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Microbial efficiency to degrade Carbol fuchsin and Malachite green dyes

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ABSTRACT

Carbol fuchsin and Malachite green dyes are extensively used in textile dyeing, paper, printing and other industries. Textile effluent released from industries is a complex mixture of many polluting substances including dyes and must be treated before discharged into environment because of their recalcitrant nature and potential toxicity to animals and humans. Biological treatment offers a cheaper and environment friendly alternative to dye decolorization and wastewater reutilization in industrial process. In the present studies bacteria were isolated from textile effluent and dyes decolorization assay were performed in the basal nutrient medium. The most efficient bacterial isolate was used for further optimization studies. The morphological and biochemical studies revealed the isolated organism as Enterococcus spp. The strain showed 100% and 92% decolorization of the Malachite green and Carbol fuchsin (0.02g/L) respectively within 24 h. The optimum pH and temperature for the decolorization was 7.0 and 37^{0}C respectively. Phytotoxicity study demonstrated no toxicity of the biodegraded product. The results suggest that the isolated Enterococcus spp. can be a useful tool to treat waste water containing dyes.

Keywords*: Bacteria, Decolorization, Dyes, Phytotoxicity, Textile effluent*

INTRODUCTION

Triphenylmethane dyes are aromatic xenobiotic compounds used extensively in many industrial processes such as dye stuff manufacturing, paper printing, as a biological stain and as a textile dye in textile processing industry [5]. Carbol fuchsin and Malachite green are triphenylmethane dyes are extensively used in textile dyeing and dyestuff manufacturing industries, as a biological stain and in printing paper [4].The textile industries discharge millions of liters of dye containing effluent into the environment. The release of colored compound into water bodies is undesirable. They may significantly affect photosynthesis activity in aquatic life because of reduced light penetration. These dyes are toxic, carcinogenic and genotoxic [3, 6].

Currently various chemical and physical treatment methods including adsorption, oxidation, precipitation, bleaching, photo degradation and membrane filtration were used to remove the dye. Because of the high cost, disposal problems and generation of toxic products most of the chemical and physical methods are not widely applied in the textile industries [5, 7, 9]. In the last few years, Bioremediation is becoming more attractive methods for decolorization because of its cost effective, environmentally friendly and less sludge producing nature [11].

Several microorganisms have been found to decolorize and degrade many structurally different dyes efficiently [1]. Bacterial decolorization is associated with involvement of various enzymes such as lignin peroxidase, Laccase, Azoreducatase and biotransformation enzymes. A wide range of aromatic amines are aerobically biodegraded rapidly and hence they are unlikely to remain in the environment for a long time. Therefore microbiological treatment of textile waste water is the best way for detoxification [8].

The present study deals with the isolation, identification of Carbol fuchsin and Malachite green dye degrading bacteria and determination of different optimal conditions required for dye decolorization.

MATERIALS AND METHODS

2.1 Sample collection

The textile effluent samples were collected from small scale dyeing industries located in Kalyan-Dombivali MIDC area, Kalyan, Dist. Thane. The samples were transported to the laboratory in sterile container and stored at 4°C.

2.2 Chemicals and Media

Dyes Carbol fuchsin (CF) and Malachite green (MG), microbiological media and individual medium ingredients were purchased from Himedia laboratories (Mumbai, Maharashtra, India).

2.3 Enrichment, Isolation and screening of dye decolorizing bacteria

The collected effluent samples were enriched by inoculating 5ml of sample in Nutrient broth containing 0.02gm/lit each CF and MG. The flasks were incubated at Room temperature (28ºC±2ºC) under shaking conditions with 150 rpm. After 48 hrs of incubation, 1 ml of the culture broth was appropriately diluted and plated on Nutrient agar containing 0.02 gm/L MG and CF dye separately. Plates were incubated at 37°C for 24 hrs. After incubation, morphologically distinct bacterial isolate showing clear zone around their colonies due to decolorization of dyes were selected for further studies.

2.4 Dye decolorization assay

Decolorization activity of isolated bacteria was performed in 100ml Nutrient broth containing 0.02 gm/L CF and MG individually. The flasks were inoculated with 5 ml of 24 hrs. old bacterial culture and incubated at 37°C on a shaker (Make : Remi) with 150 rpm up to 7 days maximum. Medium without dye was used as blank. Uninoculated dye medium served as control. After incubation 10 ml medium was centrifuged at 5000 rpm for 15 min. and supernatant was removed.

