Bioremediation of Tannery Effluents and Chromium Containing Wastes Using Cyanobacterial Species

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Abstract

Heavy metal pollution of ground and surface waters by industrial effluents has become a serious threat to the environment especially in developing countries. One example of heavy metal pollution is the high chromium containing liquid effluents discharged from tanneries. Though many conventional physico-chemical methods are currently being practiced, biotechnological methods are becoming attractive alternatives, as they are economical and eco-friendly. In this context, the search for innovative and eco-friendly biotechnologies to remove toxicants from effluents has focused attention on the detoxification capacity of a variety of microbes especially cyanobacteria. In the present work, preliminary studies were carried out using two species of cyanobacteria for the treatment of tannery effluents with special reference to trivalent chromium. The effects of different concentrations of chromium on the growth response of these species in terms of biomass and chlorophyll-a were studied in addition to their capacity to accumulate chromium. These species were very effective in removal of trivalent chromium (95 - 100%) besides reducing BOD and COD of the effluent. SDS-PAGE and FTIR studies indicated the formation of a complex with protein. Better performance of the species in the field conditions gives positive indication of their usefulness in treatment of tannery effluent.

Abstracto

Contaminación por metales pesados de los suelos y aguas superficiales se ha convertido en una amenaza seria al medio ambiente sobretodo en países en vía de desarrollo. Un ejemplo de la contaminación por metales pesados son los efluentes de alto contenido de cromo descargados por las curtiembres. Aun que muchos métodos fisicoquímicos se practican en la actualidad, los métodos biotécnicos se han convertido en atractivas alternativas, ya que son económicos y ecológicamente aceptables. En este contexto, la búsqueda de biotecnologías innovadoras y ecológicas para remover materias tóxicas de los efluentes ha enfocado la atención sobre la capacidad desintoxicante de varios microbios especialmente cianobacterias. En el presente trabajo, estudios preliminares se efectuaron utilizando dos especies de cianobacteria en el tratamiento de efluentes de curtiduría con especial hincapié en el cromo trivalente. Los efectos de las diferentes concentraciones del cromo sobre la capacidad de desarrollo de las especies en términos de biomasa y clorofila-a fueron estudiados en adición a su capacidad para acumular cromo. Estas especies fueron muy efectivas en remover cromo trivalente (95-100%) como también en reducir la DBO y la DQO del efluente. Análisis "SDS-PAGE" y "FTIR" indican la formación de un complejo con proteína. Un mejor rendimiento de las especies bajo condiciones industriales nos da una indicación positiva de su utilidad en el tratamiento de los efluentes de curtiembres.

Introduction

In recent years, the "planet earth" has been experiencing increased anthropogenic activities due to rapid industrialization. Effluents released from some of these industries cause concern in developing countries like India. These industries are dyeing, textile, distillery, and tanning industries. Many of these industries discharge liquid effluents containing toxic chemicals and heavy metals. When such effluents are discharged on to land and water bodies, they contaminate water source causing adverse effects on living organisms.[1]

The tanning industry, one of the oldest cottage industries in India, has long been recognized as a major contributor to water pollution due to high concentrations of organic and inorganic chemical substances present in their effluents.

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There are more than 2500 tanneries in India of which about 80% of them are engaged in chrome tanning with a total processing capacity of 6,000,000 tons of hides/skins per year.[2] About 80,000 m^3/day of liquid effluents are released after different tanning processes, polluting the environment.[3] The composite effluents are often characterized by obnoxious odors, high pH and toxicants such as chlorides, sulphides, sulphates, tannins, high concentrations of oxidizeable organic matter (BOD and COD) and chromium.[4-6] Lead, zinc, iron, copper, chromium and magnesium are the most common metals present in tannery effluents after tanning and finishing. It is reported that a typical tannery discharge contains 75 - 200 mg/L total chromium in the composite effluent. However, the concentration of chromium in the exhaust chrome liquor is typically 1500 ppm and may reach 3500 ppm on rare occasions.

