# Chromium Removal from Tannery Effluent by Microbial Biomass

**Thesis**
**Submitted in fulfillment**

**of the requirement for the award of the degree of**

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In

## Biotechnology & Environmental Sciences


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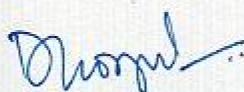
# Certificate

Certified that the thesis "Chromium removal from tannery effluent by microbial biomass" which is submitted by Ms. Indu Sharma, in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy in the Department of Biotechnology & Environmental Sciences, Thapar University, Patiala, is a record of candidate's own independent and original research work carried out by herself under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.



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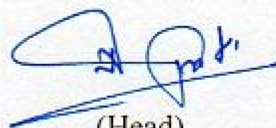

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# Candidate's Declaration

I, hereby declare that the work presented in the thesis entitled "Chromium removal from tannery effluent by microbial biomass" in fulfillment of the requirement for the award of the Degree of Doctor of Philosophy, Department of Biotechnology & Environmental Sciences, Thapar University, Patiala, is an authentic record of my own work during the period from July 2004 to July 2009, under the supervision of Dr. Dinesh Goyal, Professor, Department of Biotechnology & Environmental Sciences, Thapar University. The report has not been submitted for the award of any other degree or certificate in this or any other university.

Place: Patiala

Date: 1-2-2010 Indu Sharma

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Place: Patiala

Date: 1-2-2010 Indu Sharma

# List of Research Publications

## Published Research Papers

### International

1. **Indu Sharma** and Dinesh Goyal "*Chromium (III) removal from tannery effluent by Streptomyces* sp. *(MB2) waste biomass of fermentation process*" in International Journal of Integrative Biology. ISSN No. 0973-8363, Vol. 6(3): 2009, pp. 148-153.

2. **Indu Sharma** and Dinesh Goyal "*Adsorption kinetics: Bioremoval of trivalent chromium from tannery effluent by Aspergillus* sp. *biomass*" in Research Journal of Environmental Sciences. ISSN No. 1819-3412, 2009, Vol. 4(1): 2009, pp. 1-12. 2010 Academic Journal Inc.

3. **Indu Sharma** and Dinesh Goyal "Isolation and identification of chromium (VI) reducing bacteria from tannery effluent" *Raoultella* sp. IS1 EU980037, *Citrobacter* sp. IS2 EU980036 published in National Center for Biotechnology Information (NCBI) Genebank. 2008 http://www.ncbi.nlm.nih.gov

4. **Indu Sharma** and Dinesh Goyal "Isolation and identification of chromium (VI) reducing bacteria from chrome sludge" *Bacillus cereus* IS3 EU980034, *Citrobacter freundii* IS4 EU980035 published in National Center for Biotechnology Information (NCBI) Genebank. 2008 http://www.ncbi.nlm.nih.gov

### National

5. **Indu Sharma** and Dinesh Goyal "*Kinetic Modelling: Chromium (III) removal from aqueous solution by microbial waste biomass*" published in Journal of Scientific and Industrial Research. Vol. ISSN No. 0022-4456, 68: July 2009, pp. 640-646.

6. **Indu Sharma** and Dinesh Goyal "*Removal of Chromium (III) from aqueous solution by pretreated microbial waste biomass"* published in Research Journal of Chemistry and Environment. ISSN No. 0972-0626, Vol. 13(2): June 2009, pp. 29-33.

7. **Indu Sharma** and Dinesh Goyal *"Chromium (III) removal from aqueous solution by microbial waste from fermentation industry*" published in Indian Journal of Environmental Protection. ISSN No. 0253-7141, Vol. 29(3): March 2009, pp. 224-230.

## Papers Communicated

### International

1. **Indu Sharma,** Monika Bansal and Dinesh Goyal "*Reduction of Chromium (VI) by aerobic bacterial consortium*" communicated to Bioremediation Journal 2009.

2. **Indu Sharma** and Dinesh Goyal "*Adsorption dynamics for the removal of trivalent chromium from aqueous solution by microbial biomass*" communicated to Engineering in Life Sciences, 2009.

3. **Indu Sharma** and Dinesh Goyal "*Chromium removal from industrial effluent by Eucalyptus bark*" communicated to Asian Journal of Experimental Sciences 2009.

## Papers presented in Conferences

1. Presented a poster entitled "*Biotransformation of hexavalent chromium by bacteria isolated from tannery effluent*" by **Indu Sharma** and Dinesh Goyal in MICROCON 2009 "Microbes for the Sustainability of Mankind" International conference held at Microbial Biotechnology, CEAST, Panjab University, Chandigarh during March 3-4, 2009.

2. Presented a paper entitled "*Bioremoval of hexavalent chromium by waste biomass from chrome effluent*" by **Indu Sharma** and Dinesh Goyal in 11$^{th}$ Punjab Science Congress held at Thapar University, Patiala during February 7-8, 2008.

3. Presented a poster entitled "*Removal of Cr, Zn and Pb from aqueous solution by microbial biomass*" by Manoj Chandra Garg, **Indu Sharma** and Dinesh Goyal in 48$^{th}$ AMI National Annual Conference held at IIT Madras, Chennai during December 18-21, 2007.

4. Presented a poster entitled "*Removal of hexavalent chromium by microbial biomass from chrome effluent"* by **Indu Sharma** and Dinesh Goyal in 48$^{th}$ AMI National annual conference held at IIT Madras, Chennai during December 18-21, 2007.

5. Abstract entitled "*Bioremoval of trivalent chromium from tannery effluent using microbial biomass from fermentation industrial waste*" by **Indu Sharma** and Dinesh Goyal. II International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2007), 28 November- 1 December 2007 held at Campus Reina Mercedes, University of Seville, Seville, Spain (Accepted).

6. Presented a poster entitled "*Removal of heavy metals by non-living biomass of Paceilomyces variotii*" by Sarabjeet Singh Ahluwalia, **Indu Sharma** and Dinesh Goyal in 47$^{th}$ AMI National annual conference held at Barkatullah University, Bhopal during December 6-8, 2006.

7. Presented a poster entitled "*Chromium removal by bacterial consortium isolated from tannery effluent*" by **Indu Sharma** and Dinesh Goyal in 47$^{th}$ AMI National annual conference held at Barkatullah University, Bhopal during December 6-8, 2006.

8. Presented a poster entitled "*Chromium removal from industrial effluent by Eucalyptus bark*" by **Indu Sharma** and Dinesh Goyal in 46$^{th}$ AMI National annual conference held at Osmania University, Hyderabad during December 8-10, 2005.

# Synopsis


## Abstract

In the present study bacterial isolates and consortium isolated from tannery effluent (CT) and chrome sludge (CS) were analyzed for Cr(VI) reduction. Molecular identification based on 16S rDNA sequence analysis led to the characterization of isolates as *Raoultella* sp. (CT4) and *Citrobacter* sp. (CT5) from tannery effluent and *Bacillus cereus* (CS7) and *Citrobacter freundii* (CS8) from chrome sludge. Studies revealed that the bacterial isolate *Raoultella* sp. (CT4) completely transformed Cr(VI) to Cr(III) whereas *Bacillus cereus* (CS7), *Citrobacter freundii* (CS8) and *Citrobacter* sp. (CT5) showed 94.81%, 95.8% and 95.2% of reduction respectively.

Since biowastes from industrial fermentation units can serve as an economical and constant supply of biomass for biosorption of metal ions therefore two types of microbial biomass, which are generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy (fermentation industry) Paonta Sahib, Himachal Pradesh, India and were characterized for physical and chemical parameter such as, pH, moisture, ash content, bulk density, CHN analysis and calorific value. Removal of Cr(III) from aqueous solution and tannery effluent was carried out using microbial biomass (MB1 and MB2) and various parameters including adsorbent dosage (0.25-2%), pH (2-6) and chromium concentration (5-50 mg/L) and contact time were standardized in batch mode and to investigate the mechanism of metal uptake by Fourier transform infrared (FTIR) and X-ray diffraction (XRD) analysis was done. Fourier transform infrared studies with microbial waste biomass revealed the involvement of C=N, C=C, C-H and C-O functional groups in chromium binding. Langmuir and Freundlich adsorption isotherms were predicted from the equilibrium sorption data. Correlation coefficient ($r^2$) values indicate that the adsorption pattern for heavy metals followed both the Langmuir ($r^2 > 0.988$) and Freundlich ($r^2 > 0.993$) isotherms. A comparison of kinetic models applied to the biomass such as Lagergren, Ho and McKay, Elovich and Morris-Weber kinetic models indicates that adsorption of Cr(III) on microbial biomass follows a best Ho and McKay pseudo second-order rate equation and correlation coefficient ($r^2$) correlated with the experimental data.

Removal of Cr(III) from aqueous solution and tannery effluent by microbial biomass (MB1 and MB2) led to the development of a cost effective and eco-friendly process for removal of chromium from tannery effluent without generating toxic chemical sludge having industrial application. Maximum chromium removal capacity of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) was 99.6 and 70.9% from tannery effluent in column mode. The high efficiency of the biosorption and elution, low biomass damage and stability over a prolonged operation time makes the new biosorption process an effective alternative for chromium pollution control.

## Introduction

Chromium is an important heavy metal widely used in metallurgic, refractory, tannery, electroplating, water cooling, pulp producing, ore and petroleum refining processes. More than 1,70,000 tons of chromium wastes are discharged annually into the environment through industrial and manufacturing activities. Chromium is present in the effluent primarily in the form of chromium (III) and (VI) forms. Cr(III) is classified as a hard acid and forms relatively strong complexes with oxygen and donor ligands. Cr(III) is an essential trace element in mammalian metabolism for reducing blood glucose levels and is used to control certain cases of diabetes, whereas Cr(VI) is toxic, carcinogenic and mutagenic in animals and plants. In humans, it causes skin irritations, eye diseases, and respiratory problems.

Tanning is one of the oldest and fastest growing industries in India with about 2161 tanneries excluding cottage industries, which processes 500,000 tonnes of hides and skins annually discharging 9,420,000 $m^3$ of wastewater. The effluent from tanning industries has become one of the most vital problems due to the high rate of chromium discharge into the environment. Wastewater originating from wet processing in the beam house, tanyard and processing in the post tanning operation has a complex composition containing large amount of organic and inorganic compounds in addition to specific pollutants such as chromium and sulphide. The untreated wastewater containing 20 to 80 cubic metric per tonne ($m^3$/t) of hide or skin, is turbid, colored and foul smelling. It consists of acidic and alkaline liquors with chromium, total suspended solids, sulphides, chloride, nitrogen, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) with pathogens and pesticide residues at significant levels with co-generation of sludge. The maximum tolerance limit for total chromium is fixed at 0.05 and 0.1 mg/L in drinking water and inland surface water respectively, whereas chromium in industrial effluents ranges from 0.1 to 400 mg/L. Therefore, it becomes necessary to treat the wastewater for pollution abatement in order to keep the environment clean.

Conventional methods such as precipitation, reverse osmosis, membrane filtration, ion exchange and adsorption used for effluent treatment are expensive and not eco-friendly requiring high energy and large quantities of chemicals. Increasing demand of eco-friendly technologies has led to the search for low-cost alternatives. Biological approach appears to be efficient, economical and cost-effective for effluent treatment. Biosorption is a rapid phenomenon of passive metal sequestration by non-growing biomass and may involve utilization of inexpensive dead or live microbial biomass to remove metals from industrial effluent. It mainly involves cell surface complexation, ion exchange and micro precipitation. Different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal-binding capacities. Biosorbents derived from dead biomass are considered the cheapest, most abundant and environmentally friendly option. Industrial fermentation process can serve as an economical and constant source for the supply of biomass for metal sorption.

In the present study, bacteria and bacterial consortium isolated from tannery effluent and chrome sludge were studied for biotransformation and removal of chromium (VI) from aqueous solution. To scale up the process of biosorption, it is not feasible to cultivate microorganisms on large scale therefore, microbial biomass which are generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy fermentation industry and used as an adsorbent for the removal of trivalent chromium from aqueous solution as well as tannery effluent in batch and continuous flow sorption mode. A comprehensive study was under taken with the following objectives.

**Objectives**

1. Characterization of tannery effluent and microbial biomass from different industries.
2. Isolation and characterization of chromium reducing microbes from tannery effluent.
3. Development of a biosorbent from microbial biomass for removal of chromium from tannery effluent.
4. Process optimization in batch and continuous-flow biosorbent column.

## 1. Characterization of the tannery effluent and microbial biomass from different industries

Tannery effluent was collected from AV tanneries, Kapurthala, Punjab, India, was characterized for different physico-chemical parameters such as pH, temperature, conductivity, salinity, total suspended solids (TSS), total dissolved solids (TDS), colour, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and heavy metals Fe, Cr, Ni, Zn, Pb were analyzed by AAS. Tannery effluent was acidic (pH 3.5), greenish in colour with 1700.9 mg/L of Cr(III), 96,000 mg/L total solids, 80,000 mg/L total dissolved solids and was having chemical oxygen demand (COD) of 332.8 mg/L and biochemical oxygen demand (BOD) of 290 mg/L and with traces of other metals such as Fe, Pb Co, Cu, Cd, and secondary elements like Ca, Mg and Na etc.

Microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics were collected from Ranbaxy Paonta Sahib, Himachal Pradesh, India and characterized for physical and chemical parameter such as, pH (5-6), moisture (5-7%), ash content (5-6%), bulk density (0.5-0.7%), CHN analysis and calorific value (16-17 MJ/kg) and heavy metals Fe, Cr, Ni, Zn, Pb. Both type of microbial biomass had acidic pH with ash content ranging from 5-6%. The CHN analysis showed that *Streptomyces* sp. (MB2) had high nitrogen content than *Aspergillus* sp. (MB1). Both exhibited calorific value of nearly 16-17 MJ/kg, which coincides with the relative calorific value (17.5 MJ/kg) of solid fuels such as biomass, municipal waste, industrial waste, peat and brown coal.

## 2. Isolation and characterization of chromium reducing microbes from tannery effluent

### Isolation of bacterial consortium and isolates

Chromium reducing bacterial consortium and isolates were isolated from tannery effluent and chrome sludge according to the method of Philip et al., (1998) and Camargo et al., (2003). Complete media (M1) amended with 0.25 mM Cr(VI) was used for the isolation of bacterial consortium. For enumeration of bacteria, the consortium samples were serially diluted and plated on Luria Bertani (LB) agar. Bacteria with different colony morphologies were streaked on separate agar slants and incubated at 28°C for 24 h and were checked for chromium transformation/removal in Minimal media (M2) and thereafter, growth curves were determined in the presence and absence of different concentration of Cr (Philip et al., 1998).

**Transformation and removal of Cr by bacterial consortium**

Bacterial consortium from tannery effluent (CT) and chrome sludge (CS) were analyzed for Cr(VI) reduction. The percentage decrease in the exponential growth of bacterial consortium from tannery effluent (CT) was 11 %, 19 %, 21 % whereas of bacterial consortium from chrome sludge (CS) was 30 %, 34 %, and 36% at 3.6, 7.9 and 14.18 mg/L of Cr(VI), respectively. Cr(VI) removal and transformation by bacterial consortia was investigated at concentration of 3.6 to 14.18 mg/L Cr(VI). Transformation of Cr(VI) to Cr(III) showed that there was gradual increase in the concentration of Cr(III) and decrease in Cr(VI) concentration and the rate of increase in Cr(III) concentration coincides exactly with rate of decrease in Cr(VI) concentration. For the first 2 h at a concentration of 3.6 mg/L rate of transformation was 1.5 mg/h and 1 mg/h by CT and CS consortia respectively, with about 90% transformation was observed by CT and 60% by CS consortia. The rate decreased to approximately 0.06 mg/h and 0.125 mg/h for next four hour. After 12 h of incubation both the consortia showed similar trend in percentage transformation which was 90 % > 80 % > 70 % at 3.6, 7.9 and 14.18 mg/L respectively and 100 % transformation was observed at 3.6 mg/L and 7.9 mg/L concentrations after 22 and 36 h of incubation, respectively.

Reduction of Cr(VI) by resting cells of consortium from tannery effluent (CT) was 30% more than consortium from chrome sludge (CS). The percentage transformation by CT consortium after 24h in saline, phosphate buffer and deionised water was 69.2%, 44.4%, 19.6% and by CS consortium it was 41%, 40%, 23.2% respectively. Under aerobic conditions cell free extract of CT and CS bacterial consortium showed reduction of Cr(VI) with no change in concentration of total chromium in the solution which suggest that transformation of Cr(VI) takes place by extracellular enzymes, however no change in the concentration of total chromium suggested that it is a phenomenon involving only either live or dead cells. The study demonstrated that indigenous bacterial isolates present in chromium contaminated wastes were able to catalyze the removal of toxic and soluble Cr(VI) from media, most likely reducing it to relatively non-toxic and insoluble Cr(III). Growth inhibition by Cr(VI) was evident in all the bacterial cultures, although Cr(VI) did not completely inhibited bacterial growth. Among the three suspension media saline was best suited for Cr(VI) transformation.

**Transformation and removal of Cr by bacterial isolates**

36 bacterial isolates 18 each from tannery effluent and chrome sludge were isolated by serial dilution plating method and based on their Cr removal efficiency, 4 isolates viz., CT4, CT5, CS7 and CS8 were selected. Morphological character and gram staining study reveals all four isolates were gram negative rods. The molecular identification based on the 16S rDNA sequence analysis led to the characterization of these isolates from tannery effluent as CT4 (*Raoultella* sp. IS1) and CT5 (*Citrobacter* sp. IS2), whereas from chrome sludge as CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4). The chromium removal and its transformation by bacterial isolates differed at varying concentrations of Cr(VI) in minimal media. With increase in concentration of Cr(VI) there was decrease in reduction, which may be due to toxicity of Cr(VI) to the growing cells. However, the rate of transformation increased to 0.5 mg/h and 100% transformation was observed after 22 h of incubation.

## 3. Development of a biosorbent from microbial biomass for removal of chromium from tannery effluent

**Adsorbent preparation**

Microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) after collection form industry was ground in a blender and sieved to pass through a 2.0 mm sieved in order to obtain uniform particle size. Further they were washed with distilled water and then dried at 80ºC for overnight or till the moisture percentage was below 5% and was used as biosorbent.

**Batch studies**

Removal efficiency of Cr(III) from aqueous solution and tannery effluent by microbial waste biomass was studied in batch mode. Various parameters including biomass dosage (0.25-2%), pH (2-6) of the solution, different concentration of chromium (5-50 mg/L) and contact time were standardized. 97.81% of Cr(III) removal was observed by *Aspergillus* sp. (MB1) and 72.38% by *Streptomyces* sp. (MB2) at pH 4.0. Increase in contact time from 0.08 to 4 h led to an increase in Cr(III) removal from 40-70% in both MB1 and MB2 microbial biomass. Maximum 65% of Cr(III) removal was observed within first 2 h, which represents the time at which equilibrium of chromium biosorption is presumed to have been attained.

**Pretreatment of biomass**

Microbial biomass were pretreated with different inorganic solvents such as sodium hydroxide, hydrochloric and sulfuric acid and organic solvents such as acetone, chloroform, diethyl ether and methanol for modification of the cell wall and cell surface sequestration in order to enhance the chromium binding capacity of the biomass. Increase in adsorption capacity of Cr(III) was observed after treatment with sodium hydroxide, hydrochloric and sulfuric acid respectively. Percentage Cr(III) removal efficiency was improved by pretreated *Aspergillus* sp. (MB1) biomass with certain chemicals which was in the order of sodium hydroxide (96.21%) > sulfuric acid (70.9%) > acetone (68%) > methanol (64%) > chloroform (63%) > hydrochloric acid (62.%) > diethylether (54.3%), whereas by *Streptomyces* sp. (MB2) it was in the order of hydrochloric acid (77.7%) > sodium hydroxide (76.7%) > sulfuric acid (75.8%) > diethylether (74.5%) chloroform (69.1%) > methanol (59%). Both alkali and acid treatment of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) biomass showed significant removal of Cr(III).

**Adsorption isotherms**

Langmuir and Freundlich adsorption isotherms were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by microbial biomass. Correlation coefficient ($r^2$) values indicate that the adsorption pattern for chromium removal by microbial biomass followed both the Langmuir ($r^2 > 0.98$) and Freundlich ($r^2 > 0.98$) isotherms.

**Adsorption kinetics**

Kinetic modelling of Cr(III) study describes the solute uptake rate and this rate controls the residence time of adsorbate uptake at the solid-solution interface by using Lagergren, Ho and McKay, Elovich and Morris-Weber equation (Demisrbas et al., 2004). A comparison of kinetic models applied to the biomass indicates that adsorption of Cr(III) follows best of Ho and McKay pseudo second-order rate equation and correlation coefficient ($r^2$) correlated with the experimental data. A relatively high $r^2$ value indicates that the model successfully described the kinetics of Cr(III) adsorption.

**FTIR and XRD analysis**

To ascertain the chemical nature of binding sites for Cr(III), Fourier transform infrared spectral analysis (FTIR) of native, solvent treated, Cr(III), laden biomass were carried out. FTIR analysis of microbial biomass showed that C=N, C=C, C-H and C-O functional groups play important role in chromium binding. The X-ray diffraction analysis of *Aspergillus* sp. (MB1) biomass showed peaks that indicated its crystalline structure while *Streptomyces* sp. (MB2) showed amorphous nature. After Cr(III) adsorption by both microbial biomass the surface structures were changed to amorphous nature.

## 4. Process optimization in batch and continuous-flow biosorbent column

Batch studies with *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were conducted at varying initial concentrations (5-50 mg/L) of tannery effluent. Removal of Cr(III) from tannery effluent containing 25 mg/L Cr(III) by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) biomass was 64.48 and 88.28%, respectively. The pseudo second-order dynamics model was found to correlate with the experimental data.

Both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were used as an adsorbent for chromium removal from aqueous solution as well as tannery effluent in continuous flow sorption system. Chromium sorption capacity of microbial biomass *Aspergillus* sp. (MB1) was found to be 96% where as of *Streptomyces* sp. (MB2) it was 82%. Break through curves concluded that microbial biomass could be used for Cr(III) removal from aqueous solution and tannery effluent. Microbial biomass could be desorbed with dilute HCl and regenerated for its subsequent reuse as metal sorbent and there was no change in chromium removal efficiency up to five sorption cycles. The present study significantly implies that microbial biomass is more effective for removal of chromium in successive cycles which is applicable for treatment of tannery effluent at large scale.

## Salient findings

1. Tannery effluent collected from A.V. Tanneries, Kapurthala, Punjab was acidic, greenish in colour and contained highest concentration of TS, TDS, TSS, BOD, COD and nitrogen. Chromium concentration in the effluent was 1700 mg/L besides this effluent also contained other heavy metals.

2. Microbial waste biomass (*Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) generated as a byproduct of pharmaceutical fermentation industry had acidic pH with ash content ranging from 5-6%. The CHN analysis showed that *Streptomyces* sp. (MB2) had high nitrogen content than *Aspergillus* sp. (MB1).

3. Bacterial consortium developed from chrome sludge (CS) was more efficient than tannery effluent consortium (CT) in transforming Cr(VI) to Cr(III). Growth inhibition by Cr(VI) was evident in all the bacterial culture, although the Cr(VI) did not completely arrest bacterial growth. The percent decrease in the growth of bacterial consortium (CT) was 11%, 19% and 21% whereas in bacterial consortium (CS) it was 30%, 34% and 36% at 3.6, 7.9 and 14.18 mg/L of Cr(VI) respectively. Biotransformation of Cr(VI) by resting cells of tannery consortium (CT) was 30% more than by chrome sludge consortium (CS). Among the three suspension media saline was best suited for Cr(VI) transformation.

4. Four isolates showing highest Cr(VI) removal were selected and identified on the basis of 16S rDNA sequencing as *Raoultella* sp. (CT4) and *Citrobacter* sp. (CT5) from tannery effluent and *Bacillus cereus* (CS7) and *Citrobacter freundii* (CS8) from chrome sludge.

5. High Cr(VI) transformation or reduction efficiency was observed for all the bacterial isolates at 4 and 8 mg/L of Cr(VI) which was nearly 90% whereas CT4 (*Raoultella* sp. IS1) showed complete reduction after 22 h of incubation. The time for reduction of Cr(VI) increased with increased in incubation time at high concentration (~ 18 mg/L).

6. Removal of Cr(III) by microbial biomass *Aspergillus* sp. (MB1) obtained from pharmaceutical fermentation industry was higher than *Streptomyces* sp. (MB2). Maximum Cr(III) removal capacity of *Aspergillus* sp. (MB1) was 94.6% and 64.48% whereas by *Streptomyces* sp. (MB2) biomass was 68.2% and 73.9% from aqueous solution and tannery effluent respectively.

7. Pretreatment of microbial biomass with alkali improved removal of Cr(III). Cr(III) removal by *Aspergillus* sp. (MB1) biomass was 96.21% after treatment with alkali and by *Streptomyces* sp. (MB2) it was 77.73% after treatment with acid.

8. Adsorption isotherms and adsorption kinetices were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by microbial biomass. A comparison of kinetic models (Lagergren, Ho & McKay, Elovich & Morris-Weber) applied to microbial biomass (MB1 and MB2) indicates that adsorption of Cr(III) on biomass follows best Ho & McKay pseudo second-order rate equation and correlation coefficient ($r^2$) values correlated with experimental data.

9. Fourier transform infrared spectral analysis of microbial biomass revealed that C=N, C=C, C–H and C–O functional groups are involved in chromium binding which was additionally supported by XRD analysis that showed change in the surface of microbial biomass after biosorption.

10. Removal of Cr(III) from tannery effluent by microbial biomass from fermentation industry implies that it is a cost effective and eco-friendly process for bioremediation of chromium from industrial effluent.

## List of Symbols/Abbrevations

| | |
|---|---|
| $q_e$ | Specific metal uptake |
| $R^2$ | Correlation coefficient |
| K | Maximum amount of the metal ion per unit weight of biomass |
| $K_f$ | Indicators of adsorption capacity |
| n | Indicators of adsorption intensity |
| $C_i$ | Initial metal concentration |
| $C_f$ | Final residual metal concentration |
| h | Hours |
| d | Days |
| rpm | Revolution per minute |
| g | Gram |
| L | Litre |
| V | Volume of the solution |
| W | Dry weight of biomass |
| $\mu S\ cm^{-1}$ | Microsiemens per cm |
| mL | Millilitre |
| µL | Microlitre |
| mg/L | Milligrams per liter |
| m/g | Milligram per gram |
| % | Percentage |
| min | Minute |
| KBr | Potassium Bromide |
| C | Carbon |
| H | Hydrogen |
| N | Nitrogen |
| Pb | Lead |
| Cr | Chromium |
| Cr(VI) | Hexavalent chromium |
| Cr(III) | Trivalent chromium |
| mg/mL | Micrograms per millilitre |
| µg/g | Microgram per gram |
| Mn | Manganese |
| DPC | diphenylcarbazide |
| FTIR | Fourier-transform Infrared |
| AAS | Atomic Absorption Spectroscopy |
| XRD | X-Ray Diffraction |
| e.g. | For example |
| et al., | et alii (and others) |
| $H_2SO_4$ | Sulfuric acid |
| HCl | Hydrochloric acid |
| LB | Luria Broth |
| NaOH | Sodium hydroxide |
| nm | Nanometer |
| OD | Optical density |
| °C | Degree Celsius |

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# Chapter 1
# Introduction

Chromium is an important heavy metal widely used in the metallurgic, refractory, tannery, electroplating, water cooling, pulp producing, ore and petroleum refining processes (Barnhart, 1997). More than 1,70,000 tons of chromium wastes are discharged annually into the environment through industrial and manufacturing activities (Gadd and White, 1993). The effluent from these industries contain both Cr(VI) and Cr(III) in concentrations ranging from tens to hundreds of mg/L. Cr(VI) is known to be toxic to both plant and animals due to strong oxidizing characteristic (Costa, 2003). Hexavalent chromium [Cr VI] has many industrial applications and often causes environmental contamination in marine and freshwater sediments from urban and industrial discharges (Losi et al., 1994a). Chromate ($CrO_4^{2-}$) is a strong oxidizing agent that is reduced intracellularly to $Cr^{5+}$ which reacts with nucleic acids and other components to produce mutagenic and carcinogenic effects on biological systems (McLean and Beveridge, 2001). Although reduction of Cr(VI) is responsible for chromate toxicity, further reduction to trivalent chromium leads to the formation of stable, less soluble and less toxic Cr(III).

Trivalent chromium is classified as a hard acid and forms relatively strong complexes with oxygen and donor ligands. Cr(III) is an essential trace element in mammalian metabolism for reducing blood glucose levels and is used to control certain cases of diabetes. Chromium deficiency causes changes in the metabolism of glucose and lipids and may be associated with maturity-onset diabetes, cardiovascular diseases and nervous system disorders (Anderson, 1993, 1995), whereas acute exposure to Cr(VI) causes nausea, eye diseases, diarrhoea, liver and kidney damage, dermatitis, internal hemorrhage and respiratory problems (Mohan and Pittman Jr, 2006). Inhalation may cause acute toxicity, irritation and ulceration of the nasal septum and respiratory sensitization (asthma) (Kimbrough et al., 1999; Mohan et al., 2005, 2006; Mohan and Pittman Jr, 2006).

Tanning is one of the oldest and fastest growing industries in India with about 2161 tanneries excluding cottage industries, which processes 500,000 tonnes of hides and skins annually discharging 9,420,000 $m^3$ of wastewater. The effluent from tanning industries has become one of the most vital problems due to the high rate of chromium discharge into the environment (Mohan et al., 2005). Tanneries generate effluents that are typically high in organic and inorganic pollutants. Their effluents are complex in

nature and with variation in characteristics from time to time, process-to-process and tannery-to-tannery and a wide range of raw materials. The untreated wastewater containing 20 to 80 cubic metric per tonne ($m^3/t$) of hide or skin, is turbid, coloured and foul smelling. It consists of acidic and alkaline liquors with chromium, suspended solids, sulphides, chloride, nitrogen, BOD, COD and it may also contain residues of pesticides and pathogens at significant levels with co-generation of sludge. The maximum permissible limit for total chromium has been fixed at 0.05 mg/L and 0.1 mg/L in drinking water and inland surface water, respectively. Whereas chromium in industrial effluents ranges from 0.1 to 400 mg/L. Therefore, it becomes almost necessary to treat the wastewater for pollution abatement in order to keep the environment clean.

Conventional methods such as precipitation, reduction, reverse osmosis, membrane filtration, ion exchange and adsorption used for effluent treatment are expensive and not eco-friendly requiring high energy and large quantities of chemicals (Komori et al., 1990a; Volesky, 2001). Increasing demand of eco-friendly technologies has led to the search for low-cost alternatives. Biological approach appears to be efficient, economical and cost-effective for effluent treatment. Biosorption is a rapid phenomenon of passive metal sequestration by non-growing biomass and may involve utilization of inexpensive dead or live microbial biomass to remove metals from industrial effluent. It mainly involves cell surface complexation, ion exchange and micro precipitation. Different microbes have been found to vary in their affinity for different heavy metal(s) and hence differ in their metal-binding capacities.

Biosorbents derived from industrial or natural sources may be considered as cheapest, efficient, most abundant and environmentally friendly option (Kratochvil et al., 1998; Cabatingan et al., 2001; Ahluwalia and Goyal, 2007). Industrial fermentation process can serve as an economical and constant supply source of biomass for metal sorption. Because of these advantages, there has been extensive research exploring appropriate biosorbents which are able to effectively remove chromium such as sawdust (Yu et al., 2003), agricultural by product (Gardea-Torresdey et al., 2000; Chun et al., 2004), fungi (Merrin et al., 1998; Sag et al., 2001), bacteria (Nourbakhsh et al., 1994; Ozdermir et al., 2003; Zouboulis et al., 2004), microalgae (Aksu et al.,1997; Dönmez 1999; Aksu and Açikel, 2000) seaweeds (Kratochvil et al., 1998; Lee et al., 2000) and brown seaweed (Park et al., 2004). Feasibility and efficiency of a biosorption process depends not only on the properties of the biosorbent but also on the composition of the wastewater as majority of the industrial effluents contain more than one toxic metal. So far, such biological potentials have been exploited only to a limited extent.

A wide range of microorganisms exhibits an exceptional capacity to detoxify Cr(VI) by converting it to less soluble and less toxic Cr(III) (Michel et al., 2001). Most of them have been isolated from tannery sludge, industrial sewage, evaporation ponds, and discharge water (Losi and Frankenberger, 1994). Bacteria such as *Enterobacter* sp. (Wang et al., 1990) *Escherichia coli* (Shen and Wang, 1993) *Bacillus* sp. (Compos et al., 1995) *Pseudomonas* sp. (Oh and Choi, 1997), *Microbacterium* (Pattanapipitpaisal et al., 2001), *Desulfovibrio* (Michel et al., 2001).

In the present study, bacteria and bacterial consortium isolated from tannery effluent and chrome sludge were characterized and studied for biotransformation and removal of Cr(VI) from aqueous solution. To scale up the process of biosorption, it is not feasible to cultivate microorganisms on large scale therefore, microbial biomass which are generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy fermentation industry and used as an adsorbent for the removal of trivalent chromium from aqueous solution as well as tannery effluent in batch and continuous flow sorption mode. Therefore to address the problem of chromium contamination in waste water, a comprehensive study was under taken with the following objectives:

## Objectives

1. Characterization of tannery effluent and microbial biomass from different industries.
2. Isolation and characterization of chromium reducing microbes from tannery effluent.
3. Development of a biosorbent from microbial biomass for removal of chromium from tannery effluent.
4. Process optimization in batch and continuous-flow biosorbent column.

Approach adopted to meet these objectives

1. Tannery effluent was collected from AV tanneries, Kapurthala, Punjab, India, and microbial waste biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) generated as byproduct of pharmaceutical fermentation industry were collected from Ranbaxy Paonta Sahib, Himachal Pradesh, India and were characterized for physical and chemical parameters.

2. Chromium reducing bacterial consortium (CT and CS) and isolates (CT4, CT5, CS7 and CS8) were isolated from tannery effluent and chrome sludge. Morphological, biochemical and molecular characterization of the isolates was done. Growth, transformation of Cr(VI) to Cr(III) and removal of Cr(VI) by bacterial cells and cell free extract in minimal medium were also analyzed.

3. Microbial waste biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) after collection from industry was developed as biosorbent. Removal of Cr(III) from aqueous solution and tannery effluent by microbial waste biomass was studied in batch mode and various parameter including adsorbent dosage, pH, different concentration of chromium and contact time were standardized. Microbial biomass was pretreated with alkali, acids and different organic solvent for modification of the cell wall in order to enhance chromium binding capacity and mechanism of metal binding by Fourier transform infrared spectral (FTIR) and X-Ray diffraction (XRD) analysis was explored. Adsorption isotherms (Langmuir and Freundlich) and kinetic models (Lagergren, Ho and McKay, Elovich and Morris-Weber) were used for the analysis of experimental data.

4. To scale up the process, microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) from fermentation industry were used for removal of Cr(III) from aqueous solution and tannery effluent in continuous flow sorption columns packed with microbial biomass.

# Chapter 2
# Review of Literature

Chromium was discovered in 1797 by French chemist, Louis-Nicolas Vaquelin (1763-1829) in a mineral known as Siberian red lead. The element was named after the Greek word 'chromium' meaning 'color' because many chromium compounds have a distinctive color, ranging from purple to black to green to orange to yellow (Young, 2000; Mohan and Pittman Jr, 2006). Chromium is the earth's 21$^{st}$ most abundant element and the sixth most abundant transition metal. It is a hard, steel gray, shiny metal that breaks easily. It has a melting point of 1900$^{\circ}$C, a boiling point of 2642$^{\circ}$C and a density of 7.1 g $cm^{-3}$. A physical property that greatly adds to chromium's commercial importance is that it can be polished to a high shine. Chromium is a relatively active metal that does not react with water but does react with most metals. It combines slowly with oxygen at room temperature to form chromium oxide ($Cr_2O_3$). The chromium oxide formed acts as a protective layer, preventing the metal from reacting further with oxygen (Young, 2000). The principal chromium ore is ferric chromite, $FeCr_2O_4$, found mainly in South Africa (with 96% of the world's reserves), Russia and the Philippines. Less common sources include crocoite, $PbCrO_4$ and chrome ochre, $Cr_2O_3$.

Chromium occurs in $2^+$, $3^+$ and $6^+$ oxidation states but Cr(II) is unstable and very little is known about its hydrolysis. It produces mononuclear species $CrOH^{2+}$, $Cr(OH)^{2+}$, $Cr(OH)^{4-}$, neutral species $Cr(OH)_3^0$ and polynuclear species $Cr_2(OH)_2$ and $Cr_3(OH)_4^{5+}$ (Radovic et al., 2000, Mohan et al., 2005; Mohan and Pittman Jr, 2006). The hydrolysis of Cr(VI) produces only neutral and anionic species, predominately $CrO_4^{2-}$, $HCrO_4^{2-}$, $Cr_2O_7^{2-}$. At low pH and high chromium concentrations, $Cr_2O_7^{2-}$ predominates while at a pH greater than 6.5, Cr(VI) exists in the form of $CrO_4^{2-}$ Cr(III) is classified as a hard acid and forms relatively strong complexes with oxygen and donor ligands (Mohan et al., 2005; Mohan et al., 2006; Mohan and Pittman Jr, 2006).

## 2.1 Chromium distribution in environment

Chromium is found naturally in rock, soil, surface water, ground water, fresh water seawater, plants, animal, sediment and air contaminant (Krishnamurthy and Wilkens, 1994). Though chromium can exist in oxidation states ranging from 2 to 6, only chromium (VI) and chromium (III) are normally found in various environmental systems Table 1. The trivalent forms are relatively immobile, more stable and

much less toxic than hexavalent forms (Ross et al., 1981, Katz and Salem, 1994; Losi et al., 1994). The recommended guidelines for fresh life are 1µg/L for Cr(VI) and 8µg/L for Cr(III), for marine life 1µg/L for Cr(VI) and 50µg/L for Cr(III) and for irrigation water 8µg/L for Cr(VI) and 5µg/L for Cr(III) and for drinking water is 50µg/L for Cr(VI) (Krishnamurthy and Wilkens, 1994; Pawlisz, 1997; Zayed and Terry, 2003).

Table 1: Chromium concentration in different environmental components

| **Environmental Component** | **Concentration** |
|---|---|
| Continental crust | 80-200 mg/kg |
| Soil | 10-150 mg/kg |
| Fresh water | 0.1-6.0 mg/L |
| Sea water | 0.2-50.0 mg/L |
| Drinking water | 0.05 mg/L |
| Air Samples | 0.015-0.03 mg/m$^3$ |

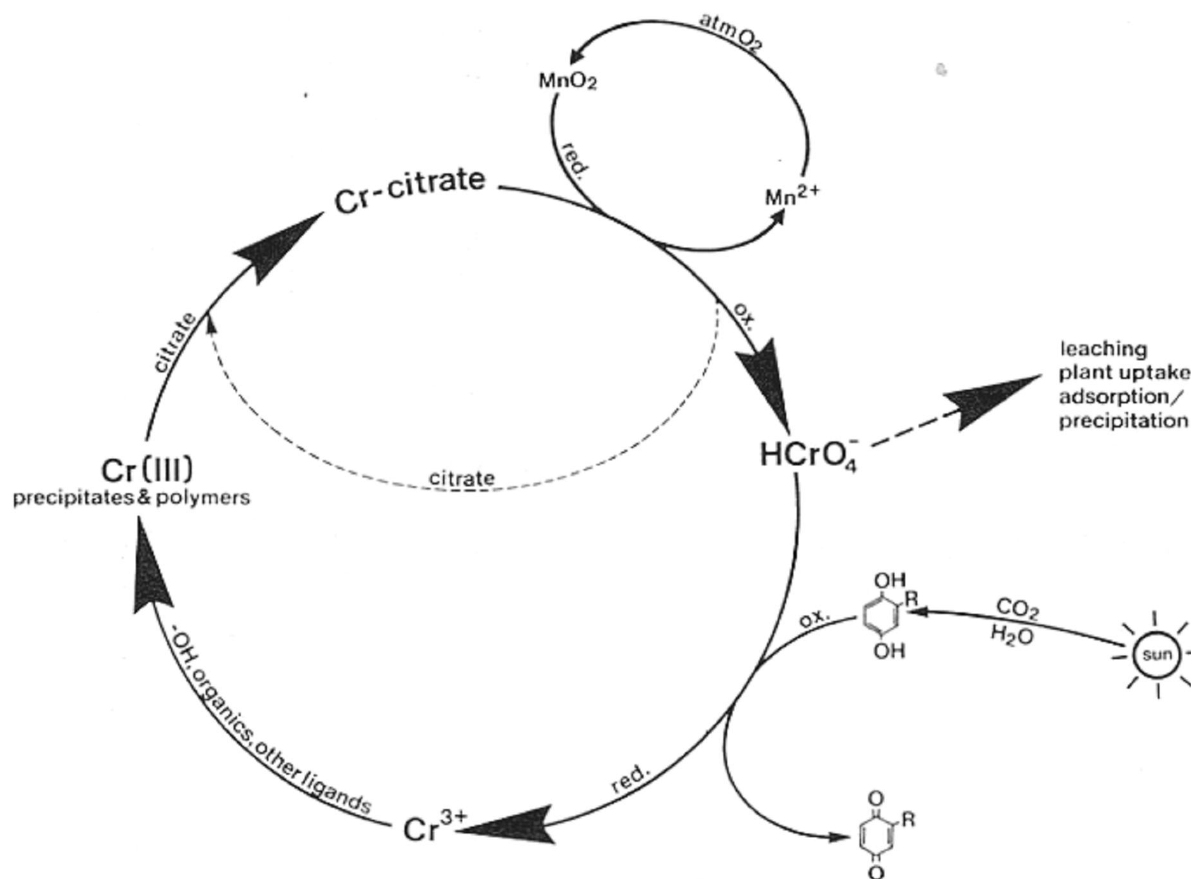
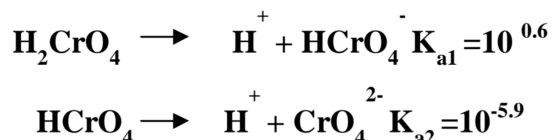


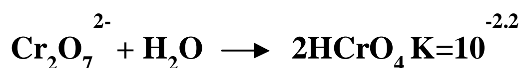

Fig. 1: Chromium cycle in natural environment (Yassi and Nieboer, 1988)

### 2.1.1 Environmental chemistry

Chromium can exist in oxidation states ranging from 0 to $6^+$. The various chemical and biological changes that chromium undergoes in the environment depend on the conditions that govern its speciation and other activities. The solubility and adsorption by soil and sediments depend on the form of chromium species. Within the ranges of redox potentials and pH commonly found in soils, chromium exists predominantly as oxyanions of Cr(III) and Cr(VI). It is a strong oxidizer and exists only in oxygenated species that are soluble and pH dependant according to the following equilibria.



$H_2CrO_4^-$ is a strong oxidizing agent and dominant species below pH 2-6 (Cotton and Wilkinson, 1980). Monohydrogen chromate, $HCrO_4$- exists between the pH values of 1 and 6. $CrO_4^{2-}$ predominates at or above pH 6. $Cr_2O_7^{-2}$ dichromate ion is formed by dimerization of $HCrO_4$ ion at Cr(VI) concentrations above $10^{-2}$ M (Losi et al., 1994).



Existence of dichromate ion is unlikely in the biological systems as typical chromium concentrations in nature are considerably lower than $10^{-2}$ M, especially at physiological pH 7. Trivalent chromium Cr(III) is the more stable form. Due to its lower affinity for oxide and hydroxide ions, it is known to form numerous complexes with both organic and inorganic ligands. Due to chemical inertness, complex species of Cr(III) tend to be more stable in solution and can be isolated. The main aqueous Cr(III) species include $Cr(OH)^{2+}$, $Cr(OH)_3$ and $Cr(OH)_4^-$ (Eary and Rai, 1987). The Cr(III) species predominate at pH < 3.6 (Francoise and Bourg, 1991), whereas $Cr(OH)_4^-$ predominates at the pH >11.5 (Rai et al., 1987). At a slightly acidic to alkaline pH, ionic Cr(III) species precipitates as amorphous $Cr(OH)_3$ (Francoise and Bourg, 1991). Cr(III) can also be chelated by organic molecules that are adsorbed to mineral surfaces (James and Bartlett, 1983). In contrast, Cr(VI) compounds $CrO_4^{2-}$, $HCrO_4^-$, $Cr_2O_7^{2-}$ are very mobile in surface sediments because they are not strongly adsorbed to soils.

Both oxidation and reduction of Cr(VI) can occur in geologic and aquatic environments (Fig. 1). The oxidation and reduction of chromium in soils depends on soil structure and on the redox conditions of the soil (Kozuh et al., 2000). Studies conducted to investigate the effect of adsorption of chromate and

Cr(VI) on the clay sand mixture showed that clay was a suitable adsorbent for chromate due to its high cation exchange capacity (CEC) and strong binding capability (Ajmal et al., 1984).

### 2.1.2 Nutritional and toxic effect of chromium

Various metals are responsible for many biochemicals, immunological and physiological essential activities of the body as micronutrients but some of these can give rise to disordered functions resulting in increased susceptibility to infections, a variety of hypersensitivity reactions and neoplasia (Shrivastava et al., 2003). The reason for such toxicity arises from the possibility of free diffusion of Cr(VI) across the cell membrane and its strong oxidative potential (Turpeinen et al., 2004). Chromium is an essential trace element for living organism. Nutritionally, Cr(III) is an essential component of a balanced human and animal diet for preventing adverse effects in the metabolism of glucose and lipids (eg., impaired glucose tolerance, elevated fasting insulin, elevated cholesterol and triglycerides, and hypoglycemic symptoms (Anderson, 1995). Chromium (III) in small amounts is an important nutrient needed by the body, swallowing large amounts of Cr(III) may also cause health problems eg., lung cancer (Zhitkovich et al., 1996; Costa, 2003). Besides this, Cr(III) has been suggested as an element which can stabilize the tertiary structure of proteins and conformation of the cell RNA and DNA (Zetic et al., 2001; Marqués et al., 2000). Cr(VI) compounds have been considered to be group 'A' human carcinogen (Bai and Abraham, 2001) and are extremely toxic which may cause death to animals and humans if ingested in large doses. Routes of human exposure to Cr compounds include ingestion of food and water, inhalation of airborne particulates and contact with numerous manufactured items containing Cr compounds. Cr(VI) is highly toxic to all forms of living organisms and is mutagenic in bacteria (Losi et al., 1994a). Cr(VI) is very toxic by dermal and inhalation route and causes lung cancer, nasal irritation, nasal ulcer, lung carcinoma, hypersensitivity reactions and contact dermatitis (Gibb et al., 2000 a,b). The mechanism of the Cr(VI) induced cytotoxicity is not entirely understood. A series of *in vitro* and *in vivo* studies (Bagchi et al., 2001) have demonstrated that Cr(VI) induces an oxidative stress through enhanced production of reactive oxygen species (ROS) leading to genomic DNA damage and oxidative deterioration of lipids and proteins. Cr(VI) would bind to cellular materials and deter their normal physiological functions (Pesti et al., 2000; Cervantes et al., 2001) (Fig. 2). Under normal physiological conditions, Cr(VI) reacts spontaneously with the intracellular reductants (e.g. ascorbate and glutathione) to generate the short-lived intermediates Cr(V) and/or Cr(IV), free radicals and the end product Cr(III) (Costa, 2003; Xu et al., 2004) (Fig. 2). The National Research Council (NRC) has identified an estimated safe and adequate daily dietary intake (ESADDI) for chromium of 50-200 μg/day (NRC, 1989), corresponding to 0.71-2.9 μg/kg-day for a 70 kg adult. The Food and Drug

Administration (FDA) has selected a Reference Daily Intake for chromium of 120 µg/d (U.S. DHHS, 1995). The guideline value of total chromium as an inorganic constituent of health significance is 0.05 mg/L. This value was recommended internationally as metal concentration in drinking-water which would not adversely influence human health (Silver et al., 2001).

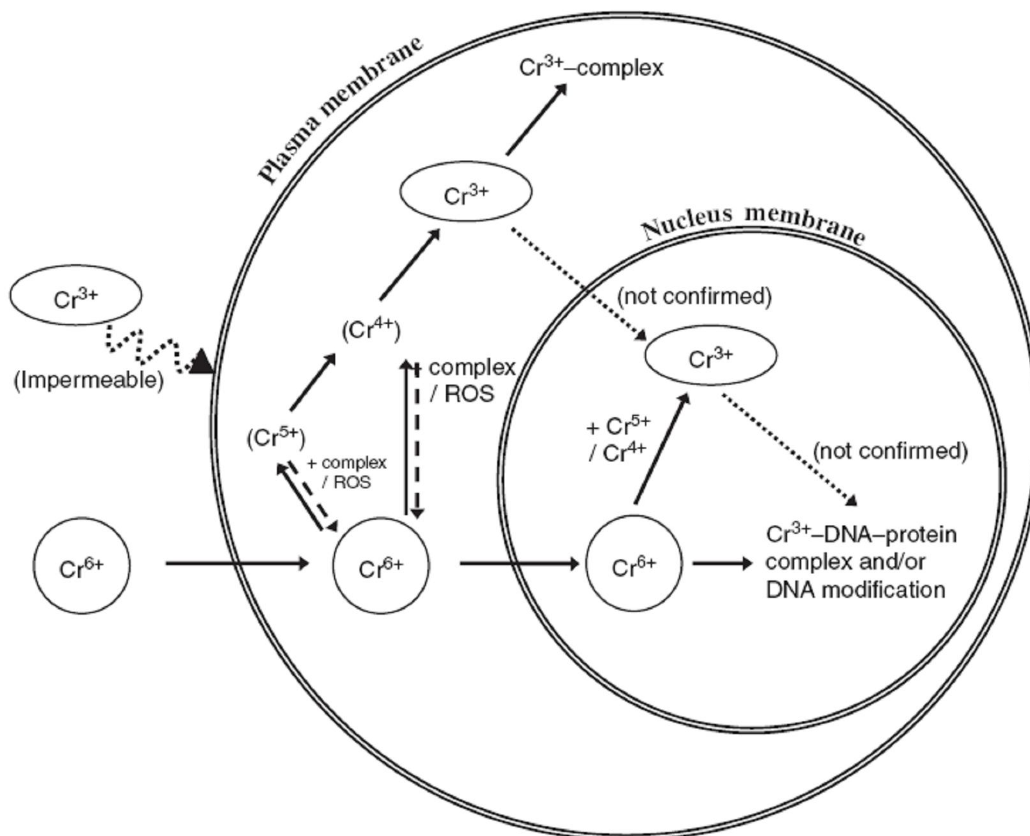



Fig. 2: Schematic diagram of toxicity and mutagenicity of Cr(VI) (modified from Vincent, 1994).

### 2.1.3 Chromium use in industries

Chromium compounds are widely used in electroplating, metal finishing, magnetic tapes, pigments, leather tanning, wood protection, chemical manufacturing, brass, electrical and electronic equipment, catalysis (Kimbrough et al., 1999). Contaminants from industrial wastewater rich in heavy metal ions remain an important environmental issue. Although control technologies have been applied to many industrial and municipal sources the total quantity of these agents released to the environment remains staggering. The most important application of chromium is in the production of steel. High-carbon and other grades of ferro-chromium alloys are added to steel to improve mechanical properties, increase hardening and enhance corrosion resistance. Chromium (II) chloride is used as reducing agent, as a catalyst in organic reactions and in chromium plating of metals. As a reducing agent, it is used to reduce alpha haloketones to parent ketones, epoxides to olefins and aromatic aldehydes to

corresponding alcohols (Patnaik, 2003). Cr(III) chloride is used for chromium plating, as textile mordant, in tanning, as a waterproofing agent and as catalyst for polymerization of olefins. Cr(III) sulfate is used as the electrolyte for obtaining pure chromium metal. It is used for chromium plating of other metals for protective and decorative purposes. Other important applications of this compound are as a mordant in the 14 textile industry, in leather tanning, to dissolve gelatin, to impart green color to paints, varnishes, inks and ceramic glazes and as a catalyst. Cr(III) oxide is used as pigment or coloring green on glass and fabrics. It is also used in metallurgy, as a component of refractory bricks, abrasives and ceramics and to prepare other chromium salts. Cr(III) fluoride is used in printing and dyeing woolens, mothproofing woolen materials, metal polishing and coloring marbles. Cr(III) hydroxide trihydrate is used as green pigment, as mordant, as a tanning agent and as a catalyst (Patnaik, 2003). Cr(VI) oxide is used for chromium plating, copper stripping, as an oxidizing agent for conversion of secondary alcohols into ketones, as a corrosion inhibitor, in purification of oil and in chromic mixtures for cleaning laboratory glassware (Patnaik, 2003).

The metallurgical, refractory and chemical industries are the fundamental users of chromium. In the metallurgical industry, chromium is used to produce stainless steels, alloy cast irons, nonferrous alloys, and other miscellaneous materials. Ferrochromiums are the main intermediates used by the metallurgical industry. Typical weight percent of chromium in stainless steel and chromium alloys ranges from 11.5% to 30%. In the refractory industry, chromium is a component in chrome and chrome-magnesite, magnesite-chrome bricks, and granular chrome-bearing and granular chromite, which are used as linings for high temperature industrial furnaces. In the chemical industry, chromium is used primarily in pigments, both Cr(III) and Cr(VI); metal finishing, Cr(VI); leather tanning, Cr(III); and wood preservatives, Cr(VI). Smaller amounts are used in drilling muds, water treatment as rust and corrosion inhibitors, chemical manufacturing, textiles, toners for copying machines, magnetic tapes, and as catalysts (CMR 1988; EPA 1984a; IARC 1990; USDI 1988a). In 1988, the U.S. chemical and metallurgical industries accounted for 83.9% and the refractory industry for 16.1% of the total domestic consumption of chromite (USDI 1988a). Chromium is used to make steel and other alloys, for chrome plating, and as an additive to limit corrosion. Named for its colored compounds, chromium has also been used to make dyes and pigments for paints, and to make bricks in furnaces, tan leather and preserve wood.

### 2.1.4 Problems of chromium contamination

In recent years, contamination of the environment by chromium, especially hexavalent chromium, has become a major area of concern. Chromium is used on a large scale in many different industries, including metallurgical, electroplating, production of paints and pigments, tanning, wood preservation, Cr chemicals production, and pulp and paper production. Often wastes from such industries (e.g., sludge, fly ash, slag, etc.) are used as a fill material at numerous locations to reclaim marshlands, for tank dikes, and for backfill at sites following demolition (Salunkhe et al., 1998). At many such sites, leaching and seepage of Cr(VI) from the soils into the groundwater poses a considerable health hazard. The tanning industry is an especially large contributor of Cr pollution to water resources; Shekar et al., (1998) estimated that in India alone about 2000 to 3200 tones of elemental Cr escape into the environment annually from the tanning industries, with a Cr concentration ranging between 2000 and 5000 mg/L in the effluent compared to the recommended permissible limit of 2 mg/L. Tannery wastewater is considered as difficult to treat wastewater since tanning process uses a series of chemicals such as surfactants, acid and metal organic dyes, natural or synthetic tanning agents and sulphonated oils and salts to transform animal hides into leather. Thus, the tanning wastewater has high chemical oxygen demand (COD), colour and chromium etc.

## 2.2 Tannery Industry

Tanning, in particular chrome leather production, is characterized by the production of highly polluted waste water, solid wastes and odour. The primary environmental issue facing tannery operations is the treatment of the toxic chromium rich waste in its wastewaters and sludge. Wastewater treatment systems must ensure high levels of metal removal, whilst sludge disposal is a major issue due to the limited availability and high cost of suitable disposal sites. In India, leather tanning is an important industry. Large volumes of tannery effluents containing chromium salts and other pollutants are being discharged to open fields, cultivable land, river streams and water bodies causing large scale pollution of soil, water and ecotoxicological risks. The current technology for effective chromium recovery and detoxification are practiced in modern units, but still many of the older and smaller units resort to conventional effluent treatment (Alexander et al., 1992; Covington and Alexander, 1993; James and McDougall, 1996).

### 2.2.1 Sources of contaminants to tannery effluent

The pollution load of an effluent is due to the presence of organic and inorganic materials in it. Biological method is reported to be very much effective in reduction of the pollution load of an effluent. Sastry 1986 and Fahim 2006 discussed the literature review on characteristics and treatment of wastewater from tanneries. In tanning industries, chromium is extensively used to convert raw skin/hide to leather and a large amount of chromium-laden effluent is discharged into the environment. Chromium in its trivalent form is an essential trace element when present at the micro level, whereas the same when present in excess is proven to be a potential soil, surfacewater, and air contaminant under specific condition (Rao et al., 2002). Basu and Chakraborty (1989) characterised tannery effluent for pH, total solids, suspended solids, BOD and COD and discussed various methods of treatment of tannery wastewater containing chlorides, sulphides and chromium. Contaminants in the effluent from tannery originate in several ways. The most obvious source of pollution is the drag-out of various processing baths into subsequent rinses, the amount of pollutants contributed by drag-out is a function of several factors such as the design of the racks or barrels carrying the parts to be plated, the shape of the parts, plating procedures and several interrelated parameters of the process solution, including concentration of toxic chemicals, temperature, viscosity and surface tension. With conventional rinsing techniques, drag-out losses from process solutions result in large volume of rinse water contaminated with relatively dilute concentration of cyanide and metals (Cushnie, 1985).

Leather tanning is one of the oldest professions in the world and involves the processing of hides and skins of animals for use in numerous products such as upper leather for shoes, clothing, furniture and automotive leather for upholstery in motor vehicles (Iqbal et al., 1998). Tanneries produce different types of leather products depending on customer needs. Tanning means converting the rawhide or skin, a highly putrescible material, into leather, a stable material. In this process the very sensitive hydrogen bonds of skin proteins are replaced by chemical bonds with tanning agent like chromium, aluminum or other mineral salts, vegetable or synthetic tanning agents to stabilize the material and to protect it against microbial attack. Leather production belongs to the natural products industry. The whole process involves a sequence of complex chemical reaction and mechanical processes.

### 2.2.2 Production of leather

Leather tanning is the process of converting raw hides into leather and can be divided into three different processes which are briefly described below.

**1. Beamhouse operations**

In the beamhouse hides are prepared for tanning by removing hair roots, pigment and other protein substances.

**2. Tanning process**

The hides are tanned to prevent them from being damaged by bacteria. Hides can be tanned in a number of different ways by using chrome tanning agents added as a powder or a liquid to produce wet blues, or by adding vegetable tannins to produce wet whites. Wet blues are not leather, but hides that have been tanned with chromium sulphate ($CrSO_4$). They are merely a stable form of the hide. The chrome gives the leather an initial blue grey colour. The tanned hides can be stored for long periods and are also more easily transported without fear of being damaged by bacteria (www.tanschool.co.za). Wet blues are recognized as an internationally traded commodity. The chrome tanning method is widely applied for the preparation of finished leather (Iqbal et al., 1998). Wet whites on the other hand are skins and hides that are tanned using vegetable tanning material such as aldehydes. Leather that is tanned using vegetable tannins has different properties and is usually much firmer. These hides will go through the dye house operations to convert the wet blue and wet whites into a product that has leather-like properties. The process in the dye house can be compared to the retan process used to produce crust leather.

**3. Finishing process**

Leather finishing is the final process that is carried out in a tannery to make the leather look better and stay looking good for longer while withstanding weather and wear. Leather finishing in very simple terms is the application of paint to the leather surface (ISTT, 2004).

**4. The retan process followed at the tanning facility**

Hides are retanned to level out the structure of a hide, by filling the loose and empty parts in the bellies and necks, to improve the cutting yield. Retanning procedures the highest quality. Wachsman (1999)

considers this is where retanning agents with real tanning properties are necessary and where either chrome salts, syntans, glururaldehyde, vegetable extract, polymers, resins aldehydes and metals provide these properties to the wet blues and wet whites used.

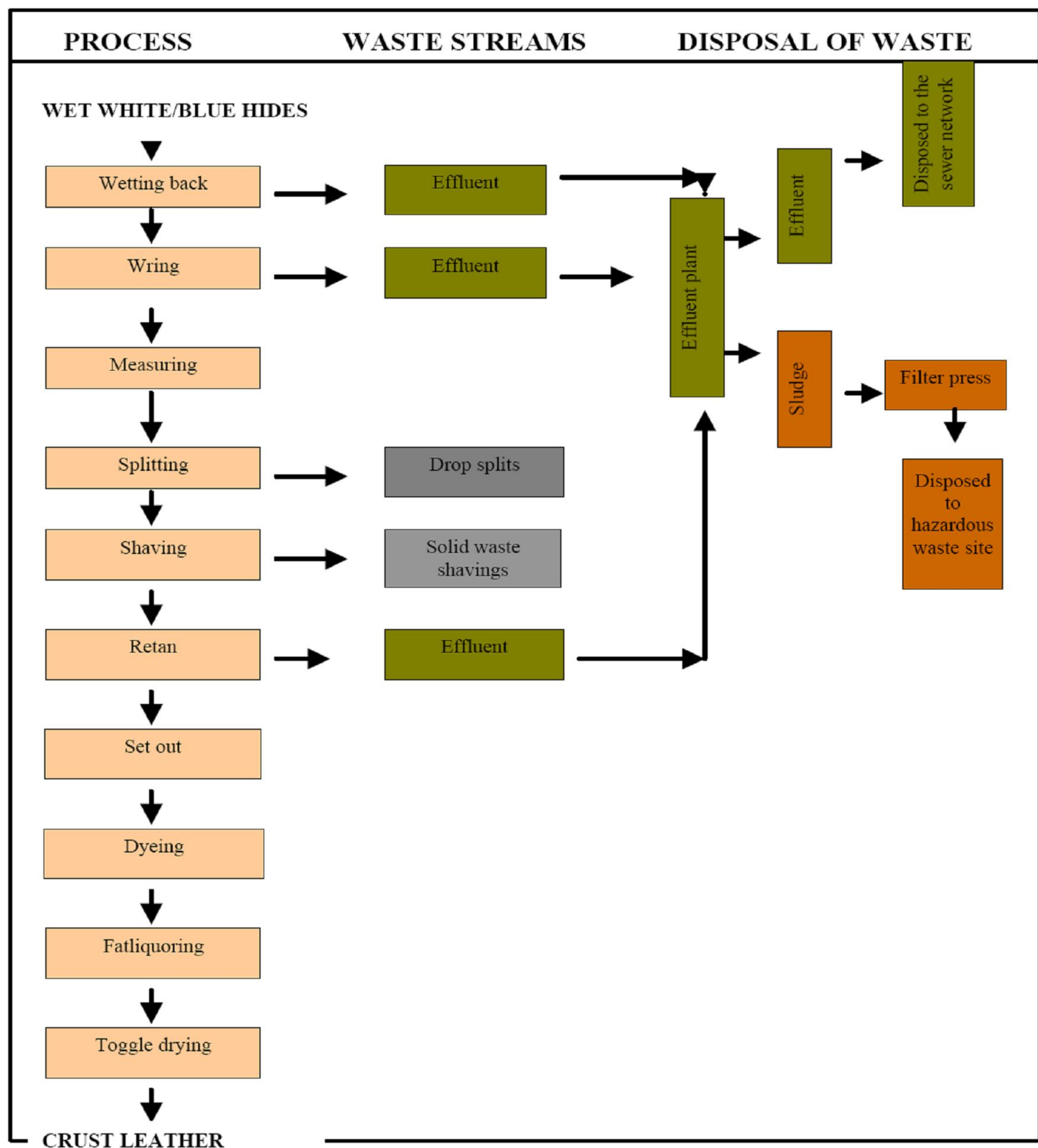



Fig. 3: Waste streams produced during the Retan process (Iqbal et al., 1998).

### 2.2.3 Waste streams generated in the retan process

During the retan process large volumes of raw materials are used in the manufacturing of crust leather. Raw materials used are chemicals, which include dyes, retanning materials, formic acids, water and the wet white or blue hides. Most of the chemicals used to prepare the hides for retanning find their way back into the environment in the form of wastes including (Iqbal et al., 1998)

1. Atmospheric waste causing air pollution
2. Liquid waste
3. Solid waste

Fig. 3 explains the process and identifies the type of waste streams generated in the retan process at the tanning facility as well as the disposal options currently adopted. Waste streams produced during the tanning process include effluent and solid waste. The effluent water originates from all the areas where water is used in the tanning process, which includes the wetting back water, water from the wringer and the water dropped during the retanning process. The partial treatment of the water results in the production of the tanning sludge and effluent that is disposed to the Municipal sewer network. Solid waste streams originate from the splitting of the hides and as a result of the effluent treatment plant. The drop splits are sold to another company for reuse, the shavings from the shaver are disposed of as general waste and the tanning sludge are disposed of to a hazardous landfill site. Although a brief description is provided for each of the wastes produced, the main focus of this dissertation is on the tanning sludge produced during the treatment of the effluent.

**1. Atmospheric waste causing air pollution**

A potential source of air pollution (atmospheric waste) may originate from the boiler. The boiler is used to produce hot water for the retan process. Possible contaminants include the release of gases. However, due to the size of the boiler used. The boiler uses less than 10 tonnes of sabufuel (a sort of paraffin) per hour and it does not use coal as an energy source. Therefore, it is expected not to have a significant impact on the environment.

**2. Liquid waste**

Tanneries are major consumers of water. The water in the wet processes and operations is used as a chemical carrier to facilitate all chemical reactions involved in leather processing. After completion of the process and operation, the water leaves the system as waste water in the same quantity as it is added to the system (Iqbal et al., 1998). Liquid waste or waste water includes all process water produced

throughout the retan process as described in Fig. 3. The waste water originates from the various stages including the wetting back, wringing and retan processes. The water volume requirements depend on each of the specific retanning processes and the specific product that needs to be manufactured at that specific time.

Effluent originating during the various stages of the process is disposed to an effluent treatment plant for partial treatment, before it is disposed of into the municipality's sewer network for further treatment. The effluent disposed of must be treated in order to comply with the local authorities' standards for disposal of industrial effluent. The water is treated through the addition of treatment chemicals (polymers, flocculants coagulants and lime), followed by dissolved air floatation of the solids. The Dissolved Air Flotation (DAF) provides successful removal of the suspended solids (SS), oil and greases, as well as any heavy metals such as chromium (Cr), Manganese (Mn), Aluminium (Al) and Iron (Fe). Tannery effluent is characterized by a high chemical oxygen demand (COD) and high salt content measured as total dissolved solids/salts (TDS). High chloride (Cl), Sodium (Na), Sulphate ($SO_4$) concentrations are also present in the waste stream due to their presence in the raw materials and this contributes to the high salinity levels of the effluent stream. It also contains a high level of suspended solids and chrome. According to Iqbal et al., (1998) this is characteristic of tannery waste water. The chrome (Cr) will only be present when wet blues are processed and chrome tanning salts are used. The chemical oxygen demand (COD) represents the oxygen consumption for chemical oxidation of organic material under strongly acid conditions. It only provides an indication of the potential oxygen depletion that may occur from discharging organic material into surface waters (Verheijen et al., 1996).

The suspended solids (SS) are insoluble organic and inorganic particles present in the waste water that originates from the hides and chemicals used. Suspended solids (SS) are mainly material that are too small to be collected as solid waste. Discharge of high concentrations of SS increases the turbidity of water and causes a long term demand for oxygen because of the slow hydrolysis rate of the organic fraction of the material. It has been estimated that using traditional tanning methods chromium salts which are not fixed to the collagen during the tanning process, are discharged as salinity and total dissolved salts (TDS). The chromium complex is relatively stable and slightly biologically available and therefore the chromium should not be oxidised to hexavalent chromium (Cr(VI)) during the retan process. The Chemical Oxygen Demand (COD) and Suspended Solids (SS) are the only constituents that are removed from the effluent through the partial treatment process, as this treatment does not

remove any salts in the effluent. The operation of the effluent plant will be discussed in the next section since the tanning sludge is generated here as a result of the removal of heavy metals, Chemical Oxygen Demand (COD) and suspended solids.

**3. Solid waste**

The solid waste produced includes the tanning sludge produced at the effluent plant, shaving buffings, raw material packaging (especially plastics) and general office waste. The majority of general solid waste is separated at source and recycled where possible. Empty drums, containers, plastics, white papers and carton boxes are recycled by outside contractors. All the effluent produced in the tanning facility reports to the effluent plant for partial treatment, to ensure that the water complies with the standards set by the Local Authority. The Dissolved Air Flotation (DAF) is designed to partially remove the Chemical Oxygen Demand (COD) and the Suspended Solids (SS). Flocculants and coagulants used to remove SS and Chemical Oxygen Demand (COD) generate the tanning sludge in this process. The Dissolved Air Flotation (DAF) plant functions on the principle of flotation. Flotation is used for the removing of suspended solids from mixed effluent. Air is dissolved into the incoming effluent under pressure. When this pressure is subsequently lowered in the treatment vessel, small air bubbles are released, carrying the suspended solids to the surface. Lime, ferrous sulphate and polyelectrolyte are usually used in order to thicken tanning sludge for easier handling (Mozes, 1995). In order to reduce the tanning sludge volumes for disposal, a filter press was introduced to dewater the tanning sludge.

## 2.3 Treatment technologies for chromium containing wastewaters

Several conventional treatment technologies have been developed to remove chromium from water and wastewater. Common methods include chemical precipitation (Atkinson et al., 1998), ion exchange (Tiravanti et al., 1997; Rengaraj et al., 2001; Rengaraj et al., 2002; Rengaraj et al., 2003; Petruzzelli et al., 1995), membrane separation (Kozlowski and Walkowiak, 2002; Shaalan, 2001), ultrafiltration (Ghosh and Bhattacharya, 2006), flotation (Matis and Mavros, 1991), electrocoagulation (Parga et al., 2005), solvent extraction (Salazar et al., 1992), sedimentation (Song et al., 2000), precipitation (Roundhill and Koch, 2002), electrochemical precipitation (Roundhill and Koch, 2002), soil flushing/washing (Roundhill and Koch, 2002), electrokinetic extraction (Roundhill and Koch, 2002), phytoremediation (Roundhill and Koch, 2002), reduction (Chen and Hao, 1998), reverse osmosis (Ozaki et al., 2002), dialysis/electrodialysis (Mohammadi et al., 2005), adsorption/filtration (Mohan et al., 2005; Mohan and Pittman Jr, 2006; Gupta et al., 1997; 1999; 2001; Babel and Kurniawan, 2003),

evaporation, cementation, dilution, air stripping, steam stripping, flocculation, and chelation (Tels, 1987; Rich and Cherry, 1987).

