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Rhizo-biodegradation of Methylene Blue Dye using Developed Mycorrhizal Soil

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ABSTRACT

The dyestuff manufacturing industry has been categorized as one of the hazardous industry by EPA under Hazardous Waste Management and Handling Rules (1989). Methylene blue is one of the commonly found compounds in hazardous waste. The present research emphasizes development of Rhizosphere bioremediation of dye compound – Methylene blue in pot culture using developed mycorrhizal soil which was grown for 3 months. Then bioremediation was carried out in mycorrhizal amended soil with dye compound of varying concentration (0, 10, 25, 50 mg/kg) for another 3 months. Rhizosphere biodegradation of Methylene blue dye was assessed by HPLC and GCMS technique in the lab where degradation in the structure of methylene blue dye was seen by formation of different metabolites in the soil which was confirmed by GCMS and 60% reduction in the quantity of dye was observed in 10 mg pot, 62% dye reduction in 25 mg pot and 64% dye degraded was seen in 50 mg pot by HPLC analysis. Hence the study focuses on formation of mycorrhizal soil which can be used as biofertilizers as well as will help to develop ecofriendly, cost-effective and efficient approaches for decontamination of recalcitrant dye compound from the soil.

Key words : *Methylene blue dye, Mycorrhiza, Greenhouse, Rhizosphere bioremediation*

Introduction

The textile dying industry is among the fastest growing industry which involves use of various dyes & chemicals and plays an important role in growth and development of manufacturing sector. Among variety of chemicals used in textile industry, most extensively used are the synthetic dyes. It has been estimated that commercially 10,000 different dyes are available for dying and printing purpose. The dyes used in textile industry are chemically diverse in nature and are broadly classified into acidic, basic, reactive, azo dye, etc.

One of the most important types of dyes are azo dyes that are extensively used in textile, printing, leather, food, cosmetics, and paper product industries due to their better steadiness, economical synthesis and greater variety of colour as compared to natural dyes. It is estimated that world production of azo dyes is around to be one million tons annually, and more than 2,000 structurally different azo dyes are currently in use (Stolz *et al*., 2001). Azo dyes are distinguished by the presence of one or more group (–N=N–) bound to large number of aromatic rings such as benzene and naphthalene.The colour of azo dyes is because of azo bond and associated chromophores (Lodha *et al.*, 2007). In general, synthetic azo dyes with replacements as part of their structure are highly resistant to degradation. Several azo dyes and their linked reductive metabolism products are toxic. Due to the presence of aryl amines which are derived from the reduction or

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transformation of azo dyes, most of the azo dyes become carcinogenic. In addition, the effluent of dye industry also contains other environmental pollutants out of which some are additives used in the dyeing process. Thus, waste generated from dye industry or effluent is a significant source of environmental pollution.

Azo dyes from industrial effluents and their breakdown products are mutagenic to humans and toxic to aquatic life, hence the removal of dyes is desirable and also for aesthetic reasons (Chung *et al.*, 1993). Without adequate treatment these azo dyes are stable and can remain in the environment for an extended period of time. Consequently, azo dyes have to be removed from wastewaters before discharge. In recent years, various chemical and physical treatment methods have been developed for the removal of azo dyes from waters and wastewaters to decrease their impact on the environment. Because of the high cost and disposal problems, many of these methods for treating azo dye-containing wastewater have not been widely applied in the textile industries.

Considering the limitation of physico-chemical treatment methods for remediation of dyestuff compound, most of the research work focuses on biotechnological approaches such as bioremediation and Phytoremediation due to their ability to efficiently degrade recalcitrant dye compounds without producing secondary pollution.

Bioremediation can be defined as a process that utilizes biological agents for complete removal of toxic substances and/or contaminants from the environment. The microorganisms undergo through a complex sets of reaction that converts contaminants from highly toxic forms into innocuous forms, that forms a part of their metabolic process. The main advantage of bioremediation includes the ability of microorganisms to degrade insitu contaminants completely by transforming them into non-toxic products like carbon dioxide, nutrients and biomass. In recent years, microbial ecologists have identified various biological agents such as bacteria, fungi, actinomycetes that can effectively degrade dyes in natural environment. The microorganisms involved in degradation of dyestuff compounds may be native to contaminated sites. Such an adaptable microbial community plays a vital role in reclamation and restoration of contaminated environment.

