Journal of Environment & Biotechnology Research

VINANIE PUBLISHERS

www.vinanie.com/jebr

Decolourization of textile industry dyes by *Calocybe indica* and *Pleurotus florida* mycelium

Loveleen Kaur, Sapna Sahota, Apurva Bhatia, Robinka Khajuria*

School of Biotechnology and Biosciences, Lovely Professional University, Phagwara, India-144402

ORIGINAL RESEARCH ARTICLE

ABSTRACT

Rapid industrialization and urbanization has led to manufacture and use of a large number of chemicals including dyes which may cause serious threat to health and environment. In the present study, the action of two fungal mycelium *Calocybe indica* and *Pleurotus florida* to decolorize textile industry effluents was studied and compared in order to devise an easy and economical decolourization and bioremediation strategy for the treatment of textile industry effluents. Solid state and broth culture studies were carried out in medium supplemented with 2.5, 5.0, 7.5 and 10 mg/100 mL of Amido-black 10-B and Sudan black dyes to study the decolourization potential of fungi. Treatment of textile industry effluent samples was also undertaken to study the change in various physicochemical properties in presence of fungal mycelium. The results indicated that *P. florida* and *C. indica* exhibited 80.29 and 80.94% reduction of Amido black 10-B, respectively, after 14 days. On the other hand, 75.65 and 71.88% reduction in Sudan black were observed in the case of *P. florida* and *C. indica*, respectively. There was a significant decrease in conductivity, total dissolved solids, biochemical oxygen demand and chemical oxygen demand of textile industry effluent sample while there was an increase in the pH of effluent sample after 14 days of incubation with both the fungal mycelia. The results showed that both the fungi have a promising potential for effluent decolourization and remediation. Though the use of *P. florida* for mycoremediation has already been undertaken by many research groups, the use of *C. indica*, which is an indigenous Indian mushroom, for the same purpose has not been explored before.

KEYWORDS

Amido black, *Calocybe indica*, decolourization, *Pleurotus florida*, Sudan black

1. INTRODUCTION

Dyes and dyestuffs are used extensively in a wide range of industries but are of primary importance to textile manufacturing. Out of 7×10^5 tons of dyes and pigments produced annually, about two-third of the production is consumed by textile industries (Vijayaraghavan et al., 2015). Effluent from these textile industries generally contain a variety of polluting substances including dyes (Rauf and Salman, 2009). About 10 to 15% of the total dye used by the industries are lost during the dyeing process and eventually released into the environment (Vijayaraghavan et al., 2015).

Textile dyes form a large group of textile chemicals and comprise over 8,000 different compounds. The textile industries mostly utilize reactive dyes, which are used in dyeing cellulose fibers. The effluent discharged from textile industries mainly comprised of residual dyes, auxiliary chemicals, surfactants, chlorinated compounds and salts (Pandey et al., 2007). Such colored effluents affect the photosynthetic processes of aquatic plants, reduce oxygen levels in water, cause damage to humans through mutagenic and carcinogenic effects and, in severe cases, results in the suffocation of aquatic flora and fauna (Crini, 2006; Puvaneshwari et al., 2006; Janaki et al., 2015). These compounds are also of concern because some of the dyes, dye precursors or

Corresponding author: **R. Khajuria**
Tel: +91-1824444264
Fax: +91-1824506111
E. mail: robinkakhajuria@gmail.com

Received: 11-05-2016
Revised: 02-06-2016
Accepted: 20-06-2016
Available online: 01-07-2016

their biotransformation products, such as aromatic amines, have been shown to be carcinogenic (Razo-Flores et al., 1997).

