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Mycodecolorization of a Toxic Dye, Malachite Green, by White Rot Fungus *Pleurotus eryngii*

Numan Yildirim* and Ummiye Gonen

Faculty of Engineering, Department of Environmental Engineering, Munzur University, Tunceli, Turkey

***Corresponding Author:** Numan Yildirim, Faculty of Engineering, Department of Environmental Engineering, Munzur University, Tunceli, Turkey.

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Abstract

Biodecolorization of a hazardous triphenylmethane dye, malachite green (MG) by white rot fungus *Pleurotus eryngii* in both solid and agitated liquid culture mediums has been studied. The fungus was used in this study isolated from Tunceli-Ovacik province of Turkey. For solid medium screening the mycodecolorization capacity of *P. eryngii*, growth and decolorization zones were determined on sabouraud dextrose agar medium containing 0.05, 0.1, 0.5 and, 1 g/L of MG. The same concentrations also were used for determination of decolorization capacity of fungus in the agitated (140 rpm) liquid culture mediums containing sabouraud dextrose broth. *P. eryngii* showed certain decolorization capacities in solid medium but not to the same extent. The decolorization in agitated medium was generally observed at low concentrations of MG. The results of in this study showed that the *P. eryngii* had maximum decolorization (23% at 0.1 g/L initial dye concentration) activities after 7 days under agitated culture conditions. This fungus could be an alternative mycoremediation tool for decolorization of MG containing textile wastewater.

Keywords: *Mycodecolorization; P. eryngii; Malachite Green*

Introduction

Dyes are intensely colored and complex organic compounds that dissolve in some environments and give permanent color to the applied material. Dyes with many natural and artificial examples are widely used in the textile, paper, leather and cosmetics industries [1]. In the textile industry, besides many auxiliary chemicals, numerous colors and types of dyes are used and consequently, a large amount of colored wastewater is released during both production and use. Today, about 10,000 different dyes and pigments are produced industrially. The annual production of these dyes worldwide is over 7 x 10^5 tons [2].

Wastewater containing dyes are discharged to receiving environments in large amounts. This situation constitutes the beginning of a process that is difficult to compensate in terms of environment and human health [3]. MG is a synthetic N-methylated diaminotriphenylmethane dye that is used extensively in aquaculture and microbiology, especially in the textile industry. Widely used in the laboratory environment, bacteria isolation, spore staining and pH indicator. MG is highly toxic to mammalian cells and has been banned by the US Food and Drug Administration [4-6].

The high cost of physical and chemical methods recommended for textile industry wastewater and the fact that they cannot be used for every dye have caused their application to be limited. Recent studies have highlighted the existence of microorganisms capable of removing many types of dyes from wastewater and highlighted biotechnological methods [7-9].

White rot fungi are capable of degrading substances such as lignin, chlorinated aromatic and aliphatic hydrocarbons and, dyes by the extracellular enzyme system. The most commonly used white rot fungus species are *Phanerochaete chrysosporium*, *Coriolus versicolor* and *Trametes versicolor* [2,10].

Aim of the Study

In this study, we have investigated fungal mycodecolorization of MG, using a white rot fungus *Pleurotus eryngii* isolated from Tunceli-Ovacik province of Turkey.

Materials and Methods

Chemicals used in this study

Chemicals used in this study were of analytical grade and obtained from Sigma and Merck Company and MG dye were purchased from a local chemical market. The chemical properties of MG were illustrated in table 1.

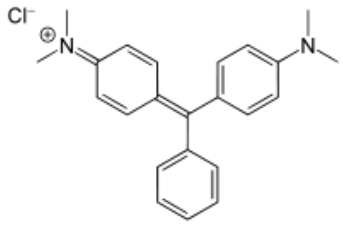
Chemical structure of MG	**Abs_{max}**	**Kimyasal sınıfı:**
	615	Triarylmethane **Molecular formula:** $C_{23}H_{25}N_2$

Table 1: *Chemical properties of MG dye.*

Fungus

White rot fungus *P. eryngii* was used in this study. The fungus was collected from Ovacik-Tunceli province of Turkey. The strains were maintained on sabouraud dextrose agar (SDA) mediums at 4°C in a refrigerator. The mycelium from stock culture was transferred to SDA plates and incubated at 25°C for 7 days. Mycelial plugs (5 mm diameter) from the peripheral region of an actively growing culture were used as inoculums for decolorization applications.

