

Research Paper

Bioremediation of dyes by fungi isolated from contaminated dye effluent sites for bio-usability

Babita Rani¹, Vivek Kumar², Jagvijay Singh³, Sandeep Bisht⁴, Priyanku Teotia⁵, Shivesh Sharma⁶, Ritu Kela¹

¹Department of Biochemistry, Division of Biosciences, College of Applied Education & Health Sciences, Meerut, India.

²Amity Institute of Microbial Technology, AMITY University, Noida, India.

³Department of Biotechnology, Division of Biosciences, College of Applied Education & Health Sciences, Meerut, India.

⁴Department of Microbiology, Uttarakhand University of Horticulture & Forestry, Bharsar, India.

⁵Department of Botany, Meerut College, Meerut, India.

⁶Department of Biotechnology, MLN National Institute of Technology, Allahabad, India.

Submitted: August 20, 2013; Approved: March 14, 2014.

Abstract

Biodegradation and detoxification of dyes, Malachite green, Nigrosin and Basic fuchsin have been carried out using two fungal isolates *Aspergillus niger*, and *Phanerochaete chrysosporium*, isolated from dye effluent soil. Three methods were selected for biodegradation, viz. agar overlay and liquid media methods; stationary and shaking conditions at 25 °C. *Aspergillus niger* recorded maximum decolorization of the dye Basic fuchsin (81.85%) followed by Nigrosin (77.47%), Malachite green (72.77%) and dye mixture (33.08%) under shaking condition. Whereas, *P. chrysosporium* recorded decolorization to the maximum with the Nigrosin (90.15%) followed by Basic fuchsin (89.8%), Malachite green (83.25%) and mixture (78.4%). The selected fungal strains performed better under shaking conditions compared to stationary method; moreover the inoculation of fungus also brought the pH of the dye solutions to neutral from acidic. Seed germination bioassay study exhibited that when inoculated dye solutions were used, seed showed germination while uninoculated dyes inhibited germination even after four days of observation. Similarly, microbial growth was also inhibited by uninoculated dyes. The excellent performance of *A. niger* and *P. chrysosporium* in the biodegradation of textile dyes of different chemical structures suggests and reinforces the potential of these fungi for environmental decontamination.

Key words: bioremediation, dyes, fungi, microbial bioassay, seed germination.

Introduction

Due to rapid industrialization and urbanization, a lot of chemicals including dyes are manufactured and are being used in day-to-day life. About 100,000 commercial dyes are manufactured including several varieties of dyes such as acidic, basic, reactive, azo, diazo, anthraquinone based meta complex dyes with an annual production of over 7×10^5 metric tons are commercially available (Campos *et al.*, 2001). Approximately 50% of the dyes are released in the industrial effluents (Zollinger, 1991). They are

used on several substrates in food, cosmetics, paper, plastic and textile industries. Some of them are dangerous to living organisms due to their potential toxicity and carcinogenicity. Dyes in wastewater often lead to calamities viz. the incidence of bladder tumors has been reported to be particularly higher in dye industry workers than in the general population (Suryavathi *et al.*, 2005). Natural pigments used for coloring textiles have been replaced by “fast colors” which do not fade on exposure to light, heat and water. These features unfortunately go with the perils of harmful

effluent quality. About 15% of the dyes used for textile dyeing are released into processing waters (Eichlerova *et al.*, 2006). Besides being unaesthetic, these effluents are mutagenic, carcinogenic and toxic (Chung *et al.*, 1992).

Commonly applied treatment methods for color removal from colored effluents consist of integrated processes involving various combinations of biological, physical and chemical decolorization methods (Galindo and Kalt, 1999; Robinson *et al.*, 2001; Azbar *et al.*, 2004), of these, approximately 10-15% of unused dyes enter the wastewater after dyeing and after the subsequent washing processes (Rajamohan and Karthikeyan, 2006). Chemical and physical methods for treatment of dye wastewater are not widely applied to textile industries because of exorbitant costs and disposal problems. Green technologies to deal with this problem include adsorption of dyestuffs on bacterial and fungal biomass (Fu and Viraraghavan, 2002; Yang *et al.*, 2009) or low-cost non-conventional adsorbents (Crini 2006; Ferrero, 2007).

A variety of physicochemical treatments have been devised previously for the dyes and textile wastewater. However, these suffered from some serious drawbacks in terms of their limited applications or their high cost. Besides, chemical treatments created an additional chemical load in water bodies that eventually resulted in sludge disposal problems. Several factors determine the technical and economic feasibility of each single dye removal technique. These include; dye type and its concentration, wastewater composition, operation costs (energy and material), environmental fate and handling costs of generated waste products. A very small amount of dye in water (10-50 mg/L) is highly visible and reduces light penetration in water systems, thus causing a negative effect on photosynthesis (Khaled *et al.*, 2010; Dhanjal *et al.*, 2013).

Recently, dye removal became a research area of increasing interest, as government legislation concerning the release of contaminated effluent becomes more stringent. Various treatment methods for removal of dyes from industrial effluents like chemical coagulation using alum, lime, ferric chloride, ferric sulphate and electro coagulation are very time consuming and costly with low efficiency. Among the numerous water treatment technologies, research interest in the fungal bioremediation due to their biomass compared to the bacteria, has increased significantly for decolorization and degradation of synthetic dyes (Shahid *et al.*, 2013).

