recording of visible absorption spectra. The uninoculated culture medium with and without dye was considered as blank and initial dye concentration for the experiments. The experiments were carried out in triplicates and the mean values were represented here. Then the decolourization percentage was calculated.

2.5 Optimization of dye decolourization

As the biological decolourization of malachite green depends on various cultural and environmental parameters the factors such as incubation time, temperature, pH, carbon and nitrogen sources were optimized.

- Incubation period:** To study the effect of incubation time on dye decolourization process, 100 ml of sterile nutrient broth amended with malachite green (0.1 mg/ml) was inoculated with 1 % overnight test bacterial suspension and incubated at 37 °C. About 3 ml of the culture medium was withdrawn at every 6 hours interval up to 24 hours from batch process. The effect of incubation period on malachite green decolourization was determined by measuring the absorbance of the cell free extract as described above.
- Temperature:** To study the effect of incubation temperature for maximum dye decolourization, the culture medium amended with malachite green (0.1 mg/ml) was inoculated and incubated at different temperatures such as 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C for 18 hours. At the end of incubation, the cell free extracts were obtained and the decolourization percentage was determined as described above.
- Initial pH:** The effect of initial pH on malachite green decolourization was studied by adjusting the initial pH of the culture medium for the batch process. Five different

Erlenmeyer flasks containing 100 ml of sterile nutrient broth amended malachite green (0.1mg/ml) was taken and the pH was adjusted to 6, 6.5, 7, 7.5, 8, 8.5 and 9 using 0.1 N HCl and 0.1N NaOH. After 18 hrs of incubation, the cell free extract was withdrawn and decolourization percentage was determined as described earlier.

- Carbon source:** To study the effect of carbon source on dye decolourization, the culture medium (pH 7) with malachite green (0.1mg/ml) was prepared and amended with different carbon sources (0.1%) additionally such as glucose, sucrose, maltose, lactose, fructose and mannitol. The medium amended with different carbon sources were inoculated with 1% overnight bacterial suspension and incubated for 18 hours at 37 °C, followed by determination of decolourization percentage using the cell free supernatant.
- Nitrogen source:** To study the effect of nitrogen sources on dye decolourization, culture medium (pH 7) with different nitrogenous sources (0.01%) such as peptone, casein, gelatin, sodium nitrite and yeast extract was prepared and amended with malachite green (0.1mg/ml). The flasks were then inoculated with 1% overnight bacterial suspension and incubated at 37 °C for 18 hours. After incubation, the cell free supernatant was collected and the decolourization percentage was calculated.

3. Results and Discussion

Malachite green is one of the extensively used dyes in dyeing and fish farming industry as food additive and fungicide ^[11]. It is found to be resistant to biodegradation and also shows toxic effects on the organs of mammals including lungs, liver, etc. Though it is banned in several countries it is still used by many industries due to its low cost and lack of suitable alternatives ^[12, 13]. Microbial decolorization of malachite green can be used as an environmentally acceptable and cost-effective alternative to complex physical and chemical processes ^[14]. Thus, the present investigation was aimed to observe the decolourization of malachite green using bacteria and its process optimization. Three different bacterial strains *Bacillus subtilis* MTCC 121, *Bacillus cereus* MTCC 430 and *Pseudomonas aeruginosa* MTCC 424 procured from IMTECH, Chandigarh, were screened for malachite green decolourization using plate method. Among the three strains, *Pseudomonas aeruginosa* NTCC 424 showed visible clear zone around the colony compared to other strains within 24 hrs of incubation and was selected for further studies.

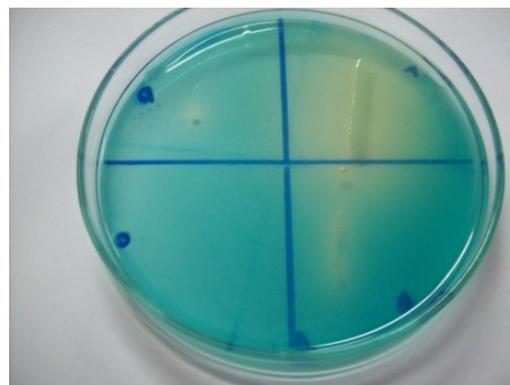


Fig 1: Plate assay for malachite green decolourization

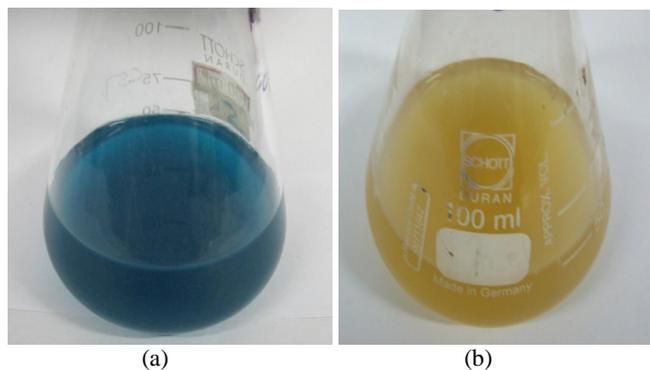


Fig 2: Broth assay - Before (a) and after (b) malachite green decolourization

The quantitative analysis of dye decolourization using *Pseudomonas aeruginosa* MTCC 424 was determined by batch process using liquid broth medium by optimizing different parameters.