The mutualistic symbiotic association of fungi and plant is known as mycorrhiza which provides effective rhizosphere for degradation of hazardous contaminants. Vesicular arbuscular mycorrhizas (VAM fungi) are vital components of ecosystem for maintenance of soil structure and re-vegetation of degraded lands (Caravaca *et al.,* 2005). VAM also controls root colonization of the host plant which alters equilibrium of microbes in the mycorrhizosphere by making changes in root exudation pattern. VAM are known to improve the growth and health of the plants by improving soil mineral nutrients thereby. Rhizosphere bioremediation encourages degradation of organic contaminants in the soil by increasing bacterial population, soil organic carbon and mycorrhizal fungi (Schoor *et al.,* 1997). Hence this research focuses on formation of mycorrhizal soil which can be used as biofertilizer as well as will help to develop ecofriendly, cost-effective and efficient approaches for decontamination of azo dye like methylene blue from the soil.

Materials and Methods

Test plant selection

Tagetes patula L. commonly called as Marigold was selected for development of mycorrhizal soil at laboratory scale. Marigold seeds were procured from local market. Before using in the experiment, the seeds were surface-sterilized for 2-3 minutes with 0.1% mercuric chloride and rinsed several times with distilled water to avoid fungal contamination. These seeds were further used for the development of mycorrhizal soil.

Soil Sampling and characterization

Soil used for the development of mycorrhizal soil, was collected from a depth of 0-15 cm along the banks of Sindhrot dam, Vadodara, Gujarat. Stones and plant remains were removed from the soil, then air dried and screened through 2 mm stainless steel and stored in plastic bag at room temperature. The soil was then characterized for physico-chemical parameters, i.e. pH, electrical conductivity, moisture content, organic carbon, kjeldahl nitrogen, phosphorus, potassium was analyzed using Standard methods described in APHA.

Greenhouse Experimental Design

For the development of mycorrhizal soil, a greenhouse experiment was set up using pot culture tech-

nique under controlled environmental condition. The experiment was carried out in 3 pots, each pot containing 3 kg sand-soil mixture. For better aeration and drainage of water; perforations were made at the base of pot. A mixture of soil and sand (3:1) was used for growing host plants to provide porosity in the soil. About 3 g VAMOZ mycorrhizal inoculum was mixed with 3 kg of sand-soil mixture per pot in 1:1 ratio. Ten sterilized marigold seeds were then sown in each pot and their growth was monitored for 3 months. The plants were watered regularly and provided with Hogland solution to provide essential nutrients. Approximately 10 ml of Hogland solution was provided per pot, after every 15 days. All pots were placed in a greenhouse with natural sunlight at temperatures of 27-28 ^{0}C. After 3 months, dense mass of roots were formed colonized with AMF and mycorrhizal spores. Further, roots of the plants were chopped and mixed in the same soil which will be our 'soil based mycorrhizal inoculum' for further experiment.

Soil sampling and analysis

The soil samples from the above experiment was collected after 3 months in order to determine soil pH, electrical conductivity, moisture content, organic carbon, kjeldahl nitrogen, phosphorus, potassium and spore count. For soil pH and EC, soil-water suspension was made and was measured in digital pH meter and conductivity meter respectively. Moisture content by oven-drying method. Organic carbon was analyzed using the Walkley-Black method, total nitrogen content by the Kjeldahl method, Phosphorus by Olson method and Potassium was determined using AAS. The spore count was done by wet-sieving method (Gerdemann and Nicholson, 1963).