Chromium is mostly present in the trivalent form. It is non-degradable, persists in nature for an extended period of time and can have deleterious effects on flora and fauna.[7,8] Chromium in the hexavalent form is toxic to living organisms, affecting various metabolic activities.[5,9] The trivalent form under certain conditions can be converted to hexavalent form. Therefore, it is essential to recover and recycle chromium from effluents before being released, as for example, improvements in tanning processes to optimize uptake of chromium by hides/skins, thereby limiting its discharge. In other words, better management of chromium by restricting its discharge at the source itself is an essential "first step". After release, there are basically two methods of chromium removal viz. physico-chemical methods and biological methods.[10]

Conventional physico-chemical methods include chemical reduction, adsorption, electrochemical reduction, ion-exchange, ion-floatation, precipitation, reverse osmosis, evaporative recovery and rhizofiltration. The general theme is removing heavy metals from waste water. But these processes seem to be uneconomical in addition to producing undesirable spin-offs.[1,11-13] An increasingly attractive solution to eco-management of chromium containing effluents may be with biotechnological methods, which seem to be more economical and eco-friendly. In recent years, a search for such innovative, eco-friendly biotechnologies to remove toxic chemicals and heavy metals from effluents has focused attention on microorganisms. In this context, biological detoxification, involving microorganisms, seems to be a viable option. In fact, efforts have been made over the last few decades towards treatment of effluents using microorganisms, especially microalgae. The microalgae, which are photosynthetic, play an important role in industrial wastewater treatment and are generally found to be very efficient, cost effective and eco-friendly.[14-17] In the present study, an attempt has been made to detoxify tannery effluents using cyanobacterial species.

EXPERIMENTAL

Culture: Pure cyanobacterial cultures of *Spirulina sp.* and *Chlorogloea sp.*, obtained from Center for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, were maintained in Erlenmeyer flasks containing sterilized standard medium used for cyanobacterial culture,[18] under controlled conditions in the growth chamber.

Chromium Source: Exhaust Chrome Liquor (ECL) was collected from CLRI tannery, Chennai, and was used for all 'chromium' studies. The chrome liquor containing 1500 ppm was used as a stock solution for further studies.

Laboratory Studies: For the laboratory and field studies, Zarrouk's medium and BG11 medium containing different concentrations of chromium (25, 50, 75, 100, 125 and 150 ppm) were prepared. 10 ml of inoculum was inoculated to Erlenmeyer flasks containing test solution (200 ml). The flasks were incubated in a growth chamber for 2 calendar weeks at 25 ± 2°C under white fluorescent lamps of 1500 lux.

Field Studies: For field studies polythene bags were used instead of Erlenmeyer flasks. The inoculated bags were kept in a nursery garden under diffused light (semi shade), which was sufficient for profuse growth of the organisms.

The cultures were checked periodically for growth by measuring OD at 560nm, which gives an indication of increased chlorophyll pigments, an indirect way of growth measurement in such studies and pH changes, if any, using a pH meter.

Microscopic study: A small amount of culture was transferred to a microscopic slide containing a drop of distilled water. The culture was teased properly to obtain isolated cells/filaments. A coverslip was placed carefully to exclude air bubbles. The cells were observed at 45 X.

Analysis of Samples: After 2 calendar weeks, the samples were analyzed for various bio-chemical parameters of the algal mass (biomass content, chlorophyll-a and protein content) and physico-chemical (BOD, COD and Chromium) characteristics of the test solutions.

Biochemical Analysis:

(i) Biomass

A known volume of the culture was centrifuged at 5000 rpm

TABLE I
Characteristics of Exhaust Chrome Liquor (Stock Solution)

Characteristics	Results
Color	Bluish green
pH	4.12
Total Solids (mg/ L)	27023
Total Suspended Solids (mg/ L)	109
Total Dissolved Solids (mg/ L)	26914
BOD (mg/ L)	271
COD (mg/ L)	920
Chloride (mg/ L)	5600
Chromium (ppm)	1500

for 15 minutes. The supernatant was discarded. The pellet was desiccated in an oven at 103 - 105°C. It was weighed before and after desiccation and biomass estimated in terms of dry weight.

(ii) Chlorophyll-a

Chlorophyll-a estimation was carried out by the Mac Kinney method[19] using methanol for extraction of chlorophyll-a.

(iii) Protein

Protein was estimated by Lowry's method.[20] For SDS-PAGE, the Laemmli method was followed.[21]

Physico - Chemical Analysis:

(iv) Total Solids, Dissolved Solids, Suspended Solids and Chlorides for Stock Solution

The characteristic analyses for the stock solution of ECL such as Total solids (TS), total dissolved solids (TDS), total suspended solids (TSS), and Chlorides were determined by the APHA Standard method.[22]

(v) BOD and COD

BOD and COD tests were performed before and after the treatment of test solutions by the APHA Standard method.[22]