Decolorization was assessed by measuring absorbance of the supernatant at wavelength maxima (λm) of respective dye. (MG = 470 nm and CF= 510 nm). Percentage of decolorization was calculated by using the formula reported earlier.

$$\% \text{ Decolorization} = \frac{(\text{Initial OD} - \text{Final OD})}{\text{Initial OD}} \times 100$$

2.5 Identification of the most potent dye decolorizing bacteria

The isolate with highest decolorization activity was identified by various morphological characteristics like staining, motility, plating on selective media and biochemical tests.

2.6 Optimization of decolorization ability for the selected isolate

Optimization medium

One gm/L yeast extract was supplemented in Mineral salt medium (MSM) used in optimization experiments to support growth and increase the degradation ability of selected bacterial isolate.

All the experiments were conducted in triplicate with standard conditions such as pH-7, incubation temp. 37°C, 0.02 gm/L dye conc., culture density of 0.6 O.D. at 600nm λ. 3 ml of cell suspension was inoculated in 100 ml of MSM broth.

Decolorization was optimized with respect to the effect of different carbon sources, nitrogen sources, pH and temperature. Uninoculated broth served as control. After incubation samples were analyzed for percent decolorization.

2.7 Phytotoxicity assay

The toxicity of dye metabolites was studied on *Trigonella foenum*. 100 ml of decolorized broth was centrifuged at 10,000 rpm for 20 min. 100 seeds were soaked in decolorized broth (supernatant), dye containing broth and Distilled water separately for overnight. Seeds in D/W were taken as control. After overnight the seeds were placed on the whatmann filter paper kept in Petri plate. Filter paper also moisture with respective water used for soaking earlier. Germination was continuously monitored day by day. After complete germination the mean of radical length of germinated seeds were measured and the results were tabulated [6].

RESULTS AND DISCUSSION

Textile dyeing industries is one of the fastest growing and a major export oriented industrial sector in India. During dyeing processes huge amount of dyes ends up in drainage. In addition, about 40-65 L of textile effluent is generated per kg of cloth produced. Among the most viable choices available for effluent treatment/decolorization, Biodecolorization has been accepted as a most promising alternative to decolorize and degrade dyes. In the present study, the potential CF and MG dye degrading bacteria were isolated from textile effluent samples. The most promising bacterial isolate was used for further studies.

3.1 Isolation and screening of dye decolorizing bacteria

Nine different morphologically distinct bacterial colonies having clear zone were isolated.

3.2 Dye decolorization assay

All the selected isolates were able to decolorize CF and MG more than 40% and 60% respectively. Out of nine, IF isolate was showing the highest decolorization activity with 82% and 94% for CF and MG within 24 hrs. And thus was selected for further studies. The resistance of the bacterial isolates against dyes suggesting their adaptability towards them. After centrifugation all bacterial strains pellets retained its original color and was not deeply colored because of adsorbed dyes. This indicates that, color removal was due to degradation and not by adsorption. There was neither growth nor decolorization in the control flasks. This clearly indicates that the decolorization was due to the metabolic activity of the bacteria.

3.3. Identification of the most potent dye decolorizing bacteria

The isolated organism was found to be Gram Negative, rod-shaped bacterium. Results of all biochemical tests were compares with Bergeys manual which suggest that the IF is belonging from *Enterobacter spp*.

3.4. Optimization of decolorization ability for the selected isolate

Various factors were optimized to enhance the decolorization activity of the selected isolate.

B2 isolate was able to decolorize the two dyes across a wide range of pH (Fig. 1) maximum degradation of 80% and 94% was observed for CF and MG, respectively, at pH 7. As seen in Fig. 2, it was observed that an increase in temp from 4°C to 37°C had positive effect on decolorization. However, optimal temperature was 37°C showing 81% and 92% decolorization of CF and MG respectively. Decolorization rate dropped sharply as the temperature increased from 37°C to 50°C. Among the different carbon sources studied, glucose showed maximum decolorization of 80% and 94% for CF and MG respectively (Fig.3). From Fig. 4, Maximum decolorization with nitrogen source was achieved with peptone (80% for CF and 92% for MG. Significant difference was not observed in percentage decolorization of dyes in presence of other carbon and nitrogen sources.

