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DECOLORIZATION OF MALACHITE GREEN BY *SPOROTRICHUM PULVERULENTUM* VARSHA

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ABSTRACT

Synthetic dyes used in the textile industry cause major environmental pollution. Present investigation aimed at studying decolorization of malachite green by three white rot fungi *Sporotrichum pulverulentum, Polystictus versicolor* and *Ptychogaster* sp. using two different media. *S. pulverulentum* showing highest decolourization in Asther broth was chosen for further work. Dye decolourization was enhanced under shaker conditions as opposed to static conditions. A pH of 4.5, use of glucose as carbon source and nitrogen limited conditions supported higher decolorization values (99%). The reuse of mycelial mat for dye decolorization showed excellent results over four cycles.

INTRODUCTION

The current worldwide production of more than 100,000 commercial textile dyes exceeds 600,000 tonnes annually. Globally, it has been estimated that 2% of the dyes produced annually are discharged in effluents from manufacturing operations whilst 10 % are discharged from textile and associated industries. (Swami, 1998; Allen *et al.* 2002). The textile dyeing process requires a large volume of wastewater of fairly high purity and it discharges equally large volume of wastewater after the dyeing process. The wastewater contains dyes at concentrations ranging from 10-200 mg / L along with other organic and inorganic accessory chemicals involved in the dyeing process (Murugesan *et al.* 2003).

A major class of synthetic dyes includes the azo, anthroquinone and triphenylmethane dyes. Triphenylmethane dyes such as malachite green, crystal violet are aromatic xenobiotic compounds, and are used extensively in textile industries. Malachite green is used extensively for dyeing silk, wool, jute, leather, ceramics as well as cotton. It is also used to treat fungal and protozoal infections. The Food and Drug Administration nominated Malachite green as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993. Malachite green and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time. Therefore, there are both environmental and human health concerns about bioaccumulation of Malachite green and leucomalachite green in terrestrial and aquatic ecosystems (Parshetty *et al.* 2006).

Due to their complex, aromatic structures and synthetic origins, most dyes are recalcitrant to microbial degradation. This results in an intensely colored discharge from water treatment facilities (Davis *et al*., 1994). The release of colored effluent into the envi-

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ronment is of growing concern as color is a visible pollutant that is increasingly being regulated in the USA, Canada and Europe. Since most dyes impart strong color at concentrations even below 1 ppm, post-treatment persistence of color is an aesthetic problem in receiving waters. Neglecting the aesthetic problem, the greatest environmental concern with dyes is their absorption and reflection of sunlight entering the water which interferes with the growth of bacteria and hinder photosynthesis in aquatic plants (Allen *et al.* 2005).

Current decolorization technologies involve expensive chemical and physical treatments which are expensive and may generate a large volume of sludge, while biological wastewater treatments have low removal efficiencies (Dubrow *et al.* 1996). Furthermore, no single conventional method has been found to be effective for all dye classes. In India, dye houses are scattered and are operated by small unit operators aggravating the problem of effluent treatment. Thus, there is a need for the development of new technologies for color removal. Biological treatment using white rot fungi is an attractive option as it could be cost-effective and environmentally friendly (Murugesan, *et al.* 2003).

Several factors make the white rot fungi particularly suitable for bioremediation applications. The non-specificity of the ligninolytic enzymes allows for the degradation of a range of pollutants, without extensive acclimation. Since the enzymes are extracellular, large molecules such as dyes can be degraded without transport across the cell wall, which often inhibits bacterial degradation. (Reddy, 1995). The global objective of this work was to develop a biodecolorization process for the removal of malachite green using a species of white rot fungus.

MATERIALS AND METHODS

Organisms - *Polystictus versicolor* NCIM 1074, *Ptychogaster* species NCIM 1074 and *Sporotrichum pulverulentum* NCIM 1106 were obtained from NCL, Pune, India. The cultures were maintained on malt agar slants at 4^0C and sub cultured every fortnight.

Chemicals - All media chemicals were of analytical grade and were purchased from Hi Media, Mumbai.

Dye - Malachite green was obtained from Hi Media and was used at predetermined minimum inhibitory concentration. Decolorization was monitored at the absorption maxima (A_{620}) on Spectronic 20.