Many authors have already worked with many microorganisms, the imperative bacteria being *Staphylococcus arlettae* (Elisangela *et al.*, 2009); Lactic acid bacteria (Khaled *et al.*, 2010); *Pseudomonas putida* (Leebana *et al.*, 2012); *Micrococcus luteus*, *Listeria denitrificans* and *Nocardia atlantica* (Hassan *et al.*, 2013) *Bacillus megaterium* (Joshi *et al.*, 2013), and fungi viz. Basidiomycetous fungi (Machado *et al.*, 2006); *Trametes pubescens* and *Pleurotus ostreatus* (Casieri *et al.*, 2008);

Aspergillus tamarii and *Penicillium purpurogenum* (Ramalingam *et al.*, 2010); *Aspergillus ochraceus* (Tisma *et al.*, 2012); and *Pleurotus ostreatus* (Siddique *et al.*, 2012); *Aspergillus niger*, *Fusarium oxysporum* and *Trichoderma lignorum* (Shahid *et al.*, 2013).

Keeping the above points in view, the main objectives of the problem were to screen and employ selected potential textile dye effluent soil fungal sp. capable to decolorize and detoxify the textile dyes using solid and liquid media under shaking and stationary conditions.

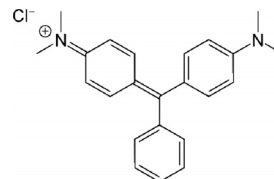
Methods and Materials

Chemicals and 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.

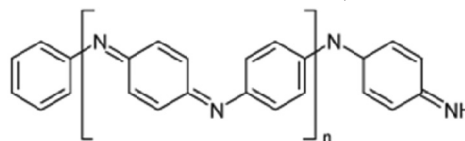
Dyes used

Malachite green (4-[(4-dimethylaminophenyl) phenyl-methyl]-N, N-dimethylaniline) Molecular formula: $C_{23}H_{25}ClN_2$,



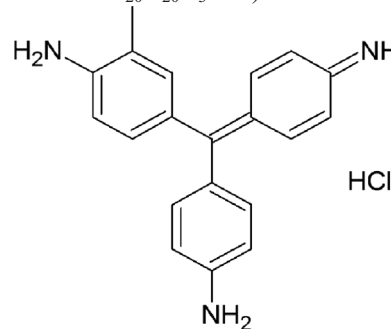
Malachite green

Nigrosin disodium; 4-amino-3-[(4-nitrophenyl) diazenyl] - 5-oxo-6- (phenylhydrazinylidene) naphthalene-2, 7-disulfonate, Molecular formula: $(C_{22}H_{14}N_6Na_2O_9S_2)$,



Nigrosin

Basic fuchsin (4-[(4-Aminophenyl)-(4-imino-1-cyclohexa-2,5-dienylidene) methyl] aniline hydrochloride): Molecular formula: $C_{20}H_{20}N_3HCl$ and mixture of all dyes.



Basic Fuchsin

Source of fungal strains

The dye effluent soil-isolated (from Meerut region, Uttar Pradesh, India) fungal strains *A. niger* and *P. chrysosporium* were maintained on Potato Dextrose Agar (Himedia, Mumbai, India) and sub cultured periodically to maintain their viability. Identification of these soil fungal strains was done previously based on their morphological characters (Yao *et al.*, 2009). These fungi have selected in present study because *A. niger* and *P. chrysosporium* has been widely studied for dye decolorization.

Screening of soil-derived fungi for dye decolorization activities

Sixty one dye effluent soil 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. Following incubation, mycelial agar plugs (~5 mm²) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) containing 5 mL of PDA overlaid with 1 mL of PDA with 0.01% (w/v) respective textile dye. All culture tubes were incubated at room temperature (~25 °C) and observed weekly for up to four weeks. Clearing of the overlaid dye indicates full decolorization (+++). Partial dye decolorization (++) was indicated by less dye intensity in comparison with the control (uninoculated PDA overlaid with PDA + 0.01% dye). All the three fungal strains were selected on the basis of full or maximum (+++) decolorization.

Decolorization of dyes in solid medium (Tube overlay method)

The two selected fungal strains were further tested for their ability to decolorize on PDA and Sabouraud Dextrose Agar (SDA) medium, Himedia, Mumbai, India. This was done to select which medium support better growth and dye decolorization activities of selected fungal isolates. Initially, all the three fungal strains were grown as previously described. Following incubation, fungal mycelial agar plugs (~5 mm²) were cut approximately 5 mm from the colony margin and inoculated on test tubes (in triplicates) each pre-filled with 2 mL of the Potato Dextrose Agar (PDA) and SDA medium, supplemented separately with either with following dye 0.01% (w/v) Malachite green, Nigrosin and Basic fuchsin, respectively (Lopez *et al.*, 2006). The culture tubes were then incubated at room temperature (~ 25 °C). The growth of the fungi and its ability to decolorize the dye were observed weekly up to four weeks. The depth of dye decolorization (in mm) indicated by clearing of the dye was then measured. Based upon growth of fungal strains and dye decolorization, for further studies PDA medium was chosen.

