Potentiality of indigenous mycoflora in bioremediation of textile dye

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ABSTRACT

The removal of color from paper, textile and leather industry waste water represents a major environmental concern. These industry wastes have a significant role in the water pollution. The discharge of the wastewater into receiving streams not only affects the aesthetic sense of nature but also interferes with transmission of sunlight into streams, thus reducing photosynthetic activity. In addition to visual effect, they have an adverse impact in terms of chemical oxygen demand, toxicity, mutagenesis and carcinogenicity. Several strategies are currently available to remove colour from the industrial effluents but these methods have some limitations. Bioremediation can be an effective tool where indigenous microorganism (bacteria, algae and fungi) are used for the treatment of industrial dye effluent. Therefore, the present investigation was focused on the isolation and characterization of fungal strains, which would efficiently decolorize the textile dye (malachite green). A total of five indigenous fungal strains were isolated from the effluents collected around the discharge site of textile industry situated in Gwalior, Madhya Pradesh, India. Effluent samples were also analyzed for their physiochemical properties. *Aspergillus niger*, *A. flavus* and *Penicillium* species were successfully identified using macroscopic and microscopic study referring relevant literature. Decolorization capabilities of these fungal species were evaluated for malachite green dye in carbon limited Czapek Dox broth carried out laboratory conditions using dyes concentrations (50 mgL^{-1}, 100 mgL^{-1}, 150 mgL^{-1}, 200 mgL^{-1}), pH (5, 6, 7 and 8), temperature (25, 30, 35 and 40), different additional carbon (glucose, sucrose, fructose and starch) at 10 gL^{-1} and nitrogen sources (ammonium sulfate, urea and ammonium nitrate) at 5 gL^{-1} to investigate the efficiency of the microbes in decolorization of the dye. The dye concentration 150mg/l showed high percentage of decolorization than other concentrations. Maximum decolourization was found using glucose as carbon and urea as nitrogen source. In different physiological conditions, optimum pH 7 and temperature 35°C showed highest percentage decolourization at 120 rpm speed after seven days of incubation in incubator shaker. The fungal isolates were found efficient in decolorization, which proves that these indigenous fungi are potential candidates for bioremediation. It is cost effective and environmental friendly and the only way for ultimate controlling of pollution generated by textile and dyestuff industries.

Keywords: decolourization, indigenous mycoflora, physiological conditions, textile dyes

INTRODUCTION

The textile industry is one of the industries that generate a high volume of waste water [1,2]. Strong color of the textile waste water is the most serious problem of the textile waste effluent [3]. Synthetic dyes are widely used in a number of industrial processes such as textile industries, paper printing and photography [4] and dye houses is characterized by high chemical and biological oxygen demands (COD and BOD), suspended solids and intense color due to the extensive use of

Research Article, Acta Biologica Indica 2013, 2(2):467-475

pISSN 2319-1244, eISSN 2279-0160

synthetic dyes. Dyes are colored substances used on several substrates in food, cosmetics, paper, plastic, and textile industries among others. They are retained on the substrates by physical adsorption, by making compounds with metals and salts by mechanical retaining and solution or by making covalent bonds. Such extensive use of colour often has problems in the form of colored waste water which need pre-treatment for colour prior to its disposal into receiving water bodies. Untreated or partially treated waste water and industrial effluent discharge into natural ecosystems pose a serious problem to the ecosystem and the life forms.

Several strategies are currently available to remove colour from the industrial effluents like precipitation, adsorption and photo degradation techniques [5]. Biological decolourization has also been studied [6] which includes degradation using bacterial [7] and fungal isolates [8,9]. Thus, more technically advanced research efforts are required for searching, exploiting new fungal species and improvement of practical application to propagate the use of fungi for bioremediation of industrial effluents and contaminated soils [10,11]. There are many variables or factors affecting enzyme production and decolorization that are expressed by different taxa and culture conditions. These features are important in the process design and optimization of fungal treatment of effluents [12].

Hence, the present study was undertaken to investigate the influence of different concentrations of dyes, various pH, various temperature, different additional carbon, and nitrogen source on percentage on decolorization of some important textile dyes like Navy Hexie, Red GF and Blue B 133 by *Phanerocheate chrysosporium* and *Pleurotus sajor-caju*.