Agar-plate screening for MG decolorization

Agar-plate screening for decolorization activity tested on SDA slants in Petri dishes. For this purpose, mycelial plugs from stock culture in 5 mm diameter were taken and then inoculated into the center of Petri dishes in 90 mm diameter containing 0.05, 0.1, 0.5 and, 1 g/L of MG, in triplicate. The SDA slants were incubated at 25ºC in the static incubator until they were completely colonized with the fungus or for a maximum period of 20 days. The diameters (cm) of the decolorization and growth halos were determined in two perpendicular directions of the plates. The plates containing the dye but not inoculated served as control.

Preparation of inoculum and submerged decolorization medium

P. eryngii were cultured at 25°C on SDA slants in glass tube. After 7 days of incubation, conidial suspensions with deionized water were prepared and this conidial suspensions used for the preparation of inoculum. 10 ml of the suspension were used as inoculum for submerged decolorization studies. This 10 ml homogenized mycelial suspension was transferred into 250 ml flasks containing SDB and 0.05, 0.1, 0.5 and 1 g/L of MG on a rotary shaker incubator at 140 rpm for 7 days at 28°C in triplicate. After incubation, all flasks filtered with filter paper and then were used in following decolorization assays.

MG decolorization assays

Decolorization of MG in submerged liquid medium was measured in culture filtrates (tree replicate flasks) after removing the mycelia by filtration through filter paper, and monitored spectrophotometrically at the maximum wavelength of absorbance (615 nm). The systems without the fungi served as abiotic controls:

Where, Ax = Absorbance of the blank (dye solution); Ay = Absorbance of the treated dyes solution after incubation.

Statistical analaysis

Statistical analyses were performed with SPSS (SPSS Inc., Chicago, IL, USA). The data presented are the averages of the results of three replicates with a standard error (SE). To compare the decolourization ability of fungus, the data were analyzed by analysis of variance (ANOVA).

Results and Discussion

In this study, the decolorization activity of white rot fungus *P. eryngii* isolated from Ovacık district of Tunceli province on MG synthetic dye was investigated in solid media and liquid agitated media.

The ability of white rot fungi to decolorize various synthetic textile dyes and wastewater has been previously studied [11-13].

In this study, micelle growth and decolorization were achieved in different rates at the end of the 20th day at low concentrations of MG in solid media. *P. eryngii* was tested for decolorization and radial growth rate on SDA plates containing 0.05, 0.1, 0.5 and 1 g/L of MG. *P. eryngii* was able to grow on solid media in the presence of the MG dye (Table 2 and figure 1 and 2).

Concentration (gL^{-1})	Day	Radial Growth (cm)	Decolorization (cm)	Mycelial density
0.05	8	4.10 ± 0.01	4.30 ± 0.01	+++
	20	8.50 ± 0.00	8.50 ± 0.00	+++
0.1	8	4.30 ± 0.12	4.50 ± 0.15	+++
	20	8.00 ± 0.15	8,50 ± 0.00	+++
0.5	8	2.50 ± 0.03	3.50 ± 0.14	+++
	20	4.30 ± 0.05	7.30 ± 0.10	+++
1	8	2.20 ± 0.22	1.50 ± 0.00	+++
	20	4.30 ± 0.15	1.50 ± 0.00	+++

