Dual biological treatment approach on Heavy Metal Chromium removal from Effluent using live *Pseudomonas* and Dead *Neurospora sp*

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ABSTRACT

Combined Microbial treatment on tannery effluent is used to remove Chromium was evaluated in this study, using chromium resistant *Pseudomonas fluorescence* and *Neurospora* sp isolated from tannery effluent site. Initially resistant strains were isolated by plating method, under 1000 ppm concentration of potassium dichromate. Efficiency of live bacterial and dead fungal mat was tested under aerobic condition. Chromium removal was noted by UV and effluent characters were tested. The results shows that reduction of chromium was moderate by bacteria and fungi alone and effective potent reduction by combined treatment and estimated as 99% chromium VI and chromium III. The concentration of Cr^{6+} was reduced as Cr^{3+} and estimated as 41 % by bacteria and 30 % by fungi alone. Further the chromium $^{6+}$ was 99% reduced by dual live bacterial-dead fungal treatment. The combined treatment also shows BOD and COD greatly reduced, compare to independent treatment. The treated effluent also showed significant result on seed germination indicates that it's free from chromium toxicity as Cr VI is reduced. This study confirms that the isolated bacteria and fungi were potent novel isolates remove hexavlant chromium efficiently.

Key words: Chromium, Pseudomonas, Fungi, toxicity

INTRODUCTION

Tanneries are the main source of environmental pollution, huge quantities of chromiumare used in leather processing and it inhibit the growth of microbes. Pollutants from tanning activities includes such as chloride, chromium, lead, zinc, formaldehyde, sulphuric acid, manganese, sulphide, phenols, synthase, tannins, protein wastes, tanned and untanned waste etc., (Hasegawa et al., 2011). Effluent contaminated by metals are difficult to remediate (Evelyne and Ravi sankar, 2014). These compounds are toxic and persist longer in the environment, it cause adverse effects to flora and fauna. Exposure to polluted water may can cause fatal diseases like cancer, neurological disordersdelayed nervous responses, mutagenic changesetc (Megharaj et al., 2003). Many toxic heavy metals among chromium is considerable environmental concern as it is widely used in electroplating, leather tanning, metal finishing and chromate preparation (Balaji and David, 2016). Chromium occur in two form one is trivalent and other one is hexavalent forms. The concentration of chromium varies from 500 to 7000 ppm in tannery effluent. Chromium metal (Cr) occurs naturally in the environment Cr exists as Cr(III) and Cr(VI) being the primary existing oxidation states in the environment and has both beneficial and potential human risks. Cr(III) is an essential nutrient for maintaining lipid, insulin, and glucose metabolism and its deficiency may lead to diabetes (Martone et al., 2013). Hexavalent chromium is toxic and carcinogen but trivalent chromium is less soluble and less toxic.

Detoxification is termed as the ability of a microbes to survive at toxic effect when it exposed to metal by means of a mechanism produced with direct response to the metal species concerned. The microbes survives at metal toxicity due to its intrinsic property called as tolerance.The microbes have potential to eliminate impurities and absorb toxic metals during treatment. Bacteria that transform hexavalent chromium to trivalent chromium reported by Jayalakshmi *et al*. (2013). Fungi termed as biological material act as a absorptive material and eradicate hexavalent chromium (Noorjahan., 2014). Bioremediation is a natural approach that involves the use microorganism or enzyme to degrade contaminants is less expensive and more sustainable. Murugan and Sohaibani (2011) reported that microorganisms have broadly researched for their ability to detoxify tannery contaminants. Biosorption mechanism is occur through physical and chemical interaction between metal and functional group present on the surface of cell wall. Two methods involved in biosorption mechanism such as metabolism dependent and non-metabolism dependent. Chromium get bound to the functional group and adsorbed into the cell wall. Fungal strains

have significant potency to adsorb chromium. Microbes play a vital role in the environment and act as a bio-degradation.