### 2.3.1 Chemical precipitation

Chemical precipitation has traditionally been the most used method. Precipitation of metals is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers. However, the large amount of sludge containing toxic compounds produced during the process is one of the main disadvantage. In chemical precipitation, chemicals such as ferrous sulfide, lime, caustic and sodium carbonate are commonly used which shows high efficiency in removing the bulk of metal ions in solutions at high or moderate concentrations. Nevertheless, a large amount of sludge is produced especially when Lime is used, disposal of which is another problem.

### 2.3.2 Reverse osmosis

It is a process in which heavy metals are separated by a semi-permeable membrane at a pressure greater than osmotic pressure caused by the dissolved solids in wastewater. The disadvantage of this method is that it is expensive (Ozaki et al., 2002).

### 2.3.3 Ion exchange

Cell wall of microbes contains polysaccharide salts and mineral ions of the polysaccharides. For example, the alginate of marine algae occurs as salts of $K^+$, $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ ions. These ions can exchange with counter ions such as $Co^{2+}$, $Cu^{2+}$, $Cu^{2+}$, $Cd^{2+}$ and $Zn^{2+}$ resulting in the biosorptive uptake of heavy metals (Kuyucak and Volesky, 1989).

### 2.3.4 Electrodialysis

In this process, the ionic components (heavy metals) are separated through the use of semi-permeable ion selective membranes. Application of an electrical potential between the two electrodes causes a migration of cations and anions towards respective electrodes. Because of the alternate spacing of cation and anion permeable membranes, cells of concentrated and dilute salts are formed. The disadvantage is the formation of metal hydroxides, which clog the membrane (Mohammadi et al., 2005).

### 2.3.5 Ultrafiltration

They are pressure driven membrane operations that use porous membranes for the removal of heavy metals. The main disadvantage of this process is the generation of sludge. Metal ions from dilute solutions are exchanged with ions held by electrostatic forces on the exchange resin. The disadvantage includes high cost and partial removal of certain ions (Ghosh and Bhattacharya, 2006).

## 2.4 Conventional treatment and disadvantages

The classical or conventional techniques like precipitation, ion exchange etc. gives rise to several problems such as unpredictable metal ions removal and generation of toxic sludge which are often difficult to dewater and require extreme caution in their disposal (Xia and Liyuan, 2002). Besides that, most of these methods also present some limitations whereby they are only economically viable at high or moderate concentrations of metals but not at low concentrations (Addour et al., 1999), meaning diluted solutions containing 1 to 100 mg/L of dissolved metal(s) (Cossich et al., 2002). Another disadvantage of using these techniques for heavy-metal removal is high cost due to extensive reagent or energy requirements (Xia and Liyuan, 2002). For these reasons, particular attention has been paid to the use of biological systems as a promising alternative method for heavy metal removal from industrial wastewaters. Many microorganisms are able to remove heavy metals from wastewaters but there is no agreement on the action mechanism of this phenomenon, which appears to be microorganism dependent.

## 2.5 Biosorption

Accumulation of dissolved substances at interfaces or between phases is defined as adsorption. Adsorbate is the dissolved substance adsorbed on solid phase that is called an adsorbent. The adsorption of dissolved substances on a microbial cell surface has been defined as biosorption.

Biosorption is one of the emerging biological methods having several advantages over the conventional method among which are: the process does not produce chemical sludge, hence nonpolluting, it is easy to operate and very efficient for removal of pollutants from dilute solutions. A major advantage of biosorption is that it can be used in situ and with proper design, it may not need any industrial process operations and can be integrated with many systems (Tewari et al., 2005). Despite the advantages mentioned earlier, biosorption of heavy metals from aqueous solutions also poses several limitations which include the fact that large-scale production of effective biosorbent materials has not been established and that this new technology has only been tested for limited practical applications (Feng

and Aldrich, 2004). Biosorption refers to many modes of nonactive metal uptake by biomass which may even be dead. Metal sequestration by different parts of the cell can occur via complexation, coordination, chelation, ion exchange, adsorption or inorganic microprecipitation. Any one or a combination of the metal-binding mechanisms may be functional to various degrees in immobilizing one or more metallic species on the biosorbent (Volesky, 1987). It is often reported that biosorptive metal uptake occurs rapidly, efficiently and sometimes as a complex phenomenon (Yong et al., 2002).

Various studies have been carried out using different types of biological materials as biosorbent of heavy-metals (Table 2). Various chemical groups have been proposed to contribute to biosorption metal binding by algae, bacteria, fungi, etc. These include hydroxyl, carbonyl, carboxyl, sulfhydryl, thoether, sulfonate, amine, imine, amide, imidazol, phosphonate and phosphodiester groups (Schiewer and Volesky, 2000). Biosorption of chromium from aqueous solutions was investigated by passive binding to nonliving biomass (Davis et al., 2003; Kapoor and Viraraghavan, 1995; Sag 2001; Gavrilescu, 2004; Volesky and Holan, 1995; Schiewer and Volesky, 2000). Bioremediation could be considered as an eco-friendly complementary device to the existing high cost technologies (Bai and Abraham, 2002).

Although living microbial populations are effective sorbents for toxic heavy-metals, available processing systems are cumbersome. Alternatively, non-living cells can also be used as biosorbent. Furthermore, it has been reported that the biosorptive capacity of non-living cells may be greater, equivalent to or less than that of living cells (Kapoor and Viraraghavan, 1995).

**Factors affecting biosorption**

The following factors affect the biosorption process:

1. Temperature seems to have no influence on the biosorption performances in the range of 20-35°C (Aksu et al., 1997).
2. pH seems to be the most important parameter in the biosorptive process as it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions (Galun et al., 1987)
3. Biomass concentration in solution seems to influence the specific uptake, for lower values of biomass concentrations there is an increase in the specific uptake (Fourest and Roux, 1992).
4. Biosorption is mainly used to treat wastewater where more than one type of metal ions would be present where in the removal of one metal ion may be influenced by the presence of other metal ions (Tsezos and Volesky, 1982; Sakaguchi and Nakajima, 1991).

## 2.6 Biosorbent material

Strong biosorbent behavior of certain micro-organisms towards metallic ions is a function of the chemical make up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties. Recent biosorption experiments have focused attention on waste materials, which are by-products or waste materials from large-scale industrial operations such as waste mycelia from fermentation processes, olive mill solid residues (Pagnanelli et al., 2002), biosolids (Norton et al., 2003) and aquatic macrophytes (Keskinkan et al., 2003) etc. Availability is a major factor to be taken into account to select biomass for clean-up purposes. The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material; bacteria, fungi, algae, seaweeds, molds, yeasts, plant biomass, agriculture waste and industrial waste biomass among other kinds of biomass have been tested for chromium biosorption.

## 2.7 Chromium removal by bacteria

A wide range of microorganisms exhibit an exceptional capacity to detoxify Cr(VI) by converting it in to less soluble and much less toxic Cr(III) (Michel et al., 2001) such as *Enterobacter* sp. (Wang et al., 1990), *Escherichia coli* (Shen and Wang, 1993), *Bacillus* sp. (Compos et al., 1995), *Pseudomonas* sp. (Oh and Choi, 1997) *Microbacterium* (Pattanapipitpaisal et al., 2001), *Desulfovibrio* (Michel et al., 2001), *Streptomyces griseus* (Ashwini et al., 2009) and several other bacteria (Losi et al., 1994a; Fude et al., 1994). Most of them have been isolated from tannery sludge, industrial sewage, evaporation ponds and discharge water (Losi and Frankenberger, 1994). Gram-positive bacteria, capable of reducing Cr(VI) as a terminal electron acceptor and with a relatively high level of resistance to chromate were isolated from tannery effluents (Shakoori et al., 1999). A chromate resistant strain of the bacterium *Serratia marcescens* was isolated from tannery effluent, which was able to reduce Cr(VI) to Cr(III), and about 80% of chromate was removed from medium (Mondaca et al., 2002). Feasibility of biotransformation of hexavalent chromium by immobilized *Bacillus coagulans* was observed in reactor with an influent hexavalent chromium concentration of 26 mg/L and retention time 24 hours (Philip et al., 1998). A number of chromium-resistant microorganisms such as *Arthrobacter* (Chaturvedi, 1992)

and *Pseudomonas aeruginosa* (Ganguli and Tripathi, 1999) were reported for treatment of the tannery effluent. *Bacillus circulans, Bacillus megaterium* and *Bacillus coagulans* strains capable of bioaccumulation of Cr(VI) were isolated from treated tannery effluent of a common effluent plant (Srinath et al., 2002; 2003). *Arthrobacter* sp. and *Bacillus* sp. isolated from soil contaminated with tannery effluent were examined for their tolerance to hexavalent chromium Cr(VI) and their ability to reduce Cr(VI) to Cr(III) (Megharaj et al., 2003).

### 2.7.1 Transformation of chromium

Chromium is known to undergo various chemical and biological reactions in the natural system that govern speciation of the metal and in turn its environmental behavior. Important reactions include oxidation/ reduction, precipitation/ dissolution and adsorption/ desorption. Both oxidation of Cr(III) and reduction of Cr(VI) can occur in geologic and aquatic environments (Ishibashi et al., 1990; Gopalan and Veeramani, 1994).

### 2.7.2 Chemical transformation

Traditionally, physico-chemical processes are used to reduce Cr(VI). Most commonly used processes include reduction-precipitation, ion exchange and reverse osmosis. However, the costs to set up the required equipment and to operate these processes are prohibitively high for large-scale treatment (Mahajan, 1985; Bhide, et al., 1996; Beleza, et al., 2001). Hexavalent chromium is a strong oxidation agent and is readily reduced in the presence of appropriate electron doners. Fe(II) actively reduced Cr(VI) and the reaction rate was dependent on the solubility of Fe compound. The presence of organic matter enhanced Cr(VI) reduction. Low oxygen status also resulted in reduction of Cr(VI) and Cr(III) (Kamaludeen et al., 2003).

### 2.7.3 Biotransformation of chromium

Biotransformation of Cr(VI) to Cr(III) has been considered as an alternative process for treating Cr(VI) contaminated wastes (Williams and Silver, 1984; Ohtake and Silver 1994; Cervantes et al., 2001). Since the discovery of the first microbe capable of reducing Cr(VI) in the 1970s (Romanenko and Korenkov, 1977), the search for Cr(VI) reducing microorganisms (both aerobic and anaerobic) has been based on recent isolation and purification of Cr(VI) reductases from aerobic bacteria and the fact that the process involved in Cr(VI) reduction occurring under anaerobic conditions is starting to be understood, biological processes for treating chromium contaminated sites are becoming very promising (Cheung and Gu, 2007).

## 2.8 Aerobic reduction of Cr(VI)

### 2.8. 1 Cr(VI) reducing aerobes

In *Pseudomonas aeruginosa,* Cr(VI) resistance is attributed to the decreased uptake and/or enhanced efflux of Cr(VI) by the cell membrane (Bopp et al., 1983; Alvarez et al., 1999; Aguilera et al., 2004). A similar mechanism of resistance has been reported for *Alcaligenes eutrophus* CH34 (Nies and Silver, 1989; Valls et al., 2000; Vaneechoutte et al., 2004). On the other hand, investigations with *P. fluorescens* show that Cr(VI) resistance does not depend upon the capacity of the organism to reduce this chemical. Thus, Cr(VI) reduction activity was found to be same in sensitive (wild-type) and resistant (mutant) strains when assayed at sub-lethal Cr(VI) levels (Bopp and Ehrlich, 1988).

Other pseudomonads capable of reducing Cr(VI) include a close relative of *P. synxantha* and an unidentified species (Gopalan and Veeramani, 1994; McLean et al*.,* 2000). A number of bacteria in other genera, viz. *Bacillus* sp., *E. coli* ATCC 33456, *Shewanella* alga BrY-MT and a few unidentified strains have also been shown to reduce Cr(VI) (Shen and Wang, 1994; Wang and Xiao, 1995; Shakoori et al., 1999, 2000; Guha et al., 2001; Camargo et al., 2003). It is noteworthy that, unlike most reported Cr(VI) reducing aerobes, which utilized reductases soluble in the cytosol, in *P. maltophilia* O-2 and *Bacillus megaterium* TKW3, Cr(VI) reduction was associated with the membrane cell fractions (Blake II et al., 1993; Cheung and Gu, 2007).

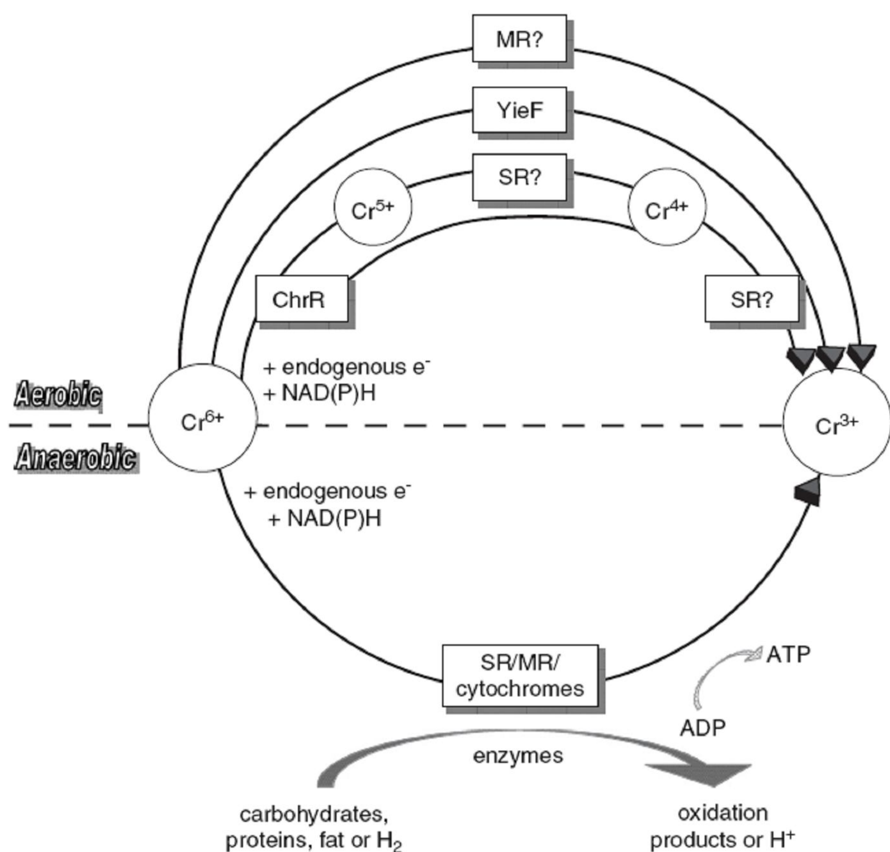



Fig. 4. Plausible mechanisms of enzymatic Cr(VI) reduction under aerobic (upper) and anaerobic (lower) conditions (modified from Wang and Shen, 1995).

### 2.8.2 Cr(VI) Reductases of aerobes

Earlier attempts have reported Cr(VI) reductase purification from pseudomonads. Ishibashi et al., (1990) partially purified a soluble Cr(VI) reductase from *P. putida* PRS2000. Suzuki et al., (1990) reported a 38-fold purification of a soluble Cr(VI) reductase from *P. ambigua* G-1. In a more recent investigation, the gene encoding this reductase was found to exhibit a high nucleotide sequence homology (58%) to a nitroreductase of *Vibrio harveyi* KCTC 2720 that was also endowed with Cr(VI)-reducing activities (Kwak et al., 2003).

Park et al., (2000) purified 600-fold a soluble Cr(VI) reductase, ChrR, from *P. Putida* MK1. The ChrR-coding gene, chrR, was identified from the genomic sequence of *P. putida* MK1, based on the known amino acid sequences of the N-terminal and internal amino acid segments of the pure enzyme (Park et al., 2002). Ackerley et al., (2004) described ChrR as a dimeric flavoprotein catalyzing the reduction of

Cr(VI) optimally at 71°C. An open reading frame, yieF, on the *E.coli* chromosome with no assigned function was found to have a high homology to chrR. This gene was cloned and the encoded protein, YieF, showed maximum reduction of Cr(VI) at 35°C (Park et al., 2002). Recently, a membrane associated Cr(VI) reductase was identified from the proteome of *B. megaterium* TKW3 detected on a two dimensional electrophoresis gel (Cheung et al., 2006).

### 2.8.3 Mechanism of aerobic Cr(VI) reduction

In the presence of oxygen, bacterial Cr(VI) reduction commonly occurs as a two- or three-step process with Cr(VI) initially reduced to the short-lived intermediates Cr(V) and/or Cr(IV) before further reduction to the thermodynamically stable end product, Cr(III). Nevertheless, it is at present unclear as to whether the reduction of Cr(VI) to Cr(V) and Cr(IV) to Cr(III) was spontaneous or enzyme mediated (Czakó-Vér et al., 1999). NADH, NADPH and electron from the endogenous reserve are implicated as electron donors in the Cr(VI) reduction process (Appenroth et al., 2000). The Cr(VI) reductase ChrR transiently reduces Cr(VI) with a one-electron shuttle to form Cr(V), followed by a two-electron transfer to generate Cr(III). Although a proportion of the Cr(V) intermediate is spontaneously reoxidized to generate ROS, its reduction through two electron transfer catalyzed by ChrR reduces the opportunity to produce harmful radicals (Ackerley, 2004). Enzyme YieF is unique in that it catalyzes the direct reduction of Cr(VI) to Cr(III) through a four electron transfer, in which three electrons are consumed in reducing Cr(VI) and the other is transferred to oxygen. Since the quantity of ROS generated by YieF in Cr(VI) reduction is minimal, it is regarded as more effective reductase than ChrR for Cr(VI) reduction (Park et al., 2002). The membrane-associated Cr(VI) reductase recently isolated from *B. megaterium* TKW3 utilized NADH as an electron donor, but the kinetics of Cr(VI) reduction is has not been characterized  (Cheung et al., 2006).

## 2.9 Anaerobic reduction of Cr(VI)

### 2.9.1 Cr(VI) reducing anaerobes

Early investigations on the biotransformation of Cr(VI) focused on the facultative anaerobes such as *Pseudomonas dechromaticans, P. chromatophila* and *Aeromonas dechromatica.* A number of chromium resistant microorganisms were subsequently isolated such as *B. cereus, B. subtilis, P. aeruginosa, P. ambigua, P. fluorescens, E.coli, Achromobacter eurydice, Micrococcus roseus, Enterobacter cloacae, Desulfovibrio desulfuricans* and *D. vulgaris* (Lovley, 1993; 1994). Sulfate-reducing bacteria (SRB) have been extensively studied for reduction of metals, including Cr(VI). For instance, Cr(VI) reduction by *D. vulgaris* was found to involve a soluble c3 cytochrome (Lovley, 1995).

In *Desulfomicrobium norvegicum*, a hydrogenase and a c-type cytochrome catalyzed Cr(VI) reduction (Chardin et al., 2002; Michel et al., 2001). *Desulfotomaculum reducens* MI-1 was capable of utilizing Cr(VI) as sole electron acceptor (Tebo and Obraztsova, 1998) this capability was only reported in another SRB consortium (Cheung and Gu, 2003). *Enterobacter cloacae* HO1 is another Cr(VI) reducing facultative anaerobe that has been extensively investigated. In this species, Cr(VI) reduction is inhibited by oxygen and the concomitant presence of other metals that inhibits Cr(VI) reduction owing to the toxicity of the metals that to the microorganism (Wang et al., 1989; Ohtake et al., 1990; Kato and Ohtake, 1991). Other reported Cr(VI) reducing anaerobes included *Microbacterium* sp. MP30 (Pattanapipitpaisal et al., 2001), *Geobacter metallireducens* (Lovley et al., 1993), *Shewanella putrefaciens* MR-1 (Myers et al., 2000), *Pantoea agglomerans* SP1 (Francis et al., 2000), *Agrobacterium radiobacter* EPS-916 (Llovera et al., 1993) and a consortium capable of simultaneously reducing Cr(VI) and degrading benzoate (Shen et al., 1996). A few species of extremophiles have been found to reduce Cr(VI). Among them are the radiation resistant *Deinococcus radiodurans* R1 (Fredrickson et al., 2000), a close relative of *Thermoanaerobacter ethanolicus* isolated from deep subsurface sediments millions of years old (Roh et al., 2002), and *Pyrobaculum islandicum* (Kashefi and Lovley, 2000) which reduced Cr(VI) at high temperatures. Several dual chemical–microbial processes based in part on the assistance of the anaerobic microbial Cr(VI) reduction have been tested for their efficiency. A fed batch bioreactor and a semi permeable dialysis bag containing a culture of *E. cloacae* HO1 were found to be effective in Cr(VI) removal (Komori et al., 1990 a, b; Fujie et al., 1996). However, cells of *D. desulfuricans* immobilized in polyacrylamide gel were less efficient than suspended cells in Cr(VI) reduction, which suggested that the gel might have limited the availability of Cr(VI) (Tucker et al., 1998).

### 2.9.2 Cr(VI) reductases of anaerobes

Both soluble and membrane-associated enzymes were found to mediate the process of Cr(VI) reduction under anaerobic conditions. Unlike the Cr(VI) reductases isolated from aerobes, the Cr(VI) reducing activities of anaerobes are associated with their electron transfer systems ubiquitously catalyzing the electron shuttle along the respiratory chains (Wang and Shen, 1995). The cytochrome families (e.g., cytochrome b and cytochrome c) were frequently shown to be involved in the enzymatic anaerobic Cr(VI) reduction. The widespread occurrence of anaerobes possessing Cr(VI) reducing activities offers great potential for in situ bioremediation of Cr(VI) contaminated sediments; which would only require the supplementation of nutrients and the modulation of physical conditions to facilitate the reaction (Turick et al., 1996).

### 2.9.3 Mechanism of anaerobic Cr(VI) reduction

Natural metabolites of anaerobes such as $H_2S$ produced by SRB, are effective chemical Cr(VI) reductants under anoxic environment (Smillie et al., 1981; Fude et al., 1994; Wielinga et al., 2001). In the past, the anaerobic reduction of Cr(VI) was considered as a fortuitous process that provides no energy for microbial growth (Wang and Shen, 1995). More recently, however, a SRB isolate was found to use for its growth the energy generated during anaerobic Cr(VI) reduction (Tebo and Obraztsova, 1998). In the absence of oxygen, Cr(VI) can serve as a terminal electron acceptor in the respiratory chain for a large array of electron donors, including carbohydrates, proteins, fats, hydrogen, NAD(P)H and endogenous electron reserves (Wang, 2000).

## 2.10 Chromium removal by fungi

Fungal biomass is capable of treating metal contaminated effluents (Kapoor and Viraraghavan, 1995; Sag, 2001). The source of raw material as a biosorbent can be a waste material produced as a by-product of large-scale fermentation processes. Inactivated biomass of filamentous fungi *Rhizopus arrhizus* was reported to be quite effective in removal of heavy metals like strontium, cadmium, copper, zinc, nickel, uranium, chromium and lead (Nourbaksh et al., 1994). *Mucor meihi,* a waste from fermentation industry was found to be effective biosorbent for removal of hexavalent chromium from tanning industrial effluents (Tobin and Roux, 1998).

Fungal biomass offers the advantages of having a high percentage of cell wall material with excellent metal binding properties particularly the genera *Rhizopus, Aspergillus*, and *Saccharomyces* (Paknikar et al., 1993). Removal of chromium (VI) from aqueous solutions was carried out in batch experiments using dead biomass of four fungal strain *Aspergillus niger* NICM-501, *Aspergillus oryzae* NICM-637, *Rhizopus arrhizus* NICM-997 and *Rhizopus nigricans* NICM-880. Out of these four *Rhizopus nigricans* and *Rhizopus arrhizus* possess high Cr(VI) specific uptake capacities (Bai and Abraham, 1998). Both living and dead fungal cells possess a remarkable ability for toxic and precious metals uptake from water/wastewater. The fungi and yeast can be grown in substantial amounts using unsophisticated fermentation techniques and inexpensive growth media. Various types of fungal biomass have been used for the removal and recovery of tri and hexavalent chromium from water/wastewater. These include *R. arrhizus* (Prakasham et al., 1999), *R. arrhizus* (Sag and Kutsal, 1996), *Penicillium chrysogenum* (Deng and Ting, 2005), dead fungal biomass (Chandrasekhar et al., 1998), *Lentinus sajorcaju* mycelia (Arıca and Bayramoğlu, 2005; Bayramoğlu et al., 2005), *R. nigricans* (Bai and

Abraham, 2001; Bai and Abraham, 2002; Bai and Abraham, 2003), *Neurospora crassa* (Tunali et al., 2005) and *Aspergillus tubingensis* (Coreño-Alonso et al., 2009). Prakasham et al., (1999) investigated Cr(VI) biosorption at pH 2.0 by non-living free and immobilized biomass from *R. arrhizus* a biphasic Cr(VI) adsorption pattern was observed. Cr(VI) removal rates were slightly higher when employing the free biomass than the immobilized state. Free and immobilized *R. arrhizus* biomass adsorption capacities were 11 and 8.63 mg/g, respectively. Park et al., (2005) reported Cr(VI) removal by *A. niger*, *R. oryzae* and *P. chrysogenum*. *R. oryzae* completely removed Cr(VI) in 48 h, while the other species needed 218-254 h for the complete Cr(VI) removal. The initial Cr(VI) removal rate by *S. cerevisiae* was faster than that by *A. niger*, but the former required more contact time than the latter to completely remove Cr(VI) from aqueous solution. Cr(VI) is reduced to Cr(III), and completely removed from aqueous solution if sufficient experimental contact time is given. Furthermore, desorption and X-ray Photoelectron Spectroscopy (XPS) studies indicated that most of the Cr bound to the biomass was Cr(III), implying that the Cr(VI) removal occurs via a "redox reaction". Park et al., (2005) proposed a new Cr(VI) removal mechanism for the dead *A. niger* fungal biomass. Aqueous Cr(VI) can be removed through the two mechanisms. In mechanism I, aqueous Cr(VI) is directly reduced to Cr(III) upon contact with the biomass. Mechanism II consists of three steps which include: (1) binding Cr(VI) to positively charged groups such as protonated amines present in the chitin and chitosan fungal cellwall components; (2) reduction of Cr(VI) to Cr(III) by adjacent functional groups having lower reduction potentials than that of Cr(VI); (3) the release of reduced Cr(III) into the aqueous solution by electronic repulsion between the positively charged groups and the Cr(III) ion. Since protons are consumed in each mechanism, the solution pH increases during Cr(VI) removal by the dead fungal biomass. Therefore, supplying protons promotes the rate and efficiency of Cr(VI) removal.

## 2.11 Chromium removal by algae

Algae include a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis. Algae are classified on the nature of the chlorophyll(s), the cell wall chemistry and flagellation. Although thousands of algal species are known, only a few of them have been investigated for their metal sorption ability and subsequent use in wastewater treatment. Metal biosorption experiments have been conducted with freshwater green algae (*Chlorella* sp., *Cladophora* sp., *Scenedesmus* sp., *Chlamydomonas reinhardii*), brown algae (*Sargassum natans*, *Fucus vesiculosus*, *Ascophyllum nodosum*, *Laminaria japonica*) and blue-green algae (*Microcystis aeruginosa* and *Oscillatoria* sp.). Micro algae are easy to grow in culture and some algal species are being grown commercially in large quantity. Metal sorption ability of algae varies greatly from species to species

and even among strains of a single species for any metal (Table 1), although this variation may also be due to variable experimental conditions in different studies. A suggestion has also been made that cells grown under different conditions vary with regard to composition of their cell wall and hence in biosorption characteristics. Some algae show a high affinity for sorbing a particular metal ion, whereas others do not show such specificity and may sorb several metal ions. Chromium biosorption in non-living biomass of *Chlorella vulgaris, Cladophora crispata, Zoogloea ramigera, Rhizopus arrhizus* and *Saccharomyces cerevisiae* has been studied (Nourbakhsh et al., 1994) and observed that initial pH (1.0-2.0) of the metal ion solution affected the metal uptake capacity of the biomass and maximum adsorption rates were at 25-35°C, which increased with increasing metal concentration. Algae used for chromium remediation include *Spirogyra* (Gupta et al., 2001), *Chlamydomonas reinhardtii* (Arica and Bayramoğlu, 2005), *Dunaliella* (Dönmez and Aksu, 2002), *Chlorella vulgaris* (Dönmez et al., 1999; Aksu and Açikel, 1999; Aksu and Açikel, 2000), *Cladophora crispata* (Nourbakhsh et al., 1994; Nourbakhsh et al., 2002), *Sargassum* sp. (Aravindhan, 2004), *Ecklonia* sp. (Park et al., 2004; Yun et al., 2001). Gupta et al., (2001) studied Cr(VI) biosorption by biomass of filamentous alga *Spirogyra* sp. Equilibrium isotherms were obtained and maximum removal of Cr(VI) was 1.47 g metal/kg of dry weight biomass at pH of 2.0. This micro algal cell wall contains a high amount of polysaccharides, some of which are associated with proteins and other components.

These bio-macromolecules contain such functional groups as amino, carboxyl, thiol, sulfydryl, and phosphate groups. At pH 1.0-2.0, protonation of cell wall amino groups enhanced the biosorption capacities of the biosorbents to Cr(VI) ions. The increased binding of Cr(VI) containing anions at low pH was due to electrostatic binding to the positively charged protonated amine groups. The Cr(VI) biosorption from saline solutions by two strains of *Dunaliella* sp. were tested as a function of pH, initial metal ion and salt (NaCl) concentrations (Dönmez and Aksu, 2002). Carmona et al., (2005) studied Cr(III) and Cr(VI) removal separately using a 23 factorial experimental design. The three factors considered were pH, temperature and metal concentration at two different levels: Cr(III), pH (2.0 and 6.0), temperature (29 and 55°C) and metal concentration (10 and 1200 mg/L); Cr(VI), pH (1.0 and 3.0), temperature (29 and 55°C), and metal concentration (10 and 1200 mg/L). The most significant effect on Cr(III) uptake was the interaction between metal concentration and pH. Algal cells have considerable potential in adsorbing anionic species of certain metals. For instance, algal cells can also adsorb Cr(VI) with considerable ease at low pH values (<2) (Kratchovil et al., 1998; Dönmez and Aksu, 2002). Removal of Cr(VI) by algae is anionic as well as through its reduction to the cationic Cr(III) under strongly acidic conditions. Biosorption of Cu(II), Ni(II) and Cr(VI) from aqueous solution on dried

algae *Chlorella vulgaris, Scenedesmus obliquus* and *Synechocystis* sp. was tested under laboratory conditions as a function of pH, initial metal ion and biomass concentration (Dönmez et al., 1999). Forty eight species of red, brown, and green algae were examined for chromate adsorption capacities and excellent adsorption was found by *Pachymeniopsis* sp., which showed high selectivity for chromate among other heavy metals ions such as cadmium and manganese. The brown seaweeds belonging to the genera *Ascophyllum* and *Sargassum* are the most exploited organisms for their high metal sorption capacities including *Spirogyra* sp., *Fucus versiculosus, Eisenia, Laminaria, Spirulina, Porphyra, Cyanidium, Chlamydomonas, Oscillatoria* (Gupta et al., 2001; Gupta and Mohapatra, 2003), *Shewanella* (Guha et al., 2003) and *Sargassum wightii* (Aravindhan et al., 2004).

## 2.12 Chromium removal by plants

Phytoremediation is the use of certain plants to clean up soil, sediment and water contaminated with metals. We can find five types of phytoremediation techniques: phytoextraction, phytotransformation, phytostabilization, phytodegradation, rhizofiltration, even if a combination of these can be found in nature (Prasad and Freitas, 2003; Acar and Malkoc, 2004; Freitas et al., 2004; Rajkumar et al., 2009). In phytoextraction process substantial amounts of chromium removed from root and translocated to the harvestable plant parts so that it can be completely removed from the contaminated site. Aquatic plant species for the phytoextraction of chromium from contaminated tannery sludge, the ability of plant species *Scirpus lacustris, Phragmites karka* and *Bacopa monnieri* to absorb, translocate and concentrate chromium in their tissues. Rhizofiltration of chromium contaminated waters has received more attention and several aquatic plant species were identified as potential chromium phytoremediators. In earlier studies, smartweed, water hyacinth and duckweed were identified as a Cr-accumualtor (Zayed and Terry, 2003). Very few plant species such as *Sutera fodina*, *Dicoma niccolifera* and *Leptospermum scoparium* have been reported to accumulate chromium to high concentration in their tissue. Chromium remediation was examined by various plants, including *Fagus orientalis* L. (Acar and Malkoc, 2004), *Agave lechuguilla* (Romero-González et al., 2005), *Atriplex canescens* (Sawalha et al., 2005), *Thuja oriantalis* cones (Oguz, 2005), *Larrea tridentate* (Gardea-Torresdey et al., 1998), *Pinus sylvestris* (Ucum et al., 2002). Cr(VI) biosorption onto *Pinus sylvestris* cone biomass was investigated by Ucun et al., (2002). Romero-González et al., (2006; 2009) reported Cr(III) biosorption onto *Agave lechuguilla* biomass. Gardea-Torresdey et al., (2000) studied Cr(VI) bioadsorption and its possible reduction to Cr(III) by *Agave lechuguilla* biomass. Sawalha et al., (2005) studied chromium adsorption by native, esterified and hydrolyzed saltbush (*Atriplex canescens*) biomass. The percentages of Cr(III) bound by native stems, leaves and flowers at pH 4.0 were 98%, 97% and 91%, respectively. On the other hand

the Cr(VI) binding by the native stems, leaves, and flowers of the native and hydrolyzed saltbush biomass decreased as pH increased. At pH 2.0 the stems, leaves, and flowers of native biomass bound 31%, 49% and 46%, of Cr(VI) respectively. Aoyama (2003) studied aqueous Cr(VI) removal by London plane leaves. Aoyama et al., (1999) had previously explored coniferous leaves for the adsorption of hexavalent chromium from dilute aqueous solution and later tested the ability of Japanese ceder (*Cryptomeria japonica*) bark for Cr(VI) remediation (Aoyama et al., (2004).

Table 2: Biosorbents for removal of chromium

| Biosorbent | Metal | Reference |
|---|---|---|
| **Fungi** | | |
| *Zoogloea ramigera* | Cr | Nourbakhsh et al., 1994 |
| *Saccharomyces cerevisiae* | Cr | Nourbakhsh et al., 1994 |
| *Mucor meihi* | Cr | Tobin and Roux 1998 |
| *Rhizopus arrhizus* | Cr | Nourbakhsh et al., 1994; Niyogi et al., 1998; Prakasham et al., 1999 |
| *Rhizopus oligosporus* | Cr | Ariff et al., 1999 |
| *Scenedesmus obliquus* | Cr, Cu, Ni | Donmez et al., 1999 |
| *Penicillium purpurogenum* | Cr(VI) | Say et al., 2004 |
| *Rhizopus nigricans* | Cr | Bai and Abraham 2001 |
| *Trametes versicolor* | Cr | Bayramoglu et al., 2003 |
| *Penicillium chrysogenum* | Cr(VI) | Deng and Ting, 2005 |
| *Aspergillus* sp. | Cr(VI) | Srivastava and Thakur, 2006 |
| **Algae** | | |
| *Cladophara crispata* | Cr | Nourbakhsh et al., 1994 |
| *Chorella vulgaris* | Cr | Nourbakhsh et al., 1994; Donmez et al., 1999 |
| *C. vulgaris* | Cu(II), Cr(VI) | Aksu and Açikel, 1999 |
| *Laurencia obtuse* | Cr(III) | Hamdy, 2000 |
| *Chlorella vulgaris* | Fe(III), Cr(VI) | Aksu and Açikel, 2000 |
| *Spirogyra* sp. | Cr | Gupta et al., 2001 |
| *Dunaliella* sp. | Cr | Donmez and Aksu, 2002 |
| brown seaweed *Sargassum wightii* | Cr | Aravindhan et al., 2004 |
| *Oedogonium hatei* | Cr(VI) | Gupta and Rastogi, 2009 |

| Bacteria | | |
|---|---|---|
| *Enterobacter cloacae* | Cr(VI) | Yamoto et al., 1993 |
| *Desulfovibrio vulgaris* | Cr(VI) | Lovley and Phillips, 1994 |
| *Staphylococcus cohnii* | Cr(VI) | Saxena et al., 2000 |
| *Pseudomonas aeruginosa* | Cr(VI) | Ganguli and Tripathi, 2000; 2001 |
| *Acinetobacter* sp. | Cr | Shrivastava and Thakur, 2003 |
| *Acinetobacter* sp. | Cr(VI) | Shrivastava and Thakur, 2007 |
| **Plant origin adsorbent** | | |
| Water hyacinth | Cr(VI) | Low et al., 1997 |
| black locust leaves | Cr(III) | Aoyama et al., 2000 |
| Larch bark | Cr(III) | Aoyama and Tsuda, 2001 |
| *Wolffia globosa* | Cd, Cr | Upatham et al., 2002 |
| *Salvinia herzogii* | Cr(III) | Maine et al., 2004 |
| *Pistia stratiotes* | Cr(III) | Maine et al., 2004 |
| *Fagus orientalis* | Cr(VI) | Acar and Malkoc, 2004 |
| *Macrophytes* | Cr | Maine et al., 2004 |
| *Agave lechuguilla* | Cr(VI) | Romero-Gonz´alez et al., 2005 |
| *Atriplex canescens* | Cr | Sawalha et al., 2005 |
| *Aegle marmelos correa* | Cr | Anandkumar and Mandal, 2009 |

## 2.13 Chromium removal by low cost sorbent

The use of low-cost sorbents has been investigated as a replacement for current costly methods of removing heavy metals from solution. Use of low cost natural resource as adsorbents for removal of chromium is constantly encouraging in preference to other conventional methods. The application of activated alumina (Gupta and Tiwari, 1985), moss peat (Sharma and Forster, 1993), wood charcoal (Deepak and Gupta et al., 1991), carbon slurry (Singh and Tiwari, 1997) and green peas skin dust (Samantaroy et al., 1998), rice straw (Samanta et al., 2000), corncob (Nigam and Rama, 2002), activated charcoal, bituminous coal and coconut shell carbon (Nagesh and Krishnaian, 2002), bagasse (Rao et al., 2003), food industrial waste (Selvaraj et al., 2003), agricultural by product (Bishnoi et al., 2004), different biowaste material (Joshi et al., 2003) and paper mill sludge (Ahluwalia and Goyal, 2004) rose waste biomass (Iftikhar et al., 2009), Bael fruit (*Aegle marmelos correa*) shell (Anandkumar and Mandal, 2009) and *Agave lechuguilla* biomass for Cr(III) removal has been explored (González et

al., 2006). Low-cost sorbents, such as bagasse fly ash (Park et al., 1999), hazelnut shell (Cimino et al., 2000), bark (Alves et al., 1993), *Eucalyptus* bark (Sarin and Pant 2006) biological wastes such as sawdust, rice husk, coirpith, charcoal and naturally occurring mineral (vermiculite) have been used for removing chromium from tannery effluent through batch and column mode and adsorption capacities of the substrates were also evaluated using isotherm test and computing distribution co-efficient (Sumathi et al., 2005).

## 2.14 Chromium removal by industrial wastes or by-products

Cr(III) and Cr(VI) adsorptive removal from water by Industrial waste or by-products has been explored. The most important of these adsorbents are fly ash (Gupta et al.,1999; Gupta and Ali 2004; Rao et al., 2002; Bayat 2002; Banarjee et al., 2004; Demirbas, 2006), blast furnace slag (Erdem et al., 2005; Srivastava et al., 1997), red mud (Gupta et al., 2001; Lopez et al., 1998), lignin (Lalvani et al., 1997, 2000; Ali et al., 2004), waste sludge (Li et al. 2004; Slevaraj et al., 2003 Aksu et al., 2002), oil shale (Shawabkeh, 2006), tea factory waste (Malkoc et al., 2006), olive industry waste waste tires (Entezari et al., 2005) and residual slurry (Namasivayam and Yamuna, 1995; Singh and Tiwari, 1997; Namasivayam and Yamuna 1999).

## 2.15 Chromium sorption mechanisms

Cr(VI) adsorption from aqueous solutions by activated carbon/low cost adsorbents/biosorbents involves mainly of two modes of uptake:

1. Hexavalent chromium species are removed by adsorption onto the interior surface of the adsorbent (carbon/biosorbent/adsorbent).
2. Chromium (VI) is reduced into Cr(III) and this trivalent state adsorbs at the external carbon surface.

The major parameters which governs the adsorption mechanism of Cr(VI) or Cr(III) are: pH, degree of mixing or velocity gradient, carbon/adsorbent dose, total chromium concentration (both Cr(III) and Cr(VI)), temperature, particle size and type of adsorbents. The majority of the sorbents reviewed herein are activated carbons, biosorbents, clays or industrial/agricultural by-products. A number of equations have been used to describe kinetic reactions in these sorbents. These include first, second order and two constant rate equations (Lagergren, 1898; Ho et al., 2004; Ho, 2004). In most of the papers discussed above the rate of the reaction was either governed by first- or second-order rate equations. Further, the adsorption of Cr(VI) and Cr(III) is governed by four consecutive steps:

1. transport in the bulk solution;

2. diffusion across the liquid film boundary surrounding the sorbent particles;

3. intraparticle diffusion in the liquid contained in the pores and in the sorbate along the pore walls; sorption and desorption within the particle and on the external surface.

To determine the exact mechanism it is necessary to carry out experiments to investigate such variables as initial concentration, sorbent particle size, solution temperature, pH, agitation time and then analyze the data for different kinetic orders or for pore/solid phase diffusion mechanisms. The mathematical treatment of Boyd et al. (1947) and Reichenberg (1953) distinguishes between diffusion in the particle, film diffusion and a mass action-controlled exchange mechanism. This treatment laid the foundations of sorption/ion exchange kinetics.

Three steps which occur in the adsorption of an adsorbate by a porous adsorbent are:

(i) transport of the adsorbate to the external surface of the adsorbent (film diffusion);

(ii) transport of the adsorbate within the pores of the adsorbent (intraparticle diffusion);

(iii) adsorption of the adsorbate on the exterior surface of the adsorbent.

Process (i) is rapid and does not represent the rate determining step in the uptake of adsorbate (1). Three distinct cases occur for the remaining two steps in the overall transport:

• case I: external transport > internal transport;

• case II: external transport < internal transport;

• case III: external transport≈internal transport.

In cases I and II, the rate is governed by diffusion in the film and in the particle, respectively. In case III, the transport rate of adsorbate to the boundary is not significant. This leads to liquid film formation with a concentration gradient surrounding the sorbent particles. External transport is usually rate limiting in systems, which have: (a) poor mixing, (b) dilute adsorbate concentration, (c) small particle size and (d) high adsorbate affinity for adsorbent. In contrast, intra-particle transport limits the overall transfer for those systems that have: (a) high adsorbate concentration, (b) good mixing, (c) large adsorbent particle size and (d) low adsorbate/adsorbent affinity.

## 2.16 Pretreatment of biomass

Biosorption of metals have been studied using pretreated biomass. Pretreatment carried out on the different types of biosorbent are chemical pretreatments such as contacting cells with acids (Ahuja et al., 1999; Arica et al., 2005; Tunali et al., 2005), alkali (Mameri et al., 1999; Selatnia et al., 2004 a, b; Liu et al., 2004; Göksungur et al., 2005) and organic compounds (Bingol et al., 2004; Göksungur et al., 2005; Seki et al., 2005). Physical pretreatment methods have been heat treatment (Ahuja et al., 1999; Arica et al., 2005 and Göksungur et al., 2005), autoclaving (Tunali et al., 2005) and boiling (Melo and D'Souza, 2004). Some researchers have used the combination of chemical and physical pretreatment such as alkali and boiling pretreatment (Tunali et al., 2005; Melo and D'Souza, 2004) and alkali and autoclaving pretreatment (Baik et al., 2002).

### 2.16.1 Effects of biomass pretreatment on metal biosorption

Most researchers have found that pretreated biomass showed higher metal adsorption than untreated biomass. Göksungur et al., (2005) reported that the three different methods of pretreatment i.e. ethanol, alkali and heat treatment had increased cadmium and lead uptake of *Saccharomyces cerevisiae*. Tunali et al., (2005) found that all the pretreatment methods i.e. acid, autoclaving and combination of alkali and boiling enhanced the adsorption capacity of *Neurospora crassa* towards Cr(VI). Baik et al., (2002) found that the combination of alkali and autoclaving pretreatment on several types of mold improved binding of copper, cadmium, nickel and zinc. Besides these, Arica et al., 2005; Seki et al., 2005; Melo and D'Souza, 2004; Liu et al., 2004; Selatnia et al., 2004b and Mameri et al., 1999 also reported on the positive effect of using pretreated biomass. On the other hand, Ahuja et al., (1999) found that the use of acid pretreated *Oscillatoria anguistissima* resulted in a decline in cobalt adsorption while metal sorption remained unchanged after alkali pretreatment. Biosorption of hexavalent chromium by raw and acid-treated green alga *Oedogonium hatei* from aqueous solutions was studied by Gupta and Rastogi, 2009.

Huang and Huang (1996) reported that the adsorption capacity of *Rhizopus oryzae* remained unchanged after acid pretreatment. It is also interesting to note that the same pretreatment on a biomass may show different effect when different metals are considered. Kapoor and Viraraghavan (1998) discovered that nickel biosorption by *Aspergillus niger* was reduced when the biomass was pretreated with sodium hydroxide, dimethyl sulphoxide, formaldehyde and detergent while improvement in lead and cadmium biosorption by these pretreatment methods. More detailed studies are required to understand why enhancement or reduction in adsorption capacity occurs under specific pretreatment conditions (Kapoor

and Viraraghavan, 1998). Arica et al., (2005) added that denaturation of protein molecules and degradation of polysaccharide compounds of the cell wall components also produce additional available binding sites. However, Galun et al., 1987 reported that *Pencillium* biomass pretreatment at 100°C for 5 minutes increased the biosorption of lead, cadmium, nickel and zinc and the increase was attributed to the exposure of latent binding sites after pre-treatment. The bioadsorption capacity of autoclaved *Mucor rouxii* decreased as compared to the live fungus, attributed to the loss of intracellular uptake (Yan and Viraraghavan, 2000). When non-viable biomass is used in the removal of heavy metals, alkali pretreatment is an effective method to improve the bioadsorption capacity for metal ions (Yan and Viraraghavan, 2000). Hence, the bioadsorption efficiency of dead biomass may be greater, equivalent to or less than that of live biomass depending on the pre-treatment method applied.

### 2.16.2 Desorption

If the biosorption process were to be used as an alternative to the wastewater treatment scheme, the regeneration of the biosorbent may be crucially important for keeping the process costs down and in opening the possibility of recovering the metals extracted from the liquid phase. For this purpose it is desirable to desorb the sorbed metals and to regenerate the biosorbent material for another cycle of application. The desorption process should: yield the metals in a concentrated form; restore the biosorbent to close to the original condition for effective reuse with undiminished metal uptake and no physical changes or damage to the biosorbent. While the regeneration of the biosorbent may be accomplished by washing the metal-laden biosorbent with an appropriate solution, the type and strength of this solution would depend on the extent of binding of the deposited metal. Dilute solutions of mineral acids like hydrochloric acid, sulphuric acid, acetic acid and nitric acid can be used for metal desorption from the biomass (De Rome and Gadd, 1990, Zhou and Kiff, 1991, Luef et al., 1991, Pagnanelli et. al., 2002, Bai and Abraham, 2003). Polysulphone immobilized *Rhizopus nigricans* were subjected to Cr(VI) recovery experiments using 0.01 N solutions of mineral acids, salt solutions, alkalies, deionised distilled water and buffer solutions. A few experiments were conducted to desorb the metal ions from the loaded waste fungal biomass of *Aspergillus* sp. (Chandrashekar et al., 1998).

### 2.16.3 Advantages and disadvantages of using living biomass

Most of the studies dealing with microbial metal remediation via growing cells describe a biphasic uptake of metals, i.e. initial rapid phase of biosorption followed by slower metabolism-dependent active uptake of metals. Application of active and growing cells offers several advantages i.e. the ability of self replenishment, continuous metabolic uptake of metals after physical adsorption and the potential

for optimization through development of resistant species and cell surface modification. Further, the metals diffused into the cells during detoxification get bound to intracellular proteins or chelatins before being incorporated into vacuoles and other intracellular sites. These processes are often irreversible and ensure less risk of metal releasing back to the environment. Apart from these, using growing cultures in bioremoval could avoid the need for a separate biomass production process e.g. cultivation, harvesting, drying, processing and storage prior to the use (Malik, 2004). Yet, there are significant practical limitations to uptake of metal by living cell systems such as sensitivity of the system to extremes of pH, high metal concentration and requirement of external metabolic energy (Malik, 2004). Sannasi et al., (2006) reviewed that the use of growing cells would be more useful if treatment involves mixed waste with potential bacterial utilization as energy and carbon source over a longer period. Malik (2004) reviewed that in toxic metal removal applications, it is important to ensure that the growing cells can maintain a constant removal capacity after multiple bioaccumulation-desorption cycles and a suitable method is required to optimize the essential operating conditions. The situation demands a multi-prong approach including strain isolation, cell development and process development in order to make the ultimate process technically and economically viable.

#### 2.16.4 Advantages and disadvantages of using non-living biomass

It has been reported that the amount of metal ion sorption from aqueous solution by non-living biomass to be the same or greater than by live biomass. The use of non-living biomass may offer several advantages over living cells (Bayramoğlu et al., 2005). Biosorption by dead biomass is a rapid process, since only passive cell wall based binding onto the cell occurs (Zouboulis et al., 1999). Another advantage of using dead biomass is the sorbed metal ions can be easily desorbed and the biomass can be reused (Selatnia et al., 2004b). On the other hand, potential for desorptive metal recovery from live biomass is restricted since metal may be intracellularly bound. The use of dead biomass also eliminates nutrient supply and the heavy metal toxicity problem which occur in living cells (Bai and Abraham, 2001b; Zouboulis et al., 2004). Besides that, killed cells may be stored or used for extended periods (Göksungur et al., 2005). Problems associated with the need to provide suitable growth condition also does not arise (Sheng et al., 2004).

One of the disadvantages of using non-living biomass is the reusability of biomass after desorption is possible only if relatively weak chemicals are used for desorption. Besides this, as metal is not taken up into the cells (just adsorbed at cell surface), thus only a small fraction of bioaccumulation capacity is exploited (Malik, 2004).

## 2.17 Characterization of biomass surface

The biomass characterization can be carried out by different ways such as potentiometric and conductometric titration, major ionic content determination, FTIR and X-Ray analysis. These analyses are useful to identify the active sites on cell wall as guidance for the choice of the possible mechanisms operating such as physical adsorption, complexation, ionic exchange or surface micro-precipitation (Mohan et al., 2006; Blázquez et al., 2009; Iftikhar et al., 2009). Besides these, the mechanisms of metal biosorption have also been elucidated on the basis of biomass treatment, BET and contact angle studies (Bayramoğlu et al., 2005).

### 2.17.1 Fourier transform infrared (FTIR) analysis

Almost any compound having covalent bonds, whether organic or inorganic, will be found to absorb frequencies of electromagnetic radiation in the infrared region of the spectrum. The infrared region of the electromagnetic spectrum lies at wavelengths longer than those associated with visible light, which includes wavelengths from approximately 400 to 800 nm but at wavelengths shorter than those associated with radio waves. For chemical purposes, the vibrational portion of the infrared region is of interest. This portion includes radiations with wavelengths between 2.5 and 15 µm (Pavia et al., 2002). As with other types of energy absorption, molecules are excited to a higher energy state when they absorb infrared radiation. Only selected frequencies (energies) of infrared radiation are absorbed by a molecule. In the absorption process, those frequencies of infrared radiation that match the natural vibrational frequencies of the molecule in question are absorbed and the energy absorbed increases the amplitude of the vibrational motions of the bonds in the molecule (Pavia et al., 2002). Most bands characteristic of functional groups appear at frequencies higher than 1200 $cm^{-1}$. The frequencies of IR bands for functional groups are reasonably characteristic and are rarely found to vary from compound to compound; however, the intensity of the absorption and the width of the band do vary. Furthermore, interaction of functional groups can lead to changes in frequency and intensity of adsorption. In the region from about 1200 to 700 $cm^{-1}$ complex band characteristic of a specific molecule (rather than a functional group) are usually observed and is called the fingerprint region. A comparison of the IR spectrum of an unknown compound with a library of spectra of known compounds can often enable the unambiguous identification of both the functional group(s) present and the specific structure. In essence, there are so many possible patterns of absorption bands in the fingerprint region that it is highly improbable that the spectra of two different compounds would be the same in all details (Mohan et al., 2006; Sarin et al., 2006).

Ngo-Thi et al., (2003) has further categorized the bacterial spectral into five different spectral regions associated to biopolymer present in a bacterium i.e. 3000-2800 $cm^{-1}$ (‘fatty acid region’), 1500-1400 $cm^{-1}$ (‘protein, fatty acid region’), 1200-900 $cm^{-1}$ (‘phosphate and carbohydrate region’) and 900-700 $cm^{-1}$ (‘fingerprint region’). The FTIR spectra of the main macromolecular building blocks present in biological samples such as nucleic acid, proteins, carbohydrate and lipid was obtained by Maquelin et al., (2002). The spectra are in accordance with those obtained by Suci et al., (1998) with additional information on bands present at 3100-2700 $cm^{-1}$ that has been categorized as the ‘fatty acid region’ by Ngo-Thi et al., (2003).

Many researchers have used the FTIR spectra as a qualitative and preliminary analysis of the chemical functional groups present on the cell wall of biomass which will provide information on the nature of the possible cell-metal ions interactions in metal biosorption by different biomass (Arica et al., 2005; Park et al., 2005b; Tunali et al., 2005). In the presence of metal, the biomass showed some changes in the IR spectra pattern such as bands disappearance after saturation of active sites (Lin et al., 2005; Yun et al., 2001 and Mameri et al., 1999), bands shifting (Lin et al., 2005; Tunali et al., 2005; Pethkar et al., 2001 and Yun et al., 2001) and bands elongation (Loukidou et al., 2004). Some researchers have reported a decrease (Tunali et al., 2005 and Sheng et al., 2004) or increase (Adhiya et al., 2002) in certain band intensity after metal binding. It has also been reported that no changes in the spectral pattern was observed after interaction with metal which indicated chemical reactions might not be involved in the biosorption of metal ions (Selatnia et al., 2004b and Pethkar et al., 2001). Selatnia et al., (2004a) has pointed out that the IR spectra may give some idea on the nature of the cell wall but further tests are necessary to give more experimental evidence such as potentiometric titration curves.

### 2.17.2 X-Ray diffraction (XRD) analyses

XRD analyses of the solid phases indicate that the solids are either amorphous or crystalline in nature. Oxidation state analyses of most of the aqueous samples by chromatographic technique described by Olsen et al., (1994) and of limited samples by x-ray absorption near-edge structure (XANES) showed that Cr(III) is the dominant oxidation state in the aqueous and solid phases. XAS experiments were conducted at Stanford Synchrotron Radiation Laboratory (beamline 4-1) to determine the local structure of trivalent chromium hydroxide solids and solutions. In contrast to a crystalline pattern consisting of a series of sharp peaks, amorphous materials produce a broad background signal. Many polymers show semi-crystalline behavior, *i.e.* part of the material forms an ordered crystallite by folding of the molecule. One and the

same molecule may well be folded into two different crystallites and thus form a tie between the two. The tie part is prevented from crystallizing. The result is that the crystallinity will never reach 100%. Powder XRD can be used to determine the crystallinity by comparing the integrated intensity of the background pattern to that of the sharp peaks. Values obtained from powder XRD are typically comparable but not quite identical to those obtained from other methods such as DSC. X-ray scattering techniques are a family of non-destructive analytical techniques which reveal information about the crystallographic structure, chemical composition and physical properties of materials and thin films. These techniques are based on observing the scattered intensity of an x-ray beam hitting a sample as a function of incident and scattered angle, polarization and wavelength or energy (Gopal and Elang 2007). X-ray diffraction finds the geometry or shape of a molecule using x-rays. X-ray diffraction techniques are based on the elastic scattering of x-rays from structures that have long range order. The most comprehensive description of scattering from crystals is given by the dynamical theory of diffraction. (XRD) is a technique used to characterize the crystallographic structure, crystallite size (grain size), and preferred orientation in polycrystalline or powdered solid samples. Powder diffraction is commonly used to identify unknown substances, by comparing diffraction data against a database maintained by the International Centre for Diffraction Data. It may also be used to characterize heterogeneous solid mixtures to determine relative abundance of crystalline compounds and, when coupled with lattice refinement techniques, such as Rietveld refinement, can provide structural information on unknown materials. Powder diffraction is also a common method for determining strains in crystalline materials.

## 2.18 Adsorption isotherms

Extensive studies have also been carried out on biosorption and its dependence on solution chemistry, ionic competition by other metals, influence of pH and ionic concentration (Bai and Abraham, 2002). Different types of adsorption isotherms have been used to quantify and contrast between the performances of different biosorbents (Davis et al., 2003). Adsorption isotherm, which are the presentation of the amount of solute adsorbed per unit of adsorbent. Among the isotherms, the Langmuir and Freundlich models are most frequently used to describe metal biosorption (Ledin, 2000). From a scientist's perspective, the field of biosorption is a challenging one, since it requires the application of first principles of organic chemistry and geochemistry. The main objectives are the elucidation of binding mechanisms, the relative affinity of heavy metals for the biomass and how both are affected by varying environmental conditions. Ultimately, the goal is the successful implementation of a remediation program. The first step towards these objectives is to quantify the capacity of a given

biomass to sequester heavy metals from an aqueous solution. This is traditionally done by characterizing the equilibrium state after the biomass has been allowed to react with an aqueous solution of the metal of interest. The reaction is commonly monitored by measuring the amount of metal remaining in solution until it becomes time invariant. The model used to describe the results should be capable of predicting heavy metal binding at both low and high concentrations. Ideally, the model should not only be predictive but should rest on our understanding of the mechanism of biosorption (Davis et al., 2003).

**Langmuir isotherm – for monolayer adsorption on a homogeneous flat surface**

Langmuir adsorption isotherm has traditionally been used to quantify and compare the performance of different biosorbents. However in order to evaluate the appropriateness of this model, we must look at its underlying assumptions. The Langmuir isotherm was originally developed to describe the gas-solid phase adsorption of activated carbon. In its formulation, binding to the surface was primarily by physical forces (i.e electrostatic or London-van der Waals forces) and implicit in its derivation was the assumption that all sites possess equal affinity for the adsorbate. Its use was extended to empirically describe equilibrium relationships between a bulk liquid phase and a solid phase. One of the simplest representations of the adsorption phenomenon calls for the migration to and the occupation of a surface site, S on a solid by an adsorbate, A. This can be represented by an equilibrium reaction: S + A SA where SA is the adsorbed complex. Surface species concentrations may be expressed as moles per liter of solution, per gram of solid, per unit area of solid surface or per mole of solid. Compliance to the Langmuir isotherm theory requires that (1) adsorption is limited to the formation of a monolayer or the number of adsorbed species, does not exceed the total surface sites and (2) all surface sites have the same energy or equal affinity for the adsorbate (Davis et al., 2003). At least one of these conditions is implicitly not met in the case of biosorption. As seen in the previous sections there exist more than one type of functional groups contributing to the biosorption process, each of which has a different affinity for the sorbing heavy metal. Furthermore, the one-to-one stoichiometry is also not complied with, since ion-exchange has been shown to be a dominant mechanism and typically approximately two protons are released upon the binding of one divalent heavy metal ion. Despite this fact, the Langmuir equation is frequently used to fit experimental data. In this case, the following form of the Langmuir equation is traditionally applied. This isotherm represents one of the first theoretical treatments of nonlinear sorption and suggested that uptake occurs on a homogeneous surface by monolayer sorption with interaction between adsorption molecules.

$$q_e = \frac{Q_l b C_e}{1 + b C_e} \qquad \text{(nonlinear form)} \qquad (1)$$

where

$Q_L$ (mg/g) and $b$ is Langmuir constants related to adsorption capacity and the energy of adsorption respectively. $Q_L$ and $b$ can be determined from the lineal plot of $1/q_e$ versus $1/C_e$

Eq (3) is usally linerized to obtain the following form

$$\frac{C_e}{q_e} = \frac{C_e}{Q_l} + \frac{1}{bQ_2} \qquad \text{(linear form)} \qquad (2)$$

where $q_e$ is the uptake and $Q_L$ is the maximal uptake of the biosorbent. $C_e$ is the final equilibrium solution concentration of the heavy metal, which is typically determined by AAS or ICPMS. In this context, b is not truly the Langmuir adsorption constant but rather a simple fitting parameter because as indicated above, the system does not comply with the assumptions of the model and cannot be related to the Gibbs free energy of a specific reaction. The parameter is nonetheless quite useful as a measure of the biosorption affinity or efficiency of different biomasses. High values of b are reflected by the steep initial slope of a sorption isotherm and indicate a high affinity for the adsorbate. In terms of implementation, biosorbents with the highest possible $Q_L$ and a high value of b are the most desirable (Davis et al., 2003).

**Freundlich isotherm – for heterogenous flat surface**

This isotherm also considers monolayer sorption with a heterogenous distribution of active sites of the sorbent. The Freundlich isotherm has also been employed to quantify equilibrium biosorption systems. Like the Langmuir isotherm, the extent of adsorption / sorption is determined as a function of the equilibrium concentration of the metal in solution without reference to pH or other ions in the same aqueous system. The Freundlich isotherm is originally of an empirical nature, but was later interpreted as sorption to heterogenous surfaces or surfaces supporting sites of varied affinities. It is assumed that the stronger binding sites are occupied first and that the binding strength decreases with increasing degree of site occupation. Specifically, the Freundlich isotherm is obtained when a log-normal affinity distribution is assumed. The Freundlich isotherm is defined by the following expression:

$$q_e = K_f C_e^{1/n} \qquad \text{(nonlinear form)} \qquad (3)$$

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \qquad \text{(linear form)} \qquad (4)$$

where $K_f$ (mg /g) strands for adsorption capacity and n for adsorption intensity
where $K_f$ and 1/n can be determined from the linear plot of log ($q_e$) versis log ($C_e$). Experimental values obtained for the adsorption capacity experiments were used to calculate the parameters where k and n are empirically determined constants, with k being related to the maximum binding capacity and n related to the affinity or binding strength (Davis et al., 2003).

## 2.19 Adsorption kinetics

Adsorption kinetics depends on the adsorbate-adsorbent interaction and system condition and has been investigated for their suitability for application in water pollution control. Two vital evaluation elements for an adsorption process operation unit are the mechanism and the reaction rate. Solute uptake rate determines the residence time required for completing the adsorption reaction and can be enumerated from kinetic analysis. Numerous attempts were made in formulating a general expression to describe the kinetics of adsorption on solid surfaces for the liquid-solid adsorption system.

Adsorption kinetics of chromium study describes the solute uptake rate and this rate controls the residence time of adsorbate uptake at the solid-solution interface. The conformity between experimental data and the models predicted values was expressed by the correlation coefficients ($r^2$ values close or equal to 1). A relatively high $r^2$ value indicates that the model successfully described the kinetics of Cr(III) adsorption. In 1898, Lagergren presented the first order rate equation for the adsorption of ocalic acid and malonic acid onto charcoal (Lagergren, 1898). Lagergren kinetics equation may have been the first one in describing the adsorption of liquid-solid systems based on solid capacity.

**The pseudo first-order equation (Lagergren, 1898)**

Lagergren kinetics equation has been most widely used for the adsorption of an adsorbate from an aqueous solution.

**Lagergren Equation (pseudo-first-order)** Sorption kinetic model Lagergren, (1898) is expressed as

$$\frac{dq_t}{dt} = k_l(q_e - q_t) \qquad (5)$$

where $q_e$ and $q_t$ (mg/g), adsorption capacity at equilibrium and at time $t$, respectively; $k_l$, rate constant of pseudo first-order adsorption process. After integration and applying initial conditions ($t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$), integrated form of Eq. (3) becomes

$$\log(q_e - q_t) = \log(q_e) - \frac{k_l}{2.303}t \qquad (6)$$

**The pseudo second-order equation**

(Ho et al., 1996; Ho and Mckay 1999; Ho et al., 2000; Demirbas et al., 2004)

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \qquad (7)$$

where $k_2$ is rate constant of Ho & McKay equation (g/mg/min). For boundary condition ($t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$), integrated form of Eq. (7) becomes

$$\frac{l}{(q_e - q_t)} = \frac{l}{q_e} + kt \qquad (8)$$

**The Elovich equation**

The Elovich model equation is generally expressed as (Chien and Clayton, 1980**;** Demirbas, et al., (2004))

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \qquad (9)$$

where:
$\alpha$ is the initial adsorption rate (mg/g.min), $\beta$ is the desorption constant (g/mg) during any one experiment. To simplify the Elovich equation, Chien and Clayton (1980) assumed $\alpha\beta t >> t$ and by applying the boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$ Eq. (9) becomes:

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \quad (10)$$

If Cr(III) adsorption fits the Elovich model, a plot of $q_t$ vs. In (t) should yield a linear relationship with a slope of (I/β) and an intercept of (1/ β) In (α β).

**Morris-Weber Equation**

Intraparticle diffusion model is expressed as by Weber and Morris (1963) and Srivastava et al., (1989)**.** Sorption kinetics of Cr(III) was also examined by using Moris-Weber equation as

$$q_t = R_{id}\sqrt{t} \quad (11)$$

where: $q_{t,}$, sorbed concentration at time $t$; $R_{id}$, rate constant of intraparticle transport. $q_t$ was plotted against $t^{1/2}$

# Chapter 3
# Materials and Methods

## 3.1 Collection and characterization of tannery effluent

Tannery effluent and chrome sludge was collected from AV tanneries, Kapurthala, Punjab, India. Characterization of effluent was performed as per procedure described by Clesceri et al., 1998 and APHA, 1998 for physico-chemical parameters such as pH, electrical conductivity, salinity, total solids (TS), total suspended solids (TSS), total dissolved solids (TDS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), colour, chlorides, sulphide, ammonical nitrogen, organic nitrogen and Total Kjeldahl Nitrogen. Besides this other heavy metals such as Cr(III), Fe, Pb, Co, Cu, Cd, Ni, Zn, and secondary trace metals Ca, Mg and Na in tannery effluent were determined based on the standared methods for water and waste water examination.

### 3.1.1 pH

pH measurement was carried out with digital pH meter (Thermo Orion, USA). pH meter was calibrated using standard buffer solution of pH 4.0, 7.0 and 9.2 at room temperature.

### 3.1.2 Electrical conductivity and salinity

Conductivity and salinity measurements were carried out with portable water analysis kit (Thermo Orion, 125 USA), which was calibrated with standard solution of 0.01 M potassium chloride (1413 $\mu S\ cm^{-1}$) and sample measurements were made at room temperature.

### 3.1.3 Determination of total solids (TS)

Total solids refer to the material residue left in the vessel after evaporation of a sample and its subsequent drying in an oven at a defined temperature which includes total suspended solids (TSS), the portion of total solids (TS) retained by a filter and total dissolved solids (TDS) the portion that passes through the filter.

**Apparatus**

- Evaporating dish or beaker
- Dishe of 100 mL capacity made of porcelain, platinum or high silica glass
- Desiccator
- Drying oven for operation at 105±2°C
- Analytical balance
- Hot plate or oven

**Procedure**

1. A clean evaporating beaker kept at 103-105°C for 1 h was cooled in a dessicator and weighed. The process was repeated until a constant weight was obtained.
2. 100 mL of well mixed sample was transferred to preweighed evaporating beaker and placed in an oven at a temperature slightly lower than boiling point to avoid splattering of sample.
3. After the complete evaporation of sample the evaporating beaker was transferred to a drying oven at 103-105°C for 24 h.
4. Beaker was cooled in a desiccator and weighed. The process of drying and cooling, dessication and weighing was repeated until a constant weight was obtained.

**Calculation**

$$\text{Total solids (mg/L)} = \frac{(A-B)}{SampleVolume(mL)} \times 1000$$

where

A= Final weight (mg) of the evaporating beaker + residue

B= Initial weight (mg) of the evaporating beaker

### 3.1.4 Total dissolved solids (TDS)

**Apparatus**

- Evaporating dish or beaker
- Whatman filter paper no. 1
- Filteration apparatus
- Drying oven for evaporation at 105°C±2°C
- Analytical balance
- Desiccator

**Procedure**

1. 100 mL of sample was well mixed and filtered through Whatman filter paper no. 1. The samples were transferred to preweighed beaker or evaporating dish and placed in oven at 103°C-105°C.
2. After complete evaporation of water from the residues, the samples were cooled in desiccator to balance temperature and weighed. This procedure is repeated until a constant weight of the sample is obtained.
3. Duplicates were also maintained at the same set of conditions. The total dissolved solid in the sample was calculated with the following formula.

**Calculation**

Total dissolved solids (TDS) mg/L $= \frac{(A-B)}{Sample Volume(mL)} \times 1000$

where

A = Final weight (mg) of the evaporating beaker + residue

B = Initial weight (mg) of the evaporating beaker

### 3.1.5 Total suspended solids (TSS)

**Apparatus**

- Glass-microfibre filter
- Filtration apparatus with suction flask
- Desiccator
- Analytical balance
- Drying oven for evaporation at 103°C-105°C
- Vacuum pump

**Procedure**

1. The whatman filter paper kept in an oven was cooled in a dessicator and weighed to note the intial weight. The above step was repeated until a constant weight of whatman filter paper was obtained.
2. 100 mL of well-mixed sample was filtered through whatman filter paper using filtration assembly.

3. Filter paper was carefully removed from filtration apparatus and transferred to oven for drying.
4. The filter paper was dried for at least 24 h at 103 to 105°C in an oven, cooled in a dedicator and weighed. The cycle of drying, cooling in desiccator and weighing was repeated until a constant weight was obtained.

**Calculations**

Suspended solids (mg/L) = Total residue - Dissolved residue

Total suspended solids (TSS) mg /L $= \frac{(A-B)}{SampleVolume(mL)} \times 1000$

where

A = Final weight (mg) of whatman filter paper and dried residue

B = Initial weight (mg) of whatman filter paper

### 3.1.6 Biochemical oxygen demand (BOD)

**Apparatus**

- 300 mL capacity BOD bottles
- BOD incubator, thermostatically controlled at 27°C with dark incubation to exclude light to prevent any photosynthetic production of DO.

**Reagents**

1. **Phosphate buffer solution:** Dissolved 8.5 g C 21.75 g $K_2HPO_4$, 33.4 g $Na_2HPO_4.7H_2O$ and 1.7g $NH_4Cl$ in about 500 mL distilled water and diluted to 1 L. The pH was adjusted to 7.2.
2. **Magnesium sulfate solution:** Dissolved 22.5 g $MgSO_4.7H_2O$ in distilled water and diluted to 1 L.
3. **Calcium chloride solution:** Dissolved 27.5 g $CaCl_2$ in distilled water and diluted to 1 L.
4. **Ferric chloride solution:** Dissolved 0.25 $FeCl_3.6H_2O$ in distilled water and diluted to 1 L.
5. **Acid and alkali solutions (1 N):**
   **Acid solution:** 28 mL $H_2SO_4$ (concentrated) was added to distilled water and diluted to 1 L.
   **Alkali solution:** Dissolved 40 g NaOH in distilled water and diluted to 1 L.
6. **Glucose-glutamic acid solution:** Reagent-grade glucose and reagent grade glutamic acid was dried at 103°C for 1 h. 150 mg glucose and 150 mg glutamic acid was added to distilled water and diluted to 1 L. It was prepared fresh.

