International Journal of Basic and Applied Sciences Vol. 2. No. 3. 2013. Pp. 88-93

Full Length Research Paper

Bioremediation of Tannery Wastewater by *Aspergillus niger* SPFSL 2 - a Isolated from Tannery Sludge

Smiley Sharma and Piyush Malaviya*
Department of Environmental Sciences, University of Jammu, Jammu (J&K)

Corresponding Author: Piyush Malaviya

Abstract
Tannery wastewaters cause a recurrent environmental pollution problem owing to high values of chemical oxygen demand (COD), color, and total dissolved solids (TDS). In the present study, tannery wastewater was subjected to biological treatment by Aspergillus niger SPFSL2-a isolated from tannery sludge. The treatment resulted in the reduction of COD and color of the wastewater in the order of 81.58% and 62.21%, respectively after six days. A major part of reduction in these parameters occurred within initial two days of the treatment, and during this period, total dissolved solids, electrical conductivity, total suspended solids and turbidity of the wastewater have also shown decline. The efficacy of treatment with this isolate was evaluated by using germination tests on Triticum aestivum L. var. Raj-3077. Germination tests confirmed the reduction of toxicity of the treated wastewater since while using treated tannery wastewater the germination percentage increased up to 60% as compared to the values obtained with untreated tannery wastewater (10%).

***Key words**: Aspergillus niger, Bioremediation, Decolorisation, Germination, Tannery wastewater, Toxicity*

Introduction

The problem of water and soil pollution due to tanneries is a serious environmental threat especially in the developing countries. In India, there are more than 2,500 tannery units, scattered all over the country, with an annual capacity of processing 0.7 million tons of hides and skins (Rajamani, 1995; Ram *et al*., 1997). Leather processing in a tannery generally comprises three categories: pre-treatment of skin/hide (beamhouse operations), chrome or vegetable tanning of skin/hide (tanning operation) and finishing operations (Stoop, 2003; 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 contains large quantities of chemical oxygen demand (COD),color, sodium sulphide, nitrate, chloride, chromium and suspended solids (SS) (Szpyrkowicz *et al*., 2001).The colored wastewaters hamper light penetration (Goncalves *et al*., 2000), whereas high COD resulted in decreased dissolved oxygen in the aquatic ecosystem (Raj *et al*., 1996). Additionally, high concentrations of dissolved solids make the possible discharge of tannery wastewaters into water bodies problematic, as they cause eutrophication and other adverse environmental effects (Durai and Rajasimman, 2011).Commonly applied treatment methods for tannery wastewaters consists of integrated processes involving various combinations of physical and chemical methods (Schrank *et al*., 2005; Malaviya and Singh, 2011). These integrated treatment methods are efficient but not cost effective in terms of energy and reagent consumption besides generating large quantity of sludge which renders waste disposal problematic (Chu, 2001).

There are some studies which have been conducted employing bacteria for remediation of components of tannery wastewater (Thacker *et al*., 2006; Srivastava and Thakur, 2006a) or fungal mycelia as bioabsorbent (Bai and Abraham, 2002; Srivastava and Thakur, 2006b). But, most of the studies are pollutant specific targeting only one or two specific pollutants mostly chromium. Moreover, fungal decolorisation studies have been performed on single model dyes at low concentrations, but these conditions are poorly predictive of the actual decolorisation efficiency in real wastewaters where dyes are usually present as a mixture and often in quite high concentrations (Couto, 2009). In the present work, *Aspergillus niger* SPFSL2-a isolated from the tannery sludge was used for bioremediation study and was found to be significantly effective in the reduction of COD and color of tannery wastewater. The fungal efficiency of the treatment was further supported by germination experiments on *T. aestivum* L. var. Raj-3077.

Materials and Methods

Tannery wastewater characterization

Tannery wastewater was collected from a tannery located in Central Leather Research Institute, (CLRI) complex, Kapurthala Road, Jalandhar (India) and stored in a refrigerator at 4°C. 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 (465nm) according to the method of Bajpai *et al.* (1993). Other parameters of the wastewater e.g. pH, electrical conductivity (EC), 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 (Model, 331E).

Isolation of fungus and bioremediation experiments

The fungal strain was isolated from tannery sludge by dilution plating method (Mohamed *et al*. 2011), and it was identified as *Aspergillus niger* by National Center of Fungal Taxonomy (NCFT), New Delhi. 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 taken and 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.

