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# UTILIZING *Aspergillus niger* FOR BIOREMEDIATION OF TANNERY EFFLUENT

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Abstract: Tannery waste water is characterized by highly toxic ammonium, sulfates, surfactants, acids, dyes, sulfonated oils and organic substances, including natural or synthetic tannins. This study was designed to study the potential of *Aspergillus niger* for bioremediation of tannery effluent. Addition of glucose as a carbon source in the tannery effluent encouraged the growth of *A. niger* but there was no change in physico-chemical parameters. The toxic effects were mostly reduced after treatment when 20% mineral salt medium was added in tannery effluent. Colour, COD, TS, TDS, TSS, chlorides, sulfides and chromium reduction were 71.9%, 72.1%, 69.0%, 65.0%, 68.1%, 66.8%, 65.7% and 57.8%, respectively.

Keywords: *Aspergillus niger*; Bioremediation; Chromium; Tannery effluent.

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## INTRODUCTION

Environmental pollution has become a global concern. The toxic pollutants include acids, alkalies, oils, fats, floating organic dissolved matter and colouring agents. There are various industries such as tannery, paper and pulp, sago, sugar, distillery, etc. which contribute to this pollution. The disposal of waste waters is of widespread national concern. Industrial activities generate a large number and variety of waste waters which are generally discharged into water streams. There are several physical and chemical treatment methods such as electrochemical method employed to remove heavy metals in the effluent. The primary and pretreatment methods were used for the removal of suspected organic and inorganic solids. But the most reliable way seems to be the biological treatment in which microorganisms serves as an efficient detoxifiers of pollutants. It is cost effective and therefore highly suitable for reduction of pollutant load of an effluent as microorganisms are capable of oxidizing the organic and inorganic constituents (Chaturvedi, 1992). The tannery industry represents an important sector in the economy of many countries. Tanneries generate wastewater with high concentrations of suspended solids, BOD, COD and tannins including chromium (Nandy *et al.,* 1999). Major problems are due to wastewater containing heavy metals, toxic chemicals, chloride, lime with high dissolved and suspended salts and other pollutants (Uberai, 2003). Tanneries are a major source of chromium pollution and release Cr(VI) ranging from 40–25,000 mg/l of wastewater. Hexavalent chromium is carcinogenic, even with a little quantity, 10mg/l can cause nausea, vomiting, skin irritation and problems related to respiratory tract, can cause lung carcinoma due to chromium toxicity (Palmer and Puis, 1994). Tannins are toxic to plants, animals and soil as well as aquatic organisms. In plants they cause stunting growth, chlorosis and reduction in yield. However, a few microorganisms degrade tannins and utilize their carbon source. *Chaetomium globosum*, *C. cupreum*, *Fusarium solani*, *Aspergillus niger* and *Trichoderma viride* utilizes tannins as carbon source. Species of *Rhizobium*, *Pseudomonas putida*, *P. solanacearum* grow luxuriantly when cultured in tannin medium (Mahadevan and Sivaswamy, 1985). A review of literature (Akthar and Mohan, 1995; Bosshard *et al.,* 1996; Andleeb *et al.,* 2010; Sharma and Gupta, 2012) revealed that *A. niger* is very potent microorganism for the degradation of various xenobiotics, therefore, the present study is aimed to reduce pollution load of tannery effluents by using *A. niger*.

## EXPERIMENTAL

**Microbial culture:** *Aspergillus niger* Tiegh. used in the current study was procured from the National Type Culture Collection (NTCC), Forest Pathology Division, Forest Research Institute, Dehradun and maintained in Potato Dextrose Agar (PDA) medium and stored at 4°C.

**Biodegradation Study:** Degradation of tannery effluent was carried out in three different conditions. The pH of effluent was adjusted to 7.0 and taken in Erlenmeyer flasks (250 ml) containing 100 ml of effluent and autoclaved. In first set of experiment only crude effluent was taken. In second set of experiment, sterilized glucose solution added in tannery effluent aseptically to maintain the final concentration 1.0 % (w/v). In another set, 20 % (v/v) of mineral salts medium (MSM) (Bekatorou *et al.,* 2007) (g/l): $MgSO_4 \cdot 7H_2O$: 0.005; $CaCl_2 \cdot 2H_2O$: 0.005; $NH_4H_2PO_4$: 0.5; $FeSO_4 \cdot 7H_2O$: 0.001; $CuSO_4 \cdot 5H_2O$: 0.02; $MnSO_4$: 0.001) sterilized separately and then mixed with tannery effluent (80 ml effluent+ 20 ml MSM) aseptically in a laminar flow. The flasks were inoculated with ten plugs from 7 days old culture of *A. niger* (5 mm diameter) grown in PDA plates and incubated in BOD incubator at 26±1ºC for 15 days. Control samples for each set of experiment were also maintained separately without inoculation of fungal discs. All experiments were carried out in triplicates. After incubation period, the treated effluents were centrifuged at 5000 rpm for 15 min. The supernatants were used for analytical determinations. The fungal biomass of *A. niger* was determined by gravimetric measurements after drying at 70°C to constant weight. Color reduction was measured at 465 nm in a UV-Vis spectrophotometer (Bajpai *et al.,* 1993). Other physico–chemical parameters like pH, TS, TDS, TSS, COD, chlorides, sulfides and chromium were carried out before and after treatment. The concentration of each of the component was determined as per the procedure outlined in APHA (American Public Health Association) (Greenberg *et al.,* 1992).

