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Bioremediation of Cotton-Textile Effluent using Fungi

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ABSTRACT
Wastewater released from textile and dye industry can cause serious environmental effects due to the presence of toxic dyes and dark coloration. Colour from this effluent makes the receiving water bodies unaesthetic affecting its water transparency and gas solubility. Over the past decade, the white rot fungi have been studied for their ability to degrade recalcitrant organo-pollutants such as polycyclic aromatic hydrocarbons, cholorphenols, and polychlorinated biphenyls. Many white rot fungi, actinomycetes and bacteria are used for the development of biological processes for the treatment of textile effluents. Many physical and chemical methods such as adsorption, coagulation, precipitation, chemical oxidation, photodegradation and filtration have been used for colour removal from wastewaters. Unfortunately, due to high operating costs and operational problems such as development of toxic intermediates, lower removal efficiency, and higher specificity for a group of dyes, among others. Apart from this, these methods produce large quantities of sludge, which again causes a problem in its disposal. Biological decolorization being simple to use and low in cost have been the main focus in recent studies, since it results in partial or complete bioconversion of pollutants to stable nontoxic end products. Many white rot fungi have been intensively studied in connection with their ligninolytic enzyme production and their decolorisation ability. The present study shows the potential of various fungi for their capacity to decolorize the textile effluent and their capability for heavy metal removal. Maximum decolorization (92%) was achieved by Trametes versicolor, whereas maximum concentration of chromium was removed by Mucor hiemalis.
***Keywords**: Textile wastewater, Decolorization, Heavy Metals*

INTRODUCTION

Textile industry is one of the oldest and largest industries of India. Not going far back in the history of textile industry in India, the East India Company started its business by cotton industry. Now-a-days, India is a major exporter of textile-finished materials. Textile industry in India is a fast growing industry [1] and its wastewater is rated as the most polluting among all industrial sectors considering both volume and composition of effluent [2]. It is a complex and highly variable mixture of many polluting substances ranging from inorganic compounds and elements to polymers and organic products [3]. It induces persistent color coupled with organic load leading to disruption of the total ecological/symbiotic balance of the receiving water stream.

The increasing trend of requirement and production of dye and dye intermediates is also associated with the anticipated generation of both liquid and solid wastes [4]. The removal of dyes from industrial effluents is becoming a major problem for the textile industry as government legislations are becoming more stringent. The effluents of wastewater in some industries such as dyestuff, textile, leather, etc., contain synthetic dyestuff and toxic chemicals [5]. About 10-20% of the dye comes in wastewater during textile processing and enters into different environmental segments [6]. Dyes have a tendency to sequester metal ions producing micro toxicity to fish and other organisms [7]. According to Inbaraj *et al.* [6], some dyes are also reported to cause allergic dermatitis, skin irritation, cancer and mutations in humans.

Moreover, the dyes without an appropriate treatment can persist in the environment for extensive periods of time and are deleterious not only for the photosynthetic processes of the aquatic plants but also for all the living organisms since the degradation of these can lead to carcinogenic substances [8-9]. The release of dyes into the environment constitutes only a small proportion of water pollution, but dyes are visible in small quantities due to their brilliance. Tightening government legislation is forcing textile industries to treat their waste effluent to an increasingly high standard. Currently, removal of dyes from effluents is being done by physico-chemical means. Such methods are often very costly and although the dyes are removed, accumulation of concentrated sludge creates a disposal problem. There is a need to find alternative treatments that are effective in removing dyes from large volumes of effluents and are low in cost, such as biological or combination systems.

Heavy metals beyond permissible limits cause direct toxicity to all living beings. Metallic effluents can have ecological impacts on water bodies leading to increased nutrient load especially if they are essential metals. These metals in effluents may increase fertility of the sediment and water column and lead to eutrophication, which leads to oxygen deficiency, algal bloom and death of aquatic life.
The microorganisms produce both constitutive and inducible enzymes to bioremediate heavy metals and chemical compounds present in wastewater. Strains of bacteria, fungi and algae can be used extensively in bioremediation of textile effluents. The majority of bioremediation studies have been concentrated upon bacterial cultures, largely excluding the fungi, even though they represent a major component of microbial life in the biosphere. For example it has been estimated that 1g of forest litter can contain as much as 1 km of fungal hyphae [10].
Microorganisms have been shown to take up heavy metals from aqueous solutions [11]. There is a need for innovative treatment technologies for the removal of heavy metal ions from wastewater. Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints. Besides the production of various relevant metabolites, fungi have been attracting a growing interest for the bioremediation of wastewater ingredients such as metals, inorganic nutrients and organic compounds [12]. White rot fungi belong primarily to the Basidiomycetes, but may also include some Ascomycetes from the order *Sphaeriales* [13]. Basidiomycetes are usually recognised by their sexual fruiting bodies (the mushrooms, toadstools and brackets) but it is the main body of the fungus, the mycelium that is of interest in bioremediation studies. The present study was undertaken to the study the biotreatment potential of various fungi in treating the textile wastewater and heavy metal removal