Assay for the dye decolorization activities of fungi in liquid media

The spores and mycelia of *A. niger* and *P. chrysosporium* were then dislodged from Petri plates using a flame-sterilized inoculating loop and mixed properly with one mL of sterile distilled water. From this mixture, 10 µL of the fungal spore and mycelium inoculum were added on culture vials (in triplicates) pre-filled with 25 mL Potato Dextrose Broth (PDB) supplemented with 0.01% of either one of the following dyes: Malachite green, Nigrosin and Basic fuchsin. Three sets were prepared and were incubated either under constant agitation/shaking (100 rpm, Yoriko Scientific Orbital Shaker) or under stationary/without shaking condition (Park *et al.*, 2007). All culture vials were incubated at room temperature (~25 °C) for 10 days and all assays were performed in triplicate. Growth and dye decolorization were noted every day. Following culture for 10 days, the culture filtrates were decanted and subjected to spectrophotometric analysis. Absorbance maxima of the tested dyes were read as malachite green-620 nm, Nigrosin-600 nm and Basic fuchsin-550 nm wavelength. The extent of dye decolorization by the soil fungal strains on liquid media was calculated using the formula below:

$$\text{Pdd (\%)} = \frac{\text{Absorbance}_c - \text{Absorbance}_i}{\text{Absorbance}_c} \times 100 \quad (1)$$

where Pdd = Percent dye decolorization, Absorbance_c = Absorbance control and Absorbance_i = Absorbance inoculated.

Finally, the mycelial biomass were harvested on clean Petri plates and observed directly. Fungal hyphae were also mounted on clean glass slides and observed under a compound light microscope, make Olympus (1500x) for the biosorption of dyes.

Enzyme assay

Laccase activity was measured by using syringaldazine as a substrate as per the method of (Valmaseda *et al.*, 1991). The activity was assayed using using 1.0 mL of 0.2 M sodium phosphate buffer (pH 5.7) and 0.2 mL syringaldazine (1.6 mg/mL) in absolute ethanol, (4.47 Mm). Reactions were initiated by the addition of syringaldazine and after mixing; incubations were conducted at 30 °C for 1 h, because after 1 h highest enzyme laccase activity was observed. The absorbance was measured in a spectrophotometer (ELICO SL 150) before (0 time) and after incubation (60 min) at 526 nm and the increase in absorbance was calculated. One unit activity was defined as the enzyme producing one absorption unit/min at 526 nm.

Seed germination bioassay

Effect of bioremediated and untreated dye solution was observed on wheat seed germination. The wheat seeds were sterilized using 0.1% HgCl₂ 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 bioremediated, 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.

Bacterial toxicity

Effect of bioremediated and untreated dye solution was observed on bacterial growth by measuring zone of inhibition. Log phase cells of *E. coli*, 0.1 mL of 10^{-8} were evenly spreaded on Petri plates and sterile filter paper discs impregnated with bioremediated, untreated dye solution and sterile distilled water were kept on the seeded bacterial cells at equidistance and pressed lightly and kept at 30 °C for 48 h, observation for zone of inhibition was observed, if any (Kumar, 2011).

Results

Out of 61 fungal isolates, two fungal isolates were selected after comprehensive screening of the textile dyes biodegradation for further studies.

In tube overlay method, highest decolorization was observed in Malachite green and Nigrocin (92.85 and 93.33% respectively), followed by Basic fuchsin (90.05%) by *A. niger*. The mixture of dye decolorization was 10.4%. In case of *P. chrysosporium* highest decolorization was observed in Malachite green 71.42%, followed by Basic fuchsin (70%) and least was Nigrocin (8.33%), while the mixture decolorization was 9.6% (Figure 1).

Dye decolorization in liquid medium exhibited different tendency as that of tube overlay method. In this method highest decolorization by *A. niger* was observed in Basic fuchsin (81.85%), followed by Nigrocin (77.47%), Malachite green (72.77%) and least by dye mixture (33.08%) under shaking conditions. While, under static conditions,

highest decolorization was observed in Basic fuchsin (79.77%), followed by Nigrocin (58.78%) and Malachite green (50.37%), least by dye mixture (24.38%) (Figure 2).

Similarly, dye decolorization in liquid medium by *P. chrysosporium* exhibited different tendency as that of tube overlay method. In this method highest decolorization was observed in Nigrosin (90.15%), followed by Basic fuchsin (89.8%), Malachite green (83.25%) and least by dye mixture (68.4%) under shaking conditions (Figure 3).

Under stationary conditions, highest decolorization was observed in Nigrosin (65.04%), followed by Malachite green (53.75%) and Basic fuchsin (52.6%) least by dye mixture (40.6%) (Figure 3).

Change in pH was also observed by *A. niger* and *P. chrysosporium* under shaking and stationary conditions. After inoculation, the pH of all the dye samples was increased to neutral or near neutral (Figures 4, 5).

Microbial bioassay study showed that untreated dye (control) inhibited the growth of *E. coli* on Petriplates by forming a zone of inhibition, while the treated dye did not show any zone of inhibition (Figure 6). Moreover, seed germination examination also showed the effect of untreated and treated dyes on germination of seeds. It was found that germination percentage was higher upto 90% by treated dye, while the untreated (control) dye inhibited the germination of wheat seeds (Figure 7). Data of zone of clearance and seed germination is not mentioned due to brevity.

