STUDIES ON BIOSORPTION OF CHROMIUM FROM SIMULATED TAN LIQUOR USING BLUE GREEN ALGAE

A PROJECT REPORT

Submitted by

DIVYAA.C.R

ROHINI RAJAMANI

VIDYA SRI.M.

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ANNA UNIVERSITY: CHENNAI 600 025

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BONAFIDE CERTIFICATE

Certified that this project report **"STUDIES ON BIOSORPTION OF CHROMIUM FROM SIMULATED TAN LIQUOR USING BLUE GREEN ALGAE"** is the bonafide work of **C.R.DIVYAA, ROHINI RAJAMANI, VIDYA SRI. M** who carried out the project work under my supervision.

SIGNATURE

Dr.P.RAJASEKARAN

HEAD OF THE DEPARTMENT

DEPARTMENT OF BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY

CHINNAVEDAMPATTI

COIMBATORE-641006

SIGNATURE

Dr.STEPHEN.V.RAPHEAL

SUPERVISOR

SENIOR LECTURER

DEPARTMENT OF BIOTECHNOLOGY

KUMARAGURU COLLEGE OF TECHNOLOGY

CHINNAVEDAMPATTI

COIMBATORE-641006

CENTRAL LEATHER RESEARCH INSTITUTE
(Council of Scientific & Industrial Research)
Adyar, Chennai - 600 020
INDIA

Dr. MAHADESWARA SWAMY
Scientist F,
Bio-Products Laboratory

Lab ☎: 24911386 Extn. 248 / 233
Fax : (91) 044-24911589
Res ☎: (044) 24404054
Email : swamy_clri@hotmail.com

Mr. M. R. SRIDHARAN,MS(Chem.Engg)
Scientist E – II,
Chemical Engineering Department

Lab ☎: 24911386 Extn. 207
Fax : (91) 044-24911589
Res ☎: (044) 24425562
Email : mrs_clri@yahoo.co.in
mrsridharan@clri.info

11th April 2008

CERTIFICATE

This is to certify that the dissertation entitled "**Studies on biosorption of chromium from simulated tan liquor using blue-green algae**" is a bonafide record work carried out by **Ms. Vidya Sri. M, Ms. Rohini Rajamani** and **Ms. Divyaa C.R** at **Central Leather Research Institute**, Adyar, Chennai, in partial fulfillment for the degree of **B.Tech in Biotechnology** for **Kumaraguru College of Technology, Coimbatore**. The work embodied in this dissertation had been done by the candidate herself under our guidance and supervision during **January 2008** to **April 2008**. This has not previously formed the basis for the award of any degree, diploma, fellowship or any other similar title.

CO-GUIDE:

(Mahadeswara Swamy)

GUIDE:

(M. R. Sridharan)

CERTIFICATE OF EVALUATION

COLLEGE : Kumaraguru College of Technology

BRANCH : Biotechnology

SEMESTER : Eighth Semester

Sl.No.	Name of the Students	Title of the Project	Name of the Supervisor with Designation
01	C.R.Divyaa Reg nos:71204214008	STUDIES ON BIOSORPTION OF CHROMIUM FROM SIMULATED TAN LIQUOR USING BLUE GREEN ALGAE	Dr.Stephen.V.Rapheal Senior Lecturer
02	Rohini Rajamani Reg nos:71204214024		
03	Vidya Sri.M. Reg nos:71204214035		

The Report of the project work submitted by the above students in partial fulfillment for the award of Bachelor of Technology degree in BIOTECHNOLGY of Anna University were evaluated and confirmed to be report of the work done by the above students and then evaluated.

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Divyaa

DIVYAA.C.R

Rohini

ROHINI RAJAMANI

Vidya Sri

VIDYA SRI. M.

ABBREVATIONS

1. BCS – Basic Chromium Sulphate
2. BGA – Blue Green Algae
3. C6 & C8 – Cyanobacterial species
4. NaCl – Sodium Chloride
5. Cd – Cadmium
6. Fe – Ferrous or ferric
7. ml – Milliliter
8. µg – Microgram
9. mg – Milligram
10. Hg – Mercury
11. Cr – Cromium
12. Cu – Copper
13. Pb – Lead
14. Na_2PO_4 – Sodium Phosphate
15. rpm – Revolutions Per Minute
16. g – Gram
17. nm – Nanometer
18. Chl-a – Chlorophyll-a
19. HCl – Hydrochloric acid
20. H_2SO_4. – Sulphuric acid

ABSTRACT

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Heavy metal pollution of ground and surface waters by industrial effluents has become a serious threat to the environment especially in developing countries. The environment is under increasing pressure from solid and liquid wastes emanating from the leather industries. Though many conventional physico-chemical methods are currently being practiced, bio-remediation methods are becoming attractive alternatives. Heavy metals like Chromium can be treated using organisms like algae, fungi and bacteria. Cyanobacteria or Blue Green Algae has been proved to have immense detoxification capacity owing to its biosorption potential and so it can be used for treatment of tannery effluents and other domestic and industrial wastes containing chromium. It provides a cheaper and efficient means to control water and land pollution because cyanobacteria is autotrophic, non-pathogenic and can be cultured in normal conditions using a simple media.

Certain parameters of interest of two Cyanobacterial species CLRI-BGA-C6 and CLRI-BGA-C8 such as Phycobillin protein content, chlorophyll-a content and the total carbohydrate content have been studied under normal and stress conditions. Similarly, the amount of chromium and sulphate adsorbed by each cyanobacterial species under various Basic Chromium Sulphate (BCS) concentrations have also been analysed. It has been observed that under various chromium stress conditions, there have been significant changes in the biomass content as well as in the individual cell parameters. These studies in turn have numerous applications in understanding the behavior of such algae in bioremedial treatments.

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1) INTRODUCTION:

1.1) Heavy metal pollution in the environment:

Heavy metal(s) are widespread pollutants of great environmental concern as they are non-degradable and thus persistent. It is well perceived that there is a permissible limit of each metal, above which they are generally toxic and some are even hazardous. Globally, in developed countries, pollution of the aquatic system is controlled by the union under the framework 'Dangerous Substances Directive' which has led to certain environmental protection acts and regulations enforced by environmental agencies. Consequently, all effluents need to be assessed and require integrated pollution documentation before their final discharge (Rani *et al.*, 2000).

In developing countries like India, wastewater treatment is of utmost importance. The degree of treatment may range from a main process for seriously polluted industrial waste to a polishing process for removing the trace concentrations which remain after the main treatment. The conventional processes used for effluent treatment are precipitation as hydroxides/ sulphides, oxidation/reduction and ion exchange. The processes are expensive and not eco-friendly. Further, the major disadvantage with conventional treatment techniques is the production of sludge. As a result, an aquatic problem is changed into solid disposal problem. Therefore, amongst the chemical adsorbents only ion exchange resins were considered as the option for remediation with least ecological problem. However, chemical resins are expensive and the increasing demand of eco-friendly technologies has led to the search of low-cost alternatives which could be considered as single use

materials. In this light, biological materials have emerged as an ecofriendly and economic option (Rani *et al.*, 2000).

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation is the gradual build up over time of a chemical in a living organism (Neff, 2002). This occurs either because the chemical is taken up faster than it can be used, or because the chemical cannot be broken down for use by the organism (that is, the chemical cannot be metabolized). Bioaccumulation need not be a concern if the accumulated compound is not harmful. Compounds that are harmful to health, such as mercury, cadmium, etc however, can accumulate in living tissues (Beek, 1999).

1.2) Leather industry:

Leather enriches our lives in numerous pleasant ways. Today it has long outgrown its practical use and is regarded more as a luxury than of a necessity. This transformation has been made possible primarily due to the induction of state of art treatment and tanning technologies to give leather a never before possible thickness, unlimited color variations, luxurious feel and silky touch.

Global leather industry has been in existence for over 4000 years. The global leather industry produces about 18 billion square feet of leather a year with an estimated value of about US $40 billion. Developing countries now produce over 60% of the world's leather needs. About 65% of the world production of leather goes into leather foot wear. There are large numbers of tanneries scattered all over the country, but the main areas of their concentration are Tamil Nadu, Uttar Pradesh and West Bengal (Covington, 2000).

1.3) Environmental Issues created by the leather industry:

Tanning industry is a byproduct industry to process the hides and skins generated from abattoirs and there are nearly three thousand tanneries in India. They process 700,000 tons of hides and skins per year. The waste water discharge from tanneries is more than 30 million m^3 / year. (Rajamani *et al.*, 2002).

The environment is under increasing pressure from solid and liquid wastes emanating from the leather industry. These are inevitable by-products of leather manufacturing process and cause significant pollution unless treated in some way prior to discharge. (Aloy *et al.*,1976). The environmental balance of Leather Production is shown in Table 1.

TABLE 1:

ENVIRONMENTAL BALANCE OF LEATHER PRODUCTION

MATERIAL GENERATED	kg/ton OF RAW SKIN/HIDE
Leather	150
Solid waste (skin trimming, fleshing, buffing, chrome shaving wastes)	850
Fleshing	400

1.3.1) Soil pollution:

The untreated wastewaters from tanneries have been applied on land merely to contain them at one place. The long-term use of land for disposal of wastes has resulted in contamination of soil. The soils holding it directly and irrigated with

contaminated groundwater have been reported to lose productivity (Mariappan, 1997).

1.3.2) Atmospheric pollution:

The tanneries are proverbially known generating malodor. The hydration of salted hides and skins generally emit odor of volatile fatty and amino acids evolved in the course of biological decomposition in the presence of water. In addition, the stench of hydrogen sulphide along with acids, fats, carbohydrates etc in liming, deliming and tanning processes is predominant within the tanneries. The venting out of malodorous substances to ambient air and subsequent transports to further distance are responsible for atmospheric pollution. Hydrogen sulphide at 20 ppm ($30mg/m^3$) in ambient air is lethal to human kind. (Caree, 1984).