7. **Standard sodium thiosulfate solution:** Dissolved 6.205 g $Na_2S_2O_3.5H_2O$ in distilled water. 1.5 mL of 6 M NaOH or 0.4 g solid NaOH was added and diluted to 1 L. It was standardized with $K_2Cr_2O_7$ solution.

**Procedure**

1. **Preparation of dilution water:**

   Desired volume of water (for 1 sample in duplicate, 5 litre of distilled water was required) and 1 mL/L each of phosphate buffer, $MgSO_4$, $CaCl_2$, $FeCl_3$ and seed was added in bottle. Dilution water was saturated with DO by aerating with organic-free filtered air for 8-10 h.

2. **Sample pretreatment; Sample containing caustic alkalinity or acidity:**

   Sample was neutralized to pH 6.5 to 7.5 with a solution of $H_2SO_4$ or NaOH of such strength that quantity of reagent did not dilute the sample by more than 0.5%.

3. **Dilution technique:**

   10 mL of seed per litre of dilution water was added in already saturated dilution water. Appropriate volume of the sample was added in the BOD bottle of known capacity. 4 BOD bottles were filled by siphoning to avoid air bubble. BOD bottles were stoppered and mixed well. Initial DO of two BOD bottles were determined by iodometric methods and remaining two bottles were incubated for 3 days at 27$^{o}$C.

4. **Determination of initial DO:**

   Iodometric method was used to determine the initial DO of all the samples and blank.

5. **Dilution water blank:**

   Dilution water was used as blank to check on quality of unseeded dilution water and cleanliness of incubation bottles. Bottle of blank sample (without sample, i.e. dilution water only) was incubated and initial and final DO was determined by the above tritimetric method.

6. **Determination of final DO:**

   After 3 days incubation determined the DO in sample and blank by iodometric method.

**Calculations** $$BOD_3 mg/L = \frac{(D_1 - D_2) - (B_1 - B_2)F}{P}$$

where

$D_1$ = Initial DO of diluted sample/standard (mg/L)

$D_2$ = Final DO of diluted sample/standard after 3 days incubation at $27^oC$ (mg/L)

P = Decimal volumetric fraction of sample used

$B_1$ = Initial DO of seed control (blank) (mg/L)

$B_2$ = Final DO of seed control after 3 days incubation at $27^oC$ (mg/L)

F = Ratio of seed in diluted sample to seed in seed control (% seed in diluted sample)/(% seed in seed control)

### 3.1.7 Chemical oxygen demand (COD):

**Reflux Apparatus**

- 500 mL of round bottom flask
- Condenser
- Hot plate/ heating mantle

**Reagents**

**Standard potassium dichromate solution (0.0415 M):** 12.259 g $K_2Cr_2O_7$ dried at 103°C for 2 h, was dissolved in distilled water and diluted to 1 L.

**Sulfuric acid reagent:** Added $Ag_2SO_4$ reagent, crystal or power to concentrated $H_2SO_4$ at the rate of 5.59 $Ag_2SO_4$ per kg $H_2SO_4$. The solution was allowed to stand for 1 to 2 days to dissolve $Ag_2SO_4$.

**Standard ferrous ammonium sulfate (FAS) titrant (0.25 M):** Dissolved 98g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water and 20 mL of concentrated $H_2SO_4$ was added and diluted to 1 L.

The above solution was standardized against standard $K_2Cr_2O_7$ solution before using as follows:

10 mL of $K_2Cr_2O_7$ standard was diluted to about 100 mL. 30 mL of concentrated $H_2SO_4$ was added. Titration was done with FAS titrant using 0.10 to 0.15 mL (2 to 3 drops) ferroin indicator.

$$\text{Molarity of FAS solution} = \frac{\text{Volume of 0.0417 M } K_2Cr_2O_7 \text{ solution (mL)} \times 0.25}{\text{Volume of FAS used in titration (mL)}}$$

**Ferroin indicator solution:** Dissolved 1.485 g 1, 10-phenanthroline monohydrate and 695 mg $FeSO_4$. $7H_2O$ in distilled water and diluted to 100 mL.

**Mercuric sulfate ($HgSO_4$) powder:**

**Potassium hydrogen phthalate (KHP) standard:** 425 mg of potassium hydrogen phthalate ($HOOCC_6H_4COOK$) was dissolved in distilled water and diluted to 1 L. KHP has a theoretical COD of 1.176 mg $O_2$/mg and this solution has a theoretical COD of 500 $\mu gO_2$/mL. This solution was stable when refrigerated for up to 3 months in the absence of visible biological growth.

**Procedure**

**Treatment of samples with COD of 750 mg $O_2$/L:**

1. 50 mL of sample was taken in 500 mL refluxing flask. 1 g of $HgSO_4$ and few glass beads were added and than 5 mL concentrated $H_2SO_4$ was added very slowly with constant stirring.
2. 25 mL of 0.09 M $K_2CrO_7$ was added and mixed with 70 mL of sulfuric acid with constant stirring. The flasks were attached to the condenser turned on cooling water and refluxed for 2 h. The sample mixture was diluted with two volumes of distilled water and cooled at room temperature.
3. The excess of $K_2CrO_7$ in sample and blank was titrated with ferrous ammonium sulphate (FAS) using 2-3 drops of ferroin indicator. The end point was the sharp color change from blue green to reddish brown and the blue green color reappear.

**Calculations**

The chemical oxygen demand was calculated with the following formula**:**

$$\text{COD as mg } O_2/\text{L} = \frac{(A-B)\times M}{Sample Volume(mL)} \times 8000$$

where

A= mL of FAS used for blank

B= mL of FAS used for sample/standard

M= Molarity of FAS

### 3.1.8 Colour

**Apparatus**

- Aspirator vacuum
- Filter papers
- Spectrophotometer

**Reagents**

**Standard solution, 500 platinum-cobalt units:**

1.246 g potassium chloroplatinate $K_2PtCl_6$ (equivalent to 500 mg metallic Pt) and 1.0 g crystallized cobaltous chloride $COCl_2.6H_2O$ (equivalent to about 250 mg metallic Co) dissolved in distilled water with 100 mL of concentrated HCl and diluted to 1 L with distilled water. This stock standard has a colour of 500 units.

**Procedure**

1. Sample pH was adjusted to 7.0-7.5 by using $H_2SO_4$ and NaOH (1N) the resulting volume change does not exceed 3%. A standard pH is necessary because of variation in colour with pH.
2. Suspended particles were removed by the filtration using Whatman No. 1 filter paper. 25 mL of distilled water was taken in the sample cell. Sample cell was placed in the cell holder of DR 2000 Spectrophotometer.

3. Absorbance as outlined in operation manual of DR 2000 was measured. The results in platinum-cobalt units were displayed. Similarly 25 mL of filtered sample was taken in sample cell and the colour unit in platinum-cobalt was noted in similar manner.

**Accuracy check**

A 250 platinum-cobalt unit standard was made by pipetting 50 mL of 500 platinum-cobalt unit standard into a 100 mL volumetric flask and diluted to volume with deionized water.

**Calculations**

Calculate colour units by the following equations:

$$\text{Colour units} = \frac{(A \times 25)}{B}$$

where

A = Colour obtained of the diluted sample

B = mL of sample taken for dilution

Note: The above procedure is for the estimation of true colour. If apparent colour has to be determined, then do not filter the sample. Proceed as such by taking 25 mL of unfiltered samples in the sample cell.

### 3.1.9 Chloride ($Cl^-$)

**Apparatus**

- Erlenmeyer flask, 250 mL
- Burette, 50 mL
- Pipettes and other usual glasswares

**Reagents**

**Standard sodium chloride 0.0141 M (0.0141 N):**

824.0 mg NaCl was dissolved (dried at 140°C) in distilled water and diluted to 1 L; 1 mL ≡ 500 µg $Cl^-$.

**Standard silver nitrate titrant 0.0141 M (0.0141 N):** 2.395 g $AgNO_3$ was dissolved in distilled water and diluted to 1 L. Standardized against NaCl and stored in a brown bottle.

**Potassium chromate indicator solution:** 50 g $K_2CrO_4$ was dissolved in a 1 L of distilled water. $AgNO_3$ solution was added until a definite red precipitate was formed. Than stand for 12 h filtered and diluted to 1 L with distilled water.

**Special reagents for removal of interference**

**Aluminum hydroxide suspension:**

125 g aluminum potassium sulphate or aluminium ammonium sulphate was dissolved in 1 L distilled water and heated at 60°C and then added 55 mL concentrated $NH_4OH$ slowly with constant stirring. It was allowed to stand for 1 h then transfered to a large bottle and washed the precipitates by successive addition of water with thorough mixing and decanting until it was free from chloride. When freshly prepared, the suspension occupies a volume of approximately 1 L.

**Phenolphthalein indicator solution:**

Sodium hydroxide, NaOH, 1(N)
Sulphuric acid, $H_2SO_4$, 1(N)
Hydrogen peroxide, $H_2O_2$, 30%

**Procedure**

**Sample preparation:**

100 mL sample or a suitable portion was diluted to 100 mL. If the sample is coloured, 3 mL of $Al(OH)_3$ was added to the suspension, mixed and kept for sometime to settle and then filtered. If sulphide, sulphite or thiosulphate were present, 1 mL $H_2O_2$ was added and stirred for 1 min.

**Titration**

Sample pH was adjusted between 7 to 10 with $H_2SO_4$ or NaOH if it was not in this range. 1 mL $K_2CrO_4$ indicator solution was added. The sample and blank were titrated with standardized $AgNO_3$ titrant to a pinkish yellow end point.

**Calculations**

$$\text{mg Cl}^-/\text{L} = \frac{(A - B) \times N \times 35.45}{Sample Volume(mL)}$$

where

A = mL titrant for sample

B = mL titrant for blank

N = Normality of $AgNO_3$

Note: For coloured samples, it is advisable to use either ion selective method or ion chromatography method.

**3.1.10 Sulphide**

**Reagents**

**Zinc acetate solution (2N):** Dissolved 220 g Zinc acetate $Zn(C_2H_3O_2)_2.2H_2O$ in 1 L.

**Sodium hydroxide (6N):** 240 g NaOH dissolved in 1 L.

**Potassium iodine solution:** A 100 g/L solution of potassium iodide was prepared in distilled water and stored in dark bottle.

**Sulphuric acid dilution:** Concentrated $H_2SO_4$ 98% (36N) was added slowly to water and diluted 3 times.

**Iodine solution (0.125N):** Dissolved 25 g of potassium iodine in 50 mL of water in a volumetric flask. Then 15.9 g of iodine and potassium iodide solution were added to 1 L flask and final volume was made up to 1 L.

**Potassium iodate (0.125N):** 4.458 g potassium iodate dissolved in 1 L distilled water.

**Sodium thiosulphate (0.125N):** Dissolved 31.2 g sodium pentahydrate, $Na_2S_2O_3.5H_2O$ in 1 L of freshly boiled and cooled water. 3 drops of chloroform was added to stabilize the solution and stored in a dark bottle.

**Starch indicator solution:** 5 g of soluble starch was added into 1 L of boilied water and allowed to cool before use.

**Procedure**

1. 500 mL sample was taken in a 1 L distillation flask. 1 mL of 2N $Zn(CH_3COO)_2$ (200g/L) and 2 mL of 1N NaOH were added.
2. 20 mL of 18N $H_2SO_4$ was added and quickly distiled the solution (Precipitate of ZnS disloves) into 200 mL of 2.2 % $Zn(CH_3COO)_2$
3. The receiver flask was disconnected, and excess of 0.05 N $I_2$ solution (10 mL) was added and acidified with $H_2SO_4$ (124 mL/500 mL deionized water).
4. After 20 min. the excess $I_2$ was titrated with 0.05 N $Na_2S_2O_3$ using starch indicator until the blue colour disappeared.

**Calculation**

mg/L Sulphide as $S^{2-} = \frac{2000 \times V_1}{V_2}$

where

$V_1$ = Volume of 0.125 N iodine consumed (mL)

$V_2$ = Volume of sample (mL)

**3.1.11 Ammonical nitrogen**

In sewage and polluted water most of the nitrogen is in the form of organic (protein) nitrogen and ammonia. As time progresses, the organic nitrogen is gradually converted to ammonical nitrogen and later on if aerobic conditions prevails it gets oxidized to Nitrate ($NO_2$-N). Nitrites are products of incomplete oxidation.

**Reagent**

**Potassium per manganate (0.32%):** 3.2 g of $KMnO_4$ was dissolved in water and final volume made up to 1 L.

**NaOH (2.5%):** 25 g of sodium hydroxide pellets was dissolved in water and volume made up to 1 L.

**boric acid (2%):** 20 g of boric acid powder was dissolved in warm $H_2O$ by stirring and diluted to 1 L.

**Mixed indicator:** 0.066 g of methyl red and 0.099 g of bromocresol green was dissolved in 100 mL of ethyl alcohol. 20 mL of the mixed indicator was added to each litre of 2% boric acid solution and final pH was adjusted to 4.5 with dilute HCl or dilutes NaOH.

**Potassium hydrogen phthalate (0.1N)**: Dissolved 20.422 g of the salt in water and diluted to 1 L.

**NaOH (0.1 N):** 4 g of NaOH was dissolved in water and diluted to 1 L and then standardized against 0.1 N potassium hydrogen pthalate solution.

**$H_2SO_4$ (0.02 N):** 0.1 N $H_2SO_4$ was prepared by adding 2.8 mL of concentrated $H_2SO_4$ to about 990 mL of distilled water. From this 0.02 N $H_2SO_4$ was made by diluting a suitable volume five times with distilled water and standardized against 0.1 N NaOH.

**Procedure**

1. 50 mL of boric acid was put in a washed and dried conical flask.
2. 500 mL of sample was taken in Kjeldahl flask containing glass beads. 2 mL NaOH (6N) was added to adjust the pH to 9.5 and 25 mL of borate buffer was added.
3. Make the volume 300 mL in conical flask of distillate titrated with 0.02N $H_2SO_4$ with mixed indicator (methylene blue, methyl red 6-7 drops) end point was green.
4. Dissolved 200 mg methyl red indicators in 100 mL of 95% ethyl or isopropyl alcohol.
5. Dissolved 100 mg methylene blue in 50 mL 95% ethyl or isopropyl alcohol combine solutions prepared monthly.
6. **Boric acid solution:** 20 g $H_3BO_3$ was dissolved in desired volume of distilled water and 10 mL of mixed indicator solution was added and volume was made upto 1 L.
7. Ammonia in distillate was titrated with standard 0.02N $H_2SO_4$ until indicator turns pale lavender.

**Calculation**

$$\text{Liquid Sample} = \frac{(A - B) \times 0.1 \times 1000 \times 14}{Sample Volume(mL)}$$

where

A= Titrant volume (0.02N $H_2SO_4$) for sample

B= Titrant volume (0.02N $H_2SO_4$) for blank

### 3.1.12 Total Kjeldahl nitrogen

Kjeldahl Nitrogen is the nitrogen in tri negative state. This includes ammonical nitrogen and organic nitrogen and is referred to as TKN (Total Kjeldahl Nitrogen). This does not account for nitrogen in the form of azides, azo, hydrazone, nitrate and other nitro derivatives. In water and wastewater TKN is a measure of nitrogen in proteins and other degenerative products like peptones and amino acids. Higher values indicate gross pollution status.

**Reagents**

**Digestion Mixture**: 1g HgO, 5 g $CuSO_4$, 100 g $K_2SO_4$, 2:1:20 ratio, 0.1N $H_2SO_4$, 2.8 mL of concentrated $H_2SO_4$ in 990 mL of $H_2O$, 0.1NaOH, 4 g of NaOH in 1 L of distilled water.

Boric acid

Phenolphatheline

**Apparatus**

- Kjeldahl flask
- Round bottom flask

**Procedure**

1. 5 mL of sample was taken in Kjeldahl flask than 10 g of $CuSO_4$, 0.3 g of $K_2SO_4$ and 30 mL of concentrated $H_2SO_4$ acid was added.
2. The flask was kept on the digestion 3-4 h.
3. The contents from Kjeldahl flask were transferred to round bottom flask after making volume upto 300 mL and added 20 mL of 40% NaOH.
4. Distillation was done in flask for 30 min and ammonia liberated was collected in the flask containing 50 mL of 0.1N $H_2SO_4$.
5. Titrate with 0.1N NaOH using phenolphatheline indicator to the end point light pink colour.

**Calculation**

$$\text{Liquid Sample} = \frac{(A-B)\times 0.1\times 1000\times 14}{Sample Volume(mL)}$$

where

A= Titrant volume (0.1N NaOH) for sample

B= Titrant volume (0.1N NaOH) for blank

### 3.1.13 Heavy metal analysis

Tannery effluent sample was acidified with 2-3 drops of concentrated HCl, filtered and centrifuged. Thereafter, lead, chromium, iron, nickel and zinc were analyzed by atomic absorption spectrophotometer (*GBC 932 AA, Australia*) using single element hollow cathode lamp with detection limits of AAS are 0.01 mg/L for lead, 0.003 mg/L for chromium, 0.005 mg/L for iron, 0.009 mg/L for nickel and 0.0005 mg/L for zinc. Analytical wavelength used for the various metals were 357.9 nm for chromium, 248.3 nm for iron, 217.0 nm for lead, 232.0 nm for nickel and 213.9 nm for zinc. Periodically the instrument response was checked throughout the analysis with known standards. Samples were read three times and a mean value and relative standard deviation were computed.

## 3.2 Collection and characterization of microbial biomass from fermentation industry

Microbial biomasses generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy (fermentation industry) Paonta Sahib, Himachal Pradesh, India. This biomass was characterized for physical and chemical parameter such as pH, electrical conductivity, moisture, bulk density, ash content, CHN analysis and calorific value as per the procedure Clesceri et al., (1998) and heavy metals Fe, Cr, Ni, Zn, Pb analyzed by AAS.

### 3.2.1 pH

Ten g of microbial biomass was placed in a 100 mL beaker and 20 mL of distilled water was added and the microbial biomass was stirred well for five minutes and kept undisturbed for some time followed by stirring again. pH was measured using a Thermo Orion Model 290 pH meter after calibration with buffers of pH 4.0, 7.0 and 9.2.

### 3.2.2 Electrical conductivity

Ten g of microbial biomass was placed in a 100 mL beaker and 20 mL distilled water was added. The biomass-water mixture was allowed to stand undisturbed until the biomass settled completely. The conductivity meter (Orion Model 125) was calibrated with 0.01 M potassium chloride (1413 µS $cm^{-1}$).

### 3.2.3 Moisture

The beaker contaning microbial biomass with moisture was weighed. Then oven dried, powdered microbial biomass sample weighed followed by overnight drying at 80º C in the oven in order to obtain constant weight.

**Calculation**

Moisture content of microbial biomass was determined as follows:

$$Moisture\ content\ (\%) = \frac{Mass\ of\ water}{Oven - Dry\ mass} \times 100$$

### 3.2.4 Bulk density

The specific gravity bottle was weighed and the volume of water, which could fill it up to the brim, was measured. The bottle was filled with biomass and weighed.

**Calculation**

Bulk density of the microbial biomass = $\frac{(W_2 - W_1)}{V}\ g\ \text{cm}^{-1}$

where

Weight of empty bottle = $W_1$

Weight of bottle and biomass = $W_2$

Weight of biomass = $W_2$-$W_1$

Volume of the biomass or volume of water needed to fill the bottle = $V$ mL

### 3.2.5 Ash

Ash analysis was carried out as per the procedure mentioned in Indian Standard IS 10158 (1982). The oven dried, powdered samples in pre-weighted crucible were incinerated at 600°C in muffle furnace for 2 h. Thereafter, residual inorganic matter/ash was recorded. Metal laden microbial biomass was dried and ignited at 600°C. 2 g of ash containing chromium was suspended in 20 mL of 0.1 N HCl leached and filtered by qualitative filter paper and final volume was made up to50 mL with milli Q water in volumetric flask. Thereafter, chromium content in ash was analyzed with atomic absorption spectrophotometer.

**Calculation**

Ash (%) = $(W_1 - W_2) \times 100 / S$ (g)

where

$W_1$= weight empty crucible

$W_2$ = weight crucible + ash
S = Sample weight

### 3.2.6 CHN analysis

A CHN Analyzer is a scientific instrument which can determine the elemental composition of a sample. The name derives from the three primary elements measured by the device carbon (C), hydrogen (H) and nitrogen (N). Sulfur (S) and oxygen (O) can also be measured. The analyzer uses a combustion process to break down substances into simple compounds which are then measured by separating out inorganic carbon using a solvent, organic carbon in a sample can be measured using this device as well.

### 3.2.7 Calorimetric analysis

Calorimetric analysis was performed on metal laden biomass samples using isothermal bomb calorimeter (Advance Research Instrument Company Okhla Industrial Phase 1 New Delhi, India). Biomass samples were filled into gelatinous capsule. Approximately 1g of material was ignited at 20 atm. oxygen in an isothermal bomb calorimeter including correction for fuse wire combustion. Benzoic acid standards tested as unknowns were recorded within 0.1% of the standard value.

**Calculation**

GCV (Gross Calorific value) = $Q (\Delta T + 0.01) - h / W$

where

Q = Water equivalent (Pre-determined value)

W = Sample weight

$\Delta T = T_2 - T_1$

h = Calorific value of the cotton thread which is used for burning of thread. The caloric unit used here is the Kilocalorie (Kcal) equal to $10^3$ gram calories (1Kcal = 4.184KJ) results were expressed as ash free caloric value (Kcal/g).

### 3.2.8 Heavy metal analysis

The microbial biomass was dried and digested with aqua-regia (hydrochloric acid: nitric acid, in the ratio of 3:1) to dryness. Heavy metals were analyzed by suspending the residue in 50% HCl and filter the solution through Whatman filter paper (42). Thereafter, chromium, lead, nickel, iron and zinc were analyzed by atomic absorption spectrophotometer (*GBC932AA, Australia*) using single element hallow cathode lamp and the detection limits of AAS are 0.0 mg/L for lead, 0.003 mg/L for chromium, 0.005 mg/L for iron, 0.009 mg/L for nickel and 0.0005 mg/L for zinc. Analylical wavelength used for the various metals was 357.9 nm for chromium, 248.3 nm for iron, 217.0 nm for lead, 232.0 nm for nickel and 213.9 nm for zinc. Periodically the instrument response was checked through out the analysis with known standards. Samples were read three times and a mean value and relative standard deviation were computed.

## 3.3 Isolation and screening of chromium reducing microbes from tannery efflluent

### 3.3.1 Isolation of bacterial consortium

Chromium reducing bacterial consortium was isolated from tannery effluent and chrome sludge as per the procedures given by Philip et al., 1998 and Camargo et al., 2003. 10 mL of effluent sample or chrome sludge was added to 100 mL of complete medium (M1) consisting of bactotryptone: 10 g; yeast extract: 5g; $NaH_2PO_4$: 6.75 g; glucose (C source): 5 g (0.5%) in 1 L of distilled water with pH 7.0 and incubated at 28±2°C for 24 h at 120 rpm in an orbital shaker. After 24 h, when there was a significant growth, 1mL of culture was transferred to 100 mL of fresh M1 medium containing 0.25 mM Cr(VI) and incubated for another 24 h and serial 10 fold dilution ($10^1$-$10^6$) were plated on LB (Luria Broth) agar plates and incubated at 28±2°C. Colonies of different morphologies were selected and streaked on LB agar plates and incubated at 28±2°C for 24 h. Growth and chromium removal efficiency of the isolated strains was checked in minimal medium (M2) consisting of yeast extract: 5 g; $NH_4Cl$: 0.03; $K_2HPO_4$: 0.03g; $KH_2PO_4$, 0.05 g; NaCl, 0.01; and $MgSO_4.7H_2O$, 0.01; pH 7.2 containing 25 mg/L chromium supplemented with 0.5% glucose as a carbon source.

### 3.3.2 Development of bacterial consortium and screening of bacterial isolates

Bacterial isolates were grown in 100 mL of M1 and M2 in 250 of Erlenmeyer flask containing different concentration 25, 50, 75, 100 mg/L of Cr(VI) along with control without chromium. The stock solution containing 1000 mg/L of Cr(VI) (2.83 g of potassium dichromate in 1000 mL of milli Q water) was prepared from analytic grade of Cr(VI). Filter sterilized Cr(VI) solution was added to the autoclaved

medium at different concentration (25, 50, 75, 100 mg/L) at the time of inoculation. Over night culture (1 OD) was used as inoculum and incubated for 24-48 h on a rotary shaker (Orbitek Scigenis Biotech) at 120 rpm in medium. The OD and residual concentration of chromium content in medium was determined.

### 3.3.3 Selection of Cr(VI) reducing bacterial isolates

Single colony isolation was carried out using streak plate technique. Culture grown in liquid medium was streaked onto agar plates aseptically and incubated at 28±2°C. Repeated streaking of each well-isolated colony was carried out to obtain a pure culture (Madigan et al., 1997).

### 3.3.4 Morphological and biochemical characterization of bacterial isolates

Bacterial isolates were characterized morphologically for the colony shape, colony size, colony elevation and for the presence of spores, capsule and gram character as per the standard protocol (Cappuccino et al., 1987).

**Morphological characterization**

**Gram staining**

Gram staining of bacterial isolates CT4, CT5, CS7 and CS8 was done and the strains were analysed microscopically (Gram, 1884).

**Requirements**

1. **Aqueous crystal Violet (1%):** 1 g crystal violet in 100 mL distilled water.
2. **Gram's iodine:** 2 g potassium iodide in 100 mL distilled water.
3. **Decolorizer:** acetone (50%) and alcohol (50%).
4. **Aqueous safranin (2%):** 2g safranin in 100 mL distilled water.

**Procedure**

1. Preparation of a fixed bacterial smear: A loopful of bacterial inoculum was placed on the slide with the help of an inoculation needle and spread evenly in the center of the slide. The smear was dried and heat-fixed.
2. The slide was placed on a staining rack and flooded with crystal violet for about 1 min.
3. The stain was washed gently with iodine solution and stained with fresh iodine solution for 1 min followed by washing in tap water or by dipping in a beaker containing water.
4. Few drops of decolorizer were added on the smear until colour ceases to come out.

5. Counter-stained with safranin for 10-30 seconds.

7. Again the slide was washed with water and dried with absorbent paper and left for drying by evaporation.

8. The dry slide is a permanent preparation, which was examined under the microscope directly without a cover slip first under low power and then under higher magnification.

**Biochemical characterization**

Biochemical tests (Cappucino et al., 1987) were performed on the isolates.

**Growth on MacConkey agar plates**

All the isolates were streaked on MacConkey Agar and incubated for 24 h at 37°C to determine their Gram character.

**Catalase test**

**Procedure**

1. One drop of 3% $H_2O_2$ was placed on a glass slide.
2. With a sterile inoculating loop, transferred several isolated colonies of the organism to the $H_2O_2$ on the microscope slide.
3. The slide was observed for the formation of gas bubbles for upto 1 min.

**Oxidase test**

**Procedure**

1. Added 1-2 drops of freshly prepared 3% tetramethyl-p- phenylene diamine solution on a filter paper.
2. Bacterial isolate was lifted with the help of sterile cotton swab and rubbed on the presoaked tetramethyl-p-phenylenediamine filter paper.
3. A colour change to purple within 30 seconds was looked for.

**Nitrate Reduction Test**

**Procedure**

1. Nitrate agar plates were inoculated with the cultures and incubated at 37°C until growth was observed.

2. To the grown bacterial colony, added few drops of sulphanilic acid and few drops of alphanaphtalamine, each.
3. The appearance of a red colour was noted.

**C.F.U. Count**

**Procedure**

1. Serial dilutions of the samples were prepared
2. 100 μL of each dilution was spread on 0.5% LB agar plates (pH 7.0).
3. The plates were incubated at 30-37°C for 24 h.
4. The colony forming units per mL were calculated for each bacterial sample according to the formula:

$$\text{C.F.U count /mL} = \frac{\text{Number of colonies counted on plate} \times \text{Dilution factor}}{\text{Volume of sample spread on the plate}}$$

### 3.3.5 Molecular identification based on 16S rDNA sequences analysis

Out of 36 isolates four isolates (CT4, CT5, CS7 and CS8) from tannery effluent and chrome sludge which showed highest Cr(VI) reduction, were sent to Institute of Microbial Technology (IMTECH), Chandigarh, India for partial 16S rDNA sequencing. The partial sequences were submitted in National Center for Biotechnology Information (NCBI) Genebank data base and phylogenetic tree was constructed using Neighbor-joining method with MEGA version 4.0 (Tamura et al., 2007).

### 3.3.6 Growth of bacteria in presence of Cr(VI)

Bacterial consortium (CT and CS) and bacterial isolates (CT4, CT5, CS7 and CS8) were grown in minimal media (M2) contatining different concentration of 0-100 mg/L of Cr(VI) and cell density was determined at regular intervals by measuring optical density (OD) at 600 nm. The growth curve was obtained by plotting the absorbance versus time (Cappuccino, 1987). Cells were harvested by centrifugation (6000 rpm, 10 min) and supernatant was analyzed for residual Cr(VI) and total chromium.

**Requirement**

1. Overnight grown bacterial cultures
2. Test tubes containing nutrient broth
3. Mechanical shaker
4. Cuvettes

5. Autopippetes

6. Spectrophotometer

**Procedure**

1. 2 LB tubes containing 5 mL of media and 100 mL of LB in a 250 mL Erlenmeyer flask were prepared and autoclave them.
2. Subculture the bacterial strain in a 5 mL LB tube.
3. Inoculated the 100 mL LB with 1 mL of the fresh overnight grown culture of each (CT4, CT5, CS7 and CS8 and incubate at an optimum temperature 28±2°C.
4. Starting from the zero hour with drawn 3 mL aliquots from the flask at regular intervals of 1 h and optical density was measure at 600 nm.
5. Continued to withdraw the aliquots until the optical density levels off.
6. The growth of isolates was measured as a function of time by plotting the absorbance against each hour.
7. Construct a plot of optical density vs. time and also the log of the cell density vs. time.
8. Generation time was calculated as follows:

During this time the increase in the number of the bacteria (N) per unit time (t) is propotional to the number of bacteria present in the culture.

$$dN/dt = kN \quad (12)$$

where k is a growth constant; on integration this yields

$$\log(N) = \log(N_0) + Kt \quad (13)$$

where K= k/2.3 and $N_0$ = the number of bacteria present when t = 0. If we plot log ($N$) or log (OD) vs time (t), then the slope K often we are interested in the generation time of the culture, the time required for the cells to double in number. In this case N = $2N_0$. Since from equation 13 we now here have log2 = Kt, generation time = t = 0.3/K. Generation time of a bacterial culture was determined from a plot of the logarithm of the optical density vs time.

### 3.3.7 Removal of Cr(VI) and its transformation by bacterial consortia and isolates

Bacterial consortia were inoculated in as above minimal media (M2) and incubated at 28±2°C, 120 rpm. Bacterial cell density of liquid cell culture was determined at regular intervals by measuring optical density at 600 nm. Cr(VI) was supplemented to the final concentration of 5 to 20 mg/L to the exponentially growing cell cultures. The samples were taken at regular intervals. Cells were removed

by centrifugation (6000 rpm, 10 min) and supernatant was analyzed for residual Cr(VI) and total chromium. Similarly bacterial isolates CT4, CT5, CS7 and CS8 were grown separately in minimal media (M2) and incubated at 28±2°C, 120 rpm. Cr(VI) was supplemented to the final concentration of 5 to 20 mg/L to the exponentially growing cell cultures of isolates. The samples were drawn at regular intervals centrifuged (6000 rpm, 10 min) and supernatant was analyzed for residual Cr(VI) and total chromium.

#### 3.3.8 Resting cell assay

The bacterial consortium from tannery effluent (CT) and chrome sludge (CS) was grown for overnight in 300 mL of minimal media (M2) supplemented with 0.5% glucose at 28±2ºC, 120 rpm. Cells were harvested by centrifugation (6000 rpm, 10 min) and were equally divided in three parts and suspended in 25 mL of phosphate buffer, 25 mL of 0.85% saline and 25 mL of deionised sterile water along with Cr(VI) at a concentration of 10 mg/L and incubated at 28±2ºC, 120 rpm and samples were taken after every two hours to evaluated the chromium removal and transformation under aerobic condition and supernatant was analyzed for the residual Cr(VI) and total chromium (Megharaj et al., 2003).

#### 3.3.9 Cell free extract assay

The supernatant obtained from resting cell experiment was supplemented with Cr(VI) to the final concentration of 10 mg/L and analyzed for the Cr(VI) reduction after every 30 min to check the presence and activity of extracellular chromium reductase (Megharaj et al., 2003).

### 3.4 Chromium biosorption studies with microbial biomass

#### 3.4.1 Development of biosorbent from microbial biomass

Microbial biomass *Aspergillus s*p. (MB1) and *Streptomyces* sp. (MB2) are by product derived from fermentation industry. Biomass was washed with water, oven dried at 70°C to 80°C for two days, pulverized using pestle and mortar, sieved by using 0.2 mm sieve in order to obtain uniform particle size and preserved in polythene bags for further chromium adsorption experiment.

#### 3.4.2 Pretreatment of microbial biomass

Microbial biomass from fermentation industry was chemically pre-treated with mineral acids (0.1 M) hydrochloric acid, sulfuric acid and alkali sodium hydroxide (0.1M) and other organic solvents (acetone, chloroform, diethyl ether and methanol). 10 g of the native biomass was mixed with 100 mL

of each solution and agitated at 120 rpm for 6 h at ambient temperature. After each pretreatment, the biomass was washed with distilled water and oven dried at 60°C for 24 h. Alkali treated biomass was washed with distilled water until the pH of the waste solution was in near neutral range (pH 6.8-7.2). Chemically pretreated biomass was used for removal of aqueous solution of chromium (III) as well as tannery effluent.

### 3.4.3 Preparation of aqueous chromium solution

Aqueous solution of chromium was prepared by dissolving required quantity of $K_2Cr_2O_7$ and $Cr_2O_{12}S3_7$ in the distilled water. Stock solution (1000 mg/L) was diluted with distilled water to obtained working solution and desired pH of the solution was adjusted with 1N NaOH and 1N HCl.

### 3.4.4 Batch adsorption studies

Batch sorption experiments were conducted with dried microbial biomass, *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) in 250 mL of Erlenmeyer flask with different biomass concentration (0.25-2% of biomass) and different concentrations (5-50 mg/L) of Cr(III) separately for each chromium along with control without any metal at pH 4 and were agitated at 120 rpm at 28±2°C. Similarly, experiments were conducted at different pH 2, 3, 4 and 5 for removal of Cr(III) from 25 mg/L aqueous solution as well as tannery effluent by 1g of microbial biomass at 28±2°C and 120 rpm. Five mL sample was drawn at different timer (0- 24h), acidified using 2-3 drops concentrated HCl, filtered using qualitative filter paper (equivalent to grade 1) and residual chromium content in sorption medium were analysed by using an atomic absorption spectrophotometer AAS (GBC 932AA Australia). Further studies on chromium removal were carried with synthetic chrome solution at pH 5 and 25 mg/L.

### 3.4.5 Adsorption isotherms

The capacity of the *Aspergillus* sp. (MB1) and *Streptomces* sp. biomass (MB2) was determined with two isotherms models at different concentration of aqueous solution and tannery effluent. Langmuir and Freundlich isotherms were used to analyze the biosorption data.

The Langmuir isotherms model is valid for monolayer adsorption on to surface containing finite number of identical sorption sites which is described by the following

$$q_e = \frac{Q_L b C_e}{1 + b C_e} \qquad (14)$$

where $q_e$ and $Q_L$ are the observed and maximum uptake capacities (mg/g biosorption); $b$ the equilibrium constant; $C_e$ the equilibrium concentration (mg/L). The Langmuir Eq. (14) can be rearranged to linear form for the convenience of plotting and determining the Langmuir constants by the following

$$\frac{C_e}{q_e} = \frac{1}{bQ_L} + \frac{C_e}{Q_L} \quad (15)$$

The Freundlich isotherm model is nonlinear sorption model is purely empirical based on heterogeneous surface, which commonly defined as

$$q_e = K_f C_e^{1/n} \quad (16)$$

where $K_f$ and n are the Freundlich constants related to adsorption capacity and intensity, respectively. The Freundlich equation can be linearized in logarithmic form for determination of Freundlich constants as

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_f \quad (17)$$

The correlation coefficient values were obtained from the Langmuir and Freundlich isotherms.

### 3.4.6 Adsorption kinetics

Adsorption kinetics of Cr(III) study describes the solute uptake rate and this rate controls the residence time of adsorbate uptake at the solid-solution interface. The conformity between experimental data and the models predicted values was expressed by the correlation coefficients ($r^2$ values close or equal to 1). A relatively high $r^2$ value indicates that the model successfully described the kinetics of Cr(III) adsorption.

**Pseudo first-order equation (Lagergren, 1898)**

The pseudo first-order equation (Lagergren, 1898) is generally expressed as follows:

$$\frac{dq_t}{dt} = k_l(q_e - q_t) \quad (18)$$

where: $q_e$ and $q_t$ are the adsorption capacity at equilibrium and at time $t$, respectively (mg/g), $k_l$ is the rate constant of the pseudo first-order adsorption process.

After integration and applying boundary conditions $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, the integrated from of Eq. (18) becomes

$$\log(q_e - q_t) = \log(q_e) - \frac{k_l}{2.303}t \quad (19)$$

The values of *log* ($q_e$-$q_t$) were linearly correlated with *t.* The plot of *log* ($q_e$-$q_t$) vs. *t* should give a linear relation from which $k_l$ and $q_e$ can be determined from the slop and intercept of the plot, respectively.

**Pseudo second-order equation (Ho et al., 2000)**

The pseudo second-order adsorption kinetics rate equation is expressed as (Ho et al., 2000)

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \quad (20)$$

where
$k_2$ is the rate constant of pseudo second-order adsorption (g/mg/min). For the boundary condition $t = 0$ to $t = t$ and $q_t = 0$ to $q_t = q_t$, the integrated form of Eq. (20) becomes:

$$\frac{l}{(q_e - q_t)} = \frac{l}{q_e} + k_2 t \quad (21)$$

This is the integrated rate law for a pseudo second-order reaction. Eq. (21) can be rearranged in a linear from as:

$$\left(\frac{t}{q_t}\right) = \frac{l}{k_2 q_e^{\ 2}} + \frac{l}{q_e}(t) \quad (22)$$

If the initial adsorption rate, *h* (mg/g/min) is:

$$h = k_2 q_e^{\ 2} \quad (23)$$

Using (23) Eq. (22) becomes as:

$$\left(\frac{t}{q_t}\right) = \frac{l}{h} + \frac{l}{q_e}(t) \quad (24)$$

The plot of ($t/q_t$) and t of the Eq. (24) should give a linear relationship from which $q_e$ and $k_2$ can be determined from the slope and intercept of the plot, respectively.

**Elovich equation**

The Elovich model equation is generally expressed as (Chien and Clayton, 1980):

$$\frac{dq_t}{dt} = \alpha \exp(-\beta q_t) \quad (25)$$

where

$\alpha$ is the initial adsorption rate (mg/g min), $\beta$ is the desorption constant (g/mg) during any one experiment. To simplify the Elovich equation, Chien and Clayton (1980) assumed $\alpha\beta t >> t$ and by applying the boundary conditions $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$ Eq. (25) becomes:

$$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta}\ln(t) \quad (26)$$

If Cr(III) adsorption fits the Elovich model, a plot of $q_t$ vs. ln ($t$) should yield a linear relationship with a slope of ($1/\beta$) and an intercept of ($1/\beta$) ln ($\alpha$ $\beta$).

**Morris-Weber equation**

The intraparticle diffusion model of Cr(III) is expressed as by Weber and Morris 1963 and Srivastava et al., 1989. The kinetics of sorption of Cr(III) was also examined by using Moris-Weber equation in the following form:

$$q_t = R_{id}\sqrt{t} \quad (27)$$

where: $q_t$, the sorbed concentration at time $t$, $R_{id}$, the rate constant of interparticle transport. The $q_t$ was plotted against, $t^{1/2}$ . The sorption follows linearity as per Eq (27) with coefficient of determination, $r^2$ (0.831). The value of $R_{id}$ computed from the slope of the plot.

### 3.4.7 Fourier transform infrared (FTIR) analysis

Infrared spectra of the native as well as metal laden biomass were obtained after drying the biomass at 70ºC for which the finely powdered samples (0.1 mg) were encapsulated with potassium bromide to prepare translucent sample disks and spectra were recorded by Fourier transform infrared spectroscope (*BOMEM Hartmann & Braun, Canada*).

### 3.4.8 X- Ray diffraction (XRD) analysis

For XRD analysis, settled mineral residue was removed from the pressure tubes to minimize liquid transfer and dried under anaerobic conditions; the dried solid was smeared on a glass slide. The slides were maintained under an anoxic atmosphere until the time of analysis. The XRD apparatus consisted of two Philips wide-range vertical goniometers with incident-beam 2-θ compensating slits, soller slits, fixed 2-mm receiving slits, diffracted beam graphite monochromators and scintillation counter detectors. The X-ray source was a Philips XRG3100 X-ray generator operating a fixed-anode, long-fine-focus Cu tube at 45 kV, 40 mA (1,800 W). Instrument control was by means of Databox NIMBIM modules (Materials Data, Inc., Livermore, California.). The microbial biomass were dried, sieved (2mm), and ground in an agate mortar for XRD. After packed in the circular cavity of holder, the XRD pattern was obtained on a Siemens D5005 instrument using Cu K α radiation equipped with a diffracted-beam monochromator in the range of 3-40°2θ.

## 3.5 Column sorption studies with microbial biomass

Biosorption of chromium from synthetic and tannery effluent was carried out in columns packed with waste biomass and various parameters such as flow rate, loading time etc were optimized. After the saturation it was regenerated with mineral acids for reuse.

### 3.5.1 Down flow continuous sorption column

Syringe column of 1cm diameter and 5 cm length was packed with 3 g dried microbial biomass to study the removal of Cr(III) in continuous flow sorption column mode. During column sorption an aqueous solution containing 25 mg/L of chromium was passed flow controlling IS unit of drip bottle from the top and the fraction of the elutant were collected at different time interval.

### 3.5.2 Up flow continuous sorption column

Glass column of 20 cm length and 2.5 cm of diameter was packed with 16 g microbial biomass. The aqueous solution of containing 25 mg/L of chromium (pH 5.0) was pumped continuously downward from the top using peristaltic pump (Cole Parmer USA) at a constant flow rate of 200 mL/h. The samples, collected from outlet of the column were analysed for residual chromium concentration by atomic absorption spectrophotometer.

### 3.5.3 Regeneration and reuse of microbial biomass

After the column became saturated, it was regenerated with dilute acid (0.1N HCl).

### 3.5.4 Determination of residual chromium (VI)

In all adsorption and desorption experiments, the residual Cr(VI) was determined spectrophotometrically using 1,5-diphenyl carbazide (DPC) reagent (250 mg of 1,5-diphenyl carbazide in 50 mL of acetone) in acid solution as complexing agent, which reacts with chromate, forming colour complex that absorbs light at 540 nm (Clesceri et al., 1998). The absorbance was measured using UV-VIS spectrophotometer (*Hitachi Japan*) and calibration curve was prepared in the range of 1-10 mg/L

### 3.5.5 Determination of total residual chromium

Sample were drawn at different time intervals and residual concentration of Cr(III) in solution was measured by atomic absorption spectrophotometer (GBC 932AA, Australia). Working standard solution of Cr(III) was prepared from stock (1000 mg/L) procured from Acros Organic Ltd, New Jersey, USA. Triplicates of each sample were analysed and mean value and relative standard deviation as given by AAS were recorded.

The (R %) removal of Cr(III) ions from aqueous solution was calculated using mass balance (Fahim et al., 2006)

$$R(\%) = \frac{(C_i - C_f)}{C_i} \times 100 \qquad (28)$$

$C_i$ and $C_f$ are the initial and final chromium concentrations in mg/L

The reduction of Cr(VI) to Cr(III) was calculated by subtracting concentration of Cr(VI) from total chromium (Ohtake and Hardoyo, 1992; Shen and Wang, 1994).

### 3.5.6 Metal uptake by microbial biomass

The metal-laden biomass was dried and digested with perchloric acid: nitric acid (in the ratio of 3:1) to dryness. Chromium was extracted by suspending the residue in HCl : $H_2O$ (1:1) and filter the solution through Whatman filter paper.

Specific metal uptake was calculated as follows:

$$q_e = \frac{(C_i - C_f)V}{m} \quad (29)$$

where: $C_i$ and $C_f$ are the initial and final chromium concentrations in mg/L respectively, V is the volume of Cr(III) solution in mL, m is the weight of biomass in g.

### 3.5.7 Statistical analysis

All data represents the mean of three independent experiments. All results are discussed by mean±SD values. All statistical analysis was done by using Microsoft Excel 2004, The correlation coefficient ($R^2$) values of the linear form of Langmuir and Freundlich isotherm, pseudo-first-order and pseudo-second-order models were also determined using statistical functions of Microsoft Excel, 2004.

**Standard deviation**

It is convenient to have a measure of variation expressed in the original units of X and this can be done by taking the square root of the variance. This quantity is known as the standard deviation and is,

$$SD = \sqrt{Variance} \quad (30)$$

**Standard error**

The standard error (SE) is a measure of the variation or dispersion of the means of a set of measurements. It is therefore; smaller than the standard deviation of a single series of measurements form the same of population. It is used to compare means with one another.

The variances $S^2$ is given as:

$$S^2 = \text{variance} = \sum\left(X_i - \overline{X}\right)^2 / (n-1) \qquad (31)$$

Therefore,

$$\text{Standard deviation} = \text{square root of variance} = \sqrt{S^2} \qquad (32)$$

$$\text{Standard error} = \sqrt{(\text{variance/sample size})} = \sqrt{(S^2/n)} \qquad (33)$$

Standard error is the standard deviation of the means of measurements. It is an indication of the magnitude of variation between sample mean values. Standard error is also called the standard deviation of the mean.

# Chapter 4
# Results

## 4.1 Characterization of the tannery effluent and microbial biomass from different industries

### 4.1.1 Characterization of tannery effluent

Tannery effluent was collected from A.V. Tanneries, leather complex, Kapurthala, Punjab, in January 2005 and was characterized (Table 3) for various parameters as per procedure given by Clesceri et al., 1998 and APHA 1998.

Table 3: The physico-chemical characteristics of tannery effluent.

| S. No | Parameters | Values | Permissible Limit |
|---|---|---|---|
| 1. | pH | 3.73 | 5.5-9.0 |
| 2. | Temperature (°C) | 22.8 | 45°C |
| 3. | Electric conductivity (mS) | 65.5 | - |
| 4. | Salinity (‰) | 43.4 | - |
| 5. | Total solids (TS) (mg/L) | 96,000 | - |
| 6. | Total dissolved solids (TDS) (mg/L) | 80,000 | 2100 |
| 7. | Total suspended solids (TSS) (mg/L) | 16,000 | 50 |
| 8. | Biochemical oxygen demand (BOD) (mg/L) | 290 | 350 |
| 9. | Chemical oxygen demand (COD) (mg/L) | 332.8 | 250 |
| 10. | Colour (pt.co) | 2150 | 25 |
| 11. | Chlorides (mg/L) | 12,300 | 1000 |
| 12. | Sulphide (mg/L) | 1.35 | - |
| 13. | Ammonical nitrogen (mg/L) | 172.2 | 50 |
| 14. | Organic nitrogen (mg/L) | 37.94 | - |
| 15. | Total Kjeldhl nitrogen (mg/L) | 210.14 | 100 |
| 16. | Cr (III) (mg/L) | 1700.9 | 2.0 |
| 17. | Ca (mg/L) | 154.1 | 200 |
| 18. | Cu (mg/L) | 0.1 | 3.0 |
| 19. | Cd (mg/L) | 0.012 | 1.0 |
| 20. | Co (mg/L) | 0.8125 | - |
| 21. | Fe (mg/L) | 110.11 | - |
| 22. | K (mg/L) | 57 | - |
| 23. | Mg (mg/L) | 115.52 | - |
| 24. | Mn (mg/L) | 0.88 | - |
| 25. | Na (mg/L) | 42.55 | - |
| 26. | Ni (mg/L) | 1.152 | 3.0 |
| 27. | Pb (mg/L) | 15.26 | 1.0 |
| 28. | Zn (mg/L) | 0.793 | 15 |
| 29. | Bacterial count (× cfu/100 mL) | $2.62 \times 10^6$ | $< 10$ |

Tannery effluent was acidic (pH 3.73), greenish in colour (2150 pt.co) and contained chlorides (12,300 mg/L), sulphide (1.35 mg/L). The data shows that 1700.9 mg/L chromium (III) is discharged from tannery. Besides this effluent also contains other heavy metals such as Fe (110.11 mg/L), Pb (15.26 mg/L), Co (0.8125 mg/L), Cu (0.1mg/L), Cd (0.012 mg/L) and secondary element that as i.e. Ca (154.1 mg/L), Mg (115.52 mg/L), Na (42.55 mg/L) etc. This effluent has highest concentration of TS (96,000 mg/L), TDS (80,000 mg/L), TSS (16,000 mg/L), BOD (290 mg/L), COD (332.8 mg/L) and total nitrogen (210.14 mg/L) because raw hides and skins are used as the starting material in tanning process (Table 3).

### 4.1.2 Characterization of microbial biomass

Table 4: Characterization of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2)

| Microbial Biomass | | | |
|---|---|---|---|
| S. No. | Parameters | *Aspergillus* sp. (MB1) | *Streptomyces* sp. (MB2) |
| 1 | pH | 5±0.52 | 6.50±0.43 |
| 2 | Carbon (%) | 46.24±0.20 | 45.77±1.20 |
| 3 | Hydrogen (%) | 6.80±0.42 | 6.79±0.22 |
| 4 | Nitrogen (%) | 3.22±0.54 | 8.53±0.34 |
| 2 | Bulk density (g/cm$^3$) | 0.58±0.35 | 0.70±0.21 |
| 3 | Ash content (%) | 5.58±0.56 | 5.70±0.60 |
| 4 | Moisture content (%) | 7.5±0.52 | 5.0±0.40 |
| 5 | Calorific value (MJ/Kg) | 16.42±1.51 | 17.25±1.30 |

Microbial biomasses which are generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy (fermentation industry) Paonta Sahib, Himachal Pradesh, India and characterized for different physico-chemical parameters (Table 4). Microbial biomass had acidic pH with ash content ranging from 5-6%. The CHN analysis showed that *Streptomyces* sp. (MB2) had high nitrogen content than *Aspergillus* sp. (MB1). Both had calorific value of nearly 16-17 MJ/kg. Processing of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) after collection from industry was ground in a blender and sieved to pass through a 2.0 mm sieve in order to obtained uniform particle size. Further they were washed with distilled water and then dried at 80°C for overnight or till the moisture percentage was below 5% and was used as biosorbent.

## 4.2 Isolation and characterization of chromium reducing microbes from tannery effluent

Isolation and characterization of chromium reducing microbes from tannery effluent and chrome sludge collected from A.V. Tanneries, Kapurthala, Punjab, India was done. Bacterial consortia (CT, CS) were developed and bacterial isolates were analyzed for Cr(VI) transformation and its removal. Bacterial isolates (CT4, CT5, CS7 and CS8) exhibiting efficient transformation were characterized for morphological, biochemical and molecular basis using 16S rDNA sequence analysis. Bacterial isolates were identified as *Raoultella* sp. IS1 (CT4), *Citrobacter* sp. IS2 (CT5), *Bacillus cereus* IS3 (CS7) and *Citrobacter freundii* IS4 (CS8). Growth of bacterial consortium and isolates was studied at different concentration of Cr(VI) and its transformation was studied by resting cell and cell free extract assay.

### 4.2.1 Development of bacterial consortium and screening of bacterial isolates

Chromium removing bacterial consortium was developed from tannery effluent (CT) and chrome sludge (CS) according to the method of Philip et al., (1998) and Camargo et al., (2003). 10 mL of effluent and sludge sample was added to 100 mL of complete medium (M1) with pH 7.0 and incubated at 28±2°C for 24 h at 120 rpm in an orbital shaker. After 24 h, when there was a significant growth, 1mL of culture was transferred to 100 mL of fresh M1 medium containing 0.25 mM Cr(VI) and incubated for another 24 h and serial 10 fold dilution ($10^1$-$10^6$) were plated on LB agar plates and incubated at 28±2°C. Colonies of different morphologies were selected and streaked on agar plates and incubated at 28±2°C for 24 h and were checked for chromium removal in minimal media (M2) in the presence of different concentration (5-25 mg/L) of Cr(VI) (Philip et al., 1998).

Bacterial consortium from tannery effluent (CT) showed 38.4, 30.31, 24.11 and 20.6% removal of Cr(Total), whereas bacterial consortium from chrome sludge (CS) showed 72.8, 61.7, 61.1 and 45.25% removal at 5, 10, 15 and 25 mg/L of Cr(Total) in minimal medium (M2) respectively (Fig. 5). Chromium removing bacterial isolates from bacterial consortium (CT and CS) from tannery effluent and chrome sludge were screened for removal of Cr(VI). All 36 bacterial isolates from tannery effluent and chrome sludge bacterial consortium were able to remove Cr(Total) (Table 5; Fig. 6, 7). Chromium removal by all the 18 isolates (CT1-CT18) from tannery effluent was in the range of 21-38%. Similarly by bacterial isolates (CS1-CS18) from chrome sludge it was in the range of 6-38% (Table 5).

Four isolates CT4, CT5 and CS7, CS8 showed highest Cr(VI) removal in minimal medium (M2) contaning 25 mg/L of Cr(VI). CT4 and CT5 showed 38.73%, 34.10% removal of Cr(VI), whereas removal of Cr(VI) by CS7 and CS8 was 37.33% and 38.83 respectively as compared to other isolates (Table 5, Fig. 5, 6). These were selected for further characterization and biotransformation of Cr(VI).

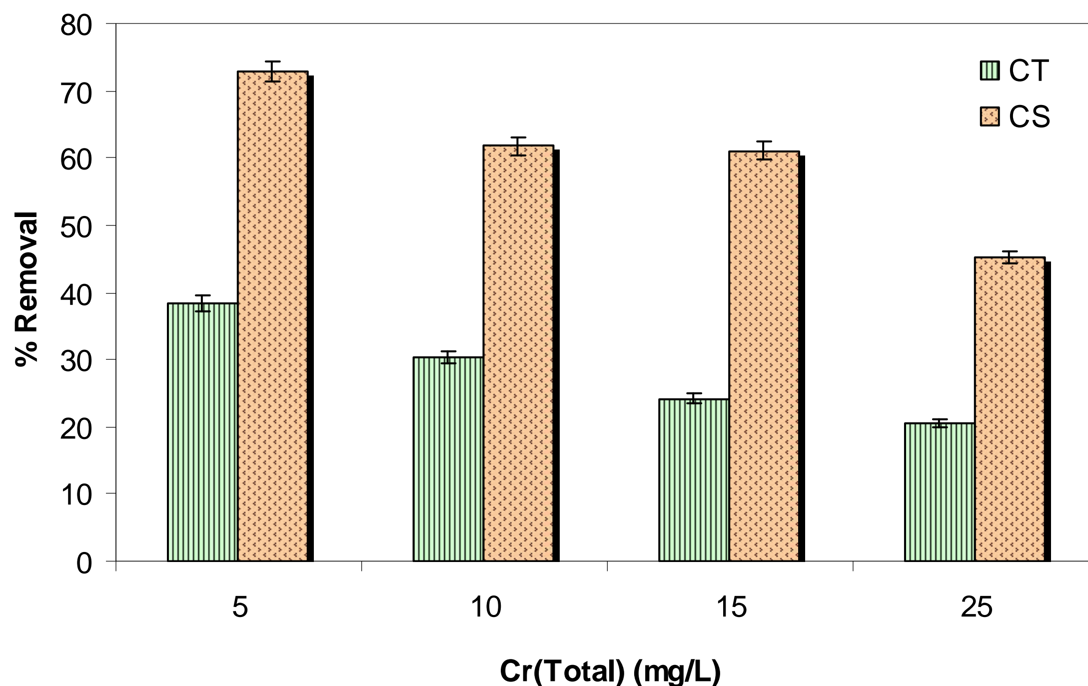



Fig. 5: Removal (%) of Cr(Total) by bacterial consortium from tannery effluent (CT) and chrome sludge (CS) from minimal media (M2) supplemented with different concentration of Cr(VI) after 24 h of incubation.

Table 5: Removal of Cr(total) by bacterial isolates from tannery effluent and chrome sludge consortia.

| S.No. | Bacterial Isolates (tannery effluent) | % Removal | Bacterial Isolates (chrome sludge) | % Removal |
|---|---|---|---|---|
| 1 | CT 1 | 27.05 | CS 1 | 32.43 |
| 2 | CT 2 | 32.10 | CS 2 | 33.03 |
| 3 | CT 3 | 33.93 | CS 3 | 27.00 |
| 4 | **CT 4** | **38.73** | CS 4 | 6.53 |
| 5 | **CT 5** | **34.10** | CS 5 | 16.10 |
| 6 | CT 6 | 29.77 | CS 6 | 25.13 |
| 7 | CT 7 | 27.07 | **CS 7** | **37.33** |
| 8 | CT 8 | 21.23 | **CS 8** | **38.83** |
| 9 | CT 9 | 27.73 | CS 9 | 26.93 |
| 10 | CT 10 | 33.13 | CS 10 | 21.97 |
| 11 | CT 11 | 33.40 | CS 11 | 4.23 |
| 12 | CT 12 | 31.10 | CS 12 | 20.77 |
| 13 | CT 13 | 26.20 | CS 13 | 37.00 |
| 14 | CT 14 | 28.53 | CS 14 | 35.03 |
| 15 | CT 15 | 25.10 | CS 15 | 32.83 |
| 16 | CT 16 | 22.87 | CS 16 | 31.73 |
| 17 | CT 17 | 26.30 | CS 17 | 22.47 |
| 18 | CT 18 | 30.87 | CS 18 | 20.87 |

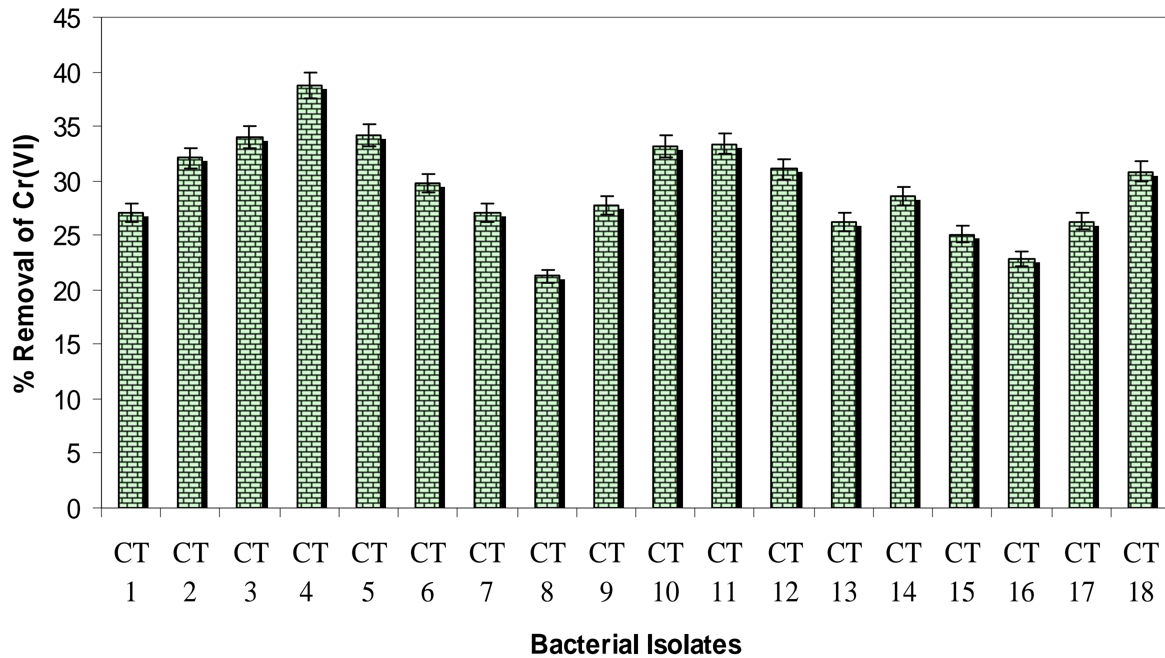



Fig. 6: Cr(VI) removal by different bacterial isolates from tannery effluent.

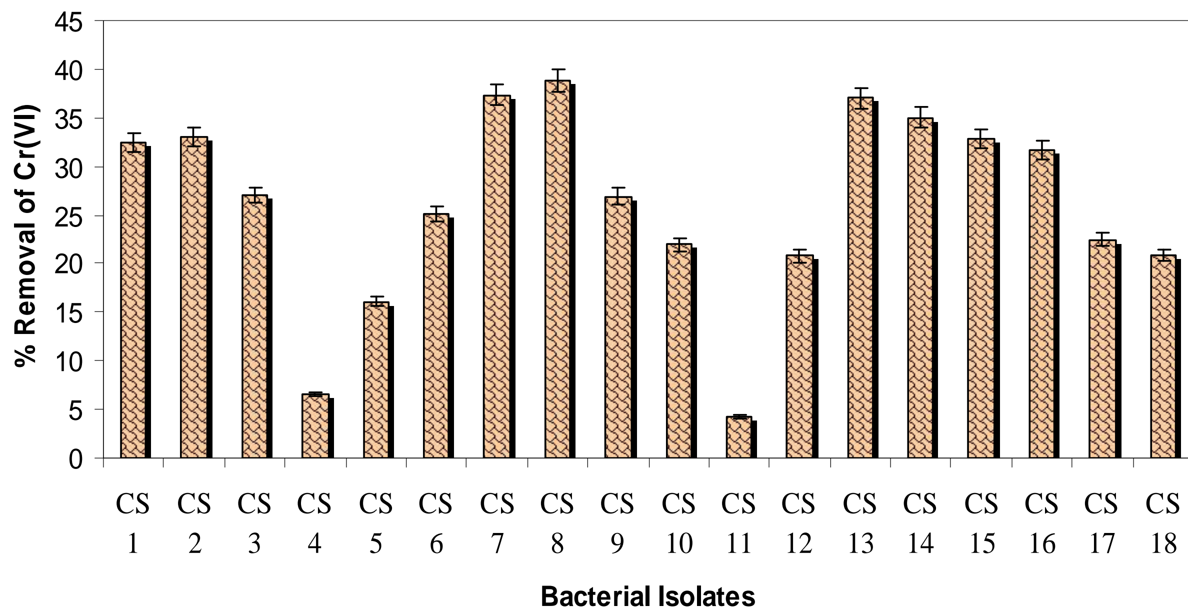



Fig. 7: Cr(VI) removal by different bacterial isolates from chrome sludge.

### 4.2.2 Morphological and biochemical characterization of bacterial isolate

Bacterial isolates were characterized for colony shape, size and elevation and for the presence of spores, capsule, catalase, oxidase and Gram character as per the standard protocol (Cappuccino et al., 1987). The colonies of all the four isolates were circular and convex. CT4, CT5 and CS8 were Gram negative short rods, whereas CS7 was Gram positive short rods. None of the isolate was found to have capsule and only CS7 formed spores (Table 6).

Table 6: Morphological and biochemical characterization of bacterial isolates

| Type of characterization | Bacterial Isolates | | | |
|---|---|---|---|---|
| Morphological | CT4 | CT5 | CS7 | CS8 |
| Colony shape | Circular | Circular | Circular | Circular |
| Colony elevation | Convex | Convex | Convex | Convex |
| Colony size | 1 mm | 1 mm | 0.8 mm | 0.8 mm |
| Cell shape | Short rods | Short rods | Short rods | Short rods |
| Gram staining | – | – | + | – |
| Capsule staining | – | – | – | – |
| Spore staining | – | – | + | – |
| Cultural | | | | |
| Growth on McConkey's Agar | + | + | – | + |
| Biochemical | | | | |
| Catalase | + | + | + | + |
| Oxidase | – | + | – | + |
| Nitrate Reduction | – | + | – | + |

+: positive reaction; –:negative reaction;

The Gram character was also confirmed by MacConkey agar test. MacConkey agar is an enrichment medium on which only Gram negative bacteria can grow and the growth of bacterial isolates (CT4, CT5, CS8) was a further indication of their Gram negative character. Biochemical characterization indicated that all the isolates were catalase positive whereas only CT5 and CS8 were oxidase positive and nitrate reducers (Table 6).

**16S rDNA sequences of the bacterial isolates obtained from Institute of Microbial Technology (IMTECH), Chandigarh**

**Cr(VI) reducing bacteria from tannery effluent**

>*Raoultella* sp, Isolate IS1, 688 bp, partial 16S rDNA

GCGCACGCAGGCGGTCTGTTAAGTCAGATGTGAAATCCCCGGGCTCAACCTGGGAACT
GCATTTGAAACTGGCAGGCTTGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGG
TGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGA
CTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCA
CGCTGTAAACGATGTCGACTTGGAGGTTGTTCCCTTGAGGAGTGGCTTCCGGAGCTAAC
GCGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGAC
GGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTT
ACCTACTCTTGACATCCAGAGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTG
AGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGC
AACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATTCGGTCGGGAACTCAAAGGAGACT
GCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAG
TAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGAC

>*Citrobacter* sp, Isolate IS2, 626 bp, partial 16S rDNA

GGAGGAATACCGGTGGCGAAGGCGGCCCCCTGGACAAAGACTGACGCTCAGGTGCGAA
AGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGA
CTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGACCGCCT
GGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGG
TGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAG
AGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCT
GTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC
CTTTGTTGCCAGCGGTTCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAG
GAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCT
ACAATGGCATATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTAT
GTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGT

**Cr(VI) reducing bacteria from chrome sludge**

>*Bacillus cerrus*, Isolate IS3, 640 bp, partial 16S rDNA

GGCTCAGGATGAACGCTGGCGGCGTGCCTAATACATGCAAGTCGAGCGAATGGATTAAGA
GCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAA
GACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATAACATTTTGAACCGCATGGTT
CGAAATTGAAAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTT
GGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCA
CACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCA
ATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAA
CTCTGTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTA
TCCGGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTAAGTCTGATGTGAAAGCCCA
CGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGAC

>*Citrobacter freundii*, Isolate IS4, 606 bp, partial 16S rDNA

CACATGCAAGTCGAACGGTAGCACAGAGGAGCTTGCTCCTTGGGTGACGAGTGGCGGACG
GGTGAGTAATGTCTGGGAAACTGCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTA
ATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTTCGGGCCTCTTGCCATCGGATGT
GCCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGACGATCCCTAGCT
GGTCTGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGG
CAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGA
AGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGAGGAGGAAGGCGTTGTGGTTAATAACC
GCAGCGATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGT
AATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCT
GTCAAGTCGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCGAAACTGGCAGGC
TAGAGT

### 4.2.3 Molecular identification based on 16S rDNA sequence analysis

16S rDNA sequences of the bacterial isolates as given above were obtained from MTCC, IMTECH, Chandigarh. The partial sequences of all the 16S rDNA of bacterial isolates were BLAST compared with already existing sequences in the database to identify the most probable similarity with high expect value. Multiple sequence alignment of all the sequences obtained with existing sequences in databank was carried out using the program MULTALIN (http://prodes.toulouse.inra.fr/multalin). The phylogenetic tree was constructed based on the sequences of 16S rDNA gene and those obtained from the GenBank database after BLAST analysis using the Neighbor-joining method of MEGA4 software. Branch lengths and scale bar corresponding to the evolutionary distances (% 100) was assigned by MEGA4, measured by the number of nucleotide substitutions between sequences. The BLAST results revealed that the partial sequencing of isolate CT4 (*Raoultella* sp. IS1) and CT5 (*Citrobacter* sp. IS2) had 99-100% homology with *Raoultella* sp. and *Citrobacter* sp. respectively. Further neighbor joining (NJ) tree depicted (Table 7a-7b; Fig. 8) that *Raoultella* sp. AF129441 closely clustered with *R. ornithinolytica* clade with 100% bootstrapping. Likewise, CT5 (*Citrobacter* sp. IS2) clustered with *C. freundii* clade with 99% bootstrapping. 16S rDNA gene (1500 bp) of CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) showed maximum homology with that of *Bacillus* sp. and *Citrobacter* sp. respectively and showed homology with sequences of *Bacillus cereus,* strain RIVM and *Bacillus cereus* NS58, but the best results are shown with *Bacillus sp.* KR076 and probability for CS8 (*Citrobacter freundii* IS4) to be *Citrobacter freundii* strain IRB3 is good enough (Fig. 9). 16S rDNA gene of CS7 (*Bacillus cereus* IS3) showed 99-100 % homology with 16S rDNA gene of *Bacillus cereus* strain RIVM BC00075 (Table 7c, 7d; Fig. 9).

