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Biodegradation of Textile Dyes Using Fungal Isolates

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ABSTRACT

The textile industries produces considerably high amount of aquatic toxicity which is discharged directly into the environment before treated properly. The waste generation volume and load produced is hazardous in nature. Thus, this study explores the role of fungal biomass against pollution due to textiles dyes as degrading agent. This study will be beneficial for treating water effluent from textile industry and will decrease the pollution form environment with advanced technology for future use. In this study the evaluation of fungal species for the decolourization and degradation of textile dye has been carried. Four potential fungal strains (NS-1, NS-2, NS-9 and NS-10) were exploited after screening for the decolourization of Rubine Toner-12 dye under aerobic condition. Growth associated decolorization studies were carried out in Potato Dextrose Broth (PDB) supplemented with Rubine Toner-12. About 99% percent decolorization was achieved on supplementation with 10 mg L^{-1} of dye. Comparative spectrophotometric analysis of control and fungus inoculated medium supplemented with rubine toner-12 showed almost 100% decolorization in inoculated flasks. The fungus was identified to be *Aspergillus niger*. Maximum decolorization of Rubine Toner-12 was observed at pH 6. It is a better technique to check environmental pollution.

Key words: Fungal, biodegradable, environment, decolorization, pollution, rubine

INTRODUCTION

Many scientist in the field of biology and chemistry have given significant contribution to science and technology by utilizing the natural resources (Chauhan and Kaith, 2012; Aan *et al*., 2011; Abd El-Hady and Abd El-Baky, 2011; Abdi *et al*., 2010; Raja and Thilagavathi, 2011; Issaoui *et al*., 2011; Abd El-Hady 2011; Das *et al*., 2011; Rocco, 2011; Adedayo, 2012). Rapid industrialization had introduced a lot of chemicals including dyes into the environment polluting the entire ecosystem. Coloration of the natural water bodies is not only undesirable from aesthetic point of view, but it also affects the aquatic flora and fauna by reducing the transmission of sunlight through the water surface. Azo dyes are the largest group of synthetic dyes and pigments with industrial application and over the worldwide production of 100,000 commercially available dyes. These methods cause a significant amount of sludge or may easily cause secondary pollution due to excessive chemical usage. High cost and disposal problems of the chemical and physical methods for treating dye wastewater make them inefficient to cop up pollution caused due to the textile industries (Mazmanci *et al*., 2009; Ghoreishi and Haghighi, 2003; Robinson *et al*., 2001). Microbial enzymes, such as laccase, lignin peroxidase, manganese peroxidase and azo reductase are assumed to play an important role in the degradation of lignin in their natural lignocellulosic substrates. As reported by Raghukumar *et al*. (2008), the white-rot fungus degrades dye along with

lignin. Most of the xenobiotic compounds and dyes are degraded with ligninolytic system in white-rot fungi. Their enzymes producing activity makes them effective decolorizers and the bio-accessible groups are present in the lignin structure, seem to be access points to the fungus ligninolytic enzymes produced by fungi.

This study explores the role of various fungal species against pollution due to textiles dyes by serving as degrading agent. The work is new and remains unnoticed for its viability.

MATERIALS AND METHODS

The selection of dye for present study started on 13th August, 2010 it was made on the basis of its solubility in water. Rubine Toner-12 was found fairly soluble in water and hence used to explore the potential of fungal isolates towards decolorization of textile dyes. Spectrum scan of the dye was obtained using Shimadzu Spectrophotometer and λ_{max} of dye was obtained. The study completed in December, 2011.

Isolation of fungal strains: For isolation of potential strains, eight different types of samples from various environments (sludge samples from industrial effluent, waste water samples from Buddha Nala, effluent sample from alcohol, dye shops of Patiala and textile dyeing plant in Ludhiana) were collected and preserved at 4°C till further use. Soil/sludge samples were air dried and sieved after grinding followed by serial dilutions of the samples prepared in the saline (0.85% NaCl). Different dilutions were spread over potato dextrose agar (PDA) followed by incubation at 28°C for 72°C. Isolated fungal colonies were repeatedly sub cultured on fresh PDA plates to ensure the axenic nature of the isolates. Pure cultures were preserved at 4°C till further use.