MATERIALS AND METHODS

Collection of Sample

Samples were collected in airtight brown bottle around the discharge site of textile industry situated in Gwalior (MP), India and stored at 4°C temperature for further experiment. Effluent samples were used for the isolation of fungus culture as well as physiochemical analysis.

Physicochemical analysis of effluent sample

Temperature, pH, colour and odour of the effluent were recorded on the spot. Sample collected from the discharge site was filtered through Whatman no. 1 filter paper and their biological oxygen demand (BOD), chemical oxygen demand (COD), total solids (TS) and total dissolved solids (TDS) was determined using standard procedures [13].

Dye and chemicals

The textile dye malachite green used for decolourization is an organic compound that is used as a dyestuff. Malachite green is traditionally used as a dye for materials such as silk, leather, and paper. All media/chemicals used in the study were of analytical grade and purchased from Hi-Media Laboratories (Mumbai, India).

Determination of absorption maxima (λmax) of malachite green dye

The absorption maxima (λmax) were determined by using UV-Vis spectrophotometer Elico-169 model. Optical density of dye solution was observed in water solution at different wavelength between (340–700 nm). The maximum wavelength, for malachite green was determined 620 nm.

Isolation and identification of dye decolorizing fungi

The fungus cultures were isolated on Czapeck Dox Agar (CDA) using a dilution plate technique. The medium composition is as follows: (K_2HPO_4- 1.0 g/l; $NaNO_3$- 30.0 g/l; KCl, 0.5 g/l; $MgSO_4.7H_2O$- 0.5 g/l; $FeSO_4.7H_2O$- 0.01 g/l; Yeast extract, 5.0 g/l; Sucrose 30.0 g/l; Rose Bengal, 0.03 g/l; Agar, 15.0 g/l). The fungus cultures were isolated and identified using photomicrograph taken with steriobinocular microscope and with the help of taxonomic guides and standard procedure [14,15]. The identified fungus cultures were preserved on CDA slants at 4°C in a refrigerator and were served as stock cultures.

Screening of fungal cultures for decolourization on solid media

The isolated fungi were tested against dye malachite green in Czapek Dox (CD) broth medium. After sterilization of media, dye was added aseptically at the concentration of 100 mg/l. For this a disc of inoculums was inoculated into the flask and an uninoculated flask was also run as control. All these flasks were incubated at 25±2°C and observed for decolourization using spectrophotometer at 620 nm.

Preparation of fungal spore suspension

Mycelium about 1 cm diameter obtained from 5 days old fungus culture slant were transferred to 50 ml potato dextrose broth in a 250 ml conical flask and incubated at 27°C temperature for 5 days.

Optimization of decolourization conditions

Decolourization were performed in different culture conditions, i.e. dyes concentrations (50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l), pH (5, 6, 7 and 8), temperature (25, 30, 35 and 40), different additional carbon (glucose, sucrose, fructose and starch) at 10 g/l and nitrogen sources (ammonium sulfate, urea and ammonium nitrate) at 5 g/l to investigate the efficiency of the fungi on dye decolourization. The culture flasks were run on incubator shaker at 120 rpm for seven days.

Decolorization assay

The ability of fungal strains to decolorize textile dyes was carried out in C-limited Czapek-Dox broth (5 g/l) amended with reactive blue MR (200 mg/l). Erlenmeyer flasks contained 100 ml sterile media with dye and were inoculated with fungal disc (8 mm) separately. The flasks were incubated at 25± 2°C for 10 days in static condition. Samples were withdrawn aseptically on alternate days, centrifuged at 5000 rpm for 10 minute and the supernatant was scanned in a spectrophotometer at λmax (620 nm) of malachite dye. Two control flasks were also maintained for each fungal strain. One flask contained media (without dye) and inoculated with fungal biomass and second flask contained media with dye and no fungal biomass. Percent decolorization was calculated by applying the formula [16]:

$$\text{Decolorization \%} = \frac{Ao - At}{Ao} \times 100$$

where Ao - initial absorbance of sample, At - the absorbance at different time intervals. Effect of different carbon sources (sucrose, glucose and fructose) and dye concentration (100-300 mg/l) in the liquid C-limited Czapek-Dox medium were determined using the same protocol.

