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Bioremediation of Tannery Wastewater by *Aspergillus flavus* SPFT2

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A B S T R A C T

Aspergillus flavus SPFT2 was isolated from untreated tannery wastewater. The isolate exhibited minimum inhibitory concentration (MIC) for Cr (VI) as 500 ppm. The physico-chemical analysis revealed heavy pollution load of tannery effluent. After exposure to fungal treatment, the effluent exhibited 55.46, 61.50, 77.27, 89.80, 98.02 and 56.50% reduction in COD, color, Cr (VI), TSS, turbidity and NO_3^-, respectively after six days of treatment.

Introduction

The problem of pollution of aquatic environments due to industrial contaminants is a serious environmental concern. The tanneries are considered as highly water intensive industries. Leather processing in a tannery generally comprises three categories: pretreatment of skin/hide (beam house operations), chrome or vegetable tanning of skin/hide (tanning operation) and finishing operations (Thanikaivelan *et al.*, 2004). Nearly 30 m^3 of wastewater is generated during processing of one tonne of raw skin/hide (Suthanthararajan *et al.*, 2004). These wastewaters contain large quantities of chemical oxygen demand (COD), color, sodium sulphide, nitrate, chloride, chromium and suspended solids (SS) (Sharma and Malaviya, 2014).

These colored wastewaters hamper light penetration, whereas high COD results in decreased dissolved oxygen in the aquatic ecosystem. Similarly, chromium toxicity is also one of the major causes of environmental hazards caused by tannery effluents. Chromium exists in several oxidation states (I–VI), more stable as Cr (III). Cr (VI) is the toxic form of the element which causes severe diarrhoea, ulcers, eye and skin irritation, kidney dysfunction and probably lung carcinoma (Malaviya and Singh, 2011).

Bioremediation is a set of technologies that uses living organisms in order to degrade or transform contaminants into their less toxic forms (Vidali, 2001). It is based on the existence of microorganisms with capacity to enzymatically attack the recalcitrant

compounds. Unlike bioremediation, the physical and chemical methods are not cost effective in terms of energy and chemical consumption besides generating large quantity of sludge which renders waste disposal problematic (**M**alaviya and Singh, 2014). Therefore, in the present study an attempt has been made to remediate the tannery wastewater by *Aspergillus flavus* SPFT2 isolated from the untreated tannery effluent.

Materials and Methods

Sample Collection

The tannery effluent samples used in the study were collected in acid-rinsed polyethylene containers from the tannery located in Central Leather Research Institute (CLRI) complex, Kapurthala Road, Jalandhar, India. The collected samples were brought to the laboratory and stored in a refrigerator at 4°C to be used in further studies.

Isolation of Fungus from Effluent

For the isolation of fungal strain the tannery effluent was serially diluted, centrifuged at 900 rpm for five minutes and the supernatant was used for further enrichment in modified Lee**'**s minimal medium without glucose (0.25% KH_2PO_4, 0.20% $MgSO_4$, 0.50% $(NH_4)_2SO_4$ and 0.50% NaCl) containing tannery sludge as sole source of carbon in Erlenmeyer flasks for three days (150 rpm, 28 ºC).

This process was repeated several times with fresh sludge amended minimal salt medium (MSM). The final set of flasks were used for subsequent plating and isolation of fungi on sludge containing MSM plates. The pH of the medium was maintained at 5.3 with 100 mM L^{-1} citrate-phosphate buffer (Cardenas-Gonzalez and Rodrguez, 2010). The inoculated petriplates were incubated at 28ºC for seven days. The fungal colonies appearing on MSM were picked and purified by repeated culturing on Potato Dextrose Agar (PDA) and were further identified by National Center of Fungal Taxonomy (NCFT), New Delhi. Out of the isolated fungi, *Aspergillus flavus* was used for the treatment of tannery wastewater (Fig. 1).

Determination of Minimum Inhibitory Concentration (MIC) for Fungal Strain

The Cr-resistance of fungal isolate was evaluated on modified Lee**'**s minimal medium (with 0.25% glucose) supplemented with 100, 200, 300, 400, 500, 600, and 700 ppm concentrations of hexavalent chromium. The petriplates were inoculated with 8 mm agar plugs from young fungal colonies, pre-grown on PDA and incubated at 28ºC for seven days (Ezzouhri *et al.,* 2009)**.**

The fungal growth was used as a measure of viability and was determined by measuring the change in mycelia length with the help of measuring scale at 24 hours interval from the 3rd day to the 7th day post-inoculation (Shugaba *et al.,* 2010). The minimum inhibitory concentration for Cr (VI) [MIC Cr (VI)] was defined as the concentration of hexavalent chromium that inhibits visible growth of the fungal isolate.

