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Bioremediation of malachite green and crystal violet dyes by *Phanerochaete chrysosporium* white rot fungus

Sheetal V Jadhav and Nandini Math

Abstract
Phanerochaete chrysosporium, a fungal isolate, has been used to biodegrade and detoxify colours including Malachite green and Crystal violet. Agar overlay, liquid media, and stationary and shaking conditions at 25 °C were the three techniques used for biodegradation. Crystal violet (92%) and malachite green (95%) showed the greatest decolourization in *P. chrysosporium*. In comparison to a stationary approach, the results from the chosen fungal strains were superior under shaking circumstances. On the seventh day under typical circumstances, a maximum decolourization of 98% was accomplished. The rate of decolourization was tested at various concentrations, and it was discovered that the percentage of decolourization is suppressed as dye effluent concentration rises. The percentage of Seed Germination Study that shows germination whereas uninoculated dyes prevent it even after 6 days is better in treated dye solutions.

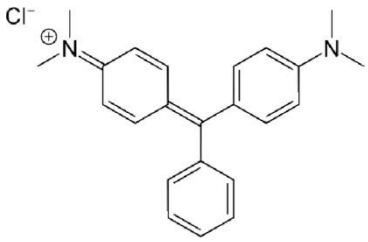
Keywords: Malachite green, crystal violet, biodegradation, *Phanerochaete chrysosporium* white rot fungus, seed germination

Introduction
The industries of paper, paint, cosmetics, food, and leather all depend on dyes in one way or another. The use of several chemicals, including dye, has increased as a result of industrialization. Water pollution results from industrial waste being disposed of in waterways. The dyes can disrupt phytoplankton photosynthesis, restrict light penetration, and make gases more soluble. Untreated dye effluent discharge into water causes changed pH, BOD, and COD, hence treating industrial effluent is essential to prevent environmental damage. All living organisms face significant health hazards as a result of environmental contamination. The biological technique is a straightforward, affordable, and environmentally beneficial process.

Fungi-based bioremediation is a growing field in wastewater treatment. According to Anjaneyulu *et al*. (2005) [1], dye and its components have always been unwanted and have been a research topic. One of the essential triphenyl group dyes, crystal violet is notorious for being mutagenic. Due to its extracellular, non-specific, and non-sterioselective enzyme system, which includes lignin peroxidase (LiP), laccase, and manganese peroxidise, fungi have been examined to breakdown contaminants (MnP). These fungi have been chosen for the current investigation. The ability of *P. chrysosporium* to remove dye has received much research. *Phanerochaete chrysosporium*, a type of fungus, is a successful microorganism for detoxifying dyes (Faraco *et al*., 2009a, Knapp *et al*., 1995, Swamy and Ramsay., 1999) [5, 9, 11]. The industrial effluent containing dyes can also be decoloured using *P. chrysosporium*. (Assadi *et al*., 2001, Asamudo *et al*., 2005; Wesenberg *et al*., 2003; Gomma *et al*., 2008; Faraco *et al*., 2009a, 2009b) [3, 2, 12, 7, 5, 6] Determining which fungi are capable of detoxifying and decolorizing colours using solid and liquid media while in stationary and shaking conditions is the primary objective of the current study.

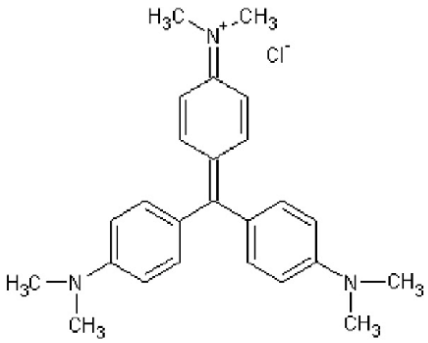
2. Methods and Material
2.1. Media
All the chemicals, dyes and media such as Potato Dextrose Agar (PDA), Potato Dextrose broth (PDB) and Nutrient agar (NA) were procured from Himedia, Mumbai, India.

2.2. Dyes used
Malachite green (4-[(4-dimethylaminophenyl) phenyl-methyl]-N, N-dimethylaniline) Molecular formula: $C_{23}H_{25}C_lN_2$,



Malachite green

Crystal violet Tris (4-(dimethylamino)phenyl)methylium chloride $C_{25}N_3H_{30}Cl$



2.3 Source of culture

The isolated culture of *Phanerochaete chrysosporium* was obtained from College of Agriculture, Vijayapura. Karnataka and maintained on Potato Dextrose Agar (Himedia, Mumbai, India) and sub cultured periodically.