Table 7: Sequences of bacterial isolates which showed homology to the 16S rDNA gene of other bacteria

Table 7 (a): CT4 (*Raoultella* sp. IS1)

| Accession No. | Description | Max score | Total score | Query coverage | E value | Max identity |
|---|---|---|---|---|---|---|
| EF474096.1 | *Raoultella ornithinolytica strain* KNUC188 16S ribosomal DNA gene, partial sequence | 1271 | 1271 | 100% | 0.0 | 100% |
| AB004756.2 | *Raoultella ornithinolytica* 16S ribosomal DNA gene, partial sequence | 1271 | 1271 | 100% | 0.0 | 100% |
| EF057396.1 | *R. ornithinolytica* strain D70 activated sludge gene for 16S ribosomal DNA, partial sequence | 1271 | 1271 | 100% | 0.0 | 100% |
| U78182.1 | KOU78182 *Klebsiella ornithinolytica* 16S ribosomal DNA gene, partial sequence | 1271 | 1271 | 100% | 0.0 | 100% |

Table 7 (b): CT5 (*Citrobacter* sp. IS2)

| Accession No. | Description | Max score | Total score | Query coverage | E value | Max identity |
|---|---|---|---|---|---|---|
| DQ347839.1 | *Citrobacter freundii* isolate Cfr_IIT-BTL139 16S ribosomal DNA gene, partial sequence | 1157 | 1157 | 100% | 0.0 | 100% |
| DQ068814.1 | *Uncultured bacterium* clone f6h4 16S ribosomal DNA gene, partial sequence | 1157 | 1157 | 100% | 0.0 | 100% |
| AJ233408.1 | *Citrobacter freundii* (strain DSM 30039) 16S ribosomal DNA gene, partial sequence | 1157 | 1157 | 100% | 0.0 | 100% |
| AB210978.1 | *Citrobacter freundii* strain: SSCT56 16Sribosomal DNA gene, partial sequence | 1157 | 1157 | 100% | 0.0 | 100% |

Table 7 (c): CS7 (*Bacillus cereus* sp. IS3)

| Accession No. | Description | Max score | Total score | Query coverage | E value | Max identity |
|---|---|---|---|---|---|---|
| AY920248.1 | *Bacillus cereus* strain UB 1020 16S ribosomal DNA gene, partial sequence | 1182 | 1182 | 100% | 0.0 | 100% |
| AJ577282.1 | *Bacillus cereus,* strain RIVM BC00067 partial 16S rDNA gene partial sequence | 1182 | 1182 | 100% | 0.0 | 100% |
| AY920251.1 | *Bacillus cereus* strain F4810/72 gene for 16S rDNA, partial sequence | 1182 | 1182 | 100% | 0.0 | 100% |
| AY920250.1 | *Bacillus cereus* strain RIVM BC00075 16S ribosomal DNA gene, partial sequence | 1182 | 1182 | 100% | 0.0 | 100% |
| AJ577274.1 | *Bacillus cereus* partial 16S rDNA gene, strain NS 58 gene for 16S rDNA, partial sequence, | 1182 | 1182 | 100% | 0.0 | 100% |

Table 7 (d): CS8 (*Citrobacter freundii* IS4)

| Accession No. | Description | Max score | Total score | Query coverage | E value | Max identity |
|---|---|---|---|---|---|---|
| DQ068808.1 | *Uncultured bacterium* clone f5s4 16Sribosomal DNA gene, partial sequence | 1120 | 1120 | 100% | 0.0 | 100% |
| AM268137.1 | *Uncultured Citrobacter* sp. clone OTU21 16S ribosomal DNA gene, partial sequence | 1120 | 1120 | 100% | 0.0 | 100% |
| AM184281.1 | *Citrobacter freundii* strain WAB1942 gene for 16S rDNA, partial sequence | 1120 | 1120 | 100% | 0.0 | 100% |
| EF679196.1 | *Uncultured Citrobacter* sp. clone ASP-42 16S rDNA gene, partial sequence | 1120 | 1120 | 100% | 0.0 | 100% |
| AY870315.1 | *Citrobacter freundii* strain IRB3 gene for 16S rDNA, partial sequence, | 1120 | 1120 | 100% | 0.0 | 100% |

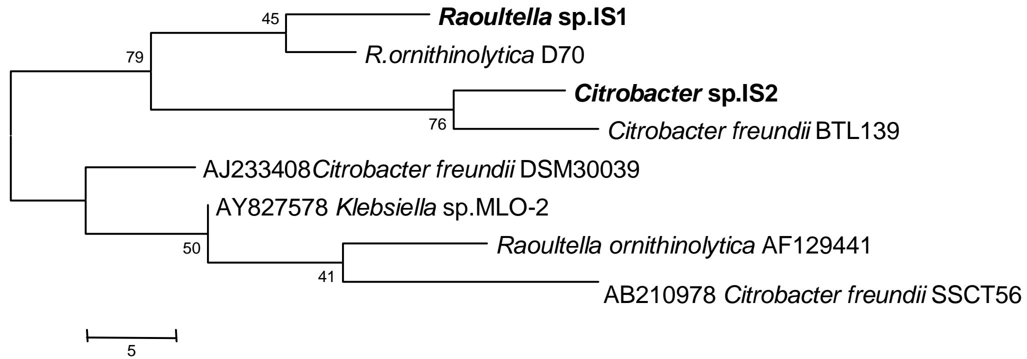



Fig. 8: Phylogenetic tree constructed using maximum parsimony. Number above the branches represents the bootstrap value.

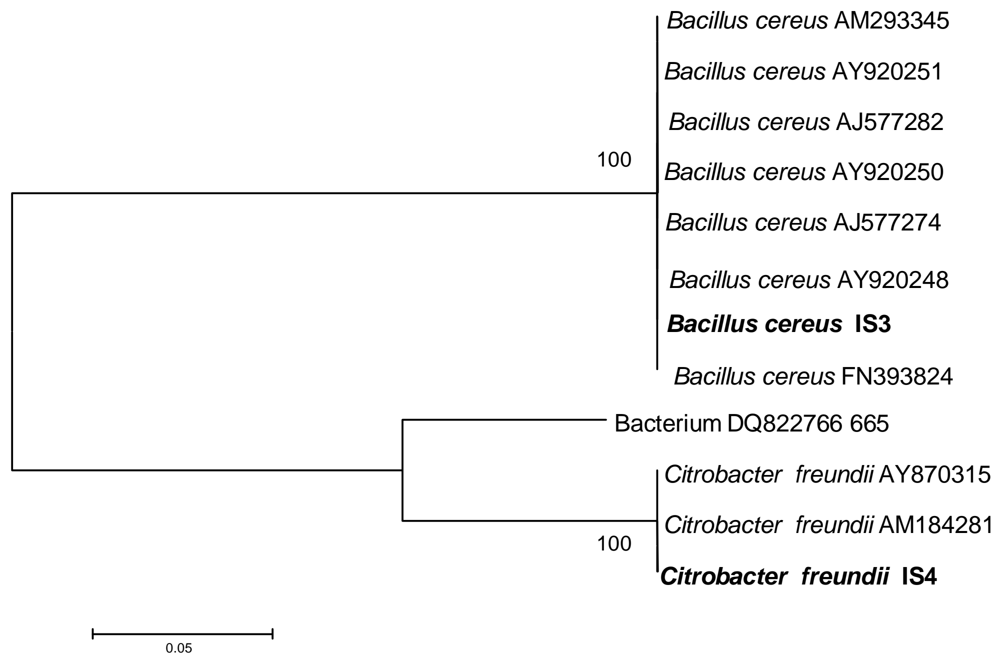



Fig. 9: Phylogenetic tree constructed using maximum parsimony. Number above the branches represents the bootstrap value.

The partial 16S rDNA sequences of all the four isolates were submitted to NCBI database with the help of software 'Sequin' and their corresponding accession numbers alongwith other details are given in Table 8.

Table 8: Accession numbers of bacterial isolates from NCBI

| **Strain** | **Isolates name** | **Accession number** |
|---|---|---|
| CT4 | *Raoultella* sp. IS1 | EU980037 |
| CT5 | *Citrobacter* sp. IS2 | EU980036 |
| CS7 | *Bacillus cereus* IS3 | EU980034 |
| CS8 | *Citrobacter freundii* IS4 | EU980035 |

GenBank: EU980037.1

**Raoultella sp. IS1 16S ribosomal RNA gene, partial sequence**

```
LOCUS       EU980037 688 bp DNA linear BCT 10-SEP-2008
DEFINITION  Raoultella sp. IS1 16S ribosomal RNA gene, partial sequence.
ACCESSION   EU980037
VERSION     EU980037.1  GI:198042136
KEYWORDS    .
SOURCE      Raoultella sp. IS1
  ORGANISM  Raoultella sp. IS1
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Raoultella.
REFERENCE   1 (bases 1 to 688)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Isolation and identification of chromium(VI) reducing bacteria from
            tannery effluent
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 688)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-AUG-2008) Department of Biotechnology and
            Environmental Sciences, Thapar University, Thapar Technology
            Campus, Patiala, Punjab 147004, India
FEATURES             Location/Qualifiers
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                     /organism="Raoultella sp. IS1"
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                     /strain="IS1"
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      121 aatgcgtaga gatctggagg aataccggtg gcgaaggcgg ccccctggac aaagactgac
      181 gctcaggtgc gaaagcgtgg ggagcaaaca ggattagata ccctggtagt ccacgctgta
      241 aacgatgtcg acttggaggt tgttcccttg aggagtggct tccggagcta acgcgttaag
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      361 acaagcggtg gagcatgtgg tttaattcga tgcaacgcga agaaccttac ctactcttga
      421 catccagaga acttagcaga gatgctttgg tgccttcggg aactctgaga caggtgctgc
      481 atggctgtcg tcagctcgtg ttgtgaaatg ttgggttaag tcccgcaacg agcgcaaccc
      541 ttatcctttg ttgccagcga ttcggtcggg aactcaaagg agactgccag tgataaactg
      601 gaggaaggtg gggatgacgt caagtcatca tggcccttac gagtagggct acacacgtgc
      661 tacaatggca tatacaaaga gaagcgac
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GenBank: EU980036.1

**Citrobacter sp. IS2 16S ribosomal RNA gene, partial sequence**

```
LOCUS       EU980036 626 bp DNA linear BCT 10-SEP-2008
DEFINITION  Citrobacter sp. IS2 16S ribosomal RNA gene, partial sequence.
ACCESSION   EU980036
VERSION     EU980036.1  GI:198042135
KEYWORDS    .
SOURCE      Citrobacter sp. IS2
  ORGANISM  Citrobacter sp. IS2
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Citrobacter.
REFERENCE   1 (bases 1 to 626)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Isolation of chromium(VI) reducing bacteria from tannery effluent
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 626)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-AUG-2008) Department of Biotechnology and
            Environmental Sciences, Thapar University, Thapar Technology
            Campus, Patiala, Punjab 147004, India
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                     /product="16S ribosomal RNA"
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      241 tgtggtttaa ttcgatgcaa cgcgaagaac cttacctact cttgacatcc agagaactta
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      361 tcgtgttgtg aaatgttggg ttaagtcccg caacgagcgc aacccttatc ctttgttgcc
      421 agcggttcgg ccgggaactc aaaggagact gccagtgata aactggagga aggtggggat
      481 gacgtcaagt catcatggcc cttacgagta gggctacaca cgtgctacaa tggcatatac
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      601 gagtctgcaa ctcgactcca tgaagt
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GenBank: EU980034.1

**Bacillus cereus strain IS3 16S ribosomal RNA gene, partial sequence**

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LOCUS       EU980034 640 bp DNA linear BCT 10-SEP-2008
DEFINITION  Bacillus cereus strain IS3 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   EU980034
VERSION     EU980034.1  GI:198042133
KEYWORDS    .
SOURCE      Bacillus cereus
  ORGANISM  Bacillus cereus
            Bacteria; Firmicutes; Bacillales; Bacillaceae; Bacillus; Bacillus
            cereus group.
REFERENCE   1 (bases 1 to 640)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Isolation and identification of chromium(VI) reducing bacteria from
            chrome sludge
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 640)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-AUG-2008) Department of Biotechnology and
            Environmental Sciences, Thapar University, Thapar Technology
            Campus, Patiala, Punjab 147004, India
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      421 actctgttgt tagggaagaa caagtgctag ttgaataagc tggcaccttg acggtaccta
      481 accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg tggcaagcgt
      541 tatccggaat tattgggcgt aaagcgcgcg caggtggttt cttaagtctg atgtgaaagc
      601 ccacggctca accgtggagg gtcattggaa actgggagac
```

GenBank: EU980035.1

**Citrobacter freundii strain IS4 16S ribosomal RNA gene, partial sequence**