Selection of the most efficient isolate - 1 mL suspension of 10^8 spores / ml prepared from 7 days old cultures grown on malt agar slants were added in Asthana and Hawker broth (Glucose 5g, $MgSO_4.7H_2O$ 1.75g, KNO_3 3.5g, $KH_2PO_4.7H_2O$ 1.75g in1L distilled water, pH 4.5) and Asther broth (Glucose 10g, Aspargine 1g, $(NH_4)_2 NO_3$ 0.5g, Yeast Extract 1.0g in 1L distilled water, pH 4.2) in two different sets of 100ml medium in 500mL Erlenmeyer flasks. The inoculum was incubated for 6 days at 30°C at 200 rpm on a rotary shaker. Malachite green was added to the culture as aliquots of concentrated stock solutions separately at predetermined minimum inhibitory concentration (MIC). The flasks were again incubated under similar conditions as used for growth. Decolorization in liquid medium was measured spectrophotometrically at the maximum visible wavelength of absorbance for the dye. Complete decolorization was taken to be the total decolorization of the medium, with no visible sorption to the biomass. The medium and the organism showing highest decolorization after 48 hours were chosen for further study (Chao *et al*. 1994).

Effect of static and shaking conditions on dye decolorization - Two sets of 100mL of the selected medium were inoculated with the most efficient isolate. One set of inoculated shake flask was incubated statically, while the other was incubated with rotary agitation at 200 rpm for 6days at 30°C. Dyes were added after day 6 and decolorization was studied as described earlier.

Effect of pH on dye decolorization - 100 mL of the chosen medium varying in pH from 3.0 to 7.0 at an interval of 0.5 units was inoculated with the culture and dye decolorization was studied as described earlier.

Effect of carbon source on dye decolorization - Dye decolorization was studied in 100 mL medium by replacing glucose from the medium by equal concentration of sucrose, lactose, fructose, starch and carboxy methyl cellulose.

Effect of nitrogen source on dye decoloration - The original nitrogen sources in the selected medium were replaced by 0.5g / L (nitrogen limited) and 5g / L (nitrogen rich) of various nitrogen sources like NH_4Cl, $NaNO_3$, $(NH_4)_2SO_4$, urea and yeast extract. The dye decolorization was studied as described above under optimized conditions.

Efficiency of reusing the mycelial mat for dye de-

colorization - The dye decolorization was carried out under all the optimized conditions. The mycelial mat that had decolorized malachite green in 100 mL Asther broth for 48 hours was removed, rinsed with sterile saline and added into fresh broth containing malachite green at a concentration of 5 mcg / mL. This step was repeated thrice. The efficiency of dye decolorization was monitored after every step at the end of 48 hours.

RESULTS AND DISCUSSION

The decolorization of textile waste water depends on the fungal strain used. To achieve the biodegradation of environmentally hazardous compounds, white rot fungi appear as a valuable alternative because they are capable of oxidizing compounds of complex structure such as lignin. The capability of oxidation is based on the ability of white rot fungi to produce oxidative enzymes such as laccase, manganese peroxidase (MnP), and lignin peroxidase (LIP). These oxidases and peroxidases have been reported as excellent oxidant agents to degrade dyes (Lopez *et al*. 20004). Parshetty *et al.* (2006) showed efficiency of white rot fungi in decolorization of malachite green.

In the present study, all the three white rot fungi showed dye decolorization.to varying extent. *Sporotrichum pulverulentum* 1106, however, was found to be the most efficient isolate showing 82.00±0.9% decolorization of malachite green at 5mcg / mL in Asther broth (Table 1). Hence, it was chosen for further work using Asther broth.

When one set of inoculated shake flasks was incubated statically, the cultures formed filamentous "mats" at the surface of the growth medium by day 4. However, when a second set of flasks was incubated with rotary agitation at 200 rpm after inoculation, uniform mycelial pellets, 2 to 3 mm in diameter were formed. Malachite green was added to both sets of flasks on day 6 .With statically grown culture, decrease in dye absorbance was accompanied by visible sorption of the dyes to the fungal mat. This has been previously reported in a study of dye decolorization by static cultures of different white rot fungi, including *T. versicolor,* (Heinfling *et al*. 1997) and in several studies with *P. chrysosporium* (Bumpus and Brock, 1988; Cripps *et al*. 1990; Spadaro *et al*. 1992). In contrast, no mycelial sorption of dye was detected in agitated cultures. Xinjiao (2003) reported superior and increased performance of the agitated cultures due to physiological state of the fungi and increased mass and oxygen transfer between the cells and medium due to mixing. In the present work, agitated culture displayed greater decolorization ability (82.00±0.9%) than statically grown culture (20.30±0.60%).

pH is a critical factor for dye decolorization. Murugesan (2003) suggested that pH range for optimum growth of fungi was 4.3 to 4.8 and decolorization was greatly retarded below pH 4.0 or above pH 5.0.The results obtained in the present study are in accordance with these results showing maximum dye decolorization at pH 4.5 as shown in Fig. 1.

