International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.5, No.5, pp 2177-2185, July-Sept 2013

Detoxification And Bioremediation Of Chromium (VI) From The Tannery Effluents

Katragadda Sai Priya[1], Kondate Roja[1], Sakunthala Priya.A[1], Arvind Sivasubramanian[2] and Meenakshi sundaram Muthuraman[1]*

[1]Department of Biotechnology, School of Chemical and Biotechnology, SASTRA University, Thanjavur-613 401, Tamilnadu, India

[2]Department of Chemistry, School of Chemical and Biotechnology, SASTRA University, Thanjavur-613 401, Tamilnadu, India

***Corres. author: msundar77@yahoo.com**
Mobile: +91-9894475375

Abstract: [Cr(III)] is used in tannery processing and when left untreated, it is converted to its toxic form [Cr(VI)], due to oxidation. The toxic hexavalent chromium [Cr(VI)] is a known carcinogen and a mutagen. Extensive use of hexavalent chromium [Cr(VI)] in various industrial applications has caused substantial environmental contamination. The present work involves the evaluation of two bacterial strains, *Ochrobactrum anthropi* and *Chromobacterium violacieum*, for their hexavalent chromium reduction activity. Samples were collected from tanneries during the stages of animal hide processing, chrome tanning and final processing. Initial parameters like COD, BOD and pH were measured in the samples. In this study, strains were experimented on the final tannery effluent sample and on the nutrient broth amended with 50 and 36mg/l of chromium (VI) concentrations and their comparative efficiency for detoxification of chromium(VI) were analysed and compared. *Aspergillus niger* and other precipitating agents were experimented on chrome tanning effluent samples and their efficiencies for the removal of trivalent chromium were analysed and compared.
Keywords: Chromium, Detoxification, Tolerance, Bioremediation.

1. Introduction:

Chromium is a blue-white metal that is hard, brittle and corrosion resistant. Chromium compounds are used to anodize aluminum, a process which coats aluminum with a thick, protective layer of oxide. Chromite, chromium's primary ore, is used to make molds for the firing of bricks because of its high melting point, moderate thermal expansion and stable crystal structure. So it is released into the environment by a large number of industrial operations including chrome plating, petroleum refining, leather tanning, wood preservation, textile manufacturing and pulp processing. Chromium has been widely used in tannery industries. The most stable forms are [Cr(VI)], [Cr(III)] . [Cr(III)] is a less toxic , less soluble and thus has a lesser problem when used for processing. But it is converted to its toxic form [Cr(VI)] due to oxidation when left untreated. Toxic [Cr(VI)] is a known carcinogen and mutagen[1]. The deposition of metallic chromium materials imparts a refractory nature on such materials thus rendering them resistant to microbial attack and flexible over extended periods. Acute exposure of Cr(VI) causes contact dermatitis, nasal perforations, rhinitis, kidney damage and abnormalities in liver and reproductive system. One main major source of chromium pollutant

releasing industry is the tannery industries. Permissible [2] limit of total chromium in Indian tanneries is 2mg/l. Hence the major task is to detoxify and remove the polluting chromium from tannery effluents. Several physical and chemical methods exist to remove chromium from the environment. However these methods are reported to be impractical due to the high operational cost and an subsequent generation of solid waste which is difficult to treat[20]. Hence microbial tolerance to hexavalent chromium has practical importance because it can serve as a basis for selecting organism that can be used to detoxify chromium in the environment. The present work deals with the bioremediation of hexavalent chromium with bacterial strains like *Ochrobactrum anthropi* and *Chromobacterium violacieum* and removal of trivalent chromium with *Aspergillus niger* and other precipitating agents were experimented, analysed and compared.