Toxicity testing of tannery wastewater

For toxicity assessment, seeds of *T. aestivum* L. var. Raj-3077 were surface sterilized with 2.0% $HgCl_2$ solution (Ahsan *et al*., 2007) and ten seeds were placed in glass petridishes lined with two Whatman No.1 filter paper discs. These filter discs were moistened with 5 ml of tap water for control and with the same volume of untreated and treated tannery wastewater samples followed by incubation at 25±1°C in a BOD incubator (Scigenics Biotech, India) for a period of six days. The visible protrusion of radical from seed coat was taken as criterion of seed germination. Various germination indices adopted from Czabator (1962), Rao *et al*. (1979) and Zucconi *et al*. (1981) were used to record the germination parameters and seedling growth.

Results and Discussion

Tannery wastewater characterization

The physico-chemical analysis of untreated tannery wastewater has shown alkaline pH, high COD, color, TDS, TSS, chloride, sodium and nitrate as mentioned in Table 1.The electrical conductivity was also high due to the presence of inorganic substances and salts whereas elevated amount of COD may be due to high amount of organic compounds which are not affected by the bacterial decomposition (Nagrajan and Ramachandramoorthy, 2002). These results are in agreement with the previous studies (Alvarez-Bernal *et al*., 2006; Mishra *et al*., 2009).

Table 1: Physico-chemical characteristics of tannery wastewater

S.No.	Parameters	Values*
1.	pH	9.16±0.20
2.	TDS (mg/l)	17650±20.10
3.	TSS (mg/l)	1694±11.20
4.	Turbidity (NTU)	505±2.0
5.	COD (mg/l)	5776±30.10
6.	Color (CU)	1984.85±12.80
7.	EC (mS /cm)	35.3±0.25
8.	Cr(VI) (mg/l)	9.86±0.18
9.	Na^+ (mg/l)	3080±35.60
10.	Cl^- (mg/l)	4700±40.10
11.	NO_3^- (mg/l)	600±5.0

*=Mean ±SD (n=3)

Treatment of tannery wastewater

There was significant reduction in COD and other physico-chemical parameters of tannery wastewaters following six day treatment with *A. niger* SPFSL2-a (Table 2). The results of the present study revealed that the COD removal was highly influenced by fungal treatment of tannery wastewater because there was significant reduction of COD values (60.52%) within two days of fungal treatment, whereas final COD reduction on sixth day was 81.58%. This was ascribed to the uptake of organic matter as a carbon source by the fungus (Pechsuth *et al*., 2001), which was confirmed by increase in fungal biomass during the treatment period (Fig. 1)**.** Mohamed *et al*. (2011) reported only 19.42% COD reduction of tannery wastewater after three day treatment with *A. niger*, the high COD reduction in the present study might be attributed to competency of *A. niger* SPFSL2-a as it was directly isolated from the

contaminated site (tannery sludge). In similar studies, 52.5-95% COD reduction was reported in distillery wastewater treatment with *A. niger* (Miranda *et al*., 1996; Pal and Vimala, 2012), while, 60% decrease in COD by the same isolate was reported during treatment of poplar alkaline peroxide mechanical pulping wastewater (Liu *et al*., 2011). The color in tannery wastewater is a contribution of dissolved solids, tannins and synthetic dyes etc. The color in wastewaters not only affects the aesthetics and water transparency but also affects gas solubility of water bodies (Yuxing and Jian, 1999). In the present study, 62.21% decolorisation of tannery wastewater was achieved after six days of fungal treatment. The decolorisation was possibly achieved by oxidative degradation of the dye molecules (Mohorcic *et al*., 2006). Several authors (Miranda *et al*., 1996; Pal and Vimala, 2012; Liu *et al*., 2011) have also reported 43-69% color reduction in distillery wastewater and poplar alkaline peroxide mechanical pulping wastewater by *A. niger*.

High level 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). Presence of total suspended solids in water leads to turbidity resulting in poor photosynthetic activity in the aquatic system (Goel, 2000) and clogging of gills and respiratory surfaces of fishes (Alabaster and Lloyd, 1980). After six days of fungal treatment there was 92.55% and 99.00% reduction in TSS and turbidity, respectively because filamentous fungi entrap the suspended solid particles in the wastewater (Alam *et al*., 2001; Fakhrul-Razi and Molla, 2007). The reduction in NO_3^-, Na^+ and Cl^- ions might be attributed to utilization of these ions for growth by the fungal isolate (Mert and Dizbay, 1977).