Spiking of dye in the soil

Mycorrhizal inoculum obtained from green house experiment was used over here. 4 pots were filled with mycorrhizal soil spiked with methylene blue dye at various concentrations viz. 0 (control), 10, 25, 50 mg/kg. 10 marigold seeds were sown per pots amended with methylene blue dye. This was carried out in triplicate set (total 12 pots)and pots were kept in green house with temperature 27-28 ^{0}C with the natural light for 3 months as shown in Fig 1(a) & (b). After this soil samples were collected at an interval of 1 month to evaluate degradation of dye. Rhizosphere biodegradation of methylene blue dye was assessed by HPLC and GCMS technique in the lab.

Results and Discussion

Physico-chemical properties of the developed mycorrhizal soil had better pH, moisture holding capacity, OC, N, K than in the collected original soil. Comparison between normal soil and mycorrhizal soil has been depicted in Table 1.

The pH of the mycorrhizal soil was found to be

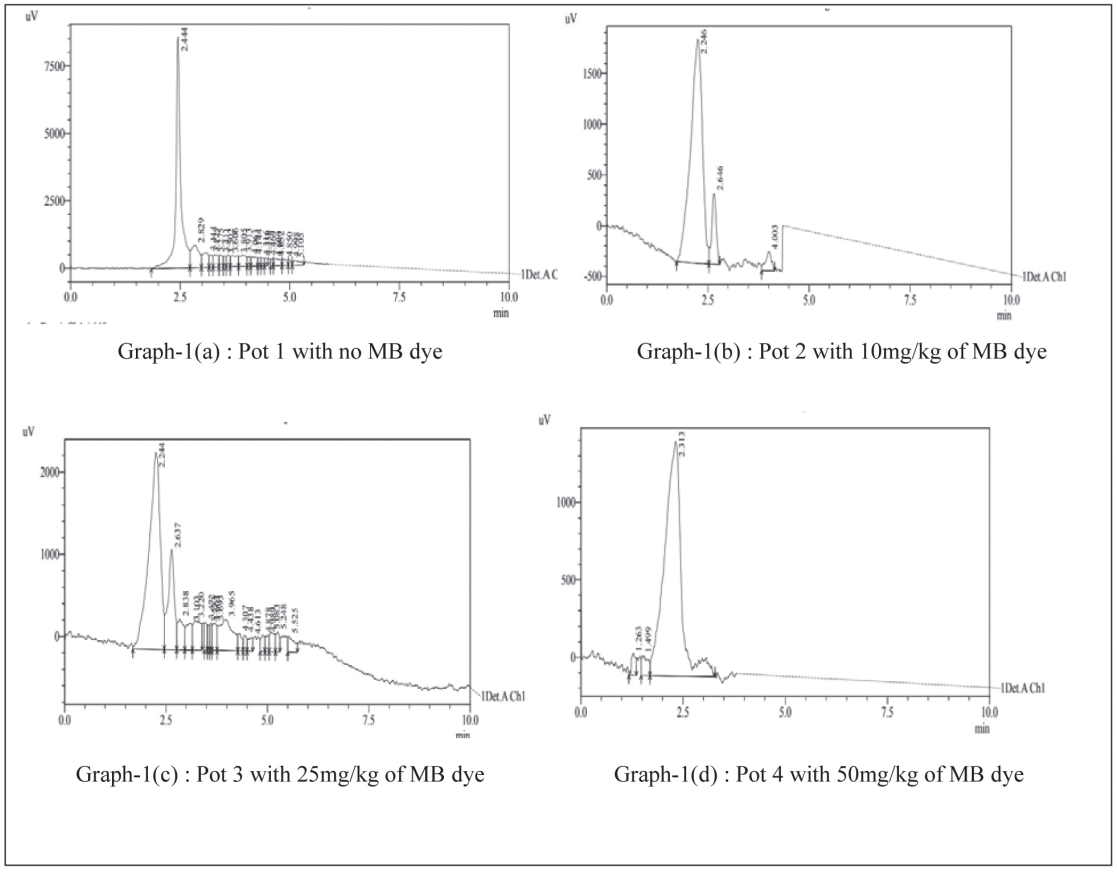
Table 1. Comparison between normal soil and mycorrhizal soil after 3 months.