(vi) Chromium Uptake and Valency State

Chromium uptake was estimated as per a Colorimetric method[23] after digesting the biomass with ternary acid mixture (sulphuric acid, nitric acid and perchloric acid in the ratio of 3.5 : 5 : 11.5).[8] To find out the valency state, biomass samples containing chromium were ashed in a muffle furnace at 500°C for 2 hrs. and allowed to cool. 5ml of HCl (1:1) were added and transferred to a test tube and shaken well. Diphenyl carbazide reagent was added and allowed to stand for 5 minutes. Presence of pink color indicates the presence of hexavalent chromium and non-development of pink color indicates the presence of trivalent chromium.[27]

(vii) Chromium Removal from Exhaust Chrome Liquor

The test solutions containing different concentrations of chromium were analyzed before and after treatment by the Colorimetric method.[23]

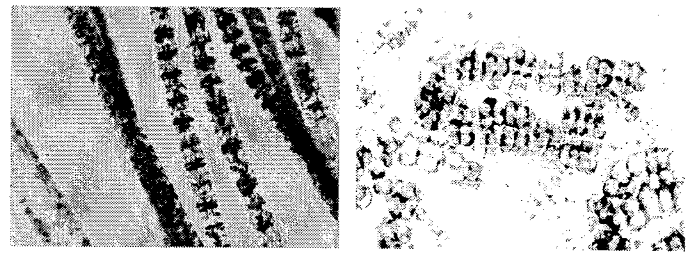


Figure 1. - *Spirulina sp.* at 45 X *Chlorogloea sp.* at 45 X

TABLE II
Biomass and Chlorophyll-a in the Laboratory Trial

Chromium concentration	*Spirulina species* Biomass (g/L)	Growth Inhibition %	Chlorophyll-a (µg/ml)	*Chlorogloea species* Biomass (g/L)	Growth Inhibition %	Chlorophyll-a (µg/ml)
Control	2.9	-	2.3	0.5	-	3.1
25 ppm	2.5	12	2.1	0.45	10	2.6
50 ppm	1.9	35	1.7	0.4	20	1.9
75 ppm	1.5	49	1.5	0.3	40	1.5
100 ppm	1.4	53	0.8	0.2	60	1.0
125 ppm	1.4	53	0.7	0.2	60	0.9
150 ppm	0.5	84	0.6	0.1	80	0.8
Biomass and Chlorophyll-a in the Field Trial						
	Spirulina species			*Chlorogloea species*		
Control	2.9	-	2.3	0.6	-	3.7
25 ppm	2.7	9	1.8	0.5	16	3.3
50 ppm	2.0	32	1.5	0.5	16	2.5
75 ppm	1.5	49	1.4	0.4	33	1.8
100 ppm	1.4	51	0.8	0.3	50	1.4
125 ppm	1.4	53	0.7	0.2	66	1.2
150 ppm	0.7	76	0.5	0.2	66	1.2

TABLE III
Protein Estimation of *Chlorogloea sp.*

Chromium Concentration	Total Protein (µg/100ml of fresh culture)
Control	780
100 ppm	360
150 ppm	240

(viii) IR Spectrum
5mg of biomass samples containing chromium were ground with 500mg KBr using a mortar and pestle and formed into a pellet. The infrared (IR) spectra were taken in a Nicolet Impact 400 Fourier Transform Infrared Spectrometer.[24] They were compared with the spectra of control samples.

RESULTS AND DISCUSSION

Basic chromium sulphate is the source of trivalent chromium in tanneries for chrome tanning and the unutilized fraction is released in the exhaust chrome liquor after chrome tanning. The Exhaust Chrome Liquor has been used for our studies. The results of analysis of the stock solution have been given in Table I. The efficiency of cyanobacterial species in chromium removal from media containing chromium has been studied, using test solutions of different concentrations ranging from 0 - 150 ppm. They truly represented the chromium contents in the composite effluents discharged from tanneries.

Microscope studies:
1) *Spirulina sp.*: Order - Nostocales; Family -

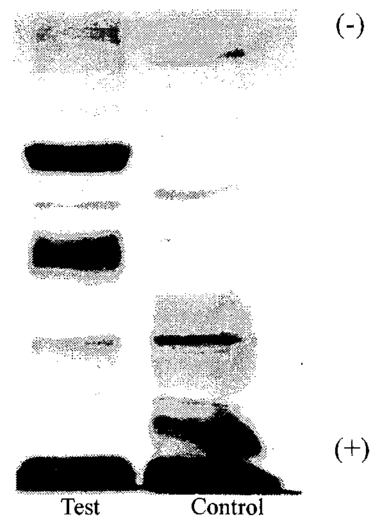


Figure 2. - SDS - PAGE (Qualitative Analysis)