2.4 Screening fungi for dye decolourization activities

Fungal strains were screened for their ability to degrade dyes using the tube overlay method. Initially, the fungal strains were grown on culture plates pre-filled with Potato Dextrose Agar (PDA) and incubated at room temperature for 14 days.

For liquid culture PDB media was used. PDA plates were incubated statically at 25 °C simultaneously for one week. Erlenmeyer flask (250 ml) containing sterilized liquid dye (100 ml) containing medium inoculated with 10mm agar plugs taken from fungal colony growing on PDA plates and incubated in aerobic condition in an incubator shaker at 150 rpm for 7 days.

2.5. Decolourization Assay

Effect of pH studied for 4, 5, 6, 7 on dye degradation. Effect of temperature was studied for 25, 30, 35; 40 °C. Different concentration of dye 50, 100, 150, 200 and 250mg/l were prepared and studied. For the purposes of this experiment, liquid cultures were grown for 7 days in a shaker incubator. The supernatant of samples centrifuged at 4000 rpm. Observe absorbance for Malachite green 660 nm and Crystal violet 588nm on a spectrophotometer on alternate days were observed. The experiments were all run in two replications, with the controls remaining dye-free. Compared to stationary cultures, the rate of dye degradation is increased in shaking conditions due to improved oxygen transport (McMullan *et al*., 2000; Kaushik and Malik., 2009) [8, 10]

$$\%\text{Decolourization} = \frac{\text{Initial Absorbance - Absorbance at particular time Interval}}{\text{Initial Absorbance}} \times 100$$

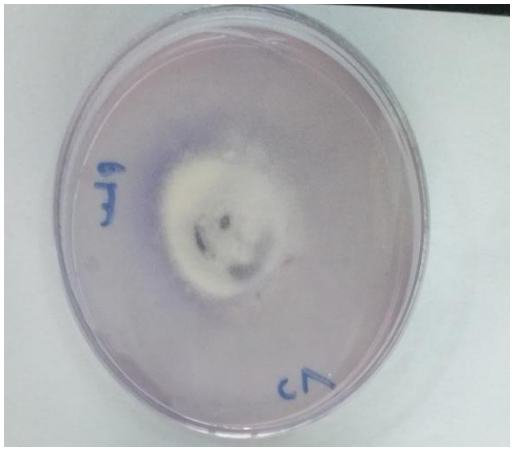
2.6. Seed germination bioassay

Effect of bio remediated and untreated dye solution was observed on wheat seed germination. The wheat seeds were sterilized using 0.1% $HgCl_2$ solution for 50 s, washed 6–7 times with sterile distilled water to remove traces of $HgCl_2$. In sterile Petri plates sterile filter paper was kept soaked in bio-remediated, untreated dye solution and with sterile distilled water soaked filter paper as control, respectively. Ten wheat seeds were kept in each Petri plate and the experiment was conducted in triplicate. Observation on seed germination was taken for four days. The experiment was conducted at room temperature of 25 ± 1 °C.

Result and Discussion

Detoxification of all the dyes was finally confirmed by the wheat seed germination and bacterial growth bioassay. The untreated dyes inhibited the wheat seeds germination after four days of incubation, while the seed germination was observed after 48 h in treated dyes treatments. Similarly, filter paper discs impregnated with untreated dye solution exhibited zone of inhibition of microbial growth, while the discs impregnated with treated dyes showed no zone of inhibition. The results of this study suggest that potentially competent fungal strains can be efficiently used for detoxification and bioremediation of harmful dyes.

