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Biological Decolorization of Malachite Green by a Carotenoid Producing Bacterium

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Abstract—*Biological decolorization of dye is a promising alternative since it is a low cost, highly effective and environmental friendly method. The present study focuses on the potential of a carotenoid producing bacterium in decolorization of Malachite Green (MG), a triphenyl methane dye. MG is used extensively in textile industries and it alone accounts for two-third of the total production of dye containing wastewater. Though it has been banned due to its carcinogenicity and toxicity, bioremediation of MG is essential for maintaining environmental sustainability. Kocuria marina DAGII is a gram positive carotenoid producing cocci. Batch decolorization study was carried out by supplementing the growth medium containing Glucose (7.5 g/L), Maltose (10 g/L), Yeast Extract (10 g/L), Peptone (5 g/L) and NaCl (4 g/L) with increasing concentrations of MG (10-60 ppm). The experimental flasks was inoculated with 1% (v/v) culture and incubated at 25°C on a rotary shaker (150 rpm) for 8h. The rate of decolorization was found to decrease with increasing MG concentration. After 8 h, 99% decolorization was observed up to 20 ppm. Only 36%, 29%, 21% and 14% decolorization was observed at 30, 40, 50 and 60 ppm, respectively. The effect of increasing inoculum percentage (1-3%) on decolorization was also studied. It was found that 98% decolorization took place at 40 and 50 ppm with 2% and 3% inoculum, respectively. In addition, Two-way ANOVA was carried out and the effect of MG concentration on decolorization was found to be more significant (p value = 0.0076) than that of inoculum percentage (p value = 0.0219). Till date, this is a first time report on Kocuria marina DAGII as a potential strain for MG decolorization.*

1. INTRODUCTION

As a consequence of rapid industrialization, dyes have become the major source of water pollution. Removal of dye by traditional wastewater treatment methods like coagulation, ozonation, ion-exchange, reverse osmosis, etc., are not only less effective and expensive but also produces high amount of sludge [1]. As an alternative, biological processes have received considerable importance in recent times due to its low cost, effectiveness, ability to produce less sludge and environmental sustainability [2]. In last few decades, a wide variety of microorganisms capable of removing wide range of dyes have been reported [3].

Malachite Green (MG) is a triphenylmethane dye, commonly known as Basic green 4. MG is an important component of textile industry. It alone accounts for the two-third of the total production of dye containing wastewater [4]. Due to its high tinctorial value (less than 1ppm), MG is highly preferred for dyeing materials like jute, silk, wool, cotton, ceramics, acrylic fibres and paper [5]. But toxic effects of MG are not only limited to human beings but highly affect the aquatic life on acute or chronic exposure [6]. The Food and Drug Administration nominated MG as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993 [7]. Though MG has been banned worldwide since 2002, it is still being used in some areas due to its low cost and lack of suitable alternatives [6]. Therefore, bioremediation of MG is of paramount importance for maintaining the environmental integrity.

Different microorganisms have exhibited high potential in decolorizing MG containing aqueous solution. *Kocuria marina* DAGII, a gram positive carotenoid producing cocci, was isolated in our laboratory during routine screening of pigment producing microorganisms. The present study focuses on the capability of the isolated strain in decolorizing aqueous solution containing MG. The decolorization was studied by varying the MG concentration and inoculum percentage. Statistical analysis by Two-way ANOVA was also done to identify the significant parameter for MG decolorization.

2. MATERIALS AND METHODS

2.1. Chemicals

MG used in this study was obtained from Merck Specialities Private Limited, India. Ingredients of the fermentation media were purchased from Himedia Laboratories, India**.**

2.2. Organism and culture condition

The strain *Kocuria marina* DAGII, a yellow colored gram positive carotenoid producing bacterium was previously isolated from soil in our laboratory. It was maintained on Brain Heart Infusion Agar (BHIA) at 4°C and subcultured every month. The bacterium was cultured as described by Mitra et al. (2015) [8]. The pure culture of the bacterium was

grown in a 250mL Erlenmeyer flask, containing 50mL of fermentation medium (g/L : Glucose 7.5, Maltose 10.0, Yeast Extract 10.0, Bacteriological Peptone 5.0 and NaCl 4.0). The initial pH was adjusted to 7.9. The experimental flasks were incubated at 25°C on a rotary speed of 150 rpm (New Brunswick Innova®incubator shaker; NJ, USA).

2.3. Inoculum preparation

Inoculum was prepared by inoculating a loopful of pure cultures of *Kocuria marina* DAGII from BHIA into test tubes containing 5ml of the fermentation medium. Incubation was done at 25°C(New Brunswick Innova®incubator shaker; NJ, USA) at 150rpm for 24h. 1mL from the culture tube was transferred into 250ml Erlenmeyer flask containing 50ml of the fermentation medium and incubated. The inoculum was standardized by measuring the absorbance (optical density) at 600nm using a spectrophotometer (U-2800, Hitachi). 1% (v/v) of the inoculum with an optical density of 0.4-0.6 was used to inoculate the fermentation medium.