1.3.3) Solid wastes:

Several researchers are carrying out study in solid wastes for the reduction of pollution problem in leather processing. The composition of solid wastes generated in Leather industry shown in Table 2.

TABLE 2:

SOLID WASTES GENERATED IN LEATHER INDUSTRY

TYPES OF SOLIDS WASTES GENERATED	PERCENTAGE
Hair	2-5%
Fleshing	50-60%
Chrome shavings, chrome splits and buffing dust	35-40%
Skin trimmings	5-7%

Thus out of 1000 kg of raw hides, nearly 850 kg of the raw materials is generated as solid wastes in leather processing. Only 150 kg of the raw material is converted into leather. The solid wastes in the leather processing constitute 80% from beam house, 19% from tanning and 1% from finishing. The solid wastes can be hydrolyzed and used as a useful by product in many ways. Several technologies have been explored for the utilization of solid wastes generated in leather industry. Among the solid wastes, keratin plays an important role because of the difficulty in hydrolyzing it. The disulfide bond plays an important role in safe guarding the keratin. It is highly resistant towards enzymes and most of the chemicals. It is rich in cysteine containing amino acids. The chrome shavings and fleshing also play a major source of proteins commercially utilizable if processed properly. (Practise and Thurston, 1961).

1.3.4) Liquid Effluents: (Trivedi, 2004)

Most of the constituents present in a tannery effluent are of highly damaging character irrespective of whether they are discharged into ocean, river, stream, sewer or on land.

Effects on Streams:

1) The dull brown color of the effluent which is very difficult to bleach is imparted to the receiving streams and results in an unsightly appearance. The color persists for a longer stretch.

2) The highly disagreeable foul odor is transmitted to the water.

3) The individual effluents and combined effluents are either acidic or alkaline and these extremities results in corrosion and deterioration of concrete, metallic pipes etc. The water becomes unsuitable for any use.

3) Sulphide, the most damaging constituent sulfide is usually present in excess concentrations. It is toxic to fish and other aquatic life. The concentration of sulfides and oxygen demand are so high that they deplete the oxidized dissolved oxygen rapidly resulting in massive fish kills.

4) Another detrimental factor is the excess concentration of sodium chloride. The sodium and chloride in any form make the water unsuitable for irrigation. Further they corrode the treatment structures etc.

5) The dissolved constituents such as proteins and albumins putrefy slowly evolving highly offensive odors.

6) The suspended solids, mostly hair, fleshing and calcium carbonate, settle to the bottom affecting the fish spawning grounds and also interfere with the aeration and photosynthetic activities of aquatic plants.

7) Chromium is highly toxic to fish and other aquatic life and interferes with the natural purification systems.

8) The effluent may also contain a considerable amount of pathogen.

Deterioration of Ground Water Quality:

As already stated, the abnormal concentration of sodium chloride and chromium affect ground water quality seriously within a short period where the effluent discharged on land or if stored in unlined tanks. If the ground water is affected once, it cannot be rectified. It is an irreparable damage. There is no other alternative expecting abandoning the source. It is to be noted that in places where tanneries are concentrated, no single source of ground water is available either for human consumption or for irrigation. So many acres of land became infertile due to the seepage caused by stagnation of these effluents.

Effect on Sewers:

The two major constituents that affect sewers are suspended solids and sulfides. The lime suspended gets precipitated as calcium carbonate by carbon di oxide produced during the decomposition of organic matter. This calcium carbonate, with the hair (which acts as a binding agent) adhere to the surfaces of the sewer gradually reducing the passage and results in complete choking. Further due to 'Crown Corrosion' of sulfides, the concrete structures crumble.

Effect on Human Beings:

In humans, exposure to hexavalent chromium salts for periods of 2 to 26 years has been implicated as a cause of cancer of the digestive tract. High levels

of chromium and zinc in soil have been correlated with regional incidences of stomach cancer. Based on exposure to chromium via inhalation, the International Agency for Research on cancer has categorized "chromium and certain chromium compounds" in Group 1, sufficient evidence for carcinogenicity in humans and animals (IARC, 1990).

Chromate compounds, mainly sodium and potassium chromate and dichromate, causes irritation to skin and ulcers may develop at the site of skin damage. Teratogenicity (Gale, 1978), Mutagenicity (Petrilli and Flora, 1977) and carcinogenicity(Gruber and Jennette, 1978) from chromium exposure have been reported for human subjects. A mutagenic potency is shown for potassium dichromate and therefore cannot be excluded for chromates in the chromate-using industries.

Exposure to trivalent chromium does not produce such effects. Certain persons manifest allergic skin reactions to hexavalent and possibly trivalent chromium (Andre , 1997). Skin reaction through dermal exposure to chromium is often described. Chromium present in industrial wastes primarily in the hexavalent form as chromate and dichromate acts as a oxyanion that is actively transported into cells by the sulphate transport system (Srinath *et al.*, 2001)

1.4) CHROMIUM AND ITS USE:

Chromium is found chiefly in chrome-iron ore. It is used to harden steel, manufacture stainless steel, and to form many useful alloys. Much is used in electroplating to produce a hard surface. Chromium compounds frequently are added to cooling water for corrosion control (APHA, 1992). Chromium gives glass an emerald green color and is widely used as a catalyst. The refractory

industry has found chromite useful for forming bricks and shapes, as it has a high melting point, moderate thermal expansion, and stability of crystalline structure.

Chromium has been widely used in various industries. Hexavalent chromium (Cr^{6+})is a priority toxic (Louis *et al.*, 1996), mutagenic and carcinogenic chemical, whereas its reduced trivalent form (Cr^{3+}) is much less toxic and insoluble. Hence, the basic process for chromium detoxification is the transformation of Cr^{6+} to Cr^{3+}. A number of aerobic and anaerobic microorganisms are capable of reducing Cr^6 (Bosnic *et al.*,2000).

1.5) CHROMIUM IN TANNING INDUSTRY:

Chromium is the buzzword to both tanners and the environmental scientists.80-90% of all leathers today is tanned with chromium salts due to several advantages of chrome tanning over other tanning agents. In spite of obvious advantages, chrome tanning has come under severe criticism owing to ecotoxicological objections. The accepted fact in leather industry is that 1000 kilograms of raw hide yields approximately 150 kg leather along with other split waste; solid waste and 30 cubic metres of effluent containing 400 kg dissolved and suspended solids. In such huge tannery waste, chromium salt is considered to be the 'whipping boy'. In India, it is estimated that around 40,000 tonnes of BCS salts are used in leather industry annually. (Buddhadeb , 2000).

Currently adopted methods of Cr removal from tannery effluents generally afford uptake of 50-70% of chrome used in tanning operations, whilst the rest is wasted thereby incurring a loss of nearly 400 million rupees per year. Besides it has been found that a load of 1500-3000 milligram per liter of Cr^{3+} in the untreated waste water from chromium tanning operation units pollute

environment. Wastage of this valuable non-renewable resource on one hand and its toxicity to biotic system on the other, poses a serious ecological treat (Mukesh and Anil 2005). Since 1950, many suggestions have been made to recover and reuse chromium from tannery spent liquor. In developing countries, especially for the small tanneries, cheap cost effective measures are yet to be explored. The recovery measures should not involve much space and man power, must not reduce unmanageable volumes of slurry or sludge and look forward towards a significant separation of chrome salts from the spent chrome liquor making the salts available for reuse. (Trivedi, 1989).

REVIEW OF LITERATURE

2) REVIEW OF LITERATURE:

2.1) TREATMENT OF CHROMIUM IN EFFLUENTS:

There are many methods by which chromium could be treated in tannery wastes. The various conventional methods like physical methods, chemical methods and biological methods for detoxification of tannery wastes are being observed.

Chemical adsorption of chromium into resins such as amberlite by redox adsorption approach follows Langmiur model. Processes such as Ion exchange Recovery has been developed for removal of trivalent chromium from segregated wastewater from tannery operations (Fred *et al.*,1958). Physical means such as high temperature and pressure are effective as well to separate out chromium from both BCS solution and spent chrome liquor containing varying concentrations of chromium (Buddhadeb , 2000).

2.2) BIOREMEDIATION OF TANNERY EFFLUENT: (Chaudhry, 2003)

Various biological methods are being observed for recovery and reuse of chromium in tanned effluents. Bioremediation is the use of biological agents to reclaim soils and waters polluted by hazardous substances. The biological agents used for bioremediation are most frequently the intact micro organisms themselves. Isolating micro organisms that have a desired function is the first step in developing a bioremediation process. The processes by which micro organisms interact with toxic metals enabling their removal and recovery are biosorption, bioaccumulation and enzymatic reduction.

2.3) USE OF VARIOUS MICROBES FOR BIOSORPTION OF CHROMIUM:

Among micro-organisms, fungal biomass offers the advantage of having a high percentage of cell wall material which shows excellent metal-binding properties. Many fungi and yeast have shown an excellent potential of metal biosorption, particularly the genera *Rhizopus, Aspergillus, Streptoverticillum* and *Saccharomyces*. Among bacteria, *Bacillus* sp. has been identified as having a high potential for metal sequestration and has been used in commercial biosorbent preparation. Besides there are reports on the biosorption of metal(s) using *Pseudomonas* sp, *Zoogloea ramigera* and *Streptomyces* sp. Among photoautotrophs marine algae became the candidate of interest due to bulk availability of their biomass from water bodies. *Sargassum natans* and *Ascophyllum nodosum* in this group have shown very high biosorptive capacities for various metal(s) (**Rani** *et al.*, 2000).

Survival and Chromate Reducing Ability of *Pseudomonas aeruginosa* in industrial effluents was studied by Ganguli and Tripathi (2004).The ability of a chromate-reducing *Pseudomonas aeruginosa* strain, isolated from tannery effluent, to survive and reduce chromate in the effluent of a tannery and an electroplating unit was evaluated. The test strain survived in the native tannery effluent but numbers fell sharply in the native electroplating effluent. Supplementation with a carbon (C), nitrogen (N) and phosphorus (P) source supported bacterial multiplication and chromate reduction in both types of effluents with almost equal efficiency. Chromate reduction, however, was not observed in the absence of C, N or P supplement, or in the chromate-reducing strain. Also, Konovalova, (2003) studied Chromium (vi) reduction in a membrane bioreactor with immobilized *Pseudomonas* Cells.