Screening of isolates: For screening of potential fungal strains, a cylindrical bit of preserved culture was cut with cork borer and revived on fresh PDA plates. A bit of 8 mm dia was picked and inoculated in 250 mL Erlenmeyer's flask containing 100 mL potato dextrose broth (PDB). Then flasks were incubated on shaker at 120 rpm at 28°C for 72 h. The fungal beads thus formed were thoroughly washed several times with phosphate buffer (pH-6.0) at regular intervals of four hours with continuous shaking between the washing to remove traces of media. Washed fungal beads were added into Czapek-dox media containing Rubine Toner-12 (100 mg L^{-1}) and incubated at 28°C for 72 h on an orbital shaker at 120 rpm to check sorption of dye in the fungal beads. Potential cultures were used to carry out further studies.

Decolourization studies and statistical analysis: Decolourization studies were carried out by supplementing dye before (Treatment I) and after autoclaving (Treatment II) at 100 mg L^{-1} dye concentration along with control flasks (without inoculation). Four potential strains were inoculated in each flask except control. Flasks were kept on agitator at 28°C and 120 rpm. Decolorization of dye was monitored after regular interval of 48 h using spectrophotometer except first observation which was taken after 24 h of inoculation. Decolorization assay was carried out in terms of extent of decolorization using the method as described (Ali and Muhammad, 2008). After optical density measurement, percent decolorization was calculated using the following equation:

$$\text{Percent decolorization} = \frac{(AI-AF)}{AI} \times 100\%$$

where, AI is initial absorbance and, AF is final absorbance.

Morphological characterization of fungi: Morphological characterization of positive isolates was carried out on PDA plates by visual observation of mycelial growth along with microscopic features after staining with lactose phenol cotton blue staining using optical microscope.

Effect of pH on growth and decolorization of dye: From decolorization studies NS-10 was further used to study the effect of pH on growth of fungus as well as decolorization. Potato dextrose broth was prepared and maintained at different pH, starting from extreme acidic to neutral followed by extreme basic pH (4, 6, 8 and 10). A bit (8 mm) of sporulating culture was cut with the help of cork borer and inoculated followed by incubation at 28°C for 120 h and determined decrease in optical density.

RESULTS AND DISCUSSION

Variety of fungal growth was observed in different samples collected from different sites. As the samples were collected from sites with contamination of textile dyes, these fungal strains were supposed to be efficient dye degrading strains. On the basis of different growth patterns on PDA plates, ten different strains were screened for biosorption of dye in the biomass. Out of these ten isolates only four cultures (NS-1, NS-2, NS-9 and NS-10) were observed positive for biosorption. The three *Bacillus* sp. isolates were screened (*Bacillus* sp. strain SF, *Bacillus* sp. strain LF and *Bacillus pallidus*) on petri-dish to obtain visually decolorized colony (Maier *et al*., 2004). While no decrease in colour was observed in the incubated positive and negative controls. More than 99% of dye was sorbed. On the basis of decolorization of dye in supernatant, four isolates were selected and then characterized on the basis of colony morphology on PDA plates along with microscopic examination as shown in Table 1. All these strains were found viable and fairly tolerant to prolonged exposure to dye as indicated by appearance of extending mycelia followed by sporulation on PDA plates after exposure.

Growth Associated Decolorization of dye: Four selected strains were explored for growth associated decolorization of dye with respect to time. Aliquots of samples were subjected to spectrum scan after regular interval of 24 h in Fig. 1a, interval 72 h in Fig. 1b and Interval 120 h in Fig. 1c in the visible range to ensure degradation of dye along with characterization of metabolites. Strain NS-10 showed maximum decolorization after 24 h whereas an intermittent decolorization was observed with NS9 and NS2 strains, Fig. 1a. There was little or no decolorization as neghgible reduction in optical density was observed at characteristic wavelength in NS-1 inoculated flask after 24 h of incubation. After 120 h of incubation, almost 100% decolorization of dye was observed as optical density was neghgible with respect to control were no

Table 1: Morphological and microscopic characterization of selected fungal isolates

Strain	Morphological observations	Microscopic observations
NS-1	Black colored spore attached to hyphae	Sporangiosphores arising from a foot cell. Conidia are present in chains and arises from vesicles
NS-2	Creamish-pink colored cottony conidia growth	Sickle-shaped transversely septate arising from conidiophores
NS-9	Greenish-brown colored spores	Conidia in long chains on repeatedly branched, with brush like appearance
NS-10	Yellow colored having cream	Conidia in long chains, repeatedly branched and conidiophores resembling brush like head