```
LOCUS       EU980035 606 bp DNA linear BCT 10-SEP-2008
DEFINITION  Citrobacter freundii strain IS4 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   EU980035
VERSION     EU980035.1  GI:198042134
KEYWORDS    .
SOURCE      Citrobacter freundii
  ORGANISM  Citrobacter freundii
            Bacteria; Proteobacteria; Gammaproteobacteria; Enterobacteriales;
            Enterobacteriaceae; Citrobacter.
REFERENCE   1 (bases 1 to 606)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Characterization of chromium(VI) reducing bacteria from chrome
            sludge
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 606)
  AUTHORS   Sharma,I. and Goyal,D.
  TITLE     Direct Submission
  JOURNAL   Submitted (04-AUG-2008) Department of Biotechnology and
            Environmental Sciences, Thapar University, Thapar Technology
            Campus, Patiala, Punjab 147004, India
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                     Patiala, Punjab"
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      421 gcagcgattg acgttactcg cagaagaagc accggctaac tccgtgccag cagccgcggt
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      541 gtcaagtcgg atgtgaaatc cccgggctca acctgggaac tgcatccgaa actggcaggc
      601 tagagt
```

### 4.2.4 Growth of bacteria in presence of Cr(VI)

**End point growth studies**

**Bacterial Consortium**

The present study demonstrates growth of indigenous bacteria from tannery effluent and chrome sludge in the presence of Cr(VI) in minimal media supplemented with 0.5% glucose. End point growth of bacterial consortium (CT and CS) in presence of graded concentration (25-100 mg/L) of Cr(VI) was studied up to 24 h of incubation at pH 7.0, agitation 120 rpm, 28±2°C temperature (Fig. 10). With increase in Cr(VI) concentration, growth of bacterial consortium was decreased. However, the growth of CS was higher than CT at all concentrations of Cr(VI). There was almost 50% reduction in the growth of CT at 25 mg/L of Cr(VI) where as in CS it was at 50 mg/L of Cr(VI). Therefore further growth studies were carried out at lower range (5-25 mg/L) of Cr(VI). At lower range also as the concentration was increased from 5 to 25 mg/L there was decrease in the growth and the effect was more pronounced in CT as compared to CS (Fig. 11).

**Bacterial isolates**

With increasing concentration of Cr(VI) there was reduction in the growth of all the bacterial isolates. Growth of bacterial isolate *Roultella* sp. IS1 (CT4) from tannery effluent was reduced by 31.27, 42.99, 59.94 and 71.76 % at 5, 10, 15 and 25 mg/L of Cr(VI) in minimal media M2 after 24 h of incubation (Fig. 12), whereas in *Citrobacter* sp. IS2 (CT5) the trend in growth inhibition was 44.68, 50.19, 55.08 and 61.17% at incorresponding increasing concentration of Cr(VI). Both the isolates gave different response at different concentration. However at low concentrations of 5-10 mg/L of Cr(VI) *Roultella* sp. IS1 (CT4) appears to be more tolerant than *Citrobacter* sp. IS2 (CT5).

Growth of bacterial isolate *Bacillus cereus* IS3 (CS7) from chrome sludge was reduced by 36.11, 42.13 47.22 and 57.40% at 5, 10, 15 and 25 mg/L of Cr(VI) in minimal media M2 after 24 h of incubation (Fig. 12), whereas in *Citrobacter freundii* IS4 (CS8) the trend in growth inhibition was 30.67, 38.04, 40.60 and 49.51% at corresponding increasing concentration of Cr(VI). Similarly both the isolates gave different response at different concentration. However at low concentrations of 5-10 mg/L of Cr(VI) *Citrobacter freundii* IS4 (CS8) appears to be more tolerant than *Bacillus cereus* IS3 (CS7) (Fig. 12).

The results also indicates that at higher concentration (25 mg/L) of Cr(VI) higher tolerance is exhibited by *Citrobacter freundii* IS4 (CS8) (49.51%) followed by CS7 (57.40%) CT5 (61.17%) and CT4 (71.76%) (Fig. 12).

**Effect of Cr(VI) on exponentially growing bacteria**

**Bacterial consortium**

Bacterial consortium (CT and CS) had a lag phase of 6-8 h, which was increased to 10 and 12 h in presence of Cr(VI), similarly log phase was extended from 8-10 to 14-18 h and stationary phase from 16-18 to 20-22 h (data not shown). Effect of Cr(VI) on exponentially growing bacterial consortia (CT and CS) and bacterial isolates {CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4)} at graded concentration (0, 3.6, 7.9, 14.18 mg/L) of Cr (VI) was studied. Overnight grown cultures were inoculated in fresh minimal media (M2) supplemented with 0.5% glucose and Cr(VI) was added during the exponential phase after 6 h of incubation. The growth was retarded with the addition of Cr(VI) and was more pronounced with increasing concentration from 3.6 to 14.18 mg/L. The log phase of both the consortia (CT and CS) was drastically reduced from approximately 16 h to 9 h in the presence of Cr(VI). On addition of Cr(VI) sharp decline in growth was observed after 9 h of incubation in CS consortium. This decrease was followed by slight increase in growth soon after which bacteria entered into stationary phase. The percentage decrease in the exponential growth of bacterial consortium from tannery effluent (CT) was 11%, 19% and 21% (Table 9; Fig. 13), whereas of bacterial consortium from chrome sludge (CS) was 30%, 34%, 36% at 3.6, 7.9 and 14.18 mg/L of Cr(VI), respectively (Table 10, Fig. 14). Pronounced effect of Cr(VI) on generation time was observed in bacterial consortia (CS) from chrome sludge. Generation time was found to double from 5 to 10 h at 3.6 mg/L of Cr(VI) no further negative effect on generation time was observed with increase in Cr(VI) concentration. For tannery consortia (CT) only a slight increase in generation time was observed in presence of Cr(VI) which increased from 4 h (0 mg/L) to 4.5 h (at 3.6 mg/L), ~ 4.8 h (at 7.9 mg/L), ~ 5 h (at 14.18 mg/L) (Table 9, 10; Fig. 13, 14).

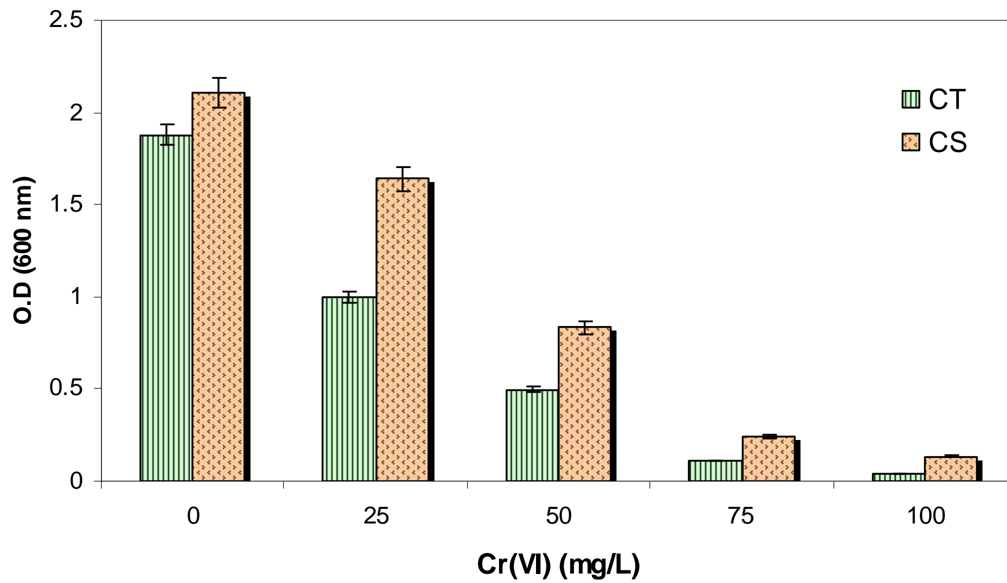



Fig. 10: End point growth of bacterial consortium from tannery effluent (CT) and chrome sludge (CS) in presence of graded concentration (25-100 mg/L) of Cr(VI) after 24 h of incubation.

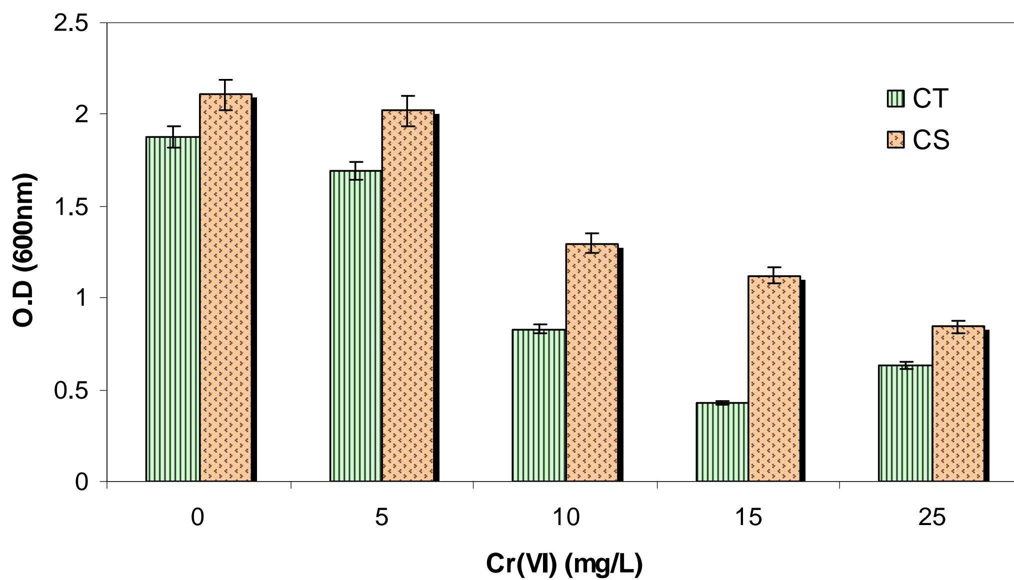



Fig. 11: End point growth of bacterial consortium from tannery effluent (CT) and chrome effluent (CS) in presence of graded concentration (5-25 mg/L) of Cr(VI) after 24 h of incubation.

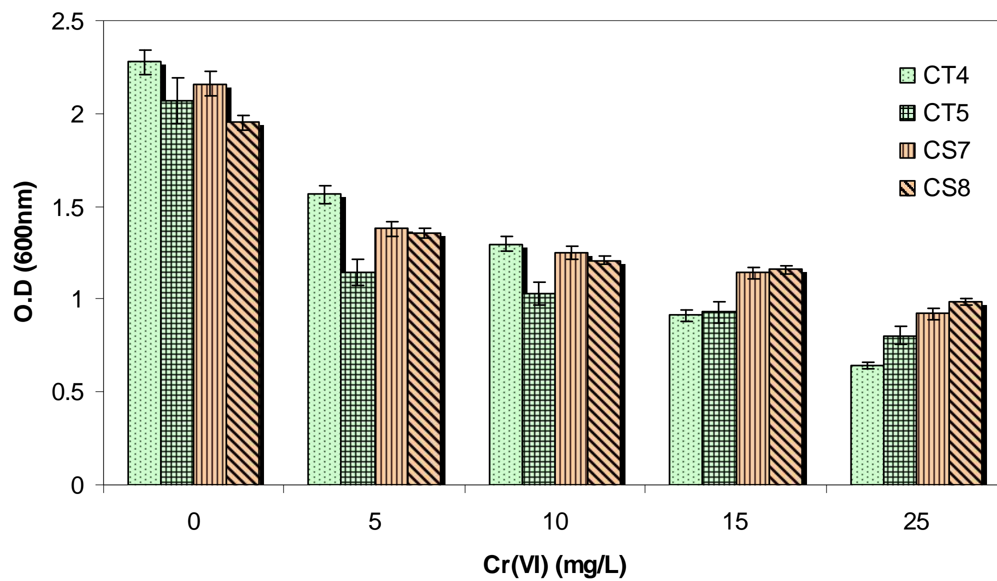



Fig. 12: End point growth of bacterial isolates (CT4, CT5, CS7 and CS8) in presence of graded concentration (5-25 mg/L) of Cr(VI) after 24 h of incubation.

Table 9: Effect of Cr(VI) on the growth of exponentially growing bacterial consortium (CT) from tannery effluent Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time (h) | 0 mg/L | 3.6 mg/L | 7.9 mg/L | 14.18 mg/L |
| 0 | 0.02±030 | 0.02±0.30 | 0.02±0.14 | 0.02±0.01 |
| 2 | 0.09±0.20 | 0.09±0.07 | 0.09±0.03 | 0.09±0.07 |
| 4 | 0.50±0.25 | 0.50±0.09 | 0.50±0.54 | 0.50±0.15 |
| 6 | 0.72±0.02 | 0.72±0.07 | 0.72±1.02 | 0.72±0.20 |
| 8 | 0.83±0.20 | 0.81±0.15 | 0.81±0.02 | 0.80±0.06 |
| 10 | 0.88±0.07 | 0.83±0.20 | 0.80±0.15 | 0.80±1.20 |
| 12 | 0.95±0.14 | 0.87±1.20 | 0.83±0.75 | 0.80±0.07 |
| 14 | 1.02±0.16 | 0.91±0.21 | 0.83±1.00 | 0.81±0.01 |
| 16 | 1.04±0.15 | 0.91±0.07 | 0.84±0.20 | 0.82±0.54 |
| 18 | 1.08±0.07 | 0.99±0.15 | 0.84±0.07 | 0.83±0.15 |
| 20 | 1.09±0.85 | 1.01±0.06 | 0.86±0.60 | 0.83±0.70 |
| 22 | 1.07±0.21 | 1.02±1.20 | 0.84±0.16 | 0.78±0.82 |
| 24 | 1.06±0.01 | 0.99±0.08 | 0.83±1.08 | 0.79±0.64 |
| 26 | 1.04±0.14 | 0.97±0.70 | 0.80±0.05 | 0.79±0.07 |
| 28 | 1.06±1.20 | 1.01±0.54 | 0.83±0.23 | 0.81±0.16 |
| 30 | 1.08±0.65 | 1.04±0.02 | 0.83±0.15 | 0.83±1.20 |
| 32 | 1.05±0.15 | 0.99±1.20 | 0.83±0.12 | 0.81±0.60 |
| 34 | 0.98±0.03 | 0.92±0.85 | 0.76±0.07 | 0.76±1.20 |
| 36 | 0.96±0.02 | 0.90±0.04 | 0.76±0.02 | 0.78±0.06 |

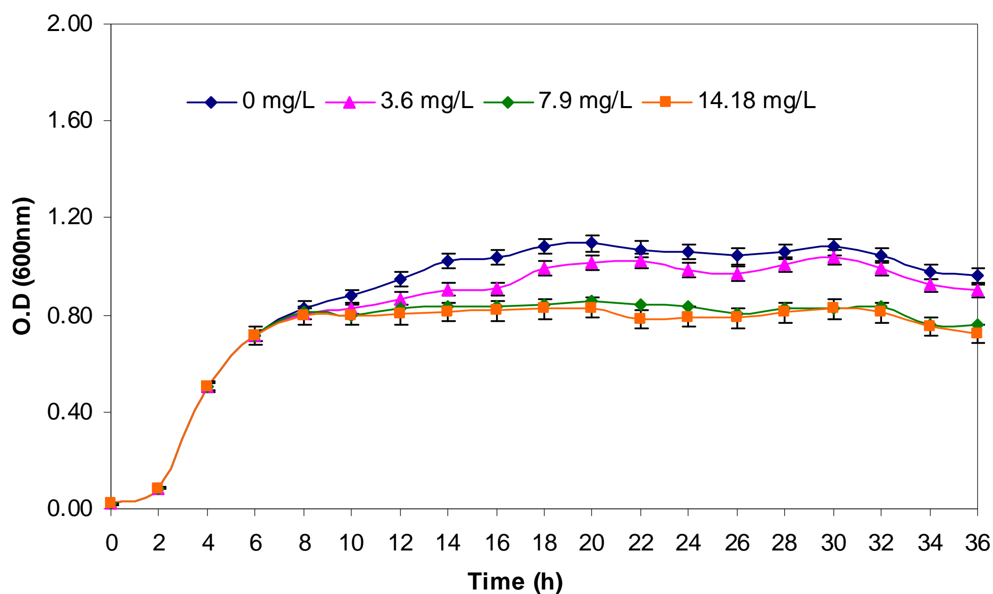



Fig. 13: Effect of Cr(VI) on the growth of exponentially growing bacterial consortium (CT) from tannery effluent Cr(VI) added after 6 h of incubation.

Table 10: Effect of Cr(VI) on the growth of exponentially growing bacterial consortium (CS) from chrome sludge Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time (h) | 0 mg/L | 3.6 mg/L | 7.9 mg/L | 14.18 mg/L |
| 0 | 0.05±0.02 | 0.05±0.07 | 0.05±0.04 | 0.05±0.01 |
| 2 | 0.47±0.54 | 0.47±0.03 | 0.47±0.06 | 0.05±0.02 |
| 4 | 0.86±0.07 | 0.86±0.14 | 0.86±0.02 | 0.86±0.04 |
| 6 | 0.94±0.16 | 0.94±0.04 | 0.94±0.01 | 0.94±0.06 |
| 8 | 1.13±0.02 | 1.06±0.16 | 1.03±0.12 | 1.00±0.10 |
| 10 | 1.23±0.70 | 1.08±0.54 | 1.03±0.08 | 1.01±0.08 |
| 12 | 1.32±0.15 | 1.01±0.01 | 0.93±0.04 | 0.92±0.06 |
| 14 | 1.37±0.30 | 0.97±0.02 | 0.91±0.02 | 0.88±0.04 |
| 16 | 1.62±0.15 | 1.14±0.12 | 1.07±0.06 | 1.04±0.02 |
| 18 | 1.76±0.02 | 1.12±0.04 | 1.05±0.02 | 1.04±0.04 |
| 20 | 1.78±0.16 | 1.14±0.16 | 1.06±0.04 | 1.06±0.02 |
| 22 | 1.72±0.54 | 1.06±0.51 | 0.99±0.12 | 0.98±0.04 |
| 24 | 1.78±0.60 | 1.13±0.14 | 1.04±0.15 | 1.03±0.14 |
| 26 | 1.61±0.18 | 0.98±0.02 | 0.93±0.02 | 0.91±0.02 |
| 28 | 1.62±0.01 | 0.98±0.04 | 0.92±0.01 | 0.89±0.04 |
| 30 | 1.64±0.07 | 1.02±0.07 | 0.93±0.04 | 0.94±0.08 |
| 32 | 1.80±0.20 | 1.02±0.06 | 0.97±0.02 | 0.98±0.06 |
| 34 | 1.88±1.20 | 0.98±0.02 | 0.94±0.01 | 0.96±0.04 |
| 36 | 1.84±0.06 | 0.98±0.04 | 0.92±0.02 | 0.94±0.01 |

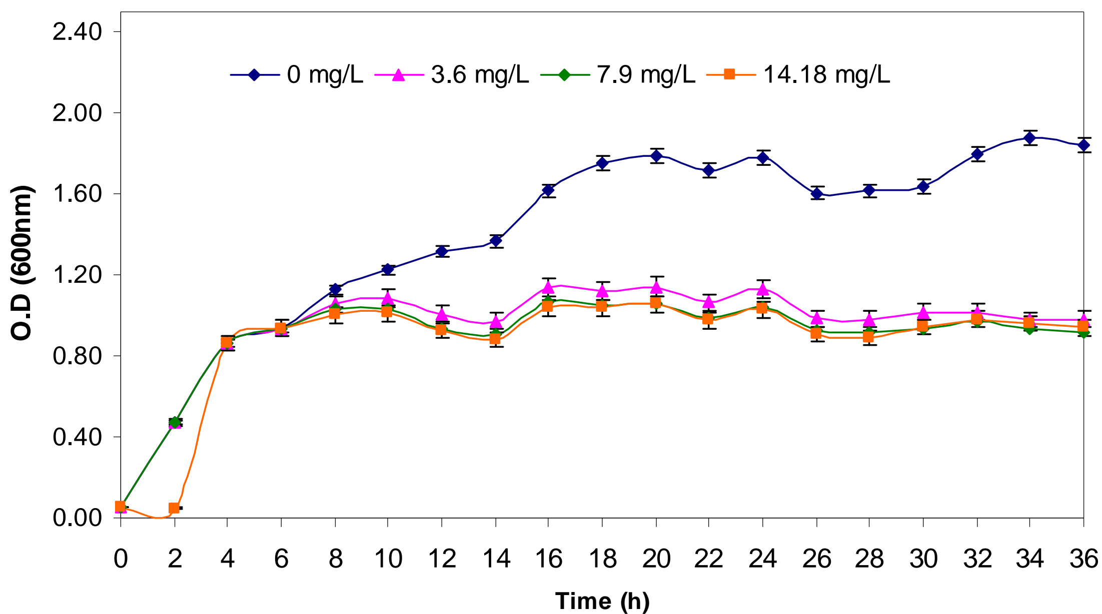



Fig. 14: Effect of Cr(VI) on the growth of exponentially growing bacterial consortium (CS) from chrome sludge. Cr(VI) added after 6 h of incubation.

**Bacterial isolates**

Bacterial isolates (CT4, CT5, CS7 and CS8) also showed similar lag phase of 6-8 h and it increased to 10 and 12 h in minimal media with Cr(VI) and log phase was extended from 8-10 to 14-18 h and stationary phase from 16-18 to 20-22 h (data not shown). However bacterial isolates took longer time in acclimatizing in minimal media as compared to consortium CT and CS.

Bacterial isolates from tannery effluent CT4 (*Raoultella* sp. IS1) exhibited 15% decrease in growth at 3.24 mg/L of Cr(VI) and CT5 (*Citrobacter* sp. IS2) showed 23% reduction in growth at 4.46 mg/L of Cr(VI) when added after 6 h of incubation during the exponential growth phase (Table 11, 12; Fig. 15, 16). At higher concentration of 6.24 and 14.94 mg/L Cr(VI), 37 and 39% decrease in growth was observed for CT4 (*Raoultella* IS1) respectively whereas reduction of 50% and 43% was observed in CT5 (*Citrobacter* sp. IS2) at 8.04 and 16.46 mg/L Cr(VI). For both the chrome sludge isolates CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) a decrease in growth by 30% at 4-4.5 mg/L Cr(VI) was observed. Further increase in Cr (VI) concentration did not retard the growth much, since only 27 and 34% decrease in growth was observed for CS7 (*Bacillus cereus* IS3) and 32% and 36% for CS8 (*Citrobacter freundii* IS4) at ~9 and ~19 mg/L respectively (Table 13, 14; Fig. 17, 18).

In all the isolates with the addition of Cr(VI) to the exponentially growing bacteria there was increase in doubling time with concomitant reduction in growth. For tannery isolate CT4 (*Raoultella* sp. IS1) a gradual increase in the generation time was observed from 3 to 5.6 h with increase in Cr(VI) concentration. A similar trend was observed for CT5 (*Citrobacter* sp. IS2) (Table 11, 12; Fig. 15, 16). For sludge isolates CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) generation time was almost doubled from 5 h to 10 h upon addition of Cr(VI) (Table 13, 14; Fig. 17, 18).

Table 11: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CT4 (*Raoultella* IS1) Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time (h) | 0 mg/L | 3.24 mg/L | 6.24 mg/L | 14.94 mg/L |
| 0 | 0.06±0.01 | 0.06±0.02 | 0.06±0.01 | 0.06±0.01 |
| 2 | 0.20±0.04 | 0.20±0.04 | 0.20±0.06 | 0.20±0.04 |
| 4 | 0.37±0.06 | 0.37±0.12 | 0.37±0.02 | 0.37±0.02 |
| 6 | 0.76±0.02 | 0.76±0.54 | 0.76±0.08 | 0.76±0.12 |
| 8 | 0.95±0.06 | 0.95±0.08 | 0.95±0.02 | 0.95±0.06 |
| 10 | 1.35±0.14 | 1.10±0.02 | 1.03±0.06 | 0.98±0.01 |
| 12 | 1.50±0.52 | 1.13±0.06 | 1.05±0.01 | 0.99±0.02 |
| 14 | 1.61±0.04 | 1.31±0.04 | 1.08±0.04 | 1.09±0.04 |
| 16 | 1.71±0.06 | 1.46±0.02 | 1.08±0.06 | 1.07±0.06 |
| 18 | 1.76±0.04 | 1.58±0.12 | 1.08±0.02 | 1.07±0.02 |
| 20 | 1.80±0.02 | 1.68±0.04 | 1.06±0.01 | 1.05±0.04 |
| 22 | 1.82±0.08 | 1.69±0.15 | 1.06±0.04 | 1.04±0.01 |
| 24 | 1.85±0.02 | 1.65±0.24 | 1.05±0.06 | 1.14±0.02 |
| 26 | 1.87±0.01 | 1.69±0.02 | 1.06±0.02 | 1.12±0.04 |
| 28 | 1.88±0.25 | 1.70±0.01 | 1.08±0.01 | 1.13±0.02 |
| 30 | 1.86±0.04 | 1.72±0.06 | 1.10±0.12 | 1.12±0.08 |
| 32 | 1.87±0.02 | 1.79±0.02 | 1.12±0.04 | 1.10±0.04 |
| 34 | 1.89±0.01 | 1.78±0.01 | 1.14±0.02 | 1.11±0.10 |
| 36 | 1.89±0.06 | 1.80±0.04 | 1.16±0.06 | 1.12±0.02 |

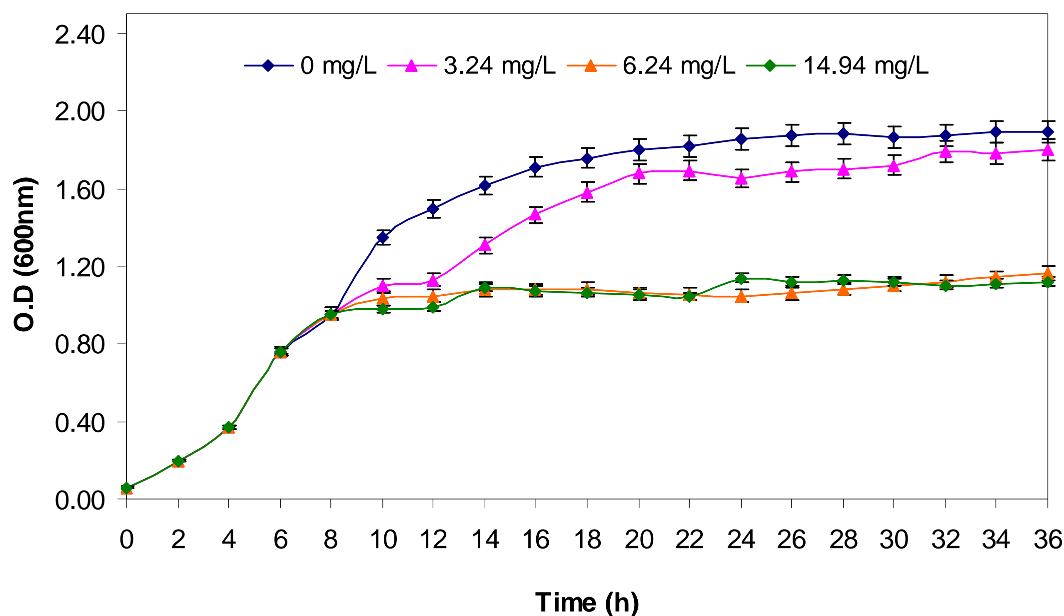



Fig. 15: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CT4 (*Raoultella* sp. IS1) Cr(VI) added after 6 h of incubation.

Table 12: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CT5 (*Citrobacter* sp. IS2) Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time (h) | 0 mg/L | 4.46 mg/L | 8.04 mg/L | 16.46 mg/L |
| 0 | 0.04±0.01 | 0.04±0.01 | 0.04±0.02 | 0.04±0.01 |
| 2 | 0.09±0.02 | 0.09±0.02 | 0.09±0.02 | 0.09±0.02 |
| 4 | 0.53±0.01 | 0.53±0.01 | 0.53±0.01 | 0.53±0.01 |
| 6 | 1.15±0.04 | 1.01±0.06 | 0.90±0.06 | 1.01±0.02 |
| 8 | 1.20±0.02 | 1.09±0.03 | 0.92±0.02 | 1.04±0.04 |
| 10 | 1.48±0.03 | 0.91±0.02 | 0.71±0.01 | 0.83±0.02 |
| 12 | 1.55±0.02 | 1.18±0.12 | 0.91±0.04 | 1.03±0.08 |
| 14 | 1.63±0.04 | 1.25±0.02 | 0.94±0.06 | 1.10±0.12 |
| 16 | 1.75±0.12 | 1.39±0.24 | 0.95±0.08 | 1.10±0.06 |
| 18 | 1.93±0.04 | 1.50±0.02 | 0.96±0.04 | 1.11±0.08 |
| 20 | 1.94±0.02 | 1.65±0.04 | 0.96±0.06 | 1.12±0.06 |
| 22 | 1.95±0.06 | 1.69±0.02 | 0.93±0.04 | 1.20±0.02 |
| 24 | 1.97±0.12 | 1.70±0.09 | 0.91±0.08 | 1.09±0.24 |
| 26 | 1.99±0.02 | 1.74±0.02 | 0.90±0.04 | 1.08±0.10 |
| 28 | 2.03±0.03 | 1.90±0.06 | 0.89±0.02 | 1.17±0.04 |
| 30 | 2.00±0.06 | 1.87±0.02 | 0.89±0.01 | 1.16±0.02 |
| 32 | 2.10±0.02 | 1.89±0.01 | 0.91±0.02 | 1.17±0.14 |
| 34 | 2.12±0.04 | 1.88±0.04 | 0.89±0.04 | 1.18±0.02 |
| 36 | 2.11±0.01 | 1.90±0.06 | 0.92±0.01 | 1.18±0.06 |

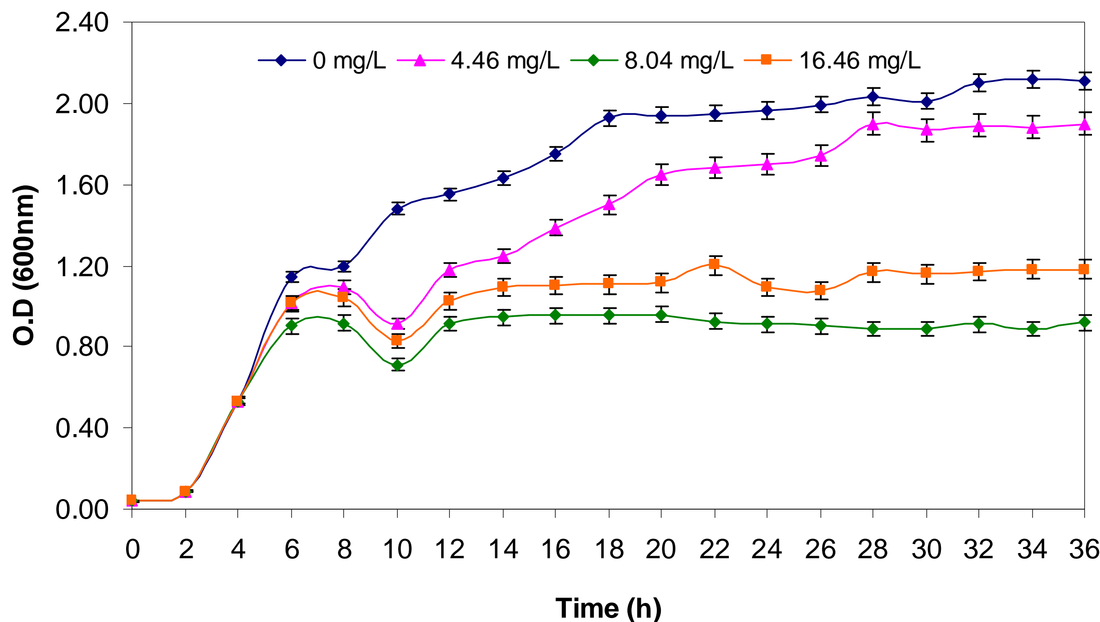



Fig. 16: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CT5 (*Citrobacter* sp. IS2) Cr(VI) added after 6 h of incubation.

Table 13: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CS7 (*Bacillus cereus* IS3) Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time (h) | 0 mg/L | 4.15 mg/L | 8.97 mg/L | 18.56 mg/L |
| 0 | 0.11±0.15 | 0.11±0.01 | 0.11±0.02 | 0.11±0.06 |
| 2 | 0.49±0.04 | 0.49±0.05 | 0.49±0.15 | 0.49±0.02 |
| 4 | 0.80±0.01 | 0.80±0.16 | 0.80±0.02 | 0.80±0.12 |
| 6 | 1.04±0.03 | 1.04±0.08 | 1.04±0.07 | 1.04±0.04 |
| 8 | 1.42±0.04 | 1.21±0.24 | 1.31±0.16 | 1.19±0.01 |
| 10 | 1.65±0.02 | 1.25±0.04 | 1.35±0.06 | 1.21±0.14 |
| 12 | 1.77±0.44 | 1.21±0.01 | 1.36±0.44 | 1.20±0.06 |
| 14 | 1.81±0.16 | 1.25±0.08 | 1.33±0.02 | 1.21±0.24 |
| 16 | 1.83±0.07 | 1.26±0.06 | 1.36±0.06 | 1.24±0.02 |
| 18 | 1.84±0.02 | 1.28±0.12 | 1.37±0.41 | 1.24±0.01 |
| 20 | 1.88±0.01 | 1.30±0.04 | 1.47±0.04 | 1.30±0.06 |
| 22 | 2.01±0.06 | 1.25±0.15 | 1.38±0.02 | 1.30±0.04 |
| 24 | 2.1±0.10 | 1.26±0.24 | 1.36±0.10 | 1.31±0.02 |
| 26 | 2.14±0.04 | 1.28±0.06 | 1.41±0.26 | 1.32±0.06 |
| 28 | 2.12±0.24 | 1.25±0.02 | 1.38±0.12 | 1.30±0.16 |
| 30 | 2.0±0.12 | 1.27±0.14 | 1.36±0.04 | 1.34±0.08 |
| 32 | 2.1±0.04 | 1.27±0.08 | 1.39±0.08 | 1.30±0.10 |
| 34 | 2.2±0.02 | 1.28±0.06 | 1.34±0.02 | 1.32±0.14 |
| 36 | 2.2±0.06 | 1.30±0.10 | 1.35±0.01 | 1.34±0.04 |

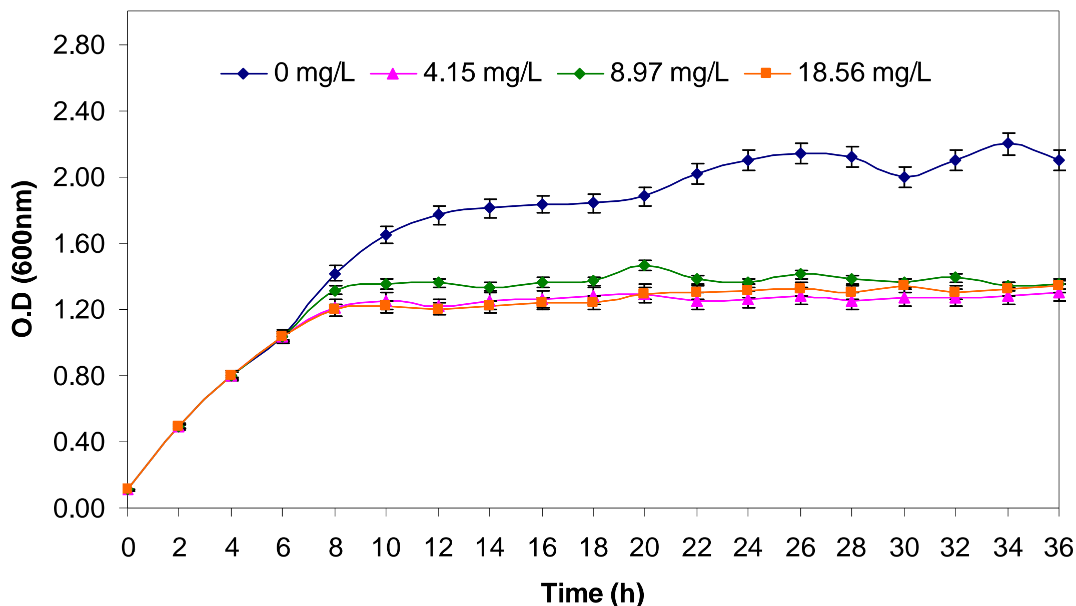



Fig. 17: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CS7 (*Bacillus cereus* IS3) Cr(VI) added after 6 h of incubation.

Table 14: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CS8 (*Citrobacter freundii* IS4) Cr(VI) added after 6 h of incubation.

| Growth (600 nm) at different concentration of Cr(VI) | | | | |
|---|---|---|---|---|
| Time(h) | 0 mg/L | 4.46 mg/L | 8.82 mg/L | 18.85 mg/L |
| 0 | 0.10±0.01 | 0.10±0.01 | 0.10±0.02 | 0.10±0.01 |
| 2 | 0.47±0.04 | 0.47±0.04 | 0.47±0.04 | 0.47±0.02 |
| 4 | 0.84±0.02 | 0.84±0.05 | 0.84±0.05 | 0.84±0.10 |
| 6 | 1.12±0.06 | 1.12±0.12 | 1.12±0.01 | 1.12±0.04 |
| 8 | 1.41±0.01 | 1.24±0.08 | 1.22±0.02 | 1.12±0.14 |
| 10 | 1.65±0.15 | 1.27±0.04 | 1.24±0.04 | 1.15±0.06 |
| 12 | 1.78±0.08 | 1.23±0.02 | 1.21±0.12 | 1.14±0.04 |
| 14 | 1.81±0.16 | 1.26±0.06 | 1.24±0.02 | 1.16±0.03 |
| 16 | 1.82±0.12 | 1.26±0.01 | 1.25±0.06 | 1.17±0.01 |
| 18 | 1.83±0.04 | 1.26±0.02 | 1.25±0.02 | 1.17±0.02 |
| 20 | 1.84±0.08 | 1.28±0.04 | 1.29±0.02 | 1.22±0.05 |
| 22 | 1.98±0.04 | 1.25±0.06 | 1.26±0.01 | 1.21±0.07 |
| 24 | 1.96±0.21 | 1.26±0.08 | 1.28±0.04 | 1.21±0.10 |
| 26 | 1.92±0.16 | 1.28±0.10 | 1.29±0.21 | 1.24±0.08 |
| 28 | 1.89±0.08 | 1.27±0.24 | 1.30±0.14 | 1.23±0.14 |
| 30 | 1.90±0.12 | 1.26±0.16 | 1.28±0.10 | 1.22±0.12 |
| 32 | 1.95±0.06 | 1.29±0.12 | 1.27±0.04 | 1.24±0.06 |
| 34 | 1.94±0.10 | 1.28±0.06 | 1.29±0.02 | 1.26±0.18 |
| 36 | 1.94±0.04 | 1.27±0.08 | 1.28±0.10 | 1.24±0.24 |

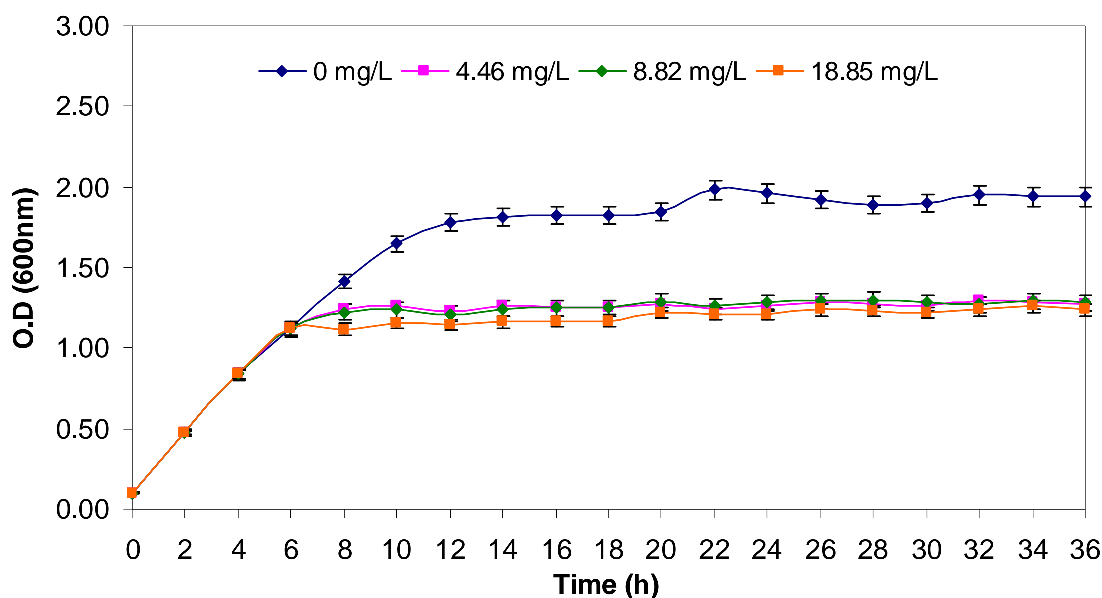



Fig. 18: Effect of Cr(VI) on the growth of exponentially growing bacterial isolate CS8 (*Citrobacter freundii* IS4) Cr(VI) added after 6 h of incubation.

### 4.2.5 Biotransformation of Cr(VI) to Cr(III)

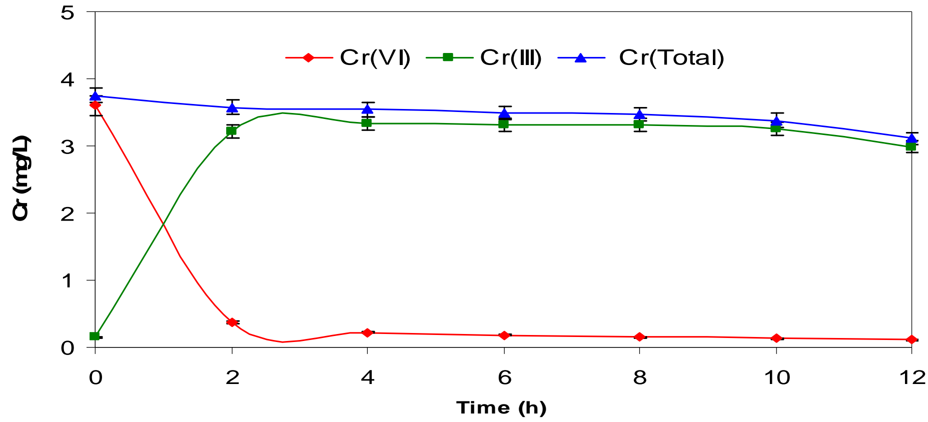
Removal of Cr(VI) and its transformation to Cr(III) by bacterial consortia (CT and CS) and isolates CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) at different concentration (3-15 mg/L) of Cr(VI) in minimal media by actively growing bacteria was studied. Chromium removal and its biotransformation by bacterial consortium and isolates was different at varying concentrations of Cr(VI) in minimal media (M2). With increase in concentration of Cr(VI) there was decrease in transformation, which may be due to toxicity of Cr(VI) to the growing cells. Biotransformation of Cr(VI) to Cr(III) showed that there was gradual increase in the concentration of Cr(III) and decrease in Cr(VI) concentration and the rate of increase in Cr(III) concentration coincides exactly with rate of decrease in Cr(VI) concentration. In the first 2 h at 3.6 mg/L Cr(VI), the rate of transformation was 1.5 mg/h by CT consortium (Table 15; Fig. 19A) and 1 mg/h by CS consortium (Table 16; Fig. 20A) which corresponded to 90% transformation by CT and 60% by CS consortium. The rate decreased to approximately 0.06 mg/h and 0.125 mg/h respectively in the next 4 h. After 12 h of incubation both the bacterial consortia showed similar trend in percentage transformation, which was 90%, 80% and 70% at 3.6, 7.9 and 14.18 mg/L Cr(VI) respectively. Nearly 100% transformation was observed at 3.6 mg/L and 7.9 mg/L Cr(VI) concentrations after 22 and 36 h of incubation respectively by both CT and CS consortia.

The % removal of Cr(VI) was determined to cross check the ability of bacterial consortium to transform Cr(VI) to Cr(III). Percentage removal of Cr(VI) with reference to the original concentration in the minimal medium (M2) inoculated with exponential growing consortium is given in Table 15, 16. In 24 h of incubation at 3.60, 7.90 and 14.8 mg/L Cr(VI), CS showed 100%, 99.19% and 95.51% removal of Cr(VI) and 38.4% 30.31% and 24.11% removal of Cr(Total) (Table 15; Fig. 19). Similarly CT showed 100%, 99.4% and 92.19% removal of Cr(VI) and 72.8%, 61.74%, 61.11% removal of Cr(Total) (Table 16; Fig. 20).

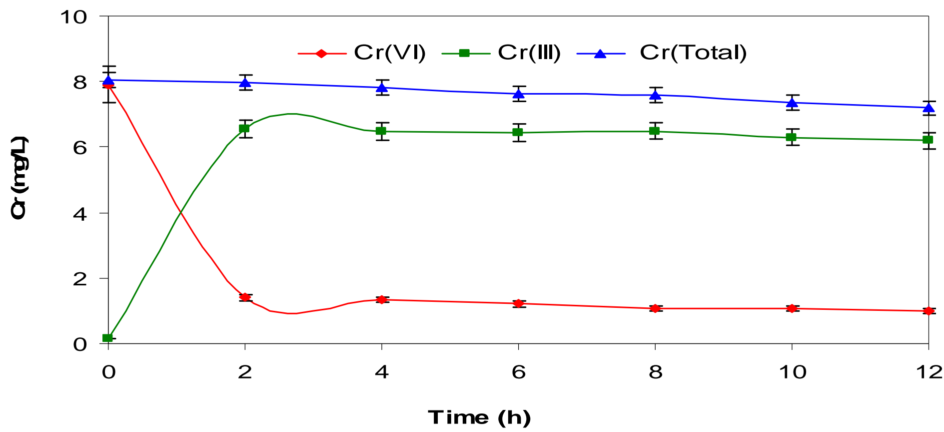
Bacetrial consortium (CT) showed 100%, 99.2%, 95.5% removal of Cr(VI) and 38.4%, 30.3%, 24.1% of Cr(Total) from minimal media (M2) (Table 15; Fig. 21) at 3.6, 7.9 and 14.18 mg/L concentration of Cr(VI) respectively, whereas bacterial consortium (CS) showed 100%, 99.4%, 99.1% removal of Cr(VI) and 72.8%, 61.7% and 61.1% of Cr(Total) from minimal medium (M2) at the end of 36 h of incubation (Table 16; Fig 22).

Table 15: Removal (%) of chromium by exponentially growing bacterial consortium (CT) from tannery effluent.

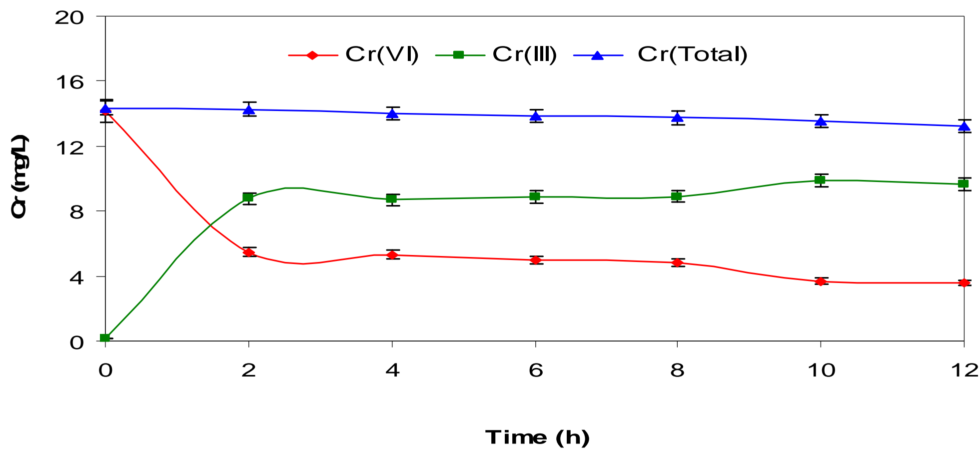
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 3.60±0.15 | - | - | 3.75±0.02 | - | - | 0.15 | - |
| | 7.90±0.70 | - | - | 8.05±0.16 | - | - | 0.15 | - |
| | 14.18±0.16 | - | - | 14.35±0.21 | - | - | 0.17 | - |
| 2 | 3.60±0.15 | 0.37±0.01 | 89.81 | 3.75±0.02 | 3.57±0.14 | 4.72 | 0.15 | 3.21 |
| | 7.90±0.70 | 1.41±0.06 | 82.14 | 8.05±0.16 | 7.97±0.06 | 0.97 | 0.15 | 6.56 |
| | 14.18±0.16 | 5.47±0.12 | 61.44 | 14.35±0.21 | 14.25±0.14 | 0.70 | 0.17 | 8.78 |
| 4 | 3.60±0.15 | 0.22±0.02 | 94.01 | 3.75±0.02 | 3.54±0.06 | 5.52 | 0.15 | 3.33 |
| | 7.90±0.70 | 1.34±0.04 | 82.99 | 8.05±0.16 | 7.81±0.04 | 2.98 | 0.15 | 6.47 |
| | 14.18±0.16 | 5.32±0.06 | 62.51 | 14.35±0.21 | 14.00±0.24 | 2.44 | 0.17 | 8.68 |
| 6 | 3.60±0.15 | 0.18±0.02 | 94.95 | 3.75±0.02 | 3.49±0.06 | 6.85 | 0.15 | 3.31 |
| | 7.90±0.70 | 1.21±0.06 | 84.70 | 8.05±0.16 | 7.64±0.02 | 5.09 | 0.15 | 6.43 |
| | 14.18±0.16 | 4.98±0.01 | 64.88 | 14.35±0.21 | 13.85±0.08 | 3.48 | 0.17 | 8.87 |
| 8 | 3.60±0.15 | 0.15±0.02 | 95.88 | 3.75±0.02 | 3.47±0.04 | 7.44 | 0.15 | 3.32 |
| | 7.90±0.70 | 1.09±0.12 | 86.19 | 8.05±0.16 | 7.58±0.06 | 5.84 | 0.15 | 6.49 |
| | 14.18±0.16 | 4.83±0.04 | 65.95 | 14.35±0.21 | 13.74±0.16 | 4.25 | 0.17 | 8.91 |
| 10 | 3.60±0.15 | 0.13±0.06 | 96.35 | 3.75±0.02 | 3.38±0.10 | 9.81 | 0.15 | 3.25 |
| | 7.90±0.70 | 1.06±0.08 | 86.62 | 8.05±0.16 | 7.36±0.07 | 8.57 | 0.15 | 6.30 |
| | 14.18±0.16 | 3.67±0.10 | 74.14 | 14.35±0.21 | 13.54±0.04 | 5.64 | 0.17 | 9.87 |
| 12 | 3.60±0.15 | 0.11±0.09 | 95.17 | 3.75±0.02 | 3.11±0.12 | 17.09 | 0.15 | 2.99 |
| | 7.90±0.70 | 0.99±0.02 | 80.79 | 8.05±0.16 | 7.19 ±0.04 | 10.68 | 0.15 | 6.20 |
| | 14.18±0.16 | 3.58±0.15 | 74.74 | 14.35±0.21 | 13.20±0.06 | 8.01 | 0.17 | 9.62 |
| 22 | 3.60±0.15 | 0.00±0.00 | 100 | 3.75±0.02 | 3.01±0.02 | 19.73 | 0.15 | 3.01 |
| | 7.90±0.70 | 0.87±0.02 | 88.96 | 8.05±0.16 | 6.98±0.04 | 13.29 | 0.15 | 6.11 |
| | 14.18±0.16 | 2.96±0.06 | 79.13 | 14.35±0.21 | 12.85±0.24 | 10.45 | 0.17 | 9.89 |
| 24 | 3.60±0.15 | 0.00±0.00 | 100 | 3.75±0.02 | 2.83±0.07 | 24.53 | 0.15 | 2.83 |
| | 7.90±0.70 | 0.59±0.04 | 92.58 | 8.05±0.16 | 6.30±0.06 | 21.70 | 0.15 | 5.72 |
| | 14.18±0.16 | 2.77±0.14 | 80.43 | 14.35±0.21 | 12.04±0.02 | 16.10 | 0.17 | 9.27 |
| 26 | 3.60±0.15 | 0.00±0.00 | 100 | 3.75±0.02 | 2.71±0.01 | 27.79 | 0.15 | 2.71 |
| | 7.90±0.70 | 0.16±0.02 | 97.91 | 8.05±0.16 | 6.17±0.04 | 23.39 | 0.15 | 6.00 |
| | 14.18±0.16 | 2.32±0.06 | 83.64 | 14.35±0.21 | 11.75±0.03 | 18.12 | 0.17 | 9.43 |
| 28 | 3.60±0.15 | 0.00±0.04 | 100 | 3.75±0.02 | 2.60±0.02 | 30.64 | 0.15 | 2.60 |
| | 7.90±0.70 | 0.10±0.02 | 98.76 | 8.05±0.16 | 6.07±0.21 | 24.57 | 0.15 | 5.97 |
| | 14.18±0.16 | 1.61±0.14 | 88.63 | 14.35±0.21 | 11.57±0.16 | 19.37 | 0.17 | 9.96 |
| 30 | 3.60±0.15 | 0.00±0.00 | 100 | 3.75±0.02 | 2.57±0.02 | 31.47 | 0.15 | 2.57 |
| | 7.90±0.70 | 0.08±0.01 | 98.98 | 8.05±0.16 | 5.81±0.04 | 27.80 | 0.15 | 5.73 |
| | 14.18±0.16 | 1.56±0.24 | 88.98 | 14.35±0.21 | 11.38±0.06 | 20.70 | 0.17 | 9.82 |
| 32 | 3.60±0.15 | 0.00±0.00 | 93.66 | 3.75±0.02 | 2.45±0.01 | 34.67 | 0.15 | 2.45 |
| | 7.90±0.70 | 0.06±0.01 | 98.75 | 8.05±0.16 | 5.78±0.06 | 28.20 | 0.15 | 5.72 |
| | 14.18±0.16 | 0.67±0.04 | 97.57 | 14.35±0.21 | 11.07 ±0.04 | 22.86 | 0.17 | 10.40 |
| 36 | 3.60±0.15 | 0.00±0.00 | 100.00 | 3.75±0.02 | 2.31±0.02 | 38.40 | 0.15 | 2.31 |
| | 7.90±0.70 | 0.06±0.01 | 99.19 | 8.05±0.16 | 5.61±0.04 | 30.31 | 0.15 | 5.55 |
| | 14.18±0.16 | 0.64±0.02 | 95.51 | 14.35±0.21 | 10.89±0.06 | 24.11 | 0.17 | 10.25 |




(A)




(B)

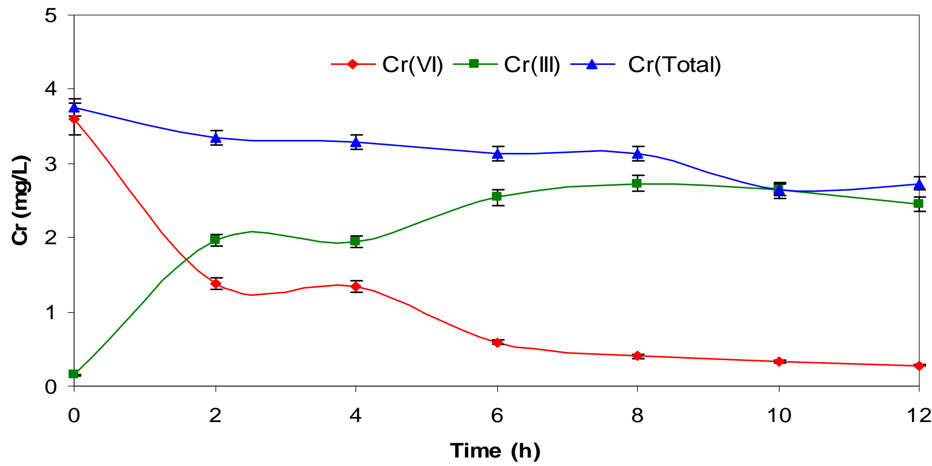



(C)

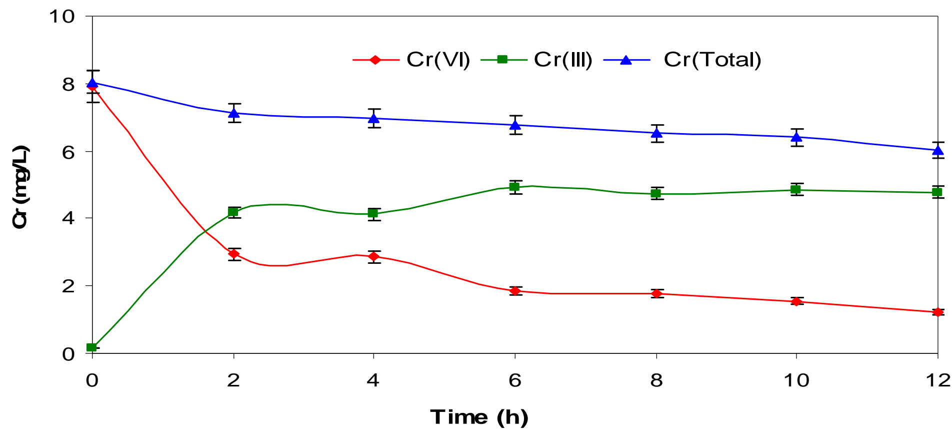
Fig. 19: Transformation of Cr(VI) to Cr(III) by bacterial consortium from tannery effluent (CT) {$C_i$ : Cr(VI): 14.18 mg/L}{A: $C_i$ Cr(VI): 3.6 mg/L; B: 7.9 mg/L; C: 14.18 mg/L}.

Table 16: Removal (%) of chromium by exponentially growing bacterial consortium (CS) from chrome sludge.

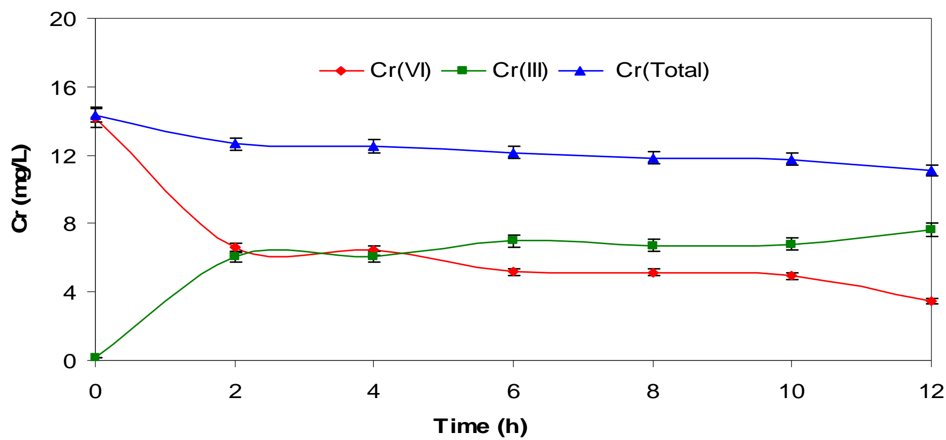
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 3.60±0.02 | - | - | 3.75±0.02 | - | - | 0.15 | - |
| | 7.90±0.12 | - | - | 8.05±0.06 | - | - | 0.15 | - |
| | 14.18±0.24 | - | - | 14.35±0.10 | - | - | 0.17 | - |
| 2 | 3.60±0.02 | 1.38±0.04 | 61.75 | 3.75±0.02 | 3.34±0.02 | 10.99 | 0.15 | 1.96 |
| | 7.90±0.12 | 2.95±0.06 | 62.06 | 8.05±0.06 | 7.12±0.08 | 11.55 | 0.15 | 4.17 |
| | 14.18±0.24 | 6.58±0.12 | 53.60 | 14.35±0.10 | 12.65±0.12 | 11.85 | 0.17 | 6.07 |
| 4 | 3.60±0.02 | 1.34±0.02 | 62.68 | 3.75±0.02 | 3.28±0.01 | 12.53 | 0.15 | 1.94 |
| | 7.90±0.12 | 2.86±0.04 | 63.82 | 8.05±0.06 | 6.98±0.02 | 13.29 | 0.15 | 4.12 |
| | 14.18±0.24 | 6.43±0.08 | 54.67 | 14.35±0.10 | 12.52±0.04 | 12.75 | 0.17 | 6.09 |
| 6 | 3.60±0.02 | 0.59±0.01 | 83.73 | 3.75±0.02 | 3.13±0.01 | 16.64 | 0.15 | 2.54 |
| | 7.90±0.12 | 1.85±0.04 | 76.60 | 8.05±0.06 | 6.76±.0.06 | 16.02 | 0.15 | 4.91 |
| | 14.18±0.24 | 5.16±0.14 | 63.58 | 14.35±0.10 | 12.14±0.02 | 15.40 | 0.17 | 6.98 |
| 8 | 3.60±0.02 | 0.40±0.01 | 88.87 | 3.75±0.02 | 3.13±0.07 | 16.56 | 0.15 | 2.73 |
| | 7.90±0.12 | 1.78±0.02 | 77.45 | 8.05±0.06 | 6.52±0.12 | 19.01 | 0.15 | 4.74 |
| | 14.18±0.24 | 5.13±0.04 | 63.81 | 14.35±0.10 | 11.85±0.08 | 17.42 | 0.17 | 6.72 |
| 10 | 3.60±0.02 | 0.33±0.01 | 90.74 | 3.75±0.02 | 2.98±0.02 | 20.64 | 0.15 | 2.64 |
| | 7.90±0.12 | 1.55±0.04 | 80.44 | 8.05±0.06 | 6.41±0.05 | 20.37 | 0.15 | 4.86 |
| | 14.18±0.24 | 4.93±0.10 | 65.24 | 14.35±0.10 | 11.74±0.24 | 18.19 | 0.17 | 6.81 |
| 12 | 3.60±0.02 | 0.28±0.02 | 92.14 | 3.75±0.02 | 2.73±0.04 | 27.20 | 0.15 | 2.45 |
| | 7.90±0.12 | 1.23±0.03 | 84.49 | 8.05±0.06 | 6.01±0.12 | 25.40 | 0.15 | 4.78 |
| | 14.18±0.24 | 3.48±0.06 | 75.45 | 14.35±0.10 | 11.12±0.11 | 22.51 | 0.17 | 7.64 |
| 22 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 2.41±0.01 | 35.73 | 0.15 | 2.41 |
| | 7.90±0.12 | 0.59±0.01 | 92.58 | 8.05±0.06 | 5.27±0.02 | 34.52 | 0.15 | 4.69 |
| | 14.18±0.24 | 3.30±0.04 | 76.75 | 14.35±0.10 | 9.47±0.06 | 34.01 | 0.17 | 6.17 |
| 24 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 2.23±0.02 | 40.43 | 0.15 | 2.23 |
| | 7.90±0.12 | 0.55±0.02 | 93.01 | 8.05±0.06 | 5.12±0.03 | 36.40 | 0.15 | 4.57 |
| | 14.18±0.24 | 3.04±0.01 | 78.53 | 14.35±0.10 | 8.41±0.06 | 41.39 | 0.17 | 5.37 |
| 26 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 1.72±0.02 | 54.08 | 0.15 | 1.72 |
| | 7.90±0.12 | 0.20±0.01 | 97.49 | 8.05±0.06 | 4.72±0.03 | 41.37 | 0.15 | 4.52 |
| | 14.18±0.24 | 2.91±0.06 | 79.48 | 14.3±0.10 | 6.86±0.06 | 52.23 | 0.17 | 3.95 |
| 28 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 1.34±0.02 | 64.16 | 0.15 | 1.34 |
| | 7.90±0.12 | 0.10±0.00 | 98.76 | 8.05±0.06 | 3.60±0.14 | 55.32 | 0.15 | 3.50 |
| | 14.18±0.24 | 2.34±0.04 | 83.52 | 14.35±0.10 | 6.10±0.21 | 57.53 | 0.17 | 3.76 |
| 30 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 1.21±0.01 | 67.73 | 0.15 | 1.21 |
| | 7.90±0.12 | 0.08±0.02 | 98.98 | 8.05±0.06 | 3.54±0.04 | 56.02 | 0.15 | 3.46 |
| | 14.18±0.24 | 2.24±0.04 | 84.23 | 14.35±0.10 | 5.98±0.02 | 58.33 | 0.17 | 3.74 |
| 32 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 1.14±0.01 | 69.60 | 0.15 | 1.14 |
| | 7.90±0.12 | 0.06±0.01 | 99.19 | 8.05±0.06 | 3.32±0.03 | 58.76 | 0.15 | 3.26 |
| | 14.18±0.24 | 1.98±0.10 | 86.01 | 14.35±0.10 | 5.58±0.02 | 61.11 | 0.17 | 3.60 |
| 36 | 3.60±0.02 | 0.00±0.00 | 100.00 | 3.75±0.02 | 1.02±0.01 | 72.80 | 0.15 | 1.02 |
| | 7.90±0.12 | 0.05±0.01 | 99.40 | 8.05±0.06 | 3.08±0.04 | 61.74 | 0.15 | 3.03 |
| | 14.18±0.24 | 1.11±0.06 | 92.19 | 14.35±0.10 | 5.58±0.07 | 61.11 | 0.17 | 4.47 |




(A)




(B)




(C)

Fig. 20: Transformation of Cr(VI) to Cr(III) by bacterial consortium from chrome sludge (CS) {A: $C_i$ Cr(VI): 3.6 mg/L; B: 7.9 mg/L; C: 14.18 mg/L}.

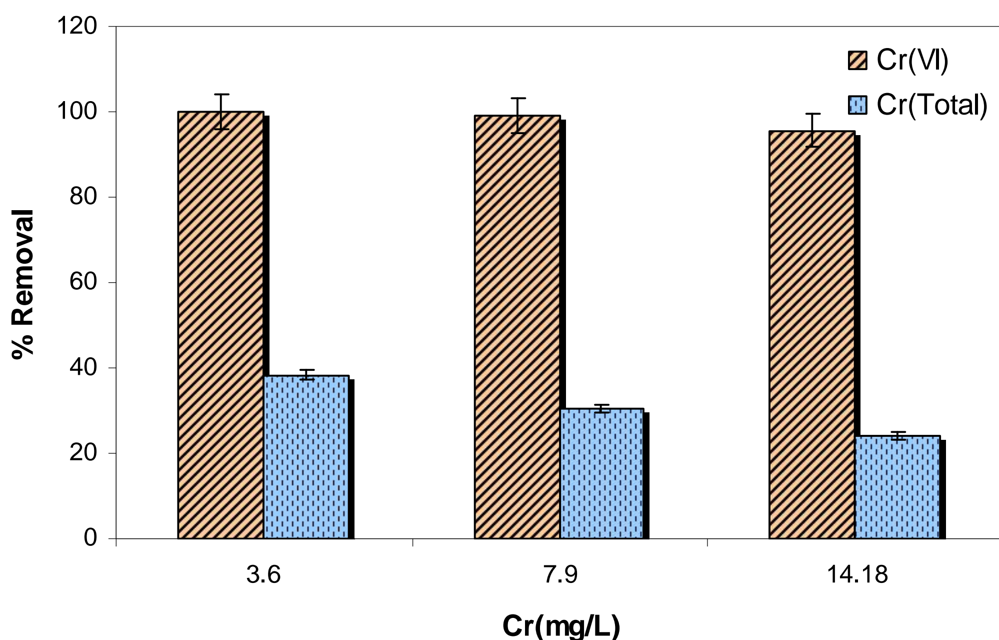



Fig. 21: Removal (%) of Cr(VI) and Cr(Total) by bacterial consortium from tannery effluent (CT) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

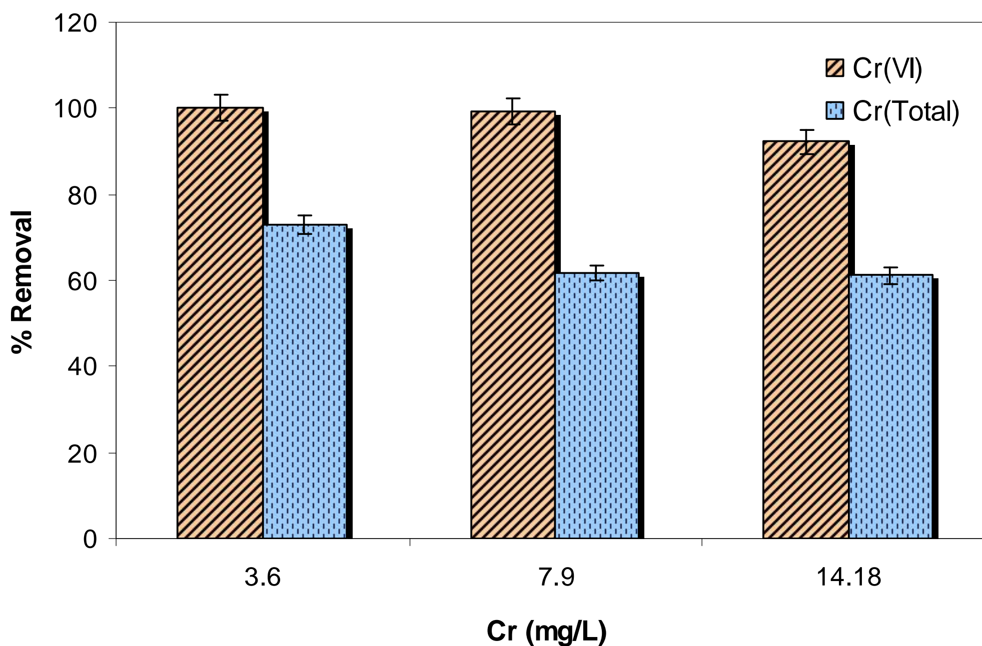



Fig. 22: Removal (%) of Cr(VI) and Cr(Total) by bacterial consortium from chrome sludge (CS) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

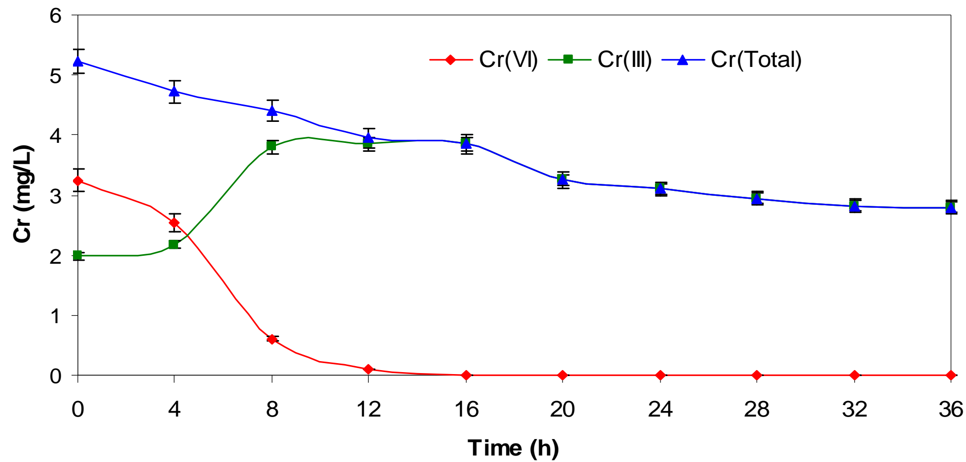
Chromium removal and its transformation by bacterial isolates (CT4, CT5, CS7, CS8) are shown in Table 17-20 and Fig. 23-26. As found with bacterial consortia, similar decrease in reduction rate was with observed with increase in concentration of Cr(VI). In the first 4 h CS7, CS8 and CT5 exhibited a transformation rate of approximately 1 mg/h, which was nearly ten times the transformation rate of CT4. Gradually over next 6 h the rate of transformation increased to 0.5 mg/h and 100% transformation was observed after 22 h of incubation (Fig. 23-26). Complete reduction of Cr(VI) to Cr(III) was observed by CT4 after 22 h of incubation whereas CS7 (*Bacillus cereus* IS3), CS8 (*Citrobacter freundii* IS4) and CT5 (*Citrobacter* sp. IS2) showed 94.81%, 95.81% and 95.17% reduction after 24 h of growth respectively. Reduction of Cr(VI) to Cr(III) was observed to be growth associated to avoid toxic effect of Cr(VI) as it is converted to Cr(III). In the first four hours reduction of Cr(VI) to Cr(III) at approximately 4 mg/L Cr(VI) concentration was 21.69%, 88.75%, 91.17% and 94.41%, by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) (Table 17-20; Fig. 23-26) respectively.

Fig. 23-26 shows the percentage removal of Cr(VI) with reference to the original concentration in the minimal medium (M2) inoculated with exponentially growing bacterial isolates. Removal efficiency differed at varying concentrations of Cr(VI) in minimal medium. Bacterial isolate CT4 (*Raoultella* sp. IS1) showed 100%, 93.58%, 76.37% removal of Cr(VI) and 46.46%, 30.53% 15.36% of Cr(Total) from minimal media (M2) (Table 17) at 3.25, 6.24 and 14.95 mg/L concentration of Cr(VI) respectively, whereas CT5 (*Citrobacter* sp. IS2) showed 93.66%, 98.75%, 97.57% removal of Cr(VI) and 66.02%, 25.18%, and 23.14% of Cr(Total) from minimal medium (M2) at the end of 36 h of incubation (Table 18; Fig. 27, 28).

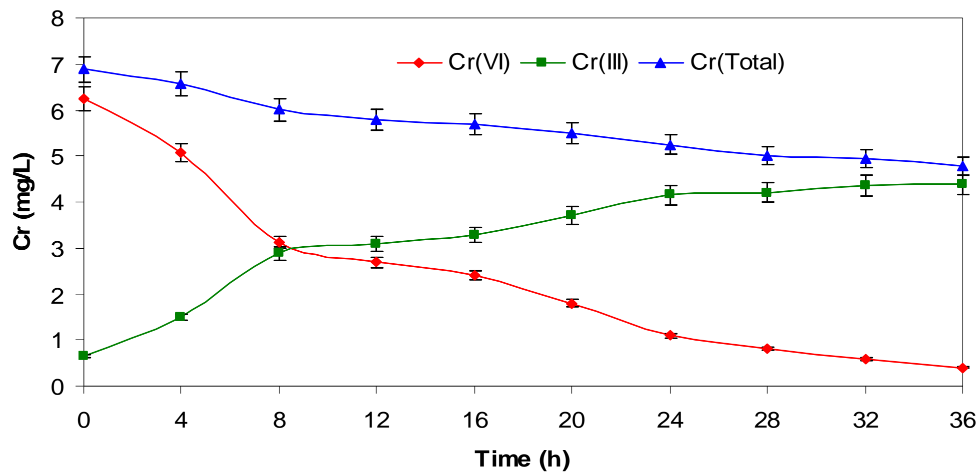
Bacterial isolates from chrome sludge CS7 (*Bacillus cereus* IS3) showed 94.81%, 94.97%, 79.8% removal of Cr(VI) and 27.42%, 9.66% and 5.29% removal of Cr(Total) from minimal media (Table 19), whereas CS8 (*Citrobacter freundii* IS4) showed 95.18%, 98.8%, 98.5% Cr(VI) and 20.47%, 11.05% and 4.46 % of Cr(Total) removal respectively (Table 19; Fig. 29, 30).

Table17: Removal (%) of chromium by exponentially growing bacterial isolate CT4 (*Raoultella* sp. IS1).

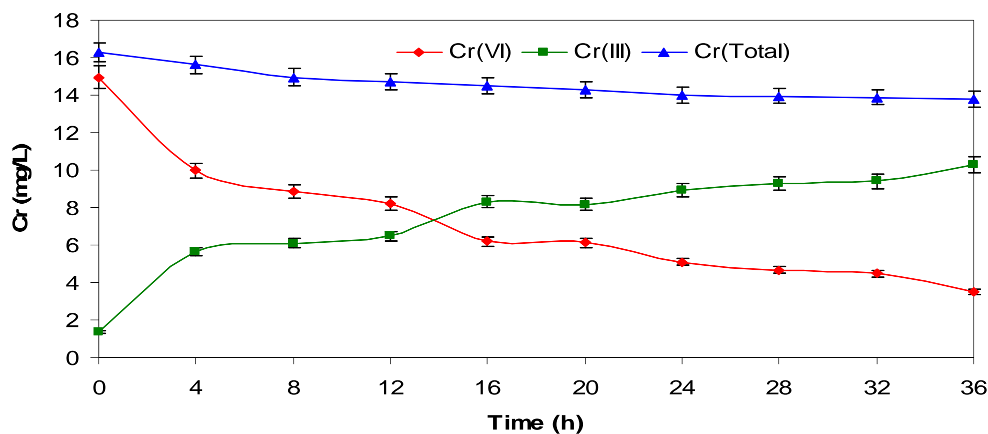
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 3.25±0.01 | - | - | 5.23±0.04 | - | - | 1.98 | - |
| | 6.24±0.06 | - | - | 6.88±0.70 | - | - | 0.64 | - |
| | 14.95±0.12 | - | - | 16.31±0.54 | - | - | 1.36 | - |
| 4 | 3.25±0.01 | 2.54±0.01 | 21.69 | 5.23±0.04 | 4.72±0.02 | 9.75 | 1.98 | 2.18 |
| | 6.24±0.06 | 5.08±0.06 | 18.61 | 6.88±0.70 | 6.58±0.12 | 4.37 | 0.64 | 1.50 |
| | 14.95±0.12 | 9.98±0.21 | 33.23 | 16.31±0.54 | 15.62±0.54 | 4.20 | 1.36 | 5.64 |
| 8 | 3.25±0.01 | 0.60±0.16 | 81.48 | 5.23±0.04 | 4.40±0.08 | 15.87 | 1.98 | 3.80 |
| | 6.24±0.06 | 3.13±0.06 | 49.89 | 6.88±0.70 | 6.01±0.15 | 12.66 | 0.64 | 2.88 |
| | 14.95±0.12 | 8.87±0.02 | 40.66 | 16.31±0.54 | 14.95±0.16 | 8.31 | 1.36 | 6.08 |
| 12 | 3.25±0.01 | 0.10±0.70 | 96.89 | 5.23±0.04 | 3.95±0.02 | 24.47 | 1.98 | 3.85 |
| | 6.24±0.06 | 2.69±0.65 | 56.90 | 6.88±0.70 | 5.78±0.04 | 16.00 | 0.64 | 3.09 |
| | 14.95±0.12 | 8.21±0.04 | 45.06 | 16.31±0.54 | 14.70±0.12 | 9.84 | 1.36 | 6.49 |
| 16 | 3.25±0.01 | 0.00±0.00 | 100.00 | 5.23±0.04 | 3.85±0.44 | 29.34 | 1.98 | 3.85 |
| | 6.24±0.06 | 2.40±0.02 | 61.49 | 6.88±0.70 | 5.70±0.18 | 17.16 | 0.64 | 3.30 |
| | 14.9±0.12 | 6.19±0.69 | 58.57 | 16.31±0.54 | 14.50±0.06 | 11.07 | 1.36 | 8.31 |
| 20 | 3.25±0.01 | 0.00±0.60 | 100.00 | 5.23±0.04 | 3.25±0.04 | 37.86 | 1.98 | 3.25 |
| | 6.24±0.06 | 1.80±0.04 | 71.20 | 6.88±0.70 | 5.50±0.06 | 20.07 | 0.64 | 3.70 |
| | 14.95±0.12 | 6.13±0.10 | 59.02 | 16.31±0.54 | 14.30±0.04 | 12.30 | 1.36 | 8.18 |
| 24 | 3.25±0.01 | 0.00±0.52 | 100.00 | 5.23±0.04 | 3.10±0.08 | 40.73 | 1.98 | 3.10 |
| | 6.24±0.06 | 1.09±0.18 | 82.52 | 6.88±0.70 | 5.25±0.12 | 23.70 | 0.64 | 4.16 |
| | 14.95±0.12 | 5.10±0.04 | 65.89 | 16.31±0.54 | 14.01±0.03 | 14.08 | 1.36 | 8.91 |
| 28 | 3.25±0.01 | 0.00±0.00 | 100.00 | 5.23±0.04 | 2.95±0.02 | 43.60 | 1.98 | 2.95 |
| | 6.24±0.06 | 0.81±0.06 | 87.11 | 6.88±0.70 | 5.01±0.06 | 27.19 | 0.64 | 4.21 |
| | 14.95±0.12 | 4.66±1.15 | 68.82 | 16.31±0.54 | 13.96±0.14 | 14.38 | 1.36 | 9.30 |
| 32 | 3.25±0.01 | 0.00±0.00 | 100.00 | 5.23±0.04 | 2.82±0.25 | 46.08 | 1.98 | 2.82 |
| | 6.24±0.06 | 0.59±0.02 | 90.62 | 6.88±0.70 | 4.94±0.10 | 28.21 | 0.64 | 4.35 |
| | 14.95±0.12 | 4.49±0.02 | 69.95 | 16.31±0.54 | 13.89 ±0.09 | 14.81 | 1.36 | 9.40 |
| 36 | 3.25±0.01 | 0.00±0.00 | 100.00 | 5.23±0.04 | 2.80±0.04 | 46.46 | 1.98 | 2.80 |
| | 6.24±0.06 | 0.40±0.04 | 93.58 | 6.88±0.70 | 4.78±0.06 | 30.53 | 0.64 | 4.38 |
| | 14.95±0.12 | 3.53±0.16 | 76.37 | 16.31±0.54 | 13.80±0.12 | 15.36 | 1.36 | 10.27 |




(A)




(B)

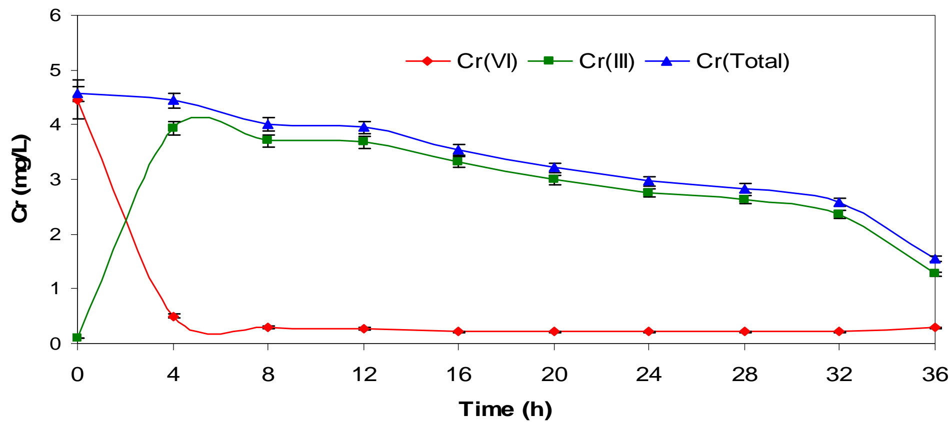



(C)

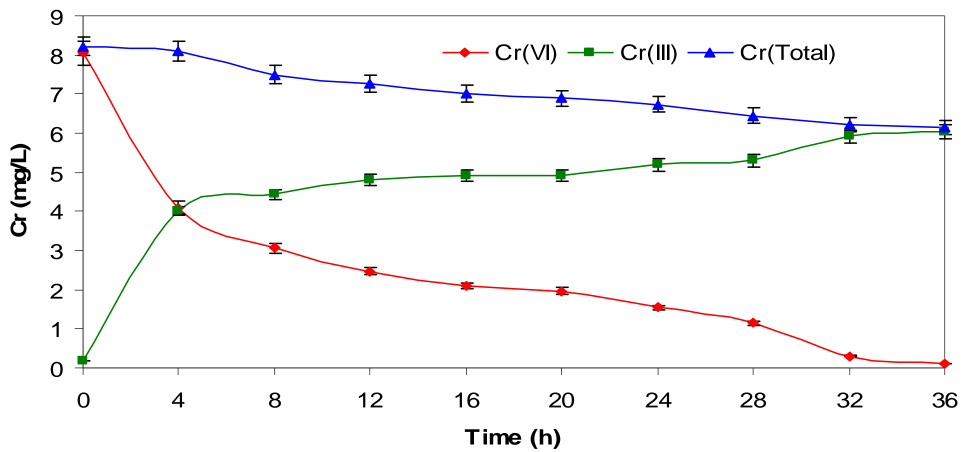
Fig. 23: Transformation of Cr(VI) to Cr(III) by CT4 (*Raoultella* sp. IS1) from tannery effluent {A: $C_i$ Cr(VI): 3.25 mg/L; B: 6.24 mg/L; C: 14.95mg/L}.

Table18: Removal (%) of chromium by exponentially growing bacterial isolate CT5 (*Citrobacter* sp. IS2)

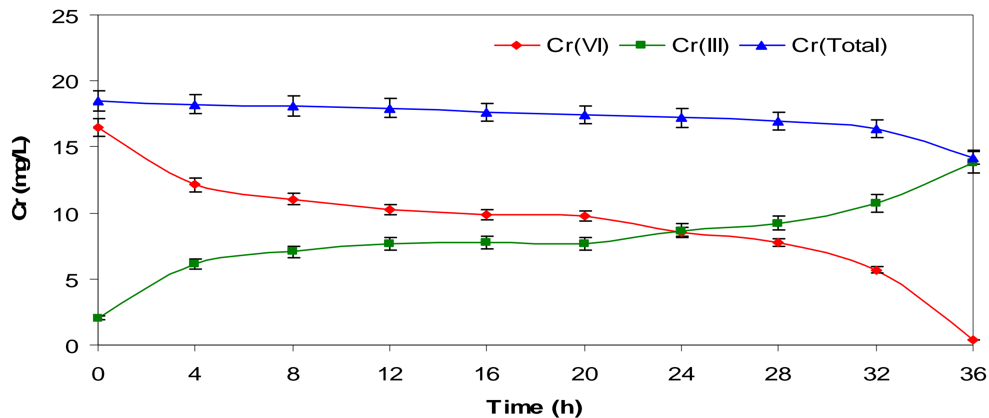
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 4.46±0.04 | - | - | 4.56±0.70 | - | - | 0.10 | - |
| | 8.04±0.15 | - | - | 8.22±0.16 | - | - | 0.18 | - |
| | 16.46±0.30 | - | - | 18.50±0.04 | - | - | 2.04 | - |
| 4 | 4.46±0.04 | 0.50±0.01 | 88.75 | 4.56±0.70 | 4.44±0.02 | 2.61 | 0.10 | 3.94 |
| | 8.04±0.15 | 4.09±0.04 | 49.18 | 8.22±0.16 | 8.10±0.05 | 1.46 | 0.18 | 4.01 |
| | 16.46±0.30 | 12.12±0.06 | 26.39 | 18.50±0.04 | 18.23±0.04 | 1.49 | 2.04 | 6.11 |
| 8 | 4.46±0.04 | 0.30±0.08 | 93.28 | 4.56±0.70 | 4.0±0.70 | 12.10 | 0.10 | 3.71 |
| | 8.04±0.15 | 3.06±0.12 | 61.95 | 8.22±0.16 | 7.50±0.12 | 8.76 | 0.18 | 4.44 |
| | 16.46±0.30 | 11.06± 0.54 | 32.83 | 18.50±0.04 | 18.10±0.14 | 2.17 | 2.04 | 7.04 |
| 12 | 4.46±0.04 | 0.27±0.02 | 94.03 | 4.56±0.70 | 3.95±0.02 | 13.42 | 0.10 | 3.68 |
| | 8.04±0.15 | 2.47±0.12 | 69.28 | 8.22±0.16 | 7.28±0.70 | 11.44 | 0.18 | 4.81 |
| | 16.46±0.30 | 10.27±0.10 | 37.64 | 18.50±0.04 | 17.94±0.12 | 3.03 | 2.04 | 7.67 |
| 16 | 4.46±0.04 | 0.22±0.04 | 95.17 | 4.56±0.70 | 3.54±0.15 | 22.40 | 0.10 | 3.33 |
| | 8.04±0.15 | 2.10±0.06 | 73.88 | 8.22±0.16 | 7.01±0.54 | 14.72 | 0.18 | 4.91 |
| | 16.46±0.30 | 9.83±0.08 | 40.30 | 18.50±0.04 | 17.62±0.04 | 4.76 | 2.04 | 7.79 |
| 20 | 4.46±0.04 | 0.22±0.04 | 95.17 | 4.56±0.70 | 3.21±0.04 | 29.64 | 0.10 | 3.00 |
| | 8.04±0.15 | 1.97±0.02 | 75.56 | 8.22±0.16 | 6.89±0.02 | 16.18 | 0.18 | 4.92 |
| | 16.46±0.30 | 9.76±0.01 | 40.70 | 18.50±0.04 | 17.45±0.06 | 5.68 | 2.04 | 7.69 |
| 24 | 4.46±0.04 | 0.22±0.04 | 95.17 | 4.56±0.70 | 2.97±0.05 | 34.90 | 0.10 | 2.76 |
| | 8.04±0.15 | 1.55±0.54 | 80.79 | 8.22±0.16 | 6.74±0.16 | 18.01 | 0.18 | 5.20 |
| | 16.46±0.30 | 8.55±0.12 | 48.07 | 18.50±0.04 | 17.21±0.15 | 6.98 | 2.04 | 8.66 |
| 28 | 4.46±0.04 | 0.22±0.02 | 95.17 | 4.56±0.70 | 2.84±0.07 | 37.75 | 0.10 | 2.63 |
| | 8.04±0.15 | 1.14±0.01 | 85.81 | 8.22±0.16 | 6.45±0.04 | 21.53 | 0.18 | 5.31 |
| | 16.46±0.30 | 7.76±0.08 | 52.87 | 18.50±0.04 | 16.99±0.08 | 8.17 | 2.04 | 9.23 |
| 32 | 4.46±0.04 | 0.22±0.01 | 95.17 | 4.56±0.70 | 2.58±0.14 | 43.45 | 0.10 | 2.37 |
| | 8.04±0.15 | 0.30±0.12 | 96.28 | 8.22±0.16 | 6.21±0.06 | 24.45 | 0.18 | 5.91 |
| | 16.46±0.30 | 5.69±0.12 | 65.45 | 18.50±0.04 | 16.41±0.05 | 11.30 | 2.04 | 10.72 |
| 36 | 4.46±0.04 | 0.28±0.06 | 93.66 | 4.56±0.70 | 1.55±0.08 | 66.02 | 0.10 | 1.27 |
| | 8.04±0.15 | 0.10±0.04 | 98.75 | 8.22±0.16 | 6.15±0.05 | 25.18 | 0.18 | 6.05 |
| | 16.46±0.30 | 0.40±0.18 | 97.57 | 18.50±0.04 | 14.22±0.02 | 23.14 | 2.04 | 13.82 |




(A)




(B)

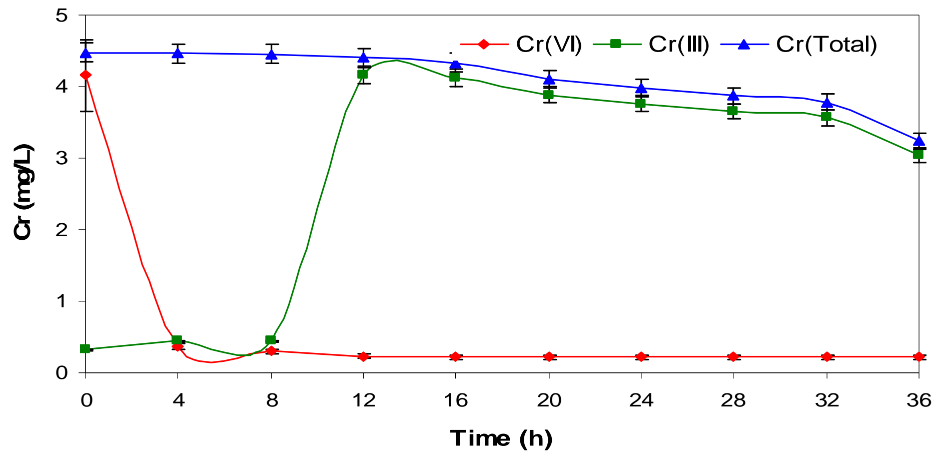



(C)

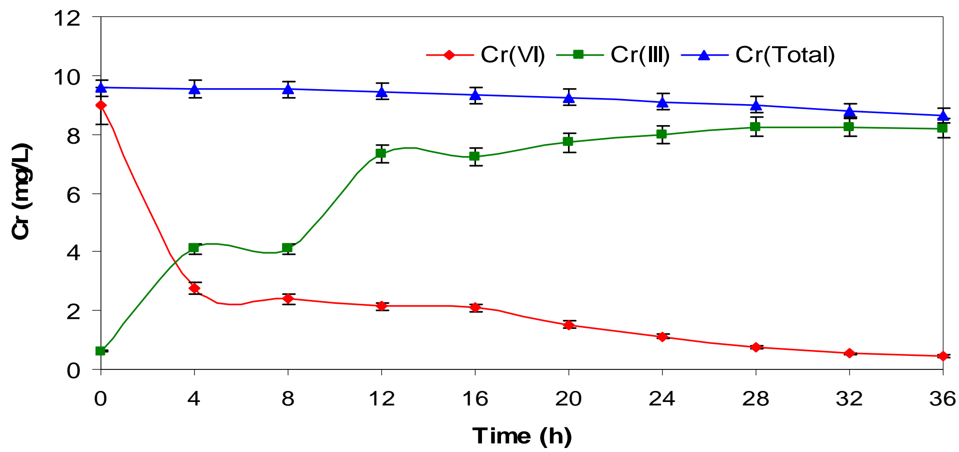
Fig. 24: Transformation of Cr(VI) to Cr(III) by CT5 (*Citrobacter* sp. IS2) from tannery effluent {A: $C_i$ Cr(VI): 4.46 mg/L; B: 8.22 mg/L; C: 16.46 mg/L}.