Discussion

The use of these fungi, thus could offer a much cheaper and efficient alternative treatment of wastewaters contaminated heavily with textile dyes. However, even though qualitative assays using the tube overlay method are powerful tools in screening fungi for extracellular enzyme production, they are not conclusive in that a negative reaction is not an absolute confirmation of a species' inability to produce a particular enzyme (Abdel-Raheem and Shearer, 2002). Hence, the tube agar overlay method (Lo-

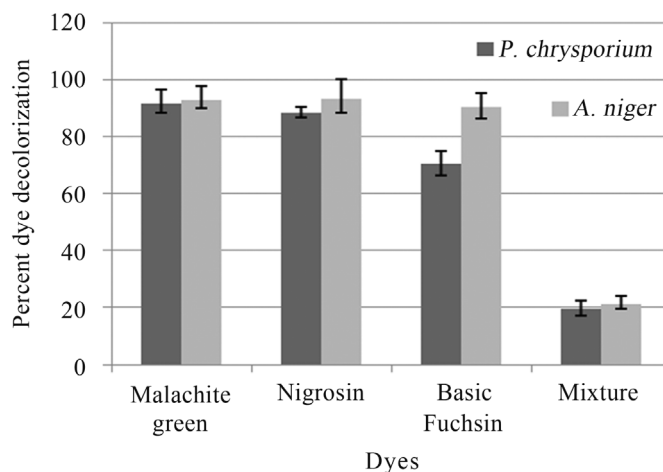


Figure 1 - Dye decolorization by soil fungi in tube overlay method.

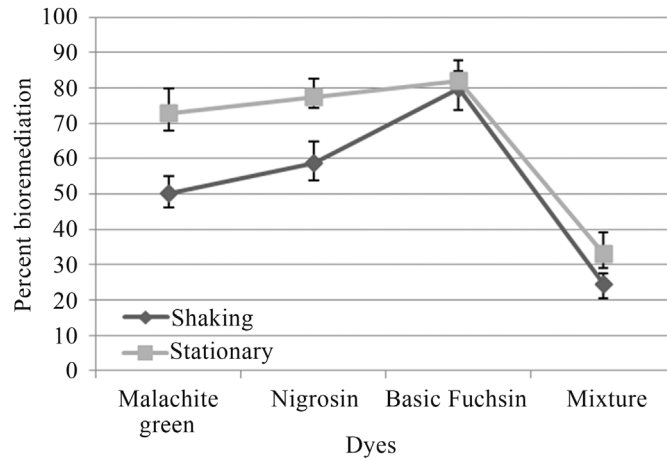


Figure 2 - Percent bioremediation of textile dyes by *A. niger*.

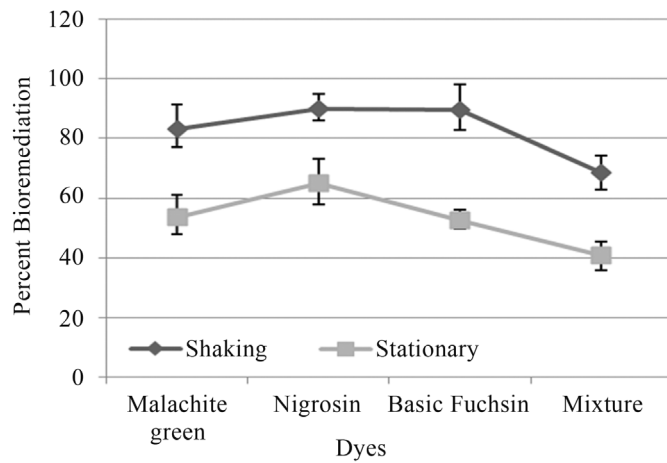


Figure 3 - Percent bioremediation of textile dyes by *P. chrysosporium*.

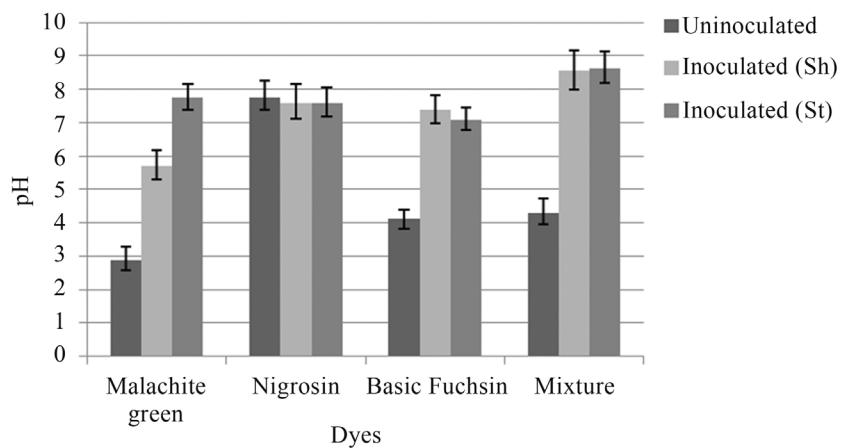


Figure 4 - Change in pH after inoculation with *A. niger* under shaking (Sh) and stationary (St) conditions.

pez *et al.*, 2006) only provides an easier and quicker method to screen a large number of fungal isolates for further studying dye decolorization activity.

