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Microbial Remediation of Tannery Effluent Using *Alternaria Brassicae* and Its Physico-Chemical Characterization

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Abstract

Industrial effluents are mainly contains huge amount of pollutants including heavy metals like chromium. In the present study, an attempt was made to isolate chromium resistant fungi from tannery effluent and was identified as Alternaria brassicae CR04. The fungi was studied for the removal of heavy metals from tannery effluent and also observed for the reduction of various pollutant levels. The dead fungal biomass of Alternaria brassicae CR04 was found to reduce the levels of BOD, COD, and other vital parameters affect the water quality. The results obtained revealed the potential of the fungi, Alternaria brassicae CR04, in the bioremediation of industrial effluents containing higher concentration of heavy metals.

Keywords: Tannery effluent, Heavy metal, Chromium, BOD

INTRODUCTION

Tannery industry is considered as one of the major source of pollution which is of potential environmental concern (Eye and Lawrence, 1971). The effluent released from tannery industries are by high biological and chemical oxygen demand, color and other inorganic impurities, dissolved and suspended solids, etc. (Rajalo and Petrovskaya, 1996; Sharma and Malaviya, 2013). The strong color hinders the light penetration and high COD results in the decreased dissolved oxygen in the aquatic environment (Raj et al., 1996). Heavy metal pollution of aquatic ecosystem is one of the resultant of industrialization. Microorganisms are possessing ability to produce a wide variety of extra cellular metabolites including organic acids, enzymes and their biomass might be used for removal or reduction or detoxification of pollutants in the industrial effluents.

The use of industrial effluents in agriculture has been practiced in recent years due to the

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presence of high concentration of major and micronutrients. Such industrial effluent contains high concentration of heavy metals which might enter into humans and animals through food chain. Therefore, it is necessary to remove such heavy metals from effluents. The conventional methods including reverse osmosis, lime coagulation, ion exchange precipitation, etc. are found to be either less efficient or quiet costly (Peters et al., 1985; Donmez and Aksu, 2001) Hence, an efficient and cheap method is required for the removal of heavy metals from the industrial effluents.

In recent years, the micro-organisms has been reported as adsorbents for the removal of heavy metals from effluents in an eco-friendly way (Veglio and Beolcmi, 1997). Several studies were available for the biosorption of heavy metals using live and dead fungal biomass. In addition, living biomass may subject to toxic effect of heavy metals at elevated concentration. To overcome the disadvantages; non-viable or dead biomass is preferred (Butter et al., 1998). Recent studies showed that the bio-sorption capacity of the heat treated cells might be greater, equivalent or less than that of their living counterparts (Arıca et al., 2003; Ahmad and Ansari, 2006). Heavy metal resistant microorganisms are mostly present in heavy metal contaminated source. Hence, it is required to isolate the heavy metal tolerant fungi from heavy metals contaminated source like tannery effluent. Against these backdrops, the present study aimed to isolate heavy metal tolerant fungi and to evaluate their efficiency in the remove heavy metals from tannery effluent under laboratory conditions.

METHODOLOGY

Effluent Sample

The tannery effluent from the final discharge unit of a tannery industry located in Chennai was collected in polyethylene containers. The collected effluent samples were brought to the laboratory and stored in a refrigerator at 4 °C till their utilization.

Microorganism

For the isolation of chromium resistant fungi, the tannery effluent sample was serially diluted and plated on PDA plates amended with potassium dichromate at 0.1 mg/ml concentration. The inoculated petriplates were incubated at 28 ºC for seven days. The chromium resistant fungal colony grown on PDA plate was picked and

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purified by repeated culturing on Potato Dextrose Agar (PDA) and further identified as *Alternaria brassicae* CR04.

Fungal biosorbent preparation

The fungal biosorbent was prepared by incubating the inoculum of *Alternaria brassicae* CR04 onto 250 ml erlenmeyer flasks containing 100 ml of potato dextrose broth (PDB) and incubated at 30 ºC for 5 days in. The mycelium obtained was filtered, air-dried and powdered using mortar and pestle. Fungal biosorbent (2% w/v) were inoculated in combined tannery effluent amended with 0.1% glucose and 0.1% ammonium nitrate. The pH was maintained at 5.0 and the flasks were incubated at 30ºC in a shaker for five days at 150 rpm. The wastewater samples were collected at different time intervals (3d, and 5d) and reduction in COD, color, Cr(VI) and other pollution parameters were measured (Smiley and Piyush, 2014).