Oscillatoriaceae. Thallus: Blue green trichomes slightly constricted at the cross-walls with sheath, 6 - 8µm broad, regular spirally coiled. Spiral 26 - 36µm wide, cells nearly as long as broad, 2 - 6µm long.
2) *Chlorogloea sp.*: Order - Chroococcales; Family - Entophysalidaceae. Thallus: Deep blue green crust of indefinite size, composed of rounded or irregular cell packets; cells arranged in vertical or horizontal rows, rounded or angular without evident mucilage envelops with pale blue-green, usually 6 - 8µm diameter. Single or in groups of two or more cells separating for propagation. Spherical, formed singly within the cells and liberated by the rupture of the membrane on germination forming a uniserate filament of 3 - 12 cells.[25] Photomicrographs of the cyanobacteria are shown in the Figure 1.

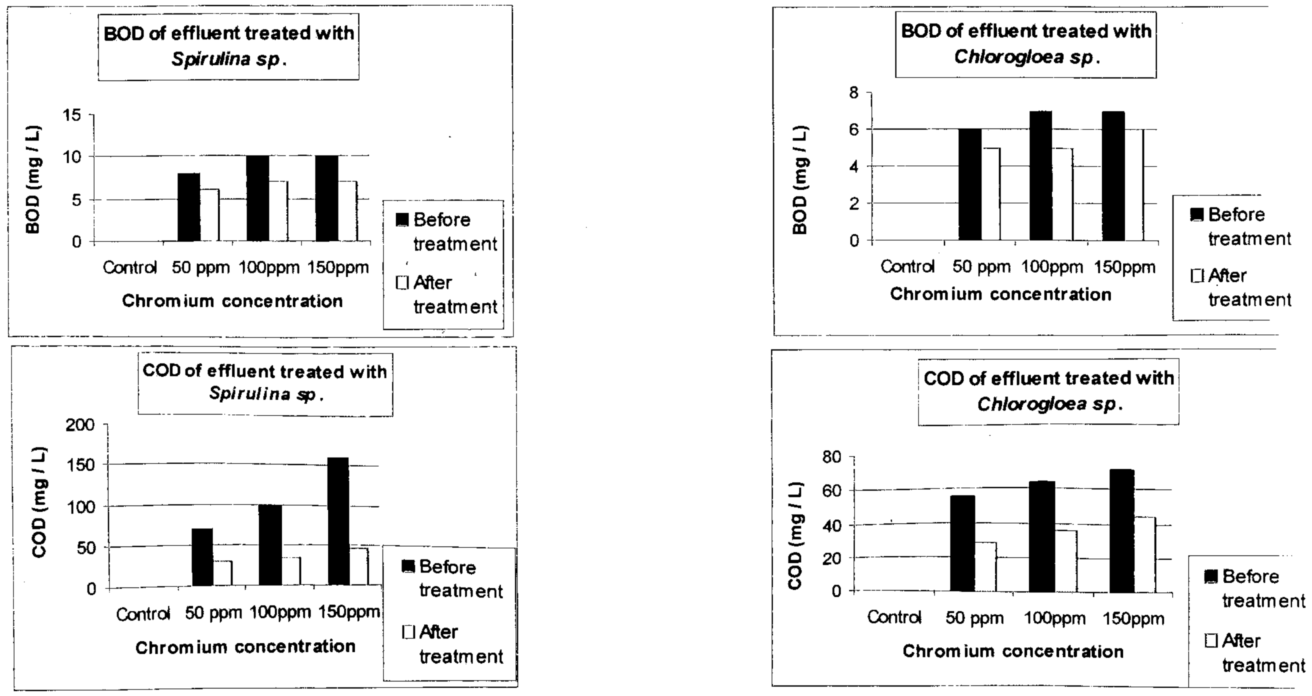


Figure 3. - The effect of *Spirulina sp.* and *Chlorogloea sp.* on COD and BOD in effluent