The removal of the dye color is vital in the potential ap-plication of effluent soil fungal organisms as bioremediation agents in wastewater treatment plants and in runoff waters. Thus, it is essential to test dye contaminated soil

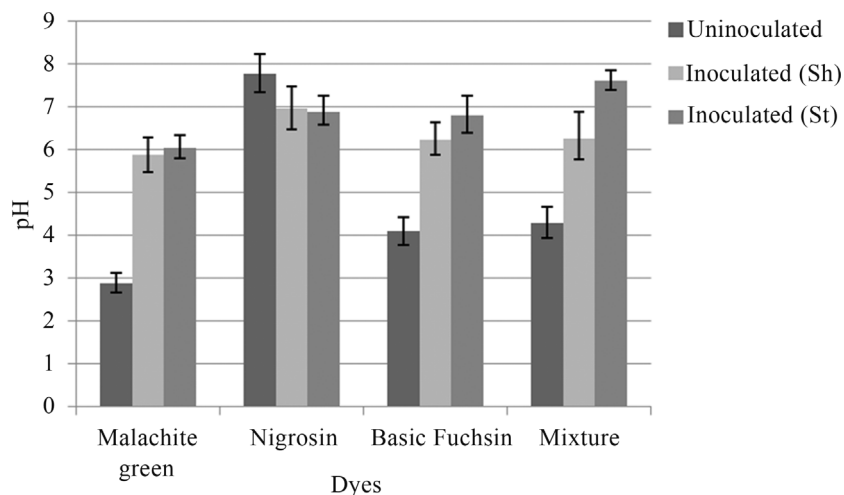


Figure 5 - Change in pH after inoculation with *P. chrysosporium* under shaking (Sh) and stationary (St) conditions.

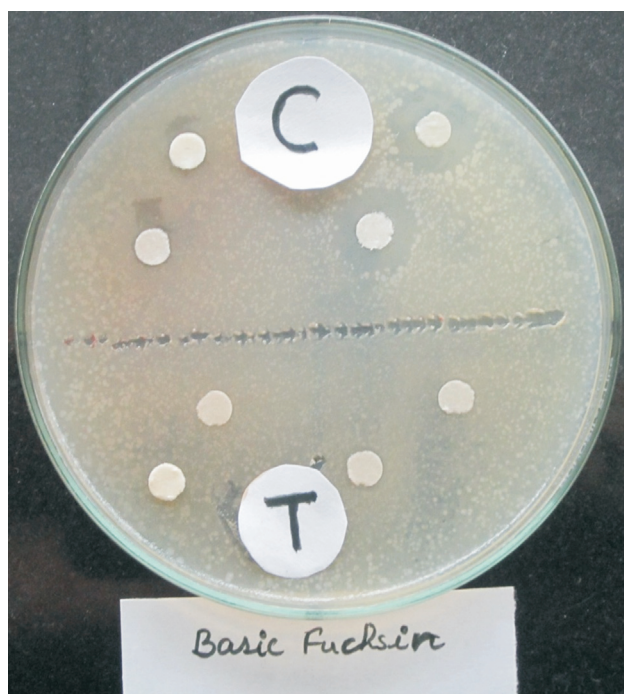


Figure 6 - Antimicrobial assay of Basic fuchsin (C = control dye; T = treated dye).

fungal strains for dye decolorization in liquid medium. *Aspergillus niger* and *P. chrysosporium* bioremediated Nigrosin, Basic fuchsin and Malachite green within 6 days up to 90%. Kanmani *et al.* (2011) reported that Nigrosin was a substrate for the ligninolytic enzyme lignin peroxidase.

Microbes have developed enzyme systems for the decolorization of azo dyes, moreover, dye molecules display a high structural variety, and they are degraded by only few enzymes. These biocatalysts have one common mechanistic feature, they are all redox-active molecules and thus,

exhibit relatively wide substrate specificities (Mester and Tien 2000). In the case of enzymatic remediation of azo dyes, azo reductases and laccases seem to be the most promising enzymes. These enzymes are multicopper phenol oxidases that decolorize azo dyes through a highly nonspecific free radical mechanism forming phenolic compounds, thereby avoiding the formation of toxic aromatic amines (Wong and Yu, 1999). Fungal systems appear to be most appropriate in the treatment of azo dyes (Ezeronye and Okerentugba, 1999). The capacity of fungi to reduce azo dyes is related to the formation of exo enzymes such as peroxidases and phenol oxidases. Peroxidases are hemo-proteins that catalyze reactions in the presence of hydrogen peroxide (Duran *et al.*, 2002). Laccase oxidizes the phenolic group of the phenolic azo dye, with the participation of one electron generating a phenoxy radical which is sequentially followed by oxidation to a carbonium ion. A nucleophilic attack by water on the phenolic ring carbon bearing the azo linkage to produce 3-diazenyl-benzenesulfonic acid (III) and 1, 2-naphthoquinone then takes place (Camarero *et al.*, 2005).