Currently, removal of dyes from effluents is carried out using physical and chemical methods like adsorption, chemical precipitation, flocculation, photolysis, chemical oxidation and reduction, electrochemical treatment and ion-pair extraction (Zhang et al., 2002; Ansari and Thakur, 2006). Most of the physical methods, however, simply accumulate and concentrate the dyes and create solid waste. Among the low cost and viable alternatives available for effluent treatment, biological processes are recognized by their capacity to reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Several microorganisms, including fungi, bacteria, yeasts and algae, can decolorize and even completely mineralize azo dyes under certain environmental conditions. In recent years, many white rot fungi e.g. *Pleurotus ostreatus*, *Pleurotus sajor-caju*, *Tinea versicolor*, *Phanerochaete chrysosporium*, *Lentinus strigosus*, *Daedalea flavida* have been intensively studied in connection with their ligninolytic enzyme production and their decolourization ability (Kumaran and Dharani, 2011; Rani et al., 2011; Theerachat et al., 2012; Vaithanomsat et al., 2012). Production of enzymes like lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13) and laccase (EC 1.10.3.2) is one of the reasons behind the ability of white rot fungi to decolorize various dyes (Sumit and Vimla, 2012).

In the present investigation, mycelia of two edible fungi *Pleurotus florida* and *Calocybe indica* were used for the treatment of textile effluents containing dyes. *Pleurotus* spp. (dhingri) is one of the most commonly grown oyster mushroom, and it is widely appreciated for its unique flavor, texture, color, high shelf life and poor substrate specificity. *Pleurotus* spp. are also considered as medicinal mushrooms (Cohen et al., 2002), exhibiting haematological, antiviral, anti-tumor, antibiotic, antibacterial, hypocholesterolic and immunomodulatory activities.

Calocybe indica, an indigenous Indian species, also known as milky mushroom grows well on agricultural residues like wheat straw and paddy straw. First attempt on the induction of fruit bodies of *C. indica* in culture was made almost two decades ago (Purkayastha, 1982). *C. indica* is rich in protein, lipids, fibre, carbohydrates, vitamins and also contains an abundant amount of essential amino acids and low fat product (Amin et al., 2010). These qualities make it suitable for food supplement in diet. This is the first instance where *C. indica* is used as a mycoremediation agent for treating textile dyes as most of the studies have concentrated on nutrition analysis, improvement of biological efficiency, strain improvement etc. in relation with *C. indica*.

Agriculture-intensive states of India such as Punjab and Haryana produce tons of agricultural residues which are burnt in open fields, thereby accentuating the problem of environmental pollution. The present research approach which uses mushroom mycelia for bioremediation of textile wastes may serve as an alternative for burning the agriculture residues in fields and thereby preventing environmental pollution

2. MATERIALS AND METHODS

2.1. Microorganism used

Mycelial cultures of *Pleurotus florida* and *Calocybe indica* were obtained from Punjab Agriculture University Ludhiana, Punjab, India. The fungal mycelia were maintained on potato dextrose agar (PDA) medium by frequent sub-culturing and incubated at 25±2 °C and 30±2 °C, respectively.

2.2. Chemicals used

Two synthetic dyes Amido black 10-B and Sudan black, purchased from HiMedia (Mumbai, India), were used in the study. The dyes evaluated in the present study were chosen as representatives of diazo dyes which are widely used by the local industries in Punjab. The textile effluents were collected from the industrial area of Ludhiana from the point source of textile industries.

2.3. Radial growth studies in the presence of industrial dyes

PDA medium was supplemented with four different concentrations (2.5, 5.0, 7.5 and 10 mg/100 mL) of Amido black 10-B and Sudan black. 8 mm bits of mycelial culture were then aseptically transferred to petriplates and incubated at 25±2 °C and 30±2 °C for *P. florida* and *C. indica*, respectively. Radial growth in terms of colony diameter was measured after 7 and 14 days of incubation.

2.4. Dye decolorization test in aqueous medium

About 100 mL of potato dextrose broth (PDB) was supplemented with different concentrations viz, 2.5, 5.0, 7.5 and 10 mg of Amido black 10-B and Sudan black. The flasks were inoculated with single 8 mm bits of *P. florida* and *C. indica* and incubated at optimum temperatures of the two fungi. The absorbance was measured at 400 and 590 nm for Amido black 10-B and Sudan black, respectively and the percent decolourization of textile dyes in the presence of mycelia was calculated according to the method of Tripathi and Srivastava (2011).