Biochemical Analysis: The growth response of the cyanobacterial species has been studied in both laboratory and field conditions in respect of biomass and chlorophyll - a. The results are given in Table II.
(i) Biomass
There was a tendency of decreased biomass with increased concentration of chromium under both laboratory and field conditions, when compared to the control. LC50 in terms of biomass has been found to be at around 100 ppm where 50% of inhibition was observed in both the species. It is interesting to note that there was a marginal increase in the biomass of both the species when grown under field condition.
(ii) Chlorophyll-a
The chlorophyll-a content after 15 days showed a gradual decrease with increasing concentrations of chromium in the growth media. The chlorophyll-a content increased in lower concentrations and was highest in 25 ppm and steadily decreased with increasing concentrations of chromium in the media. Furthermore, samples from field experiments showed equal or slightly higher concentrations of chlorophyll-a, when compared to their counterparts in laboratory studies.
(iii) Protein content
The results of protein estimated for *Chlorogloea sp.* are given in Table III and qualitative analysis is shown in the bands of SDS-PAGE (Figure 2).The electrophoretic pattern of proteins extracted from cyanobacterial biomass (*Chlorogloea sp.*) treated with chromium-containing effluent showed that the number of bands corresponding to chromium treatments was in excess of control. Hence, there

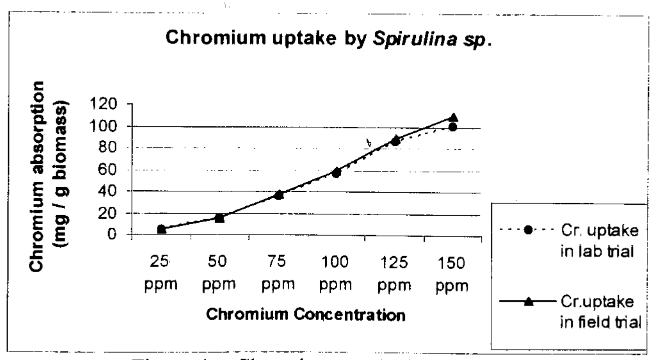


Figure 4. - Chromium uptake by *Spirulina sp.*

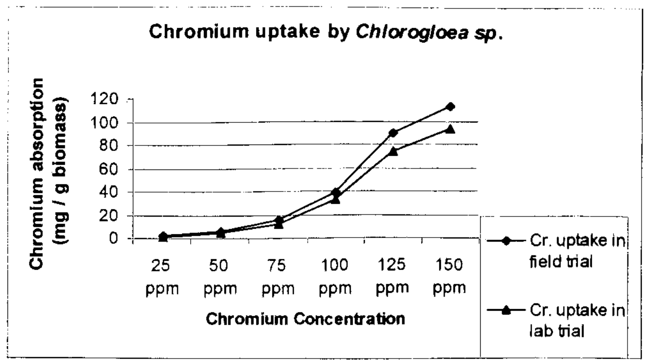


Figure 5. - Chromium uptake by *Chlorogloea sp.*

TABLE IV
Recovery of Chromium Using *Spirulina sp.* in Lab Trial

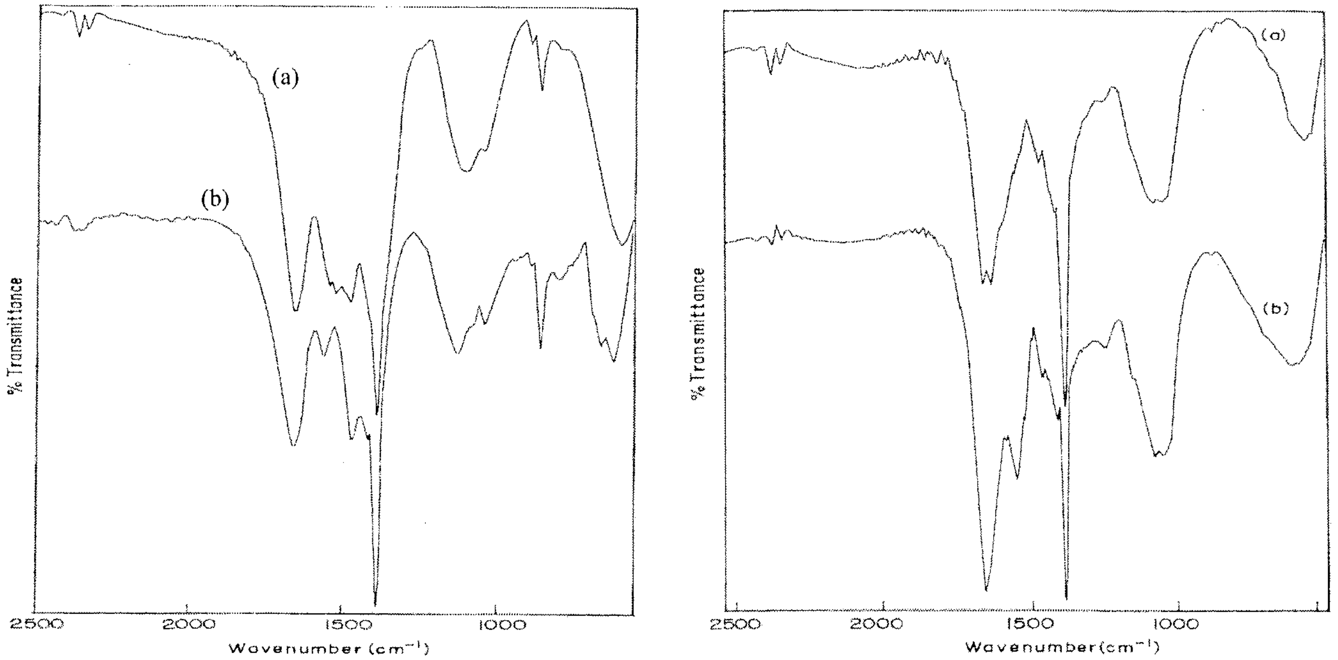
Chromium concentration	Concentration of chromium after treatment (ppm)	Chromium recovery from biomass
25	0.2	99.9
50	0.5	99.6
75	0.8	98.9
100	1.2	98.8
125	1.0	97.1
150	2.7	96.4