Statistical analysis

Data were statistically analyzed and the mean of triplicates ± standard deviation (SD).

RESULTS AND DISCUSSION

Physicochemical characteristics of the textile effluent sample

The effluent discharge sample cause serious problem of ground water pollution. Physicochemical analysis of textile sample represents the pollution level. Therefore, the physicochemical parameters were examined. Table 1 showed that effluents have dark black colour with pungent smell, high temperature (measured by lab thermometer), pH 8.5, BOD (465 mg/l), COD (1240 mg/l) TDS (62000 mg/l). The colour of the effluent is black due to the mixture of different chemicals and dyes used in the industrial processes [17]. High ph is mainly due to use of carbonate, bicarbonate, H_2O_2, and NaOH during bleaching process in the textile [18]. Soil permeability also gets affected, which results in polluting the underground resources of water [19]. Elevated temperature tends to decrease the solubility of gases in water which is expressed as high BOD/COD. TDS value of textile effluent sample was found higher than the permissible limits [20]. High TDS value reduces the light penetration into the water and ultimately decreases the photosynthesis in aquatic flora. This causes the reduction in dissolved oxygen level of water bodies.

Table 1. Physicochemical characteristics of the textile effluent sample.

Parameters	Effluent
Colour	Dark black
Odour	Pungent
Temperature (°C)	40
pH	8.5
TDS (mg/l)	6200
BOD (mg/l)	465
COD (mg/l)	1240

Identification of fungus cultures

Morphologically total five different fungal cultures were recovered from the effluents collected around the discharged site by employing spread plate technique. Out of the five fungus cultures, three were identified after staining with lactophenol cotton blue and microscopic analysis, *viz*. *Aspergillus niger*, *A. flavus* and *Penicillium* species. These fungal strains may be much adapted to the polluted sites and are utilizing the dyes as a substrate. The occurrence of fungi in the polluted water depends on the availability of nutrient, oxygen, biological, physical and chemical characteristics of the pollutants. Several researchers also reported that *Aspergillus* species are well adapted to textile waste water and are frequently isolated from effluents and dye contaminated soils [17,21].

Decolorization assay

Initial screening of the isolated fungus was done on the basis of percentage decolourization using UV-Vis spectrophotometer at 620 nm wavelength. It was found that maximum decolorization efficiency shown by *A. niger* followed by *A. flavus* and least by *Penicillium* species in stationary condition after seven days of incubation with the dye (100mg/l) and glucose as a carbon source [22,23].

Effect of dye concentration on decolorization

The decolorization efficiency of *Aspergillus allhabadii*, *A. niger* and *A. sulphureus* were analyzed at 100-200 mgl^{-1} in liquid media containing glucose as a carbon source. It was found that highest decolorization shown by *Aspergillus niger* (92.2±0.11%) and *Aspergillus flavus* (88.04±0.25%) with 150 mgl^{-1} after seven days of incubation at 12 rpm (Figure 1). Generally, the concentration of color compounds found in the effluent or rivers ranged as low as 12 to 16 mg/L. It is reported that higher dye concentration strongly inhibits decolorization, which may be due to desorption or toxic effects. The ability of enzyme for recognizing the substrate efficiently at very low concentrations may be present in some waste water [24,25]. The desorption of the dyes from the fungal cells, especially at higher dye concentrations may be due to higher molecular mass, structural complexity [26].

Effect of carbon source on decolorization

Carbon sources such as glucose, sucrose, fructose and starch were used at 5.0g/l to investigate their effect on the decolorization efficiency of the fungus cultures. It was found that highest decolorization shown by *Aspergillus niger* (94.13±0.12%), *Aspergillus flavus* (78.01±0.25%) and minimum by *Penicillium* species (66.20±.21 %) in glucose supplemented medium. The results of the present investigation depicted that all the tested fungus cultures were efficient in decolorization with glucose supplemented media (Figure 2). The primary mechanism of decolorization is due to dye adsorption/degradation by mycelium of fungi with reduction of dye intensity in solution because of changes caused by them [27,28]. Growth media enhances the growth and adsorption/degradation rate by fungi and on addition of carbon or other nutrient sources further increases decolorization process [29].