2.Materials and methods:

2.1 Microorganisms employed for detoxification of Cr(VI)

Pure cultures of *Ochrobactrum anthropi* (MTCC No-9026) and *Chromobacterium violaceum* (MTCC No-8071) were purchased from MTCC, Chandigarh, India and were immediately transferred to sterile agar slants of nutrient agar media. The strains were grown in nutrient media: Yeast Extract 2.0g, Beef extract 1.0g, Peptone 5.0g, NaCl 5.0g, Distilled water 1.0L. Bacterial cells were cultivated at 37°C with repeated sub-cultures to ensure activity of cells. The Bacterial Biomass was transferred to 100ml of Nutrient broth and was incubated at 37 °C for 24 hours in an oribital shaker at 120 rpm. These bacterial cells are preserved in glycerol stock at -20°C for long term use. Starter culture for the experiment is prepared by inoculating a loop full of bacteria from the preserved cultures in two different 250ml flasks containing 100ml of nutrient broth and incubated at 37°C, for 24hrs.

2.2 Chromium(VI) reduction analysis in nutrient growth media

Prepare nutrient broth amended with Cr(VI) at 50mg/l and 36mg/l concentrations in 250 ml of conical flasks and keep it for sterilization .Then inoculate it with 2% of starter cultures of *Ochrobactrum anthropi*[7] and *Chromobacterium violaceum*[7] in each of conical flasks containing different concentration of chromium. For growth analysis OD at 600nm is observed for samples taken from all conical flasks at definite intervals of 24,48,72,96,120 hrs. For observing Cr(VI) reduction draw the aliquots at the above pre defined intervals and centrifuge at 7000 rpm for 5mins then supernatant is collected and analysed for Cr(VI).Hexavalent chromium was determined by 1,5-diphenyl carbazide method using UV-VIS spectrophotometer[6].

2.3 Hexavalent chromium reduction analysis in final tannery effluent

100ml of sterilized and filtered tannery effluent was taken in two conical flasks and are inoculated with 0.1% of each bacteria in different flasks and incubated at 37°C at 220 rpm for 24hrs.Test aliquots are withdrawn at definite intervals and Centrifuged at 7000 rpm for 5mins, supernatant is collected for Cr(VI) analysis[9].Hexavalent chromium concentration in the supernatant was determined by 1,5-diphenyl carbazide method using UV-VIS spectrophotometer.

2.4 Removal of Trivalent Chromium from Chrome Tanning Effluent

2.4.1 Microorganisms and media employed for removal of chromium (III)

Pure culture of *Aspergillus niger*[5] used in this study was purchased from MTCC, Chandigarh, India and was immediately transferred to sterile fungal slants of Czapek Dox Agar media.. The fungal Biomass was transferred to 100ml of Czapek Dox broth and was incubated at 37 °C for 4 days in an oribital shaker at 120 rpm. The pH of media was adjusted to 7.2.The cell suspension was then separated and stored for subsequent use in biosorption experiments.

2.4.2 Preparation of Live biomass for bioaccumilation

The fungus was filtered and washed several times with distilled water so as to free it from the media components. It was air dried and 1g was used for 100ml of tanning effluent.

2.4.3 Preparation of Auto claved biomass for biosorption

Known aliquot of *Aspergillus niger* was taken in excess and autoclaved at 15lb and 121°C for 15 minutes then it was filtered and washed several times with distilled water. It was air dried and 1g was used for 100ml of tanning effluent.

2.4.4 Preparation of alkali treated fungus (pre-treatment).

The adapted fungal species was boiled in 50 ml of 0.5 N sodium hydroxide for15 min, filtered and washed several times with distilled/deionised water. Washing with deionised water was meant to bring down the pH to neutral range. It was then air dried and 1.0g of this biomass was used per 100 ml of chrome tanning effluent.

2.5 Treating chrome tanning effluent by *Aspergillus niger*:

Batch experiments were carried out in conical flasks by adding known volume/weight of fungal biomass in different forms mentioned above in chrome tanning effluent. The flasks were gently incubated at 35°C and 120rpm in an orbital motion shaker. Samples were taken from the solution after 5 to 6 days for the estimation of residual Cr (III) ion concentration in the solution.

