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A Study of Phytotoxicity and Decolorization of Methyl Orange Dye by *Bacillus* Sp.

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A B S T R A C T

Azo dyes accounts for the majority of all textile dyestuffs produced and have been most commonly used synthetic dyes in the textile, paper and leather industries. Methyl Orange is one such azo dye, which is widely used in textile industries and also as an indicator in chemical laboratories. In the present study effect of NaCl salt concentration, effect of presence or absence of nitrogen source and phytotoxicity of Methyl orange and its degradation products is studied. Presence of 5g/l of NaCl did not have any negative impact on decolorization efficiency. There was slight delay in time taken for decolorization at and above 20g/l of NaCl in the media. Optimum amount of peptone for the efficient decolorization was found to be 5g/l. Presence of less than or more than 5g/l of peptone has delayed the decolorization time and reduced the decolorization efficiency.

Introduction

Approximately 10,000 different dyes and pigments are used industrially and over 0.7 million tonnes of synthetic dyes are produced annually worldwide (Palmeiri, 2005). During processing upto 15% of the used dyestuffs is lost in the industrial effluents. Azo dyes accounts for the majority of all textile dyestuffs produced and have been most commonly used synthetic dyes in the textile, paper and leather industries. Methyl Orange is one such azo dye, which is widely used in textile industries and also as an indicator in

chemical laboratories. In addition to their visual effects and their adverse impact in terms of Chemical oxygen demand, many synthetic azo dyes show their toxic, carcinogenic and genotoxic effects. Thus the textile wastewater containing Methyl Orange must be treated before release into the natural environment. These dyes have stable chemical structures and hence cannot be removed by conventional physical and chemical methods. In addition physicochemical methods are expensive, time consuming, ineffective and non eco-friendly (Eichlerova *et al.*, 2006). On the

other hand biological method has become one of the more favorable method due to their cost effective, environmental friendly lower sludge production compared to the physicochemical method (Mohana *et al.*, 2008). It is worth noting that, fungi and bacteria are widely used for the degradation of textile dyes (Amar *et al.*, 2010). Bacterial degradation of azo dyes is more advantageous as bacterial cell grow fast and their decolorization rate is also very high.

The focus of the present study was the study of effect of NaCl salt and peptone concentration on the efficiency of decolorization of Methyl orange dye by bacterium *Bacillus* sp. Strain and also phytotoxicity of Methyl orange and its degradation products was studied.

Materials and Methods

Bacterial Culture

Bacillus sp. Strain was previously isolated from dye contaminated soil sample collected around the Dyeing Industries, Solapur, India. This strain was capable of decolorizing azo dyes efficiently. This strain was grown under static condition at room temperature in Luria-Bertani (LB) broth.

Dyes and Media

The azo dye Methyl Orange was procured from sd fine chemicals ltd. Dye was checked for its color, solubility in water and absorption maximum. The mineral salt medium was prepared with following composition (g/l): K₂HPO₄ (6.3), KH₂PO₄ (1.8), NaCl (5), NH₄NO₃ (1), MgSO₄.7H₂O (0.1), MnSO₄ (0.1), CaCl₂.2H₂O (0.1), FeSO₄.7H₂O (0.1), NaMoO₇. 7H₂O (0.006). The final pH of the medium was adjusted to 7.2. The mineral salt medium was supplemented with yeast extract

(2.5g/l), peptone (5g/l) and Methyl orange dye (200mg/l). All the chemicals used in this study were of analytical grade.

Decolorization Experiments

The decolorization experiments were performed in 250ml Erlenmeyer flasks containing medium. Methyl Orange (200mg/l) was added to decolorization medium and inoculated with 2ml of cultures broth. The flasks were incubated at 40°C under static conditions till the decolorization was completed.

Analytical methods for dye decolorization studies

The samples were withdrawn at different time intervals and analyzed for decolorization efficiency. Decolorization was quantitatively analyzed by measuring the absorbance of the supernatant using a UV-Visible spectrophotometer at maximum wavelength. λ_{max} for Methyl Orange was 465nm. Decolorization percentage was calculated by using the equation

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

Study of Physico-chemical parameters

Decolorization ability of strain on Methyl Orange was studied at different pH (5-9), temperature values (15-50°C), dye concentration (200-1000mg/l) etc. The effect of these physicochemical factors was studied.

It was observed that pH 7.2 and temperature 37°C were found to be optimal for the decolorization activity. Effect of other factors was studied at pH 7.2 and temperature 37°C.

Phytotoxicity Studies

The tests were carried out on Pigeon pea (*Cajanus cajan*) seeds commonly used in agriculture. The required concentration of Methyl orange showing inhibitory effect on the growth of seeds was selected for the study.

Ten seeds were germinated on a bedded filter paper with daily watering of 10.0 ml solutions for respective samples. Simultaneously, a control set with the plain water was carried out.