2.5. Determination of various physico-chemical properties of untreated and treated textile effluents

Changes in physico-chemical parameters of textile industry effluents i.e. pH, conductivity, total dissolved solids (TDS) after treatment with *P. florida* and *C. indica* were determined using standard methods (APHA, 1998). BOD and COD of the effluent were calculated according to the method of Ademoroti (1996). Two-way ANOVA was used to calculate Critical Difference @ 5% (CD@5%) in order to find if there was a significant effect on the fungal cultures based on the change in physiochemical properties of wastewater containing dyes as compared to the control.

3. RESULTS AND DISCUSSION

3.1. Radial growth studies in the presence of industrial dyes

The results indicated that both *P. florida* and *C. indica* can grow well in the presence of Amido black 10-B and Sudan black. The fungi showed a general trend of increase in radial growth with increase in the number of days when incubated on PDA medium supplemented with different concentration of dyes. But with increase in concentration of dyes there was a decrease in the radial growth of the mycelia of the two fungi suggesting that higher concentration of dyes were harmful for the

Table 1. Determination of radial growth of *P. florida* and *C. indica* in the presence of synthetic dyes.

Amount of dye (mg/100 mL)	*Radial Growth of *P. florida* (mm)				*Radial Growth of *C. indica* (mm)			
	Day 7		Day 14		Day 7		Day 14	
	Amido black 10-B	Sudan black	Amido black 10-B	Sudan black	Amido black 10-B	Sudan black	Amido black 10-B	Sudan black
Control	52.0	52.0	81.0	86.0	35.0	71.0	84.0	90.0
2.5	47.5	47.0	76.5	84.5	35.5	63.0	84.0	89.0
5.0	43.5	43.5	74.5	81.5	33.0	55.0	82.5	88.0
7.5	41.0	41.5	72.0	81.0	28.0	52.0	81.5	87.0
10	31.0	36.5	62.0	80.0	25.5	46.0	80.0	84.0
CD@5%	A: 0.23 B: NS A x B: 0.33		A: 0.26 B: 0.17 A x B: 0.37		A: 0.30 B: 0.19 A x B: 0.42		A: 0.11 B: 0.68 A x B: 0.15	

*Average of three replicates
Incubation temperature: 25±2 °C (for *Pleurotus florida*) and 30±2 °C (*Calocybe indica*)
NS: No significant difference observed; A: CD@5% for different dye concentrations; B: CD@5% when two different types of dyes were used; A x B: CD@5% when interaction between dye concentrations and types of dyes were considered

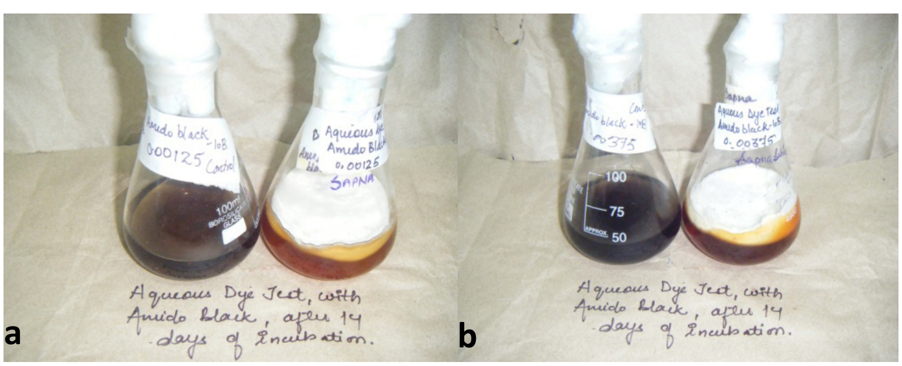

Figure 1. Decolorization of Amido black 10-B on PDB after 14 days incubation with a) *P. florida* and b) *C. indica*. The flask containing the culture of fungus has a less intense color than the control.

Table 2. % decolourization of Amido black dye, Sudan black with respect to time in presence of *P.florida* and *C.indica*.