Samples drawn was treated under ultrasonic vibrations and then centrifuged at 10000 rpm for 5minutes. The supernatant was filtered using Whattman No .42 filter paper and the biomass retained was washed with a slow stream of deionised water, and again passed through the filter assembly[5]. The net supernatant collected was diluted into volumetric flasks using 5% HC1 for estimation of Cr (III) ions by AAS[12]. The removal of Cr (III) concentration was then calculated from the difference between the initial total Cr (III) and the present value.

2.6 Treating chrome tanning effluent by precipitating agents:

2.6.1 Using sodium hydroxide:

15% sodium hydroxide was added to 100ml of tannery effluent and the pH was maintained at 9. The sample was centrifuged at 1200 rpm for 15 min and the supernatant was filtered using Whattman filter paper, and chromium concentration in the sample was analyzed using AAS. After 4hrs of settling time the sludge volume was measured [18].

2.6.2 Using lime:

3g of lime was added to 100ml of tannery effluent and the pH was maintained. The sample was centrifuged at 1200 rpm for 15 min and the supernatant was filtered using Whattman filter paper, and chromium concentration in the sample was analyzed using AAS. After 4hrs of settling time the sludge volume was measured.

Calculation:

$$\% \text{ Removal of chromium} = \frac{\text{Initial chromium value} - \text{final chromium value}}{\text{Initial chromium value}} \times 100$$

3. Results and discussions:

3.1 Initial parameters

The table 1 shows BOD, COD, pH, total chromium concentration of the final tannery effluent and chrome tanning effluent. The permissible limit of BOD and COD as stated by pollution control board of India is 30 mg/l & 250mg/l[2]. When compared with this standard value the below stated value in the table is quite high .The reason for this dramatic increase is mainly due to the difference in organic and inorganic load as it keeps differing from industries to industries. Another reason is due to the usage of cheap chemicals by the tanning industries. Basically the COD value will always be higher than the BOD value, mainly because the BOD deals only with the biodegradable substances and the COD deals with both biodegradable and non-biodegradable substances. pH value of the both effluents seems to be in alkaline condition. The chromium concentration is

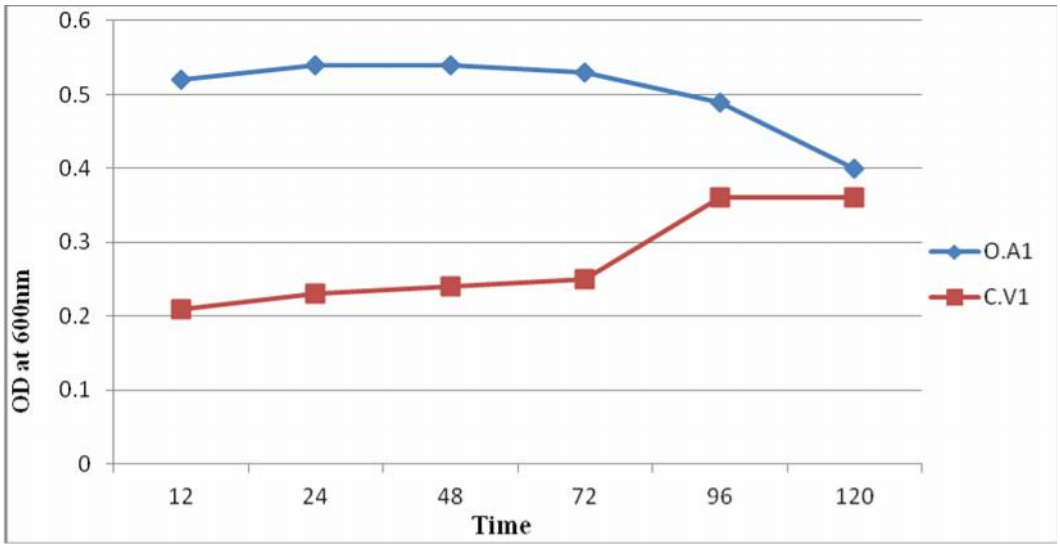
very high in chrome tanning effluent when compared with the final effluent as it was collected after the chrome tanning process in the industry.