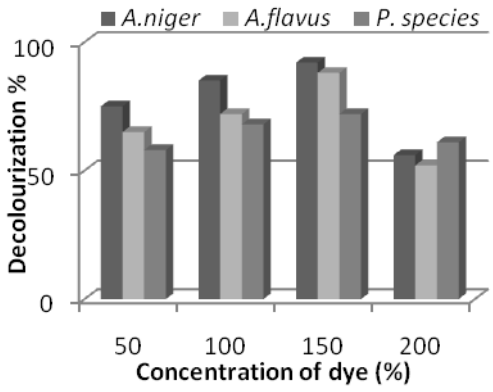

Figure 1. Effect of different dye concentrations on percentage decolourization at 120 rpm speed.

Effect of nitrogen source on decolorization

The different nitrogen sources (ammonium sulfate, urea and ammonium nitrate) also tested to check the influence in rate of decolorization of textile dyes (Figure 3). The rate of decolorization of malachite green with ammonium sulfate 84.32±1.2%, urea 92.20±.1.1% and ammonium nitrate 74.40±1.2% by *Aspergillus niger* and *Aspergillus flavus* shows decolorization with ammonium sulfate 78.2±1.4%, urea 87.4±1.2% whereas *Penicillium* species showed ammonium sulphate 70.4±1.1 %, urea 74.2±2.1 % and ammonium nitrate 64.2±1.0%. In few isolates, nitrogen sources inhibited the decolorization efficiency which is on exact line of Zhang et al. [27,30-32] used urea and ammonium nitrate as inorganic nitrogen sources for optimizing the maximum decolorization of azo dyes Reactive Black Band Reactive Orange 16. These results are in harmony with some above researchers, who also reported inhibition of dye decolorization with supplemental nitrogen. The addition of inorganic nutrients like nitrogen does not always enhance degradation of organic compounds, because there are many other factors which many decrease microbial activity [33].

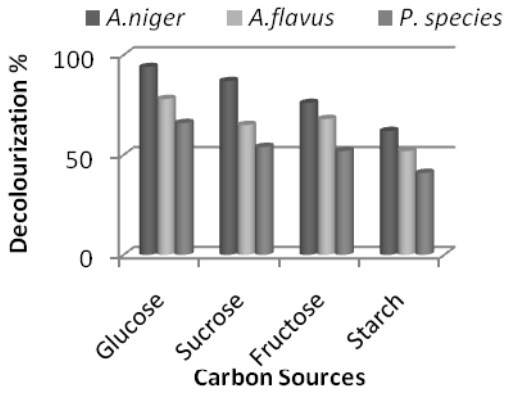

Figure 2. Effect of different carbon sources on percentage decolourization at 120 rpm speed.

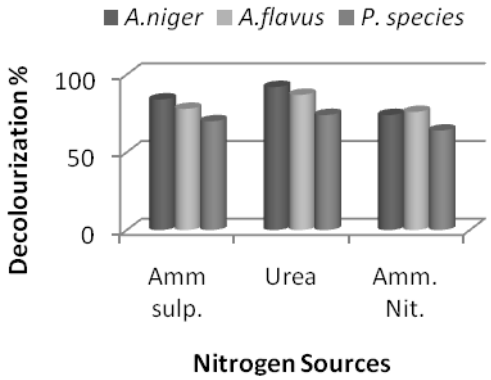

Figure 3. Effect of different nitrogen sources on percentage decolourization at 120 rpm speed.