MATERIAL AND METHODS

The fungi used were collected from National Type Culture Collection, Forest Pathology Division, Forest Research Institute**.** Samples of the effluents and / the dead organic matter (wood, leaves, etc.) floating/ lying in and around effluent affected localities were brought to the laboratory for examination and isolation of the fungi. The textile effluent was collected from Malwa Cotton Spinning Mills Ltd. (Paonta Sahib). The experiments were conducted in the year 2006.
The following fungal cultures were used in this study: *Bjerkandera adusta, Flavodon flavus, Geotrichum candidum, Merulius tremellosus, Mucor hiemalis, Oxyporus ravidus, Pycnoporus sanguineus, Penicillium* sp., *Schizophyllum commune, Trametes hirsuta, Trametes versicolor,* and *Trichoderma* sp. Of these, *Geotrichum candium, Mucor hiemalis, Penicillium* sp., *Schizophyllum commune* and *Trichoderma* sp. have been isolated from effluent and surrounding areas.
As the pH of textile effluent was alkaline pH of the effluent was adjusted between 5.5-6.0 using buffers and dilute acids to provide optimum pH for fungal growth. After inoculation of fungus into the flasks containing effluent they were incubated at required optimum temperature. Aliquots of culture supernatant were removed at regular intervals and analyzed for colour removal.
Decolourization was monitored at maximum wavelength and over a range of wavelengths in culture supernatants using a scanning spectrophotometer. Distilled water was taken as blank. For each reading, a few mL liquid was taken from the sample, and then analyzed instrumentally. The experiment was performed in duplicate and mean readings were taken. % decolorization was calculated by using the following equation.

$$\% \text{ Decolourization} = \frac{A_0 - A_t}{A_0} \times 100$$

Where A_o = initial absorbance and A_t = absorbance after time t [14].
For heavy metals estimation effluent samples after 15 days of inoculation of the selected fungi into the flasks taking one set of flasks as control (no fungus) were digested .The solutions were then aspirated into flame atomic absorption spectrophotometer for the determination of Iron, Zinc, Chromium and Manganese at 248.3nm, 213.9nm, 357.9nm and 279.5nm respectively.

RESULTS AND DISCUSSION

The results of adaptivity and decolourisation of the fungi are shown in Table 1. The isolated fungi showed more adaptivity in the textile effluent but *Geotrichum candidum and Penicillium* sp*.,* gave a poor percent of decolorization hence these two fungi along with *Flavodon flavus* (which was used as a white rot test fungi) were not used for heavy metal removal studies.

Maximum decolorization was shown by *Trametes versicolor* followed by *Schizophyllum commune* and *Oxyporus ravidus*. Mohorcic *et al.* [15] found *Bjerkandera adusta* to decolorize synthetic textile dye Reactive Black 5 from blue black to a yellow color. Murali *et al.* [16] reported effectiveness of *Trametes hirsuta* in decolorization of methyl red and congo red dyes. This study revealed that white rot test fungi were behaving in different manner for decolourization of red dye. This can be attributed to the physiological difference and the decolorization enzymes account for the difference in the decolorization ability [17].

Other workers have also established ability of *Trametes hirsuta* in decolorizing textile effluents [18]. *Trametes versicolor* was also reported to decolorize dye effluents [19]. Biodegradation of textile dyes has been classified into two groups. In one, the authors provide no information about enzyme activity during the decolorization process [20] or the information is not related to the effect [21]. Some authors explain the process in terms of bioadsorption mainly [22]. The other group of papers shows the occurrence of degradation or biotransformation in the dye and relate the decolorization with most of the ligninolytic enzymes produced [23].

