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Bio-electrochemical removal of CR III from leather effluent by *Pseudomonas aeruginosa*

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Abstract
Chromium is an essential trace metal for living organisms occurs in two major oxidation states Chromium III and chromium IV. Chromium III is more toxic when it penetrates the living tissues. So the recovery of Chromium III from leather effluent by Bioelectrochemical method is more effectively than conventional methods. In our findings various parameters of the effluent was analysed and the reduction of Chromium III in the sample was determined by various techniques such as UV vis spectroscopy, FTIR analysis, EDAX analysis was done. In our study among various microbes *Pseudomonas aeruginosa* coated bioelectrode was more efficient in chromium reduction. It reported that this organism was recommended as safe for biological treatment of leather effluent. Efforts were in progress to extend the work to other industrial effluents and determine the trace metals which were toxic to the environment.

Keywords: Chromium, *Pseudomonas aeruginosa,* effluent, bioelectrochemical, BOD

Introduction
Leather processing is an important activity in many developing countries, which are dependent on the agro economy. The leather industry has gained a negative image in society with respect to the environmental unfriendliness of its processing methods. (Gangopadhyay *et al*., 2000).

Treatment of the large volumes of effluents and their subsequent discharge into sewers, rivers etc are only a short term solution which adds to compounded problems of investment and recurring expenditure. (Sahasranaman *et al*., 2000).The name of the element is derived from the Greek word "chroma" meaning color. Chromium belongs to the 6^{th} group transition metal. The atomic number is 24 and the molecular weight is 51.9961g mol^{-1}.

Chromium is regarded with great interest because of its high corrosion resistant and hardness. Chromium is known as one of the most toxic heavy metals, being in evidence in the EPA list of human carcinogens large number of environmental samples fast and low cost analysis methods such as analysis chromium selective sensors is a promising method that gained important credibility (Singh *et al*., 2007) [10]. Hexavalent chromium (chromate CrO_4^{2-}) is toxic and mutagenic to most organisms and is known to cause irritation, corrosion of the skin and respiratory tract and lung carcinoma in humans.

Electrochemical treatment techniques are becoming an alternative waste water treatment method because many industrial processes produce toxic waste water which are not easily biodegradable and require costly physical and physiochemical pretreatment. The two effective electrochemical techniques for chromium removal are Membrane electrolysis it is one of the techniques used for the removal of hexavalent chromium from waste water. (Martinez *et al*., 2004) [5]. Electrochemical precipitation helps to maximize the removal of heavy metal from contaminated waste water, (Kurniawan *et al*., 2006) [4]. Microbial populations in metal polluted environments adapt to toxic concentrations heavy metals and become metal resistant (Prasenjit *et al*., 2005) [6]. *Pseudomonas sp.* is one of the most commonly encountered gram negative organisms and it is found in soil, water, plants as well as in domestic environments.

Chromium resistant microorganisms are *Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Pseudomonas ambigua, Pseudomonas fluorescens, Pseudomonas putida, E. coli,*

Enterobacter cloacae, etc., Among the bacterial genera, the more expressed biodegradative capacity is found in genus *Pseudomonas* (Elkarmi *et al*., 2008) [1]. Bio-transformation of hexavalent chromate to the non toxic trivalent form by biological agents therefore offers a viable alternative.(Shakoori *et al*., 2000) [9].

Materials and Methods

Sample Collection

Tannery effluent was collected from the treatment plant at CLRI, Chennai. The effluent collected was transported to CECRI Microbiology Laboratory for the further study.

Isolation of resistant microorganism

Serial dilution was performed by taking 1ml of the leather effluent and diluting it into 9ml of distilled water in a series of test tubes. Pour plate method from each dilution was performed on Nutrient Agar plates and was incubated at 37^0C for 24 hours. A loop full culture from the Nutrient Broth was streaked on a Hi Fluoro *Pseudomonas* agar base containing Pancreatic digest of gelatin - 18g,Magnesium chloride - 1.40g,Potassium sulphate-10g,Cetrimide-0.30g,Fluorogenicmixture-2.05g,Agar- 15g,Distilled water - 1000ml, Final pH at 25^0C – 7.2 ± 0.2 incubated at 25^0C for 48 hours.

Identification of Bacteria

Morphological identification was done by Gram staining test, Motility test was performed by hanging drop method and various Biochemical test analysis such as Oxidase test, Catalase test, IMVIC, Urease test, Gelatin hydrolysis test was done to determine the metabolic characteristics of an organism.(Norris and Ribbons,1972).

Characterization of leather waste

pH analysis

The collected sample was filtered using filter paper. pH of the filtered leather sample before and after treatment was measured using pH meter model "EUTECH instrument pH700".