Enzymatic dehalogenation of pentachlorophenol by *Pseudomonas fluorescens* of the microbial community from tannery effluent was studied in four different bacterial isolates obtained from a stable bacterial consortium. These isolates were capable of utilizing pentachlorophenol (PCP) as sole carbon and energy source. The consortium was developed by continuous enrichment in the chemostat. The degradation of PCP by bacterial strain was preceded through an oxidative route as indicated by accumulation of tetrachloro-rho-hydroquinone and dichlorohydroquinone. Among the four isolates, *Pseudomonas fluorescens* exhibited maximum degradation capability and enzyme production. (Shachi and Indu, 2003).

A Cr (VI) resistant bacterial strain SDCr-5, identified as *Ochrobactrum intermedium* on the basis of 16S rRNA gene sequencing, was tolerant to high concentrations of Cr(VI) up to 15 mg ml^{-1} in acetate minimal medium. *Ochrobactrum intermedium* SDCr-5 reduced Cr (VI) under a wide range of concentrations from 100 to 1500 mg ml^{-1} and reduction was optimum at 37 °C and pH 7. It reduced 200 and 721 mg ml^{-1} Cr (VI) within 72 and 96 h, respectively. The rate of Cr (VI) reduction increased with concentration from 100 to 1500 mg ml^{-1}. The presence of heavy metal cations such as Cu^{2+}, Co^{2+}, Mn^{2+} and Ni^{2+} stimulated Cr(VI) reduction. Strain SDCr-5 might be useful for Cr (VI) detoxification under a wide range of environmental conditions. (Sikander et al., 2006).

The biosorption of Cr (VI) ions from aqueous solutions on dried vegetative cell and spore–crystal mixture of *Bacillus thuringiensis* was proved effective using batch method as a function of pH, initial metal ion concentration and temperature. At optimal conditions, metal ion uptake rises with increasing

initial metal ion concentration and it was maximum at 25 °C. The adsorption data with respect to the metal provided an excellent fit to both Langmuir and Freundlich isotherms. (Yasemin et al., 2004).

The binding of Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+} Cd^{2+} and Cr^{3+} to *Spirulina platensis* is accompanied by the liberation of protons suggesting an ion exchange reaction. Similar results were obtained in Cd^{2+}, $Cu^{2+}$and Zn^{2+} binding by previously protonated biomass of *Sargassum fluitans* where metal binding was coupled with the release of H^{+} ions. Thus biosorbents can be viewed as natural ion exchange materials that contain weak acidic and basic groups (Greene *et al.*, 1986).

The dominant mechanism of Cu^{2+} biosorption by *Ecklonia radiata* as ion exchange mechanism involving exchange of Ca^{2+} and Mg^{2+} ions present in their cell walls has been reported by (Matheickal *et al.*,1997).

Characterizations of Cr ion exchange with hydrotalcite experiments were performed to characterize the removal of chromium from water with uncalcined hydrotalcite, a clay mineral ion exchange media. The process was characterized as a function of pH, temperature, contact time and hydrotalcite concentrations. A Freundlich isotherm, used to describe adsorption equilibria, was used as a model and Freundlich constants were determined. The kinetics of the ion exchange reaction were also modeled using a pseudo-first order reaction rate. Finally, an equilibrium stage process was modeled with sequential batch separations to determine if hydrotalcite ion exchange could reduce aqueous Cr levels to below the EPA limit of 0.1 mg l^{-1}. Under optimal conditions, maximum removals of chromium greater than 95% were achieved.

Sequential batch tests performed on initial Cr solutions ranging from 5 mg l^{-1} to 40 mg l^{-1}, demonstrated that the water could be purified to a level that was not statistically different than the EPA limit, thus demonstrating the applicability of hydrotalcite ion exchange. (Patricia , 2004).

A novel strain of *Arthrobacter aurescens* reduces hexavalent chromium at low temperature. Core samples obtained from a Cr(VI) contaminated aquifer at the Hanford facility in Washington were enriched in Vogel Bonner medium at 10 Centigrade with 0, 25, 50, 100, 200, 400 and 1000 mg/l Cr (VI). The extent of Cr (VI) reduction, evaluated using the diphenyl carbazide assay is up to and including 1000 mg/l Cr (VI) (**Rene** *et al.*, 2006).

Sulfur oxidizing bacteria such as *Thiobacillus ferroxidans*, *Thiobacilli*, *Thermophilic archaea* and *Potamogeton lucens* act as a biosorbent for many heavy metal ions. The chromate-reducing ability of *Pseudomonas aeruginosa* was compared in batch culture, with cells entrapped in a dialysis sac, and with cells immobilized in an agarose-alginate film in conjunction with a rotating biological contactor. In all three systems, the amount of Cr (VI) reduced by the cells immobilized in agarose-alginate biofilm was twice and thrice the amount reduced by batch culture and cells entrapped in a dialysis sac, respectively (Costa and Leite, 1991)

In the presence of oxygen, microbial reduction of Cr^{6+} is commonly catalyzed by soluble enzymes, except in *Pseudomonas maltophilia* O-2 and *Bacillus megaterium*, which utilize membrane-associated reductases. Two soluble Cr^{6+} reductases, ChrR and YieF, have been purified from *Pseudomonas putida* and *Escherichia coli*, respectively. With the advancement in technology for enzyme immobilization, it is speculated that the direct application of Cr^{6+}

reductases may be a promising approach for bioremediation of Cr^{6+} in a wide range of environments (Ehrlich, 1988).

Several facultative anaerobes including 5 isolates of *Aerococcus*, 2 isolates of *Micrococcus* and a single isolate of genus *Aeromonas*, tolerant to high levels of chromate (>400 µg/ml) and displaying various degrees of Cr^{6+} were isolated. These isolates were subjected to further characterization for possible use in Cr^{6+} detoxification and tolerance to a variety of other heavy metals as well. 70% efficiency has been obtained (Srinath *et al.*, 2004)

The potential of *Asergillus niger* MTCC 2594 to accumulate chromium as well as its biosorption capacity was tested and found to be 75-80% absorption of initial chrome content in 24-36h. Maximum adsorption of 83% for Cr at 48 and 79% of Cr at 36 h in spent chrome liquor is observed. The biosorption characteristics follow Lagergren kinetics and fit well with Langmuir and Freundlich isotherms (Sandana *et al.*, 1999).

The bioaccumulation of chromium from retan chrome liquor by *Spirulina fusiformis* is found to be very effective for detoxification of tannery effluents. 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 effective in removal of trivalent chromium (95%-100%) besides reducing BOD and COD of the effluent. Moreover the metal ion uptake is proportional to the initial metal ion concentration upto 300mg/l. SDS-PAGE, FTIR physico-chemical parameters like total solids total dissolved solids total suspended

solids, BOD,COD and biochemical studies related to biomass, chlorophyll-a and protein proved the same (Pandi *et al.*,2007).

The biosorption of trivalent chromium is excellent by both live and dry biomass of *Spirulina platensis* in free state and also when immobilized in Polyurethane foam and sodium alginate. Biosorption kinetic data on Cr^{3+} sorption onto dried biomass were analyzed using pseudo-first and pseudo-second order kinetic models in batch column experiments. The second-order equation was more appropriate to predict the rate of biosorption. The effects of height of packing and diameter of the column, concentration of the blue-green algae in varying amounts of sodium alginate, chromium concentration were studied. The results fit into both Langmiur and Freundlich isotherm models with very high regression coefficients. Furthermore, equilibirium studies using retan chrome liquor (RCL), with a chromium concentration of 1660 ppm, obtained from tannery also justify the studies (Shashirekha *et al.*, 2007).

Aspergillus niger isolated from soil and effluent of leather tanning mills had higher activity to remove chromium. The potency of *Aspergillus niger* was evaluated in shake flask culture by absorption of chromium at pH 6 and temperature 30Â°C. The results of the study indicated removal of more than 75% chromium by *Aspergillus niger* determined by diphenylcarbazide colorimetric assay and atomic absorption spectrophotometry after 7 days. (Shaikh *et al.*, 2001).

A new type of biomass, protonated brown seaweed *Ecklonia sp.*, was used for the removal of Cr (VI). When synthetic wastewater containing Cr (VI) was placed in contact with the biomass, the Cr (VI) was completely reduced to

Cr (III). The converted Cr (III) appeared in the solution phase or was partly bound to the biomass. The Cr (VI) removal efficiency was always 100% in the pH range of this study (pH 1 to approximately 5). From a practical viewpoint, the abundant and inexpensive Ecklonia biomass could be used for the conversion of toxic Cr (VI) into less toxic or nontoxic Cr (III). (Donghee et al. 2004).

Fifteen *Cyanothece* strains isolated from saline environments have been characterized with regard to exopolysaccharide (EPS) production. The polymers contained six to eight monosaccharides, with one or two acidic sugars. In some EPS samples, the additional presence of acetyl, pyruvyl, and/or sulfate groups was also detected. Cyanobacteria can also be included among the potential sources of new polymers, several species being characterized by the presence of thick capsules surrounding the cells and by the ability to release polysaccharide material into culture medium. Thus, research aimed at the isolation and characterization of new exopolysaccharide (EPS)-producing cyanobacterial strains has been carried out, focusing attention on hypersaline habitats because they are generally known to harbor a large number of EPS-producing strains (Roberto *et al.*, 1997).