Fungal Inoculum Development and Effluent Bioremediation

For bioremediation studies, the fungal inoculum was prepared in the form of pellets. Erlenmeyer flasks (250 ml capacity) containing 100 ml potato dextrose broth (PDB) and streptopenicillin (100 ppm) were inoculated with mycelial discs. These flasks were incubated at 30±1°C for 6 days in orbital shaker (Scigenics Biotech, India) at 150 rpm. The mycelium thus obtained was

filtered by cheesecloth and air-dried on sterilized petriplates. The fungal pellets (2% w/v) were inoculated in tannery wastewater amended with 0.1% glucose and 0.1% ammonium nitrate.

The pH was maintained at 5.3 and the flasks were incubated at 30±1°C in orbital shaker for six days at 150 rpm. The wastewater samples were collected at different time intervals (2d, 4d and 6d) and reduction in COD, color and other pollution parameters was measured. For evaluation of fungal growth, biomass collected by centrifugation was washed thrice with sterile distilled water and dried at 80°C till constant weight and values were recorded.

Analytical Method

The tannery wastewater samples were analyzed for physico-chemical parameters as per standard methods for wastewater analysis. 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)**.** AAS 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) were measured using Multi Parameter Water Analyzer Kit (WTW, Germany). Sodium, chloride and nitrate ions were measured by Thermo Scientific Orion DUAL STAR ion meter while turbidity was measured by Digital Turbidity Meter (Environmental and Scientific Instruments Co., India).

Results and Discussion

Isolation and Screening of Fungi

The fungal strain *Aspergillus flavus* SPFT2 was isolated from untreated tannery effluent by serial dilution technique. The isolate exhibited MIC for Cr (VI) as 500 ppm. 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)**.**

Tannery Effluent Characterization Before and After Fungal Treatment

The combined tannery effluent used in bioremediation studies was dark grayish in color with unpleasant smell. The result indicated the mean values of pH, COD, color, Cr, TSS, turbidity, Na^+, Cl^- and NO_3^- were 9.16±0.20, 5776±30.10, 1984.85±12.80, 12.260±0.556, 1694 ±11.20, 505 ±2.00, 3080 ±35.60, 4700±40.10 and 600 ±5.00 (Table 1). High BOD and COD values show that the effluent contained highly oxygen demanding wastes which cause the depletion of DO, which is a fundamental requirement for aquatic life (Kumar, 1989). The high levels of total suspended solids present in the tannery wastewater could be ascribed to their accumulation during the processing of finished leather (Deepa *et al.,* 2011). These colloidal and suspended impurities cause turbidity in the receiving streams and reduce the light penetration into water and ultimately decrease the photosynthesis (Nosheen *et al.,* 2000). Similarly, dissolved impurities like Na^+, Cl^- and NO_3^- increase salinity of the water and thus may render it unfit for irrigation or consumption.