Table1: Initial parameters of tannery effluents

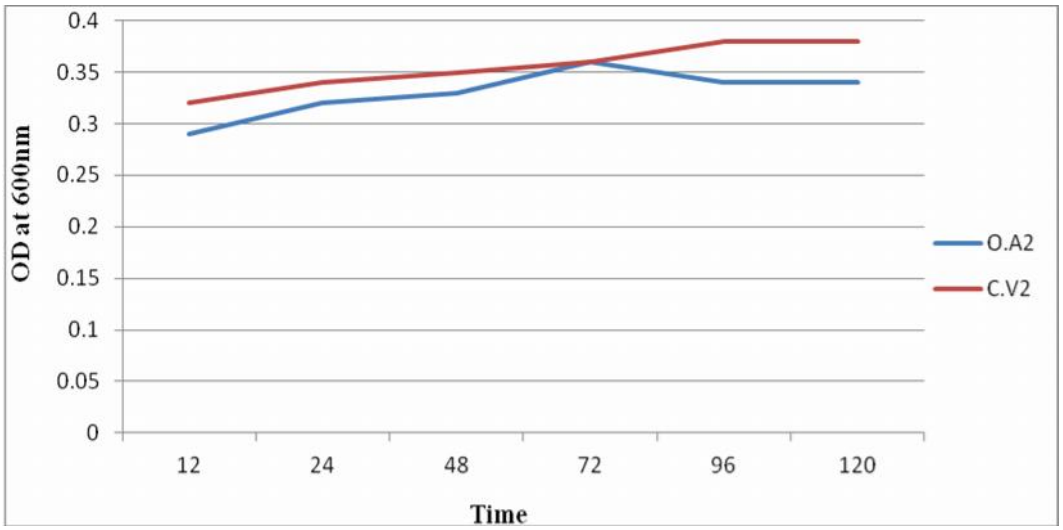
Contents	Final tannery effluent	Chrome tanning effluent
Total chromium concentration	36ppm	1050ppm
Biological oxygen demand	445ppm	5086ppm
Chemical oxygen demand	950ppm	11050ppm
pH	5.7	3.6

3.2 Growth study:

Graph1 and graph2 shows the growth curve plotted for *Ochrobactrum anthropi* and *Chromobacterium violaceum* in nutrient growth media amended with two different concentrations of hexavalent chromium 50mg/l and 36mg/l. The different phases of growth are more readily distinguished when the viable cell concentration is plotted against time. During the lag phase immediately after inoculation rate of growth is essentially zero. Cells use the lag phase to adapt to their new environment. New enzymes or structural components may be synthesized. Following the lag period growth starts in the acceleration phase and continues to the growth and decline phases. The growth phase appears as a straight line as the nutrients in the culture medium become depleted or inhibitory products accumulate slows down and the cells enter the decline phase. *Ochrobactrum anthropi* had shown growth phase till 48 hrs ,from 48hrs to 72 hrs it has shown stationary phase, from 72hrs to 120hrs and from then the decline phase persists. While *Chromobacterium violaceum* has shown growth phase till 72hrs,from 72hrs to 120hrs it has shown stationary phase in the nutrient growth media amended with 50mg/l,36mg/l of hexavalent chromium concentrations.



Graph I: Shows time course growth study of *Ochrobactrum anthropi* and *Chromobacterium violaceum* in nutrient growth media amended with 50mg/l of Cr(VI).



Graph 2: Shows Time course study of the growth of *Ochrobactrum anthropi* (O.A2) and *Chromobacterium violaceum* (C.V 2)in nutrient broth growth media amended with 36mg/L of Cr(VI).