Conductivity measurements

Conductivity of the leather sample before and after treatment was measured using the conductivity meter model "EUTECH instrument COND610".

Chloride analysis

Chloride was estimated by Mohr's method.10ml of the sample was taken in a conical flask and 3 drops of potassium chromate (as indicator) was added to the samples. Standardized $AgNO_3$ of 0.1N was used to titrate against all the samples until the color changes from yellow to brick red. The chloride concentration was estimated as follows

$$\text{Chloride} = \frac{(A-B) \times N \times \text{molecular weight of chloride}}{\text{Volume of sample}} \text{ mg/l}$$

Where, A = ml of $AgNO_3$ required for sample
B = ml of $AgNO_3$ required for blank
N = normality of $AgNO_3$ used

Sulphate analysis

Sulphate was estimated by using gravimetric method. 20ml of the sample was acidified by 5ml of 1:3 HCl. It was diluted using 80ml of distilled water and heated. 10ml of 10% $BaCl_2$ was added and stirred. The sample was kept in water bath for for 2 hrs. The sample was filtered and washed with distilled water. The precipitate was kept in a furnace at 900^0C and weighed as barium sulphate $BaSO_4$.
Sulphate was calculated as follows

$$\text{Sulphate} = \frac{96.06 \times \text{weight of precipitate} \times 1000}{233.4 \times \text{ml of sample}} \text{ mg/l}$$

Total organic carbon (TOC)

5ml of sample was taken in flask. 10ml of 1N $K_2Cr_2O_7$ was added to the sample and was mixed with conc. H_2SO_4 for 1 minute. After 30 minutes 200ml of distilled water, 10ml of phosphoric acid and 1ml of diphenylamine indicator was added. The prepared sample was titrated against 0.5N ferrous ammonium sulphate. The dull green was turned into dull blue color. The final end point is the appearance of a bright green.

Total dissolved solids (TDS)

The sample was heated until only the dry sediment was obtained. The weight of the beaker along with the sediment was taken after few minutes. The sediment was collected and stored in a desiccator.
TDS = (Weight of the beaker with sample) – (Dry weight of the beaker) g/l

Chemical oxygen demand (COD)

2.5ml of the sample and 2.5ml of the blank was taken. A pinch of $HgSO_4$ was added to each sample. 3ml of $K_2Cr_2O_7$ and 3.5ml of H_2SO_4 reagent was added. The sample was kept for digestion at 150^0C for 2hrs in a COD digester model "Spectroquant TR 320". The end point was a sharp color change from blue green to reddish brown.
COD was calculated using the following formula,

$$\text{COD} = \frac{(A-B) \times N \times 8 \times 1000}{\text{Volume of sample taken}} \text{ mg/l}$$

Trace metal concentration (AAS)

100ml of filtered sample was taken. At pH 2 add HNO_3. Then sample was poured into a separating funnel (No. 1) and 1ml of Ammonium pyrrolidinedithiocarbamate (APDC) was added to the sample and was shaken well. 15ml of Methylisobutylketone (MIBK) solvent was added to the sample and was shaken vigorously for 5 minutes. The sample was allowed for layer separation approximately for 5 minutes The volume was made up to 25ml with double distilled water and was stored in a small polyethylene bottle until analysis in AAS. Various elements in the sample were analyzed using atomic absorption spectrophotometer (AAS) model "variant Spectra 220".

Electrochemical study and bioelectrochemical study:

- CELL - Two compartment cell
- MEMBRANE - Proton exchange membrane (Nafion 117')
- ANODE - TSIA electrode
- CATHODE - Titanium electrode
- ANOLYTE - HCl
- CATHOLYTE - Leather effluent
- BIOCATHOLYTE - Leather effluent enriched with Microorganisms

The electrodes were fixed in the 2 compartment membrane containing cell. Anolyte and the catholyte were added in specific anode and cathode compartments. The electrolytic cell was runned for 1 hr and the foam generated was simultaneously collected and dissolved in the distilled water. The electrolyzed product from the electrolytic cell was further analyzed for chromium reduction (Fig. 16).

Analysis of electrolyzed product

UV-Visible analysis

Liquid sample was used to analyze the presence of chromium (VI) and Chromium (III) in 200-800nm range. The analysis was performed in an UV-Visible spectrophotometer model "Thermo scientific evolution 201".

FT-IR analysis

Bruker, Tensor 27 model Fourier Transform Infrared Spectroscopy (FTIR) system was used for the analysis of the direct sample and precipitate obtained after running EK cell. The spectrum was taken in the mid IR region of 400 – 4000 cm^{-1} with 16 scan speed.