The effects of temperature and salinity on the carbohydrate accumulation profiles of a range of unicellular cyanobacteria have been studied. Four isolates of *Synechocystis sp.* which usually accumulated glucosyl-glycerol produced a second carbohydrate, sucrose, when grown in a seawater-based medium at high temperature (35°C). In contrast, three sucrose-accumulating isolates of *Synechococcus*, grown over a range of temperatures and salinities, did not produce a second detectable low molecular weight carbohydrate in any experimental treatment. The interaction of temperature and salinity was further

examined in *Synechocystis* PCC 6714. Sucrose production was found to be favoured by high temperature and low salinity. The rate and extent of sucrose accumulation differed in cells grown at 20 and 37° C reaching an upper asymptote 12 h after upshock at 37°C and 24 h after upshock at 20°C followed by a decrease to an approximately constant level after 96 h. This is the first demonstration of the accumulation of sucrose as a second osmotically active carbohydrate in cyanobacteria. (Warr *et al.*, 1985).

The unicellular cyanobacterial strain 16Som2, isolated from a Somaliland saltpan and identified as *Cyanothece* sp., is characterized by cells surrounded by a thick polysaccharidic capsule, the external part of which dissolves into the medium during growth, causing a progressive increase in culture viscosity. In spite of this, the thickness of the capsule remained almost constant under all the culture conditions tested, demonstrating that the processes of its synthesis and solubilization occurred at a similar rate. The synthesis of carbohydrates was neither enhanced by increasing salinity (sea-water enriched with NaCl in the range 0 to 2.0 M) nor by Mg^{2+}, K^{+} or Ca^{2+} deficiencies. In contrast, N-limitation and, to a lesser extent, P-limitation induced a significant enhancement of carbohydrate synthesis; in particular, N-deficiency stimulated the synthesis of all the carbohydrate fractions (intracellular, capsular and soluble). The soluble polysaccharide, separated from the culture medium and hydrolyzed with 2N trifluoroacetic acid, showed a sugar composition consisting of glucuronic acid: galacturonic acid: galactose: glucose: mannose: xylose: fucose in a molar ratio of 1: 2: 2.4: 6.8: 4.8: 2.9: 1.6.(Roberto *et al.*, 1993).

At least one group of prokaryotes is known to have circadian regulation of cellular activities—the cyanobacteria. Their "biological clock"

orchestrates cellular events to occur in an optimal temporal program, and it can keep track of circadian time even when the cells are dividing more rapidly than once per day. Growth competition experiments demonstrate that the fitness of cyanobacteria is enhanced when the circadian period matches the period of the environmental cycle. Three genes have been identified that specifically affect circadian phenotypes. These genes, *kaiA, kaiB*, and *kaiC*, are adjacent to each other on the chromosome, thus forming a clock gene cluster. The clock gene products appear to interact with each other and form an autoregulatory feedback loop. (Carl, 1999).

2.4) BIOSORPTION:

The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of adsorption (Gadd, 1998). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986). The major advantages of biosorption over conventional treatment methods include (Kuyucak and Volesky, 1998)

- Low cost
- High efficiency
- Minimization of chemical and or biological sludge
- No additional nutrient requirement
- Regeneration of biosorbent and
- Possibility of metal recovery

Here dead microbial biomass functions as an ion exchanger by virtue of various reactive groups available on the cell surface such as carboxyl, amine, imidazole, phosphate, sulfhydryl, sulfate and hydroxyl. The process can be made economical by procuring natural bulk biomass or spent biomass from various fermentation industries. The performance of a biosorbent can further be improved by various physical and chemical treatments. The pretreatments modify the cell surface either by removing or masking the groups or exposing more metal binding sites. Immobilized biomass of these microbes offers the continuous sorption–desorption system in a fixed bed reactor. Various commercial microbial biosorbents available are AlgaSorb, AMT-Bioclaim and Bio-fix. The economics of these sorbents merit their commercialization, over chemical ion exchangers (**Rani** *et al.*, 2000).

2.5) BIOSORPTION MECHANISMS:

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria.

According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

1) Metabolism dependent
2) Non-metabolism dependent

According to the location where the metal removed from solution is found, biosorption can be classified as

* Extra cellular accumulation or precipitation
* Cell surface sorption or precipitation

* Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. (Costa *et.al.*, 1991).This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal. Biosorption mainly involves cell surface complexation, ion exchange and microprecipitation. (**Rani** et al., 2000).

During non-metabolism dependent biosorption, metal absorbed is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cell's metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988).

In the case of precipitation, the metal absorbed may take place both in the solution and on the cell surface (Ercole *et al.*, 1994). Further, it may be dependent on the cell's metabolism if, in the presence of toxic metals, the microorganism produces compounds that favor the precipitation process.

OBJECTIVES

OBJECTIVES:

The objectives of the present study are:

1) To reduce environmental pollution caused due to the discharge of chromium in tannery effluents by sorption of chromium onto cyanobacteria.

2) To find out the biosorption potential of cyanobacteria to accumulate chromium and sulphate from BCS solutions of varying strengths.

3) To test parameters like the content of Phycobillin protein, carbohydrate, chlorophyll-a under normal and stress conditions of chromium.

MATERIALS AND METHODS

4) MATERIALS AND METHODS:

4.1) CYANOBACTERIAL CULTURES:

Pure cyanobacterial cultures of 2 species were maintained in Erlenmeyer flasks containing sterilized media (Rippka *et al.*, 1979), under controlled conditions in the growth chamber (Fig 3) under white fluorescent tubes (4000-5000 lux) at a temperature of 25 – 35°C. Regular growth monitoring of culture was done by optical density measurement, pH monitoring and microscopic observation. Cultures were also maintained in plastic tubs in the garden at CLRI for pilot scale studies (Fig 1 and Fig 2).

FIG 1:

CLRI-BGA-C6

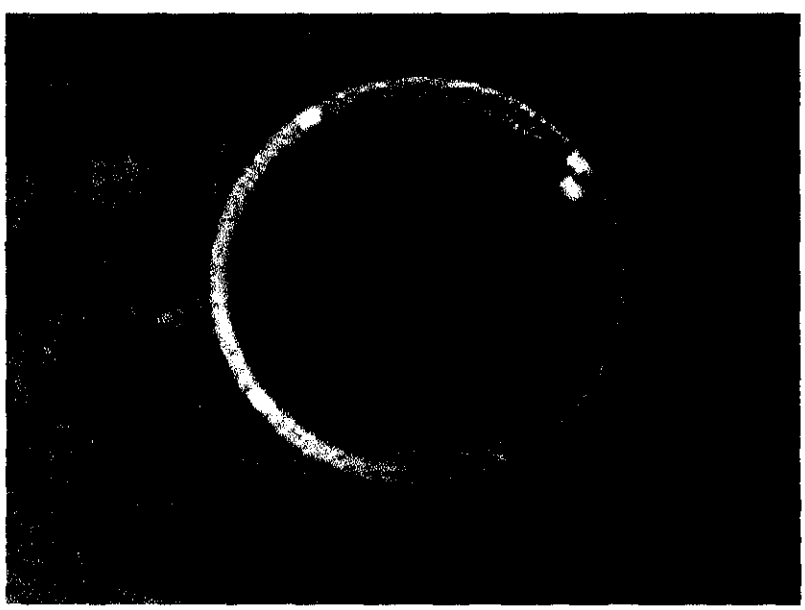


FIG 2:

CLRI-BGA-C8

4.2) MICROSCOPIC STUDY:

A small quantity of culture was transferred to the microscopic slide containing a drop of distilled water. The culture was teased properly to obtain isolated cells, filaments. A coverslip was placed carefully without letting in air bubbles. The cells were observed at 20X and 40X.

4.3) CHROMIUM SOURCE:

Basic chromium sulphate (BCS):

BCS was collected from the tannery department, CLRI.

4.4) MEDIA PREPARATION:

BG-11 MEDIUM COMPOSITION: (pH 7.5)

COMPONENTS	CONCENTRATION/LITRE
Sodium nitrate	1.5 g
Dipotassium Hydrogen Phosphate	0.04 g
Magnesium Sulphate	0.075g
Calcium Chloride	0.036g
Sodium carbonate	0.020g
Citric acid	0.006g
Ferric Ammonium Citrate	0.006g
EDTA	0.001g
Trace metal mix	1 ml
Distilled water	1000 ml

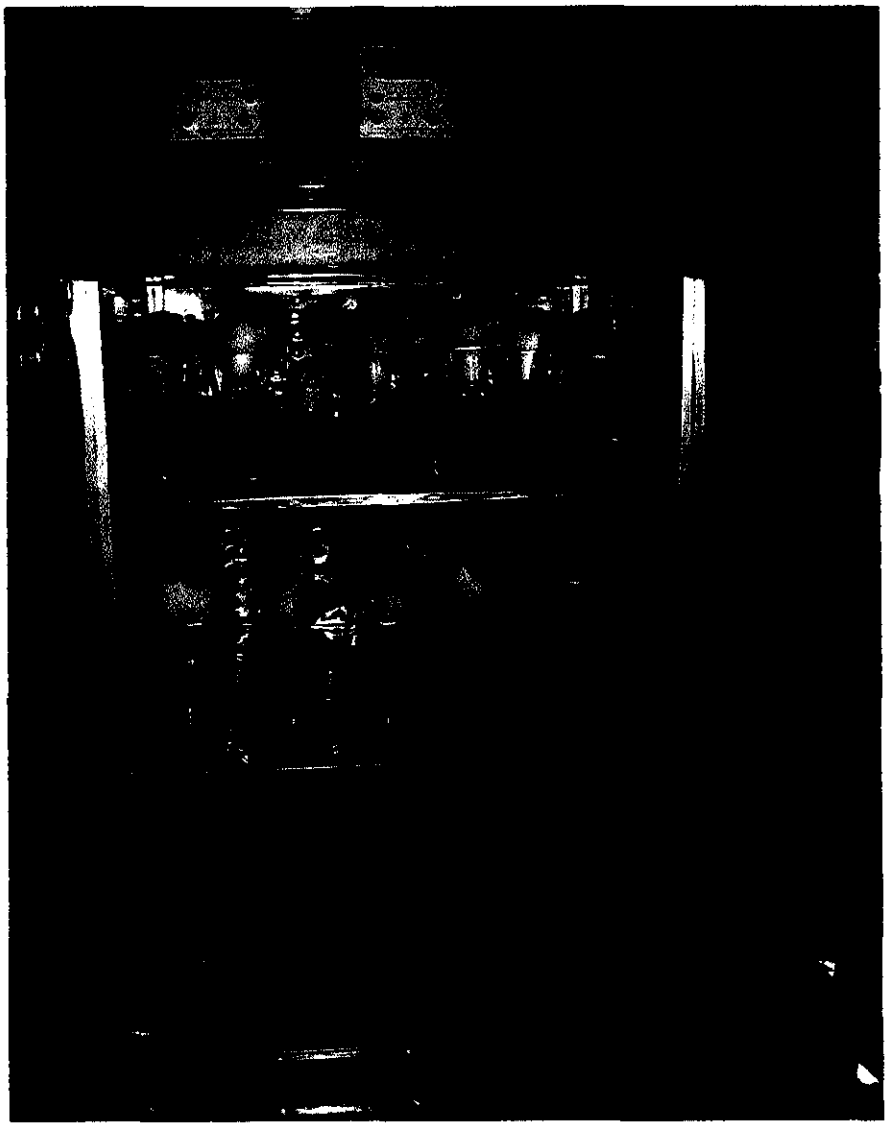
Trace metal mix composition:

COMPONENTS	CONCENTRATION/LITRE
Boric acid	2.86g
Manganous chloride	1.81g
Zinc sulphate	0.22g
Sodium molybdate	0.079g
Cobalt nitrate	0.0474g
Distilled water	1000 ml

The dissolved components of the media were taken in 2 separate erlenmeyer flasks and sterilized. The media is then inoculated with the cyanobacterial species; CLRI-BGA-C6, CLRI-BGA-C8. The cultures were allowed to grow in growth chambers (Fig 3) and harvested after 18 days.

FIG 3:

ALGAL GROWTH CHAMBER



4.5) BIOMASS ESTIMATION:

A known volume of the culture was centrifuged at 5000 rpm for 15 minutes. The supernatant was discarded and the pellet was desiccated in a hot air oven at 103-105°C. The biomass was weighed after desiccation and the dry weights were recorded.

4.6) ESTIMATION OF PHYCOBILIN PROTEINS:

Phycobilins are complex photoreceptor pigments, open-chain tetrapyrroles that are structurally related to mammalian bile pigments.These phycobiliproteins are aggregated in a highly ordered protein complex called a phycobilisome (PBS), which are attached to the cytosol (stromal) face of the thylakoid. Extending into the cytosol, the phycobilisomes consist of a cluster of phycobilin pigments including phycocyanin and phycoerythrin attached by their phycobiliproteins. These particles serve as light-energy antennae for photosynthesis. Phycobilisomes preferentially funnel light energy into photosystem II for the splitting of water and generation of oxygen. Both phycocyanin and phycoerythrin fluoresce at a particular wavelength. The light produced by this fluorescence is distinctive and reliable, so phycobilins may be used as chemical tags.

REAGENTS USED:

0.15 M NaCl, 0.01 M Na_2PO_4.

PROCEDURE:

1) Take 2 ml of culture into individual centrifuge tubes and rinse with distilled water.

2) Centrifuge at 5000 rpm for 10 minutes. Discard the supernatant and measure 200 g of the pellet. To the pellet, add 3ml of NaCl and 3ml of Na_2PO_4.
3) Sonicate the mixture till the solution becomes clear without any suspended particles.
4) Centrifuge for atleast 15 minutes at 5200 rpm.
5) Take out the supernatant and the measure the absorbance at various values (562nm, 615nm, 652nm) to find out the concentration of various types of proteins (Phycocyanin, Allophycocyanin, Phycoerythrin).The results are tabulated.

CALCULATION:

Phycocyanin (PC) : $\frac{A_{615}-0.474(A_{652})}{5.34}$

Allophycocyanin(APC): $\frac{A_{652}-0.208(A)_{615}}{5.09}$

Phycoerythrin(PE): $\frac{A_{562}-2.41(PC)-0.849(APC)}{9.2}$

4.7) CHROMIUM ANALYSIS (APHA,1992):

PRINCIPLE:

The method is predominantly used to analyse liquors containing chromium in very low concentrations. Chromium in its hexavalent state is treated with diphenyl carbazide and the resultant solution is analysed

spectrophotometrically at 540 nm. This principle is used in preparing a standard graph. The standard graph is obtained by plotting the absorbance values of solutions containing concentrations of Cr (VI) to their respective concentrations.

The linear nature of the graph makes it possible for us to obtain concentrations of Cr (VI) present in the solution for any absorbance value.

REAGENTS

1) Stock Chromium solution:

0.1414g of potassium dichromate dissolved in 1 liter. (1ml=50µg).

2) Standard Chromium Solution:

10ml of stock chromium solution was diluted to 100ml with distilled water.

3) Potassium Permanganate:

40g of Potassium permanganate was dissolved in one liter with distilled water.

4) Sulphuric acid:

Make to 1:1 ratio with distilled water.

5) Diphenyl Carbazide:

Dissolve 250 mg of 1,5 Diphenyl carbazide in 50 ml of acetone and store it in a brown bottle.

PROCEDURE:

1. Solutions containing chromium in the trivalent state have to be oxidized to the hexavalent state using the tri-acid mixture digestion. This will determine the concentration of chromium in the solution.

2. Tri-acid mixture digestion is performed using a mixture of three acids namely sulphuric acid (3.5 ml), perchloric acid (11.5 ml) and nitric acid (5 ml) along with a known volume of the sample.
3. The resultant solution is heated on a hot plate at around 95°C.
4. The end of the reaction is marked by a change in color from green to orange.
5. In order to quantify the chromium present in the solution, 5 ml of the digested sample is taken and made upto 10 ml using distilled water.
6. 2 ml of 4% potassium permanganate helps in oxidizing the unoxidized Cr (III) to Cr (VI).
7. After heating, 1 ml of 0.5% sodium azide solution is added and the solution turns colourless.
8. The resultant solution is made upto 18 ml using distilled water and 2 ml of diphenyl carbazide solution (250 mg dissolved in 50 ml of acetone) is added.
9. Absorbance of the solution is measured at 540 nm.

PREPARATION OF CALIBRATION CURVE:

1) Pipette out chromium working standard solution of varying concentrations ranging from 5-500 (μg).
2) Make up the solution to 10ml.
3) Add one drop of sulphuric acid and two drops of potassium permanganate.
4) The violet color solution so formed was boiled for 2 minutes.
5) Add 1 ml of sodium azide and shake thoroughly till color is decolorized.
6) Add three drops of orthophosphoric acid and make up to 18 ml with distilled water.

7) Add two ml of diphenyl carbazide.

8) Read the absorbance at 540 nm. The readings are tabulated and a calibration curve is obtained.

4.8) ESTIMATION OF CHLOROPHYLL-a (Mackinney, 1941):

PRINCIPLE:

Cyanobacteria have only Chl-a, which can be completely extracted in solvents like acetone or methanol and absorbance, was measured at 663nm.

REAGENT:

80% Methanol

PROCEDURE:

A known volume of homogenous cyanobacterial suspension was centrifuged at 5000 rpm for 10 minutes. The pellet was washed, weighed for 200g and suspended in 4ml of methanol and vortexed thoroughly in a test tube. The test tube was covered with aluminum foil and incubated in a water bath at 60°C for one hour under dark condition. The tube was cooled and centrifuges again at 5000 rpm for 5mins.The supernatant was transferred to another tube and once again the extraction was done by adding 4ml of the solvent to pellets. The complete extraction was ensured by repeating the process with 2ml of the solvent. All the supernatants were pooled and made up to 10 ml using methanol and absorbance measured at 663nm using UV visible spectrometer.

CALCULATION:

$$\text{Chlorophyll-a} = \frac{A_{663} \times 12.63 \times \text{Volume of sample}}{\text{Volume of methanol}} \quad \mu g/ml$$

Where,

A_{663} = Absorbance at 663 nm,

12.63 = Correction factor.

4.9) DETERMINATION OF TOTAL CARBOHYDRATE BY ANTHRONE METHOD (Sadasivam and Manikam,1991):

Carbohydrates are the most important components of storage and structural materials in the plants. They exist as free sugars and polysaccharides. The basic units of carbohydrates are the monosaccharides which cannot be split by hydrolysis into simpler sugars. The carbohydrate content can be measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides.

PRINCIPLE:

Carbohydrates are first hydrolysed into simple sugars usong dilute hydrochloric acid. In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This compound forms with anthrone a green coloured product with an absorption maximum at 630 nm.

MATERIALS:

- 2.5 N HCl
- Anthrone Reagent: Dissolve 200mg anthrone in 100ml of ice cold 95% H_2SO_4. Prepare fresh before use.

- Standard glucose: Stock – Dissolve 100mg in 100ml of distilled water. Working standard is 10 ml of stock diluted to 100ml with distilled water. Store refrigerated after adding few drops of toluene.

PROCEDURE:

1) Weigh 100mg of the sample into a boiling into a boiling tube.
2) Hydrolyze by keeping it in a boiling water bath for three hours with 5ml of 2.5 N – HCl and cool to room temperature.
3) Neutralize it with sodium carbonate until the effervescence ceases.
4) Make up the volume to 100ml and centrifuge.
5) Collect the supernatant and take 0.5 and 1ml aliquots for analysis.
6) Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard. '0' serves as a blank.
7) Make up the volume to 1ml in all the test tubes including the sample tubes by adding distilled water.
8) Then add 4ml of anthrone reagent.
9) Heat for eight minutes in a boiling in a boiling water bath.
10) Cool rapidly and read the green to dark green color at 630nm.
11) Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
12) From the graph calculate the amount of carbohydrate present in the sample tube.