Physico-chemical characterization of tannery effluent

The tannery effluent samples before and after treatment were analyzed for physico-chemical parameters as per standard methods. Chemical oxygen demand (COD) and total suspended solids (TSS) were determined according to American Public Health Association (APHA) methods (Greenberg et al., 1995). Color was measured spectrophotometrically (465 nm) according to the method of Bajpai *et al.* (1993). The hexavalent chromium [Cr(VI)] was determined colorimetrically using the diphenylcarbazide (DPC) method (Greenberg et al., 1995). Other parameters of the wastewater e.g. pH, electrical conductivity (EC), and total dissolved solids (TDS), sodium, chloride and nitrate ions were also measured using standard methods.

RESULTS AND DISCUSSION

The fungal strain *Alternaria brassicae* CR04 was isolated from tannery effluent by serial dilution technique. The results indicated that some native fungi have a marked adaptation to heavy metals under constant metal stress for a long time, and the toxic metals were even used as micronutrients by these growth stimulated fungi (Zhang et al., 2008). Similar to our findings, *Aspergillus niger*, *Aspergillus lentulus*, *Penicillium* sp., and *Fusarium solani* were also isolated from contaminated sites have been reported (Srivastava and Thakur, 2007; Fukuda et al., 2008; Sen et al., 2012). The tolerance to

Cr(VI) occur by various mechanisms such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions (Malik, 2004)

The tannery effluent used was dark grayish in color with unpleasant smell. The high values of pH, EC, COD, color, TSS and total dissolved solids (TDS) were recorded in the raw effluent before treatment (Table 1). Elevated amount of COD was ascribed to high amount of organic compounds which were not affected by the bacterial decomposition. The high electrical conductivity and TDS were due to the presence of inorganic substances and salts (Nagarajan and Ramchandramoorthy, 2002). The concentration of sodium, potassium, calcium, chloride and nitrate ions were also found to be present in moderate level. Various heavy metals present in the combined tannery effluent were Cr, Pb, Cu, Zn and Mn. The raw tannery effluent characteristics were found to be in agreement with the previous studies (Mishra et al., 2009; Lofrano et al., 2013.

The microorganisms including bacteria and fungi could develop potential to biodegrade the recalcitrant pollutants when they are exposed to polluted environment (Chandra and Singh, 2012). In this study, the treatment of tannery wastewater with *Alternaria brassicae* CR04 isolated from tannery effluent resulted in significant reduction of pollution parameters after five days. The final reduction in COD, color, Cr(VI), TSS, turbidity, Na+, Cl-, and NO3- was observed after fungal treatment of tannery effluent (Table 2). The decrease in COD within first three days was attributed to utilization of organics by the fungus during initial growth phase. Similarly, decolorization of tannery wastewater was achieved which might be due to the oxidative degradation of the dye molecules (Mohorcic et al., 2006).

The tannery effluent also registered reduction in pH to 4.2, due to release of organic acids by the fungi. The acidic environment might facilitated the bisorption of Cr(VI) ions. In aqueous solution, chromium exist in two oxidation states, trivalent (Cr^{3+}) and hexavalent (Cr^{6+}). At low pH, Cr^{6+} is likely to be attracted by the positively charged functional groups present on the fungal cell surface. Similarly, the reduction in TSS, turbidity, NO^{3-}, Na^{+} and Cl^{-} was might be attributed to utilization of these ions for growth by the fungi (Mert and Dizbay, 1977).

CONCLUSION

In the present investigation, the treatment of tannery effluent with *Alternaria brassicae* CR04 resulted in the reduction of COD, color, chromium, total suspended solids (TSS), turbidity, Na^{+}, Cl^{-}, and NO^{3-} after five days of duration. As the bioremediation activity is determined by the cell metabolism, which in turn depended on media components and other process parameters like pH, temperature and aeration. Thus, future detailed studies are necessary to conduct process parameter optimization studies for enhancing the bioremediation efficiency of *Alternaria brassicae* CR04.

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List of tables

Table 1. Raw effluent characteristics

Parameters	**Values**
pH	8.93
Electrical conductivity (mS cm^{-1})	33.2
COD (mg L^{-1})	5643
Turbidity (NTU)	487
Total suspended solids (mg L^{-1})	1572
Total dissolved solids (mg L^{-1})	17540
Sodium (mg L^{-1})	2985
Chloride (mg L^{-1})	4520
Nitrate (mg L^{-1})	560
Chromium (mg L^{-1})	11.5
Lead (mg L^{-1})	1.03
Zinc (mg L^{-1})	0.47

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Manganese (mg L^{-1})	0.35

Table 2. Effect of fungal mycelium on bioremediation of effluent

Parameters	**3rd Day**	**5th Day**
pH	4.6	4.2
Turbidity (NTU)	415	360
COD (mg L^{-1})	1720	1435
Total suspended solids (mg L^{-1})	1095	980
Sodium (mg L^{-1})	2750	2635
Chloride (mg L^{-1})	3350	3140
Nitrate (mg L^{-1})	257	214
Chromium (mg L^{-1})	1.53	0.00