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BIOREMEDIATION OF TANNERY WASTEWATER BY CHROMIUM RESISTANT FUNGAL ISOLATE

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ABSTRACT

Bioremediation process was approached to cleanse the effluent water pollution caused by local leather processing leads to seeping of heavy metal results the contamination of ground water, surface water and other sources of water in the selected area. Aim of the work is to bioremediation of the water treated with ***Aspergillus niger* KF514876** isolated from tannery effluent water collected from Tenali and Guntur Andhra Pradesh. Screened for various physicochemical parameters and heavy metals especially focused on chromium contamination and bioremediation of the water. Whereas results shown that parameter wise Cr (VI) and also predicted results shown that the treatment (6 days) of tannery wastewater with ***Aspergillus niger KF514876*** in shake flask experiment. Gradual decrease has seen in the consecutive six days duration of treatment.

Key words: Bioremediation, Chromium, Fungi, effluent, Wastewater and chromium.

The wastewater emanating from tanneries is characterized by a strong color and is heavily polluted with high COD and biochemical oxygen demand (BOD) and inorganic impurities (sodium, calcium, nitrate, ammonia, sulfide, and chloride), dissolved and suspended solids, and other specific pollutants such as vegetable and/or synthetic tannins, sulfonated oils, chromium, Pentachlorophenol and surfactants. 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) and Cr(VI). Cr(VI) is the toxic form of the element6 which causes severe diarrhoea, ulcers, eye and skin irritation, kidney dysfunction and probably lung carcinoma. Conventional methods for removing toxic Cr(VI) include chemical reduction followed by the precipitation under alkaline conditions, ion exchange, and adsorption on activated coal, alum, kaolinite, and ash. Most of these methods have major disadvantages; for instance, a need for high energy use and large quantities of chemical reagents, incomplete metal removal and generation of large quantity of toxic waste sludge. Furthermore, such processes may be ineffective or extremely expensive when the initial heavy metal concentrations are in the range of 10-100 mg L-1 as well as high organic load in the effluent (Zhao, J., Wu, Q., and Tang, Y. *et al.(2022)*).

Microorganisms (bacteria/fungi) are the most important eco-friendly agents for the degradation and detoxification of industrial pollutants during the biological treatment of industrial wastewaters. Therefore, bioremediation is an alternative to conventional chemical and physical methods for wastewater treatment. Fungi are known to tolerate heavy metals. They are a versatile group, as they can adapt and grow under various extreme conditions of pH, temperature and nutrient availability, as well as high metal concentrations. They offer the advantage of having cell wall material which shows excellent metal-binding properties. Generally, microbial biomasses have evolved various measures to respond to heavy metals stress via processes such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules, as well as precipitation and transformation of metals. There are some studies which have been conducted employing bacteria for remediation of components of tannery wastewater or fungal mycelia as bioabsorbent. But, most of the studies are pollutant specific targeting only one or two specific pollutants mostly chromium. We could not find even a single study on remediation of COD, color and major ions of tannery wastewater (Vijayaraj et al., 2018).

Therefore, the aim of the present study was to isolate and select fungal strain from tannery effluent enriched soil which can be able to remove the COD, color, Cr(VI) and other major ions from the leather industry wastewater.

MATERIALS AND METHODS

Sample collection: triplicate effluent water samples were cbrought to the laboratory and stored in a refrigerator at 4°C till their utilization. The fungal colonies grown on the Potato Dextrose Agar (PDA) and were further identified and characterized by by IMTECH Chandigarh. Named and deposited in NCBI was ***Aspergillus niger KF514876*** used for the treatment of tannery wastewater.

D e t e r m i n a t i o n o f m i n i m u m i n h i b i t o r y 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 8mm agar plugs from young fungal colonies, pre-grown on PDA and incubated at 28°C for seven days. The fungal growth was used as a measure of viability and it 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. 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 preparation: For tannery waolstewater bioremediation studies, the fungal inoculum was prepared in the form of mycelial 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 °C for 5 days in orbital shaker at 150 rpm. The mycelium thus obtained was filtered by cheesecloth and air-dried on sterilized petriplates. Fungal pellets were prepared by cutting in approximately 1.5-2.0 mm size. The fungal pellets (2% w/v) were inoculated in combined tannery effluent amended with 0.1% glucose and 0.1% ammonium nitrate. The pH was maintained at 5.30 and the flasks were incubated at 30 °C in a 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, Cr(VI) and other pollution parameters were measured.

Physico-chemical analysis of the tannery wastewater: 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. Color was measured spectrophotometrically (465 nm) according to the method of Bajpai et al.,. The hexavalent chromium [Cr(VI)] was determined colorimetrically using the diphenylcarbazide (DPC) method.

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). The data obtained in the study were analyzed by Duncan’s Multiple Range Test using SPSS Inc. (v 17.0) software. The differences between means were considered significant at values of pd"0.05.

RESULTS AND DISCUSSION

Isolation and Screening of fungi:

The fungal strain ***Aspergillus niger KF514876*** was isolated from tannery effluent enriched soil by serial dilution technique. The isolate exhibited MIC for Cr(VI) as 500ppm. 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. Similar to our findings, ***Aspergillus niger, Aspergillus lentulus, Penicillium sp., and Fusarium solan*** isolated from contaminated sites have been reported to tolerate 1000ppm Cr(VI) (EL-Tayieb, Marwa (2017)). 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.