CALCULATION:

Amount of carbohydrate present in 100mg of the sample

= (mg of glucose / volume of the test sample) × 100

4.10) DETERMINATION OF SULPHATE (APHA, 1992):

Sulphate is precipitated as Barium Sulfate in the presence of Hydrochloric acid. The precipitated barium sulfate is filtered, dried and weighed as barium sulfate (addition of HCl to the sample prevents the formation of Barium Carbonate with highly alkaline wastes and waters). The precipitating agent Barium Chloride is added in excess to produce sufficient common ion to precipitate sulfate ion as completely as possible. Dilution of the sample after precipitation (at 90°C) is carried out to convert the colloidal precipitate to crystalline form otherwise precipitation becomes difficult.

CHEMICALS REQUIRED:

1. Hydrochloric acid – 5ml
2. 5% Barium Chloride :

   5 g of barium chloride salt was weighed and made up to 100ml with distilled water.

PROCEDURE:

1. 100ml of the given sample was acidified with 5ml of concentrated HCl.
2. It was heated on a water bath and the volume was reduced.
3. 20ml of boiling solution of barium chloride was added. The beaker was left over the water bath for an hour for crystals of barium sulphate to

grow.

4. It was then filtered and washed with distilled water.
5. The precipitate was dried in a hot air oven for two hours and weighed.
6. These weights are converted into percentage reduction of sulphate in the treated BCS biomass.

CALCULATION:

ppm of sulphate: [(W × 96 × 1000) / (Volume of the sample taken × 233)] ppm

RESULTS and DISCUSSION

5) RESULTS AND DISCUSSION:

5.1) INTRODUCTION:

To standardize the quantity of inoculum, 2g of 18 day old culture of BGA-CLRI-C6 and BGA-CLRI-C8 were inoculated in flasks having 200ml of BCS solutions of varying Cr concentrations i.e. 0 ppm (control), 25 ppm, 50ppm, 75ppm, 100ppm, 125ppm, 150ppm, 200ppm, 300ppm, 500ppm, 750ppm respectively. The flasks were incubated at 30°C. The cultures inoculated were harvested after 18 days.

5.2) POST TREATMENT PROCESS:

After a period of 18 days, the inoculated samples were taken & centrifuged at 12000 rpm at 4°C for 20 minutes in ultra centrifuge (Hitachi high speed refrigerated centrifuge) to separate biomass from the liquor. The separated bio mass and the treated liquor were subjected to various analyses. The change in parameters such as the carbohydrate content, Phycobillin protein content and chlorophyll-a content were found with respect to varying concentrations of BCS with the treated biomass. The increases in chromium and sulphate sorption with increasing concentrations of BCS were also determined.

5.3) ESTIMATION OF WEIGHTS OF TREATED BIOMASS:

As mentioned above, 2 g of samples CLRI-BGA-C6 and CLRI-BGA-C8 were used for inoculation. After the 18 day period, the dry weights of treated biomass were noted. The control sample did not have any BCS and thereby the initial weights of samples inoculated are the same after 18 days. In the case of other samples inoculated in BCS solutions of varying concentrations, there is a

constant decrease in biomass with increasing chromium concentrations as shown in Table 3 and Table 4.

TABLE 3:

WEIGHTS OF TREATED BIOMASS: CLRI-BGA-C6

Cr CONCENTRATION (ppm)	WEIGHT OF TREATED BIOMASS (g)
0	2.07
25	1.99
50	1.39
75	1.30
100	1.13
125	1.12
150	1.19
200	1.18
300	1.13
500	1.09
750	1.07

TABLE 4:

WEIGHTS OF TREATED BIOMASS: CLRI-BGA-C8

Cr CONCENTRATION (ppm)	WEIGHT OF TREATED BIOMASS (g)
0	1.73
25	1.63
50	1.43
75	1.37
100	1.34
125	1.24
150	0.90
200	0.82
300	0.80
500	0.78
750	0.77

This may be due to increased stress conditions of chromium when loss of chlorophyll-a and Phycobillin proteins are anticipated.

5.4) BIOSORPTION STUDIES BY VARYING THE Cr CONCENTRATIONS WITH CONSTANT BIOMASS QUANTITY:

The amount of chromium adsorbed by the cyanobacterial species CLRI-BGA-C6 and CLRI-BGA-C8 are analyzed and tabulated in Table 5 and Table 6 below.

TABLE 5:

AMOUNT OF Cr ADSORBED BY CLRI-BGA-C6:

INITIAL Cr CONCENTRATION (ppm)	ADSORBED Cr CONCENTRATION (ppm)	% Cr ADSORBED
0	0	0
25	22.5	90.0
50	38.0	76.0
75	45.0	60.0
100	53.4	57.5
125	57.5	46.0
150	62.7	32.5
200	78.4	26.5
300	93.0	31.0
500	115.0	23.0
750	125.0	16.7

TABLE 6:

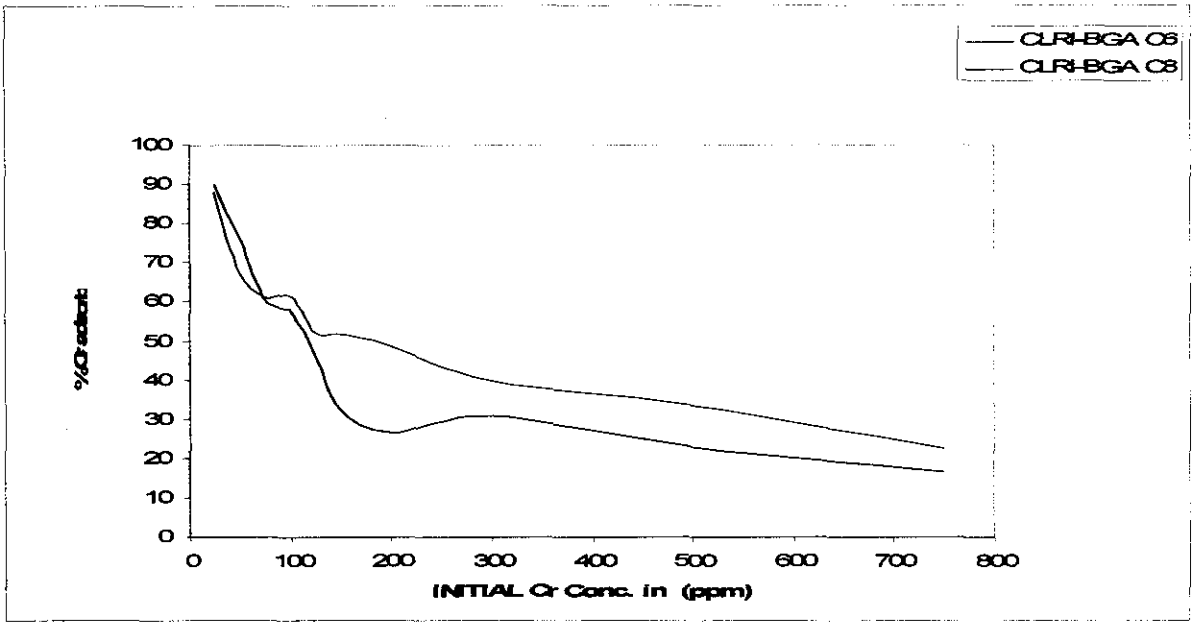
AMOUNT OF Cr ADSORBED BY CLRI-BGA-C8:

INITIAL Cr CONCENTRATION (ppm)	ADSORBED Cr CONCENTRATION (ppm)	% Cr ADSORBED
0	0	0
25	21.8	87.9
50	33.5	67.0
75	45.9	61.2
100	51.5	61.5
125	65.0	52.0
150	77.6	51.7
200	97.5	48.7
300	122.8	40.0
500	153.6	33.5
750	178.9	22.6

It is observed that the percentage adsorption of chromium by treated biomass decreases as concentrations of chromium increases. This is also shown in the Fig 4 below.

FIG 4:

PERCENTAGE ADSORPTION OF CHROMIUM BY TREATED BIOMASS



However, the amount of chromium adsorbed increases periodically with increasing Cr concentrations as shown in Table 7 and Table 8 (Fig 5 and 6).

TABLE 7:

PERIODIC ADSORPTION OF CHROMIUM BY CLRI-BGA-C6

INTIAL Cr CONC. (ppm)	ADSORBED Cr CONC. ON 6^{TH} DAY (ppm)	ADSORBED Cr CONC. ON 12^{TH} DAY (ppm)	ADSORBED Cr CONC. ON 18^{TH} DAY (ppm)
0	0	0	0
25	6.4	17.8	22.5
50	12.1	28.0	38.0
75	4.2	10.5	45.0
100	17.0	32.3	57.5
125	14.0	38.0	57.5
150	15.0	28.0	48.8
200	17.0	31.0	53.0
300	42.0	73.0	93.0
500	60.0	90.0	115.0
750	70.0	102.0	125.0

FIG 5:

PERIODIC ANALYSIS OF Cr ADSORPTION IN CLRI-BGA-C6

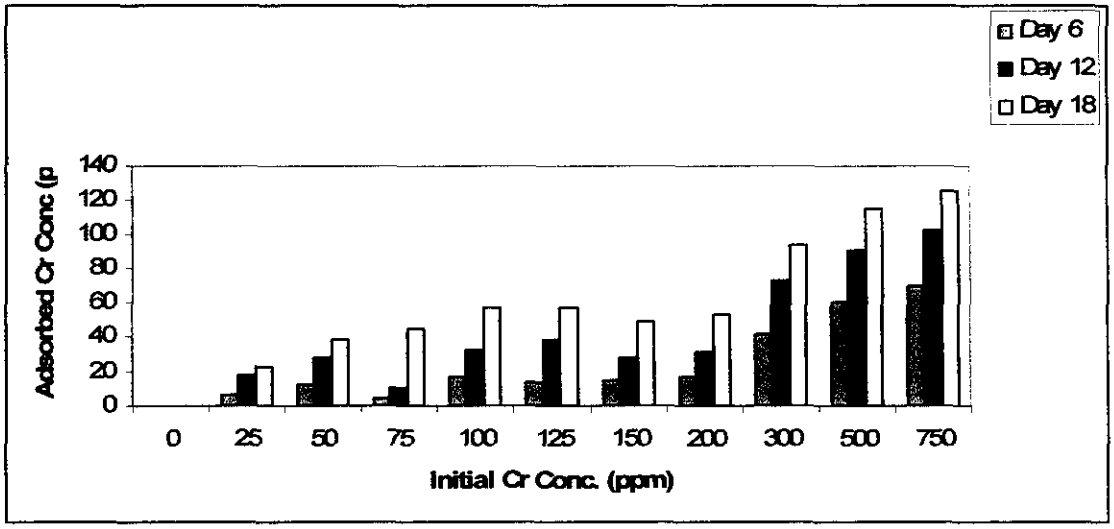


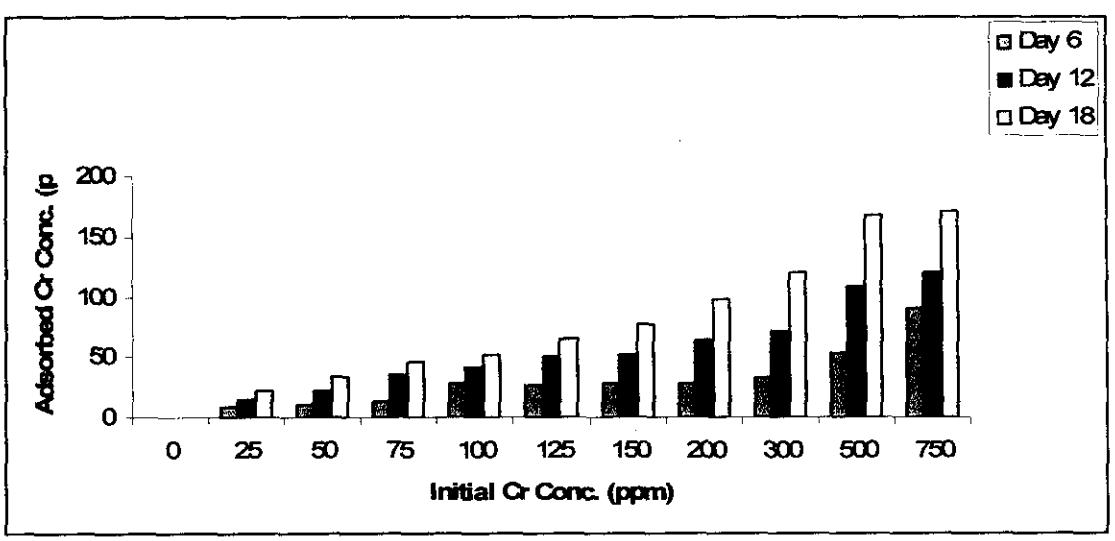
TABLE 8:

PERIODIC ADSORPTION OF CHROMIUM BY CLRI-BGA-C8

INTIAL Cr CONC. (ppm)	ADSORBED Cr CONC. ON 6TH DAY (ppm)	ADSORBED Cr CONC. ON 12TH DAY (ppm)	ADSORBED Cr CONC. ON 18TH DAY (ppm)
0	0	0	0
25	9	15.5	21.98
50	10.4	22.5	33.75
75	13.0	35.0	45.90
100	27.5	41.7	51.50
125	26.5	50.1	65.00
150	27.5	51.5	77.63
200	27.5	64.2	97.50
300	33.2	71.5	120.00
500	54.0	107.5	167.50
750	90.0	120.0	170.00

FIG 6:

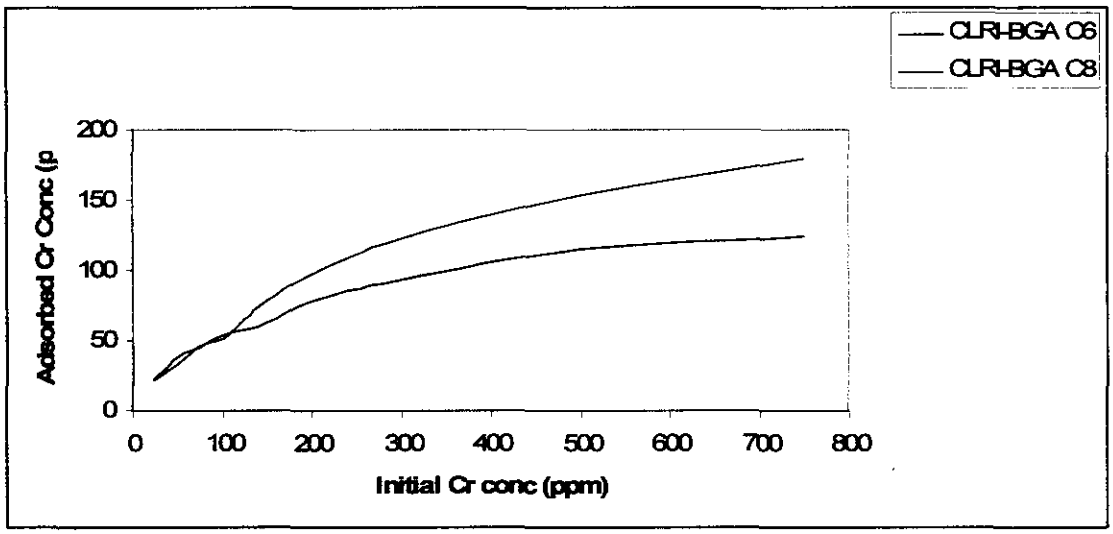
PERIODIC ANALYSIS OF Cr ADSORPTION in CLRI-BGA-C8



From the tabulated values and figures, it can be inferred that although there is a decrease in biomass, the amount of Cr adsorbed has increased despite increasing Cr stress conditions (Fig 7).

FIG 7:

LEVELS OF Cr ADSORPTION BY CLRI-BGA-C6 AND CLRI-BGA-C8



Samples of untreated and treated BCS liquors of concentrations (125 ppm,300 ppm,750 ppm) are shown in Fig 8a,8b,8c.

FIG 8a: CLRI-BGA C6 AND C8- UNTREATED AND TREATED BCS OF Cr-125 ppm.

FIG 8b: CLRI-BGA C6 AND C8- UNTREATED AND TREATED BCS OF Cr-300 ppm.

FIG 8c: CLRI-BGA C6 AND C8-UNTREATED AND TREATED BCS OF Cr-750 ppm.

5.5) SULPHATE ANALYSIS OF TREATED SAMPLES:

From the results obtained (Table 9, 10 and Fig 9), it is found that the amount of sulphate adsorbed has increased with increasing sulphate concentrations in the BCS liquor.

TABLE 9:

PERCENTAGE ADSORPTION OF SULPHATE IN TREATED CLRI-BGA-C6

INITIAL Cr CONC. (ppm)	CONC. OF SULPHATE IN BCS LIQUOR SAMPLE (ppm)	CONC. OF SULPHATE IN TREATED BGA SAMPLE (ppm)	% SULPHATE ADSORBED
0	0	0	0
25	350.21	45.32	87.06
50	412.02	156.56	62.01
75	690.13	360.10	47.82
100	1371.61	821.15	40.13
125	1930.71	1309.39	32.18
150	2309.77	2006.11	13.15
200	2703.24	2382.69	11.86
300	3038.21	2725.49	10.29
500	3468.36	3291.61	5.10
750	4788.87	4622.01	3.48

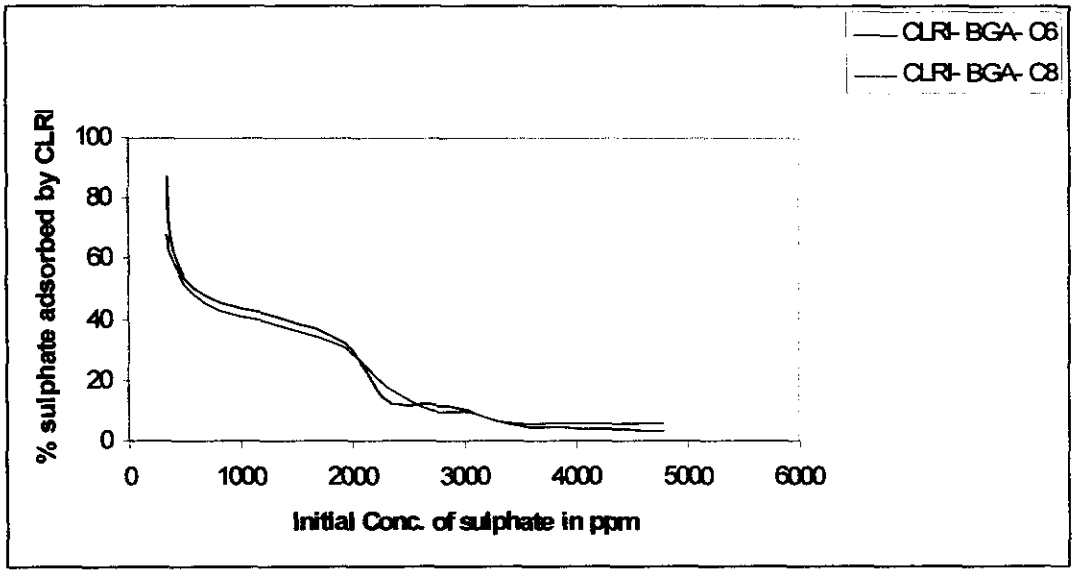
TABLE 10:

PERCENTAGE ADSORPTION OF SULPHATE IN TREATED CLRI-BGA-C8

INITIAL Cr CONC. (ppm)	CONC. OF SULPHATE IN BCS LIQUOR (ppm)	CONC. OF SULPHATE IN TREATED BGA SAMPLE (ppm)	% SULPHATE ADSORBED
0	0	0	0
25	350.21	113.30	67.64
50	412.02	168.93	59.00
75	690.13	376.58	45.43
100	1371.61	856.99	37.52
125	1930.71	1339.06	30.64
150	2309.77	1899.81	17.75
200	2703.24	2418.13	10.55
300	3038.21	2748.98	9.52
500	3468.36	3264.82	5.87
750	4788.87	4513.24	5.76

FIG 9:

PERCENTAGE SULPHATE ADSORBED BY CLRI-BGA-C6 AND CLRI-BGA-C8



This may be due a stimulated increase in Cr adsorption which equally facilitates sulphate adsorption as chromium does not exist freely in BCS but in combination with sulphate.