Sr. No.	Parameters	Normal Soil	Mycorrhizal Soil
1	pH	6.88	7.42
2	Electrical conductivity (ìmhos/cm)	8.0	8.4
3	Moisture content (%)	25.92	26.10
4	Total organic carbon (%)	0.36	0.50
5	Kjeldahl nitrogen (%)	0.20	0.31
6	Phosphorus (%)	0.028	0.011
7	Potassium (%)	0.33	0.39
8	Spore count (per 100 g soil)	-	320

7.42 which is considered as neutral. Electrical conductivity was 8.4 of mycorrhizal soil which indicates good movement of ions. Moisture content of mycorrhizal soil was 0.18 % more than normal soil which depicts it can retain more water as compared to normal soil. The organic carbon of the developed mycorrhizal soil was found to be higher (0.50%) than in the collected soil (0.36%) which indicates higher organic matter for the survival of various microbial populations. Nitrogen content was found to be 0.11% more in mycorrhizal soil as compared to normal soil which is essential for chlorophyll molecule for generating food for the plant. The phosphorous content did not vary much during development of mycorrhizal soil and was found to be lower. Soil potassium levels were 0.06% increased in mycorrhizal soil in comparison with normal soil which will trigger activation of more ATP molecules and beneficial enzymes.

HPLC chromatogram (graph – 1(a),(b),(c),(d)) confirmed the degradation of methylene blue dye in all the three samples of pot (except the control one). Pot-1 consisted of only mycorrhizal soil (control) with no MB dye in it which produced major peak at retention time of 2.444. Pot-2 consisted of mycorrhizal soil + 10 mg/kg of MB dye which produced major peak at retention time of 2.246 indicating 60% dye degradation. Pot-3 consisted of mycorrhizal soil + 25 mg/kg of MB dye which produced major peak at retention time of 2.244 indicating 62% dye degradation. Pot-4 consisted of mycorrhizal soil + 50 mg/kg of MB dye which produced major peak at retention time of 2.313 indicating 64% dye degradation.

Gas chromatography and mass spectra (GC–MS) analysis was done to investigate the metabolites formed during the biodegradation process. GC–MS analysis showed formation of these compounds, viz. oxalic acid (molecular weight = 314, m/z = 29), formic acid (molecular weight = 289, m/z = 41), carbonic acid (molecular weight = 212, m/z = 43), acetic acid (molecular weight = 197, m/z = 69), octanol (molecular weight = 158, m/z = 70) after degradation as shown in graph-2.