Effect of incubation time was studied and the results revealed that decolourization process gradually increases after 12 hours and reaches maximum at 24 hours of incubation with a decolourization percentage of 68% using *Pseudomonas aeruginosa* MTCC 424. Decolourization percentage was considered as the function of time. It has been reported earlier by Mali *et al.* [15] that decolourization percentage gets accelerated after 15 hrs of incubation which indicates that the bacteria requires some time to acclimatize in the environment.

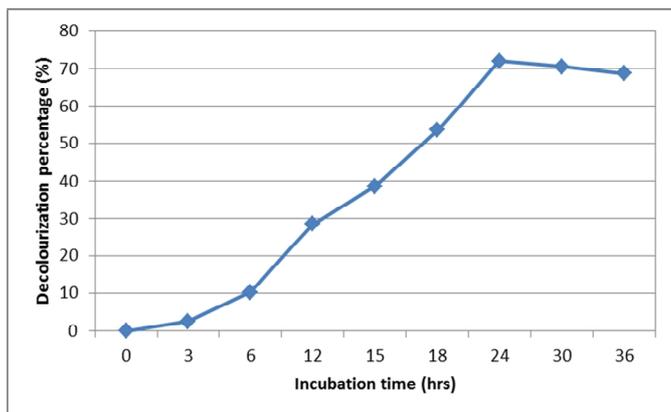


Fig 3: Effect of incubation time on decolourization of MG by *Pseudomonas aeruginosa* MTCC 424

After optimizing incubation time, experiments are carried out to optimize the temperature by incubating the culture flasks at different temperatures ranging between 25 °C and 50 °C. After 18 hrs of incubation, the absorbencies and thereby, decolourization percentage were calculated. It can be observed that *Pseudomonas aeruginosa* MTCC 424 could decolorize malachite green at different extent with respect to temperature. About 44.5% malachite green decolourization percentage was observed at 25 °C and attained the maximum of 74.5% at 35 °C. As the temperature increased beyond 35 °C, the decolourization percentage was decreased considerably up to 28.6% at 50 °C. A similar mesophilic optimized temperature of 37 °C was portrayed by Xuying *et al.* [16]. for the decolourization of malachite green by *Pseudomonas putida*.

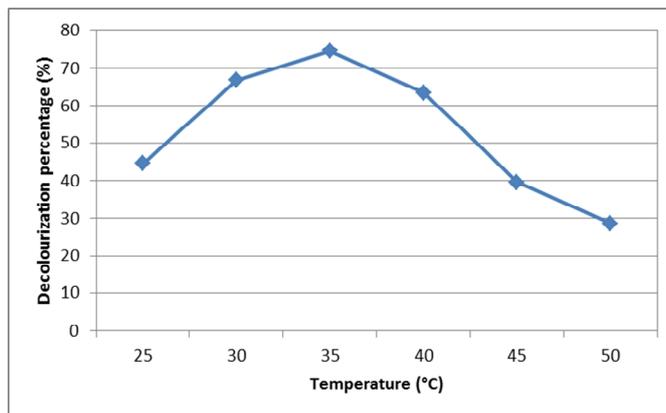


Fig 4: Effect of temperature on decolourization of MG by *Pseudomonas aeruginosa* MTCC 424

As the pH always plays a role in the ionization and binding of substrates, solubility of the nutrients required by the bacteria for their growth and decolourization ability, it is necessary to be optimized. For the determination of optimal pH, experiments were carried out at different pH ranging from 6 to 9. The bacteria were able to grow and decolorize the dyes in all pH range, but the decolourization percentage was found to be high of 76.8% at the pH of 7 and decreases beyond and ahead of pH 7. An optimum pH for malachite green was varied with respect to the bacterial strain used by different investigators. Earlier, Wu *et al.* [17] reported similar observations for decolourization of a triphenylmethane dye by *Pseudomonas otitidis*. Generally, *Pseudomonas* sp. required optimum pH of 8.0 [18] for malachite green whereas, *Sphingomonas* sp. required pH 9.0 [19, 20].