Table19: Removal (%) of chromium by exponentially growing bacterial isolates CS7 (*Bacillus cereus* IS3).

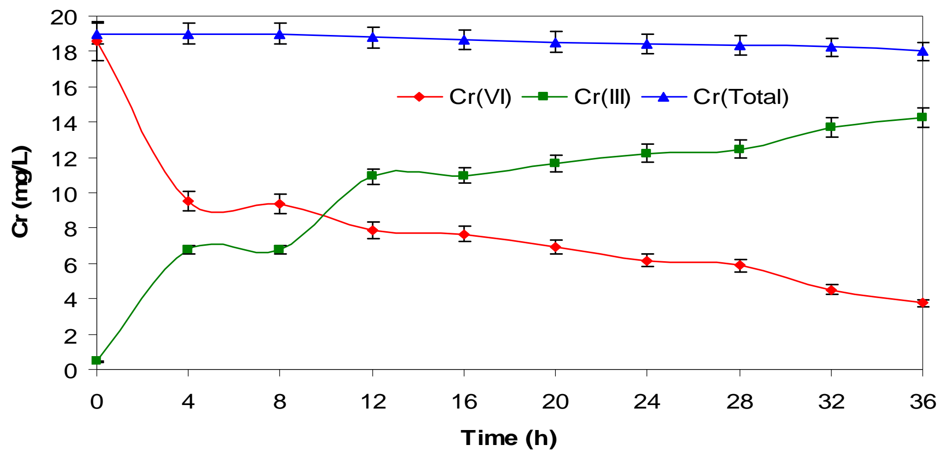
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 4.16±0.06 | - | - | 4.48±0.02 | - | - | 0.32 | - |
| | 8.97±0.14 | - | - | 9.58±0.06 | - | - | 0.61 | - |
| | 18.57±0.70 | - | - | 19.01±0.04 | - | - | 0.44 | - |
| 4 | 4.16±0.06 | 0.37±0.01 | 91.17 | 4.48±0.02 | 4.46±0.16 | 0.40 | 0.32 | 0.44 |
| | 8.97±0.14 | 2.77±0.06 | 69.07 | 9.58±0.06 | 9.55±0.04 | 0.27 | 0.61 | 4.09 |
| | 18.57±70 | 9.51±0.02 | 47.61 | 19.01±0.02 | 19.01±0.08 | 0.01 | 0.44 | 6.78 |
| 8 | 4.16±0.06 | 0.30±0.50 | 92.79 | 4.48±0.02 | 4.45±0.04 | 0.58 | 0.32 | 0.44 |
| | 8.97±0.14 | 2.39±0.04 | 73.39 | 9.58±0.06 | 9.52±0.24 | 0.58 | 0.61 | 4.09 |
| | 18.57±70 | 9.36±0.08 | 49.60 | 19.01±0.02 | 19.00±0.16 | 0.04 | 0.44 | 6.78 |
| 12 | 4.16±0.06 | 0.23±0.02 | 94.41 | 4.48±0.02 | 4.40±0.02 | 1.72 | 0.32 | 4.17 |
| | 8.97±0.14 | 2.14±0.06 | 76.20 | 9.58±0.06 | 9.45±0.16 | 1.31 | 0.61 | 7.32 |
| | 18.57±70 | 7.88±0.04 | 57.58 | 19.01±0.02 | 18.79±0.2 | 1.14 | 0.44 | 10.92 |
| 16 | 4.16±0.06 | 0.22±0.6 | 94.81 | 4.48±0.02 | 4.33±0.06 | 3.31 | 0.32 | 4.12 |
| | 8.97±0.14 | 2.08±0.09 | 76.77 | 9.58±0.06 | 9.32±0.14 | 2.67 | 0.61 | 7.24 |
| | 18.57±70 | 7.67±0.02 | 58.67 | 19.01±0.02 | 18.65±0.18 | 1.88 | 0.44 | 10.98 |
| 20 | 4.16±0.06 | 0.22±0.01 | 94.81 | 4.48±0.02 | 4.10±0.06 | 8.44 | 0.32 | 3.89 |
| | 8.97±0.14 | 1.53±0.01 | 82.96 | 9.58±0.06 | 9.24±0.02 | 3.51 | 0.61 | 7.71 |
| | 18.57±70 | 6.92±0.04 | 62.75 | 19.01±0.02 | 18.54 ±0.16 | 2.46 | 0.44 | 11.62 |
| 24 | 4.16 ±0.06 | 0.22±0.15 | 94.81 | 4.48±0.02 | 3.98±0.06 | 11.12 | 0.32 | 3.77 |
| | 8.97±0.14 | 1.13±0.14 | 87.46 | 9.58±0.06 | 9.11±0.02 | 4.86 | 0.61 | 7.99 |
| | 18.57±70 | 6.18±0.06 | 66.74 | 19.01±0.02 | 18.41±0.06 | 3.13 | 0.44 | 12.24 |
| 28 | 4.16±0.06 | 0.22±0.16 | 94.81 | 4.48±0.02 | 3.87±0.18 | 13.58 | 0.32 | 3.66 |
| | 8.97±0.14 | 0.75±0.70 | 91.59 | 9.58±0.06 | 9.00±0.04 | 5.99 | 0.61 | 8.25 |
| | 18.57±70 | 5.89±0.04 | 68.28 | 19.01±0.02 | 18.37±0.02 | 3.38 | 0.44 | 12.48 |
| 32 | 4.16±0.06 | 0.22±0.16 | 94.81 | 4.48±0.02 | 3.78±0.01 | 15.65 | 0.32 | 3.56 |
| | 8.97±0.14 | 0.54±0.01 | 94.03 | 9.58±0.06 | 8.78±0.02 | 8.31 | 0.61 | 8.25 |
| | 18.57±70 | 4.51±0.08 | 75.72 | 19.01±0.02 | 18.23±0.16 | 4.09 | 0.44 | 13.72 |
| 36 | 4.16±0.06 | 0.22±0.76 | 94.81 | 4.48±0.02 | 3.25±0.42 | 27.42 | 0.32 | 3.04 |
| | 8.97±0.14 | 0.45±0.26 | 94.97 | 9.58±0.06 | 8.65±0.06 | 9.66 | 0.61 | 8.20 |
| | 18.57±70 | 3.75±0.04 | 79.80 | 19.01±0.02 | 18.00±0.12 | 5.29 | 0.44 | 14.25 |




(A)




(B)

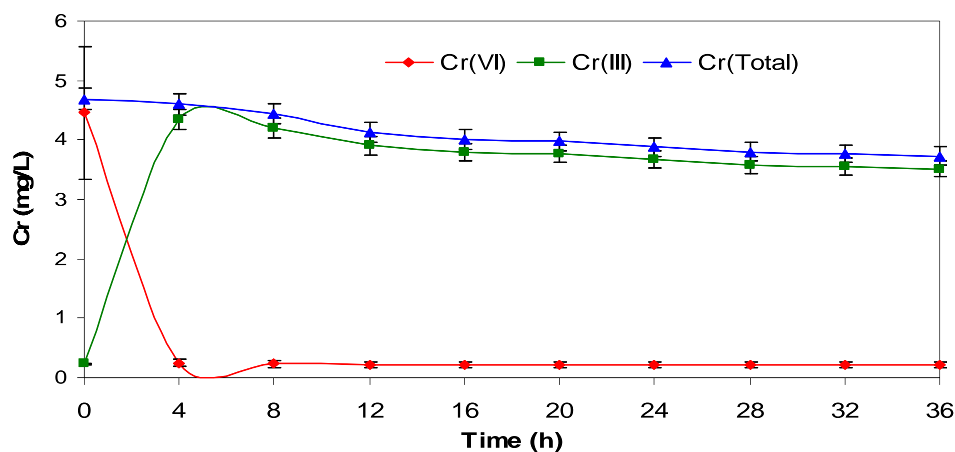



(C)

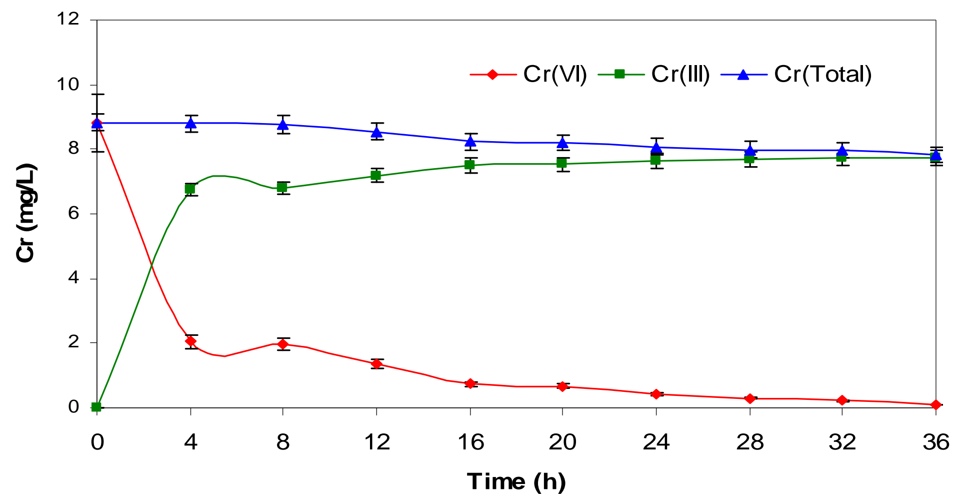
Fig. 25: Transformation of Cr(VI) to Cr(III) by CS7 (*Bacillus cereus* IS3) from chrome sludge {A: $C_i$ Cr(VI): 4.16 mg/L; B: 8.97 mg/L; C: 18.57 mg/L}.

Table 20: Removal (%) of chromium by exponentially growing bacterial isolate CS8 (*Citrobacter freundii* IS4).

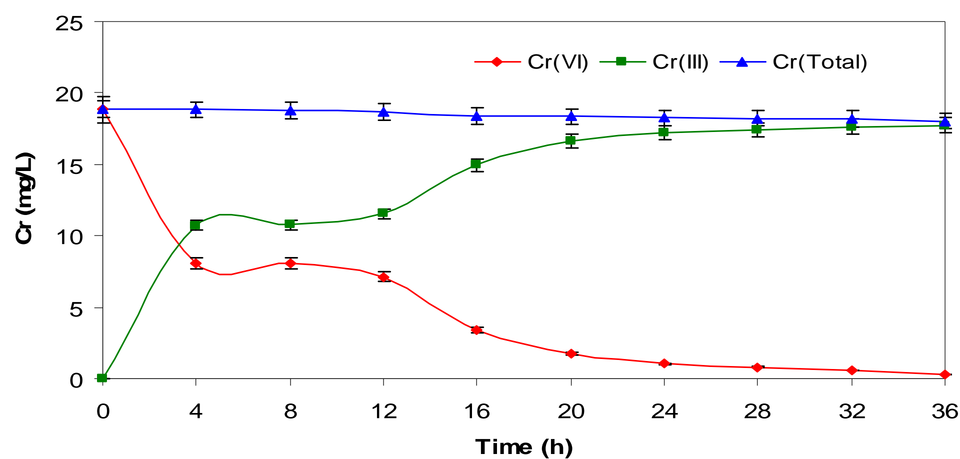
| Cr(VI) (mg/L) | | | | Cr(Total) (mg/L) | | | Cr(III) (mg/L) | |
|---|---|---|---|---|---|---|---|---|
| Time (h) | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ | % Removal | $C_i$ | $C_f$ |
| 0 | 4.46±0.21 | - | - | 4.69±0.16 | - | - | 0.23 | - |
| | 8.82±0.04 | - | - | 8.83±0.04 | - | - | 0.00 | - |
| | 18.85±0.19 | - | - | 18.89±0.20 | - | - | 0.04 | - |
| 4 | 4.46±0.02 | 0.25±0.02 | 94.41 | 4.69±0.07 | 4.60±0.07 | 1.96 | 0.23 | 4.35 |
| | 8.82±0.16 | 2.05±0.05 | 76.75 | 8.83±0.14 | 8.80±0.20 | 0.27 | 0.00 | 6.75 |
| | 18.85±0.19 | 8.09±0.04 | 57.06 | 18.89±0.1 | 18.83±0.6 | 0.13 | 0.04 | 10.73 |
| 8 | 4.46±0.02 | 0.23±0.01 | 94.79 | 4.69±0.06 | 4.44±0.12 | 5.33 | 0.23 | 4.21 |
| | 8.82±0.13 | 1.97±0.16 | 77.71 | 8.83±0.04 | 8.76±0.10 | 0.69 | 0.00 | 6.80 |
| | 18.85±0.5 | 8.04±0.06 | 57.33 | 18.89±0.10 | 18.80±0.16 | 0.27 | 0.04 | 10.76 |
| 12 | 4.46±0.02 | 0.22±0.01 | 95.18 | 4.69±0.07 | 4.12±0.07 | 12.07 | 0.23 | 3.91 |
| | 8.82±0.8 | 1.36±0.06 | 84.58 | 8.83±0.05 | 8.55± 0.19 | 3.16 | 0.00 | 7.19 |
| | 18.85±0.17 | 7.12±0.04 | 62.24 | 18.89±0.04 | 18.68±0.03 | 0.93 | 0.04 | 11.56 |
| 16 | 4.46±0.16 | 0.22±0.02 | 95.18 | 4.69±0.07 | 4.01±0.05 | 14.52 | 0.23 | 3.79 |
| | 8.82±0.06 | 0.74±0.01 | 91.64 | 8.83±0.16 | 8.23±0.16 | 6.71 | 0.00 | 7.50 |
| | 18.85±0.16 | 3.41±0.12 | 81.89 | 18.89 ±0.19 | 18.38±0.08 | 2.50 | 0.04 | 14.97 |
| 20 | 4.46±0.05 | 0.22±0.08 | 95.18 | 4.69±0.07 | 3.98±0.17 | 15.18 | 0.23 | 3.76 |
| | 8.82 ±0.02 | 0.67±0.02 | 92.40 | 8.83±0.06 | 8.20±0.05 | 7.12 | 0.00 | 7.53 |
| | 18.85±0.17 | 1.73±0.06 | 90.82 | 18.89±0.08 | 18.37±0.14 | 2.56 | 0.04 | 16.64 |
| 24 | 4.46±0.05 | 0.22±0.07 | 95.18 | 4.69±0.07 | 3.88±0.09 | 17.27 | 0.23 | 3.67 |
| | 8.82±0.02 | 0.43±0.02 | 95.08 | 8.83±0.20 | 8.08±0.18 | 8.44 | 0.00 | 7.65 |
| | 18.85±0.17 | 1.06±0.16 | 94.39 | 18.89±0.05 | 18.26±0.16 | 3.14 | 0.04 | 17.20 |
| 28 | 4.46±0.24 | 0.22±0.12 | 95.18 | 4.69±0.3 | 3.80±0.03 | 18.98 | 0.23 | 3.59 |
| | 8.82±0.12 | 0.30±0.1 | 96.60 | 8.83±0.2 | 7.99±0.04 | 9.46 | 0.00 | 7.69 |
| | 18.85±0.3 | 0.79±0.02 | 95.82 | 18.89±0.8 | 18.21±0.2 | 3.40 | 0.04 | 17.42 |
| 32 | 4.46±0.04 | 0.22±0.03 | 95.18 | 4.69±0.7 | 3.77±0.11 | 19.64 | 0.23 | 3.55 |
| | 8.82±0.16 | 0.22±0.2 | 97.48 | 8.83±0.2 | 7.95±0.05 | 10.82 | 0.00 | 7.73 |
| | 18.85±0.2 | 0.57±0.08 | 96.98 | 18.89±0.5 | 18.20±1.2 | 3.46 | 0.04 | 17.63 |
| 36 | 4.46±0.02 | 0.22±0.04 | 95.18 | 4.69±0.12 | 3.73±0.4 | 20.47 | 0.23 | 3.52 |
| | 8.82±0.17 | 0.11±0.6 | 98.80 | 8.83±0.3 | 7.85±0.8 | 11.05 | 0.00 | 7.74 |
| | 18.85±0.6 | 0.28±0.4 | 98.50 | 18.89±0.6 | 18.01±0.14 | 4.46 | 0.04 | 17.73 |




(A)




(B)




(C)

Fig. 26: Transformation of Cr(VI) to Cr(III) by CS8 (*Citrobacter freundii* IS4) from chrome sludge {A: $C_i$ Cr(VI): 4.46 mg/L; B: 8.82 mg/L; C: 18.85 mg/L}.

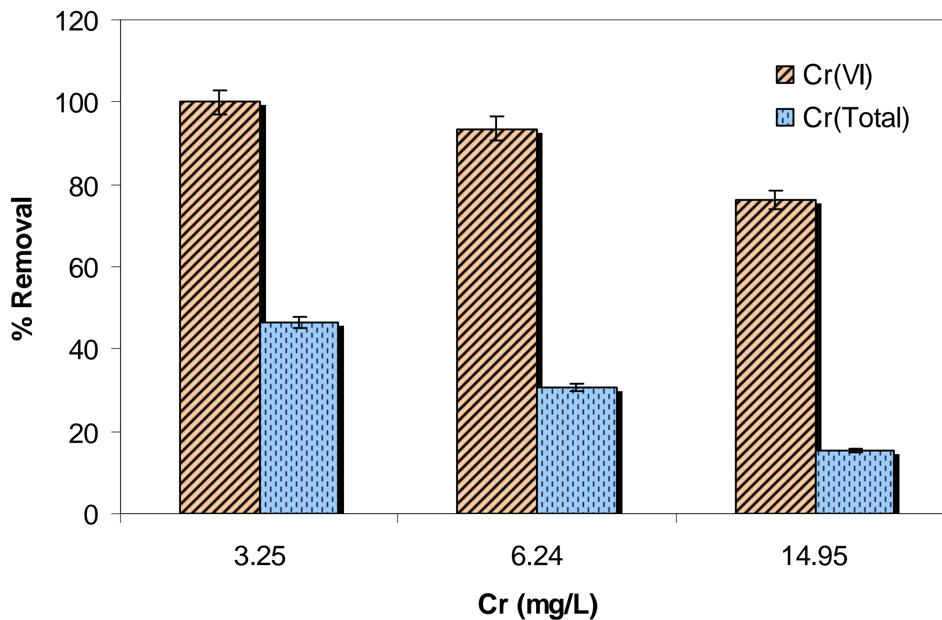



Fig. 27: Removal (%) of Cr(VI) and Cr(Total) by CT4 (*Raoultella* sp. IS1) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

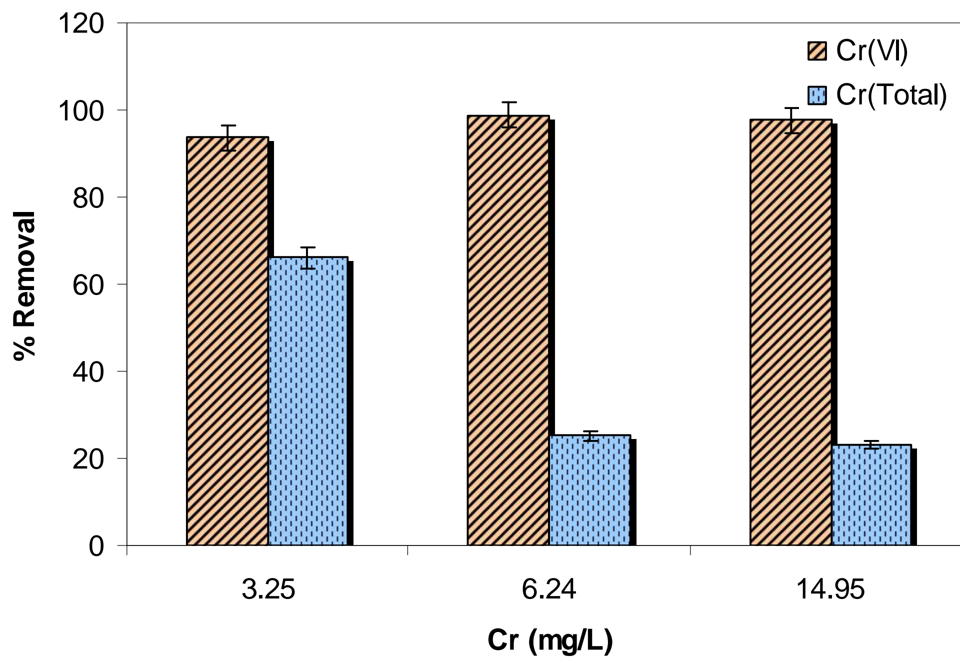



Fig. 28: Removal (%) of Cr(VI) and Cr(Total) by CT5 (*Citrobacter* sp. IS2) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

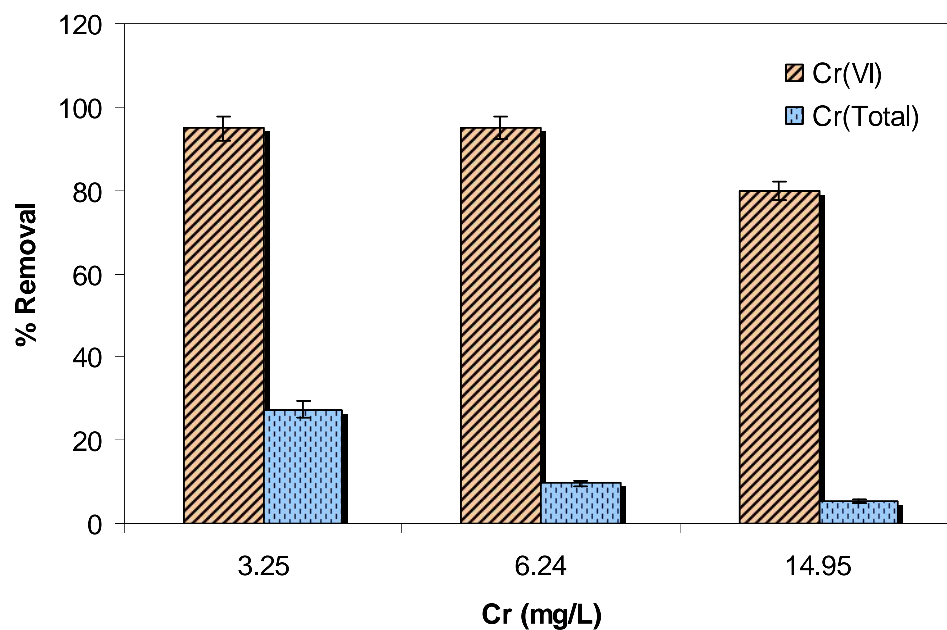



Fig. 29: Removal (%) of Cr(VI) and Cr(Total) by CS7 (*Bacillus cereus* IS3) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

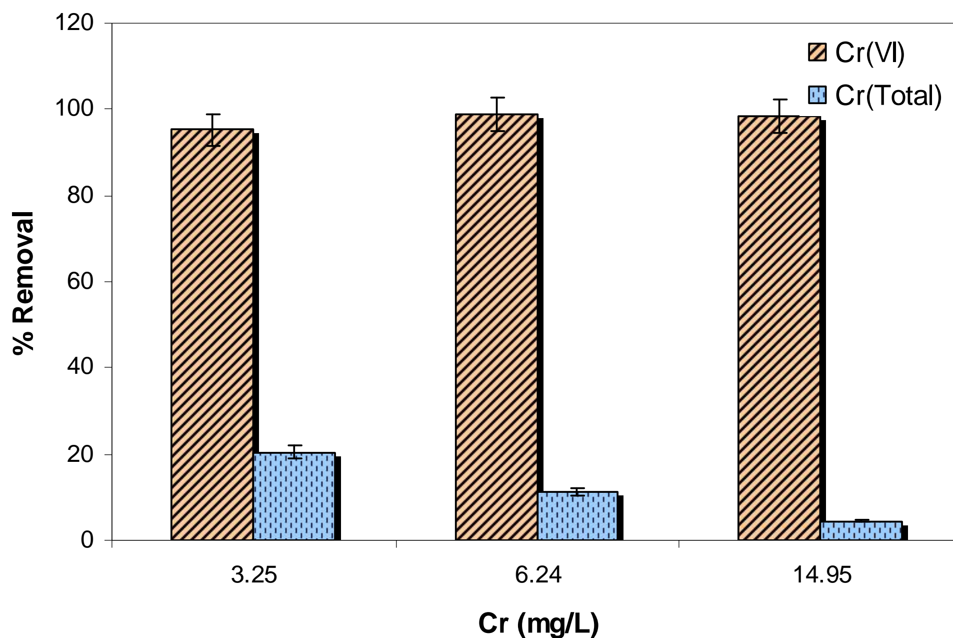



Fig. 30: Removal (%) of Cr(VI) and Cr(Total) by CS8 (*Citrobacter freundii* IS4) from minimal media (M2) supplemented with different concentration of Cr(VI) after 36 h of incubation.

**Comparison of chromium removal and transformation by bacterial isolates**

Chromium removal and its transformation by four different bacterial isolates after 4 h (Fig. 31), 8 h (Fig. 32) and 12 h (Fig. 33) of incubation in minimal media M2 supplemented with 0.5% glucose containing 3.25-4.46 mg/L Cr(VI) shows comparison among different isolates. With the passage of time there was decrease in residual concentration of Cr(VI) with concomitant increase in the concentration of Cr(III), which is confirmed from removal of Cr(VI) and its conversion or transformation to Cr(III). In the intial phase of growth the rate of transformation was higher in CT5, CS7 and CS8 and lowest by *Raoultella* sp. IS1 (CT4), however with increase in incubation time the removal and transformation was almost same by all the isolates. In the first 4 h of incubation reduction of Cr(VI) to Cr(III) at 4.46 mg/L of Cr(VI) was 21.69%, 88.75% 91.16%, and 94.41% by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) respectively (Fig. 31). The residual concentration of Cr(VI) was decreased to 2.54, 0.50, 0.37 and 0.25 mg/L, whereas of Cr(III) was increased to 2.18, 3.94, 4.09 and 4.35 mg/L by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) respectively.

After 8 h of incubation 81.48, 93.28, 91.16 and 94.79% transformation of Cr(VI) to Cr(III) at 4.46 mg/L of Cr(VI) by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) was observed respectively (Fig. 32). The residual concentration of Cr(VI) was decresed to 0.63, 0.3, 0.3 and 0.23 mg/L, whereas concentration of Cr(III) was increased to 3.8, 3.7, 4.15 and 4.2 mg/L by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) respectively (Fig. 32).

After 12 h of incubation 96.89, 94.03, 94.41 and 95.18% transformation of Cr(VI) to Cr(III) at 4.46 mg/L of Cr(VI) by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) was observed respectively (Fig. 33). The residual concentration of Cr(VI) was decresed to 0.1, 0.26, 0.23 and 0.21 mg/L, whereas concentration of Cr(III) was increased to 3.85, 3.6, 4.17 and 3.9 mg/L by CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4) respectively (Fig. 33). All the isolates showed approximately similar trend in removal of Cr(VI).

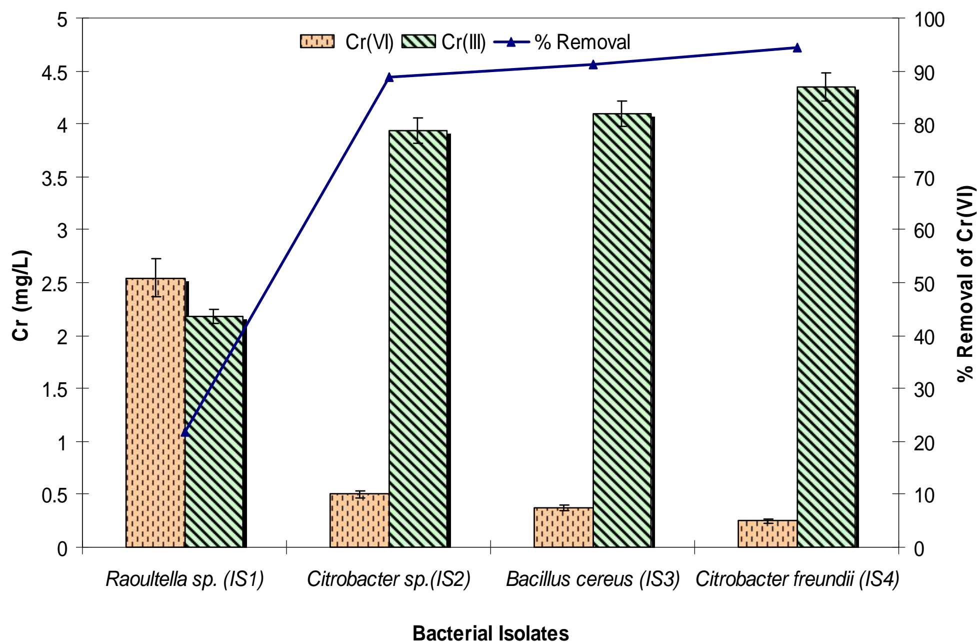



Fig. 31: Chromium removal by bacterial isolates in minimal media (M2) after 4 h of incubation. {*Raoultella* sp. (IS1) $C_i$: 3.25; *Citrobacter* sp. (IS2) $C_i$: 4.46; *Bacillus cereus* (IS3) $C_i$: 4.16; *Citrobacter freundii* (IS4) $C_i$: 4.46}.

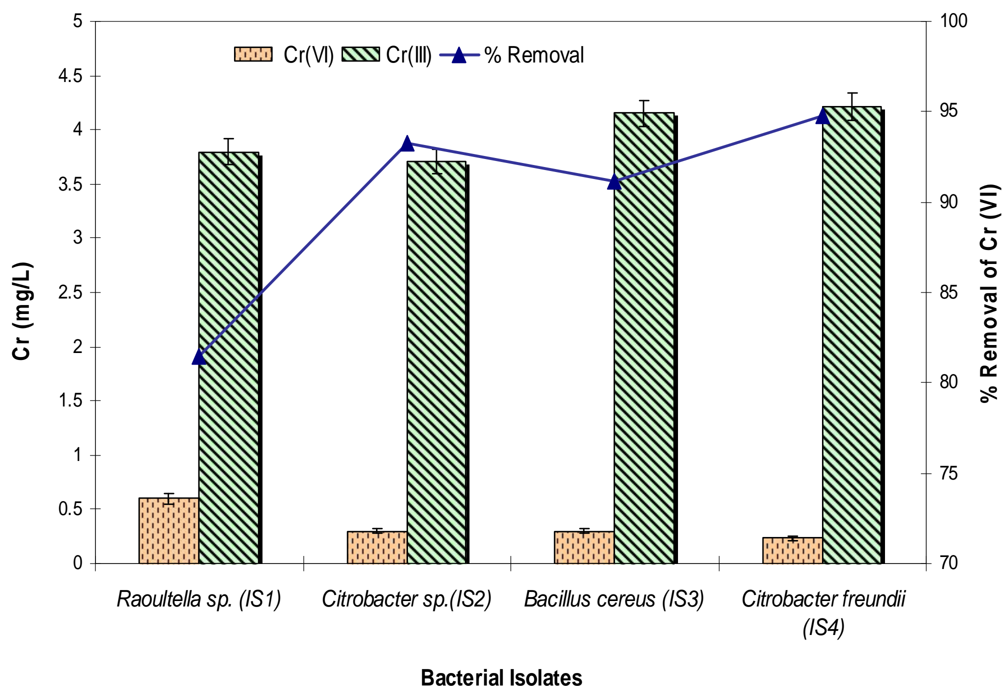



Fig. 32: Chromium removal by bacterial isolates in minimal media (M2) after 8 h of incubation. {*Raoultella* sp. (IS1) Ci: 3.25; *Citrobacter* sp. (IS2) $C_i$: 4.46; *Bacillus cereus* (IS3) $C_i$: 4.16; *Citrobacter freundii* (IS4) $C_i$ 4.46}.

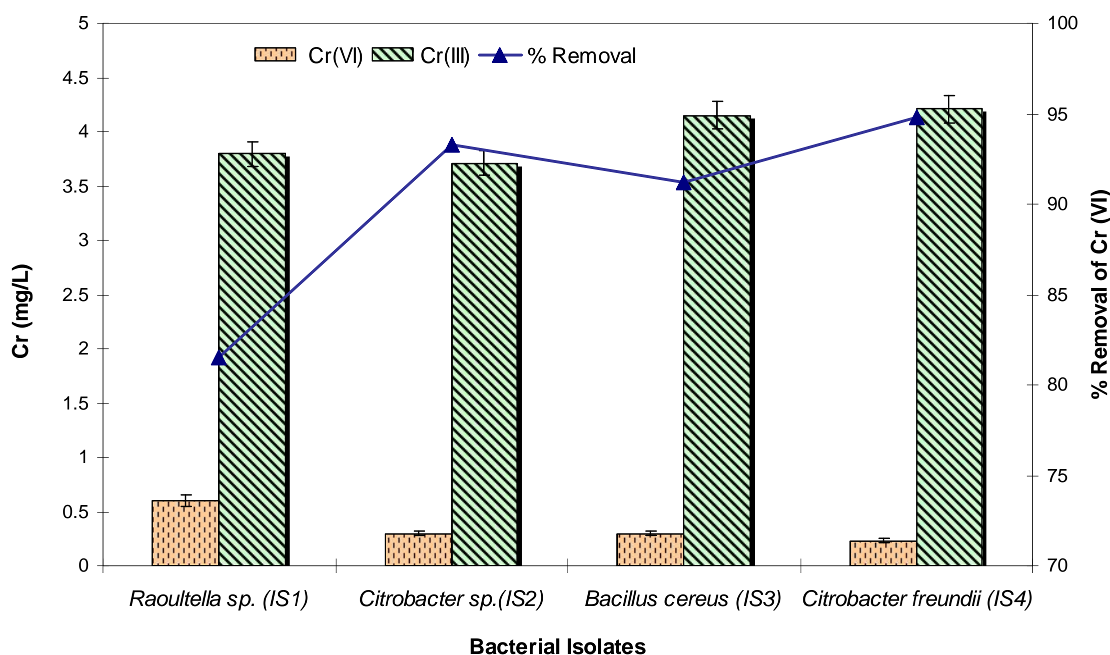



Fig. 33: Chromium removal by bacterial isolates in minimal media (M2) after 12 h of incubation. {*Raoultella* sp. (IS1) $C_i$: 3.25; *Citrobacter* sp. (IS2) $C_i$: 4.46; *Bacillus cereus* (IS3) $C_i$: 4.16; *Citrobacter freundii* (IS4) $C_i$: 4.46}.

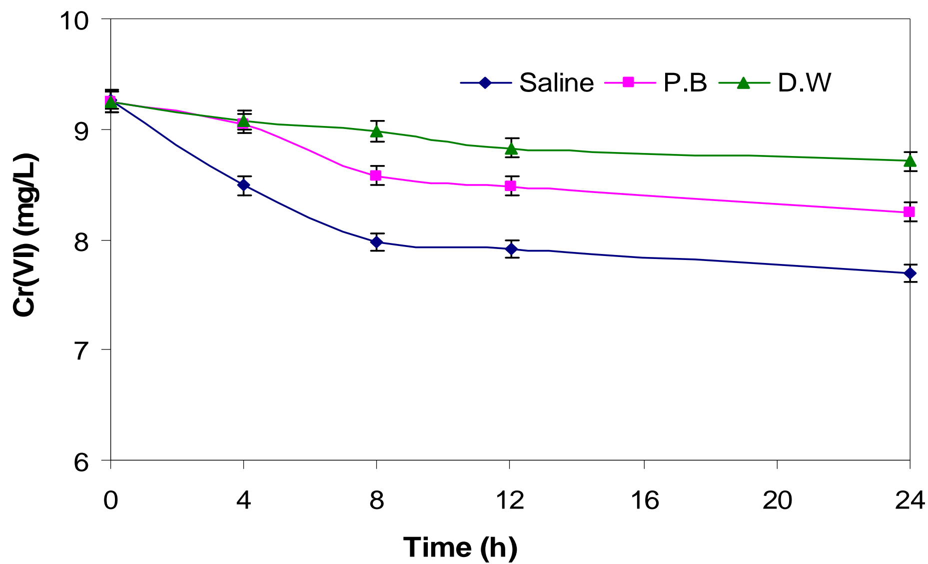
### 4.2.6 Transformation of Cr(VI) by resting cells and cell free extract

**Removal of Cr(VI) by resting cells**

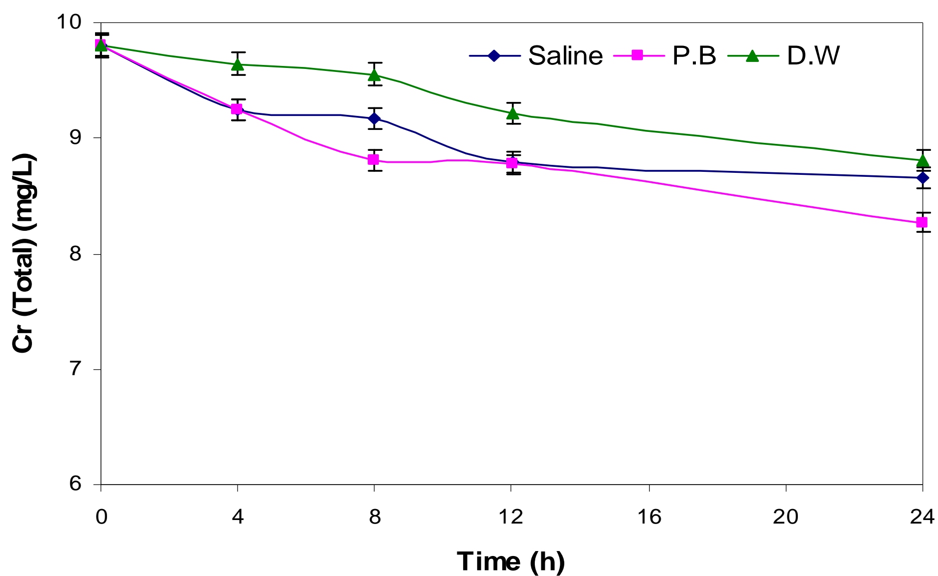
Since the effect of Cr(VI) at higher concentration (15 and 25 mg/L) was more pronounced transformation of Cr(VI) by resting cells and cell free extract was studied in different suspension media containing 9.5-9.8 mg/L of Cr(VI). The bacterial consortia (CT, CS) were grown for overnight in 300 mL of minimal media (M2). Cells were harvested and divided equally in three parts and suspended in 25 mL of phosphate buffer, 0.85% saline and deionised sterile water respectively containing Cr(VI) and incubated. The supernatant obtained was separately assayed for Cr(VI) transformation. Samples were drawn after every two hours till 24 h of incubation and the supernatant was analyzed for the residual Cr(VI) and total chromium. The data on Cr(VI) reduction by resting cells of both the bacterial consortium are presented in Table 21, Fig. 34, 35, 36). The order of percentage transformation by bacterial consortium from tannery effluent (CT) after 24 h of incubation as observed for saline, phosphate buffer and deionised water was 69.16% > 44.44% > 19.56% and by CS consortium it was 41% > 40% > 23.18% respectively (Fig. 34, 35, 36). Among the three suspension media saline was best suited for Cr(VI) transformation. With transformation of Cr(VI) there was decrease in the concentration of total chromium (Table 21, Fig. 34, 35).

Table 21: Transformation of Cr(VI) to Cr(III) by resting cells of bacterial consortia from tannery effluent (CT) and chrome sludge (CS) using different suspended media.

| Bacterial consortia (CT) from tannery effluent | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Saline (S) | | | Phosphate Buffer (PB) | | | Deionised Water (DW) | | |
| Time (h) | Cr (Total) | Cr(VI) | Cr(III) | Cr (Total) | Cr(VI) | Cr(III) | Cr (Total) | Cr(VI) | Cr(III) |
| 0 | 9.80 | 9.27 | 0.53 | 9.81 | 9.25 | 0.56 | 9.80 | 9.25 | 0.55 |
| 4 | 9.24 | 8.49 | 0.75 | 9.24 | 9.05 | 0.19 | 9.64 | 9.08 | 0.56 |
| 8 | 9.17 | 7.97 | 1.20 | 8.81 | 8.58 | 0.23 | 9.55 | 8.98 | 0.57 |
| 12 | 8.79 | 7.92 | 0.87 | 8.77 | 8.48 | 0.29 | 9.22 | 8.83 | 0.39 |
| 24 | 8.66 | 7.70 | 0.96 | 8.27 | 8.25 | 0.02 | 8.81 | 8.71 | 0.10 |
| Bacterial consortia (CS) from chrome sludge | | | | | | | | | |
| | Saline (S) | | | Phosphate Buffer (PB) | | | Deionised Water (DW) | | |
| Time (h) | Cr (Total) | Cr(VI) | Cr(III) | Cr (Total) | Cr(VI) | Cr(III) | Cr (Total) | Cr(VI) | Cr(III) |
| 0 | 9.55 | 9.12 | 0.43 | 9.54 | 9.09 | 0.45 | 9.54 | 9.10 | 0.44 |
| 4 | 7.53 | 6.93 | 0.59 | 7.85 | 7.89 | 0.00 | 7.69 | 7.12 | 0.57 |
| 8 | 6.74 | 6.36 | 0.38 | 7.33 | 6.11 | 1.22 | 7.51 | 7.10 | 0.41 |
| 12 | 6.54 | 6.26 | 0.28 | 5.82 | 5.65 | 0.17 | 7.49 | 7.04 | 0.45 |
| 24 | 5.46 | 5.40 | 0.06 | 5.77 | 5.44 | 0.33 | 7.45 | 6.99 | 0.46 |

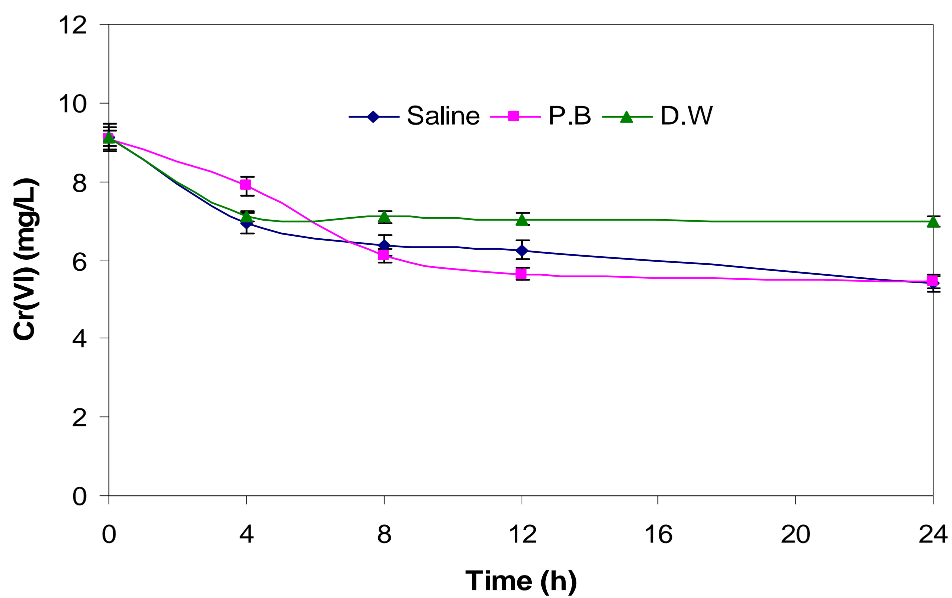



(A)

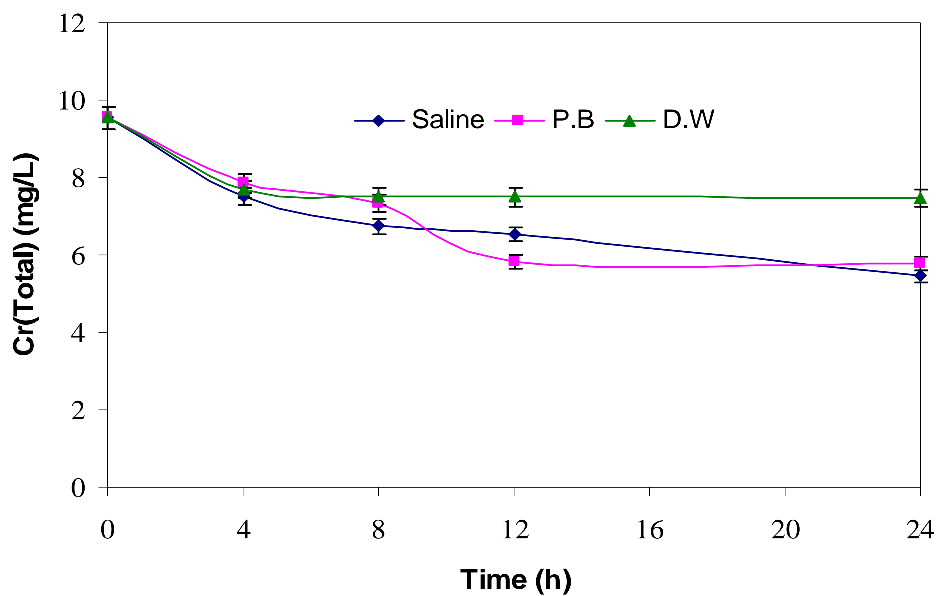



(B)

Fig. 34: Removal of chromium by resting cells of bacterial consortia (CT) from tannery effluent (A) Cr(VI); (B) Cr(Total) {S: Saline; PB: Phosphate buffer; DW: Deionised water}.




(A)




(B)

Fig. 35: Removal of chromium by resting cells of bacterial consortia (CS) from chrome sludge. (A) Cr(VI); (B) Cr(Total) {S: Saline; PB: Phosphate buffer; DW: Deionised water}.

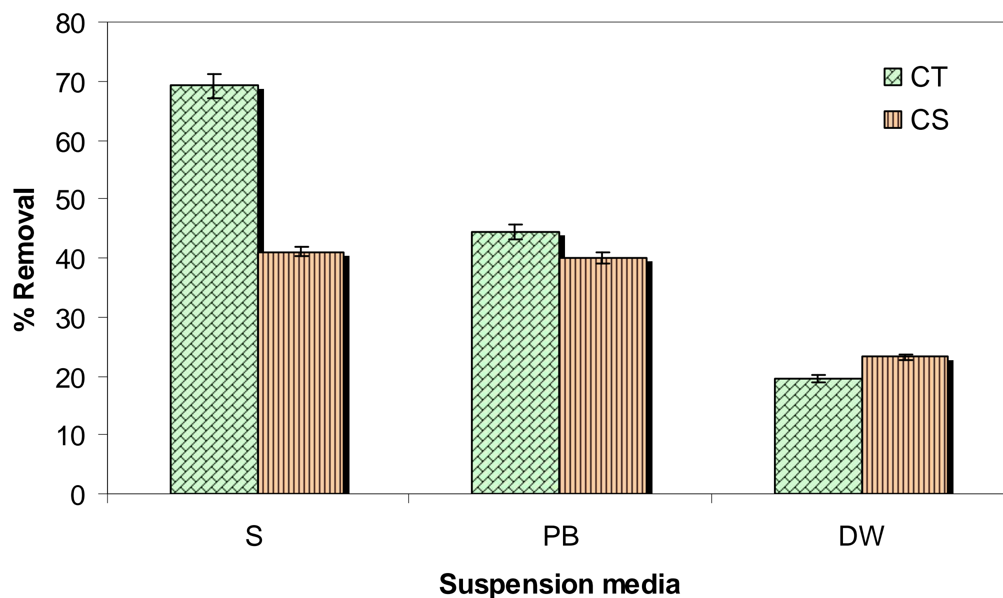



Fig. 36: Cr(VI) removal (%) by bacterial consortium isolated from tannery effluent (CT) and chrome sludge (CS) in different suspension media {S: Saline; PB: Phosphate buffer; DW: Deionised water}. after 24 h of incubation.

**Removal of Cr(VI) by cell free extract**

The supernatant representing cell free extract from overnight grown bacterial consortium in minimal media M2 obtained after removing the cells was directly supplemented with 1.2-1.9 mg/L of Cr(VI) and incubated and samples were drawn after every 30 min. and analysed for Cr(VI). Supernatant from bacterial consortium (CS) from chrome sludge demonstrated higher Cr(VI) transformation after 6 h of incubation at 1.19 mg/L of Cr(VI) as it reduced approximately 90% of Cr(VI), whereas percentage transformation was 58% by bacterial consortia (CT) from tannery effluent. Higher percentage transformation by cell free extracts of both the consortia as compared to resting cells confirms the presence of extracellular chromium reductase activity (Table 22, Fig. 37).

Table 22: Transformation of Cr(VI) by cell free extract of bacterial consortia (CT and CS) from tannery effluent and chrome sludge.

| Time (h) | Cr(VI) mg/L | |
|---|---|---|
| | CT | CS |
| 0 | 1.89±0.04 | 1.19±0.02 |
| 0.5 | 1.79±0.01 | 1.08±0.01 |
| 1 | 1.69±0.02 | 0.97±0.02 |
| 1.5 | 1.59±0.01 | 0.96±0.03 |
| 2 | 1.54±0.03 | 0.93±0.02 |
| 2.5 | 1.50±0.05 | 0.70±0.04 |
| 3 | 1.39±0.02 | 0.68±0.06 |
| 3.5 | 1.28±0.04 | 0.66±0.01 |
| 4 | 1.25±0.03 | 0.61±0.02 |
| 4.5 | 1.16±0.02 | 0.62±0.03 |
| 5 | 1.08±0.01 | 0.49±0.02 |
| 5.5 | 1.04±0.02 | 0.42±0.01 |
| 6 | 0.92±0.01 | 0.26±0.02 |
| 6.5 | 0.78±0.01 | 0.13±0.03 |

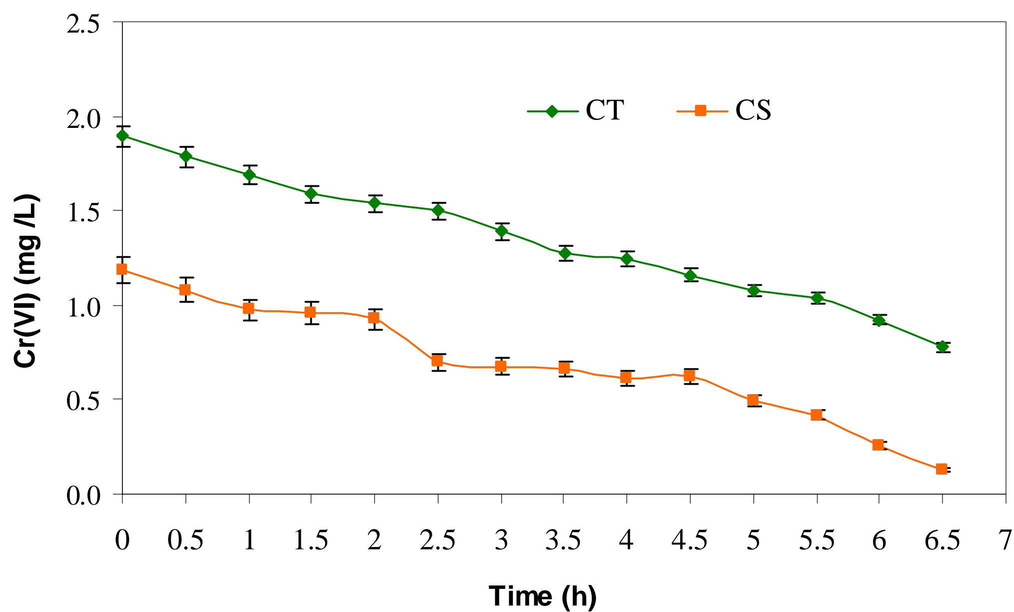



Fig. 37: Transformation of Cr(VI) to Cr(III) by cell free extract of bacterial consortium from tannery effluent (CT) and chrome sludge (CS).

## 4.3 Development of a biosorbent from microbial biomass for removal of chromium from tannery effluent

Microbial waste biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) generated as a waste byproduct of pharmaceutical fermentation industry in production of antibiotics and are available in large quantity were developed as biosorbent to study removal of Cr(III) from aqueous solution and tannery effluent respectively. The biomass was processed and used to study the effect of different experimental parameters such as adsorbent dosage, pH, initial concentration of Cr(III) and contact time on removal of chromium. The processed biomass was also pretreated with alkali, acids and organic solvents and studied for metal removal. Experimental data was analyzed by mathematical modeling such as adsorption isotherms (Langmuir and Freundlich) and kinetic models (Lagergren, Ho and McKay, Elovich and Morris-Weber). Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) analysis was done to study chemical changes that took place on the surface of the microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) before and after different pretreatment.

### 4.3.1 Development of biosorbent from microbial biomass

Microbial waste biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) obtained as a waste byproduct of pharmaceutical fermentation industry was air dried, grinded in a blender, sieved (size, 2.0 mm) and washed with distilled water and dried at 80°C for overnight or till moisture was reduced to below 5%, before being used as a adsorbent for removal of Cr(III) from aqueous solution and tannery effluent.

Batch sorption studies on Cr(III) removal from aqueous solution and tannery effluent by microbial waste biomass (MB1 and MB2) was carried out to optimize various parameters including biomass dosage (0.25-2%), pH (2-6) of the solution, different concentration of chromium (5-50 mg/L) and contact time (0.08-24 h).

### 4.3.2 Optimization of Cr(III) removal from aqueous solution and tannery effluent in batch mode

Effect of adsorbent dosage, initial pH, chromium concentration on the removal of Cr(III) by the microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) from aqueous solution was investigated.

**Effect of adsorbent dosage**

Different adsorbent dosage were taken ranging from 0.25-2% and an increase in percent removal of Cr(III) with increase in the adsorbent dosage was observed (Table 23). Cr(III) removal by *Aspergillus* sp. (MB1) biomass increased from 76.73-81.62%, 83.58-88.35%, 90.73-96.29% and 97.48-98.36%, whereas by *Streptomyces* sp. (MB2) it was from 70.38-71.99%, 78.32-89.87%, 75.13-91.30 % and 88.35-94.43% by 0.25, 0.5, 1.0 and 2 g biomass dosage respectively (Table 23; Fig. 38, 39). Maximum removal of Cr(III) from aqueous solution by both the biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) was observed during first 0.08-2 h of contact after that it reached equilibrium (Table 23; Fig. 38, 39). The removal increased up to 1% of biomass dosage and thereafter it remained constant, therefore 1% dosage of dried pulverized biomass of *Aspergillus* sp (MB1) and *Streptomyces* sp. (MB2) was chosen as an optimum for Cr(III) removal.

**Effect of pH**

The biosorption capacity of biomass is strongly influenced by the pH of the solution. The effect of pH was evaluated within a pH range of 2-6 and not beyond pH 7.0 to avoid precipitation of chromium. The effect of the initial pH on Cr(III) removal by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) is shown in (Table 24; Fig. 40, 41). 96.66% of Cr(III) removal was observed by *Aspergillus* sp. (MB1) and 71.35% by *Streptomyces* sp. (MB2) at pH 4-5. Increase in contact time from 0.08 to 4 h led to an increase in Cr(III) removal from 40-70% by both *Aspergillus* sp (MB1) and *Streptomyces* sp. (MB2) (Fig. 40, 41). 60-90% of Cr(III) removal was observed within first 2 h, which represents the time at which equilibrium of chromium biosorption is presumed to have been attained. *Aspergillus* sp (MB1) was most effective for Cr(III) removal (96.7%) than *Streptomyces* sp. (MB2) (71.35%) (Table 24; Fig. 40, 41). Adsorption of Cr(III) was pH dependent and showed maximum removal at an optimum pH 4.0 with adsorbent dosage of 1%. Increase in the initial Cr(III) concentration and contact time were found to increase Cr(III) removal.

**Effect of chromium concentration**

Chromium removal by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) was studied at varying initial concentrations (5-50 mg/L) of Cr(III) in aqueous solution. Cr(III) removal was 41.39-90.46%, 11.92-89.59%, 52.62-93.03% and 24.77-60.24 by *Aspergillus* sp. (MB1) and 43.20-91.69%, 28.29-80.32%, 9.43-72.40% and 25.39-68.15% by *Streptomyces* sp. (MB2) at 5, 10, 20 and 50 mg/L of Cr(III) respectively (Table 25; Fig. 42, 43). Maximum Cr(III) removal capacities of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) from aqueous solution were 60.24-93.03 % and 68.15-91.69% respectively at 5-50 mg/L chromium concentration in batch mode (Table 25). Equilibrium uptake of Cr(III) by both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) biomass reached after 2 h of contact time (Table 25; Fig. 42, 43).

**Effect of contact time**

With increase in contact time (0.08-4 h), Cr(III) removal from aqueous solution was increased (40-90%) by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2). Maximum Cr(III) removal from aqueous solution was observed within first 2 h of contact time than equilibrium was reached in 2-10 h of contact time. Removal of Cr(III) by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) microbial biomass from aqueous solution was in the order of 41.39-90.46% by *Aspergillus* sp. (MB1) and 28.29-80.32% *Streptomyces* sp. (MB2) (Table 23, 24, 25; Fig. 38-43). Among both the biomass *Aspergillus* sp. (MB1) showed maximum removal of 96.6% from aqueous solution at pH 4, containing 25 mg/L of Cr(III) by 1% of biomass dosage at 28±2°C with agitation rate of 120 rpm.

Table 23. Time course removal of Cr(III) from aqueous solution by microbial biomass (*Aspergillus* sp. MB1 and *Streptomyces* sp. MB2) at different adsorbent dosage ($C_i$: 23.68 mg/L (MB1); $C_i$: 25.62 mg/L (MB2); pH 4; Temp: 28±2°C; Agitation rate: 120 rpm).

| Biomass | Adsorbent dosage (g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0.25g | | 0.5g | | 1.0g | | 2.0g | |
| | Time(h) | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal |
| *Aspergillus* sp. (MB1) | 0 | 23.68±0.00 | 0.00 | 23.68±0.00 | 0.00 | 23.68±0.00 | 0.00 | 23.68±0.00 | 0.00 |
| | 0.08 | 5.51±0.05 | 76.73 | 3.89±0.16 | 83.58 | 2.20±0.08 | 90.73 | 0.60±0.02 | 97.48 |
| | 0.25 | 6.88±0.02 | 70.93 | 4.78±0.11 | 79.82 | 1.63±0.01 | 93.13 | 0.47±0.03 | 98.03 |
| | 0.5 | 4.91±0.03 | 79.28 | 1.94±0.05 | 91.82 | 1.13±0.01 | 95.22 | 0.45±0.02 | 98.51 |
| | 1 | 5.28±0.04 | 77.72 | 1.54±0.04 | 93.50 | 0.93±0.07 | 96.10 | 0.45±0.06 | 98.09 |
| | 2 | 2.84±0.01 | 88.01 | 2.05±0.01 | 91.34 | 0.93±0.07 | 96.10 | 0.33±0.02 | 98.61 |
| | 4 | 3.08±0.04 | 86.99 | 2.49±0.04 | 89.49 | 0.71±0.11 | 97.00 | 0.63±0.03 | 97.34 |
| | 6 | 3.51±0.05 | 85.19 | 2.70±0.07 | 88.58 | 0.70±0.07 | 97.03 | 0.49±0.04 | 97.93 |
| | 8 | 4.55±0.05 | 80.79 | 2.75±0.08 | 88.38 | 0.77±0.03 | 96.76 | 0.38±0.03 | 98.38 |
| | 10 | 4.35±0.06 | 81.62 | 2.76±0.05 | 88.34 | 0.88±0.05 | 96.29 | 0.39±0.03 | 98.36 |
| | 24 | 5.70±0.12 | 75.94 | 3.88±0.13 | 83.60 | 1.10±0.03 | 95.34 | 0.54±0.05 | 97.70 |
| | | | | | | | | | |
| *Streptomyces* sp. (MB2) | 0 | 25.62±0.00 | 0.00 | 25.62±0.00 | 0.00 | 25.62±0.00 | 0.00 | 25.62±0.00 | 0.00 |
| | 0.08 | 7.59±0.03 | 70.38 | 5.56±0.38 | 78.32 | 6.37±0.04 | 75.13 | 2.99±0.02 | 88.35 |
| | 0.25 | 8.44±0.02 | 67.05 | 4.78±0.11 | 81.35 | 6.33±0.04 | 75.28 | 2.33±0.03 | 90.91 |
| | 0.5 | 8.02±0.11 | 68.68 | 2.27±0.04 | 91.13 | 5.66±0.01 | 77.91 | 2.27±0.02 | 91.14 |
| | 1 | 7.64±0.04 | 70.19 | 2.00±0.08 | 92.19 | 4.61±0.07 | 81.99 | 2.26±0.06 | 91.19 |
| | 2 | 6.42±0.01 | 74.94 | 2.22±0.03 | 91.35 | 4.61±0.07 | 81.99 | 1.65±0.02 | 93.56 |
| | 4 | 7.08±0.17 | 72.38 | 2.32±0.07 | 90.93 | 3.56±0.11 | 86.11 | 3.15±0.03 | 87.70 |
| | 6 | 7.25±0.05 | 71.69 | 1.75±0.01 | 93.16 | 2.44±0.05 | 90.48 | 2.45±0.04 | 90.44 |
| | 8 | 7.90±0.11 | 69.16 | 2.92±0.04 | 88.61 | 2.69±0.02 | 89.51 | 1.82±0.03 | 92.91 |
| | 10 | 7.18±0.06 | 71.99 | 2.60±0.06 | 89.87 | 2.23±0.01 | 91.30 | 1.94±0.03 | 92.43 |
| | 24 | 7.85±0.12 | 69.37 | 2.45±0.01 | 90.45 | 2.05±0.07 | 92.00 | 1.54±0.02 | 94.00 |

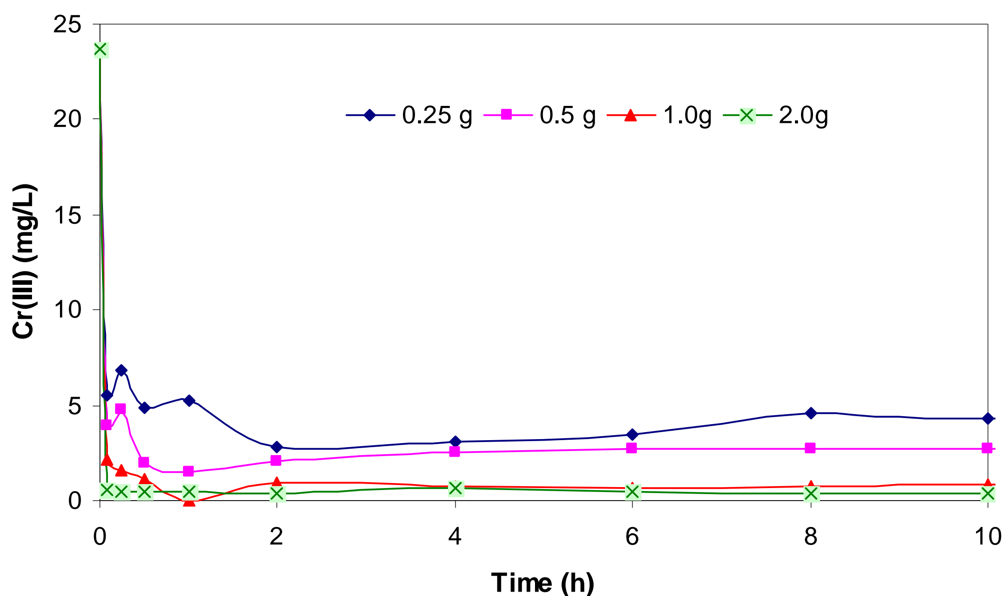



Fig. 38: Time course removal of Cr(III) from aqueous solution by microbial biomass *Aspergillus* sp. (MB1) at different adsorbent dosage ($C_i$: 23.68 mg/L; pH4; Temp: 28±2°C; Agitation rate: 120 rpm).

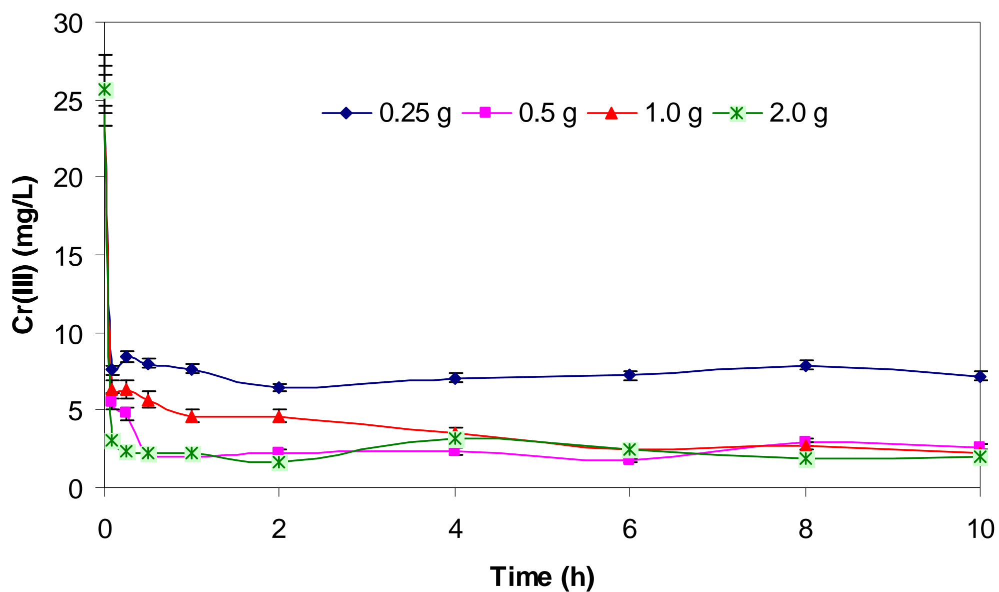



Fig. 39: Time course removal of Cr(III) from aqueous solution by microbial biomass *Streptomyces* sp. (MB2) at different adsorbent dosage ($C_i$: 25.62; pH 4; Temp 28±2°C; Agitation rate: 120 rpm).

Table 24. Time course removal of Cr(III) from aqueous solution by microbial biomass (*Aspergillus* sp. MB1 and *Streptomyces* sp. MB2) at different pH ($C_i$: 24.85 mg/L(MB1); $C_i$: 24.43 mg/L (MB2); Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

| Biomass | pH | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | pH2 | | pH3 | | pH4 | | pH5 | |
| | Time (h) | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal |
| *Aspergillus* sp. (MB1) | 0 | 24.85±0.00 | 0.00 | 24.85±0.00 | 0.00 | 24.85±0.00 | 0.00 | 24.85±0.00 | 0.00 |
| | 0.08 | 18.95±0.05 | 23.74 | 19.49±0.39 | 28.90 | 3.45±0.13 | 86.13 | 3.58±0.11 | 85.58 |
| | 0.25 | 18.09±1.32 | 27.19 | 17.67±0.06 | 13.64 | 2.34±0.52 | 90.58 | 2.90±0.16 | 88.35 |
| | 0.5 | 15.51±0.20 | 37.59 | 21.46±1.15 | 41.79 | 2.35±0.17 | 90.56 | 2.98±0.18 | 88.00 |
| | 1 | 13.39±0.26 | 46.11 | 14.47±0.05 | 39.11 | 1.57±0.08 | 93.70 | 3.20±0.04 | 87.13 |
| | 2 | 16.63±2.13 | 33.07 | 15.13±0.78 | 23.99 | 1.25±0.15 | 94.98 | 2.70±0.01 | 89.13 |
| | 4 | 14.77±1.72 | 40.57 | 18.89±0.18 | 22.36 | 1.35±0.10 | 94.58 | 2.89±0.39 | 88.38 |
| | 6 | 14.47±0.99 | 41.76 | 19.29±0.18 | 36.41 | 1.51±0.15 | 93.94 | 2.61±0.05 | 89.49 |
| | 8 | 13.91±0.24 | 44.01 | 15.80±0.70 | 41.38 | 0.87±0.17 | 96.49 | 2.40±0.01 | 90.35 |
| | 10 | 13.14±0.78 | 47.11 | 14.57±0.65 | 46.10 | 0.55±0.04 | 97.81 | 1.38±0.07 | 94.44 |
| | 24 | 11.06±0.31 | 55.51 | 13.40±0.76 | 21.56 | 0.47±0.04 | 98.11 | 0.83±0.01 | 96.66 |
| | | | | | | | | | |
| *Streptomyces* sp. (MB2) | 0 | 24.43±0.00 | 0.00 | 24.43±0.00 | 0.00 | 24.43±0.00 | 0.00 | 24.43±0.00 | 0.00 |
| | 0.08 | 18.88±0.09 | 22.73 | 21.35±0.06 | 12.63 | 13.94±0.01 | 42.93 | 11.64±0.05 | 52.34 |
| | 0.25 | 16.71±0.03 | 31.59 | 20.91±0.14 | 43.98 | 12.22±0.04 | 50.00 | 11.06±0.06 | 54.75 |
| | 0.5 | 17.43±0.09 | 28.64 | 19.31±0.05 | 48.27 | 10.92±0.03 | 55.30 | 10.58±0.07 | 56.71 |
| | 1 | 17.90±0.29 | 26.75 | 17.10±0.05 | 54.18 | 9.79±0.02 | 59.92 | 9.65±0.04 | 60.49 |
| | 2 | 17.03±0.03 | 30.28 | 13.00±0.13 | 65.17 | 8.92±0.02 | 63.50 | 9.35±0.01 | 61.73 |
| | 4 | 17.50±0.13 | 28.37 | 12.71±0.11 | 65.96 | 8.58±0.01 | 64.90 | 9.71±0.01 | 60.26 |
| | 6 | 15.41±0.03 | 36.91 | 14.52±0.03 | 61.11 | 7.97±0.05 | 67.38 | 9.05±0.06 | 62.96 |
| | 8 | 15.93±0.00 | 34.79 | 13.14±0.16 | 64.80 | 7.58±0.02 | 68.99 | 7.56±0.46 | 69.06 |
| | 10 | 16.59±0.18 | 32.11 | 12.83±0.05 | 65.64 | 7.93±0.06 | 67.56 | 7.73±0.44 | 68.35 |
| | 24 | 16.06±0.07 | 34.27 | 12.85±0.11 | 65.56 | 7.49±0.10 | 69.36 | 7.00±0.02 | 71.35 |

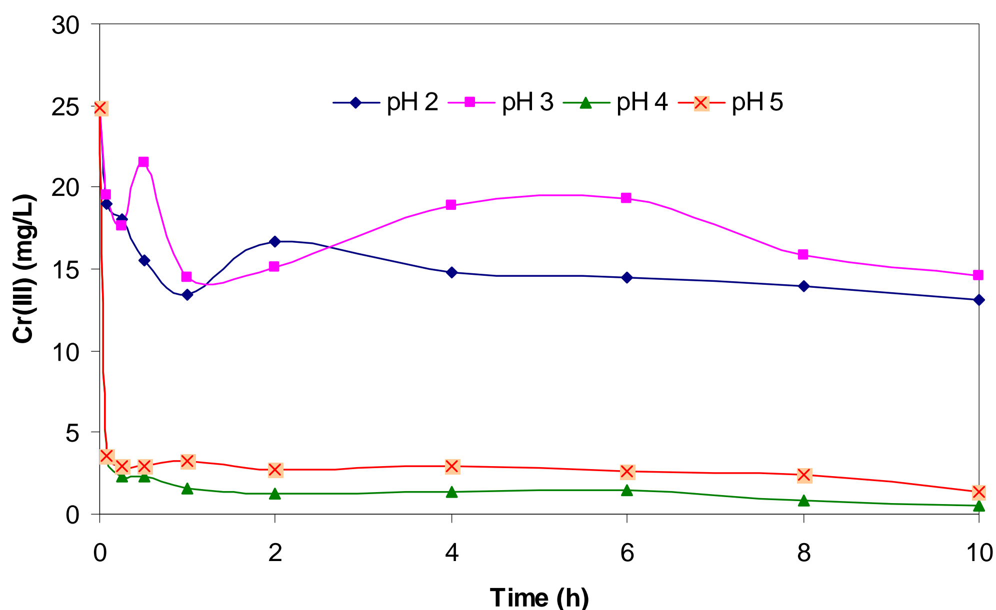



Fig. 40: Time course removal of Cr(III) from aqueous solution by microbial biomass *Aspergillus* sp. (MB1) at different pH ($C_i$ 24.85; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

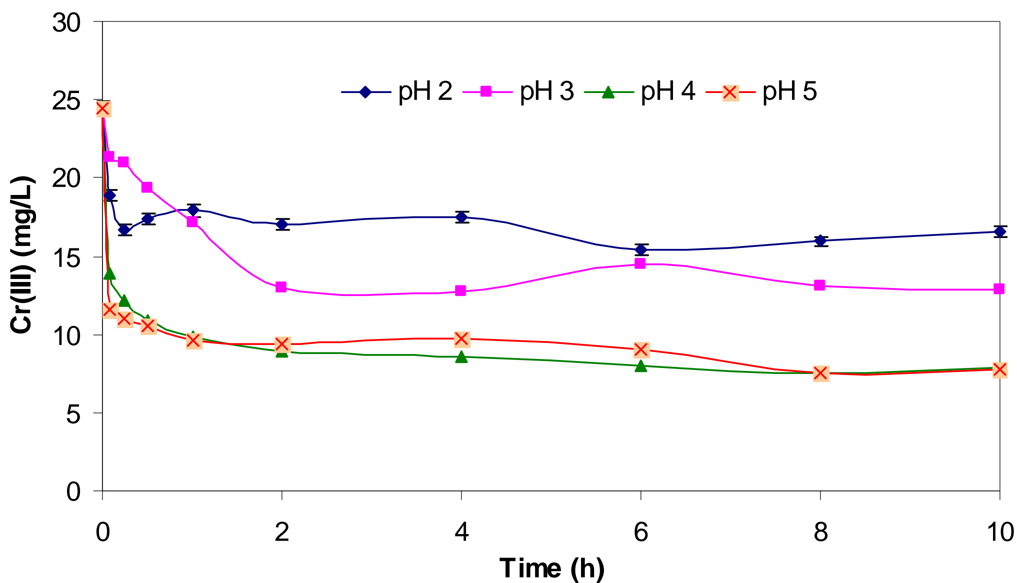



Fig. 41: Time course removal of Cr(III) from aqueous solution by microbial biomass *Streptomyces* sp. (MB2) at different pH ($C_i$: 24.43 mg/L; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

Table 25. Time course removal of Cr(III) from aqueous solution by microbial biomass (*Aspergillus* sp. MB1 and *Streptomyces* sp. MB2) at graded concentration (5-50 mg/L) (pH 4; Adsorbent dosage: 1%; Temp: 28±2°C, Agitation rate: 120 rpm).

| Biomass | | Chromium (mg/L) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Time(h) | 5 mg/L | | 10 mg/L | | 20 mg/L | | 50 mg/L | |
| | | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal |
| *Aspergillus* sp. (MB1) | 0 | 5.62±0.07 | 0.00 | 10.80±0.15 | 0.00 | 20.59±0.08 | 0.00 | 51.32±0.52 | 0.00 |
| | 0.08 | 3.29±0.06 | 41.39 | 9.51±0.22 | 11.92 | 9.76±0.19 | 52.62 | 38.61±0.35 | 24.77 |
| | 0.25 | 3.12±0.18 | 44.50 | 9.47±0.21 | 12.26 | 9.60±0.05 | 53.38 | 37.46±0.29 | 27.02 |
| | 0.5 | 2.73±0.11 | 51.46 | 8.76±0.19 | 18.91 | 8.98±0.31 | 56.37 | 36.34±0.25 | 29.19 |
| | 1 | 2.46±0.00 | 56.22 | 7.81±0.31 | 27.71 | 6.54±0.26 | 68.25 | 36.71±0.12 | 28.46 |
| | 2 | 2.28±0.21 | 59.38 | 7.47±0.08 | 30.81 | 5.52±0.25 | 73.18 | 35.87±0.03 | 30.11 |
| | 4 | 2.22±0.20 | 60.49 | 7.36±0.02 | 31.88 | 4.22±0.03 | 79.49 | 31.40±0.52 | 38.82 |
| | 6 | 1.45±0.03 | 74.16 | 6.50±0.01 | 39.84 | 2.36±0.62 | 88.55 | 26.50±0.34 | 48.37 |
| | 8 | 1.38±0.01 | 75.52 | 5.58±0.33 | 48.31 | 2.20±0.46 | 89.30 | 24.84±0.34 | 51.59 |
| | 10 | 0.83±0.35 | 85.32 | 3.31±0.04 | 69.38 | 1.75±0.39 | 91.50 | 24.34±0.50 | 52.58 |
| | 24 | 0.54±0.15 | 90.46 | 1.12±0.12 | 89.59 | 1.44±0.63 | 93.03 | 20.40±0.32 | 60.24 |
| | | | | | | | | | |
| *Streptomyces* sp. (MB2) | 0 | 4.25±0.00 | 0.00 | 7.24±0.04 | 0.00 | 20.74±0.37 | 0.00 | 42.46±0.56 | 0.00 |
| | 0.08 | 2.41±0.06 | 43.20 | 5.19±0.07 | 28.29 | 18.79±0.04 | 9.43 | 31.68±0.19 | 25.39 |
| | 0.25 | 2.69±0.17 | 36.75 | 4.88±0.28 | 32.66 | 17.30±0.08 | 16.59 | 29.40±0.08 | 30.76 |
| | 0.5 | 2.61±0.20 | 38.54 | 4.56±0.00 | 37.01 | 14.18±0.02 | 31.63 | 26.15±0.38 | 38.41 |
| | 1 | 2.02±0.02 | 52.50 | 3.74±0.13 | 48.34 | 12.36±0.60 | 40.41 | 23.21±0.30 | 45.35 |
| | 2 | 1.63±0.02 | 61.61 | 2.37±0.12 | 67.25 | 10.04±0.47 | 51.62 | 21.38±0.10 | 49.65 |
| | 4 | 1.59±0.04 | 62.49 | 2.09±0.02 | 71.11 | 10.71±0.26 | 48.36 | 18.95±0.73 | 55.38 |
| | 6 | 1.48±0.02 | 65.27 | 2.13±0.04 | 70.55 | 9.27±0.25 | 55.30 | 17.15±0.18 | 59.61 |
| | 8 | 1.35±0.04 | 68.30 | 1.68±0.09 | 76.84 | 8.51±0.49 | 58.98 | 15.24±0.24 | 64.11 |
| | 10 | 1.02±0.05 | 75.99 | 1.49±0.06 | 79.45 | 7.27±0.16 | 64.95 | 14.42±0.36 | 66.05 |
| | 24 | 0.35±0.04 | 91.69 | 1.43±0.00 | 80.32 | 5.73±0.06 | 72.40 | 13.53±0.10 | 68.15 |

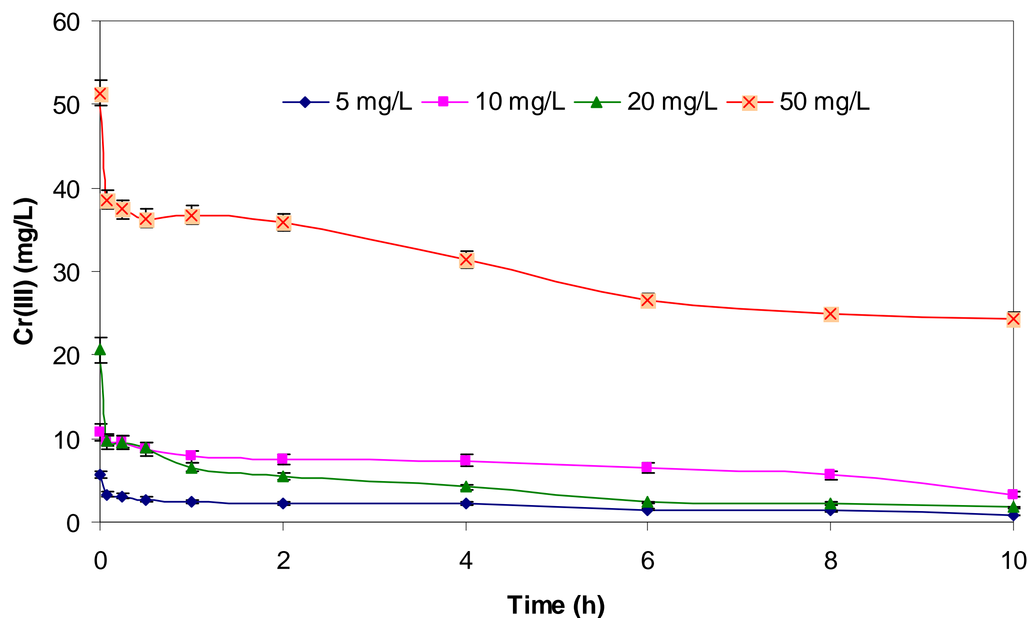



Fig. 42: Time course removal of Cr(III) from aqueous solution by microbial biomass *Aspergillus* sp. (MB1) and at graded concentration (5-50 mg/L) (pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

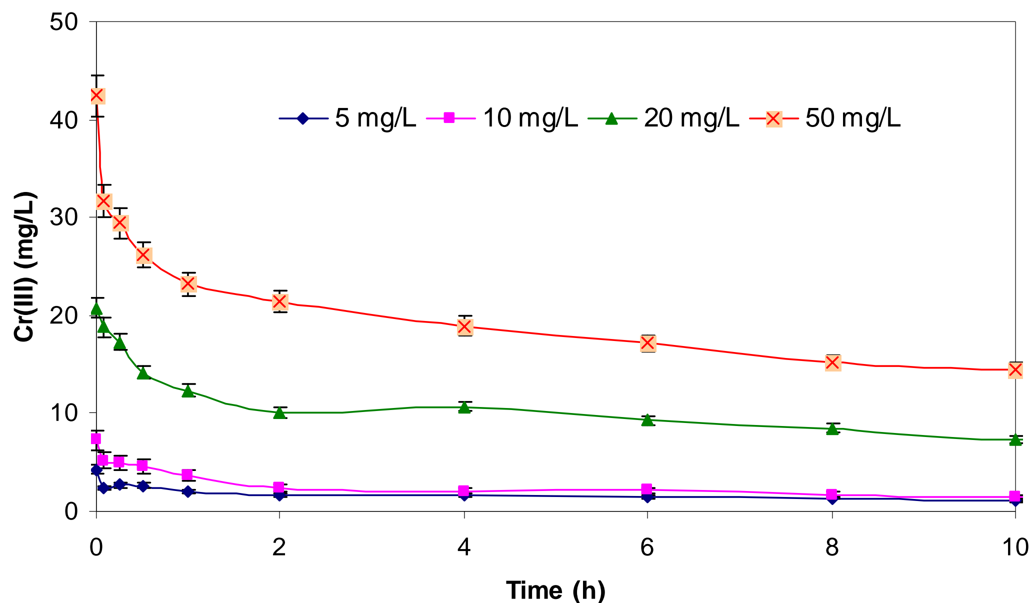



Fig. 43: Time course removal of Cr(III) from aqueous solution by microbial biomass *Streptomyces* sp. (MB2) at graded concentration (5-50 mg/L) (pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

**Removal of Cr(III) from tannery effluent**

Batch studies with *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were carried out by optimizing various parameters such as adsorbent dosage (0.25-2%), pH (2-5) and varying initial concentrations of (5-50 mg/L) Cr(III). Futher experiment were carried out with different concentration (5-50 mg/L) of tannery effluent, 1g of native MB1 and MB2 biomass at ambient temperature, 120 rpm for 24 h. Removal of Cr(III) from tannery effluent containing 25 mg/L Cr(III) by MB1 and MB2 biomass was 64.48 and 88.28 %, respectively (Table 26, 27; Fig. 44, 45).

Cr(III) removal was 22.15-64.48%, 13.38-54.92%, 13.61-61.39%, 6.60-42.03% and 10.62-30.09% by *Aspergillus* sp. (MB1) biomass and 41.00-86.04%, 42.27-88.28%, 19.8-86.51%, 11.58-87.60% and 11.36-74.00% by *Streptomyces* sp. (MB2) at 5, 10, 20 and 50 mg/L Cr(III) in tannery effluent respectively (Table 26, 27; Fig. 44, 45). Maximum Cr(III) removal capacities of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) from tannery effluent was 6.60-64.48% and 11.58-87.60% respectively at 5-50 mg/L Cr(III) in tannery effluent. Equlibrium uptake of Cr(III) from tannery effluent by both the *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) reached after 2 h of contact time (Table 26, 27; Fig. 44, 45). *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) showed 64 and 88.28% removal of Cr(III) from tannery effluent at respectively.

Table 26. Time course removal of Cr(III) from tannery effluent by microbial biomass *Aspergillus* sp. (MB1) at graded concentration ( pH 4, Temp: 28±2°C, Agitation rate: 120 rpm).

| | Chromium (mg/L) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 mg/L | | 10 mg/L | | 15 mg/L | | 20 mg/L | | 50mg/L | |
| Time(h) | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | %Removal | $C_f$ (mg/L) | %Removal | $C_f$ (mg/L) | %Removal | $C_f$ (mg/L) | %Removal |
| 0 | 3.03±0.06 | 0.00 | 11.40±0.44 | 0.00 | 16.27±0.24 | 0.00 | 21.93±0.24 | 0.00 | 88.96±0.68 | 0.00 |
| 0.08 | 2.36±0.14 | 22.15 | 9.88±0.42 | 13.38 | 14.06±0.44 | 13.61 | 21.76±0.22 | 6.60 | 79.51±0.33 | 10.62 |
| 0.25 | 2.44±0.01 | 19.52 | 8.82±0.03 | 22.70 | 10.65±0.37 | 34.53 | 16.40±0.00 | 24.40 | 73.78±1.77 | 17.06 |
| 0.5 | 2.23±0.17 | 26.37 | 6.85±0.89 | 39.97 | 10.78±0.09 | 33.72 | 17.71±0.06 | 18.37 | 73.52±0.60 | 17.36 |
| 1 | 1.93±0.10 | 36.36 | 8.20±0.01 | 28.09 | 12.03±0.78 | 26.04 | 19.12±0.54 | 11.84 | 72.36±1.32 | 16.42 |
| 2 | 2.38±0.02 | 21.48 | 7.45±0.70 | 34.64 | 9.77±0.07 | 39.98 | 14.47±0.36 | 33.28 | 74.41±0.22 | 16.36 |
| 4 | 1.81±0.16 | 40.37 | 6.52±0.30 | 42.83 | 7.52±0.45 | 53.81 | 16.28±0.93 | 24.93 | 63.97±0.19 | 28.09 |
| 6 | 1.53±0.00 | 49.56 | 7.60±0.17 | 33.38 | 9.46±0.78 | 41.87 | 16.92±0.90 | 22.01 | 62.59±0.08 | 29.64 |
| 8 | 1.95±0.11 | 35.90 | 6.48±0.21 | 43.21 | 8.52±0.76 | 47.62 | 20.58±0.36 | 5.093 | 59.50±0.85 | 33.12 |
| 10 | 1.52±0.00 | 49.9 | 5.14±0.02 | 54.92 | 9.07±0.37 | 44.28 | 17.80±0.60 | 17.95 | 63.89±0.97 | 28.19 |
| 24 | 1.08±0.00 | 64.48 | 5.80±0.42 | 49.17 | 6.28±0.13 | 61.39 | 12.57±0.07 | 42.03 | 62.20±0.29 | 30.09 |

Table 27. Time course removal of Cr(III) from tannery effluent by microbial biomass *Streptomyces* sp. (MB2) at graded concentration (pH 4; Temp: 28±2°C, Agitation rate: 120 rpm).

| | Chromium (mg/L) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 mg/L | | 10 mg/L | | 15 mg/L | | 20 mg/L | | 50 mg/L | |
| Time(h) | $C_f$ (mg/L) | %Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal | $C_f$ (mg/L) | % Removal |
| 0 | 4.05±0.10 | 0.000 | 12.23±0.05 | 0.00 | 15.61±0.12 | 0.00 | 20.55±0.76 | 0.00 | 60.47±0.43 | 0.00 |
| 0.08 | 2.39±0.01 | 41.00 | 7.06±0.18 | 42.27 | 12.52±0.60 | 19.8 | 18.17±0.30 | 11.58 | 54.00±0.39 | 11.36 |
| 0.25 | 1.98±0.12 | 51.17 | 6.37±0.39 | 47.94 | 8.54±0.48 | 45.29 | 16.45±0.53 | 19.92 | 44.62±0.36 | 26.22 |
| 0.5 | 1.86±0.05 | 54.18 | 4.40±0.08 | 64.02 | 6.69±0.11 | 57.12 | 12.28±0.36 | 40.24 | 44.28±0.18 | 26.77 |
| 1 | 1.93±0.03 | 52.37 | 3.86±0.11 | 68.42 | 7.13±0.14 | 54.34 | 10.29±0.19 | 49.93 | 41.46±0.11 | 31.44 |
| 2 | 1.36±0.06 | 66.38 | 3.79±0.22 | 69.03 | 6.38±0.47 | 59.13 | 9.19±0.07 | 55.29 | 36.43±0.41 | 39.76 |
| 4 | 1.04±0.02 | 74.40 | 3.12±0.09 | 74.47 | 4.27±0.48 | 72.62 | 8.764±0.39 | 57.35 | 35.29±1.10 | 41.64 |
| 6 | 0.88±0.01 | 78.30 | 2.18±0.19 | 82.20 | 3.23±0.20 | 79.32 | 6.90±0.37 | 66.41 | 25.27±1.01 | 58.22 |
| 8 | 0.89±0.07 | 78.09 | 1.78±0.02 | 85.42 | 2.87±0.13 | 81.62 | 4.86±0.35 | 76.37 | 21.80±1.06 | 63.96 |
| 10 | 0.79±0.04 | 80.55 | 1.86±0.07 | 84.76 | 3.14±0.04 | 79.90 | 3.72±0.16 | 81.90 | 19.39±0.09 | 67.94 |
| 24 | 0.57±0.01 | 86.04 | 1.43±0.06 | 88.28 | 2.11±0.10 | 86.51 | 2.55±0.23 | 87.60 | 15.73±0.41 | 74.00 |

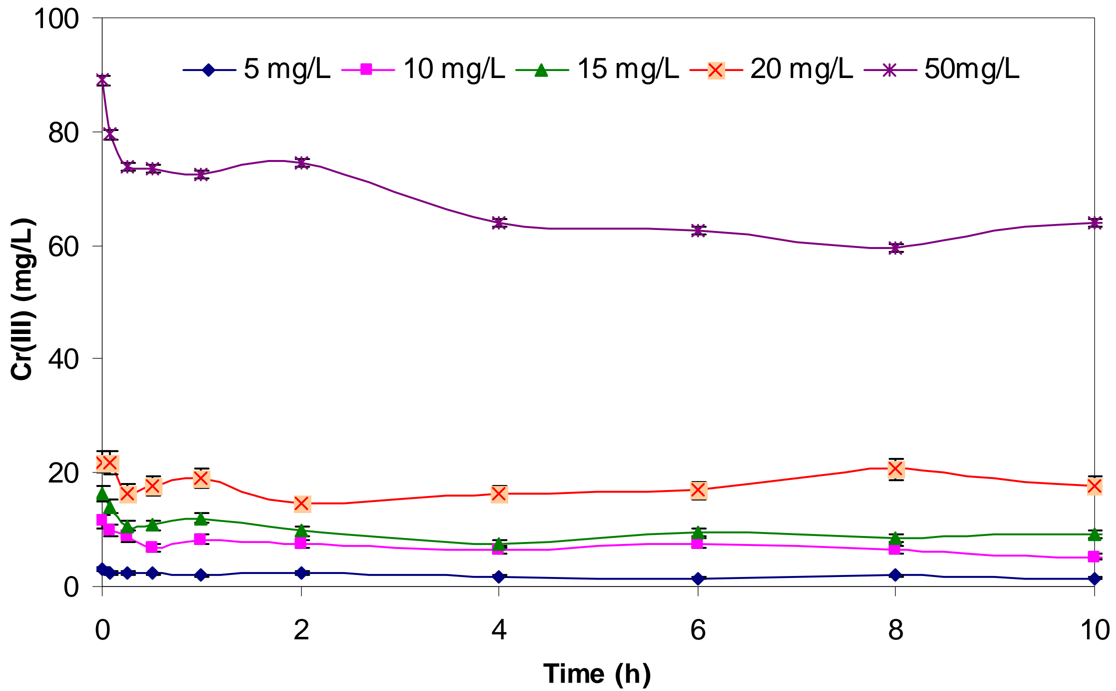



Fig. 44: Time course removal of Cr(III) from tannery effluent by microbial biomass *Aspergillus* sp. (MB1) and at graded concentration (5-50 mg/L) (pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

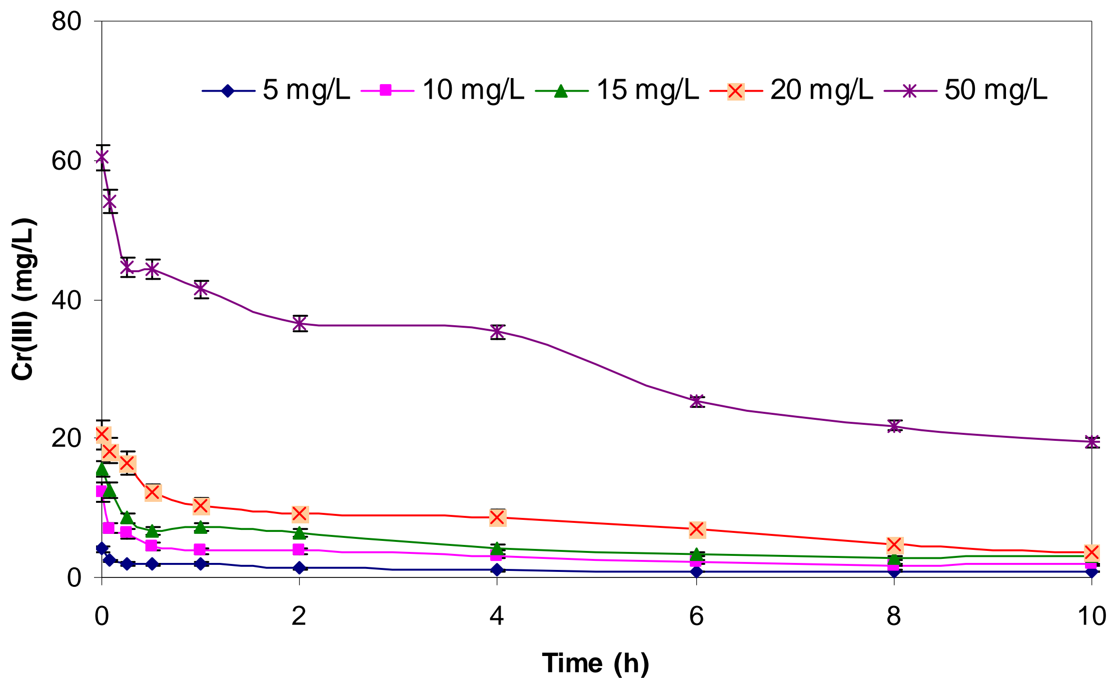



Fig. 45: Time course removal of Cr(III) from tannery effluent by microbial biomass *Streptomyces* sp. (MB2) at graded concentration (5-50 mg/L) (pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

### 4.3.3 Adsorption isotherms

Langmuir and Freundlich adsorption isotherms were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by microbial biomass. Correlation coefficient ($r^2$) values indicate that the adsorption pattern for chromium removal by microbial biomass followed both the Langmuir ($r^2 > 0.98$) and Freundlich ($r^2 > 0.98$) isotherms.