TABLE V
Recovery of Chromium Using *Chlorogloea sp.* in Lab Trial

Chromium concentration	Concentration of chromium after treatment (ppm)	Chromium recovery from biomass
25	0	100
50	0	100
75	0	100
100	0.1	99.5
125	0.2	99.2
150	0.4	98.6

was a clear evidence of change in the protein bands perhaps due to stress induced by chromium treatments/interference. Such changes in protein bands have been reported in rice seedlings treated with heavy metals like $ZnSO_4$, $CuSO_4$ and $HgSO_4$[26] and basic chromium sulphate in mung bean.[27] It seems such heavy metal induced changes in protein in plant related biological systems are common.

Physico - Chemical Analysis:
(iv) Biological Oxygen Demand and Chemical Oxygen Demand
The results of BOD and COD are given in Figure 3. Analysis of the treated effluent showed a considerable reduction in BOD and COD, indicating that pollution load has been significantly reduced especially with *Spirulina sp.*
(v) Chromium Uptake
Chromium uptake efficiency is illustrated in Figures 4 and 5. The species studied absorbed chromium depending on the concentration of chromium in the growth media. Higher chromium concentrations resulted in larger chromium absorption by the test organism. Such increased uptake of chromium by cyanobacteria has been reported earlier in *Oscillatoria sp., Nostoc sp.* and *Aulosira sp.* in laboratory and field conditions.[28,29] The present study also corroborates the results obtained in earlier studies. Furthermore, analysis of the test solutions after chromium challenge



(i) (ii)

Figure 6 (i). - IR Spectra of *Spirulina sp.*, 6 (ii). - IR Spectra of *Chlorogloea sp.*
(a) Control - biomass without chromium and (b) Biomass with 100 ppm chromium

reveals that they were almost chromium free. This is an essential characteristic for effluent treatment. In other words, the growth of the organisms enhanced the chromium removal. Despite biomass reduction with increasing chromium concentration in the growth media, chromium absorption by the test organisms was higher.

(vii) Removal of Chromium from Exhaust Chrome Liquor

The analysis of the test solution after the experiment (Tables IV and V) revealed that they were almost free of chromium, which is the most desirable characteristic required for wastewater treatment. It is observed that the growth of the organisms enhanced the removal of chromium (95 - 100%) depending on the concentration of chromium in the media. It is presumed that the complete removal of chromium is achieved due to the process of chromium accumulation by these organisms.

(ix) IR Spectra

The FTIR spectrum of the biomass is given in the figure 6 (i) and (ii). IR spectra of the control experiment [6 (i) a and 6 (ii) a] showed characteristic peak for $COOH^-$ group in the 1652 - 1544 cm^{-1} range. This peak was significantly altered in the spectra related to chromium treated cyanobacteria [6 (i) b & 6 (ii) b]. It appears that both cultures have at least 2 unique peaks. The affected peaks are different for the two organisms.

In general, bioaccumulation of heavy metals is the new trend for biological sequestration of heavy metals by employing suitable organisms. Cyanobacterial species have the capacity to remove toxic metal ions and remove other harmful organic components by binding, adsorption and absorption. Removal of metal toxicant from growth media seems to be the characteristic feature of these organisms and they even utilize certain inorganic components for their own metabolism. It seems that they allow themselves to use environmental contaminants as food and/or posses the capacity to adsorb/absorb toxic compounds. They can be used to sequester the metal through uptake or bioaccumulation. Therefore this character of cyanobacteria can be employed in the bioremediation of tannery effluents from which major toxic inorganic materials can be removed efficiently.