It is conventionally accepted that carbon (C) and / or nitrogen (N) limitation triggers ligninolytic activity in white rot fungi. White rot fungi are capable of degrading the pollutants by the process of co-metabolism and glucose is the best carbon source for fungal growth. Murugesan *et al.* (2003) reported that glucose concentration had a strong effect on the decolorization of the dye stuff. A study reported the effects of various carbon sources like glucose, fructose, starch, sucrose and CMC on decolorization ability of fungi and it was found that sucrose supported the best decolorization (Demir, 2004). In the present work both glucose and sucrose gave comparable results showing 93.30±0.90 % and 91.50±0.85% decolorization respectively. Starch proved to be least effective followed by CMC as shown in Table 2. Further work was carried out using glucose as the source of carbon.

The present study also examined the effects of nitrogen concentrations on dye decolorization by *S. pulverulentum*. Murugesan and Kalichellvan (2003) reported that degradation of Congo red by *Phanerochaete chrysosporium* was inhibited by a high concentration of nitrogen and mineralization studies with several dyes have revealed that most of the dyes investigated were degraded only in a certain range of nitrogen concentration for *Phanerochaete chrysosporium* and *Trametes versicolor*. It was also shown that low nitrogen conditions proved optimum for enzyme production and dye decolorization by *L. edodes* on a solid medium, regardless of the nitrogen source used. The present work also showed that dye decolorization was much higher under nitrogen poor conditions regardless of the nitrogen sources used, though urea proved to be inefficient compared to other nitrogen sources as seen in Table 3.

Reuse of the mycelial mat for dye decolorization can be cost effective, time saving and advantageous for effluent treatment. Xinjiao *et al.* (2003) showed

Table 1
% Decolorization of malachite green by the white rot fungi

Name of the culture	% Decolorization	
	Asthana & Hawker broth	Asther broth
P. versicolor	76.00±1.20	82.00±0.9
Ptychogaster sp.	82.60±1.45	87.20±1.30
S.pulverulentum	87.00±0.85	93.40±0.95

Table 2
Effect of carbon source on dye decolorization

Carbon source	% Decolorization
Glucose	93.30±0.90
Sucrose	91.50±0.85
Lactose	80.45±1.00
Fructose	88.10±1.05
Starch	7.20±0.85
Carboxy methyl cellulose	58.25±1.10

Table 3
Effect of nitrogen source on decolorization

Nitrogen source	% Decolorization	
	Under nitrogen rich conditions	Under nitrogen poor conditions
$(NH_4)_2NO_3$	55.30±1.05	99.00±0.75
NH_4Cl	54.40±1.40	97.00±0.90
$NaNO_3$	52.60±1.05	95.50±0.85
Urea	24.60±1.35	58.20±0.85
Yeast extract	51.30±1.25	94.80±1.55

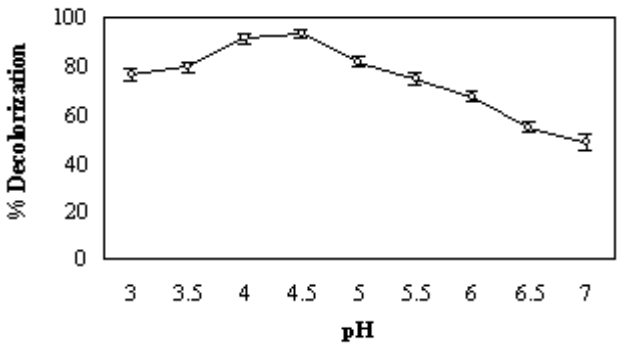

Fig. 1 Effect of pH on dye decolorization

91.1%, 87.8% and 86.4% efficiencies of dye decolorization for the first, second and third cycle respectively. In the present work higher decolorization efficiencies of 99.00±0.75%, 95 ±1.20%, 87±0.90 % and 71±1.45% for first to fourth cycles were observed respectively. The results of the present investigation show extensive potentiality of white rot fungi in treatment of dye containing effluents.

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