Microorganisms are very effective in pollution control, especially in effluent treatment (Srinivas et al., 2011). The microbes present in the effluent sample can tolerate the adverse conditions such as pH, turbidity, high BOD, COD, etc. The microbial consortium has been isolated, identified and used for the treatment. *Pseudomonas aeruginosa, Penicilium* sp and *Aspergillus* sp isolated from polluted sites from tannery and shown to be resistant to hexavalent chromium which is highly toxic reported by Awasthi et al., (2015). The application of consortium is used to eliminate chromium from polluted waste and contaminated soil (Adekunle, 2011). Mixed population of microbes degrade very high when compare to single strains. Das et al., (2010) have also stated that TDS, BOD, COD, EC, salinity, alkalinity, hardness are high in tannery effluent.The present study was carried out with chromium (VI) and chromium (III) reduction ability of bacteria and fungi isolated and screened from tannery effluents.

MATERIALS AND METHODS

Collecting site

Tannery effluent sample was collected from KMM Tannery during March 2018 located at Airport, Tiruchirappalli in a sterile bottle and processed for microbial studies. Sterilized containers were used for collection and they were transported to the laboratory within 2-4 hours and stored at 40^0C for further analysis.

Isolation of Cr tolerant *Pseudomonas sp*

For the enumeration of bacteria, samples were serially diluted and plated on Luria–Bertani (LB) agar (tryptone: 10 g l-1; yeast extract: 5 g l-1; Nacl: 10 g l-1; glucose: 0.1 g l-1) adjusted at normal pH value (7.0). The medium amended with 1000 ppm potassium di-chromate to isolate tolerant strain. Medium without chromium used as control.

Isolation of Cr tolerant *Neurospora* sp (Nagamani et al., 2006)

PDA medium with 1000 ppm of potassium di chromate was added to the media to select resistant strain. The plates were incubated at 25±2º C for five days. The fungi were identified by morphological observations. Strains are identified by lactophenol cotton blue staining technique. Medium without chromium are used as control.

Bacterial treatment in tannery effluent

About 2 L of autoclaved tannery effluent was taken in aerated tank and the pH was adjusted to seven. The effluent was enriched with 100 ppm chromium by the addition of potassium dichromates. 10% volume of freshly grown *Pseudomonas* culture was inoculated. The chromium level, BOD and COD were recorded after 24 h.

Dead fungal preparation

The fungal cells was grown at 28°C in an stirred and aerated liquid Sabourauds media containing ampicillin at a concentration of 0.1g/L (p/v). After five days of incubation, the cells were recovered by centrifugation (5000 rpm/10 min), and washed thrice with phosphate buffer and subsequently oven dried at 40°C/24 h. The biosorption of the metal by fungal dry cells was determined followed by autoclaving subsequently for 3 days.

Combined treatment in tannery effluent

In 100 mL bacterial treated effluent 5 g/L of dead mycelium was added and kept further 24-48h under shaking. After incubation BOD, COD and concentration of chromium was estimated as follows.

Chromium removal analysis

After 48 h incubation chromium absorption was monitored by recording OD between 100 to 700 nm under UV spectrophotometer along with control. For live fungal the OD was taken after 5 days incubation.

Tannery Effluent Physicochemical Properties:

The collected tannery effluent was analysed for physicochemical properties like chemical oxygen demand (COD), biological oxygen demand (BOD), chromium(VI) and chromium(III).

Plant growth promotion studies

To investigate the effect on germination using mung been seeds (*Vigna radiata* L.) was chosen for the test. Triplicate of five Seeds surface sterilized with 0.1% $HgCl_2$ and washed thrice to remove all the traces of unwanted particles. Seed germination and seedling growth

test on filter paper was carried out in glass petridishes (20 mm x 120 mm) with two layer of filter paper (125 mm in diameter, whatman No.1). Followed by a layer of cotton bed on the bottom. Seeds were soaked with treated effluent used as test and untreated effluent soaked seeds as negative control and water treated as positive control. The petridishes were covered by lid and incubated at 28^0C in dark condition for 24 hrs. The seed germination percentage, were observed in 24 hrs.