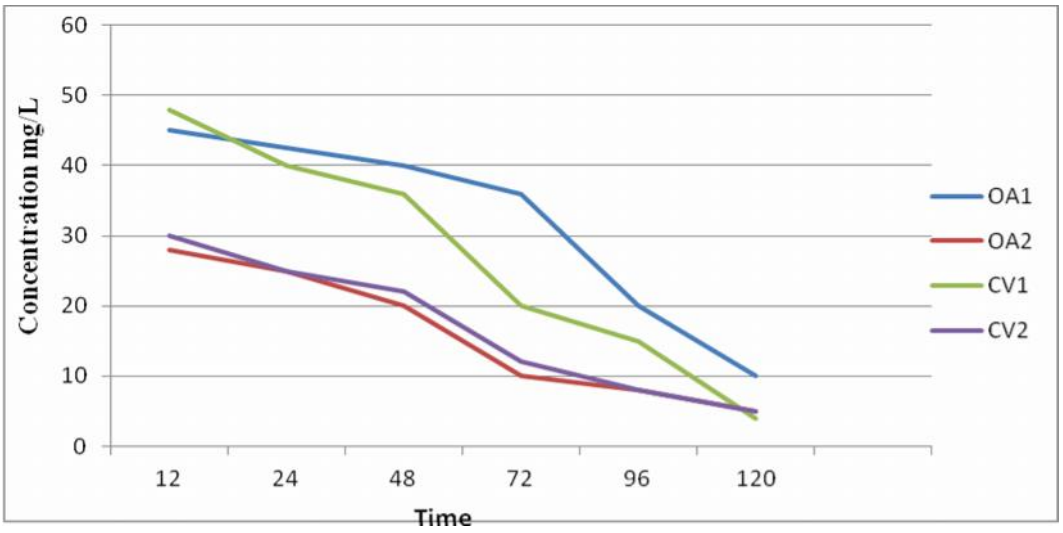
3.3 Reduction analysis in nutrient broth amended with Cr(VI):

Table 2 shows the time course study of hexavalent chromium reduction by *Ochrobactrum anthropi*(O.A1) and *Chromobacterium violaceum* (C.V1) in nutrient broth growth media amended with 50mg/L of Cr(VI).Taking the readings at different intervals of time it is found that for 50mg/l concentration *Chromobacterium violaceum* had shown maximum reduction of chromium than *Ochrobactrum anthropi*. While As per reduction analysis of hexavalent chromium by *Ochrobactrum anthropi*(O.A 2) and *Chromobacterium violaceum(*C.V 2) in nutrient broth growth media amended with 36mg/L of Cr(VI). at this chromium concentration both the bacteria has shown nonetheless the same reduction.

Table 2: Hexavalent chromium reduction analysis in nutrient growth media

Time(hours)	O.A 1 (50mg/l)	O.A 2(36mg/l)	C.V 1(50mg/l)	C.V 2(36mg/l)
12	45	28	48	30
24	42.5	25	40	25
48	40	20	36	22
72	36	10	20	12
96	20	8	15	8
120	10	5	4	5

The enzymatic reduction of Cr(VI) involves soluble and membrane bound reductases[21] such as flavin reductase, cytochromes and hydrogenases. These enzymes can be part of electron transport system and use chromate as the terminal electron acceptor. Some of the membrane bound chromate reductases are associated with cellular energy generation . The chromate reductases use NAD(P)H as electron donors to reduce Cr(VI). The SOD catalyzes the dismutation of the superoxide anion (O2*-) into hydrogen peroxide and molecular oxygen. This protein is considered to be one of the most important anti-oxidative enzymes for *Ochrobactrum anthropi* as it allows the bacterium to survive high oxygen stress environments, such as the environment produced during the reduction process of Cr(VI)[4].



Graph 3: Time course study of Cr (VI)reduction by *Ochrobactrum anthropi* and *Chromobacterium violaceum* in nutrient broth amended with 50mg/L,36mg/L Cr(VI).

3.3.1 % Reduction :

Table 3 shows that at (50mg/l) concentration *Chromobacterium violaceum* has shown maximum reduction of 92% and at (36mg/l) both the bacteria showed nonethless the same %reduction.

Table 3: Hexavalent chromium % Reduction analysis in growth media.