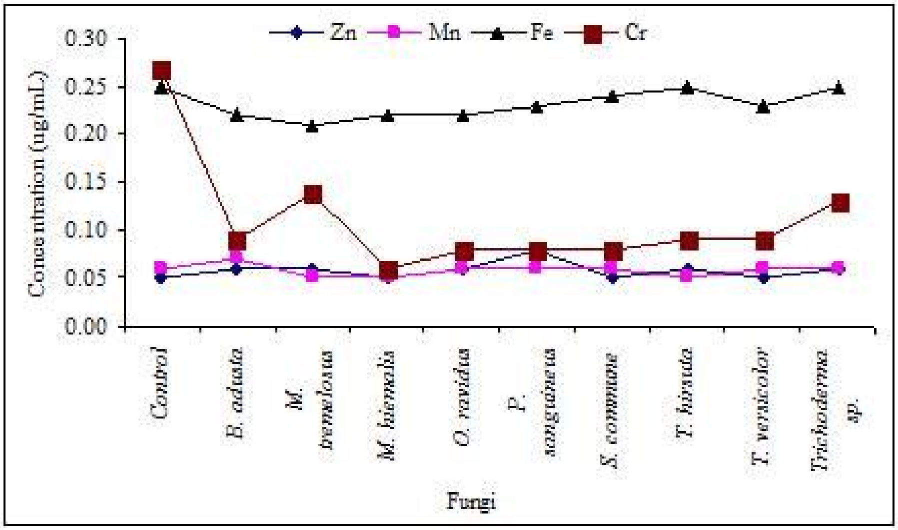
Study was also done to test the capability of fungi for heavy metal removal from effluent (Figure 1). It was observed that there was significant concentration of chromium only as compared to control. Maximum reduction in the concentration of chromium was shown by *Mucor hiemalis* followed by all other white rot test fungi. Some heavy metals were essential for the fungal metabolism, whereas others have no known biological role. Both essential and nonessential heavy metals are toxic for fungi, when present in excess. Whereas fungi have metabolic requirements for trace metals, the same metals are often toxic at concentrations only a few times greater than those required [24]. The metals necessary for fungal growth include copper, iron, manganese, molybdenum, zinc, and nickel. Nonessential metals commonly encountered include chromium, cadmium, lead, mercury and silver [25]. Mullen *et al.* [26] reported on the biosorption of Cd, Cu, Ag and La by two fungi, *Aspergillus niger* and *Mucor rouxii.* Yetis *et al.* [26] studied the adsorptive capacity of *Trametes versicolor* and found it in the order of Pb>Ni>Cr>Cd>Cu. Tham *et al.* [27] revealed that in *Ganoderma lucidum*, toxicity of heavy metals decreased in the order Hg>Cd>Cu>U>Pb>Mn=Zn. The decrease of fungal growth rate is sometimes accompanied with the increase of the lag phase. Lengthening of lag phase was also recorded on media containing Hg in case of *Pycnoporus cinnabarinus* [28].

Table 1. Preliminary screening of fungi in textile effluent and their decolourization after 30 days of incubation

Fungi	Adaptivity	Mycelial weight	Decolourization
		Gms	%
Flavodon flavus[a]	++	0.045	19.90
Geotrichum candidum[a]	+++	0.043	24.70
Bjerkandera adusta	++	0.041	69.64
Merulius tremellosus	++	0.051	74.09
Mucor hiemalis	+++	0.055	72.47
Oxyporus ravidus	++	0.043	86.64
Pycnoporus sanguineus	+	0.044	44.13
Penicillium sp[a]	+++	0.040	14.70
Schizophyllum commune	+++	0.045	88.66
Trametes hirsuta	+	0.045	80.97
Trametes versicolor	+	0.044	92.31
Trichoderma sp.	+++	0.037	72.87
SD	-	0.005	28.20
SEM±	-	0.002	11.51
CD at 5%	-	0.004	25.34

+ moderate, ++ good and +++ very good
[a] These fungi were not screened based on decolourizaton for heavy metal removal study

Figure 1. Heavy metal removal by selected fungi



CONCLUSION

In this study, the biotreatment potential and heavy metal removal from the textile wastewater using fungi were investiagted. White rot fungi show considerable promise for the decolourisation. Maximum decolorization was shown by *Trametes versicolor* followed by *Schizophyllum commune* and *Oxyporus ravidus*. Maximum reduction in the concentration of chromium was shown by *Mucor hiemalis*.
The results of the present study suggest that white rot fungi can be used for decolourising the textile wastewater. Considering the fact that, due to high operating costs and operational problems such as development of toxic intermediates, lower removal efficiency, and higher specificity for a group of dyes, among others, these methods produce large quantities of sludge, which again causes a problem in its disposal. Therefore, bioremediation using fungi can be cost effective for removing heavy metal and colour from the textile wastewater.

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