% of Seed germination= Seed germinated/ Total number of seed tested X 100

Pot study

Germinated seeds planted on pot and irrigated with treated and untreated effluent for 7 days. The morphological parameters like plant height, no. of Branches and no. of Leaves were observed and recorded. The growth effect was checked as follows

Vigour index= (mean root length+ mean soot length) x % of seed germination

RESULT AND DISCUSSION

Isolation of chromium resistant bacteria

The bacterial and fungal colonies were isolated from tannery effluent and studied based on the colony morphological characteristics. The plate without chromium showed high level of $4.6X10^7$ CFU. Plates amended with chromium showed $1.1\ X10^7$. 11 morphologically distinct colonies were selected and the frequency of Gram positive is 63% and Gram negative (36%). Based on biochemical character isolated strains were identified as *Pseudomonas fluorescens, Escherichia coli, Alcaligenes* sp, *Micrococcus* sp*, Bacillus methylotrophicus, Bacillus subtilis, E.faecalis, Pseudomonas putida, Streptococcus Anaerobius, Ruminococcus albus, Bacillus licheniformis.* The fungal strain are identified as *Neurospora* sp, *A.fumigatus*, *A. terreus*, *Fusarium* sp, *A.nidulans*, *A.niger*, *Alternaria* sp, *Mucor* sp, *Penicillium* sp and *Curvularia* sp. Presence of chromium on tannery effluent gives adaptation ability to bacteria which makes it resistant in order to give a considerably high CFU/ml (Saranraj et al., 2013).

The present study was carried out to isolate chromium resistant fungi and bacteria from tannery effluent and to evaluate the bioremediation potential. Chromium tolerant isolates was assessed by growing on Luria–Bertani (LB) agar containing concentration of chromium at 1000ppm. Among the isolates, tolerance limit was 1000 ppm recorded among *Pseudomonas fluorescens* and *Neurospora* sp. Hexavalent and trivalent chromium were reduced under permissible limit in the combined culturing treatment of tannery effluent as compared to tannery control. Figure 1 shows the UV spectrum of control of untreated effluent shows maximum of 3.8 OD between 200-350 nm. The spectrum of treated in figure 1b shows

disappearance of peak formation. The initial amount of chromium III and Cr VI was 160 and 264mg/L. The reduction of chromium III after treatment was 70 mg/L by bacteria, 50 mg/L by Fungi 12.5 mg/L followed by combined treatment. Similarly hexavalent chromium was estimated as 110≥80≥1.53 mg/L respectively for bacterial, fungal and combined treatment (figure 2). Presence of Chromium reductase in bacteria mediates resistant to chromium, which catalyse the reduction reaction of Cr (VI) to Cr (III) (Deshpande et al., 2005). Though various biological techniques used to reduce toxic substance live organisms transform remove Cr (VI) as Cr III (Pradhan et al., 2017**)** later absorbed by Fungi from water effectively remove chromium. The combined treatment shows a better reduction rate in BOD, COD(table1) and Cr reduction. Bacterial reduction shows 68% BOD and 57 % COD removal. It was further enhanced by Fungi treatment and reduced as 23% BOD and 10% COD. The seed germination test (table 2) was noted after 24 hrs. The results indicates that untreated effluent showed 30% seed germination and its vigour index was 59.6%. Treated chromium removed effluent showed 100% germination and enhanced plant growth with vigour index 2410.

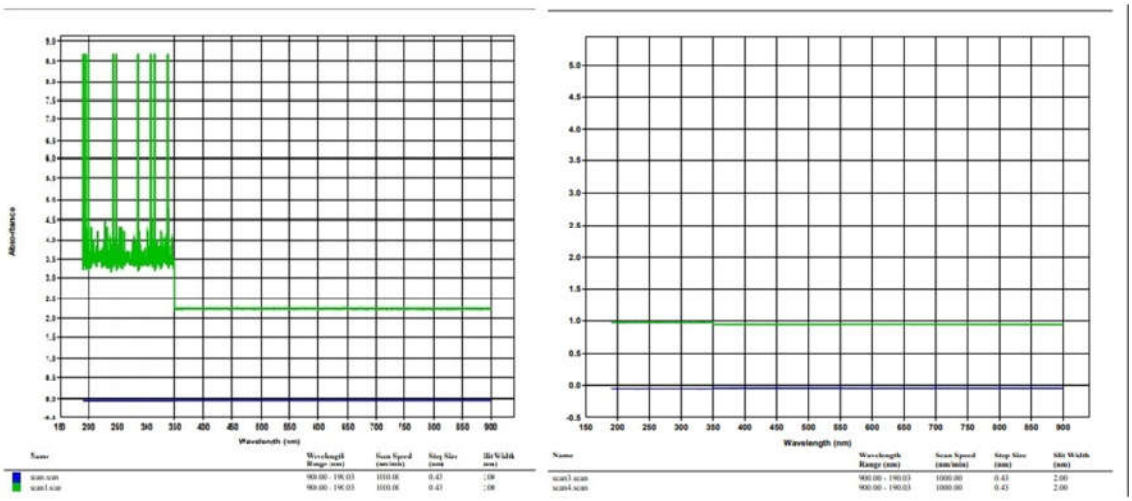
Table 1. Physiochemical analysis of effluent