## RESULTS AND DISCUSSION

Animal skin is transformed to leather through three operations: tanning, retanning and dyeing, and painting. The effluent of each step is mixed in a later step to produce a homogenized effluent, characterized as recalcitrant, which is highly pollutant. The composition of the effluent in leather industries is generally depending on the leather treatment steps. The complexity in composition of tannery effluents makes its biodegradation very difficult. The dark grayish-black colour of tannery effluents is due to the presence of organic, inorganic matter and chemicals used for the processing of leather. Various types of processes and finishing solvent and auxiliaries are used as well. It has been reported that only about 20% of the large number of chemicals used in the tanning process is absorbed by leather, the rest are released as waste.

Table 1 and Fig. 1 shows that the colour of the sample changed after treatment indicating the degradation of tannery effluent by *A. niger*. The pH was slightly changed to acidic (6.5) from the initial value of 7.0 and TDS, TSS and TS were also reduced after treatment, signifying the degradation of toxic solid components in the effluent. The decrease in level of COD indicates the reduction of biologically oxidisable and inert organic materials as result of the degradation by the fungus. Reduction in chlorides, sulfides and chromium from the effluent after treatment makes it less toxic.

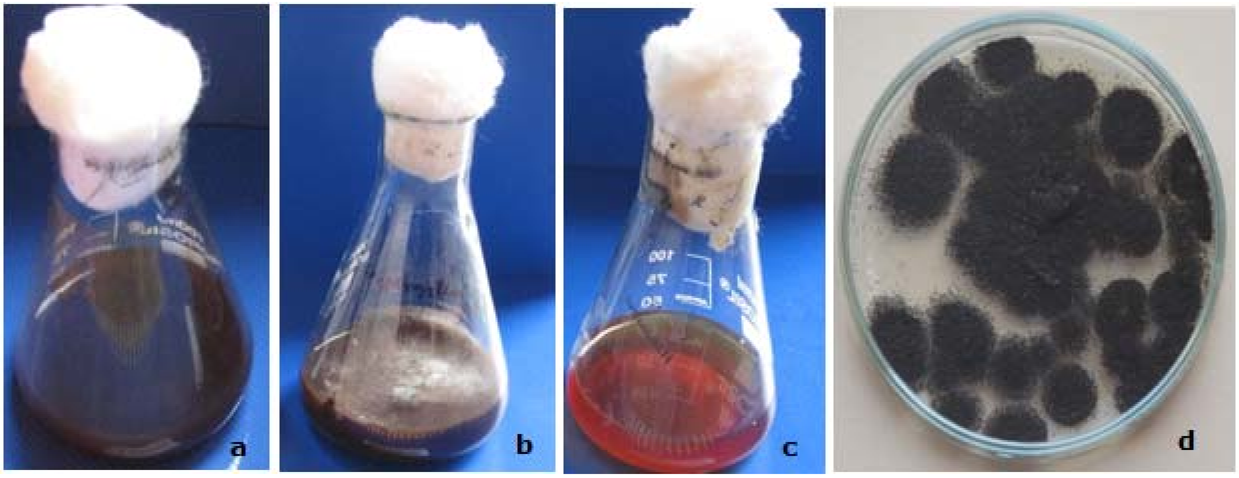


Fig. 1. Effects on tannery effluents after the growth of fungal species (a) Control, (b) Tannery effluent + *A. niger*, (c) Tannery effluent after treatment and (d) Pure culture of *A. niger*

Table 1. Physico chemical characteristics of tannery effluent and fungal biomass

| S.N | Parameters | Untreated (mg/l) | Treated with *A. niger* (% Reduction) | | |
|---|---|---|---|---|---|
| | | | Crude effluent | With glucose | With MSM |
| 1. | Fungal biomass | - | 2090 mg/l | 4550 mg/l | 3940 mg/l |
| 2. | pH | 7.0 | 6.9 | 6.8 | 6.5 |
| 3. | Colour | Black | Brown | Brown | Light yellow |
| | Absorbance at 465nm(D.F. 100X) | 0.843 | 43.2 | 52.7 | 71.9 |
| 4. | Chemical Oxygen Demand (COD) | 21560 | 52.0 | 53.2 | 72.1 |
| 5. | Total solids (TS) | 1568 | 53.3 | 55.1 | 69.0 |
| 6. | Total Dissolve soilds (TDS) | 909 | 58.2 | 59.3 | 65.0 |
| 7. | Total suspended soilds (TSS) | 715 | 60.0 | 62.2 | 68.1 |
| 8. | Chlorides ($Cl^-$) | 323 | 57.3 | 58.7 | 66.8 |
| 9. | Shulfide | 54.6 | 49.1 | 52.4 | 65.7 |
| 10. | Cromium (Cr) | 9.78 | 33.6 | 38.8 | 57.8 |