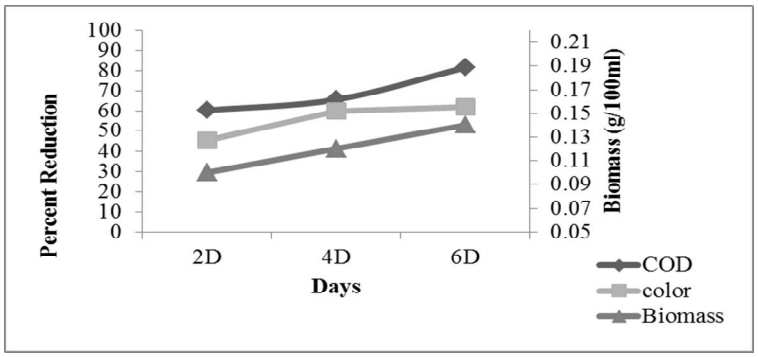

Fig1. Growth response of *A. niger* SPFSL2-a and reduction in COD and Color of tannery wastewater.

Table 2: Physico-chemical characteristics*of tannery wastewater after treatment with *A. niger* SPFSL2-a (Figures in parenthesis show % reduction)

	Treatment duration (days)		
Parameters	**2d**	**4d**	**6d**
pH	5.86±0.31	5.81±0.19	5.66±0.19
COD (mg/l)	2280±21.20 (60.52)	1976±18 (65.79)	1064±17.10 (81.58)
Color (CU)	1083.33±25.30 (45.42)	795.45±22.50 (59.92)	750 ±11.40 (62.21)
TDS (mg/l)	16600±35.10 (5.95)	15800±31.80 (10.48)	15550±29.10 (11.90)
TSS (mg/l)	818.33±12.10 (51.69)	135±3.50 (92.03)	126.20±4.0 (92.55)
Turbidity (NTU)	169±3.0 (66.53)	105±1.0 (79.21)	005±0.05 (99.00)
EC (mS /cm)	33.2 ±1.20 (5.95)	31.6±1.10 (10.48)	31.1 ±0.77 (11.90)
Na^+ (mg/l)	2560±40.0 (16.88)	2090±35.30 (32.14)	1840±20.0 (40.26)
Cl^- (mg/l)	3970±30.40 (15.53)	3790±22.20 (19.36)	3630±18.30 (22.76)
NO_3^- (mg/l)	533±5.0 (11.17)	405±4.10 (32.50)	212±2.80 (64.67)

*=Mean ±SD (n=3)

Toxicity evaluation of treated tannery wastewater

The effect of untreated and treated tannery wastewater on various germination parameters of *T. aestivum* L. var. Raj-3077 are shown in Table 3. It was observed that untreated tannery wastewater was highly toxic in nature and showed only 10% seed germination but after treatment, the toxicity of tannery wastewater was reduced significantly and showed 60% seed germination. Further, regarding the seedling growth (i.e. shoot length and root length), the present study has revealed that the seeds exposed to untreated tannery wastewater showed poor root and shoot development (0.10 and 0.30 cm) as compared to fungal treated tannery wastewater (1.5 and 1.9 cm). The inhibitory effect of untreated tannery wastewater on seed germination and seedling growth might be attributed to the high salt load and metal content, which induces high osmotic pressure and anaerobic conditions (Saxena *et al*., 1986; Ramana *et al*., 2002). The high osmotic pressure and anaerobic conditions impede various physiological and biochemical processes of seed germination and seedling growth such as movement of solute, respiration and enzymatic steps of seed germination and seedling growth (Ahsan *et al*., 2007). It was also reported that high salt load and metal content can act as inhibitor for plant hormones (amylases, auxins, gibberlines and cytokinins), which are mainly required for seed germination, seedling growth and development of plants (Ahsan *et al*., 2007).

Table 3: Effect of untreated and treated tannery wastewater on seed germination and seedling growth of *Triticum aestivum* L. var. Raj-3077*

Parameters	**Treatments**		
	Control	**UTW**	TW
Germination (%)	70	10	60
Germination index (%)	-	0.38	34.58
Speed of germination	7.00	0.33	6.00
Delay index	-	2.0	1.00
Peak value	23.33	3.30	15.00
Germination value	1633.1	33.00	900
Percent inhibition	-	60	10
Root length (cm)	3.73±0.14	0.10±0.02	1.50±0.02
Shoot length (cm)	4.17±0.9	0.30±0.01	1.90±0.04
Seedling length (cm)	7.90±0.8	0.40±0.02	3.40±0.6
Root/Shoot ratio	0.89±0.06	0.33±0.01	0.79±0.04
Seedling Vigor index	553	4.0	204

*= Mean ±SD (n=3): UTW=Untreated wastewater, TW=Treated wastewater

Conclusion

The study concluded that *Aspergillus niger* SPFSL2-a isolated from tannery sludge has ability to detoxify and decolorise the tannery wastewater. Moreover, the toxicity assessment by seed germination test on *Triticum aestivum* showed the increase in germination and seedling growth in treated tannery wastewater as compared with untreated wastewater. A more complete study should be performed on the process parameter optimization such as temperature, pH, inoculum size, agitation, etc. so as to enhance the bioremediation potency of the isolate.