Use of *A. niger* and *P. chrysosporium* as dye biodegrader or decolorizer has been studied in this report and the efficient decolorization may be attributed to either through the action of extracellular enzymes such as laccase and/or biosorption by the fungal biomass. Laccase production by the soil fungal species have been studied, this test was performed to know, whether laccase enzyme plays any role in biodegradation of dyes. Furthermore, the soil fungal strains also showed promising decolorization activities against tested dyes (Figures 3, 4). Ali *et al.* (2009) and Vasudev (2011) exhibited that Malachite green was readily degraded in liquid culture by *A. flavus*, *A. solani* and some white rot fungi within six days up to 96%, as also shown in this study. Results of the dye biodegradation by soil fungi in this study using spectrophotometric analysis were even comparable with the percent dye decolorization exhibit-ed

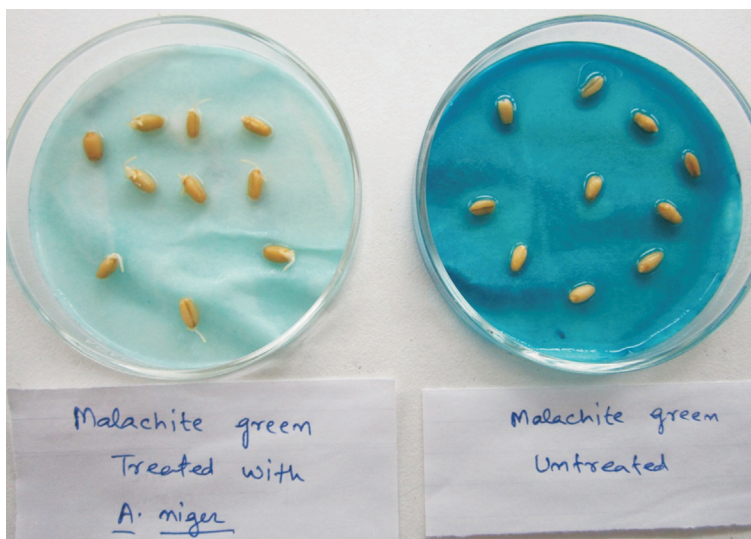


Figure 7 - Germination assay of wheat seeds by treated and untreated dye.

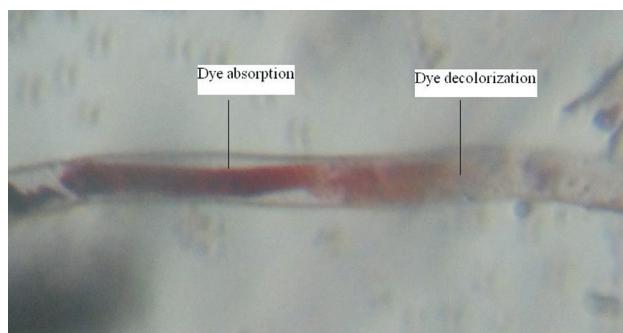


Figure 8 - Dye (Basic fuchsin) absorption and decolorization by *A. niger* hypha.

by the white-rot fungus *Trametes versicolor* and *Pleurotus ostreatus* (Yao *et al.*, 2009), and even *P. chrysosporium* (Bumpus and Brock, 1988).

Soil fungi possess ligninolytic enzymes and play an important role in the degradation of lignocellulose in soil ecosystems (Okino *et al.*, 2000). These lignin-degrading enzymes are directly involved not only in the degradation of lignin in their natural lignocellulosic substrates but also in the degradation of various xenobiotic compounds, including dyes. Moreover, ligninolytic enzymes have been reported to oxidize many recalcitrant substances such as chlorophenols, polycyclic aromatic hydrocarbons (PAHs), organophosphorus compounds, and phenols (Wesenberg *et al.*, 2003).

Additionally, it is not unusual for some species to demonstrate both enzyme-mediated degradation and biosorption in the decolorization of textile dyes (Park *et al.*, 2007; Shahid *et al.*, 2013). It is thus feasible that in addition to the production of extracellular enzymes, the ability of the dye effluent soil fungi to decolorize synthetic dyes is coupled also with their biosorption abilities (Kaushik and Ma-

lik, 2009). We have also observed dye absorption by the test fungal mycelium under light microscope (1500 x) (Figure 8). This may account for the more efficient textile dye biodegradation by the soil fungal strains (Kirby *et al.*, 2000) (Figures 1-4). It is therefore, possible that the ability of dye effluent soil-derived fungi to degrade malachite green, Nigrosin and Basic fuchsin as revealed in this study can be largely attributed to the lignin-degrading enzyme system of the organism. In addition to extracellular enzymes, it is also likely that dye decolorization activity of these fungi could also be attributed to the ability of their mycelia to adsorb/absorb the dye. Bioremediation rate of dyes was higher with individual dyes as compared to dye mixture, which could be due the reason that a mixture of dyes forms different complex structures which become resistant for biodegradation.

Biosorption of dyes occur essentially either through complexation, adsorption by physical forces, precipitation, entrapment in inner spaces of fungal mycelium, ion exchange due to surface ionization, and by formation of hydrogen bonds (Yeddou-Mezenner, 2010). Due to an increased cell-to-surface ratio, fungi have a greater physical contact with the environment. Thus, some fungi have demonstrated better dye adsorption potential exceeding that of activated charcoal (Fu and Viraraghavan, 2002).

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 po-

tentially competent fungal strains can be efficiently used for detoxification and bioremediation of harmful dyes.