To evaluate feasibility and effectiveness of the biosorption, predictions from adsorption isotherms were determined. The degree of sorption of metal on a biosorbent at equilibrium was found to be a function of metal ion concentration in solution at constant pH and temperature. The initial ($C_i$) and final ($C_f$) concentration, $Q_L$ is the Langmuir adsorption constant of chromium in batch experiments (Table 28, 29). Conventional single solute adsorption isotherm models such as Langmuir and Freundlich adsorption isotherms were predicted from equilibrium sorption data of Cr(III) by *Aspergillus* sp. (MB1) (Table 28; Fig. 46, 47) and *Streptomyces* sp. (MB2) biomass (Table 29; Fig. 48, 49). Correlation coefficient ($r^2$) value indicated good correlation between the experimental data and Langmuir and Freundlich adsorption models for Cr(III) where $r^2$ was greater than 0.9. Both Langmuir and Freundlich sorption model fitted well throughout the concentration range of Cr(III) (5-50 mg/L) with the correlation coefficient of 0.9 indicating that both physico-chemical adsorption and ion exchange interactions are involved in metal binding. Cell wall of non-living biomass consists of both organic and inorganic constituents therefore; data indicates that adsorption take place through ion exchange as well as physico-chemical interaction simultaneously.

Langmuir and Freundlich adsorption isotherm were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) microbial biomass. The correlation coefficient values obtained from the Langmuir and Freundlich isotherms (Table 28, 29; Fig. 46-49) indicates that the adsorption pattern for *Aspergillus* sp. (MB1) and *Streptomyces* (MB2) followed both Langmuir isotherms ($r^2 = 0.80$ to $0.97$) and the Freundlich isotherms ($r^2 = 0.71$ to $0.98$) at all chromium concentrations (Fig. 46-49) for both the biomass.

According to the Langmuir model, the maximum Cr(III) adsorption capacity was obtained at 50 mg/L with a value of $Q_L$ of 0.81 mg/g *Aspergillus* sp. (MB1), 0.99 mg/g *Streptomyces* (MB2) respectively represents a Cr(III) adsorption. The values obtained for Cr(III) from Freundlich model at different concentration showed a maximum adsorption capacity ($K_F$) of 191 mg/g and 54.22 mg/g at 50 mg/L concentration of Cr(III) with an affinity value (n) equal to 0.75 and 0.99 on *Aspergillus* sp. (MB1) and *Streptomyces* (MB2) biomass respectively, which represents a favorable adsorption of Cr(III) from aqueous solution.

Table 28: Model parameters for the adsorption of Cr(III) on *Aspergillus* sp. (MB1) biomass.

| Cr(III) mg/L | Langmuir isotherms | | | Freundlich isotherms | | |
|---|---|---|---|---|---|---|
| | $Q_L$ (mg/g) | b (l/mg) | $r^2$ | $K_f$ (mg/g) | n | $r^2$ |
| | | | | | | |
| 5 | 0.22 | 1.74 | 0.95 | 2.26 | 2.39 | 0.89 |
| 10 | 0.22 | 0.48 | 0.80 | 1.53 | 1.17 | 0.71 |
| 20 | 1.02 | 0.89 | 0.98 | 2.30 | 3.37 | 0.92 |
| 50 | 0.81 | 0.06 | 0.97 | 191.33 | 0.75 | 0.97 |

Table 29: Model parameters for the adsorption of Cr(III) on *Streptomyces* sp. (MB2) biomass.

| Cr(III) mg/L | Langmuir isotherms | | | Freundlich isotherms | | |
|---|---|---|---|---|---|---|
| | $Q_L$ (mg/g) | b (l/mg) | $r^2$ | $K_f$ (mg/g) | n | $r^2$ |
| | | | | | | |
| 5 | 0.14 | 1.71 | 0.92 | 3.41 | 2.25 | 0.79 |
| 10 | 0.18 | 0.79 | 0.95 | 1.23 | 1.41 | 0.94 |
| 20 | 0.27 | 0.15 | 0.82 | 4.67 | 1.57 | 0.98 |
| 50 | 0.99 | 0.10 | 0.95 | 54.22 | 0.94 | 0.94 |

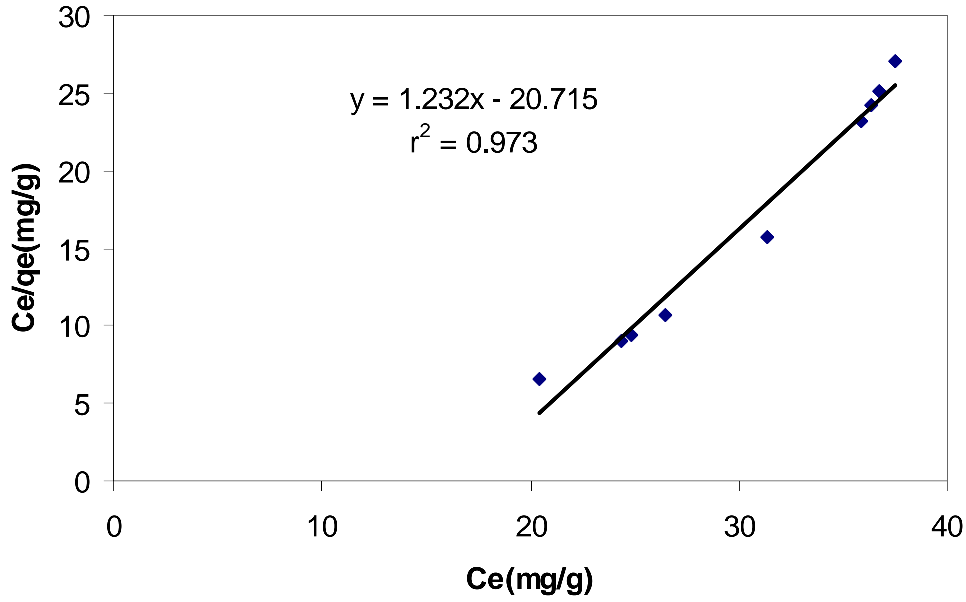



Fig. 46: Langmuir adsorption plot for the adsorption of Cr(III) on *Aspergillus* sp. (MB1) from aqueous solution.

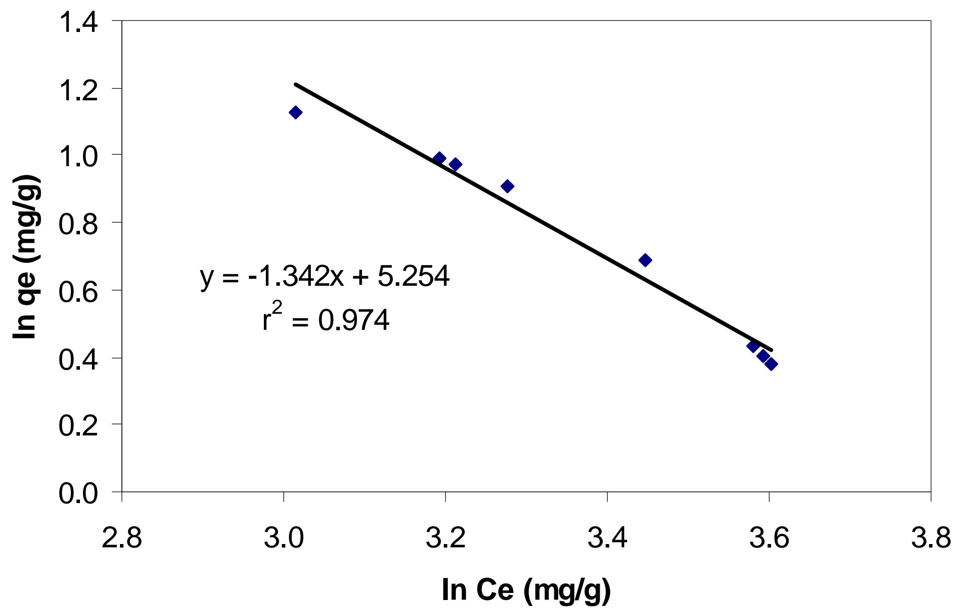



Fig. 47: Freundlich adsorption plot for the adsorption of Cr(III) on *Aspergillus* sp. (MB2) from aqueous solution.

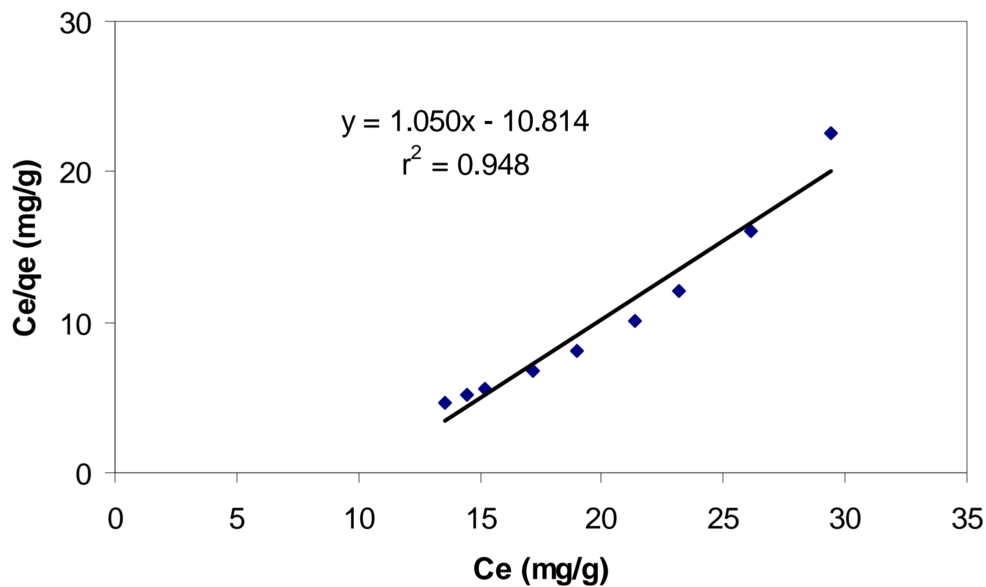



Fig. 48: Langmuir adsorption plot for the adsorption of Cr(III) on *Streptomyces* sp. (MB2) from aqueous solution.

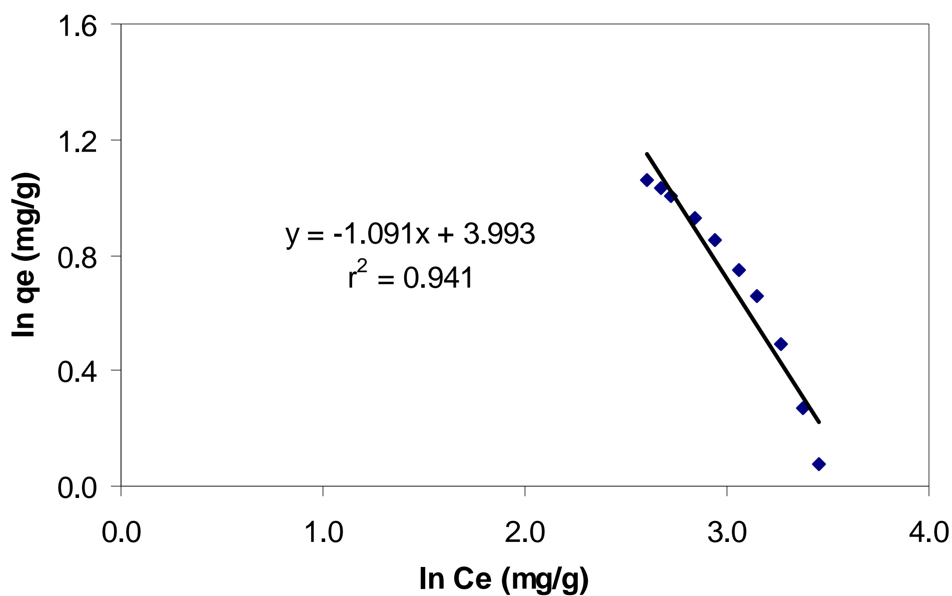



Fig. 49: Freundlich adsorption plot for the adsorption of Cr(III) on *Streptomyces* sp. (MB2) from aqueous solution.

### 4.3.4 Adsorption kinetices

Kinetic models that can describe the behaviour of batch experimental conditions are very useful for scale up studies or process optimization. A number of models which have been developed to describe the kinetic of metal biosorption in batch experiment. Kinetic modeling of Cr(III) study describes solute uptake rate, which controls residence time of adsorbate uptake at solid-solution interface. Conformity between experimental data and models predicted values was expressed by correlation coefficients ($r^2$, values close or equal to 1). A relatively high $r^2$ value indicates that model successfully described kinetics of Cr(III) adsorption. Non-linear regression analysis was applied to each set of data. A correlation coefficient ($r^2$) and a probability value (p) represent "goodness of fit" for models to the data obtained by linear as well non-linear regression by using Lagergren Equation, Ho and McKay equation, Elovich equation and Morris-Weber equation.

**Lagergren Equation (pseudo-first-order)**

Plot of log ($q_e$-$q_t$) vs $t$ should give a linear relation, from which $k_l$ and $q_e$ can be determined from slope and intercept of plot, respectively. $r^2$ of Cr(III) under different conditions were calculated from plots at different adsorbent dosage (Table 30), pH (Table 31) and Cr(III) concentration (Table 32). Results showed that adsorption reaction can be approximated with pseudo first-order kinetic model. It was observed that $k_1$ (0.03-0.05) and $q_e$ (8.64-1.42) at different adsorbent dosage (0.25-2%) by *Aspergillus* sp. (MB2) whereas $k_1$ (0.03-0.88) and $q_e$ (1.42-2.74) increased at different adsorbent dosage (0.25-2%) by *Streptomyces* sp. (MB2). The rate constant of sorption is determined from the first-order rate expressions given by the Lagergren equation log ($q$e-$q$t) was plotted versus agitation time, which gave a straight line with correlation coefficient ($r^2$) of 0.96 for *Aspergillus* sp. (MB1) and 0.71 for *Streptomyces* sp. (MB2) at pH 4 (Table 30; Fig. 50, 51). The adsorption capacity ($q_e$ and $q_t$) at equilibrium and at time $t$, and the value of first-order rate constant ($k$), computed from the slope of the linear plot.

Plot of *log* (*q*e-*q*t) versus agitation time, gave a straight line with $r^2$ of 0.97 for *Aspergillus* sp. (MB1), 0.94 by *Streptomyces* sp. (MB2) at pH 4 (Table 31). Values of $k_1$ (2.00-0.03) and $q_e$ (0.02-3.29) by *Aspergillus* sp. (MB1) whereas values of $k_1$ (-4.84-0.05) and $q_e$ (1.83-0.95) by *Streptomyces* sp. (MB2) increased at different pH (Table 31; Fig. 50, 51).

Values of $k_1$ (0.03-0.05) and $q_e$ (0.46-3.89) by *Aspergillus* sp. (MB1) whereas values of $k_1$ (0.07-0.07) and $q_e$ (0.55-1.55) by *Streptomyces* sp. (MB2) increased at different concentration of Cr(III). Plot of *log* (*q*e-*q*t) versus agitation time, gave a straight line with $r^2$ of 0.98 for *Aspergillus* sp. (MB1), 0.90 by *Streptomyces* sp. (MB2) at pH 4 (Table 32; Fig. 50, 51). Values of *qe* are found lower than experimental one. Therefore, chromium-adsorbent systems do not follow a first-order rate equation. In most of the cases, first-order equation of Lagergren did not apply throughout the contact time and is generally applicable over initial (20-30 min) sorption.

**Ho and McKay equation (pseudo-second-order)**

Plot of (*t/qt*) vs *t* should give a linear relation, from which $k_2$ and *h* can be determined from slope and intercept of plot, respectively. $r^2$ of Cr(III) under different conditions were calculated from plots at different adsorbent dosage (Table 30), pH (Table 31) and Cr(III) concentration (Table 32).

It was observed that $k_2$ (2.29-1.12) and *h* (2.44-5.07) by *Aspergillus* sp. (MB1) whereas $k_2$ (0.99-0.97) and *h* (6.32-25.38) by *Streptomyces* sp. (MB2) increase with increase in adsorbent dosage. Plot of (*t/qt*) vs *t*, gave a straight line with $r^2$ of 0.99 for *Aspergillus* sp. (MB1), 0.99 by *Streptomyces* sp. (MB2) at different adsorbent dosage.

Plot of (*t/qt*) vs *t* agitation time, gave a straight line with values of $k_2$ (1.09-2.24) and *h* (5.95-84.03) by *Aspergillus* sp. (MB1) whereas values of $k_2$ (-0.41-1.21) and *h* (-0.27-6.53) by *Streptomyces* sp. (MB2) increased at different pH with corelation coefficient $r^2$ of 1.00 for *Aspergillus* sp. (MB1) and 1.00 by *Streptomyces* sp. (MB2) respectively at different pH (Table 31; Fig. 52, 53).

Plot of (*t/qt*) vs *t*, gave a straight line with $r^2$ of 1.00 for *Aspergillus* sp. (MB1), 1.00 by *Streptomyces* sp. (MB2). Values of $k_2$ (0.32-2.77) and *h* (0.22-3.84) by *Aspergillus* sp. (MB1) whereas values of $k_2$ (0.29-2.94) and *h* (0.90-5.20) by *Streptomyces* sp. (MB2) increased with increase in initial Cr(III) ions. Values of *h* and $k_2$ were found higher for *Streptomyces* sp. (MB2) than *Aspergillus* sp. (MB1) at different concentration of Cr(III) (Table 32; Fig. 52, 53).

The data obtained separately for pseudo-second-order models from the slopes of plots showed a good compliance with the pseudo second-order equation, the $r^2$ values for the linear plots being > 0.99 showed that kinetic data fitted the pseudo second order adsorption kinetic equation $r^2$ for linear plots are found superior (in most cases $r^2$ 0.99) (Tables 30-32). Results showed that the adsorption reaction can be approximated with the pseudo second-order kinetic model. It was observed that *h* and $k_2$ values were higher for *Aspergillus* sp. (MB1) than *Streptomyces* sp. (MB2), which also showed increase with increase in adsorbent dosage, pH and Cr(III) concentration.

**Elovich equation**

Elovich model, a plot of *qt* vs ln (*t*) should yield a liner relationship with a slope of (*l/*b) and an intercept of (*1/* β) ln (±β) (Fig. 54, 55). $r^2$ were determined from plot between *qt* and ln (*t*), respectively at different pH, biomass dosage and chromium concentration (Tables 30-32). Computed value of a and b were calculated at adsorbent dosage [α (21.29-0.09), β (3.21-33.44)], pH [α (13.90-0.13) β (10.67-55.56)] and Cr(III) concentration [α (0.90-0.61) β (19.16-2.92)] by *Aspergillus* sp. (MB1), whereas adsorbent dosage α (18.18-0.26), β (9.42-34.13)], pH [α (-3.69-94.32) β (-29.71-9.45)] and Cr(III) concentration [α (7.55-78.07) β (26.25-2.85)] by *Streptomyces* sp. (MB2).

Sorption kinetics of Cr(III) examined using Elovich model gave $r^2$ of 0.93, 90 and 91 for *Aspergillus* sp. (MB1) and $r^2$ of 0.99, 96 and 99 for *Streptomyces* sp. (MB2) at adsorbent dosage, pH and Cr(III) concentration which indicates that the dynamics data fitted for both the biomass at adsorbent dosage 2g, pH 4.0, 50 mg/L concentration of Cr(III) (Table 30-32; Fig. 54,55).

**Morris-Weber equation**

The kinetics of sorption of Cr(III) was also examined by using intraparticle diffusion model. Where, *qt*,, sorbed concentration at time *t*; *Rid*, rate constant of intraparticle transport. $q_t$ was plotted against *t1/2* (Fig. 56, 57). Sorption follows linearity as per Eq with $r^2$ (0.831). Value of *Rid* was computed from slope of plot. Value of $r^2$ was (Table 30-32): pH, 0.13-0.85; adsorbent dosage, 0.74-0.59; and Cr(III) concentration, 0.69-0.83. Values of *Rid* were calculated from slope of such plots (Fig. 56, 57) and $r^2$ values led to the conclusion that intraparticle diffusion process is rate-limiting step. Higher values of *Rid* illustrate an enhancement in the rate of adsorption, whereas larger *Rid* values illustrate a better adsorption mechanism, which is related to an improved bonding between Cr(III) ions and adsorbent particles. Sorption kinetics of Cr(III) examined using intraparticle diffusion model gave correlation coefficient $r^2$ of 0.97, 90 and 84 for *Aspergillus* sp. (MB1) and $r^2$ of 0.59, 96 and 83 for *Streptomyces* sp. (MB2) at adsorbent dosage, pH and Cr(III) concentration which indicates that the dynamics data fitted for both the biomass at adsorbent dosage 2 g, pH 4.0, 50 mg/L concentration of Cr(III) (Table 30-32; Fig. 56, 57).

Table 30: The adsorption kinetic model rate constants of Cr(III) for the *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) at different adsorbent dosage.

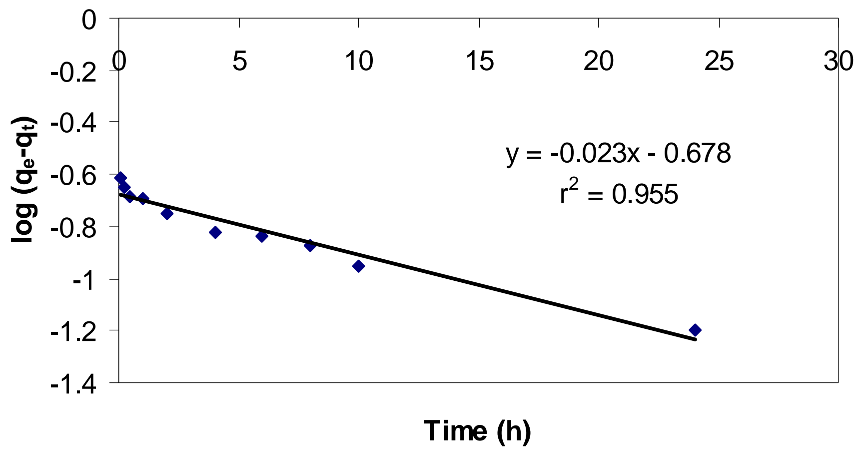
| Microbial biomass | Adsobent dosage (g) | Pseudo first-order | | | Pseudo second-order | | | Elovich model | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_1$ | $q_e$ | $r^2$ | $k_2$ | h | $r^2$ | β | α | $r^2$ |
| *Aspergillus* sp. (MB1) | 0.25 | 0.03 | 8.64 | 0.84 | 2.29 | 2.44 | 0.98 | 3.21 | 21.29 | 0.83 |
| | 0.5 | 0.02 | 3.84 | 0.79 | 1.8 | 2.61 | 0.98 | 3.24 | 9.67 | 0.93 |
| | 1 | 0.06 | 0.43 | 0.75 | 2.14 | 20 | 0.99 | 19.61 | 0.4 | 0.92 |
| | 2 | 0.05 | 0.21 | 0.96 | 1.12 | 5.07 | 0.99 | 33.44 | 0.09 | 0.93 |
| *Streptomyces* sp. (MB2) | 0.25 | 0.03 | 1.42 | 0.58 | 0.99 | 6.32 | 0.99 | 9.42 | 18.18 | 0.93 |
| | 0.5 | 0.02 | 1.34 | 0.71 | 1.01 | 7.09 | 0.99 | 13.61 | 7.41 | 0.94 |
| | 1 | 0.01 | 1.26 | 0.46 | 0.98 | 15.04 | 0.99 | 22.22 | 1.78 | 0.83 |
| | 2 | 0.88 | 2.74 | 0.44 | 0.97 | 25.38 | 0.99 | 34.13 | 0.26 | 0.81 |

Table 31: The adsorption kinetic model rate constants of Cr(III) for the *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) at different pH.

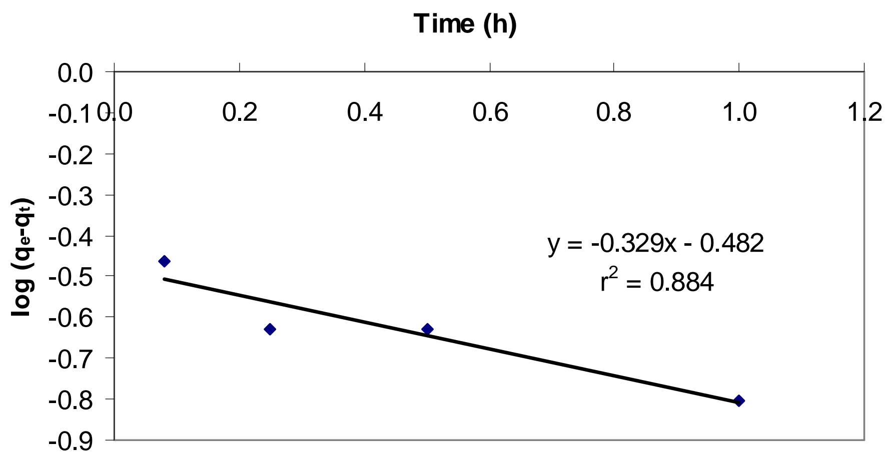
| Microbial biomass | Initial pH | Pseudo first-order | | | Pseudo second-order | | | Elovich model | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_1$ | $q_e$ | $r^2$ | $k_2$ | H | $r^2$ | β | α | $r^2$ |
| *Aspergillus* sp. (MB1) | 2 | 2.00 | 0.02 | 0.97 | 1.09 | 5.95 | 0.99 | 10.67 | 13.90 | 0.69 |
| | 3 | 0.01 | 1.95 | 0.72 | 1.04 | 1.58 | 0.99 | 10.56 | 80.60 | 0.29 |
| | 4 | 0.76 | 3.03 | 0.88 | 2.43 | 31.35 | 1.00 | 21.69 | 0.18 | 0.90 |
| | 5 | 0.03 | 3.29 | 0.71 | 2.24 | 84.03 | 1.00 | 55.56 | 0.13 | 0.75 |
| *Streptomyces* sp. (MB2) | 2 | -4.84 | 1.83 | 0.23 | -0.41 | -0.27 | 0.98 | -29.71 | -3.69 | 0.02 |
| | 3 | 0.01 | 1.66 | 0.71 | 0.02 | 0.03 | 0.84 | 26.18 | 0.51 | 0.55 |
| | 4 | 0.06 | 0.74 | 0.94 | 1.26 | 5.47 | 1.00 | 6.31 | 63.42 | 0.96 |
| | 5 | 0.05 | 0.95 | 0.78 | 1.21 | 6.53 | 0.99 | 9.45 | 94.32 | 0.95 |

Table 32: The adsorption kinetic model rate constants of Cr(III) for the *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) at different concentration of chromium.

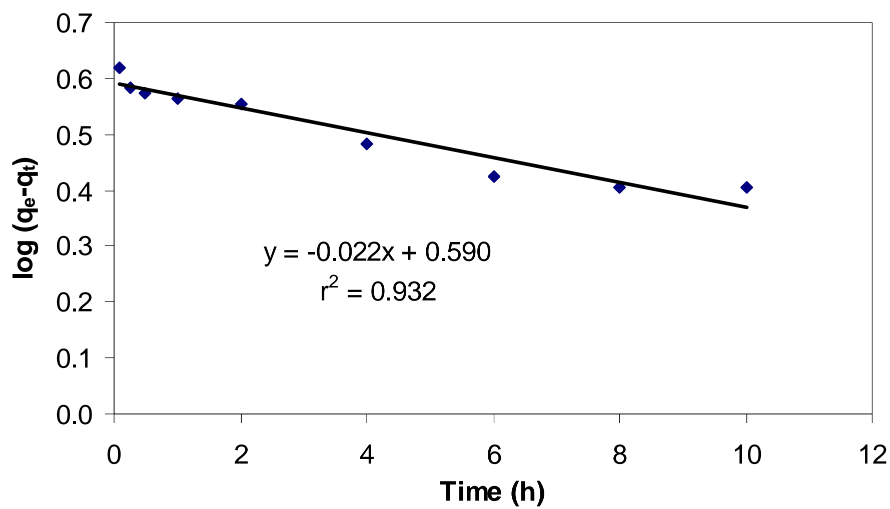
| Microbial biomass | Cr(III) mg/L | Pseudo first-order | | | Pseudo second-order | | | Elovich model | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $k_1$ | $q_e$ | $r^2$ | $k_2$ | h | $r^2$ | β | α | $r^2$ |
| *Aspergillus* sp. (MB1) | 5 | 0.03 | 0.46 | 0.68 | 0.32 | 0.22 | 0.97 | 19.16 | 0.90 | 0.89 |
| | 10 | 0.04 | 0.88 | 0.82 | 0.48 | 0.63 | 1.00 | 14.49 | 3.65 | 0.95 |
| | 25 | 0.08 | 1.92 | 0.98 | 1.56 | 0.41 | 0.98 | 4.01 | 1.62 | 0.91 |
| | 50 | 0.05 | 3.89 | 0.93 | 2.77 | 3.84 | 1.00 | 2.92 | 0.61 | 0.91 |
| *Streptomyces* sp. (MB2) | 5 | 0.07 | 0.55 | 0.51 | 0.29 | 0.90 | 1.00 | 26.25 | 7.55 | 0.86 |
| | 10 | 0.08 | 0.68 | 0.57 | 0.49 | 5.26 | 1.00 | 15.85 | 19.08 | 0.83 |
| | 25 | 0.06 | 1.20 | 0.71 | 1.19 | 1.99 | 1.00 | 5.17 | 7.26 | 0.93 |
| | 50 | 0.07 | 1.55 | 0.90 | 2.94 | 5.20 | 1.00 | 2.85 | 78.07 | 0.99 |




(A)

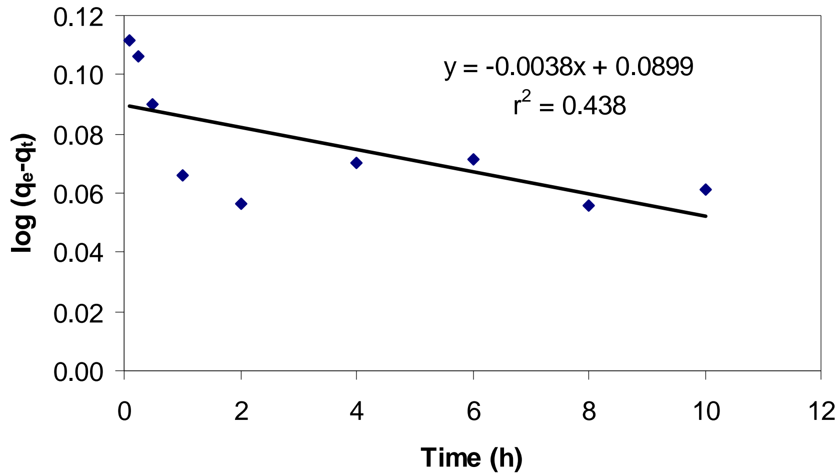



(B)

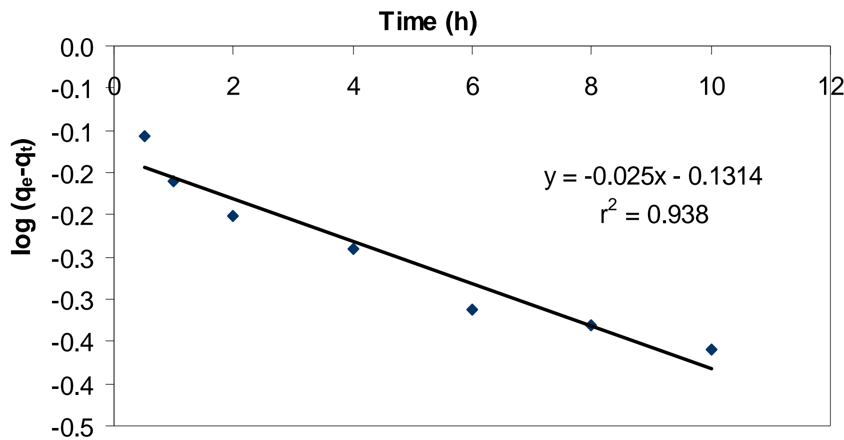



(C)

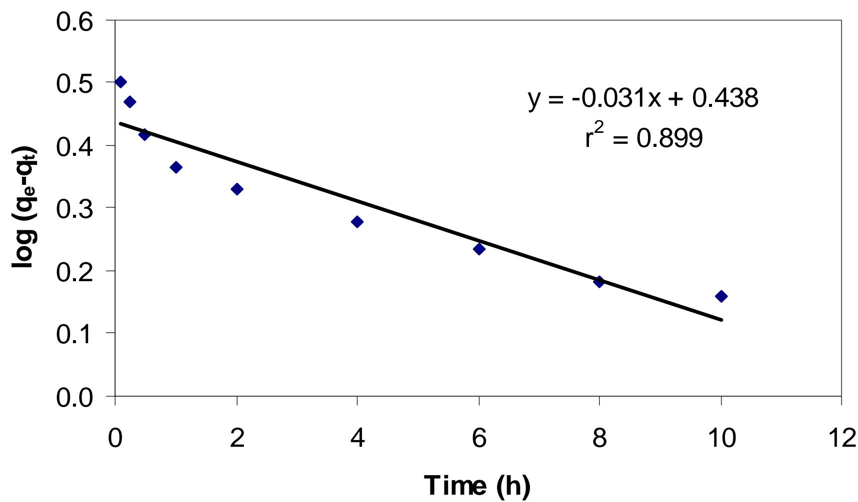
Fig. 50: Lagergren plot of Cr(III) onto microbial biomass *Aspergillus* sp. (MB1) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)

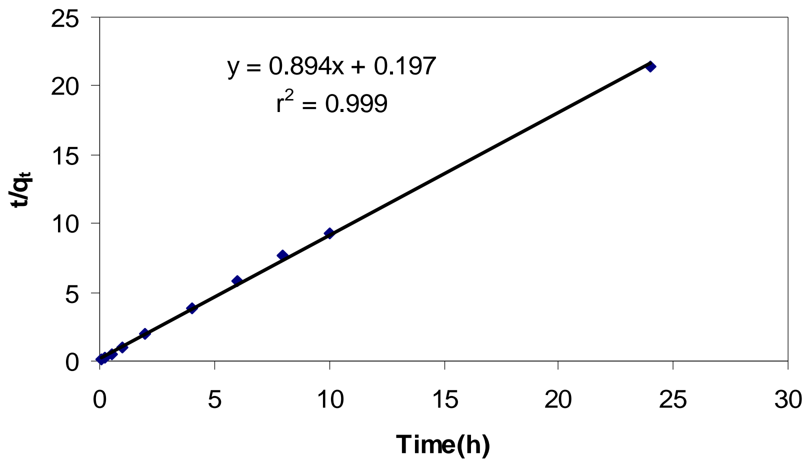



(B)

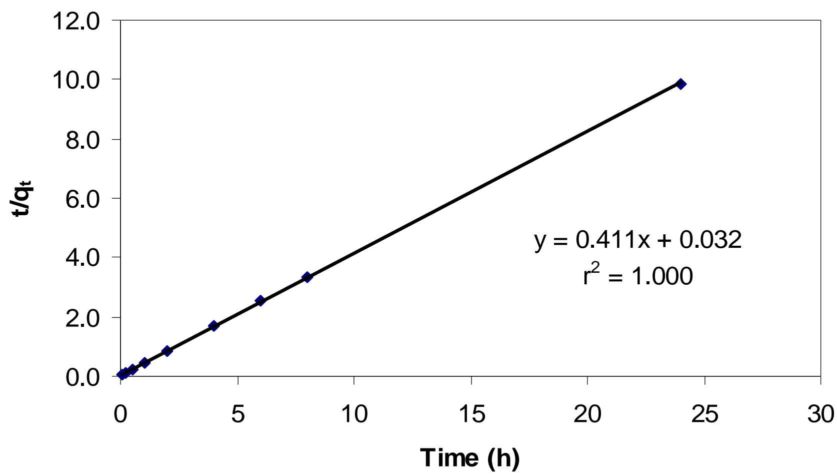



(C )

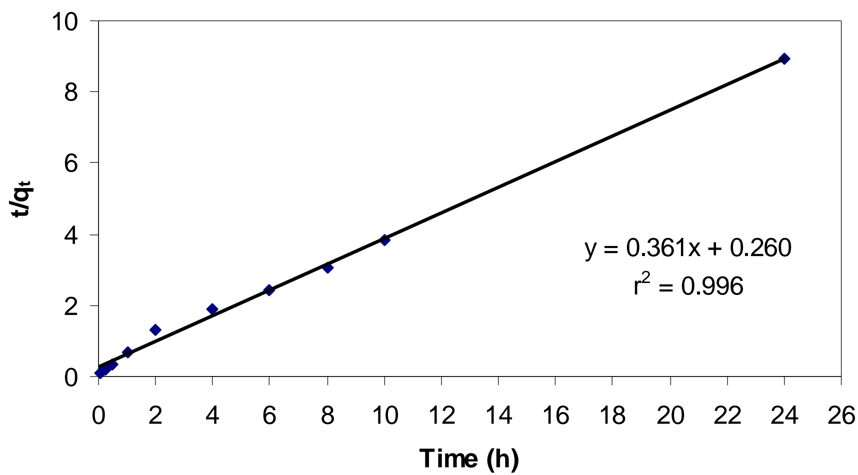
Fig. 51: Lagergren plot of Cr(III) onto microbial biomass *Streptomyces* sp. (MB2) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)

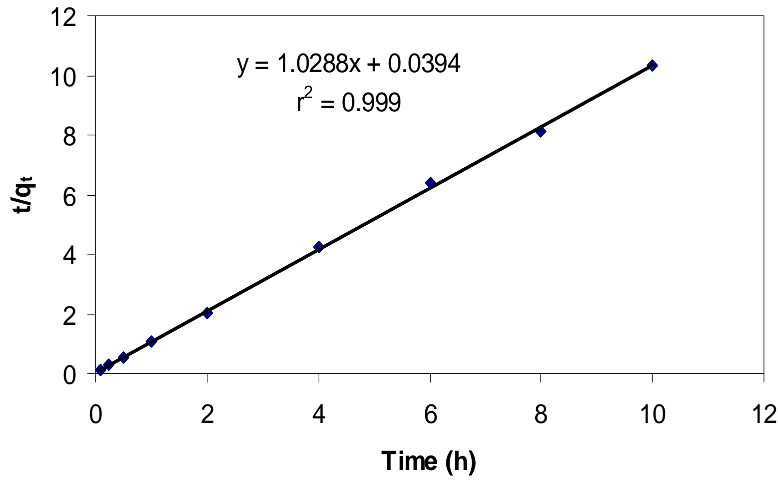



(B)

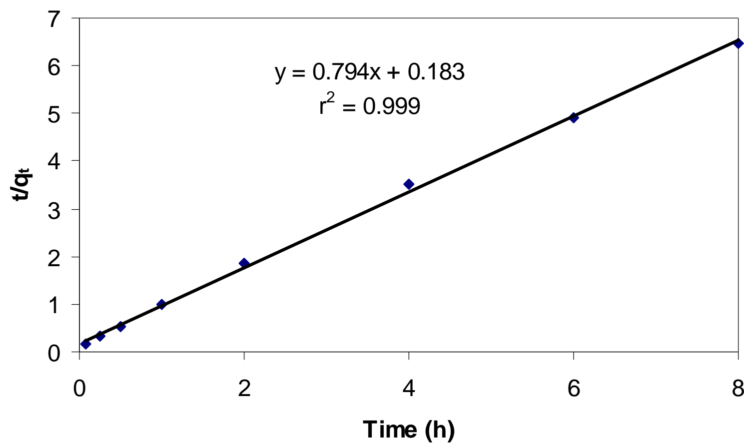



(C )

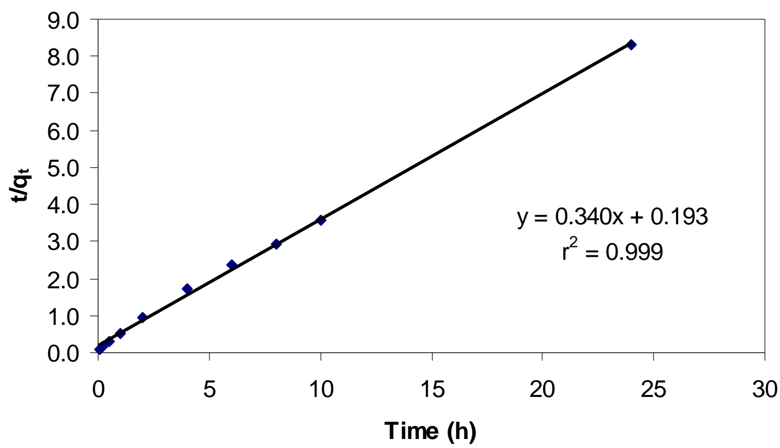
Fig. 52: Ho and Mckay equation for Cr(III) onto microbial biomass *Aspergillus* sp. (MB1) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)

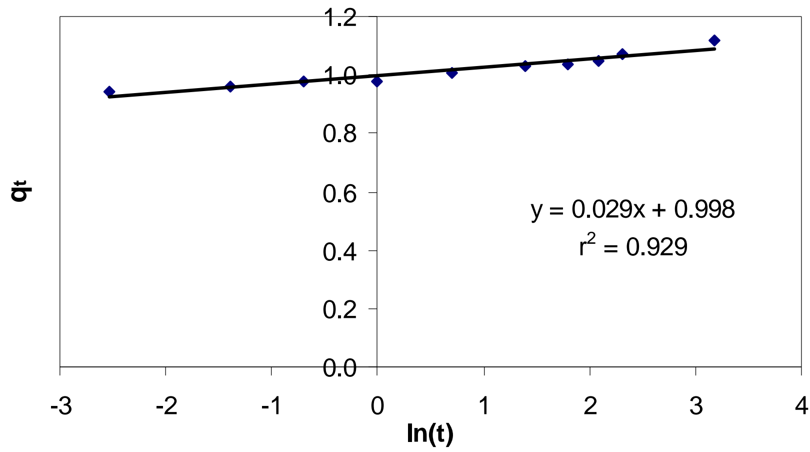



(B)

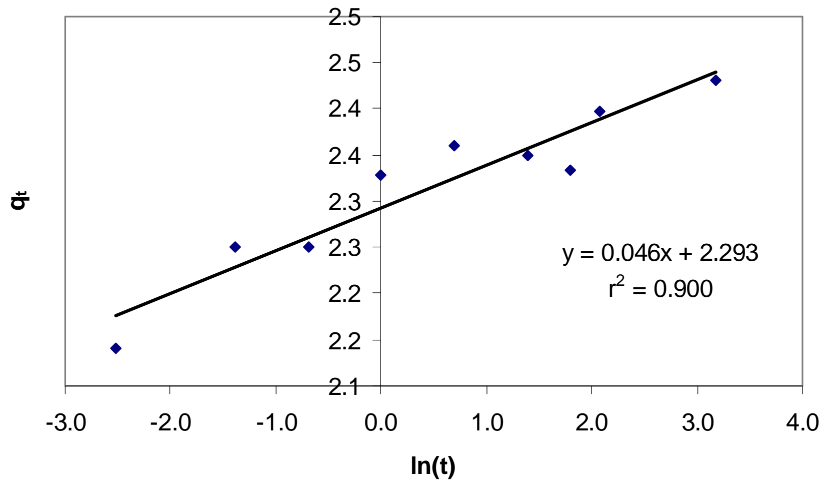



(C )

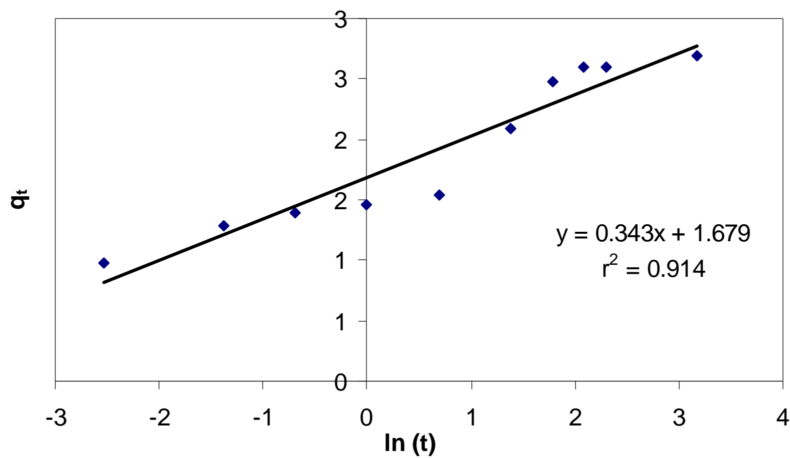
Fig. 53: Ho and Mckay equation for Cr(III) onto microbial biomass *Streptomyces* sp. (MB2) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)

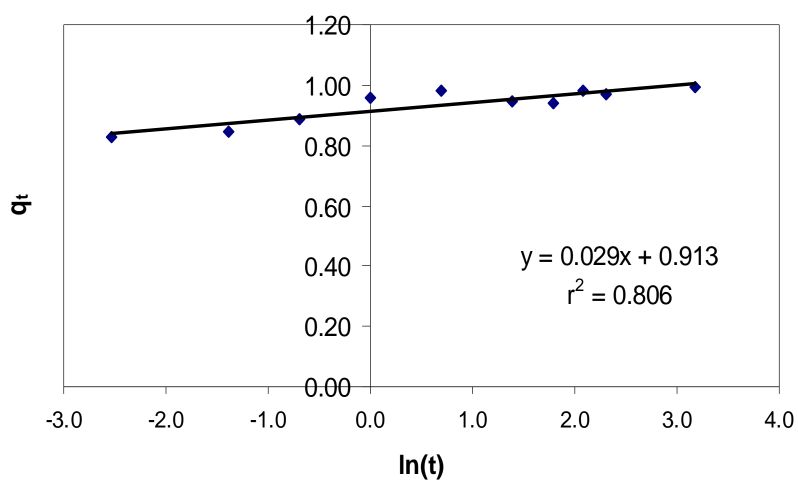



(B)

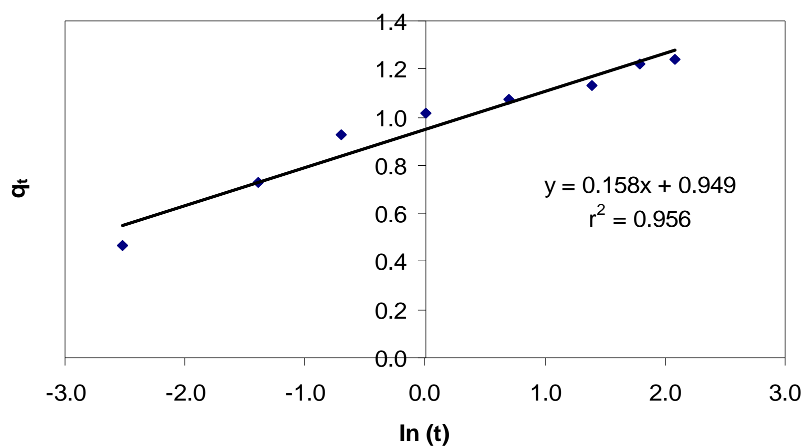



(C )

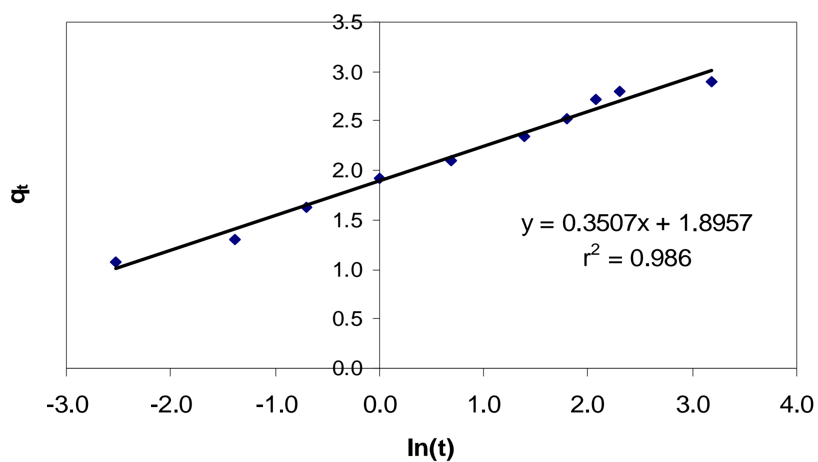
Fig. 54: Elovich equation for Cr(III) onto microbial biomass *Aspergillus* sp. (MB1) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)

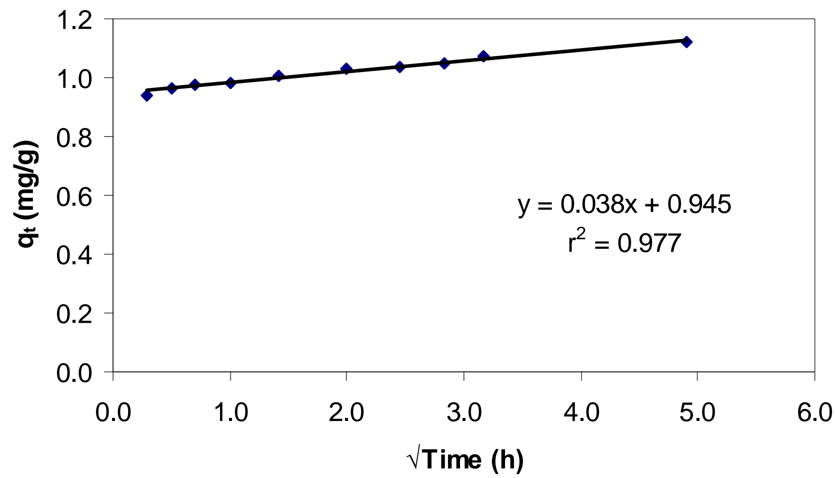



(B)

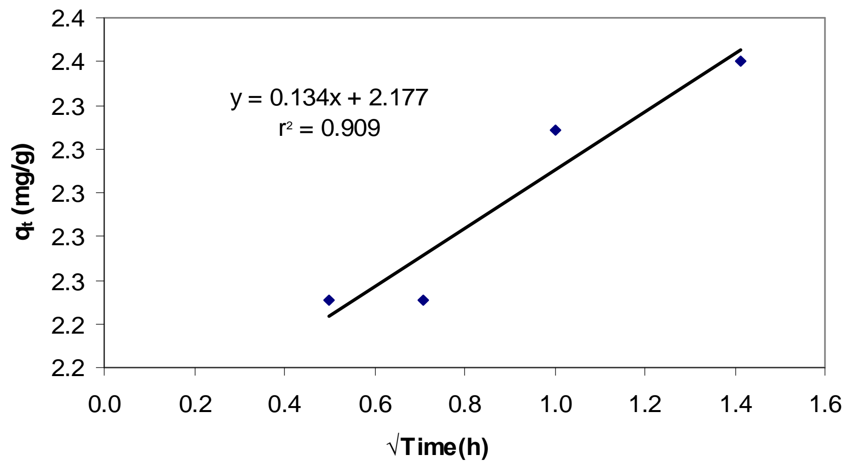



(C )

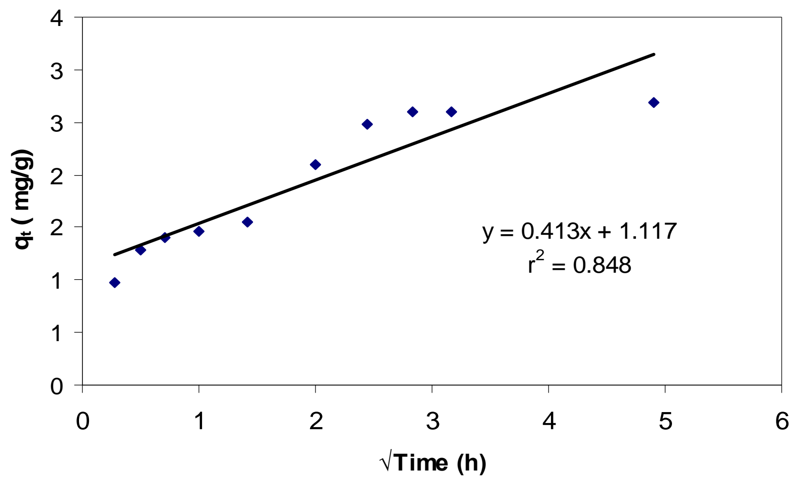
Fig. 55: Elovich equation for Cr(III) onto microbial biomass *Streptomyces* sp. (MB2) {A: 2g biomass dosage; B: pH4; C: 50 mg/L}.




(A)

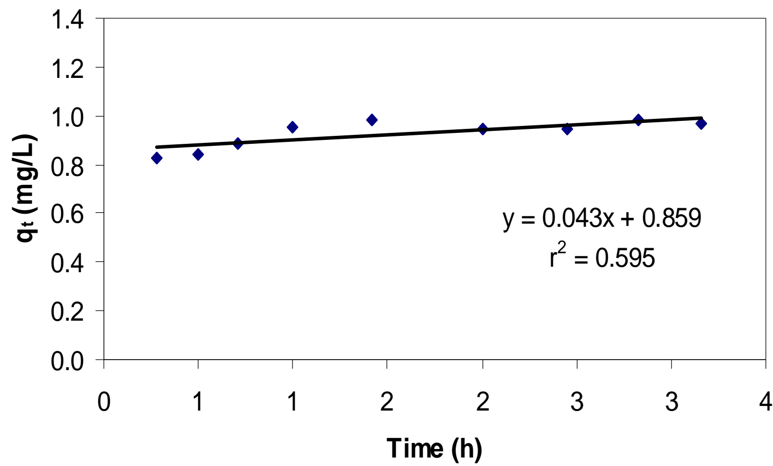



(B)

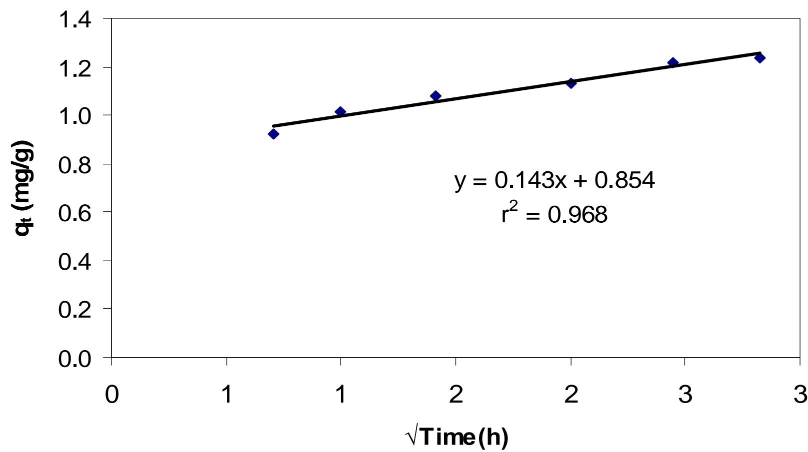



(C )

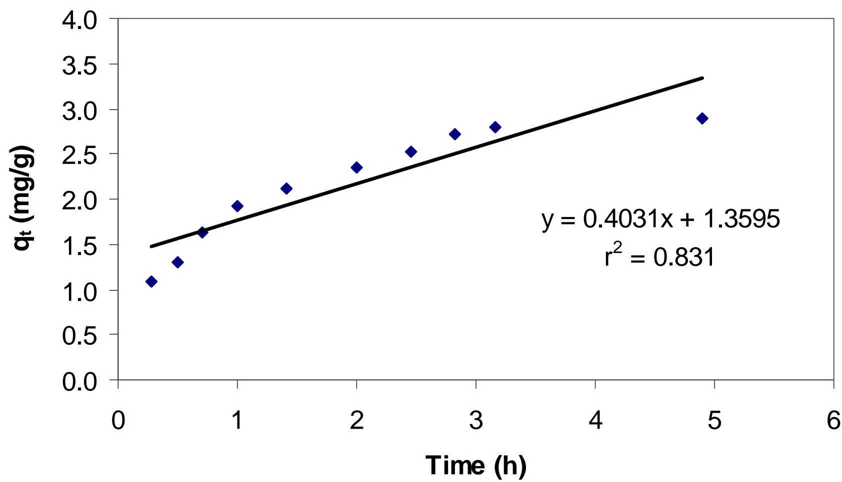
Fig. 56: Morris-Weber equation for Cr(III) onto microbial biomass *Aspergillus* sp. (MB1) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.




(A)




(B)




(C)
Fig. 57: Morris-Weber equation for Cr(III) onto microbial biomass *Streptomyces* sp. (MB2) {A: 2g adsorbent dosage; B: pH4; C: 50 mg/L}.

### 4.3.5 Removal of Cr(III) from aqueous solution by pretreated microbial biomass

Microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were pretreated with different mineral acids hydrochloric and sulfuric acid and alkali sodium hydroxide (at a concentration of 0.1M) and other organic solvents (acetone, methanol, diethyl ether and chloroform) for modification of the cell wall and cell surface sequestration in order to enhance the chromium (III) binding capacity of the biomass.

**Batch studies: Removal of Cr(III)**

Batch sorption experiments on native and chemically pretreated biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were carried out with aqueous solution of Cr(III) at pH 4, 28±2°C ambient temperature, 120 rpm for 24 h and experimental blanks were run in parallel.

**Effect of alkali and acid pretreatment**

Effect of alkali and acid pretreatment on *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) biomass is shown in Table 33, Fig. 58, 59. Treatment of *Aspergillus* sp. (MB1) biomass with NaOH showed 96.21% removal of Cr(III) removal from aqueous solution (Fig. 58). Cr(III) uptake and removal by alkali-treated *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) biomass was 2.75 (96.2%) and 1.72 (76.7%) mg/g respectively (Table 33, Fig. 58, 59). Among all the pretreatment methods alkali treatment improved the biosorption capacities of both the biomasses. Among the two acids $H_2SO_4$ (0.1M) and HCl (0.1M) pretreatment of *Streptomyces* sp. (MB2) only showed enhancement in Cr(III) removal from aqueous solution by 75.8 and 77.3 % as compared to native biomass (68.5%) (Table 33). There was a in increase in case of *Streptomyces* sp. (MB2) biomass, which rather showed decline in *Aspergillus* sp. (MB1) (Fig. 58, 59). It was observed that uptake (q values) capacity for acid pretreated biomass was higher in comparison to other treatment.

**Effect of organic solvent pretreatment**

Pretreatment by organic solvents, such as acetone, chloroform, diethylether and methanol affected the removal rate of Cr(III). As shown in Fig. 60, 61 the removal percentage of *Aspergillus* sp. (MB1) after treatment with organic solvent was in the order of acetone (68%) > methanol (64.12%) > chloroform (63.01%) > diethylether (54.28%) which was lower than the native biomass (73.98%). However pretreatment of *Streptomyces* sp. (MB2) with organic solvent was in the order of Cr(III) removal diethylether (74.49%) > acetone (71.59%) > chloroform (69.06%) which was higher than native biomass (68.11%). All the organic solvents showed the improvement of Cr(III) removal efficiency accept methanol (58.97%). Adsorption capacities of organic solvent treated microbial biomass were found to be 1.95 to 1.80 and 1.76 to 1.48 mg/L respectively (Table 34).

Table 33. Effect of alkali and acid pretreatment of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) on removal of Cr(III) from aqueous solution (Ci: 28.63; 24.59 mg/L; pH4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

| Alkali and acid pretreatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Native | | NaOH (0.1N) | | $H_2SO_4$ (0.1N) | | HCl (0.1N) | |
| Time (h) | Biomass | $C_f$ | % Removal | $C_f$ | % Removal | $C_f$ | % Removal | $C_f$ | % Removal |
| 0 | MB1 | 28.63±0.30 | 0 | 28.63±0.30 | 0 | 28.63±0.30 | 0 | 28.63±0.30 | 0 |
| | MB2 | 24.59±0.25 | 0 | 24.59±0.25 | 0 | 24.59±0.25 | 0 | 24.59±0.25 | 0 |
| 0.08 | MB1 | 17.18±0.15 | 39.99 | 3.35±0.34 | 88.30 | 27.64±0.15 | 3.46 | 19.43±0.17 | 32.13 |
| | MB2 | 16.74±0.06 | 31.93 | 6.48±0.14 | 73.64 | 14.07±0.20 | 42.79 | 11.69±0.61 | 52.46 |
| 0.25 | MB1 | 14.79±0.17 | 48.36 | 2.59±0.24 | 90.95 | 26.34±0.61 | 8.02 | 17.92±0.20 | 37.41 |
| | MB2 | 16.46±0.24 | 33.08 | 5.71±0.18 | 76.78 | 13.12±0.18 | 46.64 | 10.36±0.14 | 57.87 |
| 0.5 | MB1 | 12.87±0.17 | 55.06 | 2.34±0.21 | 91.84 | 24.89±0.26 | 13.06 | 17.45±0.18 | 39.05 |
| | MB2 | 12.88±0.23 | 47.63 | 5.53±0.15 | 77.52 | 10.38±0.23 | 57.81 | 10.24±0.07 | 58.35 |
| 1 | MB1 | 12.58±0.15 | 56.06 | 2.13±0.02 | 92.56 | 22.47±0.16 | 21.52 | 17.15±0.16 | 12.58 |
| | MB2 | 11.64±0.06 | 52.66 | 5.84±0.38 | 76.25 | 9.46±0.21 | 61.53 | 9.03±0.24 | 63.28 |
| 2 | MB1 | 12.50±0.16 | 56.36 | 2.02±0.20 | 92.95 | 22.28±0.18 | 22.18 | 16.37±0.19 | 42.82 |
| | MB2 | 11.03±0.39 | 55.15 | 5.44±0.06 | 77.90 | 7.88±0.52 | 67.96 | 7.21±0.21 | 70.70 |
| 4 | MB1 | 11.25±0.61 | 60.72 | 2.10±0.19 | 92.68 | 20.38±0.41 | 28.82 | 14.27±0.06 | 50.18 |
| | MB2 | 8.48±0.45 | 65.50 | 5.31±0.24 | 78.42 | 8.01±0.26 | 67.42 | 7.25±0.17 | 70.52 |
| 6 | MB1 | 9.38±0.20 | 67.25 | 1.85±0.15 | 93.54 | 18.33±0.52 | 35.99 | 13.97±0.08 | 51.22 |
| | MB2 | 8.33±0.14 | 66.12 | 5.43±0.17 | 77.93 | 7.31±0.04 | 70.28 | 7.27±0.02 | 70.44 |
| 8 | MB1 | 9.84±025 | 65.65 | 1.72±0.44 | 94.01 | 15.73±0.12 | 45.08 | 13.60±0.10 | 52.52 |
| | MB2 | 7.95±0.61 | 67.68 | 6.23±0.23 | 74.67 | 6.64±0.24 | 73.02 | 6.87±0.26 | 72.05 |
| 10 | MB1 | 9.66±0.21 | 66.28 | 1.69±0.15 | 94.12 | 15.38±0.19 | 46.30 | 13.22±0.14 | 53.84 |
| | MB2 | 6.87±0.07 | 72.07 | 5.74±0.24 | 76.65 | 6.77±0.23 | 72.47 | 6.52±0.02 | 73.47 |
| 24 | MB1 | 7.45±0.34 | 73.98 | 1.09±0.17 | 96.21 | 8.25±0.12 | 71.18 | 10.88±0.16 | 62.02 |
| | MB2 | 7.74±0.41 | 68.51 | 5.43±0.26 | 77.91 | 5.95±0.04 | 75.82 | 5.48±0.04 | 77.72 |

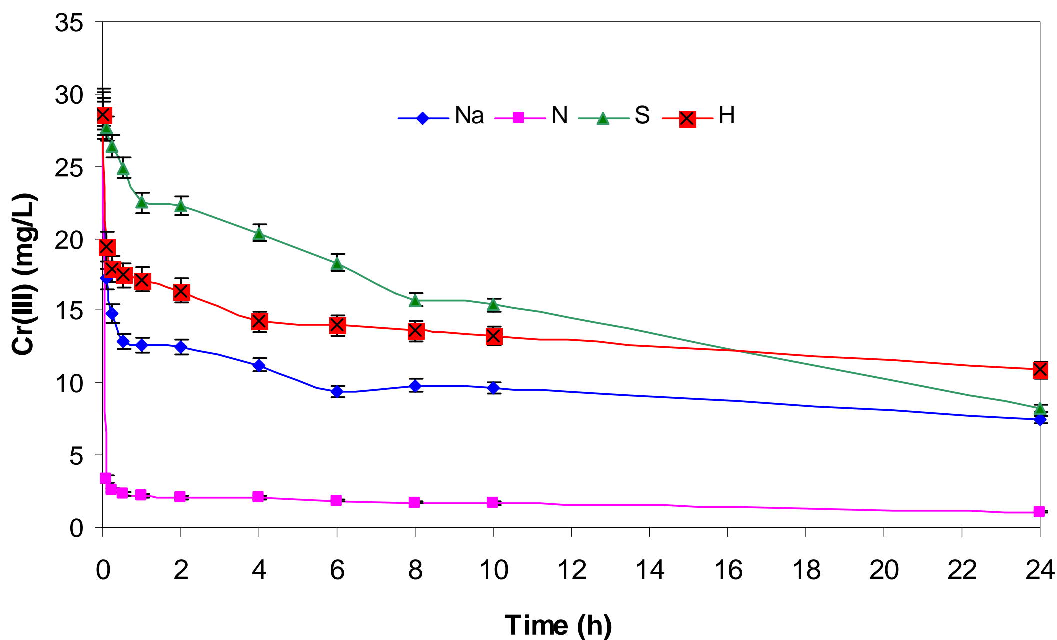



Fig. 58: The effect of alkali and acid pretreatment on Cr(III) removal by *Aspergillus* sp. (MB1) (Na: native, N: NaOH, S: $H_2SO_4$, H: HCl,) at concentration 25 mg/L; pH 4; Adsorbent dosage: 1%; Temp 28±2°C; Agitation rate: 120 rpm.

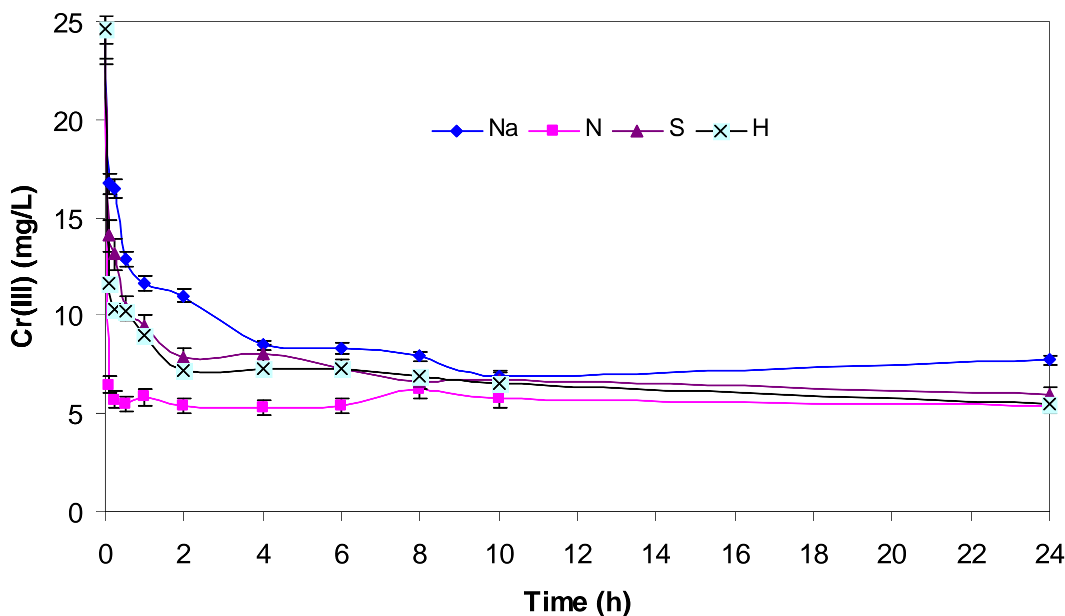



Fig. 59: The effect of alkali and acid pretreatment on Cr(III) biosorption by *Streptomyces* sp. (MB2) (Na: native, N: NaOH, S: $H_2SO_4$, H: HCl,) at concentration 25 mg/L; pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm.

Table 34. Effect of organic solvent pretreatment of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) for removal on Cr(III) from aqueous solution (Ci: 28.63, 24.59 mg/L, pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm).

| Organic solvent pretreatment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Acetone | | Methanol | | Diethyl ether | | Chloroform | |
| Time (h) | Biomass | $C_f$ | % Removal | $C_f$ | % Removal | $C_f$ | % Removal | $C_f$ | % Removal |
| 0 | MB1 | 28.63±0.30 | 0 | 28.63±0.30 | 0 | 28.63±0.30 | 0 | 28.63±0.30 | 0 |
| | MB2 | 24.59±0.25 | 0 | 24.59±0.25 | 0 | 24.59±0.25 | 0 | 24.59±0.25 | 0 |
| 0.08 | MB1 | 20.83±0.38 | 27.26 | 20.47±0.18 | 28.52 | 27.64±0.17 | 3.46 | 21.13±0.23 | 26.21 |
| | MB2 | 13.11±0.70 | 46.69 | 16.53±0.34 | 32.77 | 9.28±0.23 | 56.58 | 8.94±0.15 | 58.19 |
| 0.25 | MB1 | 20.56±0.34 | 28.21 | 18.17±0.61 | 36.55 | 26.34±0.08 | 8.02 | 19.40±0.26 | 32.24 |
| | MB2 | 13.79±0.25 | 43.94 | 13.29±0.39 | 45.94 | 8.47±0.03 | 60.40 | 9.14±0.55 | 57.24 |
| 0.5 | MB1 | 19.36±0.18 | 32.40 | 16.78±0.04 | 41.41 | 24.89±034 | 13.06 | 19.38±0.04 | 32.31 |
| | MB2 | 10.85±0.15 | 55.90 | 12.49±0.20 | 49.22 | 7.96±0.21 | 62.75 | 8.08±0.19 | 62.19 |
| 1 | MB1 | 19.36±0.25 | 32.40 | 17.01±0.15 | 40.59 | 22.47±0.18 | 21.52 | 17.72±0.24 | 38.11 |
| | MB2 | 9.50±0.04 | 61.39 | 11.41±0.20 | 53.62 | 7.40±0.25 | 65.39 | 7.25±0.17 | 66.11 |
| 2 | MB1 | 15.25±0.24 | 46.75 | 16.23±0.06 | 43.33 | 22.28±0.08 | 22.18 | 17.12±0.61 | 40.20 |
| | MB2 | 9.27±0.21 | 62.32 | 9.33±0.15 | 62.07 | 6.38±0.12 | 70.18 | 7.11±0.20 | 66.76 |
| 4 | MB1 | 14.83±0.18 | 48.22 | 14.28±0.43 | 50.12 | 20.38±0.34 | 28.82 | 16.35±0.16 | 42.91 |
| | MB2 | 8.80±0.08 | 64.23 | 9.51±0.24 | 61.32 | 5.77±0.04 | 73.02 | 6.64±0.04 | 68.94 |
| 6 | MB1 | 14.77±0.21 | 48.41 | 12.97±0.17 | 54.72 | 18.33±0.49 | 35.99 | 15.22±0.15 | 46.86 |
| | MB2 | 7.85±0.04 | 68.10 | 9.41±0.23 | 61.73 | 5.42±0.25 | 74.64 | 6.58±0.23 | 69.22 |
| 8 | MB1 | 14.60±0.15 | 49.02 | 13.66±0.23 | 52.31 | 15.73±0.34 | 45.08 | 12.79±0.55 | 55.33 |
| | MB2 | 8.10±0.06 | 67.07 | 8.47±0.17 | 65.58 | 5.81±0.24 | 72.83 | 6.26±0.20 | 70.70 |
| 10 | MB1 | 13.49±0.25 | 52.90 | 13.41±0.19 | 53.18 | 15.38±0.15 | 46.30 | 12.20±0.16 | 57.39 |
| | MB2 | 7.57±0.16 | 69.23 | 11.31±0.23 | 54.01 | 5.29±0.26 | 75.25 | 5.87±0.31 | 72.53 |
| 24 | MB1 | 9.16±0.20 | 68.01 | 10.27±0.15 | 64.13 | 13.09±0.21 | 54.28 | 10.59±0.06 | 63.01 |
| | MB2 | 6.99±0.04 | 71.59 | 10.09±0.06 | 58.97 | 5.45±0.19 | 74.49 | 6.61±0.24 | 69.06 |

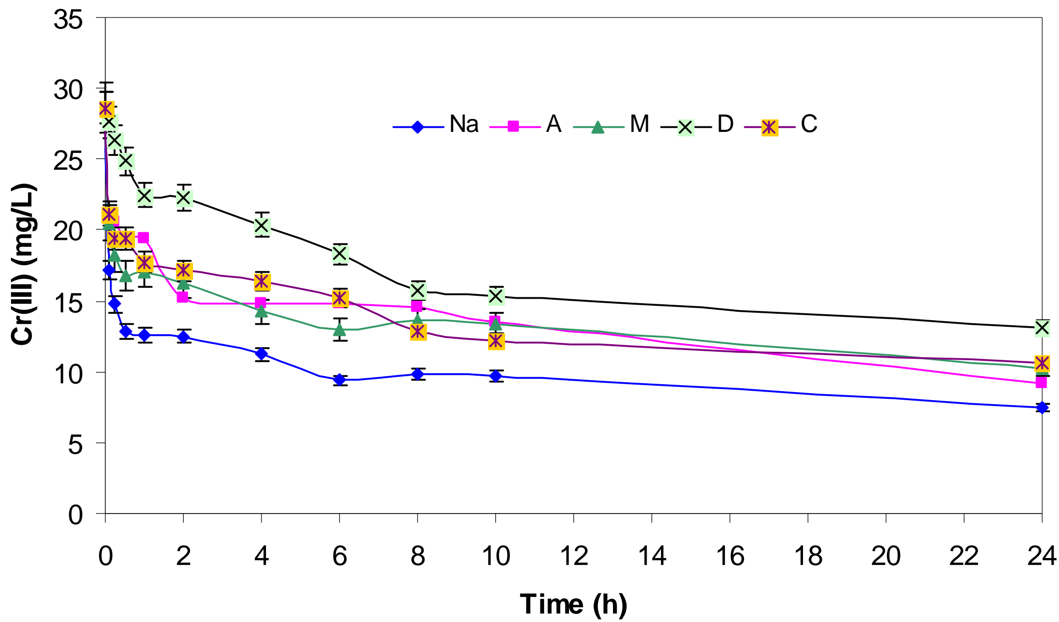



Fig. 60: The effect of organic solvent pretreatment on Cr(III) biosorption by *Aspergillus* sp. (MB1) (Na: native, A: acetone, M: methanol, D: diethylether, C: chloroform) at concentration 25 mg/L; pH 4, Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm.

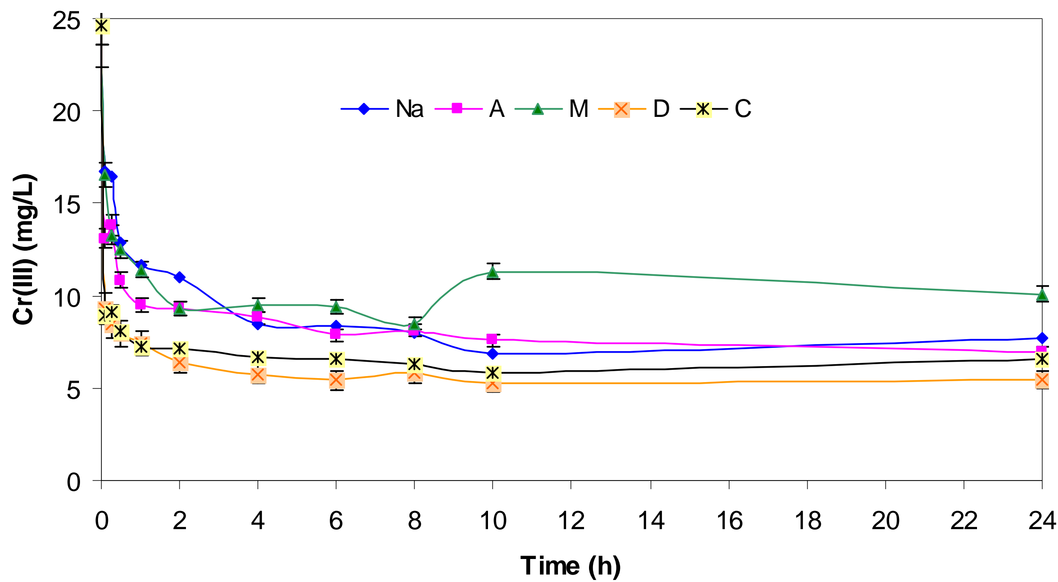



Fig. 61: The effect of organic solvent pretreatment on Cr(III) biosorption by *Streptomyces* sp. (MB2) (Na: native, A: acetone, M: methanol, D: diethylether, C: chloroform) at concentration 25 mg/L; pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm.

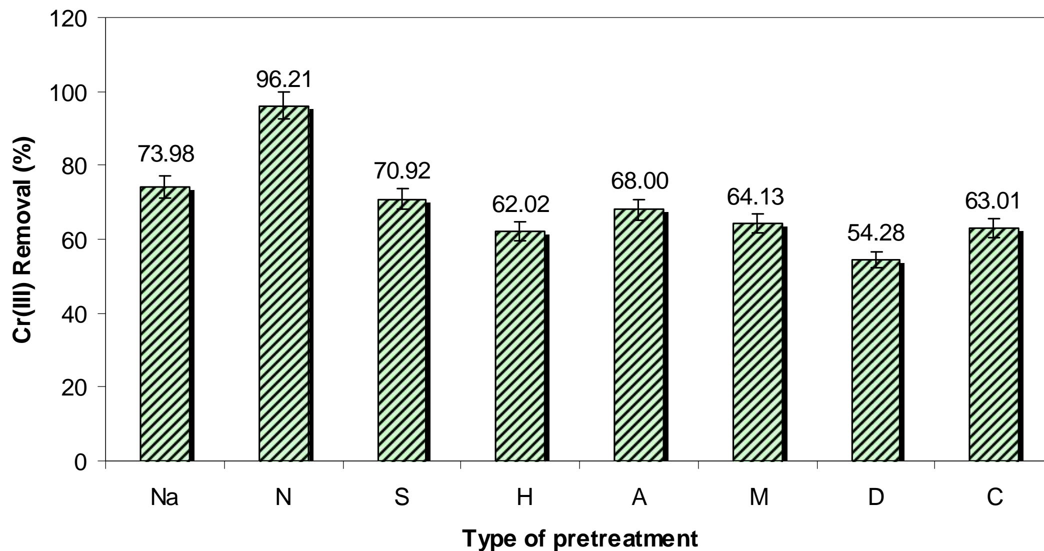



Fig. 62: The effect of pretreatment on Cr(III) removal by *Aspergillus* sp. (MB1) (Na: native, N: NaOH, S: $H_2SO_4$, H: HCl, A: acetone, M: methanol, D: diethylether, C: chloroform) at concentration 25 mg/L; pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm.

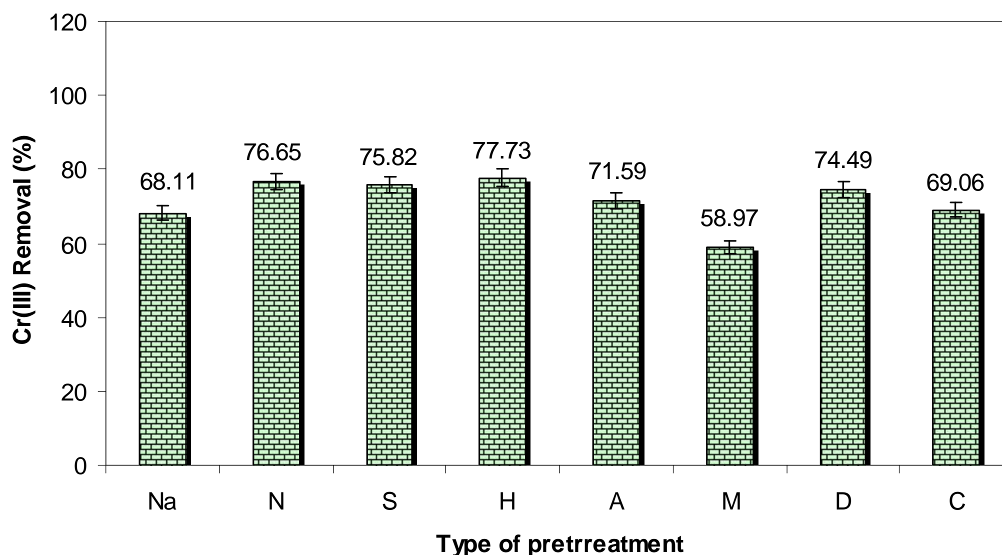



Fig. 63: The effect of pretreatment on Cr(III) removal by *Streptomyces* sp. (MB2) (Na: native, N: NaOH, S: $H_2SO_4$, H: HCl, A: acetone, M: methanol, D: diethylether, C: chloroform) at concentration 25mg/L; pH 4; Adsorbent dosage: 1%; Temp: 28±2°C; Agitation rate: 120 rpm.

**Comparison of Cr(III) removal by pretreated microbial biomass**

Increase in adsorption capacity of Cr(III) was observed after treatment with sodium hydroxide, hydrochloric and sulfuric acid respectively. Percentage Cr(III) removal efficiency was improved by pretreated *Aspergillus* sp. (MB1) biomass with certain chemicals which was in the order of sodium hydroxide (96.21%) > native (73.98%) > sulfuric acid (70.9%) > acetone (68%) > methanol (64%) > chloroform (63%) > hydrochloric acid (62.%) > diethyl ether (54.3%), whereas by *Streptomyces* sp. (MB2) it was in the order of hydrochloric acid (77.7%) > sodium hydroxide (76.7%) > sulfuric acid (75.8%) > diethylether (74.5%) > chloroform (69.1%) > native (68.5%) > methanol (59%) (Table 33, 34, Fig. 62, 63).

### 4.3.6 Fourier transform infrared (FTIR) analysis

To ascertain the chemical nature of binding sites for Cr(III), Fourier transform infrared spectral analysis (FTIR) of native, solvent treated, Cr(III) and tannery effluent, laden biomass were carried out. The FTIR spectrum of native *Aspergillus* sp. (MB1) biomass dislayed a number of adsorption peaks at 3854, 3741, 3360, 2921, 2821, 2846, 2358, 1650, 1544, 1319, 1036 and 1149 $cm^{-1}$ indicating the presence of O–H, N–H, C–H strech ($CH_2$), C=C, C=N, C=O, C–F and C–Cl groups respectively. On comparing the spectra of Cr(III) laden biomass, adsorption peaks at 3854, 3741, 3360 and 2921 $cm^{-1}$ became sharp, whereas new adsorption peaks at 2852, 2364 and 1149 $cm^{-1}$ in Cr(III) laden biomass but in tannery effluent laden biomass, adsorption peaks at 2927, 1625, 1558 and 1456 $cm^{-1}$ indicating for the involvement of C–H–$CH_2$, C=C, C=N, C=O and C–Cl alkenes, amines, alcohol, carboxylic acids groups (Fig. 64).

The FTIR spectrum of *Streptomyces* sp. (MB2) given in Fig. 65 shows peak at 3428 $cm^{-1}$ which is indicative of the existence of −OH and −NH (O−H stretching) and presence of hydroxyl and amine groups on the biomass surface. The spectra of the biomass displayed a number of absorption peaks at 2927 $cm^{-1}$ (C−H stretch), 1631 $cm^{-1}$, 1663 $cm^{-1}$ (C=C stretch), 1550, 1531 $cm^{-1}$ (C=C stretch), 1450 $cm^{-1}$, 1400 $cm^{-1}$ (C−N stretch), 1062 $cm^{-1}$ (C−O stretch), 898 $cm^{-1}$ (C−N stretch) and 698, 667 $cm^{-1}$ (C−O−H). On comparing the spectra of Cr(III) containing tannery effluent treated biomass and native biomass, some of the peaks such as 3428 $cm^{-1}$ (O–H stretch), 1631, 1550 $cm^{-1}$ (C=O, C=C stretch) are increased and becomes sharp and peak 1400 $cm^{-1}$ (C−N stretch) is disappeared whereas peak 1531 $cm^{-1}$ (C=C stretch) is reduced as a result of metal uptake by the biomass. Changes observed in the spectrum indicate possible involvement of these C=C, C=O and O–H, functional

groups in sorption process. The 1800-1540 $cm^{-1}$ band is associated with C=O stretching mode in carbonyls, carboxylic acids and lactones, while 1440-1000 $cm^{-1}$ band was assigned to the C–O stretching and O–H bending modes such as phenols and carboxylic acids (Table 35; Fig. 65).

The FTIR spectrum of *Aspergillus* sp. (MB1) treated with NaOH biomass displayed a number of adsorption peaks at 3403, 2923, 2853, 1653, 1378, 1322, 1151, and 1031 $cm^{-1}$ which is indicating the existence of −OH and −NH (O−H stretching) and presence of hydroxyl and amine and C−H stretching ($CH_3$), C=N, COOH, C−F, C−Cl and C−O groups on the biomass surface. On comparing the spectra of Cr(III) and tannery effluent laden biomass, adsorption peaks at 3403, 2923, 2853, 1653, 1378, 1322, and 1151 $cm^{-1}$ became reduced in size, whereas new adsorption peaks at 3757, 3347 $cm^{-1}$ in Cr(III) laden biomass but in tannery effluent laden biomass, adsorption peaks at 3313, 1655, 1378 and 1037 $cm^{-1}$ indicating for the involvement of N–H, C–H–$CH_2$, C=C, C=N and C=O alkenes, amines, alcohol, carboxylic acids groups (Table 35; Fig. 66).

The FTIR spectrum of *Streptomyces* sp. (MB2) treated with $H_2SO_4$ biomass displayed a number of adsorption peaks at 3282, 2927, 1655, 1533, 1442, 1239, 1161, and 1062 $cm^{-1}$ which is indicating the existence of −OH, C=N, C−O and C−O−C groups on the biomass surface. On comparing the spectra of Cr(III) and tannery effluent laden biomass, adsorption peaks at 3403, 2923, 2853, 1653, 1378, 1322, and 1151 $cm^{-1}$ became reduced in size, whereas new adsorption peaks at 3759, 3319 and 2366 $cm^{-1}$ in Cr(III) laden biomass and tannery effluent laden biomass, adsorption peaks at 3772, 3309, 1532 and 1242 $cm^{-1}$ indicating for the involvement of O–H, C–H, C=N and C=O hydroxyl, alkenes, amines, alcohol, carboxylic acids groups (Table 35; Fig. 67).

Table 35: FTIR analysis of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2).

| Microbial biomass | Treatment | Functional groups involved in chromium binding |
|---|---|---|
| *Aspergillus* sp. (MB1) | Native | Free O–H, N–H, C–H–$CH_2$, C=C, C=N, C=O, C=C, C–O stretch, C–F, C–Cl |
| | Native biomass absorbed with Cr(III) | Free O–H, N–H, C–H stretch ($CH_2$), C=C, C=N, C=O, C=C, C–O stretch, C–F, C–Cl |
| | Native biomass absorbed with tannery effluent | C–H Strech ($CH_3$), C–H stretch ($CH_2$), C=N stretch, CS–$NH_2$ |
| | NaOH treated biomass | Free O–H alcoholic, C–H Stretch ($CH_3$), C–H stretch ($CH_2$), C=N, N–H amine, COOH, C–F, C–Cl |
| | NaOH treated biomass absorbed with Cr(III) | Free O–H alcoholic, C–H stretch ($CH_3$), C–H Strech ($CH_2$), C=N stretch, COOH, C–F, C–Cl |
| | NaOH treated biomass absorbed with tannery effluent | C–H stretch ($CH_3$), N–H, C=O, COOH, C– O stretch |
| *Streptomyces* sp. (MB2) | Native | Free O–H stretch alcoholic, N–H, C–H stretch, C=O, C=C, C=N, C=C, |
| | Native biomass absorbed with Cr(III) | N–H, C–O–C stretching, C=N |
| | Native biomass absorbed with tannery effluent | N–H, C–O–C stretching, C=N C=C, C=O and O–H |
| | $H_2SO_4$ treated biomass | N–H, C–O–C stretching, C=N O–H, C–H, C=N |
| | $H_2SO_4$ treated biomass absorbed with Cr(III) | Free O–H alcoholic, C–H, C–O–C stretching, C=N |
| | $H_2SO_4$ treated biomass absorbed with tannery effluent | O–H alcoholic, C=O, C–H, N–N=O O–H, C=N |

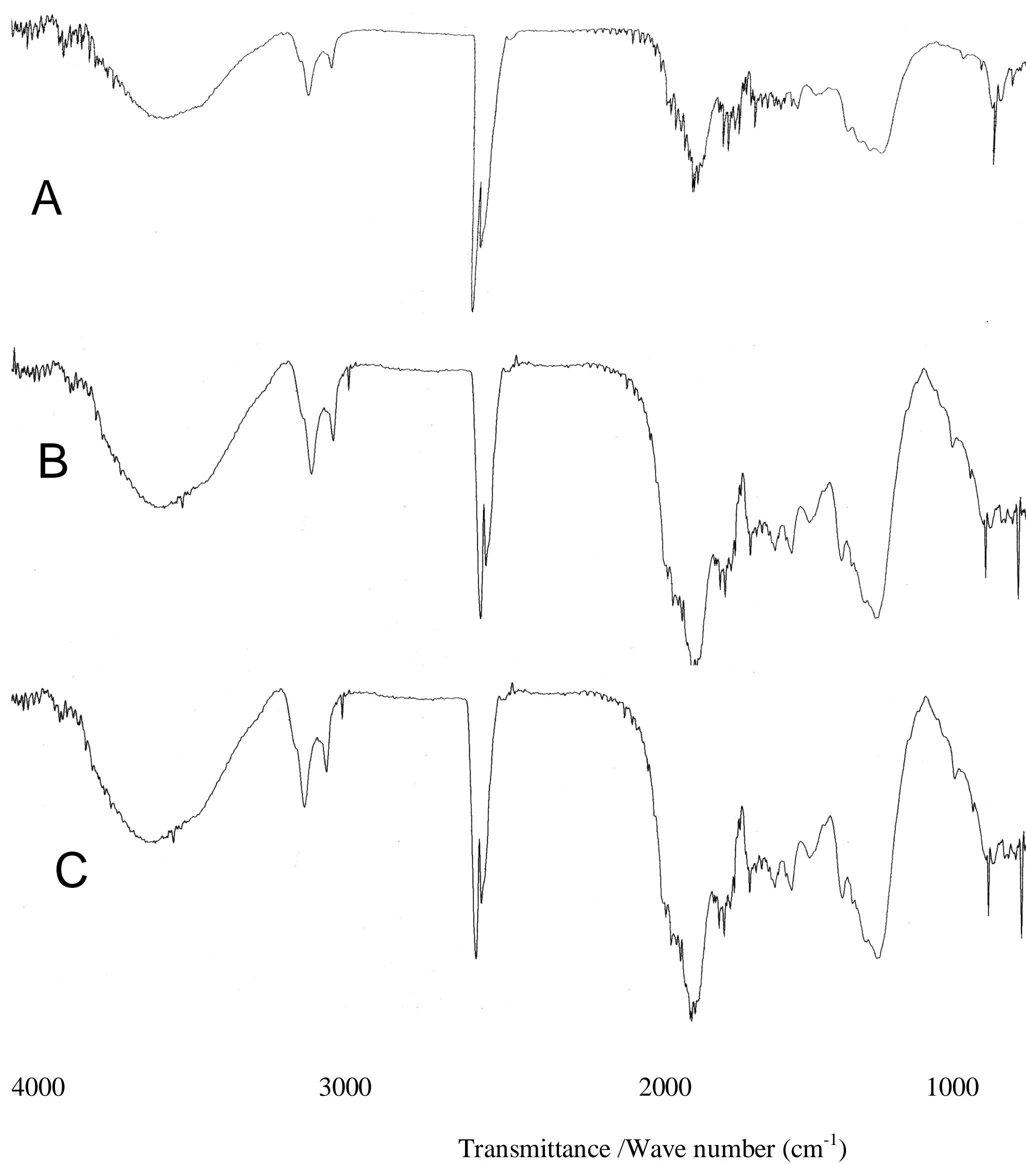



Fig. 64: FTIR spectra of microbial (*Aspergillus* sp. MB1) waste biomass {A: Native biomass; B: Cr(III) treated biomass; C: Tannery effluent treated biomass}.

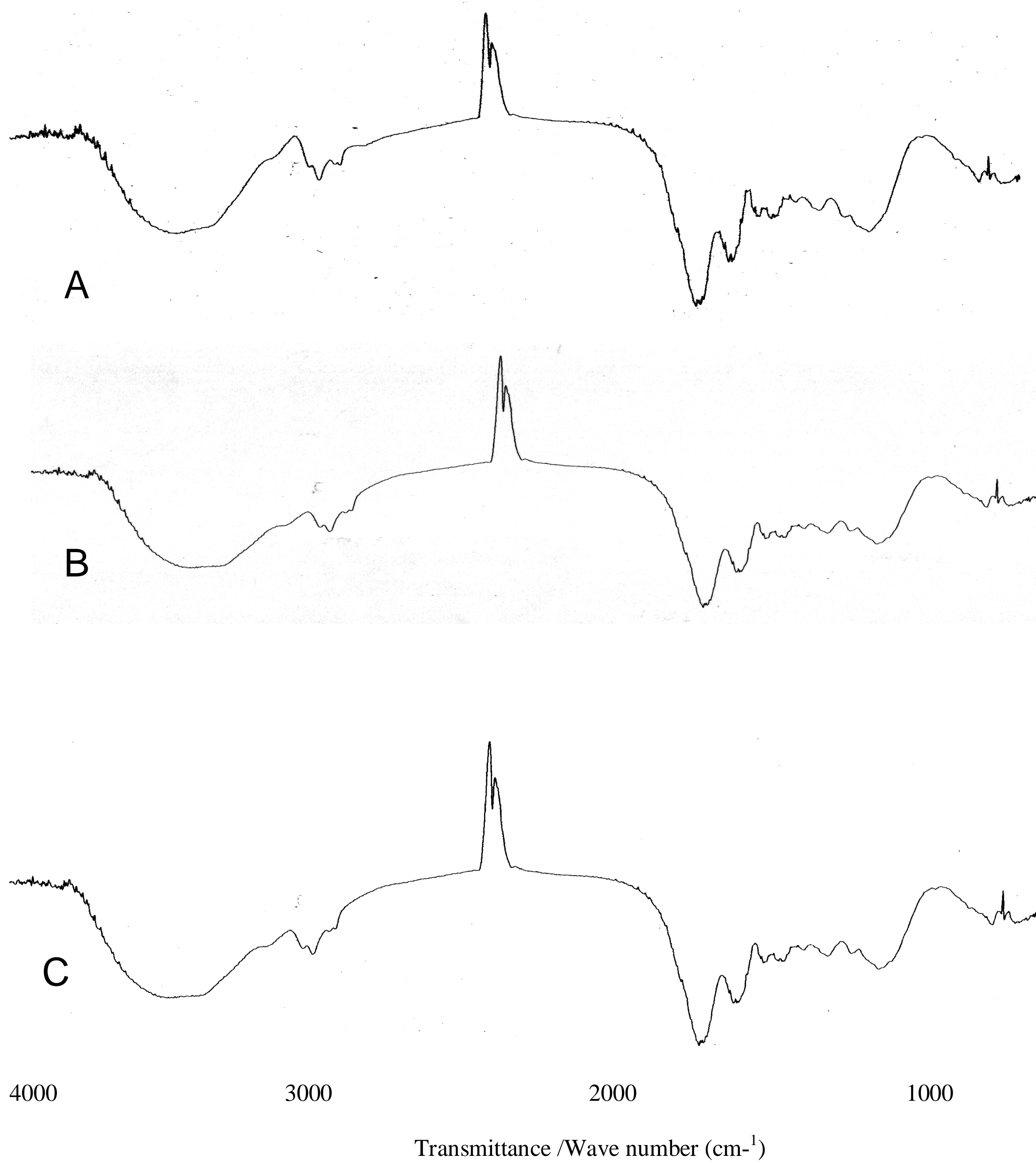
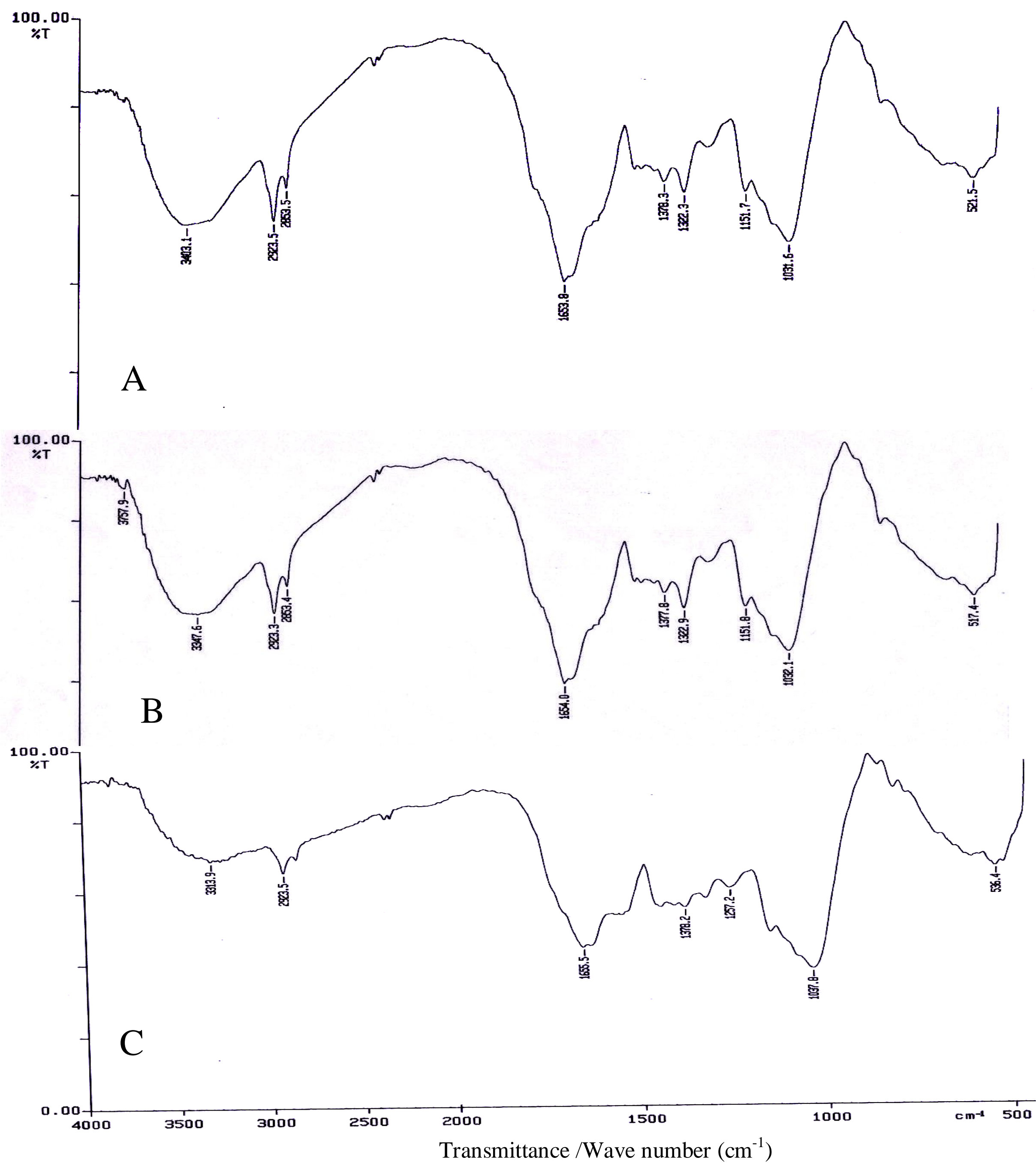



Fig. 65: FTIR spectra of microbial (*Streptomyces* sp. MB2) waste biomass {A: Native biomass; B: Cr(III) treated biomass; C: Tannery effluent treated biomass}.




Transmittance /Wave number ($cm^{-1}$)

Fig. 66: FTIR spectra of microbial waste biomass *Aspergillus* sp. (MB1) treated with NaOH. {A: Native biomass; B: Cr(III) treated biomass; C: tannery effluent treated biomass}.

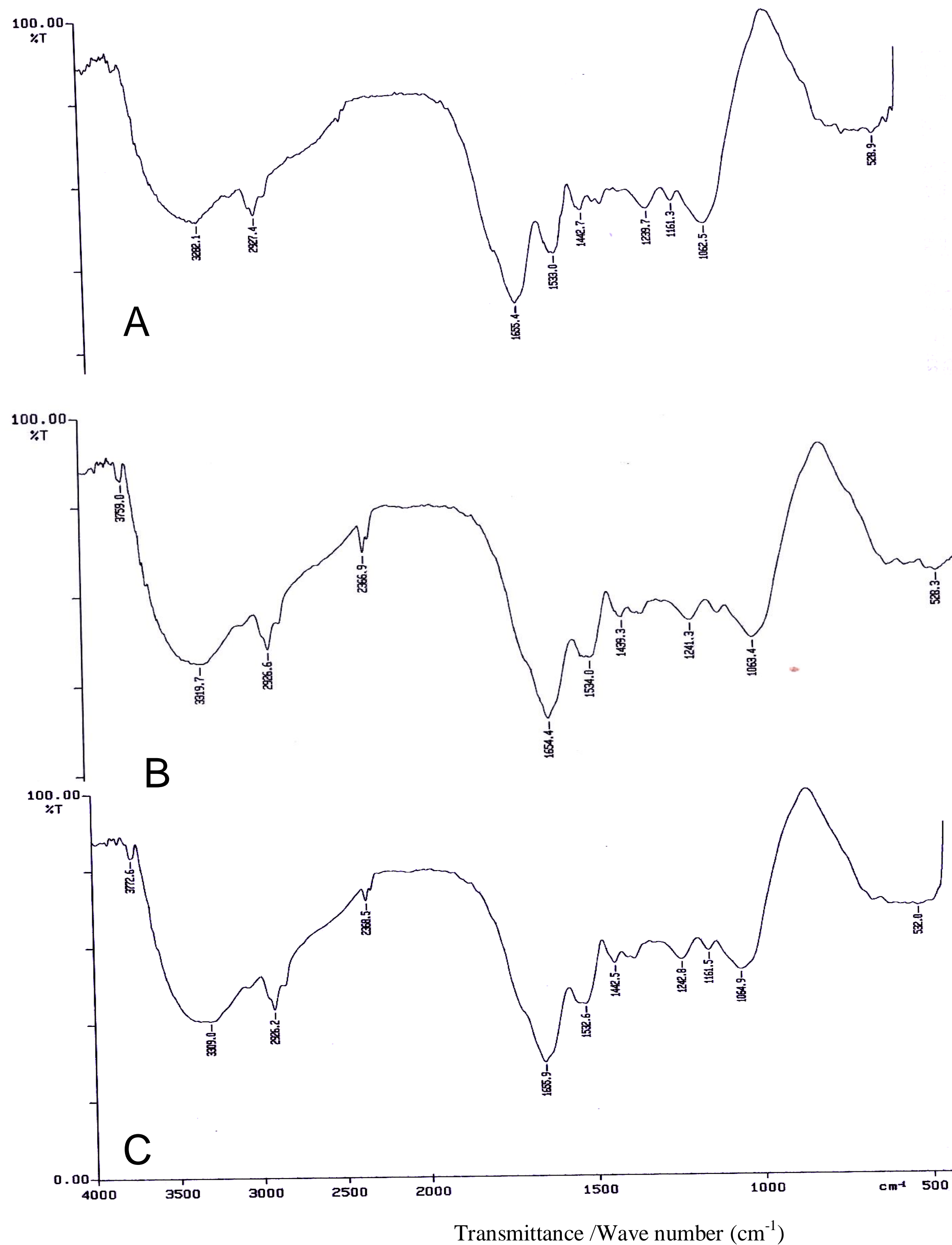



Fig. 67: FTIR spectra of microbial waste biomass *Streptomyces* sp. (MB2) treated with $H_2SO_4$ {A: Native biomass; B: Cr(III) treated biomass; C: tannery effluent treated biomass}.

### 4.3.7 X-ray diffraction (XRD) analysis

X-ray diffraction spectra of native, Cr(III) and tannery effluent treated biomass were carried out. X-Ray diffraction of both biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) before and after saturation with Cr(III) was carried out to identify the phase present on adsorbent (Table 36; Fig. 68, 69). The X-ray diffraction analysis of *Aspergillus* sp. (MB1) biomass showed peaks that indicated its crystalline structure while *Streptomyces* sp. (MB2) did not showed any peak it is showing amorphous nature of biomass. After Cr(III) adsorption by both microbial biomass the surface structures were changed to amorphous nature. The XRD analysis of *Aspergillus* sp. (MB1) biomass did not show any peak after tannery effluent treatment indicating crystaline nature is changed into amorphous nature. Amorphous and crystalline both phase in the XRD spectra, however peaks are shifted to lower 2θ values which clearly indicates that the crystalline change into the amorphous phase after tannery effluent treated biomass. The XRD data of treated adsorbent provided evidence of decreased peak intensity due to adsorption of Cr(III) on the surface of the adsorbent (Fig. 68, 69 ).

Table 36: X-ray diffraction (XRD) of microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2)

| Microbial biomass | Treatment | Nature of microbial biomass |
|---|---|---|
| *Aspergillus* sp. (MB1) | Native | Crystalline |
| | Native biomass absorbed with Cr(III) | Crystalline |
| | Native biomass absorbed with tannery effluent | Amorphous |
| *Streptomyces* sp. (MB2) | Native | Amorphous |
| | Native biomass absorbed with Cr(III) | Amorphous |
| | Native biomass absorbed with tannery effluent | Amorphous |

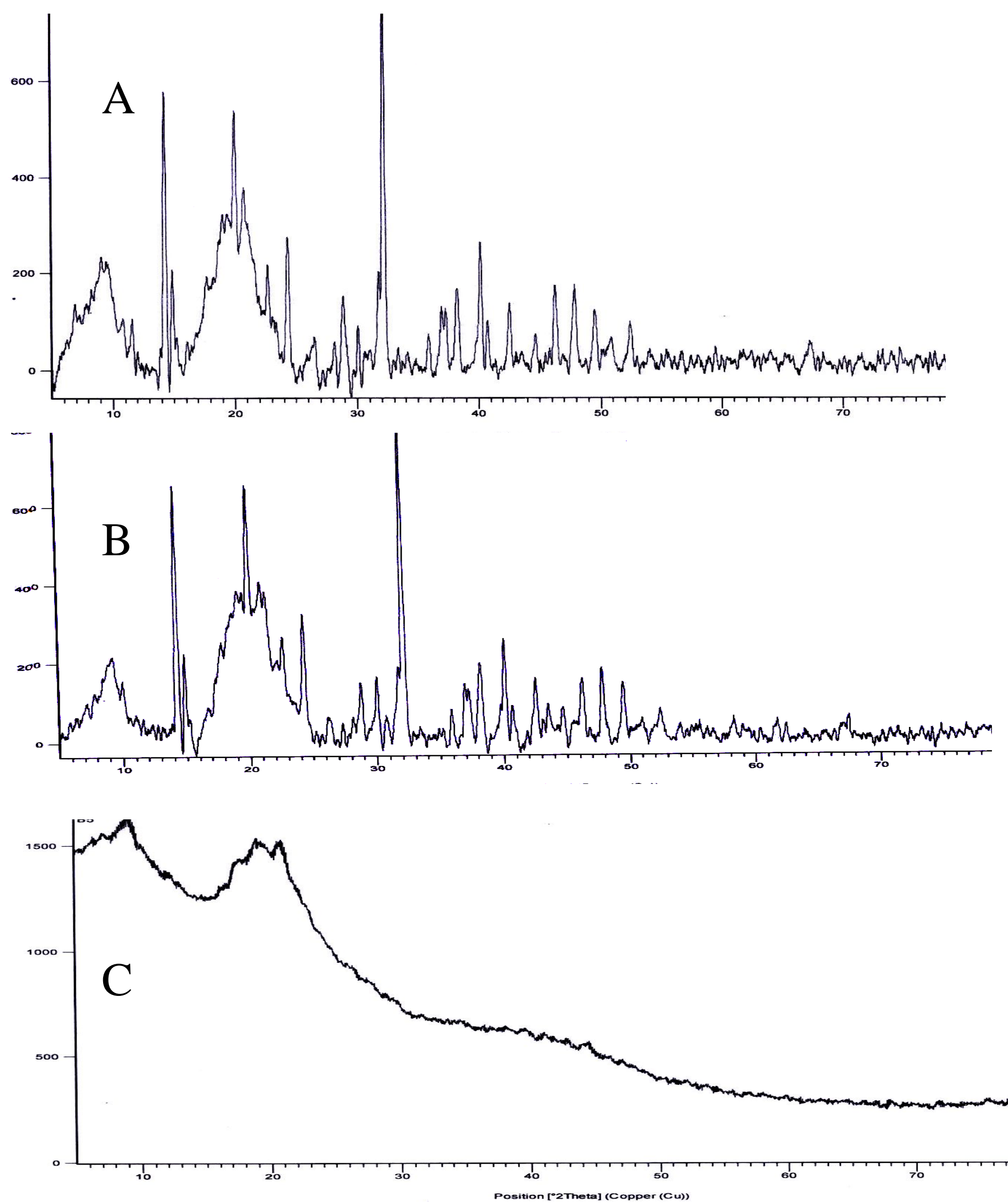



Fig. 68: X- Ray diffraction of microbial biomass *Aspergillus* sp. (MB1) {A: Native biomass; B: Cr(III) treated biomass; C: tannery effluent treated biomass}.

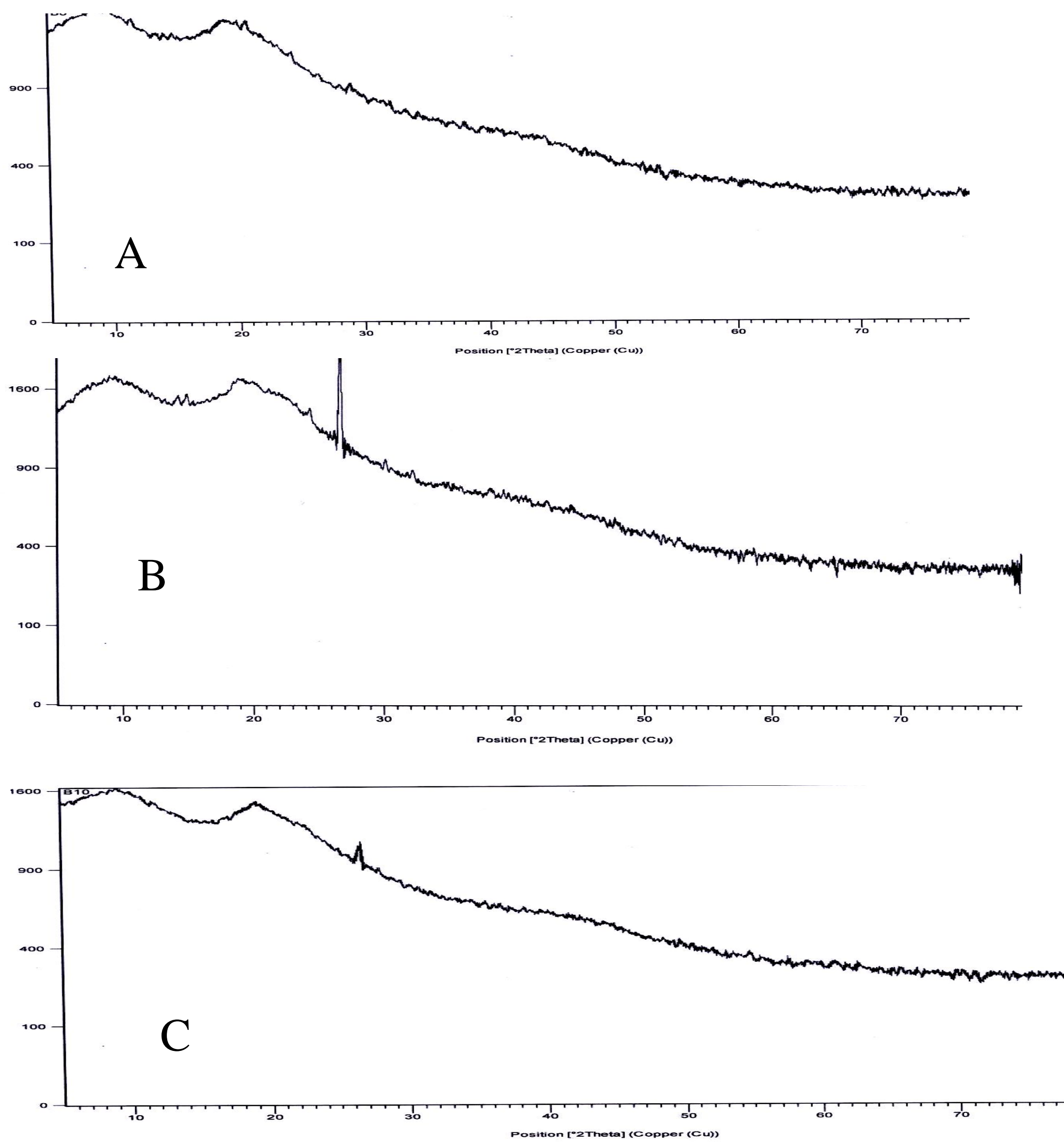



Fig. 69: X- Ray diffraction of microbial biomass *Streptomyces* sp. (MB2) {A: Native biomass; B: Cr(III) treated biomass; C: tannery effluent treated biomass}.

