International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 4 Number 5 (May-2016) pp. 172-178

Journal home page: http://www.ijcrar.com

doi: http://dx.doi.org/10.20546/ijcrar.2016.405.017

Decolorization of Sulfonated Azo Dye Methyl Orange by Bacterial Species

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KEYWORDS

Azo dyes,
Decolorization,
Degradation,
Bacillus sp.,
Textile dye.

A B S T R A C T

Synthetic dyes are extensively used in textile industries and other industries. Methyl orange is one such azo dye, which is widely used in textile industries and also as an indicator in chemical laboratories. This study focuses on the capability of *Bacillus* sp. Strain AK1 to decolorize Methyl Orange dye. Various parameters like time course, initial dye concentration, pH and temperature were optimized to achieve maximum decolorization of the dye. The bacterium exhibited remarkable color removable capability over a wide range of dye concentration, pH and temperature. The bacterial strain decolorized Methyl orange (200mg/l) upto85% in 24 hours of incubation at pH7 and temperature 37^0c. The efficiency decreased with the increase in initial dye concentration. The pH also had a great impact upon the decolorizing ability.

Introduction

Ever since the beginning of mankind, people have been using colorants for painting and dyeing their surroundings, their skins and their cloths. With the rapid industrialization, now there are more than 1,00,000 commercially available dyes and 7×10^5metric tons of dyestuffs are produced annually (Wong and Yu, 1999). Dyes are used in textile industry, leather tanning industry, paper industry, food industry, hair coloring and cosmetics. Azo dyes constitute largest group of synthetic dyes. These are extensively used for dyeing fabrics in textile industries. The textile dyeing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution (Bhatti, *et al.*, 2008), as 10-15% of dyes are lost in the effluent during the dyeing process. It is very difficult to treat textile industry effluents because of their high BOD, COD, heat, color, pH and presence of metal ions (Anjali, *et al.*, 2007). Azo dyes are used increasingly in industries. Most azo dyes are toxic, carcinogenic and mutagenic (Pinherio *et al.*, 2004). In addition to this, the dyes

may significantly affect photosynthetic activity of aquatic life by decreasing light absorption and also may be toxic due to the presence of aromatics or heavy metals (Saratale *et al.,* 2006; Vajayanant and Hemapriya, 2013). Traditional waste water treatment methods have proven to be ineffective because of the chemical stability of these pollutants. Azo bonds present in these dyes are resistant to breakdown with the potential for the persistence and accumulation in the environment (Telke Amar *et al*). A wide range of methods have been developed for the removal of synthetic dyes from waste waters. Bioremediation of textile effluents through microorganisms has been of considerable significance since it is inexpensive, eco-friendly and produces less amount af sludge. A wide variety of microorganisms are reported to be capable of decolorization of azo dyes. Considering the advantages and potential applications of bioremediation processes in textile effluent treatment, the present study deals with the bioremediation of Methyl Orange, a synthetic sulfonated textile azo dye by bacterial strain.

Materials and Methods

Bacterial Culture

Bacillus sp. Strain AK1 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): K_2HPO_4 (6.3), KH_2PO_4 (1.8), NaCl (5), NH_4NO_3 (1), $MgSO_4.7H_2O$ (0.1), $MnSO_4$ (0.1), $CaCl_2.2H_2O$ (0.1), $FeSO_4.7H_2O$ (0.1), $NaMoO_7$. $7H_2O$ (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 MS medium. Methyl Orange (200mg/l) was added to decolorization medium and inoculated with 2ml of cultures broth. The flasks were incubated at 40^oc 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. $\lambda_{max}$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 AK1 on Methyl Orange was studied at different pH

(5-9), temperature values (15-50^{o}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^{o}c were found to be optimal for the decolorization activity. Effect of other factors was studied at pH 7.2 and temperature 37^{o}C.

Results and Discussion

Time course for decolorization of Methyl Orange

At an early 6 hours of incubation 10% of decolorization was exhibited by the strain AK1. It showed 30% decolorization in 12 hours, 79% decolorization in 24 hours and 85% decolorization in 30 hours. The experiment was carried out by taking 200mg/l dye at pH 7 and temperature 37^{0}C.

Effect of initial dye concentration

The decolorization of Methyl orange was carried out at different initial dye concentrations from 200mg to 1000mg/l. The decolorization percentage of Methyl orange decreased with increase in the concentration of the dye under static conditions. Lower decolorization percentage at high dye concentration was reported. This might be due to the inhibitory effects of high dye concentration.

Effect of pH on dye decolorization

The effect of pH on the decolorization of Methyl Orange was determined over a wide range of pH from 4 to 9. The strain showed maximum dye decolorization at pH 7. At this optimum pH, the strain showed 85% decolorization of Methyl Orange. No decolorization was observed at pH 4. The strain showed 10%, 77%, 80% and 74% decolorization at pH 5, pH 6, pH 8 and pH9 respectively.