Time(hours)	O.A 1 %reduction (50mg/l)	O.A 2 %reduction (36mg/l)	C.V 1 %reduction (50mg/l)	C.V 2 %reduction (36mg/l)
12	10	22.2	4	16.6
24	15	30	20	30
48	20	44.4	28	38.8
72	28	72.2	44	66.6
96	60	77.7	60	77.2
120	80	86.1	92	86

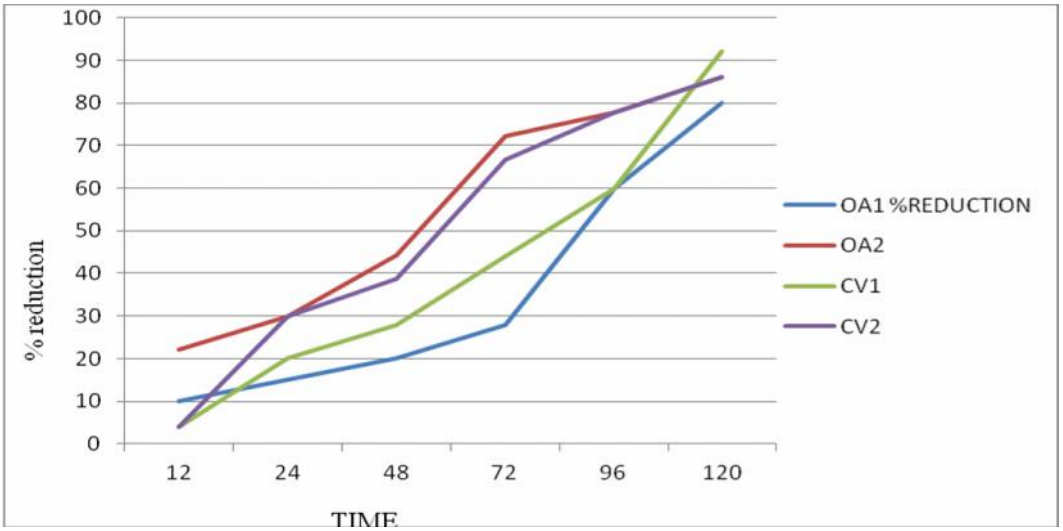


Figure 4: Time course study of %Cr(VI)reduction by *Ochrobactrum anthropi* and *Chromobacterium violaceum* in nutrient broth amended with 50mg/L,36mg/L Cr(VI)

3.4 Hexavalent chromium reduction analysis in final tannery effluent:

Table 4 shows Hexavalent chromium reduction analysis in tannery effluent .The characteristic feature of the microorganism is that it not only survives in Cr(VI) concentration but also perform function towards its reduction is important and qualifying attribute for bioreduction. The unique ability of *Ochrobactrum anthropi* and *Chromobacterium violaceum*[3] to grow and reduce chromate in final tannery effluent natural substrate such as tannery effluent without amendment and alteration of physiochemical properties has been achieved under this study. In the present study, we have studied the reduction ability of bacteria in filter sterilized absolute tannery effluent without changing its physio chemical properties which is truly conventional effluent condition. Needless to say, the progress achieved through these bacterial isolates could provide tannery effluents more efficient and sustainable way. In absolute tannery effluent *Chromobacterium violaceum* showed 84% of reduction while *Ochrobactrum anthropi* showed 80% reduction. This study reports for the first time, the ability of *Chromobacterium violaceum* to grow and reduce the Cr(VI) in absolute tannery effluent without any nutritional amendment.

Table 4: Hexavalent chromium reduction analysis in final tannery effluent

%innoculum	O.A % reduction	C.V % reduction
0.1	80	84

3.5 Removal of Cr (III) using *Aspergillus niger* from chrome tanning effluent:

Table 5 shows that *Aspergillus niger* taken in three forms live mass, autoclaves mass, alkali treated (pretreatment) which is experimented on chrome tanning effluent at two different concentrations (1000ppm and 2054ppm) after incubation period shown the following %reduction of chromium from chrome tanning effluent.