1



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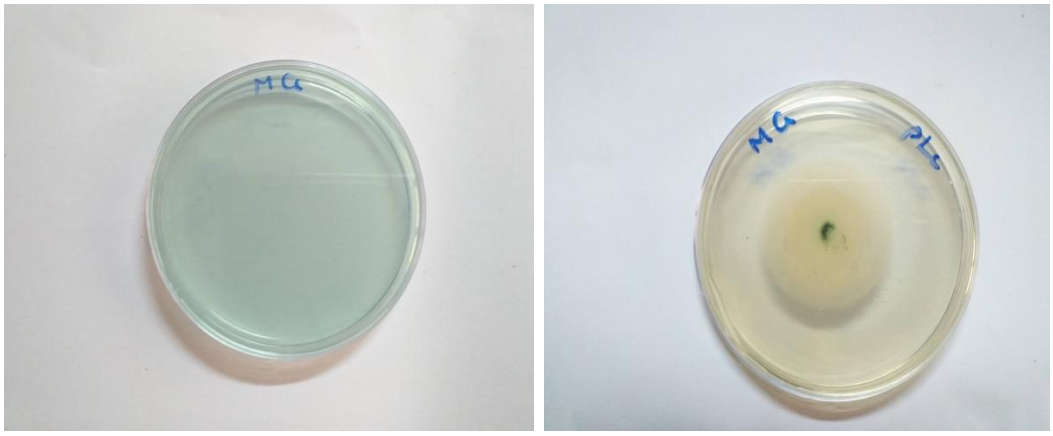

3 4

Fig 1: Dye degradation on media containing Crystal violet 1) before and 2) after Dye degradation Malachite green 3) Before and 4) after bioremediation

a) b)

Fig 2: Dye degradation in broth after 7 days

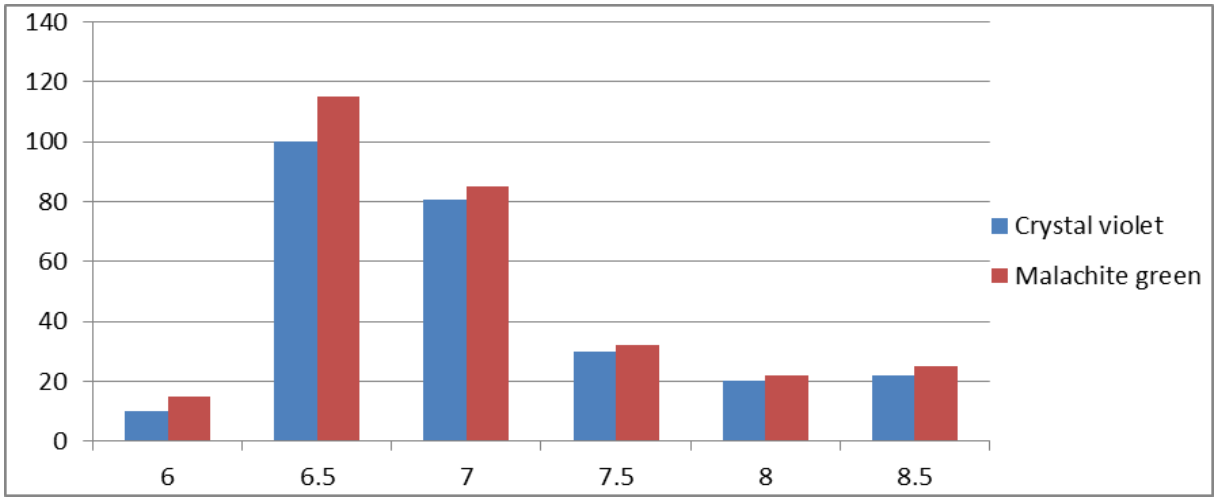

Fig 3: The effect of pH on percent of Crystal violet and Malachite green dye degradation %.