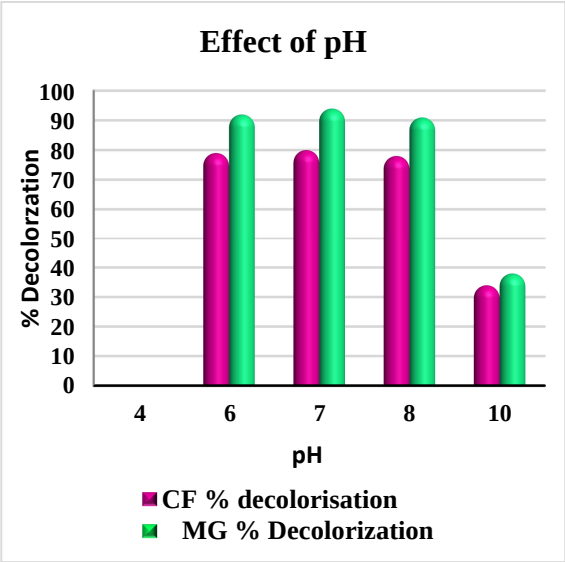

Fig: 1 Effect of pH

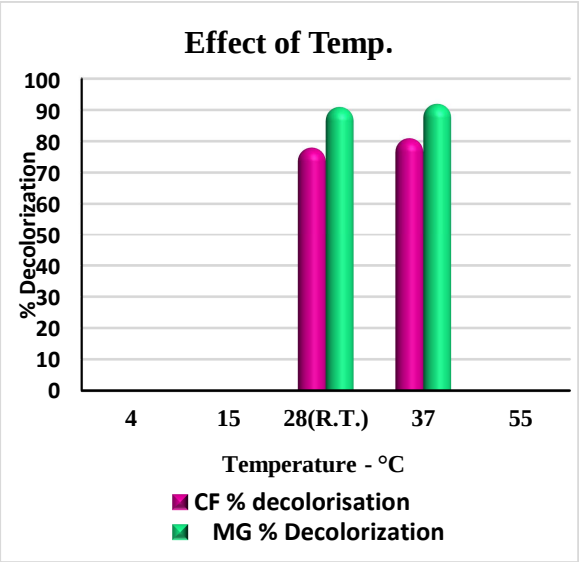

Fig : 2 Effect of Temp

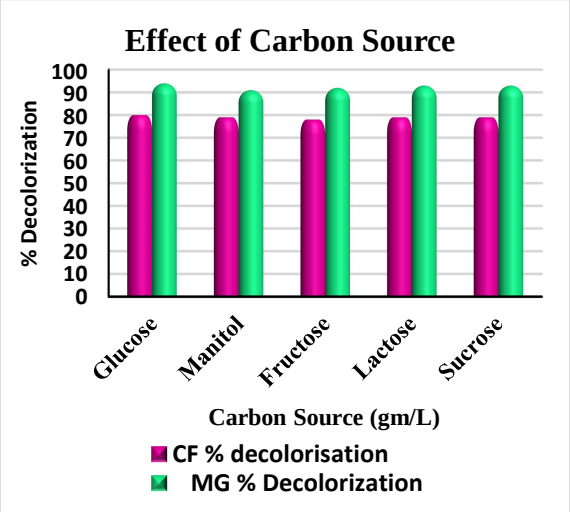

Fig: 3 Effect of carbon source

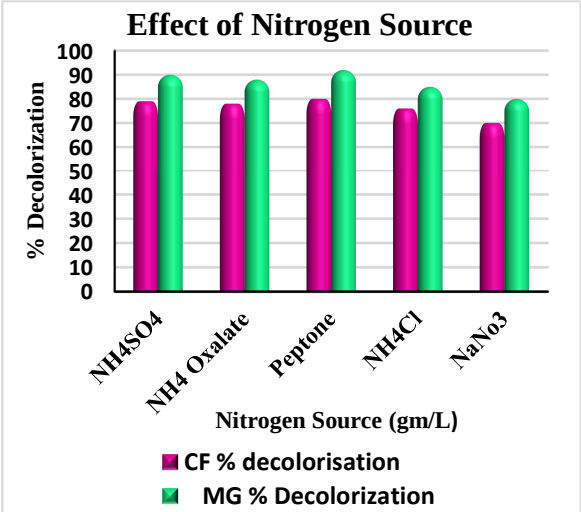

Fig: 4 Effect of Nitrogen source

3.5 Phytotoxicity assay

Seeds were successfully germinated in Decolorized broth (86%) as compare with D/W germination percentage (91%) after 48 hrs of time period. Triphenylmethane dyes effects on the germination of seeds as there is no germination was observed in seeds which were soaked in dye containing broth. 1.6 cm radical length of decolorized broth germinated seeds was observed which is very close to the length of seeds germinated in D/W (1.8 cm). Thus it was found that the metabolites formed after dye degradation was not having any toxicity for *Trigonella foenum.*

CONCLUSION

This works shows a high potential of bacteria isolated form textile effluent to decolorize triphenylmethane dyes. The isolated *Enterobacter spp*. showed excellent ability to decolorize CF and MG. The study shows that pH and temperature have a significant influence on dye removal efficiency of *Enterobacter spp*. Degradation products were non toxic for *Trigonella foenum* plant. Overall findings suggested the ability of *Enterobacter spp*. for the decolorization of triphenylmethane dye and ensured the ecofriendly degradation of dyes.

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