The toxicity was measured in terms of percent germination and lengths of plumule and radical after 10 days. Relative seed germination, relative root elongation and germination index (GI) were calculated by the following formulae.

Relative Seed Germination (%)

$$= \frac{\text{No. of seeds germinated in extract}}{\text{No. of seeds germinated in control dye}} \times 100$$

Relative root elongation (%)

$$= \frac{\text{Mean root elongation in extract}}{\text{Mean root elongation in control dye}} \times 100$$

Germination Index (%)

$$= \frac{(\% \text{ Seed germination}) \times (\% \text{ Root elongation})}{100}$$

Results and Discussion

Effect of NaCl Concentration

Dye waste waters from both the dye manufacturing and dye consuming

industries contain high salt concentration which is up to 15-20%.

The effect of NaCl concentration on the decolorization of Methyl orange by the selected strain was examined. Decolorization efficiency was not affected at NaCl concentrations of 5g/l and 10g/l. At 15g/l and 20g/l NaCl concentrations the decolorization dropped to 75% and 64% respectively. Further increase in concentration of NaCl resulted in decreased percentage decolorization.

Effect of Nitrogen Source

The percentage decolorization increased with increase in peptone concentration up to 5g/l and after which there is decrease in percentage of decolorization.

The decrease in decolorization results from nitrate or nitrite, a reducing equivalent that cells generated from peptone consumption. These metabolites of nitrate or nitrite may compete with the azo dye and result in less decolorization.

Phytotoxicity

Phytotoxicity of Methyl orange and acidic and neutral fractions of ethyl acetate extracts of the cultures grown spent medium was investigated. There was a significant change in the lengths of plumule and radical of the seeds of pigeon pea in the water, control dye (Methyl orange) and degradation products of the treated samples.

The phytotoxicity results also suggested that the degradation products of Methyl orange were much less toxic to the seeds of pigeon pea.

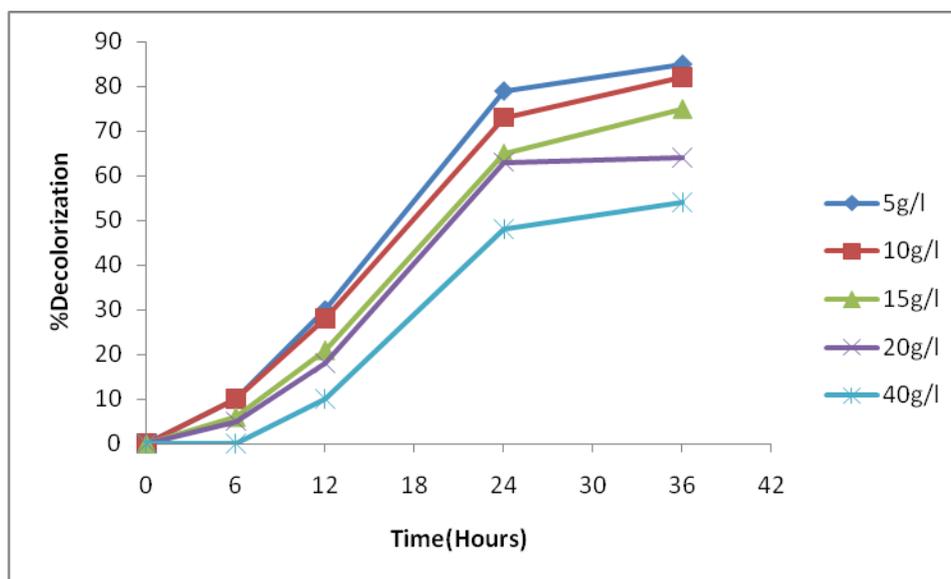


Figure.1 Effect of NaCl concentration on decolorization of Methyl orange

Table.1 Phytotoxicity of Methyl Orange and its degradation products on Pigeon pea.

Parameter	Plain water	Pigeon pea	Degradation products	
			Acidic	Neutral
Germination (%)	100	68	75	78
Plumule (cm)	12.60±0.25	4.2±0.16	9.34±0.32	10.87±0.43
Radical (cm)	11.90±0.34	4.5±0.23	9.85±0.27	10.46±0.36

Conclusion

Bacterial decolorization proves to be a very efficient method for complete decolorization of Methyl Orange. The purpose of this study is to investigate the decolorization of Methyl Orange by *Bacillus* sp. Strain. The bacteria successfully decolorize Methyl orange. Most markedly the strain could effectively decolorize the dye, Methyl orange over dye concentrations of 200-800mg/l, pH of 5-9 and temperature 20-50°C. Decolorization with *Bacillus* sp. yields high maximum decolorization activity of 85% at dye concentration 200mg/l, pH 7 and temperature 37°C. It can be concluded that *Bacillus* sp. is highly promising and suitable microorganism for use in the treatment of textile waste water.

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