Conclusions

The decolorization of dyes was studied under stationary and shaking conditions; encouraging results were obtained after 3 days, but maximum decolorization of all the dyes were obtained after 6 days. In this study we have observed higher decolorization under shaking conditions by *P. chrysosporium* and *A. niger*, which could be due to better oxygenation of the fungus and regular contact of secreted enzymes with dye molecules to decolorize it, moreover agitation also helps the fungus to grow better. Disappearance of dye color may be due to biodegradation of chromophore in dye molecule because of extracellular enzyme production by fungi along with absorption and adsorption. Due to the environmental friendly techniques it utilizes, bioremediation has been characterized as a soft technology. Its cost-effectiveness and the little disturbance in the environment render this technology a very attractive and alternative method of choice. The identification and research of new fungal strains with the aid of molecular techniques will further improve practical applications of fungi and it is anticipated that fungal remediation will be soon a reliable and competitive dye remediation technology.

Acknowledgments

The authors are grateful to the Management, College of Applied Education & Health Sciences, Meerut, India for providing research facilities to carry out this work.

References

- Abdel-Raheem A, Shearer CA (2002) Extracellular enzyme production by freshwater ascomycetes. *Fungal Diversity* 11:1-19.
- Ali H, Ahmed W, Haq T (2009) Decolorization and degradation of malachite green by *Aspergillus flavus* and *Alternaria solani*. *African J Biotechnol* 8(8):1574-1576.
- Azbar N, Yonar T, Kestioglu K (2004) Comparison of various advanced oxidation processes and chemical treatment methods for COD and color removal from a polyester and acetate fibre dyeing effluent. *Chemosphere* 55:35-43.
- Bumpus JA, Brock BJ (1988) Biodegradation of crystal violet by the white rot fungus *Phanerochaete chrysosporium*. *Applied Environ Microbiol* 54 (5):1143-1150.
- Camarero S, Ibarra D, Martínez MJ, Angel, TM (2005) Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Appl Environ Microbiol* 71(4):1775-1784.
- Camarero S, Ibarra D, Martínez MJ, Angel, TM (2005). Lignin-Derived Compounds as Efficient Laccase Mediators for Decolorization of Different Types of Recalcitrant Dyes. *Applied Environmental Microbiology* 71(4):1775-1784.
- Campos R, Kandelbauer A, Robra KH, Artur CP, Gubitz GM (2001) Indigo degradation with purified laccases from *Trametes hirsuta* and *Sclerotium rolfsii*. *J Biotechnol* 8:131-139.
- Casieri L, Varese GC, Anastasi A, Prigione V, Svobodova K, Marchisio VF, Novotny C (2008) Decolorization and detoxification of reactive industrial dyes by immobilized fungi *Trametes pubescens* and *Pleurotus ostreatus*. *Folia Microbiol* 53(1):44-52.
- Chung KT, Stevens SE, Cerniglia CE (1992) The reduction of azo dyes by the intestinal microflora. *Critical Rev Microbiol* 18:175-190.
- Crini G (2006) Non-conventional low-cost adsorbents for dye removal: a review. *Bioresour Technol* 97(9):1061-85.
- Dhanjal NIK, Mittu B, Chauhan A, Gupta S (2013) Biodegradation of textile dyes using fungal isolates. *J Env Sci Technol* 6(2):99-105.
- Duran N, Rosa MA, D'Annibale A, Gianfreda L (2002) Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. *Enzyme. Microbiol Technol* 31:907-931.
- Eichlerova I, Homolka L, Nerud F (2006) Synthetic decolorization capacity of white-rot fungus *Dichomitus sequalens*. *Bioresour Technol* 97:2153-2159.
- Elisangela F, Andrea Z, Fabio DG, Cristiano RM, Regina DL, Artur CP (2009) Biodegradation of textile azo dyes by a facultative *Staphylococcus arlettae* strain VN-11 using a sequential microaerophilic/aerobic process. *Int Biodeterior Biodegrad* 63:280-288.
- Ezeronye O U, Okerentugba PO (1999) Performance and efficiency of a yeast biofilter for the treatment of a Nigerian fertilizer plant effluent. *World. J Microbiol Biotechnol* 15:515-516.
- Ferrero F (2007) Dye removal by low cost adsorbents: Hazelnut shells in comparison with wood sawdust. *J Hazardous Mat* 142:144-152.
- Fu Y, Viraraghavan T (2002) Removal of Congo red from an aqueous solution by fungus *Aspergillus niger*. *Advance Environ Res* 7:239-247.
- Galindo C, Kalt T (1999) UV/H₂O oxidation of azo dyes in aqueous media: evidence of a structure - degradability relationship. *Dyes Pigments* 42:199-207.
- Hassan MM, Alam MZ, Anwar MN (2013) Biodegradation of textile azo dyes by bacteria isolated from dyeing industry effluent. *Int Res J Biol Sci* 2(8):27-31.
- Joshi B, Kabariya K, Nakrani S, Khan A, Parabia FM, Doshi HV, Thakur MC (2013) Biodegradation of turquoise blue dye by *Bacillus megaterium* isolated from industrial effluent. *American J Environ Protec* 1(2):41-46.
- Kanmani P, Kumar SR, Yuvraj N, Parri KA, Pethikumar V, Arul V (2011) Microbial decolorization of synthetic dyes and reactive dyes of industrial effluents by using novel fungus *Aspergillus proliferans*. *Water Env Res* 83(11):2099-2106.
- Kaushik P, Malik A (2009) Fungal dye decolorization: recent advances and future potential. *Environ Int* 35:127-141.
- Khaled E, Hassan G, Khider M, Mandour R (2010) Safe biodegradation of textile azo dyes by newly isolated lactic acid bacteria and detection of plasmids associated with degradation. *J Bioremed Biodegrad* 1(3):1-6.
- Kirby N, Marchant R, McMullan G (2000) Decolorization of synthetic textile dyes by *Phlebia tremellosa*. *FEMS. Microbiol Lett* 188(1):93-96.