Acknowledgements

P.M. gratefully acknowledges the financial support provided by Department of Biotechnology (DBT), Government of India, in the form of the research project (BT/PR10450/BCE/08/642/2008).

References

Ahsan, N.; Lee, S.H.; Lee, D.G.; Lee, H., Lee, S.W., Bahk, J.D., Lee, B.H. (2007). Physiological and protein profiles alternation of germinating rice seedlings exposed to acute cadmium toxicity. Comptes Rendus Biologies 330: 735–746.

Alabaster, J.S. and Llyod, R. (1980). Water quality criteria for fresh water fish, Butterwords London.

Alam, M. Z. and Fakhrul-Razi, A. (2001). Enhanced Settleability and Dewaterability of Fungal Treated Domestic Wastewater Sludge by Liquid State Bioconversion Process. Water Res.37: 1118-1124.

Álvarez-Bernal, D.; Contreras-Ramos, S.M.; Trujillo-Tapia, N.; Olalde- Portugal, V.; Frías-Hernández, J.T. and Dendooven, L. (2006). Effects of tanneries wastewater on chemical and biological soil characteristics. Appl. Soil Ecol. 33:269-277.

Bai, R.S.and Abraham, T.E. (2002). Studies on enhancement of Cr (VI) biosorption by chemically modified biomass of *Rhizopus nigricans*. Water Res. 36:1224-1236.

Bajpai, P.; Mehna, A. and Bajpai, P.K. (1993). Decolorization of Kraft bleach plant effluent with white-rot fungus *Tramates versicolor*. Process Biochem**.** 28: 377-384.

Chu,W. W. (2001). Dye removal from textile dye wastewaters using recycled alum sludge. Water Res. 35:3147-3152.

Couto, S.R. (2009). Dye removal by immobilisedfungi. Biotechnol. Advances. 27: 227-235.

Czabator, F. J. (1962). Germination value, an index combining speed and completeness of pine seed germination. For. Sci. 8: 386-396.

Deepa, S.; Valivittan, K.;Vincent, I. and Tharadevi, C.S. (2011). Characterisation of tannery effluent, Thirumudivakkam,Chennai, Tamil Nadu. J. basic and applied boil.5:265-270.

Durai, G. and Rajasimman, M. (2011). Biological treatment of tannery wastewater—a review. J Environ. Sci. Technol. 4:1-17.

Fakhrul-Razi, A. and Molla, A.H. (2007). Enhancement bioseparation and dewaterability of domestic wastewater sludge by fungal treated dewatered sludge. J. Hazard. Mater. 147: 350–356.

Goel, P.K. (2000). Water pollution causes, effects and control. New Age International Publication Ltd., Publ. New Delhi 269.

Goncalves, I.M.C.; Gomes, A.; Bras, R.; Ferra, M.I.A.; Amorin, M.T.P. and Porter, R.S. (2000).Biological treatment of effluent containing textile dyes. Coloration Techno. 116 : 393-397.

Greenberg, A.E.; Connors, J.J.; Jenkins, D. and Franson, M.A. (1995). Standard Methods for the Examination of Water and Wastewater, 15th ed. American Public Health Association, Washington, DC.

Liu,T.; Hu, H.; He, Z. and Ni, Y.(2011). Treatment of poplar alkaline peroxide mechanical pulping (APMP) effluent with *Aspergillus niger*. Bioresource Technol. 102:7361–7365.

Malaviya, P. and Singh, A. (2011). Physicochemical Technologies for Remediation of Chromium-Containing Waters and Wastewaters. Crit. Rev. Env. Sci. Technol. 41:1111–1172.

Mert, H.H. and Dizbay, M. (1977).The effect of osmotic pressure and salinity of the medium on the growth and sporulation of *Aspergillus niger* and *Paecilomyces lilacinum* species. Mycopathologia. 61:125-127.

Miranda, M.P.; Benito, G.G; Cristobal, N.S. and Nieto, C.H. (1996). Color elimination from molasses wastewater by *Aspergillus niger*.Bioresource Technol. 57: 229-235.