Table.1 Physico-Chemical Characteristics of Raw Tannery Effluent

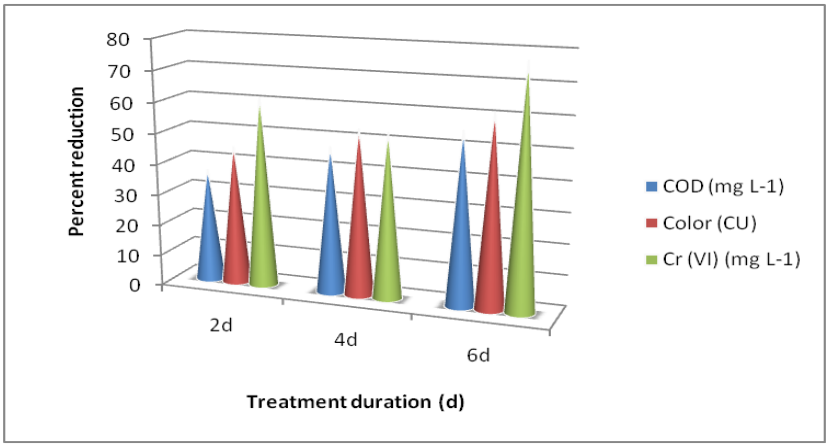
Parameters	**Values**
pH	9.16±0.20
TDS (mgL^{-1})	17650±20.10
TSS (mgL^{-1})	1694±11.20
Turbidity (NTU)	505±2.00
COD (mgL^{-1})	5776±30.10
Color (CU)	1984.85±12.80
EC ($mS\ cm^{-1}$)	35.3±0.25
Na^{+} (mgL^{-1})	3080±35.60
Cl^{-} (mgL^{-1})	4700±40.10
NO_3^{-} (mgL^{-1})	600±5.00
Ca^{2+} (mgL^{-1})	258±12.00
K^{+} (mgL^{-1})	290±11.50
Cr (VI)((mgL^{-1})	12.260±0.556
Pb(II) (mgL^{-1})	0.965±0.0140
Total Pb (mgL^{-1})	1.126±0.0131
Cu (mgL^{-1})	0.258±0.0013
Zn (mgL^{-1})	0.529±0.0028
Mn (mgL^{-1})	0.392±0.0090

Table.2 Physico-Chemical Characteristics of Tannery Effluent After Different Treatment Durations with *Aspergillus Flavus* Spft2

Parameters	**Treatment duration**		
	2d	**4d**	**6d**
pH	5.80±0.11	5.25±0.16	4.50±0.10
COD ($mg\ L^{-1}$)	3648 ±90 (36.84)	3035.66±85 (47.44)	2572.66±72 (55.46)
Color (CU)	1090.45±36.75 (45.06)	918.59±20.87 (53.72)	764.12±15.28 (61.50)
Cr (VI) ($mg\ L^{-1}$)	5.691±0.032 (61.50)	2.786±0.023 (53.28)	0.968±0.021 (77.27)
TSS ($mg\ L^{-1}$)	386±12 (77.21)	224.52±10.90 (86.75)	172.80±8.72 (89.80)
Turbidity (NTU)	112±14 (77.82)	089±8 (82.38)	010±6 (98.02)
Na^{+} ($mg\ L^{-1}$)	2340±14.80 (24.02)	2110±12.11 (31.49)	2070±17.00 (32.79)
Cl^{-} ($mg\ L^{-1}$)	3880±12.96 (17.45)	3830±16.56 (18.51)	3750±12.68 (20.21)
NO_3^{-} ($mg\ L^{-1}$)	324±3.60 (46.00)	281±2.68 (53.17)	261±6.50 (56.50)

Fig.1 *Aspergillus flavus* Spft2 Colony

Fig.2 Reduction of Cod, Color and Cr(Vi) of Tannery Effluent After Treatment with *Aspergillus flavus* Spft2



The physico-chemical characteristics of the tannery wastewater after different treatment durations with *Aspergillus flavus* SPFT2 are shown in Table 2 and Fig 2. After six days of fungal treatment, there was 55.46% reduction in COD, which was attributed to uptake and degradation of organics by the fungus. On the final day of the treatment, 61.50% decolorization of the effluent was achieved which was ascribed to oxidative degradation of the dye molecules (Mohorcic *et al.*, 2006). The tannery wastewater also marked reduction in pH from 5.30 to 4.50, due to release of organic acids by the fungal isolate. The acidic environment facilitated the biosorption of Cr (VI) ions, resulting into 77.27% reduction of Cr (VI) on final day of the treatment.

The ions like sodium, chloride and nitrate have shown 32.79, 20.21 and 56.50% reduction on sixth day of the treatment. Likewise, TSS and turbidity have shown 89.80 and 98.02% reduction, which was ascribed to entrapment of suspended solid particles by the filamentous fungi (Fakhrul-Razi and Molla, 2007).

In conclusion, *Aspergillus flavus* SPFT2 isolated from untreated tannery effluent

exhibited detoxification of tannery wastewater. The treatment of tannery effluent with *Aspergillus flavus* resulted in the reduction of COD, color, Cr(VI), total suspended solids (TSS), turbidity, Na+, Cl^- and NO_3^- in the order of 55.46, 61.50, 77.27, 89.80, 98.02, 32.79, 20.21 and 56.50%, respectively after six days of duration. For improved commercial use of the fungus, immobilization on suitable carrier is recommended.

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