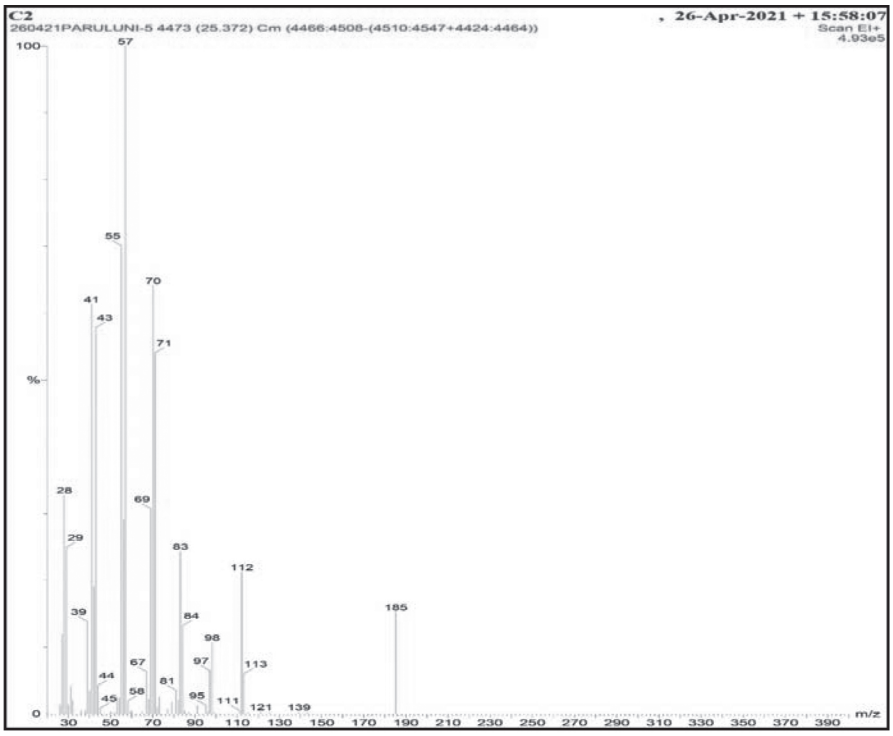
Graph-1(a) : Pot 1 with no MB dye

Graph-1(b) : Pot 2 with 10mg/kg of MB dye

Graph-1(c) : Pot 3 with 25mg/kg of MB dye

Graph-1(d) : Pot 4 with 50mg/kg of MB dye

Graph 1. HPLC Chromatogram



Graph 2. GC-MS analysis of compound formed after dye degradation

Conclusion

Discharge of textile effluent in open environment is a major environmental concern. Physical and chemical methods are effective for dye degradation but need energy and may cause pollution. Hence, economical, eco-friendly and natural techniques using rhizospheric soil can be an alternative method to treat Methylene blue dye textile effluent.

From the above study it can be concluded that physico-chemical properties of the developed mycorrhizal soil had better pH, moisture holding capacity, OC, N, K than the normal soil. Also degradation in the structure of Methylene blue dye in soil was seen by formation of different metabolites in the soil which was confirmed by GCMS and reduction in the quantity of dye was observed by HPLC analysis.

In this way efficient technologies which can be transferred from lab to land for decontamination of recalcitrant dye like Methylene blue from the soil.

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References

Atacage, E.H. 2010. Treatment of Azo Dye-Containing Wastewater Using Integrated Processes. *The Handbook of Environmental Chemistry*. Vol- 9.

Caravaca, F., Alguacil, M.M., Barea, J.M. and Roldan, A. 2005. Survival of inocula and native AM fungi species associated with shrubs in degraded Mediterranean ecosystem. *Soil Biology and Biochemistry.* 37: 227–233.

Chung, K.T. and Stevens, S.E.J. 1993. Degradation of azo dyes by environmental microorganisms and helminthes. *Environ Toxicol Chem.* 102: 2121–2132.

Eslami, H. and Khavidak, S.S. 2017. Biodegradation of methylene blue from aqueous solution by bacteria isolated from contaminated soil. *J Adv. Environ Health Res.* 5 : 10-15.

Fulekar, J., Pathak, B. and Fulekar, M.H. 2007. Development of Mycorrhizosphere Using Sorghum bicolor for Rhizosphere Biormediation. *Int. J. of Current Research Academic Review*. 5(6) : 42-48.

Gerdemann, J.W. and Nicholson, T.H. 1963. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*. 46 : 235-244.

Korade, D.L. and Fulekar, M.H. 2009. Development and

evaluation of mycorrhiza for rhizosphere bioremediation. *Journal of Applied Biosciences*. 17: 922 – 929

Korade, D.L. and Fulekar, M.H. 2009. Rhizosphere remediation of chlorpyrifos in mycorrhizospheric soil using ryegrass. *Journal of Hazardous Materials*. Elsevier. 172 : 1344–1350.

Lodha, B. and Chuaudhari, S. 2007. Optimization of Fenton-biological treatment scheme for the treatment of aqueous dye solutions. *J Hazard Mater*. 148: 459–466.

Patil, P. and Desai, N. 2007. Degradation analysis of Reactive Red 198 by hairy roots of *Tagetes patula* L. *Planta. Elsevier.* 230 : 725–735.

Schoor, J.L. 1997. Phytoremediation. Technical Evaluation Report for Ground-Water Remediation Technologies Analysis Center, Pittsburgh

Stolz A. 2001. Basic and applied aspects in the microbial degradation of azo dyes. *Appl Microbiol Biotechnology*. 56 : 69–80.