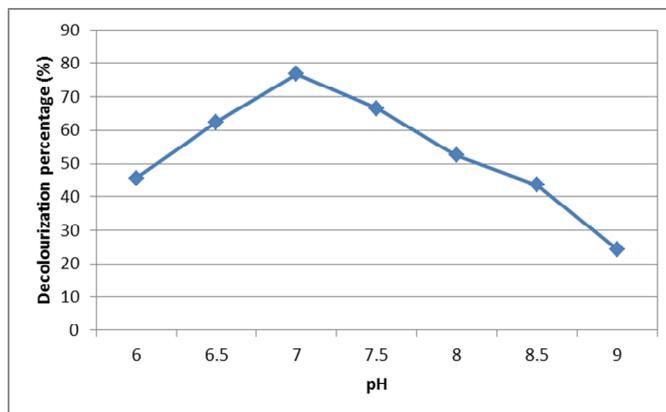


Fig 5: Effect of pH on decolourization of MG by *Pseudomonas aeruginosa* MTCC 424

Various carbon sources were used to replace original carbon source in culture medium to determine their role in malachite green decolourization. The results obtained showed that, glucose brought the highest decolourization percentage of 87.24% followed by fructose with 83.45% of decolourization when compared to other carbon sources. Hence, glucose was found to be the best carbon source for malachite green decolourization using *Pseudomonas aeruginosa* MTCC 424. Annika *et al.* [21] also reported glucose as a better carbon source for the malachite green decolourization using *Pseudomonas aeruginosa*.

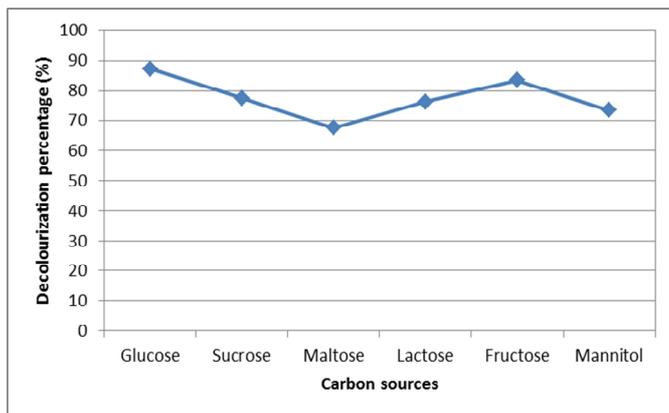


Fig 6: Effect of different carbon sources on decolourization of MG by *Pseudomonas aeruginosa* MTCC 424

Bacterial decolourization of malachite green has been found to be sensitive to repression by different nitrogen sources. Among the various nitrogen sources analyzed, peptone was found to be the best nitrogen source for malachite decolourization by *Pseudomonas aeruginosa* MTCC 424 with 92.5% of decolourization. In a similar optimization studies carried out by Ji'ai Wang *et al.* [22], peptone and beef extract were found to be enhancing the malachite green decolourizing ability of *Achromobacter xylosoxidans*.

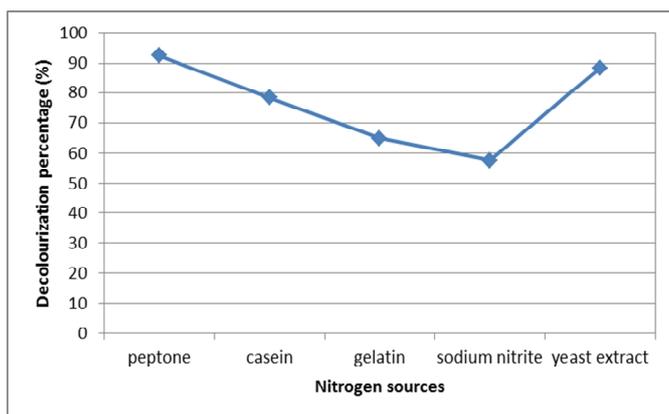


Fig 7: Effect of different carbon sources on decolourization of MG by *Pseudomonas aeruginosa* MTCC 424

From the results obtained from the present investigation, *Pseudomonas aeruginosa* MTCC 424 possesses significantly high decolorization percentage for malachite green under optimal conditions.

4. Conclusions

The present study inferred that the bacterial strain *Pseudomonas aeruginosa* MTCC 424 showed 92.5% decolourization of malachite green with the optimized physicochemical conditions. The study further recommends that the exploitation of potential bacteria is the cost effective treatment for the reactive dye polluted waste water. Further studies on pathway involved in dye decolourization and scale-up may assist in the effective decolourization of malachite green from textile effluents and water bodies polluted with such dye effluents.

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