2.4. Preparation of dye preparation

A dye stock solution of 1000mg/L was prepared by dissolving MG in distilled water. It was further diluted to obtain the desired dye concentration during experiments. The concentration of MG in the solution was measured with UV-Vis spectrophotometer (Hitachi U-2800) at 617nm (λ_{max}).

2.5. Decolorization experiment

250mL Erlenmeyer flask containing 50mL of fermentation medium was supplemented with required concentrations of MG. The resulting medium was inoculated with 1% (v/v) of the inoculum and incubated at 25°C at 150rpm. Samples were withdrawn at regular time intervals. Decolorization study was then done according to the method of Parshetti et al. (2006) [7] with minor modifications. Briefly, the samples were centrifuged at 10000 rpm for 15minutes. The supernatant was collected to measure the absorbance of MG at 617nm. The percentage decolorization was calculated as follows

% Decolorization = [(Initial absorbance - observed absorbance)/(Initial absorbance)] X 100

2.6. Statistical analysis

All the experiments were conducted in triplicates. The values represented were are the mean ± SD of three replicates which was done using Microsoft Excel 2010. Two-way analysis of variance (Two-way ANOVA) was done using Graphpad Prism 5.

3. RESULTS AND DISCUSSION

3.1. Effect of initial dye concentration on decolorization

The decolorization of MG was studied at various increasing concentrations of dye i.e., 10, 20, 30, 40, 50, 60 ppm. It was observed that rate of decolorization decreased with increasing dye concentrations. 99% decolorization of MG was observed at 10, 20ppm within 2 and 7h, respectively (Fig. 1). After 20ppm, the rate of decolorization decreased indicating reduction in decolorization with increase in dye concentration. Only 36, 29, 21, 14% decolorization was observed at 30, 40, 50 and 60 ppm, respectively after 8h (Fig. 1). Similar observation was also reported during decolorization of MG by *Kocuria rosea* MTCC 1532 [7]. These results indicated the toxicity of MG at higher concentrations.

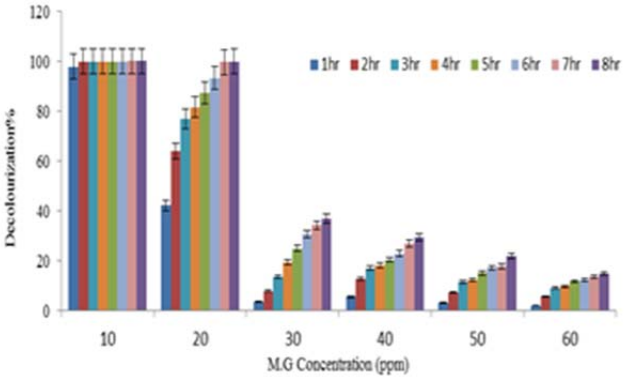

Fig. 1: MG decolorization at different initial concentration

3.2. Effect of increasing inoculum percentage on MG decolorization

Inoculum percentage was increased from 2 and 3% to study the effect of increased cell concentration on MG decolorization. Fig. 2 shows the effect of 2 and 3% inoculum on different MG concentration. 98% decolorization took place at 40 and 50ppm when the inoculum was increased from 2 and 3%, respectively (Fig. 2). At 60ppm, 50 and 60% decolorization was achieved for 2 and 3% inoculum, respectively. Thus it could be inferred that decolorization could be increased by increasing cell concentration.

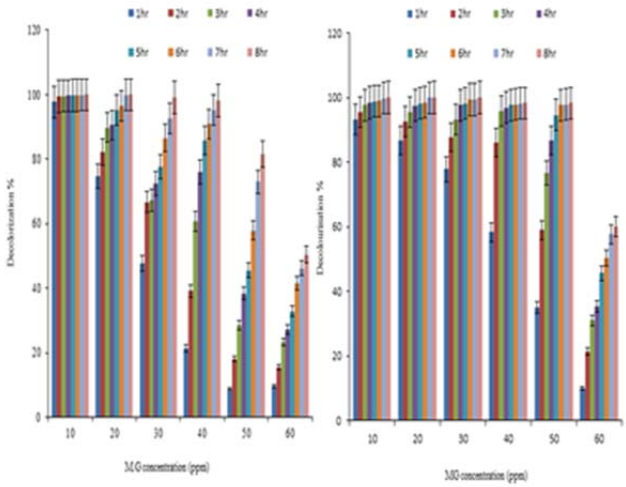

Fig. 2: Effect of inoculum percentage on MG decolorization

3.3. Statistical analysis

Two-way ANOVA was done by using Graphpad Prism 5 (California, USA) to determine the significant parameter for MG decolorization. The effect was considered significant at P value < 0.5. It was observed that the effect of initial dye concentration on MG decolorization was more significant (*p* value = 0.0076) than that of inoculum percentage (*p* value = 0.0219).

4. CONCLUSION

The study showed the ability of *Kocuria marina* DAGII to decolorize MG from its aqueous solution. It was found that on increasing the dye concentration the decolorization ability of the bacterium significantly reduced. However, on increasing the inoculum percentage, considerable amount of decolorization could be achieved at higher dye concentrations. This is a first time report on *Kocuria marina* DAGII as a potential MG decolorizer.

5. ACKNOWLEDGEMENT

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