The growth of fungus in crude effluent was slow (2090 mg/l) and 52.0 % reduction in COD was achieved. In second set of experiment, when 1 ml of glucose solution (1.0 % w/v) was added to the effluent the production of biomass increased to 4550 mg/l but COD reduction (53.2 %) was not significant. The addition of carbon source was important for fungal growth, which can be explained by the high biomass production. The COD reduction was the highest (72.1%) in third set of experiment, when 20% (v/v) MSM was added in effluent. The MSM contains microelements and the trace elements that are important for fungal growth in conjunction with the organic compounds present in the tannery effluent. However, the addition of MSM also increases the biomass production (3940 mg/l) but less as compared with addition of glucose. The other physico-chemical parameters were also reduced after treatment. Colour, TS, TDS, TSS, chlorides and sulfides reduction were 71.9%, 69.0%, 65.0%, 68.1%, 66.8% and 65.7%, respectively. *Aspergillus niger* has capability to degrade wide range of xenobiotics. It has a capability to degrade phenol and use as the sole source of carbon and energy for cell growth (Sharma and Gupta, 2012). It has been used to remove metals from the environment by either adsorption of the metals to fungal cell wall components, or complexation of the metals with organic acids produced by the fungus (Akthar and Mohan, 1995; Bosshard *et al.,* 1996). *A. niger* and *Pseudomonas aeruginosa* from distillery effluent showed significantly better variations in reducing color (87.8%), dissolved sulphites (96.8%), COD (60.7%), BOD (59.4%) and Sulphates (63.5%), respectively on 15 days of incubation when compared to these organism used alone (Pal and Vimala, 2012). *A. niger* has best degrading ability than *Gliocladium deliquescence* and *Penicillium italicum* in non harvested and harvested cell condition. However, non harvested cells recorded the higher degradative ability than harvested cells. Therefore, non harvested cells can be employed in bioremediation of Bonnylight crude oil (Ekundayo *et al.,* 2012).

*Botryosphaeria rhodina* reduced COD by 91%, total organic carbon also decreased from 4685 to 375.0 mg/l and the turbidity from 331.0 to 6.5 NTU after 5 days of incubation, indicating that the biological treatment was efficient as the fungus consumed almost all the organic compounds present in the wastewater. Authors also reported that there is no need to add an additional carbon source for the treatment, indicating that the concentration of organic compounds presented in the tannery wastewater effluent was sufficient for microorganism growth, during which the COD and TOC were reduced by about 91 and 93 %, respectively (Hasegawal *et al.,* 2011). In the current study the chromium ion reduction by *A. niger* was 57.8%, indicating that the fungus has a potential to remove chromium from tannery effluent. Tanneries are a major source of chromium pollution and release Cr(VI) ranging from 40–25,000 mg/l of wastewater (Palmer and Puis, 1994). The maximum tolerance of total Cr for public water supply has been fixed at 0.05 mg/l as per Indian standards. The environmental protection agency has formulated the maximum permissible levels of Cr(VI) into water bodies at 50 $\mu g/dm^3$ and in drinking water as 3 $\mu g/dm^3$ and that of Cr(III) as 100 $\mu g/dm^3$ (Palmer and Puis, 1994; Lee Fred and Jones, 1998). Srivastava and Thakur (2006) also isolated fungal strains from tannery effluents, and optimized some parameters of the treatment process to remove chromium with a strain of *Aspergillus* sp., which removed 70 % of the chromium after 3 days in the bioreactor. Chromium remediation by fungi and yeast might be due to the excellent potential of metal biosorption (Gupta and Ahuja, 2002).

## CONCLUSION

*Aspergillus niger* was able to grow more efficiently when MSM added to tannery effluent. No extra carbon source was necessary, indicating that the concentrations of organic compounds present in the tannery wastewater effluent were sufficient for the fungal growth and to decrease the COD values by around 72.0 %. The results revealed that the fungus is efficient enough to degrade the tannic components and detoxification of chromium in tannery effluent. The study establishes the potential use of *A. niger* in making the effluent non toxic after treatment, and the waste waters can be reused. This bioremediation study will be helpful to some extent to address the environmental pollution.

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CONFLICT OF INTEREST: Nothing