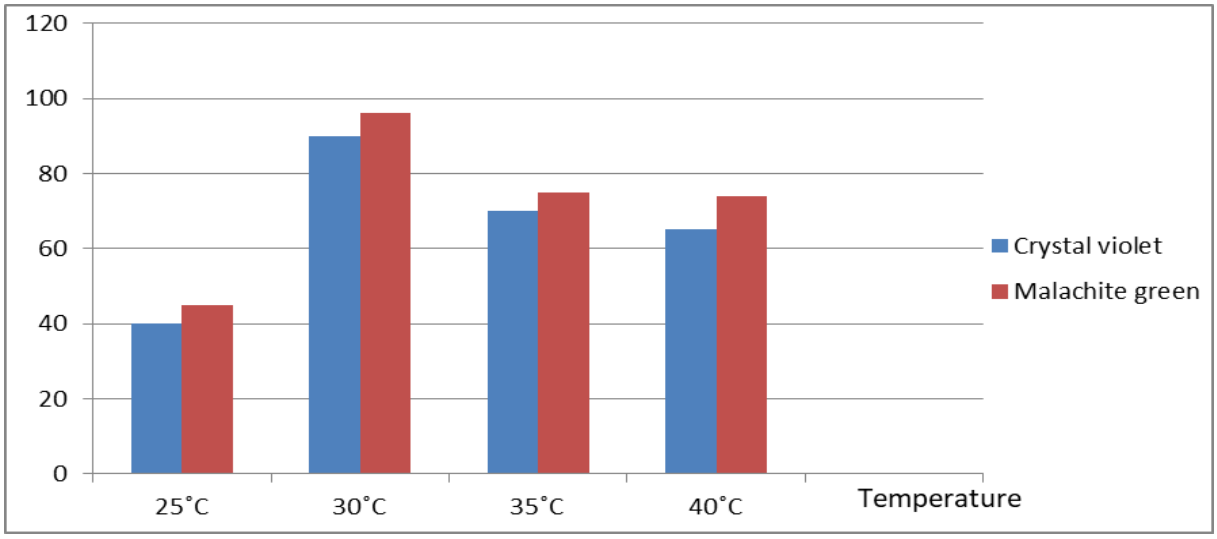

Fig 4: The effect of temperature on crystal violet and Malachite green dye degradation %.

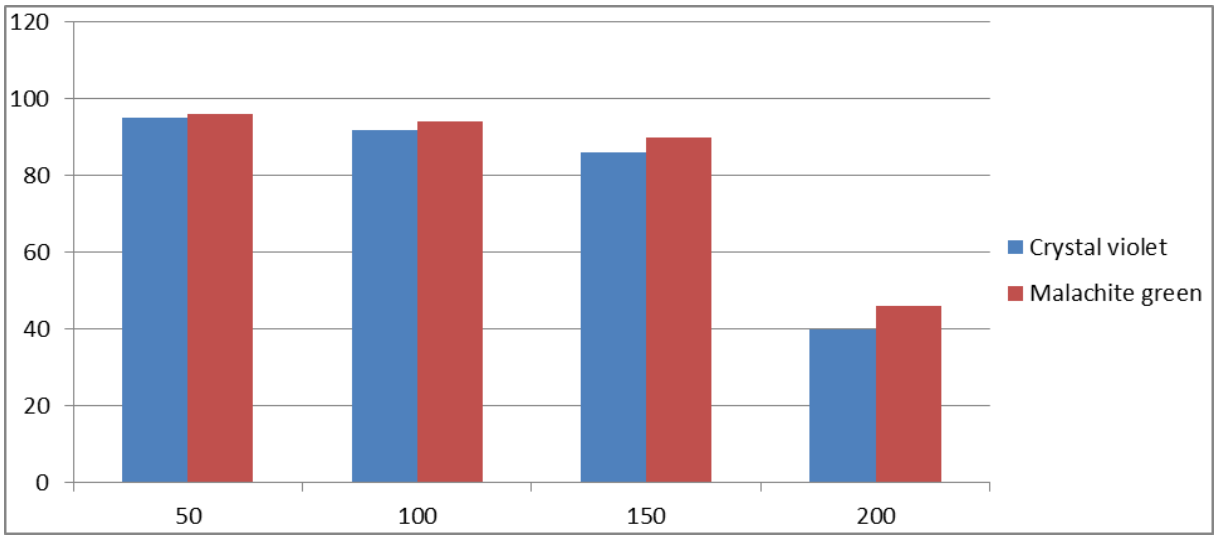

Fig 5: Effect of different concentration of dye on degradation %

Table 1: Bioassay of Dye and degraded dye toxicity

Seed type	Samples	Percentage of germination					Average shoot length (cm)	Average root length (cm)
		Day2	Day3	Day4	Day5	Day6		
Wheat seed	Control (Water)	26	62	85	100	100	15.8±0.351	3.2±0.351
	Degraded dye	21	55	57	72	78	14.8±0.833	2.4±0.436
	Dye (Malachite green)	17	30	35	42	65	13.6±0.100	2.6±0.361
	Control (water)	19	78	82	86	90	23.6±0.577	2.4±0.265
	Degrade dye	0	66	88	93	95	18.6±0.776	3.5±0.500
	Crystal violet	16	46	54	68	69	10.7±0.464	2.8±0.500

Effect of pH: In present study, degradation of crystal violet and Malachite green was found dependent on temperature and pH. This white-rot fungus was able to grow on dye effluent with high degradation efficiency from pH 5.0–7.0 (Kadpan *et al*., 2000) [8]. Fungus showed maximum degradation in slightly alkaline acidic condition. In highly acidic condition decrease in decolourization due to decrease in enzyme activity.

