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Mycoremediation of Chromium from Tannery Effluent Collected from Outskirts of Dindigul

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Abstract : In the present investigation fungal strains were employed in degrading chromium which is found to be a major contaminant in leather processing waste water. The organisms were isolated from tannery effluent situated in Sengulam in Dindigul. Isolation of fungus was carried out on SDA by serial dilution and spread plate method. Seven prominent fungal isolates were obtained and were designated as F1 to F4. The fungal isolates were further morphologically and identified as *Aspergillus fumigates, Aspergillusflavus*, *Aspergillus niger* and *Mucor*sp. The maximum Cr uptake by *Aspergillus flavus* (58%),*Aspergillus fumigatus* (37%), *Aspergillus niger*(40%), and *Mucor* sp. (31%) respectively in 72 hrs with initial Cr metal concentrations of 10ppm. *Aspergillus fumigatus* could accumulate and remove maximum Cr up to 50% from the initial conc. of 50ppm, 100ppm, 250ppm and 500ppm respectively with increase in the incubation period.
Keywords : Heavy metals, Chromium, Biosorption, *Aspergillus.*

Introduction

The leather processing is a multistage process and tanning the hide is one of the crucial processes. There are two types of tanning vegetable tanning and chrome tanning. During chrome tanning chromium is being applied to preserve the hides and make them durable after which the hides are sent to finishing stage. Tanning industries are expelling large quantities of waste water due to the repeated treatment of soaking and wringing of the hides. The waste water from leather industry possesses high COD and chromium concentration. These pollutants are responsible for the contamination of all nearby surface and groundwater systems with severely high levels of chromium[1].

Among the heavy metals chromium levels in the environment has been found to increase due to anthropogenic activity. Cr (VI) levels found in the environment are found to affect the indigenous microbial population [2].Chromium (Cr) is one of the extensively used metals in leather industries, electroplating, metal processing, ferrochrome, pigments, stainless steel welding, wood preserving, textiles, and dyes industries [3]. The toxicity and the environmental issues resulting from chromium have been reported for decades and it is crucial to remove chromium from the effluents before it is being discharged into the environment. There are various methods like ion-exchange, chemical reduction, reverse osmosis etc have been done to remove the high concentrations of different heavy metals. These methods are expensive and are not effective. An alternative method which has gained attention all around the world is biological treatment which employs microorganisms, algae and plants. The major forms of biological process of removal of toxicants from the environment are bioleaching, biodegradation, bioaccumulation and biosorption.

There are reports about fungal strains possessing the ability to remove heavy metals like Cr, Ni, Pb, Cd and Cu, from the contaminated environment. Fungus arethe versatile organisms which can withstand,adapt and thrive under various extreme conditions like pH, temperature and high metal concentrations[4]. Sahoo et al (2010)[5] have observed organisms like *Desulphovibrio*, *Aspergillus* sp., *Bacillus* sp., *Pseudomonas*, *Rhizopus* sp. and *Penicillium* sp. with the potential to remove metal and accumulate them. Shivkumar and Thippeswamy (2011) [6]have conducted a study with *Aspergillus niger, Aspergillus flavus, Rhizopus* sp., *Penicillium* sp. and *Saccharomyces* sp. and confirmed their ability to utilize heavy metals in industrial effluents. Among the five fungus *Saccharomyces* sp. qualified as a better candidate for remediation of heavy metals like Cd, Cr, Zn, Pb, Mn, Hg. *Penicilliums*p showed promising effect in uptake of chromium and *A. flavus, A.niger* and *Rhizopus* sp. demonstrated uptake of lead as 81%, 75% and 69 % respectively. Fungus isolated from mercury contaminated sites is found to be capable in removing mercury [7].

Tanneries discharge copious amount of waste water with mixture of chemicals like sodium, chloride, chromium sulphate, calcium salts, ammonium salts, sodium sulphide, acids, alkalis, fat, liquor and organic dyes. In the present study, the effluent samples were collected from four different areas of Sengulam of Dindigul district situated in South India, were 68 leather industries are located around the outskirts of the town.

Methodology

Collection of sample

The sampling wasdone inSengulam situated in Begambur of Dindigul district. Tannery waste was collected in tanks in Dindigul. These tanks have been rendered completely unfit for public or irrigational use due to continuous discharge of untreated effluent by tanneries. The total number of sampling done was on four sites in Sengulam. Each sample was a homogenous mixture of another four sub samples. The effluent samples were collected in sterile screw capped test tubes and bottles and transported to laboratory and further processing was done.