EDAX analysis

Sample obtained was dried at 100^0C and powdered and was used to determine the nature of the element using EDAX model: Nazron system SIX (Thermo electron corporation).

Determination of total Chromium

The total chromium concentration was analyzed using Atomic Absorption Spectroscopy (AAS).

Determination of Chromium (VI)

Chromium (VI) formed complex with 1, 5-Diphenyl carbazide (DPC) and therefore the chromium (VI) concentration was identified by using spectrophotometric analysis. A blank and a standard solution were analyzed with UV- Visible spectrophotometer with 1cm quartz cell which was used and the absorbance measurements was performed in the range of 300 to 800nm.

Determination of Chromium (III)

Chromium (III) could be calculated by subtracting the amount of Chromium (VI) from the total chromium. The amount of chromium (III) could be calculated as follows,

Amount of Cr (III) = (Total Cr)-(Concentration of Cr (VI))

Result and Discussion

The effluent sample was collected from treatment plant at CLRI and transported to the laboratory for further studies. Serial dilution was performed in order to decrease the load of microorganism in the leather effluent. From various dilutions pour plate was performed to isolate individual colonies. The isolated colony was inoculated into *Pseudomonas sp.* specific media. Various staining technique, motility test and biochemical tests were performed. The obtained result was presence of gram negative rod shaped motile organism and the organism was citrate, catalase, oxidase and gelatine hydrolysis positive. It was proved that the isolated organism was *Pseudomonas aeruginosa.* This organism was resistant to chromium (VI). *Pseudomonas aeruginosa* culture was added to the leather effluent for enriching the microorganism. Various possible metals that could be present in the leather effluent were identified using Atomic Absorption Spectrometery (AAS). Chromium, calcium, lead, iron, magnesium, copper and sodium were present. Among all these elements chromium was found to be in higher concentration nearly 1,88, 762mg/l (Subramanyan *et al*., 2009) [11].

Various other parameters such as color, pH, conductivity, TDS, chloride, sulphate, TOC, COD, chromium were analyzed before treatment and after treatment both electrochemically and Bioelectrochemical. Color reduction was observed after electrochemical and bioelectrochemical treatment (Table 1).

Table 1: Characteristics of leather waste before and after treatment

S. No.	Parameters	Before Treatment	After Treatment	
			Electrochemical method	Bioelectrochemical method
1.	Colour	Dark green	Green	Green
2.	pH	5.40	1-2	1-2
3.	Conductivity	80.88mS/cm^2	94.90mS/ cm^2	83.04mS/ cm^2
4.	TDS	75.28ppt	87.91ppt	77.17ppt
5.	Chloride	53780mg/l	Nil	Nil
6.	Sulphate	6150mg/l	12680mg/l	13910mg/l
7.	TOC	6137mg/l	6137mg/l	6137mg/l
8.	COD	7040mg/l	4480mg/l	6720mg/l
9.	Chromium	1,88762mg/l	1,43765mg/l	1,32405mg/l

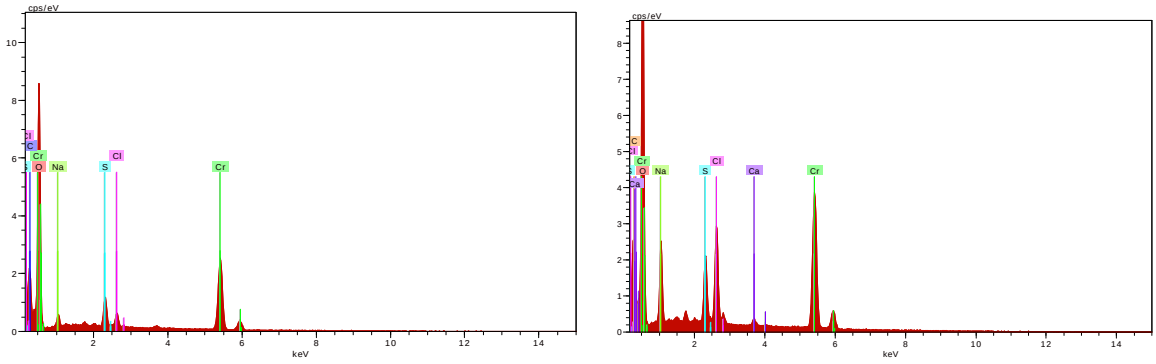
Then intensity of color reduction was merely same in both electrochemical and bioelectrochemical process. Chemical oxygen demand (COD) which was 7040mg/l before treatment decreased after treatment 4480mg/l for electrochemical process and 6720mg/l for bioelectrochemical process. The chloride 53780mg/l before treatment was nil after treatment.