Physico-chemical characteristics of the tannery wastewater before and after treatment with ***Aspergillus niger KF514876.*** The combined tannery effluent used in bioremediation studies 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 effluent (Smiley Sharma and Piyush Malaviyas (2018)). **(Table 1).** The high electrical conductivity and TDS were due to the presence of inorganic substances and salts whereas elevated amount of COD was ascribed to high amount of organic compounds which were not affected by the bacterial decomposition. Besides, significant concentration of sodium, potassium, calcium, chloride and nitrate ions were also observed. Various heavy metals present in the combined tannery effluent were Cr(VI), Total Cr, Pb(II), Total Pb, Cu, Zn and Mn. The tannery effluent characteristics were found to be in agreement with the previous studies (Tesfaye Admassu; Adey Desta and Fassil Assefa (2020)).

Table-1: Physico-chemical characteristics of untreated tannery wastewater.

Sl.No	Parameters	Values
1	pH	9.16±0.20
2	TSS (mg L-1)	1694±11.20
3	TDS (mg L-1)	17650±20.10
4	Turbidity (NTU)	505±2.00
5	COD (mg L-1)	5776±30.10
6	Color (CU)	1984.85±12.80
7	EC (mS cm-1)	35.3±0.25
8	Na+ (mg L-1)	3080±35.60
9	Cl- (mg L-1)	4700±40.10
10	NO3- (mg L-1)	600±5.00
11	Ca2+ (mg L-1)	258±12.00
12	K+ (mg L-1)	290±11.50
13	Cr(VI) (mg L-1)	9.86±0.180
14	Total Cr (mg L-1)	12.260±0.556
15	Pb(II) (mg L-1)	0.965±0.0140
16	Total Pb (mg L-1)	1.126±0.0131
17	Zn (mg L-1)	0.529±0.0028
18	Mn (mg L-1)	0.392±0.0090
19	Cu (mg L-1)	0.258±0.0013
* = Mean±SD of three replicates		

Many microorganisms could develop potential to biodegrade the recalcitrant pollutants when they are exposed to polluted environment extended time duration. On this line, the treatment of tannery wastewater with ***Aspergillus niger KF514876*** isolated from tannery wastewater affected soil resulted in significant reduction of pollution parameters after six days, concomitant with the increase in fungal dry weight (Smiley Sharma and Piyush Malaviya (2013)). **(Table-2).** The final reduction in COD, color, Cr(VI), TSS, turbidity, Na+, Cl-, and NO3- was 71.80, 64.69, 100, 36.47, 22.77, 11.69, 27.87 and 62.33%, respectively after fungal treatment of tannery wastewater. The steep decline in COD within first two days (69.51%) was attributed to utilization of organics by the fungus during initial growth phase. Similarly, rapid decolorization of tannery wastewater was possibly achieved by oxidative degradation of the dye molecules. The tannery wastewater also registered reduction in pH from 5.30 to 4.60, due to release of organic acids by the fungal isolate. The acidic environment facilitated the bisorption of Cr(VI) ions (Smiley Sharma and Piyush Malaviya. (2016)).

Many researchers have also reported highly acidic pH for Cr(VI) biosorption. In aqueous solution, chromium ions generally exist in two stable oxidation states, trivalent and hexavalent. The former exists as Cr3+, Cr (OH)2+ and Cr (OH)2+ in the pH range 1.0–6.0 and starts precipitating as Cr(OH)3 at a pH value > 6.0, while Cr6+ forms H2CrO4 and HCrO4 " species at pH 2.0–3.035. Thus, at low pH values oxyanionic species of Cr6+ is likely to be attracted by the positively charged functional groups present on the fungal cell surface. Similarly, the reduction in TSS and turbidity was ascribed to entrapment of suspended solid particles by the filamentous fungi. Whereas, reduction in NO3 -, Na+ and Cl- ions might be attributed to utilization of these ions for growth by the fungal isolate (Abba, et al., 2019).

Table. 2: Physico-chemical characteristics* of tannery wastewater after different treatment durations (2d, 4d, and 6d) with ***Aspergillus niger KF514876***.

	Treatment duration		
Parameters	**2nd day**	**4th day**	**6th day**
pH	4.80a±0.11	4.65b±0.20	4.60b±0.12
COD(mgL-1)	1761.00a±15.71(69.51)	1759.66a±20.79(69.53)	1628.66b±14.29(71.80)
Color (CU)	1129.34a±24.00(43.10)	793.44b±19.97(60.02)	700.75c±35.80(64.69)
Cr(VI) (mg L-1)	5.620a±0.241(43.00)	1.220b±0.210(87.63)	0.00c(100)
TSS (mg L-1)	1121.31a±22.32(33.81)	1098ab±12.65(35.17)	1076.12b±18.46(36.47)
Turbidity (NTU)	480a±18(4.95)	430b±25(14.85)	390b±16(22.77)
Na+ (mg L-1)	3010a±11.54(2.27)	2860b±18.00(7.14)	2720c±22.00(11.69)
Cl- (mg L-1)	3690a±22.56(21.49)	3420b±12.68(27.23)	3390b±12.78(27.87)
NO3- (mg L-1)	314a±3.10(47.67)	269b±3.18(55.17)	226c±3.70(62.33)

CONCLUSION: ***Aspergillus niger KF514876*** isolated from tannery effluent enriched soil exhibited detoxification of tannery wastewater. The treatment of tannery wastewater with ***Aspergillus niger KF514876*** resulted in the reduction of COD, color, Cr(VI), total suspended solids (TSS), turbidity, Na+, Cl-, and NO3 - in the order of 71.80, 64.69, 100, 36.47, 22.77, 11.69, 27.87 and 62.33%, respectively after six days of duration. As the bioremediation activity is highly regulated by cell metabolism, which in turn is controlled by media components such as carbon and nitrogen sources and their ratio in addition to other process parameters like pH, incubation temperature and aeration. Thus, in a future there is at need to conduct process parameter optimization studies to improve the bioremediation efficiency of the isolate.

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