Physico-Chemical characterization of effluent samples

Physicochemical parameters like pH, salinity, colour, odour, turbidity, dissolved oxygen, alkalinity, calcium and magnesium were conducted following (Heron and Mackereth, 1955)[8], total organic nitrogen (Humpheries, 1956)[9], total phosphate (Murphy and Riley, 1962)[10], ammonia was determined by Solorzano(1969)[11],Chloride was estimated by the method Strickland and Parson (1972)[12]. Potassium and Sodium (APHA, 1980)[13], Total dissolved solids were determined by (Valentine Port, 1996)[14] and Chromium (Valentine Port, 1996)[14].

Heavy Metal Analysis

The concentration of chromium present in the effluent samples were analysed by using Atomic Absorption Spectroscopy (Shimadzu, AA- 6300 within 24 hours of collection of sample. Each sample was mixed well and filtered through a filter paper of 0.45µm pore size. The filtrate was transferred to a conical flask with a capacity of 500ml and 5% concentrated nitric acid (Conc.HNO_3) was added to create a acidic condition. The flasks were heated on a hot plate and the volume of the samples was reduced up to 20- 25 ml. Then the samples were allowed to cool and transferred to an acid-rinsed volumetric flask of 25ml capacity. The volume was made up to the marked level by using Milli-Q water. The samples along with blank were analyzed by AAS.

Isolation and purification chromium tolerant fungal isolates

The effluent sample was serially diluted anddilutions of 10^{-3} and 10^{-5} dilutions were chosen for the study. Serial dilution was followed by spread plate technique and the diluted sample was plated SDA.From each dilution 100µl of sample was pipetted out on to SDA plates containing 10 µg potassium dichromate ($K_2Cr_2O_7$) per mL of the medium and uniformly spread with the help of L rod and incubated at 28^{o} C for 48-72 hrs. The spread plates were observed for fungal growth.The fungi isolates were further sub-cultured on PDA media to obtain pure culture and stored at 4^{o}C in refrigerator for further study. The total numbers of fungal isolates were counted as “colony forming unit” (CFUs).

CFU/ml = (Number of colonies /dilution factor) * 10

Cr concentrations

The stock metal solution (1000ppm) was prepared and different concentrations of chromium such as 50ppm, 100ppm, 250ppm and 500ppm were added to 100ml of Czapek's Dox medium and sterilized properly at 15psi pressure and 121°C for 15 mins. The fungal isolate D1, D2, D3 and D4 was inoculated into the liquid medium and incubated in rotary shaker at 180rpm at 30° C for 72 hours.

Metal treated filtrate medium was digested using concentrated HNO_3 (5mL) and boiling chips. The content was boiled and evaporated to 16-20mL on hot plate. Concentrated HCl (5mL) was added and boiled till sample become clear and brownish fumes were evident. Then dried container was cooled, diluted with 100mL double distilled water and filtered through Whatman No.1 filter paper. The concentration of heavy metals in the filtered solution was determined using AAS. The dried fungal matt was crushed in a pestle and mortar. The ground material was placed in a conical flask and 5:1 (nitric/perchloric acid) mixture was added [15]. The content of the flask was placed on a hot plate until the production of red nitrous fumes ceased and liquid becomes colorless. Finally the container was cooled, diluted to 100mL with double distilled water and filtered through Whatman No.1 filter paper to analyze heavy metals by AAS.

The chromium concentration was recorded at 0 hour, 24 hours, 48 hours and 72 hours respectively by AAS.

pH

For optimization study at different pH 5, 7 and 9, 100ppm metal solution from the prepared stock solution was added to 50ml of Czapek's Dox medium and sterilized at 15psi pressure and 121° C for 15 mins. The fungal isolate F-2 was inoculated into the liquid medium and incubated in rotary shaker at 180rpm at 30° C for 72 hours. The optimization study was carried out by recording the chromium concentrations at 0 hr, 24 hrs, 48 hrs and 72 hrs respectively.

Results and Discussions

The sample was collected from 4 different sites from Sengulam. Heavy metals content in the industrial effluent water samples were higher than the permissible limit. The collected samples contain different concentration of heavy metals as shown in Table- 4. The overall, from the data, it has been concluded that the concentration of chromium, varied from 2.0 to 3.5ppm. All the four samples collected were grey in colour and turbid except for sample 3 which was found to be dark brown. The pH of the effluent samples collected from Sengulam was found to within permissible limits for discharge. The physico chemical analysis of the sample is presented in table 1. The electrical conductivity of sample D1 and D3 were found to be higher with 340 and 330µS/cm respectively, followed by D2 173µS/cm and D4 120µS/cm respectively.