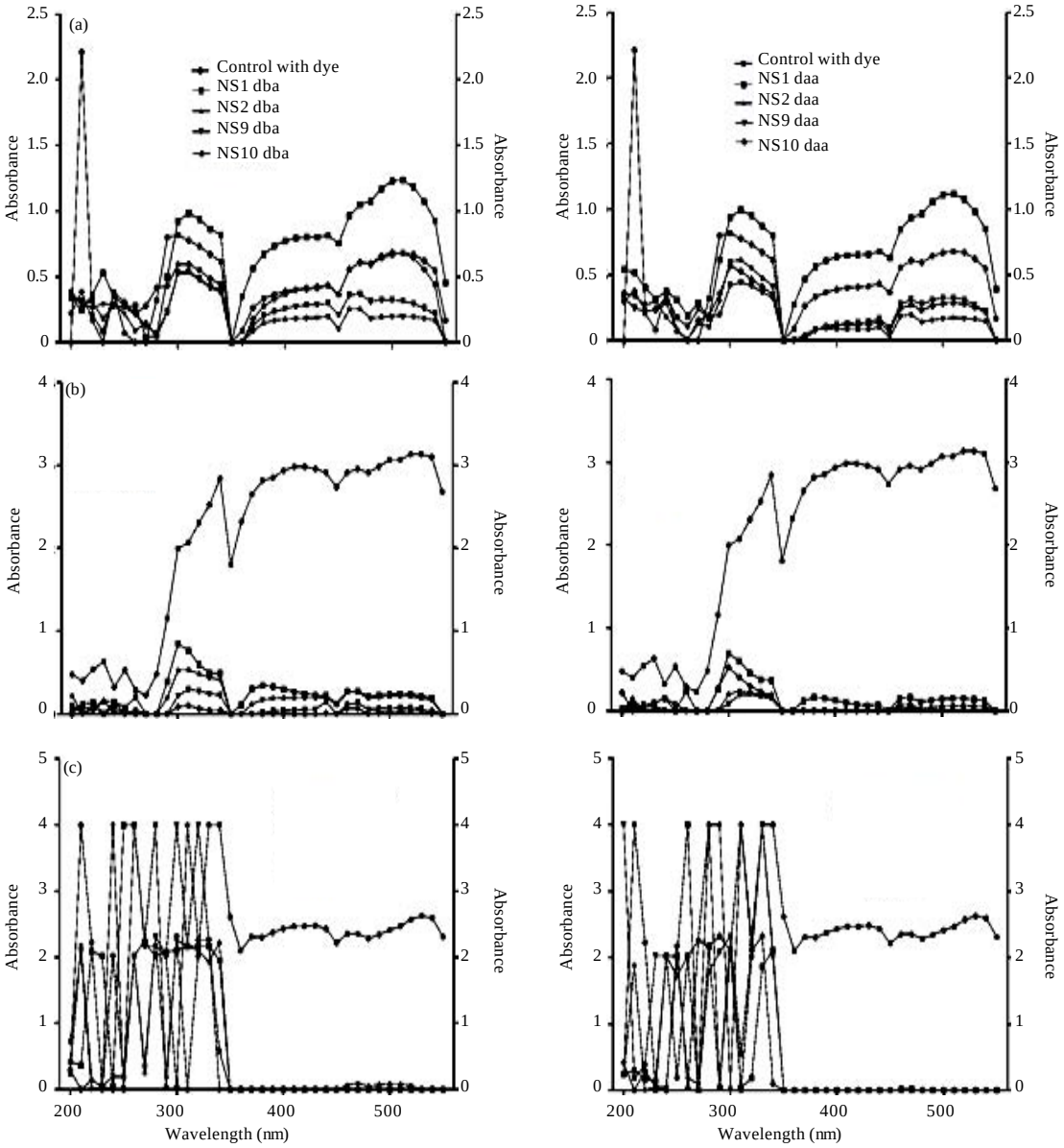

Fig. 1(a-c): Comparative overlay of biomass free supernatant after regular interval of time at (a) 24 h, (b) 72 h, and (c) 120 h

such reduction of optical density was observed, Fig. 1(c). A concomitant increase in biomass was observed that strengthen the speculation of growth associated decolorization along with biosorption. Similarly, it was reported that the decolorization of the Remazol Black B dye with *Paenibacillus azoreducens* was within 24 h (Meehan *et al*., 2001). Appearance of new novel peaks in the supernatant of fungus inoculated flasks in UV-region may be assigned to aromatic amines formed fungal metabolites produced mineralization of dyes. It was observed that 99% decolorization of Reactive Brilliant Red K-2BP (200 mg L^{-1}) with *P. rugulosa* Y-48 and *C. krusei* G-1 was in 24 h (Yu and Wen, 2005). It was described that the azo reduction activity in a novel ascomycete yeast strain during late exponential phase of growth, coincide with that observations as maximum