- Leebana VJ, Santhanum H, Geetha K, Raj SA (2012) Biodegradation of direct golden yellow, a textile dye by *Pseudomonas putida*. *Desalin Water Treat* 39:1-9.
- Lopez MZ, Guisado G, Garcia-Vargas MC, Estrella Suarez F, Moreno J (2006) Decolorization of industrial dyes by lignolytic microorganisms isolated from composting environment. *Enzyme Microbial Technol* 40:42-45.
- Machado KMG, Luciana CA, Compart LCA, Morais RO, Luiz H, Rosa LH, Santos MH (2006) Biodegradation of reactive textile dyes by Basidiomycetous fungi from Brazilian ecosystems. *Brazilian J Microbiol* 37:481-487.
- Mester T, Tien M (2000) Oxidative mechanism of ligninolytic enzymes involved in the degradation of environmental pollutants. *Int Biodeter Biodegrad* 46:51-59.
- Okino LK, Machado KMG, Fabris C, Bononi VLR (2000) Lignolytic activity of tropical rainforest basidiomycetes. *World J Microbiol Biotechnol* 16:889-893.
- Park C, Lee M, Lee B, Kim SW, Chase HA, Lee J, Kim S (2007) Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. *Biochem Eng J* 36:59-65.
- Rajamohan N, Karthikeyan C (2006) Kinetic studies of dye effluent degradation by *Pseudomonas stutzeri*. Retrieved from <http://www.eco-web.com/edi/index.htm> on 20 May 2011.
- Ramalingam S, Saraswathy N, Shanmugapriya S, Shakthipriyadarshani S, Sadasivam S, Sanmugaparakash M (2010) Decolorization of textile dyes by *Aspergillus tamari*, mixed fungal culture and *Penicillium purpurogenum*. *J Scien Ind Res* 69:151-153.
- Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in Textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource Technol* 77:247-255.
- Shahid A, Singh J, Bisht S, Teotia P, Kumar V (2013) Biodegradation of textile dyes by fungi isolated from North Indian field soil. *Env. Asia* 6(2):51-57.
- Siddique M, Mahmmod A, Sheikh M, Gafoor A, Khaliq S, Bukhai M, Yousaf K, Rehman K, Andleeb S, Nacem MM (2012) A study on the biodegradation of some reactive textile dyes by white rot fungus (*Pleurotus ostreatus*). *World Appl Sci J* 18(2):181-185.
- Suryavathi V, Sharma S, Sharma S, Saxena P, Pandey S, Grover R, Kumar S, Sharma KP (2005) Acute toxicity of textile dye wastewaters (untreated and treated) of Sanganer on male reproductive systems of albino rats and mice. *Reproductive Technol* 19:547-556.
- Tisma M, Komar M, Rajic M, Pavlovic H, Zelic B (2012) Decolorization of dyes by *Aspergillus Ochraceus* cultivated under solid state fermentation on sugar beet waste. *Chem Eng Transac.* 27:62-67.
- Valmaseda M, Martinez MJ, Marinez AT (1991) Kinetics of wheat straw solid- state fermentation with *Trametes versicolor* and *Pleurotus ostreatus*- lignin and polysaccharide alteration of related enzymatic activities. *Appl Microbiol Biotechnol* 35:817-823.
- Vasudev, K (2011) Decolorization of triphenylmethane dyes by six white rot fungi isolated from nature. *J Bioremed Biodegrad* 2(5):61-66.
- Wesenberg D, Kyriakides I, Agathos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol Adv* 22:161-187.
- Wong Y, Yu J (1999) Laccase-catalyzed decolourization of synthetic dyes. *Water Res* 33:3512-3520.
- Yang XQ, Zhao XX, Liu CY, Zheng Y, Qian SJ (2009) Decolorization of azo, triphenylmethane and anthraquinone dyes by a newly isolated *Trametes* sp. SQ01 and its laccase. *Process Biochem* 4:1185-1189.
- Yao MLC, Villanueva JDH, Tumana MLS, Caaling JG, Bunganhan ME, Dela Cruz TEE (2009) Antimicrobial activities of marine fungi isolated from seawater and marine sediments. *Acta Manilana* 57:19-28.
- Yeddou-Mezenner N (2010) Kinetics and mechanism of dye biosorption onto an untreated antibiotic waste. *Desalination* 262:251-259.
- Zollinger H (1991) Azo Dyes and Pigments. *Colour Chemistry-Synthesis, Properties and Applications of Organic Dyes and Pigments*. VCH, New York, p. 92-100.