Mishra, K.; Gupta, K. and Rai, U.N. (2009). Bioconcentration and phytotoxicity of chromium in *Eichhornia crassipes*. J. Environ. Biol.30: 521-526.

Mohamed, A.M.; Sekar, P. and John, G. (2011). Efficacy of microbes in bioremediation of tannery effluent. Int. J. of Current Res. 3: 324-326.

Mohorcic, M.; Teodorovic, S.; Golob, V. and Friedrich, J. (2006). Fungal and enzymatic decolorization of artificial textile dye baths. Chemosphere 63: 1709–1717.

Nagarajan, P. and Ramchandramoorthy, T.R. (2002). Oil and grease removal from steel industry wastewater by chemical treatment. J. Ecotoxicol. Environ. Monit. 12: 181-184.

Pal, S. andVimala, Y. (2012). Bioremediation and decolorization of Distillery effluent by novel microbial consortium. European J. Expt. Biol. 2: 496-504.

Pechsuth, M.; Prasertsan, P. and Ukita, M. (2001). Biopretreatment of palm oil mill effluent by thermotolerant polymer-producing fungi. Songklanakarin J. Sci. Technol. 23: 771-777.

Raj, E.M.; Sankaran, D.P.; Sreenath, S.K.; Kumaran, S. and Mohan, N. (1996). Studies on treated effluent characteristics of a few tanneries at Crompet, Madras. Indian. J. Environ. Protect. 16:252- 254 .

Rajamani, S. (1995). Environment management in tanneries, feasible chromium recovery and reuse system. Proc. 3rd Int. Conf. on appropriate waste management technologies for developing countries, 965-973.

Ram, B.; Bajpai, P.K. and Parwana, H.K.(1997). Characteristics and pretreatment of tannery wastewater: A case study. Ind. J. Environ. Protect. 17: 175-183.

Ramana, S.; Biswas, A.K.; Singh, A.B. and Yadav, R.B.R. (2002). Relative efficacy of different distillery wastewaters on growth, nitrogen fixation and yield of groundnut. Bioresearch Technol. 8:117–121.

Rao, J.V.S.; Rama, M.R.K.and Samba, M.S. (1979). Allelopathic effect of some weed of vegetable crops on the germination and early seedling growth of bajra. Tropical Ecol. 20: 5-8.

Saxena, R.M.; Kewa, P.F.;Yadav, R.S. and Bhatnagar, A.K. (1986). Impact of tannery effluent on some pulse crop. Ind. J. Environ. Health. 28: 345- 348.

Schrank, S. G.;Jose, H. J.; Moreira, R. F. P. M. and Schroder, H. (2005). Applicability of Fenton and H2O2/UV reactions in the treatment of tannery wastewaters. Chemosphere 60:644-655.

Srivastava, S. and Thakur, I.S. (2006b). Isolation and process parameter optimization of *Aspergillus* sp. for removal of chromium from tannery effluent. Bioresource Technol. 97:1167-1173.

Srivastava, S.and Thakur, I.S. (2006a). Evaluation of biosorption potency of *Acinetobacter* sp. for removal of hexavalent chromium from tannery effluent. Biodegradation. DOI 10.1007/s10532-006-9096-0.

Stoop, M.L.M. (2003). Water management of production systems optimized by environmentally oriented integral chain management: case study of leather manufacturing in developing countries. Technovation 23:265–278.

Suthanthararajan, R.; Ravindranath, E.; Chitra, K.; Umamaheswari, B.; Ramesh, T. and Rajamani, S. (2004). Membrane application for recovery and reuse of water from treated tannery wastewater. Desalination 164:151–156.

Szpyrkowicz, L.; Kelsall, G.; Kaul, S. and Faveri, M. (2001). Performance of electrochemical reactor for treatment of tannery wastewaters. Chem. Eng. Sci. 56:1579-1586.

Thacker, U.; Parikh, R.; Shouche, Y.andMadamwar, D. (2006). Hexavalent chromium reduction by *Providencia* sp. Process Biochem. 41: 1332-1337.

Thanikaivelan, P.; Jonnalagadda R.R.; Balachandran, N.U. and Ramasami, T. (2004). Progress and recent trends in biotechnological methods for leather processing. Trends Biotechnol. 22: 181–188.

Yuxing, W. and Jian, Y. (1999). Decolorization of synthetic dyes and wastewater from textile. Water Res. 33: 3512 -3520.

Zucconi, F.; Pera, A.; Forte, M. and De Bertoldi, M. (1981). Evaluating toxicity of immature compost. BioCycle. 22: 54-57.