Reduction in chloride may be due to the chlorine gas evolution at the anode and cathode (Rao *et al*., 2001). pH which was 5.40 before treatment reduced to 1-2 after treatment and the conductivity which was 80.88mS/cm^2 before treatment increased after treatment 94.90mS/cm^2 for electrochemical process and 83.04mS/cm^2 for bioelectrochemical process. This may be due to the protons (H^+ ions) from the anolyte. The value of sulphate increased after the electrochemical and bioelectrochemical treatment. Various papers demonstrated that electrochemical oxidation of sulfides to sulphur in tannery wastewater (Rajalo *et al*., 1996) [7]. Total dissolved solid increased after treatment. Total organic carbon (TOC) 6137mg/l was same for before treatment and after treatment. Total chromium before treatment was 188762mg/l and it reduced after treatment to 143765mg/l for electrochemical process and 132405mg/l for bioelectrochemical process.

UV-Visible combined spectrum proved that the intensity of the untreated sample was high when compared to the treated

sample. After electrolysis there was significant decrease in the intensity. Therefore the concentration of the chromium was gradually decreased after electrolysis. The peak 420 was for chromium (III) and the peak 582 was for chromium (III). With UV-Visible spectrophotometer presence of chromium (VI) was read at 540nm and was found to be in trace compared to chromium (III). In the direct sample before treatment 19.76mg/l of chromium (VI) and 188742.24mg/l of chromium (III) was the initial amount. But after electrochemical process 16.10mg/l of chromium (VI) and 188745.9mg/l of chromium (III) and after bioelectrochemical process 7.13mg/l of chromium (VI) and 1,88, 762mg/l of chromium (III) was present. Fonseca *et al*., 2009) [3].

The peak at 2925.55cm^{-1} indicated the presence of aliphatic C-H stretching. The peak at 1633.23cm^{-1} indicated the presence of carboxylate anion the formation of carboxylate anion which also enhanced chelating chromium in the effluent.(Fig 1).



(i) Electrochemically treated sample (ii) Bioelectrochemical treated sample

Fig 1: FT-IR spectrum of pure leather sample before and after treatment

The functional groups before treatment were OH structure, carboxylate anion, C-O structure and C-Cl structure. But after electrochemical treatment no OH structure was observed (Subramanyan *et al*., 2009) [11].

EDAX analysis showed that during the electrolysis process chromium was removed from the effluent in the form of foam. The foam collected during the electrochemical process was dissolved in distilled water and filtered and the precipitate was analyzed by EDAX. It was found to contain 28.5 weight % of chromium. The foam collected during the bioelectrochemical process was dissolved in distilled water and filtered and the precipitate was analyzed by EDAX and it was found to contain 28.64 weight % of chromium. Presence of other elements in trace amount were also found (Subramanyan *et al*., 2009) [11].

In both after treatment electrochemical and bioelectrochemical process reduction of the chromium (VI) was proved when compared to the total amount of chromium (VI) before treatment. At the same time increase in the chromium (III) amount was also proved when compared to the total chromium (III) before treatment. The chromium (III) during electrolysis precipitates on the cathode surface (Elsayed *et al*., 2009) [2]. The chromium (VI) may had been present in the electrolyte. The foam was formed at higher current density due to rapid gas evolution. *Pseudomonas aeruginosa* reduced the trace amount of toxic chromium (VI).Sankar Narayan Sinha *et al*., 2011) [8]. The effluent sample was found to contain large amount of chromium (III) and less amount of chromium (VI). Even at less concentration of chromium (VI) reduction was found to occur by *Pseudomonas aeruginosa* (Table 2)

Table 2: Determination of chromium (III) and chromium (VI)

S. No.	Forms of chromium	Direct sample	Electrochemically treated sample	Bioelectrochemical treated sample
1.	Total Chromium (Cr^{3+} and Cr^{6+})	1,88,762mg/l	1,88,762mg/l	1,88,762mg/l
2.	Chromium (VI)	19.76mg/l	16.10mg/l	7.13mg/l
3.	Chromium (III)	1,88,742.24mg/l	1,88,745.9mg/l	1,88,754.87mg/l

Conclusion

Chromium a transition metal was found in various forms. Tanning industries used the chromium (III) in the chrome tanning process because of its various advantages. It was also reported as nutrient source for health. This chromium (III) used in tanning was found to be released in the tannery effluent. Hence recovery method was aimed to obtain the chromium (III) from the tannery effluent and to reuse it. Presence of chromium (III) is observed in the tannery leather effluent in large concentration. Due to various use of the chromium (III) the study aimed to recover chromium (III) from the leather effluent and also to convert the trace amount of chromium (VI) which is toxic to non toxic chromium (III).

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