Table 2: *Decolorization of MG on agar plate.*

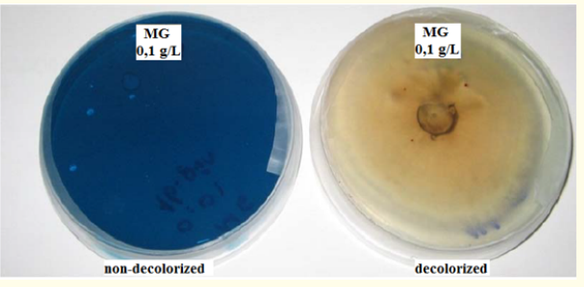


Figure 1: *Screening of P. eryngii for MG dye degradation after 20 days.*

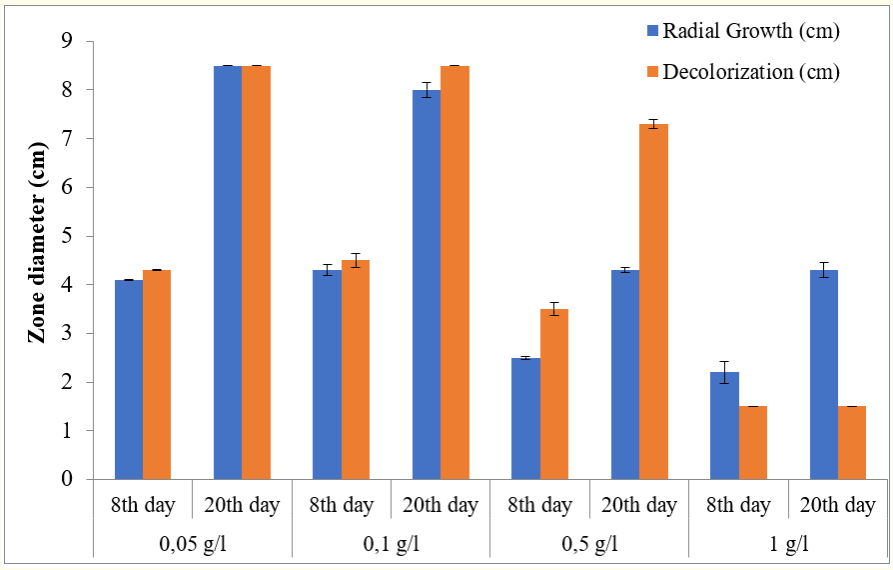


Figure 2: *The decolorization and growth zones in solid medium.*

The highest decolorization zone was found as 8.5 cm at SDA media containing 0.05 and 0.1 g/L MG dye after 20 days. Especially in medium containing high concentrations of MG (0.5 and 0.1 g/L), toxic effects of dye on fungal growth have occurred and micelle growth is suppressed by MG (Figure 2). Similarly, MG have a toxic effect on fungi has previously been reported [14]. In often, all the decolorized zones were smaller than growth zones, parallel with decolorization being a secondary metabolic activity of the mycelium. Similar results were obtained by Chiu., *et al*. (1998) [15] when evaluating white rot fungi for their ability to decolorize Poly R-478 dye.

The decolorization studies in liquid media were carried out in 250 ml flasks containing SDB and 0.05, 0.1, 0.5, and 1 g/L MG on a rotary shaker incubator at 140 rpm for 7 days at 27°C in triplicate under agitated condition. In this liquid medium at low concentrations (0.1 g/L), 23% decolorization rate was achieved by fungus. The decolorization rates of *P. eryngii* for MG in liquid medium after 3 and 7 days of cultivation are summarized in figure 3.

Mycodecolorization of MG has been investigated by a few authors. Youssef., *et al*. (2008) [16] have studied the decolorization of MG by *Acremonium kiliense*. This fungus was achieved as 95.4% decolorization rate at 5 mg L^{-1} MG concentration within 72 h. Structurally different dyes were not decolorized to the same extent. The triphenylmethane dyes (MG, crystal violet) were decolorized only poorly. It has been reported [17,18] that highly substituted triphenylmethane dyes required a longer time. Abedin 2008 was tested *Fusarium solani* for decolorization of MG and could be achieved 96% decolorization after 2 days of shaken incubation in the nutrient medium containing 2.5 mg dye/L [19]. This concentration levels significantly lower than the concentrations used in our study.

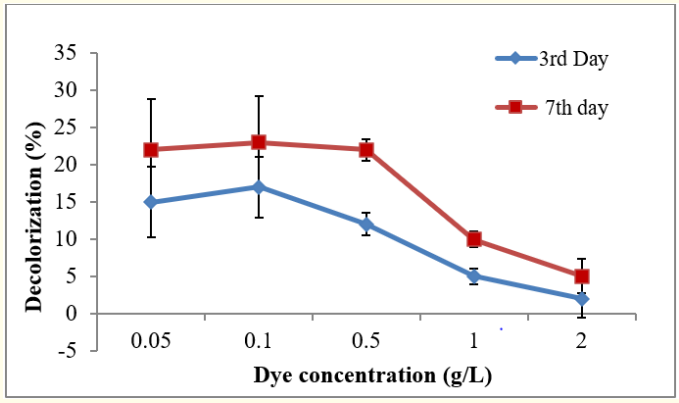


Figure 3: *The MG decolorization rates in liquid medium at 140 rpm.*

Conclusion

In this research, it can be recommended that this fungus could be an effective bioremediation agent for mycodecolorization of MG containing textile wastewater. However, further large scale applications of mycodecolorization are required to assess the field applicability of this fungus.

Conflict of Interest

The authors declare no conflict of interest.

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