Dye Conc. (mg/100 mL)	*Decolorization of Amido black 10B (%)				*Decolourization of Sudan black (%)			
	Day 7		Day 14		Day 7		Day 14	
	P. florida	*C. indica*	*P. florida*	*C. indica*	*P. florida*	*C. indica*	*P. florida*	*C. indica*
2.5	36.6	53.0	86.3	82.5	55.7	55.6	79.7	80.8
5.0	33.0	50.9	83.9	82.1	53.4	50.4	77.7	78.0
7.5	31.0	50.3	82.0	81.0	49.6	41.8	76.1	73.3
10	29.1	49.1	80.3	80.9	43.5	36.4	75.6	71.9
CD@5%	A: 0.15 B: 0.11 A x B: 0.22		A: 0.06 B: 0.04 A x B: 0.09		A: 0.05 B: 0.03 A x B: 0.07		A: 0.03 B: 0.02 A x B: 0.05	

*Average of three readings
Incubation temperature: 25±2 °C (for *Pleurotus florida*) and 30±2 °C (*Calocybe indica*)
NS: No significant difference observed; A: CD@5% for different dye concentrations; B: CD@5% when two different fungal cultures were used; A x B: CD@5% when both dye concentrations and types of fungal cultures were considered

Table 3. Effect of *P. florida* and *C. indica* on pH, conductivity and total dissolved solids (TDS) of textile industry effluent with respect to time.

Culture	Physicochemical Properties							
	pH			Conductivity			Reduction in TDS (%)	
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	Day 7	Day 14
P. florida	6.3	7.2	8.7	2.2	2.1	1.9	72.8	83.1
C. indica	6.2	7.6	8.4	2.2	2.1	1.9	68.5	84.7
CD@5%	A: NS B: 0.02 A x B: 0.02			A: NS B: $0.2*10^{-5}$ A x B: NS			A: 0.08 B: 0.08 A x B: 0.10	

Incubation temperature: 25±2 °C (for *Pleurotus florida*) and 30±2 °C (*Calocybe indica*)
NS: No significant difference observed
A: CD@5% when two different fungal cultures were used ; B: CD@5% for number of days; A x B: CD@5% when interaction between number of days and types of fungal cultures was considered

Table 4. Effect of *P. florida* and *C. indica* on the biochemical oxygen demand and chemical oxygen demand of textile industry effluent with respect to time.

Culture	Physicochemical Parameters			
	% reduction in BOD (mg/mL)		% reduction in COD (mg/mL)	
	5 Day	10 Day	5 Day	10 Day
P. florida	64.5	97.1	66.7	75.3
C. indica	55.9	87.7	33.4	85.8
CD@5%	A: 0.04 B: 0.04 A x B: 0.05		A: 0.05 B: 0.05 A x B: 0.07	

NS: No significant difference observed
A: CD@5% when two different fungal cultures were used; B: CD@5% for number of days; A x B: CD@5% when interaction between number of days and types of fungal cultures was considered

growth of the fungi. Though the growth rate in terms of increase in colony diameter was similar in both fungi, still *C. indica* showed better growth on solid medium. A radial growth of 80 mm and 84 mm for *C. indica* was observed in the medium with 10 mg/100 mL of Amido black 10-B and Sudan black respectively after 14 days of incubation (Table 1). Two-way ANOVA was used to calculate CD@5% in order to find whether the radial growth of fungal cultures differed significantly from control in the presence of different types as well as concentration of dyes.

3.2. Dye decolourization test

When aqueous culture studies were carried out, it was found that both the cultures showed better decolourization of Amido black 10 B which was more soluble in PDB as compared to Sudan black. It was also observed that with an increase in dye concentration, the decolourization efficiency of the fungi decreased. Both *P. florida* and *C. indica* showed approximately 80 percent decolourization of Amido black 10 B while there was a 75.65 and 71.88% reduction in color of Sudan black by *P. florida* and *C. indica*, respectively after 14 days of incubation (Table 2, Figure 1). Two-way ANOVA was used to calculate CD@5% in order to find whether the per cent decolourization of dyes was affected significantly in the presence of fungal cultures and when different concentration of dyes were present in effluent.

3.3. Effect of fungal mycelia on physicochemical characteristics of textile effluent

The initial pH and conductivity for the effluent were 6.2 and 2.2 µS/cm respectively, while the TDS was 4.5 mg/L (not shown in table). When physicochemical parameters of textile industry effluent treated with fungi were studied for 14 days, it was found that pH of the sample shifted towards alkalinity. This could be attributed to the potential of *P. florida* and *C. indica* to degrade acidic dyes like azo dyes. Moreover, conductivity values and TDS were also found to decrease with the increase in number of days after treatment with fungal mycelia. TDS decreased by 83.14 per cent and 84.68 percent on treatment with *P. florida* and *C. indica*, respectively after 14 days of incubation. Though *C. indica* had shown better growth in terms of colony diameter, still *P. florida* was found to a comparatively better mycoremedifying agent (Table 3).