Treatment	BOD	COD
Bacteria treated	2080 (68%)	6,400 (57%)
Combined	1607 (23%)	5800 (10%)
Control	3053	11,100

Table 2. Mean value of root and shoot length of *Vigna radiata* L. Treated with raw effluent

S. No	UNTREATED	TREATED
Stem	6.8	20.5
Root	1.76	3.6
Germination index(GI)	30	100
Vigor index (VI)	59.6	2410

Figure 1. Detection of Cr reduction after 72 h



CONTROL TREATED

Figure 2. Estimated tri and hexavalent chromium

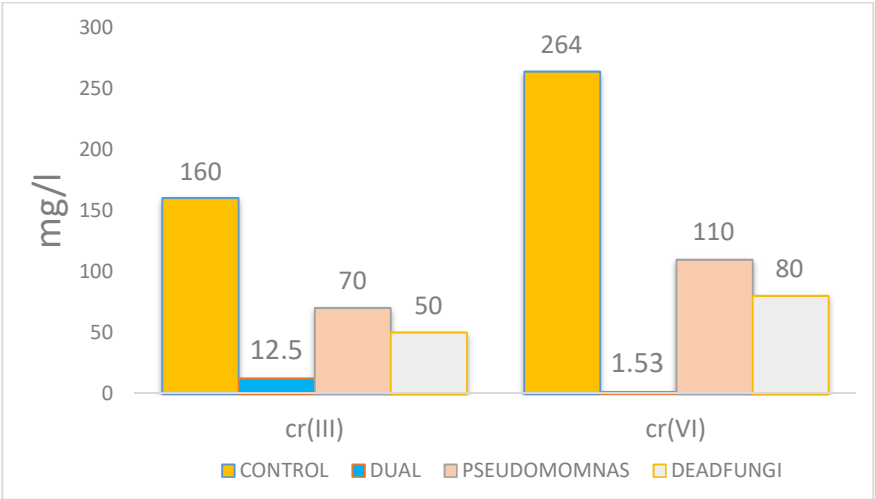
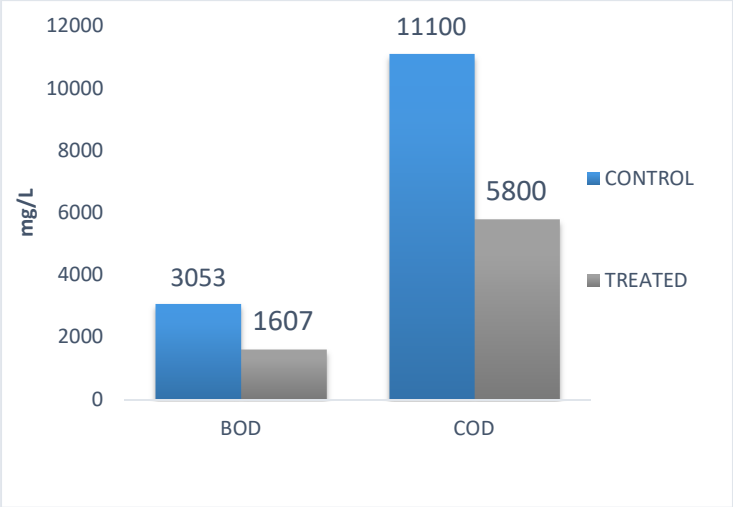


Figure 3. Physicochemical parameters



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