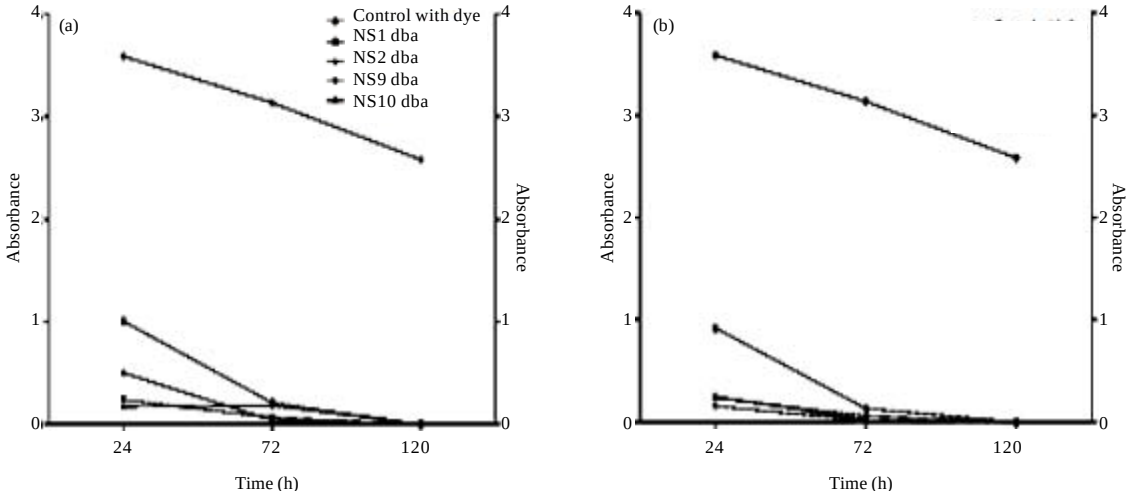

Fig. 2(a-b): Statistical analysis of percentage decolorization of Rubine Toner-12 with different Treatments, (a) Treatment 1: Dye added before autoclaving and (b) Treatment 2: Dye added after autoclaving

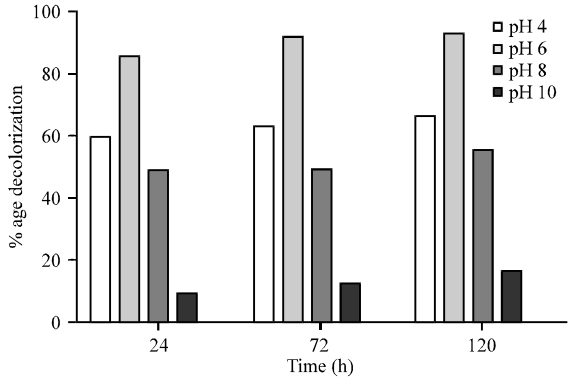

Fig. 3: Effect of pH on decolorization of Rubine Toner-12 with NS-10

decolorization observed after 72 h of incubation in the present study (Ramalho *et al*., 2004). Results reflected that the addition of dye before autoclaving and after autoclaving (Treatment (1) and Treatment (2), respectively, Fig. 2a and 2b, shows almost equal percentage of degradation by fungal strains i.e., degradation doesn't depend on dyes passed through broilers in textile industry which having mere effect on the structures of dyes. These observations indicated the inherited potential of different isolates towards degradation of dyes.

Effect of pH on growth and decolorization of dye: Effect of pH on growth and decolorization of dye was carried out for five days within a broad range of pH (4, 6, 8 and 10). Maximum decolorization was observed at slightly acidic pH (pH 6) with a substantial decolorization in acidic pH (pH 4), Fig. 3. While a significant negative impact of higher pH (pH 10) was observed on decolorization of rubine toner-12 using NS-10. These observations strengthen the basic idea of fungal metabolism where efficient absorption of nutrients and degradation of dye in acidic pH 6.

Cheriaa *et al.* (2009) observed similar results for the characterization of new algae isolated from textile wastewater plant in reducing dye pollution at pH tested (5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 and 10) with maximum decolorization at pH 8. In the present study, maximum decolorization was observed at pH 6.

CONCLUSION

Several fungal isolates were exploited to examine growth associated dye decolourization potential in this study. Out of these NS-10 was found to decolorize almost 100% dye under different set of conditions. Besides this, biosorption potential of these isolates used for initial screening further support their candidature for bioremediation of dyes contaminated sites. The fungi was identified to be *Aspergillus niger*. It could be used to check environmental pollution.

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