The BOD and COD values of freshly collected effluent were determined to be 176 mg/L and 38.4 mg/L, respectively (not shown in table). About 97.07 and 87.67% reduction in BOD was observed in the effluent sample treated with *P. florida* and *C. indica*, respectively after 10 days of incubation. A COD reduction of 75.33 and 85.79% was also observed in the effluent sample treated with *P. florida* and *C. indica*, respectively after 10 days of incubation (Table 4). It was also found that though *C. indica* showed lower rate of bioremediation of effluents during the initial 5 days but subsequently a significant increase in bioremediation potential was observed as indicated by BOD and COD values. Two-way ANOVA was used to calculate CD@5% in order to find if there was a significant effect on the fungal cultures based on change in physiochemical properties as well as BOD/COD of waste water containing dyes as compared to the control.

Various scientific groups have been able to achieve high decolourization efficiency using a consortium of fungi which included white rot fungi like P. ostreatus and certain bacterial species (Ashger et al., 2012; Devi et al., 2012; Gupta et al., 2011; Rajendran et al., 2011; Singh and Singh, 2010; Moturi and Singara, 2009; Faraco et al., 2009; Moorthi et al., 2007). The results indicated that *C. indica* performed well in comparison with other mycoremediation agents. Further work in order to determine whether the degradation products bioaccumulate in fungus is under progress.

4. CONCLUSIONS

The use of edible mushroom mycelia such as *P. florida* and *C. indica* can help to improve the quality of waste water released into stream and rivers. Use of these fungi for the decolourization of textile effluents can be an effective method of bioremediation which will additionally provide protein rich food. *C. indica* has been observed to be a very effective and potent bioremediation agent.

REFERENCES

Ademoroti, C.M.A. (1996) Standard method for water and Effluents Analysis. Foludex press Ltd, Ibadan, pp. 22-23, 44-54, 111-112.

APHA. (1998) Standard methods for the examination of water and wastewater.18th Edition. American Public health Association, Washington DC, pp. 45-60.

Amin, R., Khair, A., Alam, N. and Lee, T.S. (2010) Effect of different substrates and casing materials on the growth and yield of *Calocybe indica*. Mycobiology, 38, 97-101.

Ansari, A.A. and Thakur, B.D. (2006) Biochemical reactor for treatment of concentrated textile effluent. Colourage, 2, 27-31.

Asgher, M., Jamil, F. and Iqbal, H.M.N. (2012) Bioremediation potential of mixed white rot culture of *Pleurotus ostreatus* IBL-02 and *Coriolus versicolor* IBL-04 for textile Industry wastewater. Journal of Bioremediation and Biodegradation, S1:007 doi: 10.4172/2155-6199.S1-007

Cohen, R., Persky, L. and Hadar, Y. (2002) Biotechnological applications and potential of wood-degrading mushrooms of the genus *Pleurotus*. Applied Microbiology and Biotechnology, 58, 582-594.

Crini, G. (2006) Non-conventional low-cost adsorbents for dye removal, a review. Bioresource Technology, 97, 1061–1085.

Devi, V.M., Inbathamizh, L., Mekalai, P.T., Premalatha, S. and Divya, M. (2012) Dye decolourization using fungal laccase. Pharmacology and Life Sciences, 1, 67-71.

Faraco, V., Pezzella, C., Miele, A., Giardina, P. and Sannia, G. (2009) Bio-remediation of coloured industrial wastewaters by the white-rot fungi *Phanerochaete chrysosporium* and *Pleurotus ostreatus* and their enzymes. Biodegradation, 20, 209–220.

Gupta, N., Tripathi, A.K. and Harsh, N.S.K. (2011) Bioremediation of cotton-textile effluent using fungi. Bulletin of Environment, Pharmacology and Life Sciences, 1, 15-19.