Table 1: The physicochemical parameters of the effluent collected from four different sites

Sample Parameters	**D1**	**D2**	**D3**	**D4**
Ph	7.2	7.5	7.2	7.3
Ec µS/cm	340	173	330	120
Nitrogen µmol/L	1160.3	973. 9	1054. 5	630.20
Ammonia µmol/L	60.4	52.3	73	38.4
Nitrate µmol/L	0.32	0.17	0.23	0.17
Phosphate µmol/L	53.60	27.5	61.3	18.7
Chromium ppm	**2.722**	**2.841**	**3.452**	**3.122**

Table 2: The amount of Chromium found in the filtrate of synthetic effluent which was inoculated with *Aspergillus fumigatus*

S.no.	Chromium	24 hrs	48hrs	72hrs
1	50ppm	81.24%	78.67%	42.32%
2	100ppm	88.36%	74.68%	67.51%
3	250ppm	87.21%	74.75%	67.23%
4	500ppm	90.14%	81.24%	75.32%

The total nitrogen content in Sengulam was found to be 1160.3µmol/L in sampling site D1 and 1054.5µmol/L in D3 site with the lowest being 630.20µmol/L in D4.

A similar trend was noticed in nitrate content and total phosphate content with highest being D1 followed by D3.The level of ammonia seems to be highest in D3 followed by D1 reaching 73 µmol/Land 60.4 µmol/L respectively.

The morphological analysis of fungal isolates was conducted by performing Lactophenol cotton blue staining. The isolate F1 was found to spread rapidly in SDA, at early stages of growth it appeared white later stages started to become dull blue green colony, the surface was found to be smooth, the conidia were found to be globose in nature which was identified as *A.fumigatus.* Strain F2waspale brown in colour, the conidia displayed sub globose to globose with the conidial head dividing into poorly defined column and it was confirmed as *A.flavus.* The fungal strainF3 was identified as *A.niger* after morphological analysis which was dark in colour and displayed rough and irregular conidial surface. The fourth fungal isolate was identified as *Mucor,* F4 appeared black in colour, possessing globular conidia.

The colony forming units were found to be highest in *Aspergillus fumigates*with 4.5 x 10^2 CFU/ml, followed by *A. flavus*3 x 10^2 CFU/ml, *A.niger*2 x 10^2 CFU/ml and *Mucor* sp. recorded the least with 2.5 x 10^2 CFU/ml.

It can be inferred from the results that chromium uptake was confirmed by all the four isolates. The maximum uptake of chromium was recorded 58% by *A. fumigatus*, the accumulation was *A.flavus* 37% and 40% by and *A. niger* whereas,*Mucor* sp. recorded the least absorption which was found to be 31%.

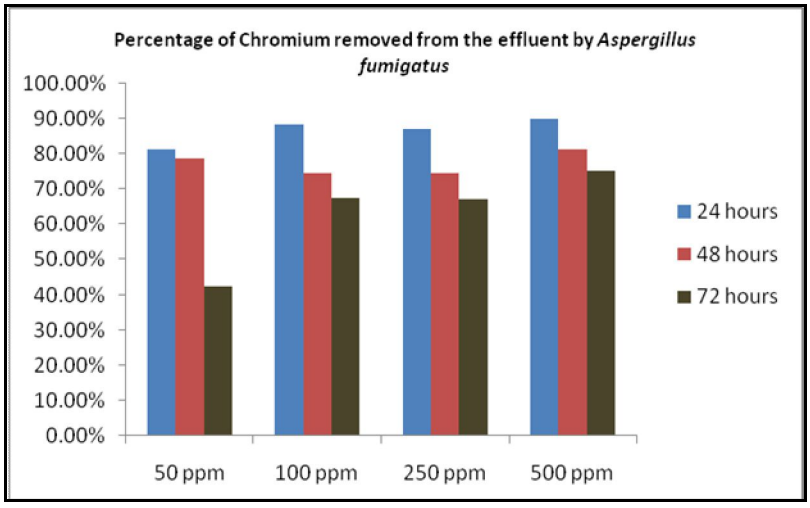

Figure 1: The amount of Chromium found in the filtrate of synthetic effluent which was inoculated with *Aspergillus fumigatus*

Thetolerance of chromium of the fungal was checked for all the strain. The fungal isolate F1 has high potential to optimize chromium at various concentrations such as 50ppm, 100ppm, 250ppm and 500ppm respectively. The initial concentrations of chromium were reduced due to the accumulation capacity of F2. At 50ppm, the initial concentration remained 82.14% at 0 hr, remained after 48 hrs were 78.67% whereas it was