Effect of Dye concentration: (50-100 mg/l) showed little changes in decolourization rate but high concentration showed inhibition on fungus thus reducing effect on dye decolourization. Dye degradation in higher concentration is difficult due to complex structure.

Effect of Temperature: Maximum dye degradation shown on 30 °C in broth culture

Effect of external aeration

Higher colour removal is observed in shaking cultures because of better oxygen transfer and nutrient distribution as compared to the stationary cultures.

Toxicity testing

According to the study's findings, it was shown that seeds exposed to low concentrations of dye were less hazardous to seed germination and seedling growth. However, additional evidence from the literature review suggested that when the dye concentration was greatly raised, germination and

shooting percent could suffer. This concurs with earlier research by Durve *et al* (2012) [4]. They found that when the dye concentration was increased from 500 to 10,000 ppm for Vigna radiata (whole moong), Triticum spp (Wheat), and Brassica juncea (Mustard) seeds, neither germination nor shooting and rooting % significantly increased**.**

Conclusion

White-rot fungi *P. chrysosporium* has demonstrated dye degradation in both soild and liquid media; hence, commercial development, application, and remediation will soon emerge in a competitive and dependable dye remediation technique. A bioreactor for a continuous process of decolorizing textile dye effluents can be designed using the results of the aforementioned investigation**.**

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Reference

1. Anjaneyulu Y, Sreedhara Chary N, Samuel Suman Raj D. Decolourization of industrial effluents – available methods and emerging technologies – a review. Reviews in Environmental Science and Bio Technology. 2005;4:245-273.
2. Asamudo NU, Daba AS, Ezeronye OU. Bioremediation of textile effluent using *Phanerochaete chrysosporium*. African Journal of Biotechnology. 2005;4:1548-1553.
3. Assadi MM, Rostami K, Shahvali M, Azin M. Decolorization of textile wastewater by *Phanerochaete chrysospovium*. Desalination. 2001;141:331-336.
4. Durve AA, Arvindand R, Sayali Naphade R. Decolourisation of Textile Dyes and Biological stains by Bacterial strains isolated from Industrial effluents Adv. Appl. Sci. Res. 2012;3(5):2660-2671.
5. Faraco V, Pezzella C, Giardina P, Piscitelli A, Vanhullec S, Sannia G. Decolourization of textile dyes by the white-rot fungi *Phanerochaete chrysosporium* and Pleurotus ostreatus. Journal of Chemical Technology and Biotechnology. 2009a;84:414-419.
6. Faraco V, Pezzella C, Miele A, Giardina P, Sannia G. Bio-remediation of colored industrial wastewaters by the white-rot fungi *Phanerochaete chrysosporium* and Pleurotus ostreatus and their enzymes. Biodegradation 2009b;20:209-220.
7. Gomaa OM, Linz JE, Reddy CA. Decolorization of Victoria blue by the white rot fungus, *Phanerochaete chrysosporium*. World Journal of Microbiology and Biotechnology. 2008;24:2349-2356.
8. Kadpan IK, Kargi F, McMullan G, Marchant R. Effect of environmental conditions on biological decolorization of textile dyestuff by C. versicolor. Enzyme Microbiol Technology. 2000;26:381-387.
9. Knapp JS, Newby PS, Reece LP. Decolourization of dyes by wood-rotting basidiomycete fungi. Enzyme Microbiol Technology. 1995;17:664-668.
10. Kaushik P, Malik A. Fungal dye decolourization: Recent advances and future potential. Environment International 2009;35:127-141.
11. Swamy J, Ramsay JA. The evaluation of white rot fungi in the decoloration of textile dyes Enzyme and Microbial Technology. 1999;24:130-137.
12. Wesenberg D, Kyriakides I, Agathos SN. White-rot fungi and their enzymes for the treatment of industrial dye effluents Biotechnology Advances. 2003;22:161-187.