As regards the mechanism of bioaccumulation, it involves several processes, which act singly or in combination. The process may be endocellular, exocellular or pericellular. The prominent processes are:

(i) Extracellular accumulation/precipitation; e.g. extracellular polysaccharides serve to chelate or bind metal ions.
(ii) Cell surface sorption or complexation; rapid binding of cations to the negatively charged groups on the cell surface. Algal surfaces contain functional groups (eg. carboxylic, amino, thio, hydroxo and hydroxy-carboxylic groups) that can interact with metal ions.
(iii) Intracellular accumulation; a subsequent metabolism-dependent intracellular uptake in live alga, wherein intracellular polyphosphates precipitate in metal sequestration. Among these mechanisms, process (i) is facilitated by using viable microorganisms, process (ii) occurs with harvested biomass, whereas process (iii) requires microbial activity.[30-34]

In essence, the cyanobacteria studied seem to be ideal candidates to combat heavy metal pollution as the biologically active (living) and inactive (non-living) cells could bind significant quantities of metals. This shows the capacity of cyanobacteria to accumulate more chromium and still survive. In this connection, it is pertinent to mention that studies using algal biomass (harvested after optimal growth) have shown that more than 90% of chromium could be removed within an hour. Further studies to optimize the algal biomass culture are underway. The "practice" of growing these organisms in the effluent media can be eliminated. In other words, the biomass can be used to remove toxicants from the effluent media directly by adopting simple and easy techniques. Alternatively, these organisms can be used in the immobilized form to remove chromium, the advantages of which are reported elsewhere.[35,36]

Conclusion

The present study reveals that the test organisms possess their own adaptive way of survival despite decreased biomass and chlorophyll-a with increased chromium concentration. The study shows that it is possible to remove chromium from tannery effluents by adopting simple biotechnological methods using microalgae. The process, once standardized, might prove economical for small and medium scale industries and can be easily adopted by developing countries. Future experiments will attempt to identify the metal ion binding sites and biochemical pathways.

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References

1. Ozer, A. and Ozer, D.; Comparative study of the biosorption of Pb(II), Ni(II) and Cr(VI) ions onto *S. cerevisiae*: determination of biosorption heats. *J. Haz. Mat.* **B(100)**, 219 - 229, 2003.
2. Rajamani, S. and Raghavan, K. V.; Mitigation of environmental impact of tannery effluents. Proceedings of 4th National Symposium on Environment, Madras, 7 - 14, February 1995.
3. John Kennedy Jawahar, A., Chinnadurai, M., Samson Ponselvan, J. K., and Annadurai, G.; Pollution from tanneries and options for treatment of effluent. *Ind. J. Env. Prot.* **18(9)**, 672 - 678, 1998.
4. Southwick, C. H. and Van Nastard, D. Industrial Effluents. Ecology and quality of our environment, New York, (1976) 2nd Edition, 14.
5. Arora, H. C.; Biodegradation of effluents from tanning industry. Proceedings of the National Workshop on microbial degradation of industrial wastes, NEERI, Nagpur, 1 - 24, 1984.
6. Kannan, N. and Vijayaraghavan, A.; Studies on removal by adsorption and recovery of chromium from chrome liquor (tannery effluent). *Ind. J. Env. Prot.* **12(1)**, 46 - 49, 1992.
7. Swamy, M. and Margaret Therasa, Y.; The effect of trivalent chromium on membrane permeability in potato tubers. *J. Curr. Biosci.* **9(3)**, 109 - 113, 1992.
8. Swamy, M.; Effects of trivalent chromium (Cr - III) on *Vigna mungo L.* seedlings. *J. Ind. Bot. Soc.* **75**, 171 - 174, 1996.
9. Suseela, K., Sivaparvathi, M. and Nandy, S. C.; Removal of chromium from tannery effluent using powdered leaves. *Leather Science* **34(7,8,9)**, 149 - 156, 1987.
10. Prasad, B. G S. and Nair, B. U.; Recovery and recycling of regenerated chromium from spent chrome liquor. *Ind. J. Envtl. Hlth.* **36** (4), 267 - 271, 1994.
11. Patterson, J. W.; Treatment technology for hexavalent chromium wastewater. *Ind. J. Envtl. Prot.* **21(12)**, 1118 - 1121, 1985.
12. Ram, B., Bajpai, P. K. and Parwana, H. K.; Kinetics of chrome tannery effluent treatment by the activated sludge system. *Process Biochem.* **35**, 255 - 265, 1999.
13. Marshall, S.; Metal and inorganic waste reclaiming encyclopedia. Wayers Beta Corp., Park - Ridge: New Jersey, (1980).
14. De la Notie, J. and Proulx, D.; Algae and wastewater. *J. Appl. Phyco.* **4**, 247 - 254, 1992.
15. Dumans, A.; Biotreatment of fish farm effluents using the cyanobacteria *Phormidium bohneri. Aquacult Engg.* **7(5)**, 68, 1988.
16. Swamy, M.; The role of cyanobacteria in removal of chromium from tannery effluent with special reference to trivalent chromium. Proceedings of the National symposium on microbes in bioremediation for eco-friendly environment in the new millennium, University of Madras, Chennai, 6-7 Jan., 2000.
17. Aravindhan, R., Madhan, B., Raghava Rao, J., Nair, B. U., and Ramasami, T.; *Environ. Sci. Technol.* **38**, 300 - 306, 2004.
18. Rippka, R., Duruelles, J., Water Bury, J. B., Heroman, M. and Stainer, R. Y.; Generic assignments, stain histories and properties of pure culture of Cyanobacteria. *J. Gen. Microbiol.* **11**, 1 - 61, 1979.
19. Mac Kinney, G.; *J. Biol. Chem.* **140**, 314 - 322, 1941.

20. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.; *J. Biol. Chem.* **193(1)**, 265 - 275, 1951.
21. Laemmli, V. K.; *Nature* **227**, 680 - 685, 1970.
22. APHA. Standard methods for the examination of water and wastewater. American Public Health Association: Washington DC, (1975) 14th edition.
23. Snell, F. D. and Snell, C. T.; Colorimetric methods of analysis. D. Van Nostrand Company, Inc., Princeton, NJ, USA, (1959) Vol. II, 1st edition, 274 - 290.
24. Ravindra Babu, P., Sastry, T. P., Rose, C. and Muralidhara Rao, N.; *J. Appl. Polym. Sci.* **65**, 555 - 560, 1997.
25. Desikachary, T. V.; Cyanophyta. Indian Council of Agricultural Research, New Delhi, (1959) 1st ed., 78 - 198.
26. Nag, P., Paul, A. K. and Mukherji, S.; *Ind. J. Exp. Biol.* **18**, 822 - 827, 1980.
27. Swamy, M.; Effects of tannery effluents on plant growth and metabolism with special reference to trivalent chromium (basic chromium sulphate) on *Phaseolus mungo L.*, Ph.D Thesis, August 1984, University of Madras, Chennai, India.
28. Gomathinayam, S., Pitchai, S., Swamy, M., and Muralidhara Rao, N.; Proceedings of the sixth National Symposium on Environment. TNAU, Coimbatore, 105 - 107, 1997.
29. Stanley Abraham, L. and Swamy, M.; Role of cyanobacteria in removal of chromium from tannery effluents. Seminar on Innovations in Leather Science and Technology, Foundation day, CLRI, Chennai, Apr. 24, 47, 2001.
30. Xue, H. B., Stumm, W. and Sigg, L.; The binding of heavy metals to algal surfaces. Wat. Res. 22, 917 - 926, 1988.
31. Gardea-Torresdey, J. L., Arenas, J. L., Francisco, N. M. C., Tiemann, K. J. and Webb, R.; *J. Haz. Subs. Res.* **1**, 2.1 - 2.18, 1998.
32. Gadd, G. M.; *Experentia* 46, 834 - 840, 1990.
33. Muraleedharan, T. R., Iyengar, L. and Venkobachar, C.; *Curr. Sci.* **61**, 121 - 125, 1999.
34. Kratochvil, D., Pimentel, P. and Volesky, B.; *Env. Sci. Tech.* **32**, 2693 - 2698, 1998.
35. Brierley, C. L., Kelley, D. P., Seal, K. J. and Best, D. J.; Biotechnology. (Eds.) Higgins, I. J., Best, D. J. and Jones, R., Black Well, Oxford, (1985) 163 - 212.
36. Shumate, S. E. and Strandberg, C. W.; Comprehensive Biotechnology (Eds.) Moo Young, Robinson, M. and Howel, J. A., Pergamon Press, New York, (1985) 235 - 247.