42.24% after 72 hrs. Similarly, at 100ppm and 250ppm initial concentrations, Cr concentration remained was about 67.32% after 72 hrs whereas it was 67.51% at 250ppm and about 75.32% of Cr remained in 500ppm after 72hrs. The initial concentration of 500ppm remained 90.128% at 24hr followed by 81.24% 48hrs respectively. From this, it was found that F1 has the potential to uptake chromium from the medium at regular intervals of time. The potential of F1 to uptake and reduced Cr concentration was about 20 % at 24 hr which increases to 29% after 48 hrs and reaching 58% at 72 hrs as shown in figure 2.

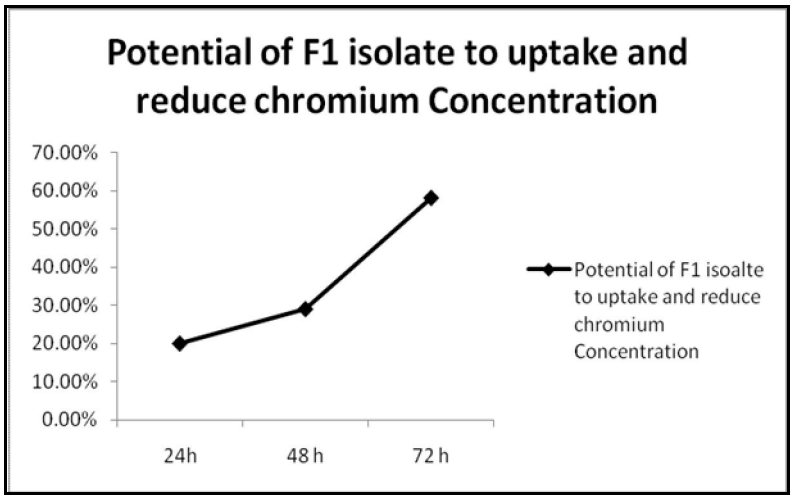

Figure 2: Potential of F1 isolate to uptake and reduce chromium

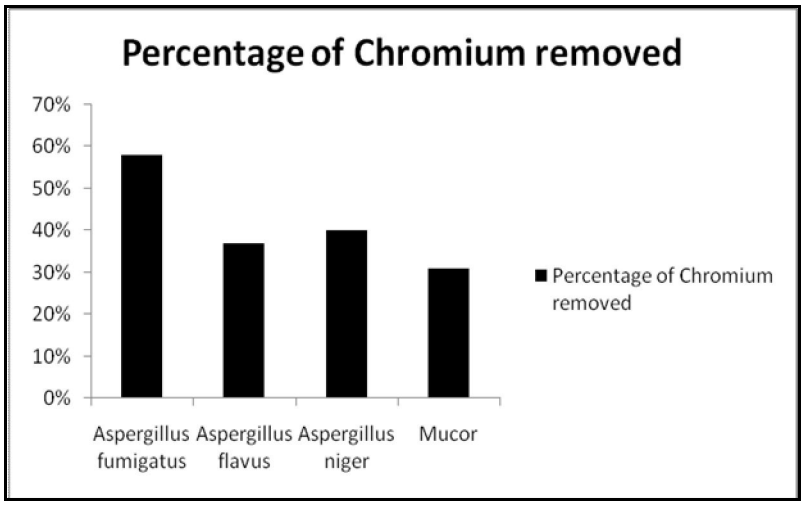

Figure 3: Percentage of Chromium removed by the fungal isolates

The chromium concentration remained in the filtrate inoculated with fungal isolate F2 *Aspergillus flavus* was found to be 37% at 72hrs of exposure to 50ppm and 85.34% to 500ppm, *Aspergillus niger* inoculated filtrate recorded 40.32% at 50ppm of Cr to a exposure of 72hrs and 87.45 % at 500ppm concentration of Cr at 72hrs. whereas, *Mucor*sp. recorded 69.21% and 87.32% of chromium in the filtrate of 50ppm and 500ppm respectively at 72hrs. The algae *Spirogyra* has also been found to be capable of removing chromium from industrial effluent but the rate of *Aspergillus* being higher than *Spirogyra* proves it to be a better way for chromium biosorption from industrial effluent [16].

Conclusion

Biosorption of chromium from tannery effluent was conducted with four strains of fungus. *Aspergillus fumigatus* has shown best biosorption capacity among the four followed by *Aspergillus flavus*, *Aspergillus niger* and *Mucor* sp.

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