Janaki,V., Shanthi,K. and Kamala-Kannan,S. (2015) Polypyrrole-A conducting nanopolymer for the treatment of simulated textile effluent. Journal of Environment and Biotechnology Research, 1, 45-51.

Kumaran, N. and Dharani, G. (2011) Decolourization of textile dyes by white rot fungi *Phanerocheate chrysosporium* and *Pleurotus sajor-caju*. Journal of Applied Technology in Environmental Sanitation, 1, 361-370.

Moorthi, S.P., Periyar, S.S., Sasikalaveni A., Murugesan, K. and Kalaichelvan, P.T. (2007) Decolourization of textile dyes and their effluents using white rot fungi. African Journal of Biotechnology, 6, 424-429.

Moturi, B. and Singara, C.M.A. (2009) Decolourization of crystal violet and malachite green by fungi. Science World Journal, 14, 28-33.

Pandey, A., Singh, P. and Iyengar, L. (2007) Bacterial decolourization and degradation of azo dyes. International Biodeterioration and Biodegradation, 59, 73-84.

Purkayastha, R.P. (1982) Cultivation of *Calocybe indica*: a new source of vegetable protein. In: Frontiers of Research in Agriculture (Ed. Roy, S.K.). Indian Statistical Institute, Calcutta. pp. 580-586.

Puvaneshwari, N., Muthukrishnan, J. and Gunasekaran, P. (2006) Toxicity assessment and microbial degradation of azo dyes. Indian Journal of Experimental Biology, 44, 618-626.

Rajendran, R., Sundaram, S.K., Prabhavathi, P., Sridevi, V. and Gopi, V. (2011) Comparative analysis of bioremediation potential of adapted and non adapted fungi on azo dye containing textile effluent. Pakistan journal of Biological Sciences, 14, 610-618.

Rani , C., Jana, A. and Bansal, A. (2011) Studies on the biodegradation of azo dyes by white rot fungi *Daedalea flavida* in the absence of external carbon source. Journal of Environmental Science and Technology, 6, 175-190.

Rauf, M.A. and Salman, S. A. (2009) Fundamental principles and application of heterogeneous photocatalytic degradation of dyes in solution. Chemical Engineering Journal, 151, 10-18.

Razo-Flores, E., Luijten, M., Donlon, B., Lettinga, G. and Field, J. (1997) Biodegradation of selected azo dyes under methanogenic conditions. Water Science and Technology, 36, 65-72.

Singh, L. and Singh, V. (2010) Biodegradation of textile Dyes, Bromophenol blue and Congo red by fungus *Aspergillus flavus*. International Journal of Environmental Science and Technology, 5, 235-242.

Sumit, P. and Vimala, Y. (2012) Bioremediation and decolourization of distillery effluent by novel microbial consortium. European Journal of Experimental Biology, 2, 496-504.

Theerachat, M., Morel, S., Guieysse, D., Remaud-Simeon, M. and Chulalaksananukul, W. (2012) Comparison of synthetic dye decolourization by whole cells and a laccase enriched extract from *Trametes versicolor* DSM11269. African Journal of Biotechnology, 11, 1964-1969.

Tripathi, A. and Srivastava, S.K. (2011) Ecofriendly treatment of azo dyes: biodecolorization using bacterial strains. International Journal of Bioscience, Biochemistry and Bioinformatics, 1, 37-40.

Vaithanomsat, P., Chedchant, J., Kreetachat, T., Kosugi, A., Apiwatanapiwat, W., Thanapase, W., Chuntranuluck, S. and Mori, Y. (2012) Improvement of lignin-degrading enzymes production from the white-rot fungus (*Lentinus strigosus*) and its application in synthetic dye removal. African Journal of Microbiology Research, 6, 267-278.

Vijayaraghavan, J., Pushpa, T.B., Basha, S.J.S., Jegan, J. (2015) Removal of a basic dye from aqueous solution by *Gracilaria corticata*. Journal of Environment and Biotechnology Research, 1, 30-36.

Zhang, F., Yediler, A., Liang, X. and Kettrup, A. (2002) Ozonation of the purified hydrolyzed azo dye reactive red 120-CI. Journal of Environmental Science and Health, 37, 707-780.