Effect of temperature on dye decolorization

The decolorization of the dye was tested for a wide range of temperatures from 15 to 50^{o}c. Increase in decolorization of Methyl Orange was observed with the increase in temperature and it was optimum at 37^{o}c. Further increase in the temperature resulted in decreased percentage of decolorization. The decrease in dye decolorizationat high temperature can be attributed to the decline in microbial activity that led to the inactivation of the enzyme and eventually loss of cell viability.

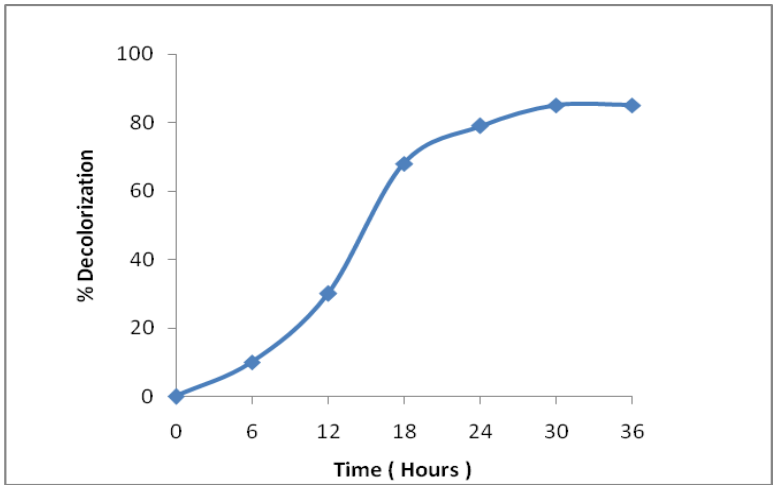

Figure.1 Effect of incubation time on decolorization of Methyl orange

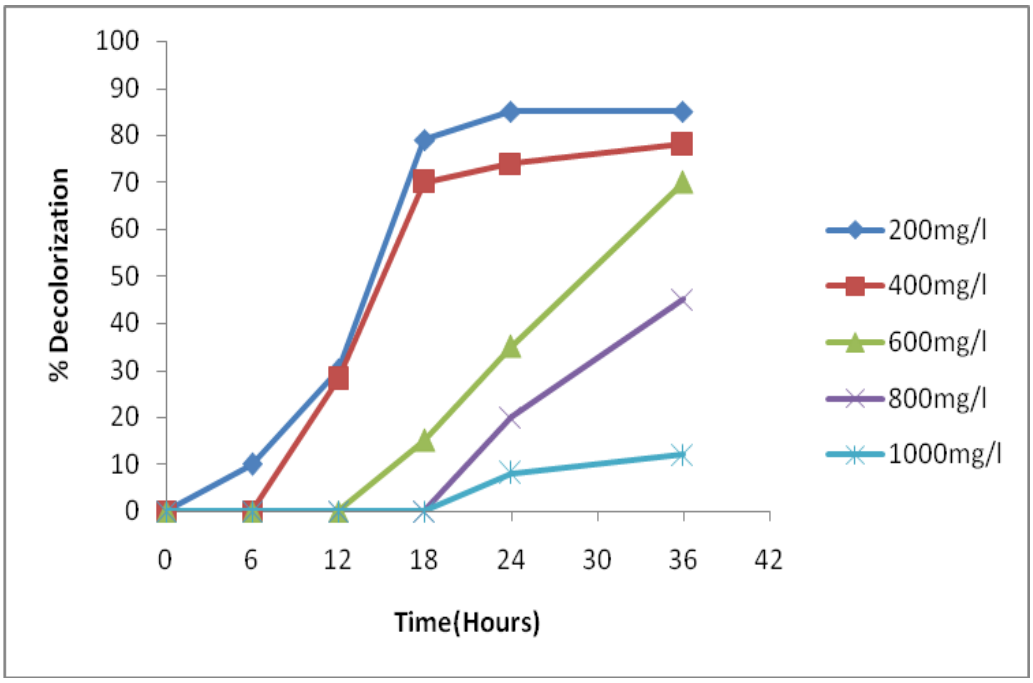

Figure.2 Effect of dye concentration on decolorization of Methyl orange

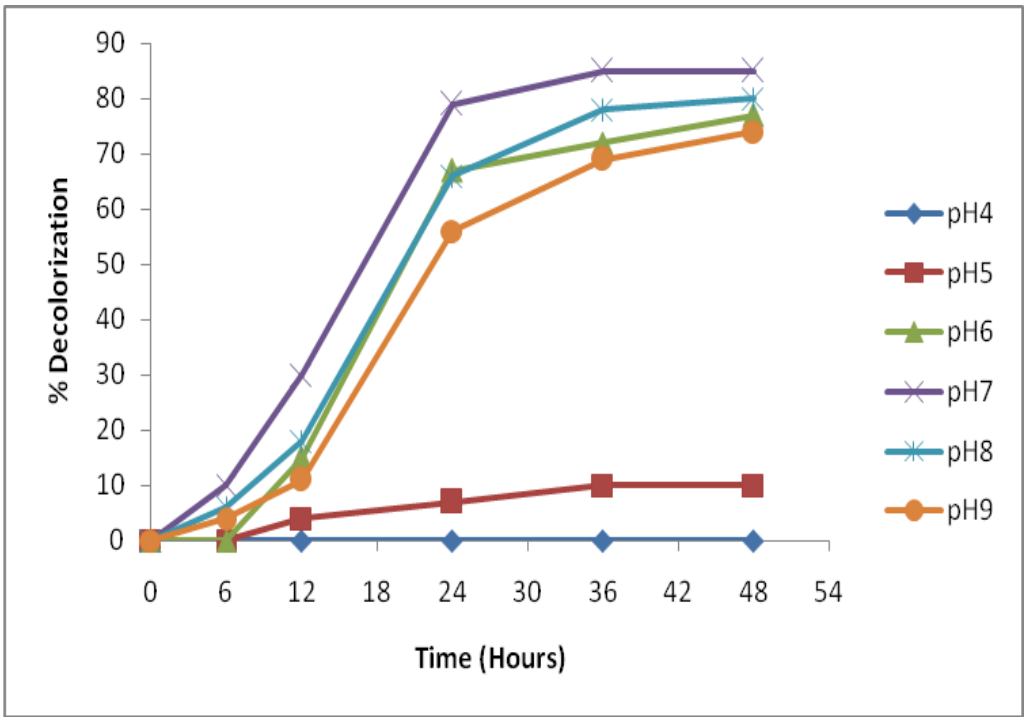

Figure.3 Effect of pH on decolorization of Methyl orange

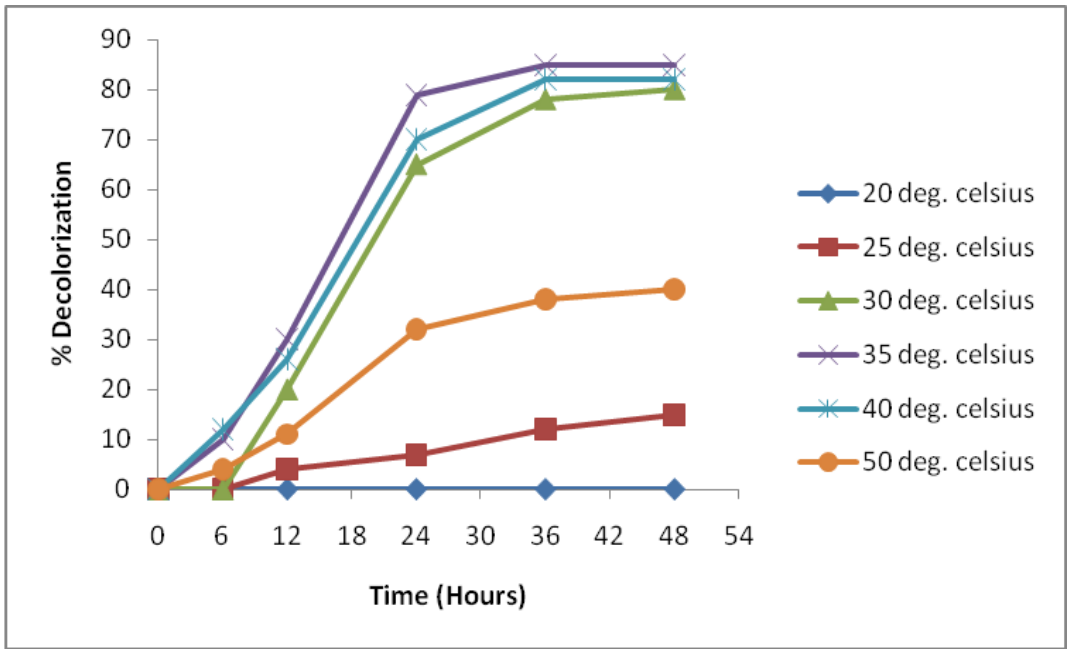

Figure 4: Effect of temperature on decolorization of Methyl orange

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 AK1. 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^{o}c. Decolorization with *Bacillus* sp. AK1 yields high maximum decolorization activity of 85% at dye concentration 200mg/l, pH 7 and temperature 37^{o}c. It can be concluded that *Bacillus* sp. AK1 is highly promising and suitable microorganism for use in the treatment of textile waste water.

Acknowledgements

The author is highly grateful to UGC for the financial support under the scheme of Minor Research Project. Also thankful to Gulbarga University, Gulbarga and Principal and management of HKES's A. V. Patil College, Aland for providing the infrastructure facility for the research work.

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How to cite this article:

Ramesh S. Masarbo. 2016. Decolorization of Sulfonated Azo Dye Methyl Orange by Bacterial Species. *Int.J.Curr.Res.Aca.Rev.*4(5): 172-178.
doi: http://dx.doi.org/10.20546/ijcrar.2016.405.017