Table 5: Removal of trivalent chromium using *Aspergillus niger*

samples	Initial chromium concentration(ppm)	final chromium concentration(ppm)	%removal of chromium
live mass	1000	103.68	89.6
live mass	2054	143.96	90.4
alkali treated	1000	87.5	92.05
alkali treated	2054	125.96	93.86
dead mass	1000	97.8	90.8
dead mass	2054	110.9	92.7

Thus from the above table *Aspergillus niger* biosorption by dead mass is 84%, live mass is 89% and when pretreated with known alkali sodium hydroxide it showed maximum efficiency of 93% of trivalent chromium removal from tannery effluent containing 2054ppm of chromium. Biosorption and bioaccumulation mechanisms of chromium onto the *Aspergillus niger* taken in three different forms is as follows:

Physical adsorption: In this category, physical adsorption takes place with the help of vander Waals' forces. Chromium biosorption by dead biomasses of algae, fungi and yeasts takes place through electrostatic interactions between the metal ions in solutions and cell walls of microbial cells.

Transport across cell membrane: Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically important ions such as potassium, magnesium and sodium. The metal transport systems may become confused by the presence of heavy metal ions of the same charge and ionic radius associated with essential ions. This kind of mechanism is not associated with metabolic activity. Basically biosorption by living *Aspergillus* biomass comprises of two steps. First, a metabolism independent binding where the metals are bound to the cell walls and second, metabolism dependent intracellular uptake, whereby metal ions are transported across the cell membrane.

When pre-treated with known alkali sodium hydroxide it showed maximum efficiency of 93% of trivalent chromium removal from tannery effluent containing 2054ppm of chromium within incubation because the efficiency of cell surfaces(negatively charged) is increased by pre-treatment as alkali neutralises the cell charge therefore absorbing increased amounts of metal ions onto it .Metal affinity to the biomass can be manipulated by pre-treating the biomass with alkalies, acids, detergents and heat, which may increase the amount of the metal sorbed. The bioadsorption capacity of autoclaved *Aspergillus niger* decreased as compared to the live fungus, attributed to the loss of intracellular uptake. The heat treatment could cause a loss of amino-functional groups on the fungal surface through the non-enzyme browning reaction. Aminofunctional groups in the polysaccharides contribute to the binding of heavy metals. However *Aspergillus niger* biomass pre-treatment at 100°C for 5 minutes increased the biosorption. Alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites[23].

3.6 Chemical Precipitation: Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. Table 6 shows in this study that we achieved precipitation of chromium from chrome tanning effluent up to 99.8% using lime. While measuring the sludge measurement for sample of volume 60 ml it resulted in the sludge of volume 40ml and sludge nature is of tooth paste. Thus large amount of sludge containing toxic compounds produced during the process is the main disadvantage. The other disadvantages like , high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal has made it imperative for a cost-effective treatment method that is capable of removing heavy metals from aqueous effluents.

Table 6: Chrome tanning effluent treatment by precipitating reagents

Samples	%Cr removal
S1 (NAOH)	99.2
S2(LIME)	99.8

4. Conclusion

This study demonstrated that *Chromobactrum violaecium* has strong potential for detoxification of chromium than *Ochrobactrum anthropi* in tannery effluents and this bacteria could be ideal for developing a sustainable green technology. The ease of growing these bacteria in tannery effluent, without any amendment ,is paving a way for technology in which the bacteria will grow directly in effluents and perform chromium reduction in a cost effective manner. This developed technology due to its cost effectiveness can be easily adapted by small and unorganized sectors to detoxify hexavalent chromium before discharging the effluent in the environment.

The main advantages of biosorption method over chemical method for the removal of trivalent chromium from chrome tanning effluent using pretreated *Aspergillus niger* includes: low cost, high efficiency,minimisation of chemical and or biological sludge.No additional nutrient requirement. Regeneration of biosorbent; and possibility of chromium metal recovery.

Hence by the above stated reasons precipitating reagents can be replaced by *Aspergillus niger* for the effective removal of trivalent chromium from chrome tanning effluents.