5.6) EFFECT OF Cr ON CARBOHYDRATE LEVELS IN CLRI-BGA C6 AND CLRI-BGA C8.

From Tables 11, 12 and Fig 10, we can see that the carbohydrate content is affected by increasing chromium concentrations.

TABLE 11:

EFFECT OF Cr CONCENTRATION ON CARBOHYDRATE LEVELS IN CLRI-BGA-C6

INITIAL Cr CONCENTRATION (ppm)	CARBOHYDRATE CONCENTRATION (ppm)	AMOUNT OF CARBOHYDRATE ($\times 10^{-2}$ µg/mg)
0	8.2	8.2
25	9.7	9.7
50	11.8	11.8
75	12.2	12.2
100	13.9	13.9
125	15.5	15.5
150	17.6	17.6
200	19.2	19.2
300	19.8	19.8
500	20.4	20.4
750	22.5	22.5

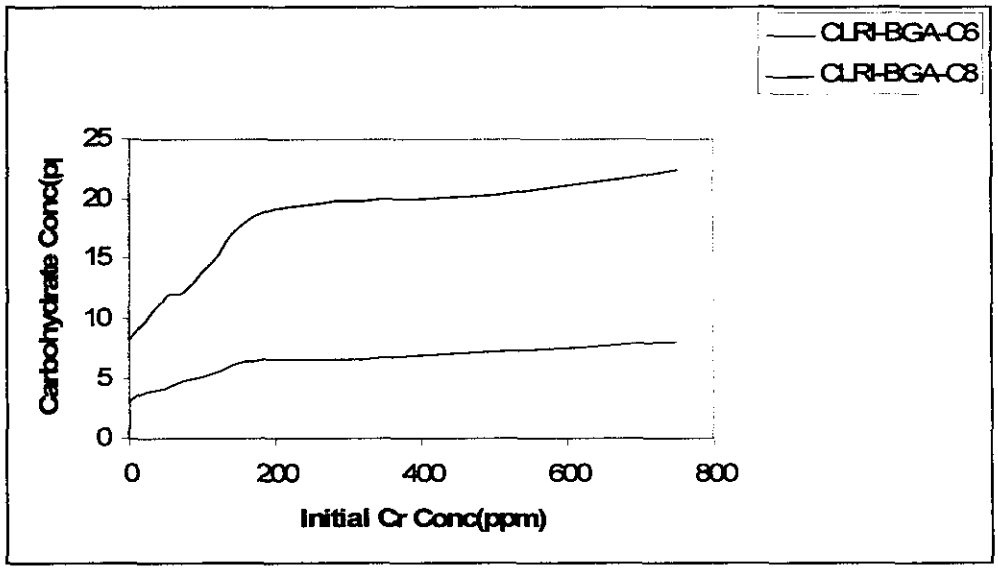
TABLE 12:

EFFECT OF Cr CONCENTRATION ON CARBOHYDRATE LEVELS IN CLRI-BGA-C8

INTIAL CR CONCENTRATION (ppm)	CARBOHYDRATE CONCENTRATION (ppm)	AMOUNT OF CARBOHYDRATE ($\times 10^{-2}$ µg/ mg)
0	3.0	3.0
25	3.8	3.8
50	4.1	4.1
75	4.8	4.8
100	5.2	5.2
125	5.6	5.6
150	6.2	6.2
200	6.6	6.6
300	6.5	6.5
500	7.2	7.2
750	8.0	8.0

FIG 10:

EFFECT OF Cr ON CARBOHYYDRATE CONTENT



Under increased sulphate and Cr stress the Blue Green algae tend to secrete polysaccharides thereby resulting in increased Carbohydrate levels.

5.7) ANALYSIS OF CHLOROPHYLL-a IN TREATED SAMPLES:

From the results shown in Table 13 and 14, we find that the chlorophyll-a concentration decreases with increase in chromium concentration. These results are also depicted in Fig 11.

TABLE 13:

ANALYSIS OF CHLOROPHYLL-a IN TREATED CLRI-BGA-C6

CONCENTRATION OF Cr (ppm)	CHLOROPHYLL-a CONCENTRATION (ppm)	AMOUNT OF CHLOROPHYLL-a ($x10^{-3}$ µg/mg)
0	2.79	13.9
25	1.98	9.94
50	1.82	9.13
75	1.54	7.72
100	1.16	5.80
125	0.83	4.15
150	0.91	4.55
200	0.65	3.26
300	0.57	2.85
500	0.44	2.19
750	0.26	1.29

At higher Cr concentrations of BCS, light is obstructed and chlorophyll is not properly synthesized. This accounts for the decrease in Chlorophyll-a content.

5.8) ESTIMATING PHYCOBILIN PROTEIN CONTENT:

From Tabulated values of control and treated samples for both CLRI-BGA-C6 and CLRI-BGA-C8 (Table 15) it is observed that there is loss of Phycobilin proteins to such an extent that it falls below spectrophotometer limits. It might be due to unavaibility of light for the organism because of higher Chromium concentration in the surrounding environment.

TABLE 15:

CONCENTRATION OF PHYCOBILIN PROTEINS IN CONTROL SAMPLES OF CLRI-BGA C6 AND CLRI-BGA C8:

SPECIES	PHYCOCYANIN CONTENT	ALLOPHYCOCYANIN CONTENT	PHYCOERYTHRIN CONTENT
CLRI-BGA -C6	0.016	0.014	0.006
CLRI-BGA -C8	0.003	0.009	0.002

CONCLUSION

6) CONCLUSION:

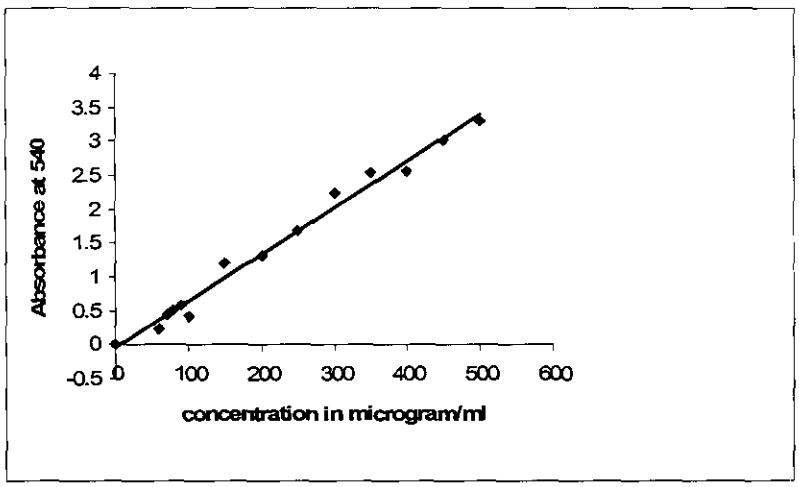
The present study reveals that CLRI-BGA-C6 and CLRI-BGA-C8 have their own adaptive way of survival despite decreased biomass and chlorophyll-a with increasing chromium concentration. The results substantiate that Blue Green Algae can be used as suitable adsorbents for the removal of Chromium from tannery effluents. It is so feasible and economical that application of Blue Green Algae for removal of Chromium from Chrome bearing tannery waste water not only gives a low cost treatment option but also recycles Chromium for leather tanning.

Further studies on the effects of increasing sizes of inoculum on the percentage of adsorption have to be performed in order to estimate the efficiency of each species for a particular concentration. Also, the adsorption potential of a particular inoculum size on higher concentrations of Chromium needs to be studied in order to find the highest concentration at which the Blue green algae of the two species become inefficient. A consortium of the two Blue green algae at different proportions can also be studied. Such studies may result in novel approaches to the biochemical route to treat tannery effluents.

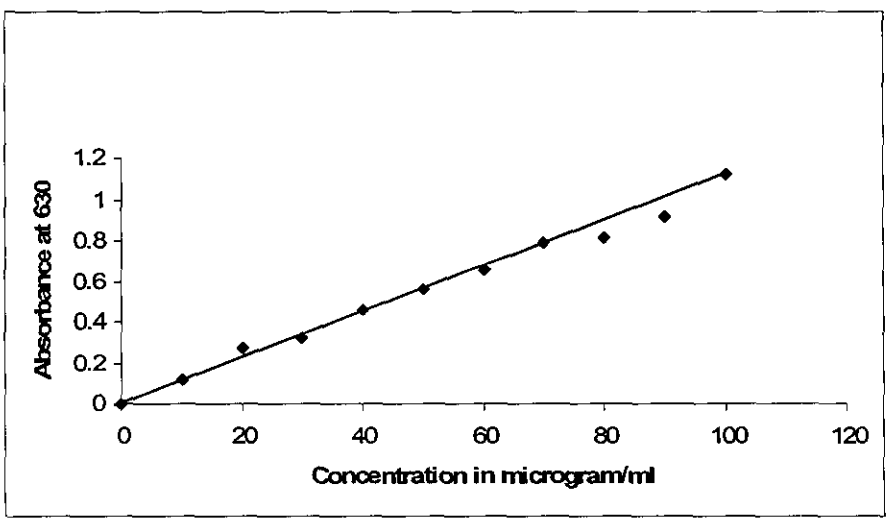
Thus the study proves to be an application oriented work with a lot of potential towards the removal of Cr (VI) that is a potent carcinogen. It is also very economical and industry focused.

APPENDICES

1. CALIBRATION CURVE FOR COLORIMETRIC ANALYSIS OF CHROMIUM



2. CALIBRATION CURVE FOR TOTAL CARBOYDRATE ESTIMATION BY ANTHRONE METHOD:



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