Effect of pH on decolorization

The different pH (5, 6, 7 and 8) used for the decolorization of dyes by the selected fungus (Figure 4). The maximum decolorization was observed in pH 7 by *Aspergillus niger*, i.e. 84.21±1.2 %. In this experiment the decolorization rate of malachite green was observed 62% at pH 5, 71% at pH 6, 84% at pH 7 and 68% at pH 8 by *Aspergillus niger*. *Aspergillus flavus* showed decolorization 60% at pH 5, 68% at pH 6, 72% at pH 7, and 65% at pH 8 while in case of *Penicillium* species 55 % at pH 5, 64 % at pH 6, 71 % at pH 7 and 68 % at pH 8. Few physical parameters influence in pH. Most of the industrial effluents contain various inorganic chemicals such as sulfides, sulfates, chlorides and carbonates [5]. A few fungi belonging to the class zygomycetes have been demonstrated to decolorize and detoxify simulated textile wastewaters of varying composition characterized by high concentrations of salts and dyes by bioadsorption [34,35]. However at higher concentrations the same carbon sources on metabolism produced organic acids, which in turn decreased the pH of media. Behavior of each strain was different for both dyes understudy due to the different chemical structure and various carbon sources as well.

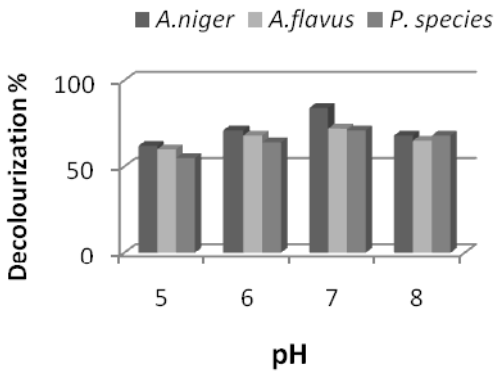

Figure 4. Effect of different pH on percentage decolourization at 120 rpm speed.

Effect of temperature on decolorization

Different temperatures (25, 30, 35 and 40°C) were used in this experiment for the optimum decolorization of dyes (Figure 5). The maximum decolorization was observed in 35°C of tested fungus cultures. The maximum decolorization of malachite green was observed 92.2±1.1% at 35°C by *Aspergillus niger* which was followed by *Aspergillus flavus*, i.e. 86.2±1.1% at the same temperature. While the maximum decolorization of tested dye was showed by *Penicillium* species at 30°C, i.e. 72.2±1.2%. Results of this study led us to consider pH and temperature of culturing. Both had significant effect on decolorization. Remazole Black B was decolorized with mix culture with optimum decolorizing temperature of 30°C [36]. Present study is thus an effort to develop an optimum condition for effective decolorizer of textile dyes. More research on the decolorization of dyes by using efficient strains is under progress. Several investigators [7,11,12] studied fungal decolourization of dye waste water. According to the results of this study and other reports, it is clear that decolorization ability of white rot fungi can be substantially increased by carefully optimizing the operational conditions such as nutrient content of the media culture, age of fungus and environmental/operational conditions. More research on the decolorization of dye industry effluents and bioremediation of dye contaminated soil using efficient strains of fungal are under progress.

CONCLUSION

The present study showed that the indigenous fungi haves the ability to remediate the dye from the effluent. It was found that at moderate dye concentration decolorization activity high as compared to higher concentration. It was also found that use of different carbon source also affects the decolorization efficiency level varying with the strains. Further, it can be suggested that dye contaminated sites can potentially be reclaimed by a low cost bioremediation process with native fungal species isolated from the dye disposal sites.

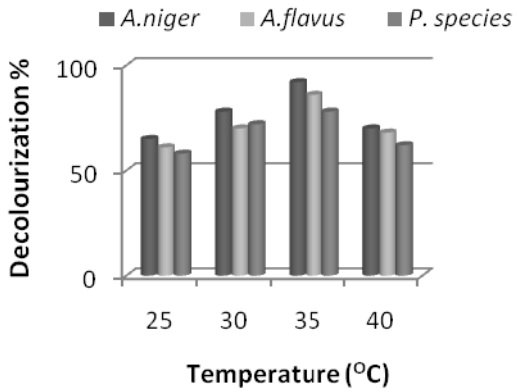

Figure 5. Effect of different temperature (°C) on percentage decolourization at 120 rpm speed.

Acknowledgements: The authors are thankful to Madhya Pradesh Council of Scientific and Technology (MPCST), Bhopal, Madhya Pradesh, India for providing the financial assistance.

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