5. References

1. Addis mekonnen, Determination of Cr(VI) andCr(III) in the tannery effluents of awash and addis ababa leather industries,Addis ababa university,department of chemistry,2006.
2. Javed A. Bhalli , Pollution Level Analysis in Tannery Effluents Collected From Three Different Cities of Punjab, Pakistan, journal of industrial pollution control board ,2004.
3. Sreeja Narayanan, Tintu Prasad, Indu C. Nair and Jayachandran K, A novel exploitable feature of *Chromobacterium violaceum:* experimental evidence for Phenol degradation, Novus International journal of Biotechnology ,2012.
4. Cheng Y, Xie Y,Identification and characterisation of Cr(VI) responding protein from a newly isolated *Ochrobactrum anthropi* CTS-32,Pub Med,2009.

5. Abhilash; Biosorption of Trivalent chromium from a model tanning solution by Adapted *Aspergillus niger*.
6. APHA: Standard Methods for the examination of water and wastewater, 18th Edn. American Public Health Association. American Water Works Association. Water Environmental Federation, Washington, DC. (1992).
7. Vera Raicevic ; Isolation of chromium resistant bacteria from bauxite mine area, African Journal of Biotechnology Vol.9(40),pp.6727-673.
8. Yangi ancheng, Bioremediation of Cr(VI) and Immobilization as Cr(III) by *Ochrobactrum anthropi*, Environ. Sci. Technol. 2010, 44, 6357–6363.
9. Seema sharma and Alok adholeya , Hexavalent chromium reduction in tannery effluent by Bacterial species Isolated from Tannery effluent contaminated soil, Journal of environmental science and Technology 5(3):142-154,2012.
10. Neha Singh, Tuhina Verma and Rajeeva Gaur, Detoxification of hexavalent chromium by an indigenous facultative anaerobic *Bacillus cereus* strain isolated from tannery effluent, African journal of Biotechnology,2012.
11. Seema Tharannu; Characterization of chromium remediating bacterium *Bacillus subtilis* isolated from electroplating effluent, International Journal of Engineering Research and Applications (IJERA) ISSN: 2248-9622 www.ijera.com Vol. 2, Issue 4, July-August 2012, pp.961-966 961.
12. A.E.Greenberg, L.S.Clesceri,Standard methods for the examination of water and waste water,18th ed.,American public health association,washington,DC,1992,P.358.
13. B. Dhal, Abhilash, K. D. Mehta & B. D. Pandey,Biological remediation of trivalent chromium from effluents,International Conference of Non-ferrous Metals Bhubaneswar 2009.
14. Ackerley DF, Gonzalez CF, Keyhan M, Blake R II & Matin A ; Mechanisms of chromate reduction by the *Escherichia coli* protein, NfsA, and the role of different chromate reductases in minimizing oxidative stress during chromate reduction. Environ Microbiol 6: 851–860,2004
15. Abdulla, chromium removal from tannery wastewater using chemical and biological techniques aiming zero discharge of pollution ,proceeding of fifth scientific environmental conference, 2010, zagazig uni., 171 - 183 .
16. B. Durga Devi*, A.J. Thatheyus and D. Ramya; Bioremeoval of hexavalent chromium, using *Pseudomonas Fluorescens*,journal of biotechnology and microbiology research,2012.
17. K.D.Mehta,V.kumaret, biological remediation of trivalent chromium from tannery effluents, International conference of Non-ferrous metals ,2009.
18. Abass Esmaeili, 2Alireza Mesdaghi nia ,Chromium (III) Removal and Recovery from Tannery Wastewater by Precipitation Process, American Journal of Applied Sciences,1471-1473, 2005.
19. Yangjian cheng (2010).environmental science technology,44,6357-6363.
20. Ezaka.E, Anyanwu.C.U (2011) chromium6 tolerance of bacterial strains isolated from sewage oxidation ditch, international jouranal pf environmental sciences Volume 1,NO 7.
21. Abhipsa Sarangi, Enzymatic reduction of hexavalent chromium in bacteria, ENVIS CENTRE Newsletter Vol.7,Issue 2 April 2009.
22. Bejoy Chandra Debnath,et.al; Isolation and Characterization of Chromium(VI)-Reducing Bacteria from Tannery Effluents ,Indian J Microbiol. 2011 January; 51(1): 76–81.
23. N. Ahalya, Biosorption of Heavy Metals, Research Journal Of Chemistry And Environment.

*****