## 4.4 Process optimization in continuous flow biosorbent column

Microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were used as an adsorbent for chromium removal from aqueous solution as well as tannery and chrome effluent in continuous flow sorption system (Plate 1-3). Chromium sorption capacity of *Aspergillus* sp. (MB1) was found to be 96% where as of *Streptomyces* sp. (MB2) it was 82%. Break through curves concluded that microbial biomass could be used for Cr(III) removal from aqueous solution and tannery effluent. Microbial biomass could be desorbed with dilute HCl and regenerated for its subsequent reuse as metal sorbent and there was no change in chromium removal efficiency up to 5 sorption cycles. The data significantly implies that microbial biomass is more effective for removal of chromium in successive cycles which is applicable for treatment of tannery effluent at large scale.

### 4.4.1 Removal of Cr(III) from aqueous solution in down flow continuous sorption column packed with microbial biomass

Syringe column of 1cm diameter and 5 cm length was packed with 3 g microbial biomass to study the removal of Cr(III) in continuous flow sorption column mode from aqueous solution at pH4. The flow of the aqueous of Cr(III) through the column was maintained at 10 to 15 mL/h. Adsorption capacity of Cr(III) by *Aspergillus* sp. (MB1) biomass is a function of contact time (Fig. 70). Removal rate of Cr(III) was rapid during the first hour of influent flow. After a rapid sorption, Cr(III) uptake increased slowly with time and reached equilibrium in 20 h in *Aspergillus* sp. (MB1) whereas *Streptomyces* sp. (MB2) showed more efficiency which increased with time (Fig. 70; Plate 2). Maximum removal of Cr(III) was in 6.08 and 0.12 mg/L by MB1 and MB2 respectively. 16 h Cr(III) removal in was in the order of 15.54, 7.15 mg/L by MB1 and MB2 respectively from influent concentration of 24.34 mg/L aqueous solution 40 h (Fig. 70). During first hour Cr(III) adsorption by *Aspergillus* sp. (MB1) ($C_f$ 4.86 mg/L) was less than *Streptomyces* sp. (MB2) Since the final concentration of Cr(III) in effluent (outlet) was 4.86 mg/L and negligible by MB1 and MB2 respectively ($C_f$ 0.00 mg/L). Cr(III) removal in 12 h was in the order of *Aspergillus* sp. (MB1) ($C_f$ 6.77 mg/L) *Streptomyces* sp. (MB2) ($C_f$ 0.11 mg/L) from influent concentration of 24.34 mg/L aqueous solution. Similarly, *Streptomyces* sp. (MB2) biomass (Fig. 70; Plate 2) showed promising removal of Cr(III) from aqueous solution up to 7 h from an influent concentration of 24.34 mg/L. Maximum Cr(III) removal after 4-6 h was by *Aspergillus* sp. (MB1) ($C_f$ 6.25mg/L) followed by *Streptomyces* sp. (MB2) ($C_f$ 8.00 mg/L.).

### 4.4.2 Removal of Cr(III) from tannery effluent in up flow continuous sorption column packed with microbial biomass

Glass column of 20 cm length and 2.5 cm of diameter was packed with 16 g microbial biomass. The diluted tannery effluent of containing 25 mg/L of chromium (pH 4.0) was pumped continuously downward from the top using peristaltic pump (Cole Parmer USA) at a constant flow rate of 30 to 45 mL/h. Removal of Cr(III) in column packed with *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) microbial biomass, 15 liters aqueous solution of 25 mg/L Cr(III) was processed before the column breakthrough point, which was arbitrarily established at 1.0 mg/L of Cr(III) in the column outlet (Fig. 71). In this column, residence time was 15 minutes and 124 mg of Cr(III) was adsorbed on 16g of *Aspergillus* sp. (MB1) biomass, which gave an overall Cr(III) sorption efficiency of 4.54 mg Cr(III)/g dry biosorbent including the only partially saturated portion of the dynamic sorption zone still inside the column breakthrough (outlet 1.0 mg Cr(III)/L. In column packed with *Streptomyces* sp. (MB2), 177 mg/L Cr(III) aqueous solution was processed before the column breakthrough point, which was arbitrarily established at 1.0 mg/L of Cr(III) in the column outlet (Fig. 71). In this case column residence time was approximately 30 min and total amount of 105 mg of Cr(III) was adsorbed on the 440g of dried biomass *Streptomyces* sp. (MB2). That gives the column overall Cr(III) biosorption capacity of 238 mgCr(III)/g including the only partial saturated portion of dynamic sorption zone still inside at the column breakthrough (outlet 1.0 mg Cr(III)/L(Fig. 72; Plate 3).

### 4.4.3 Regeneration and reuse of sorption column

Microbial saturated with chromium was regenerated with mineral acid and agan used for the next sorption cycle. There was no significant change in the removal activity of chromium up to five cycles, which indicates that both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) microbial biomass are effective for removal of Cr(III) from aqueous sosution and tannery effluent.

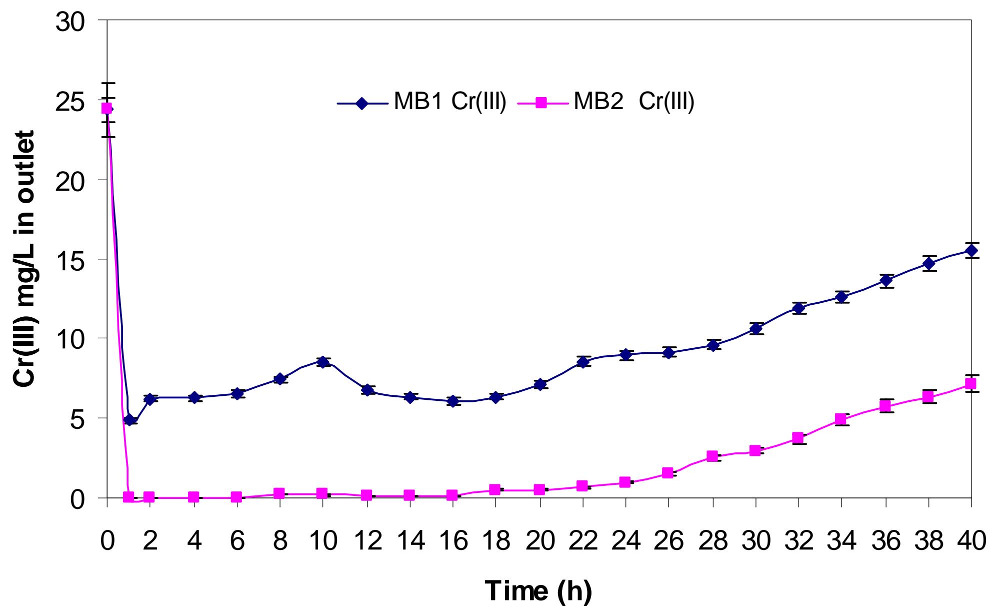



Fig. 70: Removal of Cr(III) in sorption column packed with microbial biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) (Influent concentration: 25 mg/L, Temp: 28±2°C, pH-4.0, flow rate: 10 to 15 mL/h, Dry biomass: 3g).

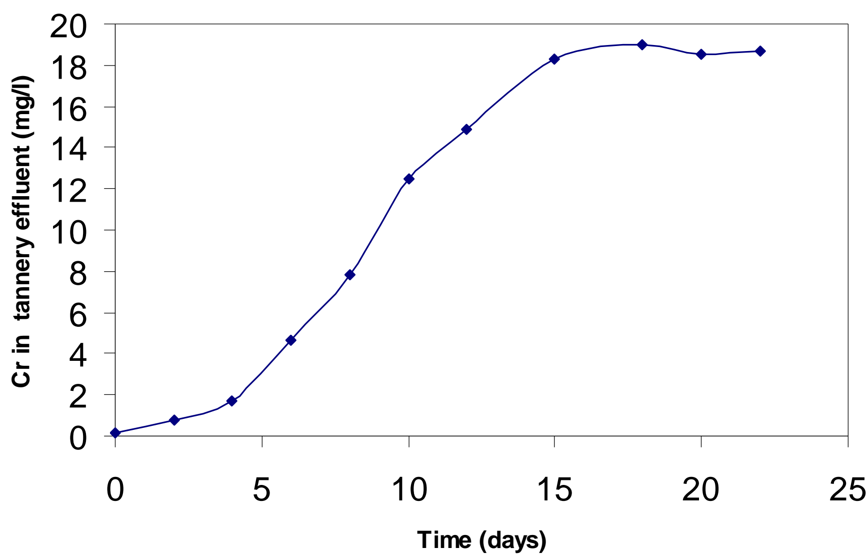



Fig. 71: Removal of Cr(III) from tannery effluent in sorption column packed with *Aspergillus* sp. (MB1) microbial biomass (Influent concentration: 25 mg/L, Temp: 28±2°C, pH: 4.0, flow rate: 30 to 45 ml/ h, Dry biomass: 16 g)

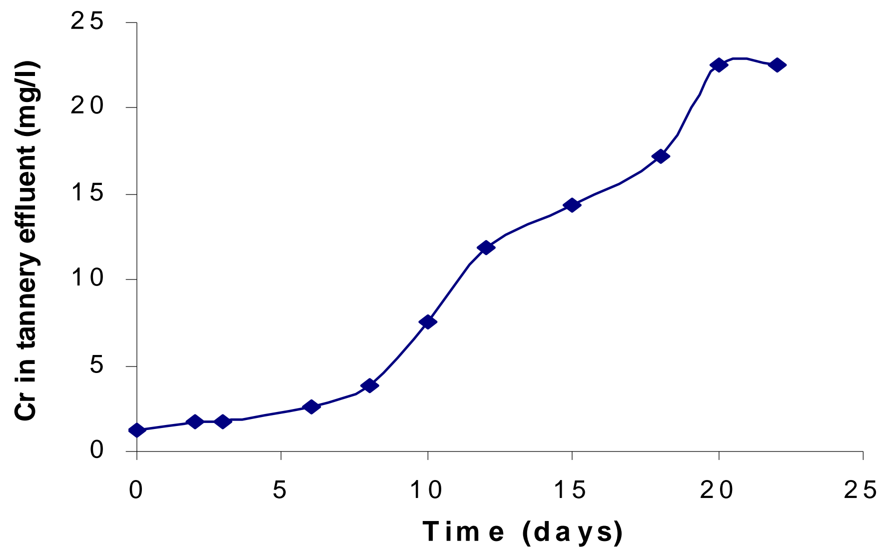



Fig. 72. Removal of chromium from tannery effluent in sorption column packed with *Streptomyces* sp. (MB2) microbial biomass (Influent concentration: 25 mg/L, Temp: 28±2°C, pH: 4.0, flow rate: 30 to 45 mL/ h, Dry biomass: 16 g)

Plate 1: Microbial waste biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2).

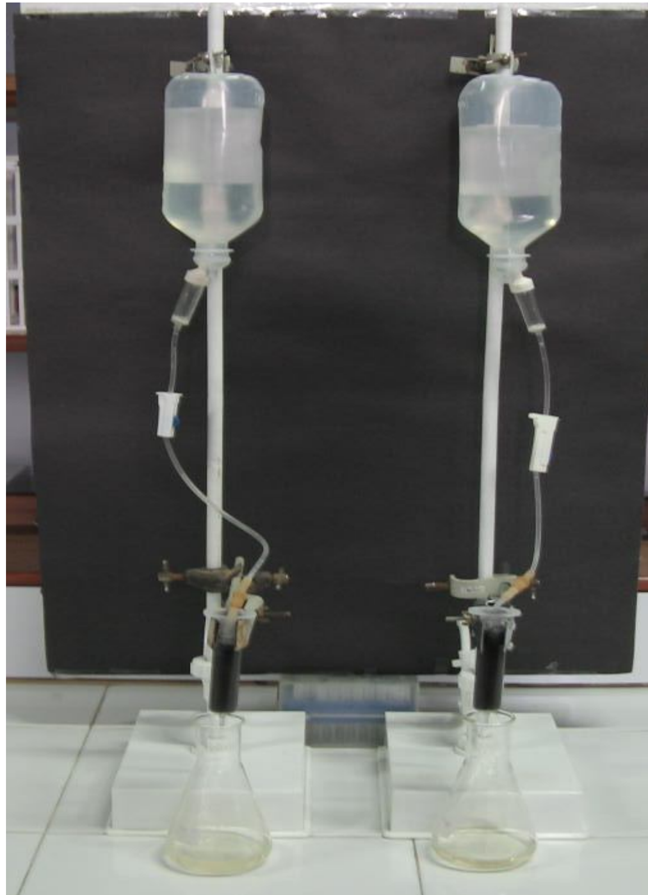


Plate 2: Down flow continuous sorption column packed with microbial biomass for the removal of Cr(III).

Plate 3: Up flow continuous sorption column packed with microbial biomass for removal of Cr(III) from tannery effluent

# Chapter 5
# Discussion

Chromium contamination in wastewater originates from various industries such as chrome plating, petroleum refining, wood preserving, textile manufacturing and leather tanning. Difficulties associated with chemical and physical techniques to remediate a chromium contaminated site along with high cost, asserts the need for alternate bioremedial measures. Studies presented in this thesis provide significant implications for bioremediation of chromium containing effluent. In the present study, bacterial consortium and bacteria isolated from tannery effluent and chrome sludge were studied for biotransformation and removal of chromium from aqueous solution. To scale up the process of biosorption, it is not feasible to cultivate microorganisms on large scale therefore, microbial biomass which are generated as a byproduct of pharmaceutical fermentation industry involving fermentative production of certain antibiotics by *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy fermentation industry and used as an adsorbent for the removal of trivalent chromium from aqueous solution as well as tannery effluent in batch and continuous flow sorption mode. Isotherms and kinetics were studied at varying pH, adsorbent dosage, initial chromium concentrations and contact time.

## 5.1 Characterization of tannery effluent and microbial biomass from different industries

### 5.1.1 Tannery effluent

Tannery effluent was collected from A.V. Tanneries, Kapurthala, Punjab, India, and characterized for various physico-chemical parameters such as pH, temperature, conductivity, salinity, total suspended solids (TSS), total dissolved solids (TDS), colour, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and heavy metals Fe, Cr, Ni, Zn, Pb. Waste generated from tanning generally contains much higher concentration of total dissolved solids (TDS), suspended solids, phenols, chromium, chlorides, ammonia, heavy metals etc (Kowalski, 1994**;** Armienta et al. 2001; Cooman et al., 2003; Esmaeili et al., 2005; Fahim et al., 2006; Verma et al., 2008). Serious concern about the toxicity of chromium compounds necessitates recovery and reuse of chromium from tanneries and other industrial wastes or rendering it to a less toxic form (Yamamoto et al., 1993; Tare et al., 2003). The color of tannery effluent was dark green due to Cr(III) cation.

Chromium forms complex with collagen proteins of hides and transforms it into leather. The tannery effluent was very acidic, since stabilization of chromium on the hide is carried out in acidic environment. The high amount of nitrogen in the effluent can be attributed to the presence of various compounds (Sumathi et al., 2005; Fahim et al., 2006).The chromium pollution is not only limited to water but sediments and soils are also greatly contaminated. The high levels of chromium found in water and sediment samples are worrying because this metal is known to be toxic (Armienta et al., 1995; Jordão et al., 1996; Jawahar et al., 1998; Chaudhry et al., 1998; Esmaeili et al., 2005).

The tannery industries produce about 20 tons of leather every day. During the manufacturing process of leather, about 1,200 kg chromium is used out of which only 60% of chromium is effectively used and remainder 40% is rejected into the waste effluents which are forwarded to effluent treatment plant (Mohan and Pittman Jr, 2006). Concentration of total chromium in the waste effluents of the tanneries in Ganga river at Kanpur (north of India) was over 150 mg/L (Khwaja et al., 2001). The contamination of food chain by chromium of waste water effluents from the tannery is undeniable because this metal is easily absorbed by roots and leaves of plants and then transported via the vascular system (Khan, 2001; Srinath et al., 2002).

**5.1.2 Microbial biomass**

Microbial waste biomass *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were collected from Ranbaxy (fermentation industry) Paonta Sahib, Himachal Pradesh, India, which had acidic pH with ash content ranging from 5-6%. The CHN analysis showed that *Streptomyces* sp. (MB2) had high nitrogen content than *Aspergillus* sp. (MB1) (Fahim et al., 2006; Sarin and Pant 2006). Both exhibited calorific value of nearly 16-17 MJ/kg, which coincides with the relative calorific value (17.5 MJ/kg) of solid fuels such as biomass, municipal waste, industrial waste, peat and brown coal (MacDonald et al., 1996). Biosorption appears to be an economically feasible means for the removal and recovery of heavy metals from industrial wastewaters (Volesky, 1995; Boddu et al., 2003).

The low cost of biosorbents is a tangible advantage over other technologies, such as ion exchange and reverse osmosis. Extensive efforts have been made to explore new types of biosorbent materials capable of effectively sequestering heavy metals (Bailey et al., 1999; Jalali et al., 2002; Ozer and Ozer, 2003; Ahluwalia and Goyal, 2007). The microbial biomass originating as byproducts from industrial bioprocesses have been tested for biosorption of heavy metal ions such as bacterial biomass *Streptomyces pimprina* (Puranik et al., 1995), *Streptomyces rimosus* (Addour et al., 1999; Chergui et al., 2007), *Streptomyces griseus* (Ashwini et al., 2009), *Aspergillus niger* (Chandrasekhar

et al., 1998), waste biomass from Brazilian alcoholic beverage (Dias et al., 2000), *Bacillus lentus*, *Aspergillus oryzae* or *Saccharomyces cerevisiae* (Vianna et al., 2000), *Rhizopus nigricans* (Kogej and Pavko, 2001), *Aspergillus niger* (Srivastava and Thakur 2006; Khambhaty et al., 2009), wine processing waste sludge (Li et al., 2004), *Corynebacterium glutamicum* (Choi and Yun, 2004), olive oil industry waste (Malkoc et al., 2006) and olive stone (Blázquez et al., 2009). The use of waste biomass from fermentation industry as a biosorbent would increase the economic competitiveness of a microbial based technology because such biomass is cheap, easily recovered at the end of fermentation and is produced in large quantities (Vianna et al., 2000).

## 5.2 Isolation and characterization of chromium reducing microbes from tannery effluent

Hexavalent chromium is a toxic heavy metal whose concentration in potable water and industrial aqueous discharges is regulated in many countries. The development of methods of Cr(VI) removal from water is currently an area of intense research. Biological detoxification of pollutants is an attractive technology that is cost-effective and ecofriendly. This study presented transformation of Cr(VI) to Cr(III) by bacterial consortium and isolates from tannery effluent and chrome sludge. Microbial reduction of hexavalent chromium has also been reported both aerobically and anaerobically (Camargo et al., 2003).

### 5.2.1 Isolation of bacterial consortium and isolates

Chromium reducing bacterial consortium (CT and CS) and isolates (CT4, CT5, CS7 and CS8) from tannery effluent and chrome sludge were screened for transformation of Cr(VI). Out of 36 Cr(VI) reducing bacterial isolates, 4 isolates were screened on the basis of their Cr(VI) removing efficiency and characterized on the basis of morphology, biochemical test and molecular approaches. Different isolates were able to reduce Cr(VI), but at different rates. They were able to reduce 70-90% of Cr(VI) aerobically at room temperature (28±2°C) within 24 h. Gram negative bacterial strain (probably a *Citrobacter* sp., *Bacillus* sp.) was isolated from tannery effluent that grew in media containing potassium dichromate up to 80 mg/L. Most of the Cr(VI) resistant microorganisms have been found to exhibit tolerance from 10 to 1500 mg/L of Cr(VI) (Losi and Frankenberger, 1994; Basu et al., 1997; Shakoori et al., 2000; McLean and Beveridge, 2001).

Many bacteria isolated from tannery effluent such as *Pseudomonas aeruginosa* (Ganguli and Tripathi 1999), *Microbacterium* (Pattanapipitpaisal et al., 2001), *Desulfovibrio* (Mabbett et al., 2002), *Bacillus* (Camargo et al., 2003), *Shewanella* (Middlton et al., 2003), *Acinetobacter* sp. (Shrivastava and Thakur 2003; 2007) and *Streptomyces griseus* (Ashwini et al., 2009) were reported to reduce Cr(VI).

### 5.2.2 Morphological and biochemical characterization of bacterial isolates

Natural habitats are generally characterized by the coexistence of a large number of toxic and nontoxic cations and presence of metal affects morphology, physiology and biochemistry of microorganisms (Verma and Singh, 1995; Lovely, 1995). All the four bacterial isolates in this study were quite efficient in transformation of Cr(VI). Futhermore, when there are other metal contaminants, it might be practical to use Cr(VI) reducing microorganisms to reduce other metal simultaneously (Lovely 1995). Recent interest in bacterial Cr(VI) reduction has been evoked by the potential use of this process with bioremediation purpose. Due to notable properties of these isolates to both tolerate and reduce Cr(VI), they represents ideal candidates to detoxify chromate from chromium-containing industrial discharges and these isolates can be exploited in Cr(VI) detoxification opration. Since then, significant progress has been made towards understanding the reduction of Cr(VI) by Gram-negative bacteria, especially those belonging to the genera *Pseudomonas, Desulfovibrio* and *Shewanella* (Chardin et al., 2003; Ackerley et al., 2004). Several Gram-positive bacteria are also known to reduce Cr(VI) including several members of the genus *Bacillus* (Campos et al., 1995; Camargo et al., 2003; Rehman et al., 2008).

### 5.2.3 Molecular identification based on 16S rDNA sequences

Chromium (VI) reducing bacteria capable of reducing Cr(VI) to Cr(III) were phylogenetically diverse. On the basis of 16S rDNA sequence analysis four isolates were identified as CT4 (*Raoultella* sp. IS1), CT5 (*Citrobacter* sp. IS2), CS7 (*Bacillus cereus* IS3) and CS8 (*Citrobacter freundii* IS4). Identification of *Arthobacter oxydans* to the species level in the previous study was performed 16S rDNA sequencing (Camargo et al., 2003). Similarly *Serratia mercascens, Pseudomonas fluorescence, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter* sp. and *Bacillus* sp. ev3 identified by 16S rDNA method (Srivastava and Thakur, 2007; Rehman et al.,

2008). Most of the chromium reducing bacteria are gram-negative and facultative anaerobes (Chardin et al. 2003; Ackerley et al. 2004). Reduction of Cr(VI) by *Bacillus* sp. and *Citrobacter* sp. has been documented previously, but no reports of Cr(VI) reduction by *Raoultella* sp. have been published so far. It still remains to be seen if the capability to reduce Cr(VI) evolved independently and specifically in some organisms or if there are some organisms that can support their growth on the reduction of Cr(VI) (Srivastava and Thakur, 2007; Rehman et al., 2008).

### 5.2.4 Growth of bacteria in presence of Cr(VI)

The present study demonstrated that indigenous microbial population present in Cr(VI) contaminated effluent and sludge are able to aerobically catalyze the removal of toxic and soluble Cr(VI) from the media, most likely by reducing it to the relatively nontoxic and insoluble Cr(III). The enriched culture and isolate consistently reduced Cr(VI) in M2 medium up to concentration of 20 mg/L. Higher concentrations inhibited reduction, due to slow growth. Both the consortium and isolates showed significant tolerance of Cr(VI) up to concentration 50 mg/L as well as measurable reduction over short period of time at concentration up to 25 mg/L. The log phase of both the consortia (CT and CS) was drastically reduced from approximately 16 h to 9 h in the presence of Cr(VI). Retarded growth was observed in the presence of Cr(VI) at concentration range of 3.6 to 14.18 mg/L. On addition of Cr(VI) sharp decrese in growth was observed after 9 h of incubation in CS consortium. This decrese was followed by slight increase in growth soon after which bacteria entered into stationary phase. Microbial reduction of soluble Cr(VI) to its insoluble Cr(III) form is a cost-effective way to prevent the mobility of Cr(VI) beyond the compliance boundaries and to eliminate the risk of health hazards to humans. Microbial reduction of Cr(VI) is controlled by many factors, including cell density, initial concentration of Cr(VI), pH and redox potential (Marsh et al., 2000; 2001)

These consortia represent adaptive capabilities of microbial populations to a changing environment, and the development of strategies, not on the part of single species, but whole communities to take advantage of a potential nutrient source or changing environmental conditions. Sorption of metals by bacteria is also sometimes a product of consortial activity. For instance, the sorption of Cr(VI) by a consortium of denitrifying bacteria. This result indicated that the removal of the chromate ion from solution may be associated with microbial metabolic processes. A number of studies suggest both growth dependent and growth independent chromium reduction (Michel et al., 2001; Chen and Cutright, 2003).

In either case, chromium reduction does seem to be biomass dependent (Rege et al., 1997; Phillip et al., 1999), which was also observed in our studies. The lag at the beginning in the reduction experiments by consortium as well as observations of increased turbidity throughout the experiments suggested that adequate cell biomass must be produced before reduction begins. It was reported earlier also that the highest rate of reduction of Cr(VI) to Cr(III) occurs during log phase of growth (Turick et al., 1996). Bopp and Ehrlich, (1998) showed that higher concentrations (1000 mg/L) of Cr(VI) produced a much longer lag phase and a significantly lower final yield of biomass than at lower concentrations. The reduced biomass would also contribute to the lack of complete reduction found at higher concentrations in many studies (Fujii et al., 1990) as well at the higher concentrations tested. Both the consortium and isolates were shown to grow and reduce Cr(VI) at 28±2°C. Similar results using chromium reducing microorganisms such as *Bacillus*, *Pseudomonas* and *Escherichia* (Shen and Wang, 1994; Wang and Shen, 1995) have been reported. The precipitation of Cr(III) might have occurred since a gradual decrease in concentration of Cr(III) was observed after 8 h of incubation as reported previously by Marisa et al., 2003. Complete reduction was observed in all experiments by both bacterial consortium and isolates at a concentration of 5-25 mg/L Cr(VI) suggesting complete reduction. Variation in the pH affected Cr(VI) reduction with highest rate at pH 7 since chromate ($CrO_4^{2-}$) is the dominant Cr(VI) species in an aqueous environment at pH 6.5 to 9.0 (Losi et al., 1994a; McLean and Beveridge, 2001). Wang et al., (1990) reported that Cr(VI) reduction by *Enterobacter cloacae* occurred at pH 6.5 to 8.5 and was strongly inhibited at pH 5.0 and 9.0. However, Losi et al., (1994b) did not observe any significant effect of pH on the rate of chromate reduction in soil amended with different manure. Cr(VI) reduction is enzyme-mediated, therefore changes in pH affects the degree of ionization of the enzyme and protein's conformation. Growth and Cr(VI) reduction by the all bacterial isolates was influenced by temperature with an optimum temperature of 28±2°C. Wang et al., (1990) reported that there was no chromate reduction at 4 and 60°C. The factors that affected Cr(VI) reduction studied here (pH, temperature, Cr concentration) are important environmental factors regulating remediation strategies for ecosystems polluted with natural or anthropogenic Cr(VI). The isolates described in this work show promise for Cr(VI) reduction and their characteristics will be useful in Cr(VI) bioremediation, which is potentially more cost-effective than traditional physical or chemical methods in the treatment of environment contaminated with Cr(VI).

### 5.2.5 Biotransformation of Cr(VI) to Cr(III)

The chromium removal and its transformation by bacterial isolates differed at varying concentrations of Cr(VI) in minimal media (M2). With increase in concentration of Cr(VI) there was decrease in reduction, which may be due to toxicity of Cr(VI) to the growing cells. Transformation of Cr(VI) to Cr(III) showed that there was gradual increase in the concentration of Cr(III) and decrease in Cr(VI) concentration and the rate of increase in Cr(III) concentration coincides exactly with rate of decrease in Cr(VI) concentration. However, the rate of transformation increased to 0.5 mg/h and 100% transformation was observed after 22 h of incubation. Biologically mediated reduction of Cr(VI) to Cr(III) is a cost effective approach in the bioremediation of Cr(VI). A number of studies have demonstrated Cr(VI) detoxification using whole cells (Cervantes et al., 2001). Ganguli and Tripathi (1999) observed similar reduction of Cr(VI) to (III) by *Pseudomonas aeruginosa* isolated from tannery effluent. Similar results were reported by Faisal and Hasain, (2004) wherein *Brevibacterium* reduced 40% of Cr(VI) aerobically. Reduction of Cr(VI) to Cr(III) was observed to be growth associated and to avoid toxic effect of Cr(VI) it is necessary to convert Cr(VI) to Cr(III). Two bacterial consortia were developed by continuous enrichment of microbial population of tannery and pulp and paper mill effluent contained *Serratia mercascens*, *Pseudomonas fluorescence, Escherichia coli, Pseudomonas aeruginosa* and *Acinetobacter* sp. The consortia evaluated for removal Cr(VI) in shake flask culture indicated pulp and paper mill consortium had more potential for removal of chromate (Srivastava and Thakur, 2007).

Complete reduction of Cr(VI) to Cr(III) was observed by *Raoultella* sp. (IS1) after 22 h of incubation whereas *Bacillus cereus* (IS3), *Citrobacter freundii* (IS4) and *Citrobacter* sp. (IS2) showed 94.81 %, 95.81 % and 95.17 % reduction after 24 h of growth respectively. It was reported earlier also that the highest rate of reduction of Cr(VI) to Cr(III) occurs during log phase of growth (Turick et al., 1996). Ganguli and Tripathi, (1999) observed similar reduction of Cr(VI) to Cr(III) by *Pseudomonas aeruginosa* isolated from tannery effluent. A similar trend of growth has been reported for *Arthrobacter oxydans*, which showed tolerance up to 50 mg/L chromate (Megharaj et al., 2004). During growth, 100% Cr(VI) reduction was observed in the medium, but enzymatic reduction showed only 50% Cr(VI) reduction for the corresponding chromate concentration.

In both the cases high concentration of Cr(VI) resulted in decreased efficiency of Cr(VI) reduction. The decrease in Cr(VI) reduction by cells was not due to bio-accumulation of chromium since the chromium was pumped out (Elangovan et al., 2006). Further comparison of treatments and control indicates that the decreased aqueous metal concentrations could be due to: (1) accumulation of metal ions by bacteria cells, (2) complexation of metal ions with nutrient compounds, and (3) pH alteration. Depending upon the initial concentration of Cr(VI), it's complete or incomplete reduction has been observed in *Enterobacter cloacae* HO1, (Komori et al., 1990a; Fujie et al., 1990). Even though a decrease in cell viability was observed in the culture on addition of Cr(VI) to the growing culture (Komori et al., 1998; Wang et al., 1989), the initial rate of Cr(VI) reduction increased with the increase in the initial rate of Cr(VI) in some cultures of *Enterobacter cloacae* (Wang et al., 1989), *E. coli* (Shen and Yi-Tin, 1994) *P. flourescens* (Wang and Xiao, 1995) and *Bacillus* sp. (Wang and Xiao, 1995). Similarly, initial specific rate of Cr(VI) reduction by cultures of *E coli* increased with increasing Cr(VI) concentrations. However, an increase in time required for complete reduction was also observed (Shen and Wang, 1994).

Cr(VI) reduction by bacterial consortia and isolates were dependent on pH (7.0), temperature (28°C) and chromium concentration (5-25 mg/L). Optimum temperature and pH conditions reported for microbial Cr(VI) reduction strongly suggest that the reduction process is related to growth. Cr(VI) reduction was observed in cultures of *Enterobacter cloacae* at pH range of 6.0-8.5, and at pH range of 3.0-8.0 in cultures of *Escherichia coli* and *Bacillus coagulans*. However, the maximum initial specific rate of Cr(VI) reduction by all three bacteria was at pH 7.0, an optimal pH for most bacterial growth. Even though Cr(VI) reduction by *E. coli* and *Enterobacter cloacae* occurred at a wide range of temperature of 10°C to 50°C, optimum temperature was found to be 30°C and 36°C respectively. Studies with sediments have shown temperature optima of 22°C and 50°C and a pH optimum of 6.8 (Marsh et al., 2000).

**5.2.6 Transformation of Cr(VI) by resting cells and cell free extract**

**Removal of Cr(VI) by resting cells**

Reduction of Cr(VI) by resting cells of consortium from tannery effluent (CT) was 30% more than consortium from chrome sludge (CS). The percentage transformation by bacterial consortium (CT) after 24 h in saline, phosphate buffer and deionised water was 69.2%, 44.4%, 19.6% and by bacterial consortium (CS) it was 41%, 40%, 23.2% respectively.

Among the three suspension media saline was best suited for Cr(VI) transformation. A similar observation was also reported by Camargo et al., (2003) where $Na^{+}$ ions stimulates chromium reductase activity.

In addition to transformation of Cr(VI) there was decrease in the total chromium concentration after 8 h of incubation which could be due to the phenomenon of contributed to phenomenon of bioaccumulation by the bacterial cells. Earlier report indicates that the amount of chromium accumulated by the cells increases with increase in concentration of chromium (Faisal and Hasnain, 2004). Many bacteria, including *Pseudomonas* sp. (Megharaj et al., 2003), *E. coli* (Ishibashi et al., 1990) and *Enterobacter* (Wang et al., 1989) can reduce Cr(VI) to the less toxic Cr(III), which readily precipitates as $Cr(OH)_3$. Cr(VI) reduction and resistance are considered to be independent processes. In the case of *P. fluorescens*, a Cr-resistant strain and a sensitive derivative reduced Cr(VI) equally (Bopp and Ehrlich, 1988). However, since Cr(VI) reduction is enzyme-mediated, change in pH will affect the degree of ionization of the enzyme, changing the protien's conformation and affecting the enzyme activity (Camargo et al., 2003).

**Removal of Cr(VI) by cell free extract**

Under aerobic conditions cell free extract of bacterial consortium (CS and CT) showed reduction of Cr(VI) with no change in concentration of total chromium in the solution which suggest that transformation of Cr(VI) takes place by extracellular enzymes, however no change in the concentration of total chromium suggested that it is a phenomenon involving only either live or dead cells. Bacterial consortium (CT) demonstrated a much higher chromium reductase activity after 6 h of incubation it reduced approximately 58% of Cr(VI) transformation by cell free extracts of the bacterial consortia as compared to resting cells confirms the presence of extracellular chromium reductase activity (Camargo et al., 2003). Though microbes of genera *Pseudomonas, Arthrobacter, Escherichia* and *Bacillus* have been reported to reduce Cr(VI) through soluble chromate reductase, only a few of these enzymes have been characterized (Park et al., 2000; Camargo et al., 2003; Megharaj et al., 2003; Bae et al., 2005). Cell free crude extract from the *Bacillus* sp.ev3 reduced Cr(VI) to Cr(III) (Rehman et al., 2008).

Bioreduction of Cr(VI) can occur directly as a result of microbial metabolism (enzymatic), or indirectly through a bacterial metabolite such as $H_2S$ (Losi et al., 1994a). Two enzymatic mechanisms are reported to reduce Cr(VI), both through metabolic activities of the chromium reducing bacteria. In aerobic conditions, most of the chromate reductase reported is soluble in the cytosol and reduce Cr(VI) to Cr(III) inside or outside the plasma membrane (Campos et al., 1995; Cervantes et al., 2001). It was reported earlier also that the highest rate of reduction of Cr(VI) to Cr(III) occurs during log phase of growth (Turick et al., 1996).

Further experimentation with cell free extract, resting cell assay showed Cr(VI) reduction activity was due to the extracellular enzyme secreted by cells in the soluble protein fraction, not the membrane bound protein (Megharaj et al., 2003). Numerous studies suggest that NADH dependant-reductase enzyme was invariably involved in reduction of Cr(VI) to Cr(III) in *E. coli* and *Pseudomonas* sp. the finding that many other bacteria can reduce Cr(VI) without any enzyme indicates that there is more than one pathway for the reduction of Cr(VI) (Camargo et al., 2003).

## 5.3 Development of a biosorbent from microbial biomass for removal of chromium from tannery effluent

Microbial waste biomass MB1 and MB2 comprising of *Aspergilus* sp. and *Streptomyces* sp. generated as a by product of pharmaceutical fermentation industry involving fermentation processes collected from Ranbaxy, Paonta Sahib, Himachal Pradesh, India were developed as biosorbent for Cr(III) removal from aqueous solution and tannery effluent. The process can be made economical by procuring natural bulk biomass or spent biomass from various fermentation industries and the performance of a biosorbent can be improved by various physical and chemical treatments.

### 5.3.1 Development of biosorbent from microbial biomass

Biosorption offers excellent potential for the removal of metals from effluents containing (Mohan and Pittman Jr, 2006; Ahluwalia and Goyal 2007). A multitude of biomass types comprising fungal biomass, bacterial biomass, algae, peat and so on have been studied for metal biosorption. Microbial biomass has emerged as an option for developing economic and ecofriendly waste water treatment processes.

### 5.3.2 Optimization of Cr(III) removal from aqueous solution and tannery effluent in batch mode

Removal of Cr(III) from aqueous solution and tannery effluent by microbial waste biomass was studied in batch mode and parameters such as adsorbent dosage (0.25-2%), pH (2-6), different concentration of chromium (5-50mg/L) and contact time were standardized. Recent comparisons have suggested that such biosorbent could be cheaper to implement than other commercially available ion exchange resins (Lloyd, 2002). Towards the development of a strategy for large scale removal of metal from wastewater, it is necessary to have the availability of biosorbent in large quantities.

**Effect of adsorbent dosage**

Biosorbent dosage has a great influence on biosorption process. Amount of biosorbent added to the solution determines the number of binding sites available for adsorption. For lower values of biomass dosage there was an increase in percentage removal of chromium. This suggests that after a certain dosage of adsorbent, the maximum adsorption sets in and hence the amount of ions bound to the adsorbent and the amount of free ions remains constant even with further addition of the dosage of adsorbent (Nomanbhay and Palanisamy, 2005). 1% biomass was found to be optimum for maximum removal of chromium. It has earlier been reported that higher specific sorption at lower biomass concentrations could be due to an increased metal to biosorbent ratio (Ariff et al., 1999; Puranik and Paknikar, 1999). The lower uptake at higher biomass concentration may be attributed to the electrostatic interactions of the functional groups at the cell surface, causing cells to agglomerate and lowering the surface area in contact with the solution and resulting in decrease in the number of available binding sites (Kapoor and Viraraghavan, 1995). Fourest and Roux (1992) however suggested reduction in metal sorption with increasing biomass concentration is due to an insufficiency of metal ions in solution with respect to available binding sites. The decrease in the sorption capacity was more pronounced at adsorbent dosage 2-4 g/L (Nomanbhay and Palanisamy, 2005; Bishnoi et al., 2007). Maximal Cr(III) uptake of 45-60 mg/g was found for the oxidized activated carbon samples (Ramos et al., 1995). The data obtained for the sorption capacities are in agreement with those referred in the literature for the most efficient heavy metals adsorbents (Dias et al., 2000; Mohan and Pittman Jr, 2006; Fahim et al., 2006).

**Effect of pH**

pH of aqueous solution is a critical parameter as it strongly affects surface charge of the adsorbent, the degree of ionization and the speciation of adsorbate species. 97.81% of Cr(III) removal was observed by *Aspergillus* sp. (MB1) and 72.38% by *Streptomyces* sp. (MB2) biomass at pH 4.0. When pH of Cr(III) solution was further increased, the insoluble precipitates are formed. Bishnoi et al., (2007) also observed maximum removal of Cr(III) at pH 5.0 by untreated and treated biosorbents. Due to further increase in pH upto 6.0, the percentage adsorption was decreased; because $OH^-$ ions increased the hindrance of diffusion as well as some of the trivalent cations may react with $OH^-$ ion and precipitated and there by decreased the free metal ions available in the solution (Mohan and Pittman Jr, 2006; Blázquez et al., 2009).

The site dissociation and solution chemistry of the heavy metals such as hydrolysis, complexation by organic or inorganic ligands, redox reactions and precipitation are strongly influenced by pH which in turn strongly influences the speciation and the biosorption availability of the heavy metals (Özer and Özer, 2003). Increase in contact time from 0.08 to 4 h led to an increase in Cr(III) removal from 40-70% by both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2). Maximum 65% of Cr(III) removal was observed within first 2 h, which represents the time at which equilibrium of chromium biosorption is presumed to have been attained. Initially, the immediate solute uptake was achieved within 2-4 h, subsequently the second stage of solute uptake continued for longer time (24 h) (Mohan and Pittman Jr, 2006; Blázquez et al., 2009). The lower adsorption values observed at low pH can be attributed to the competition between protons and Cr(III) for available binding sites of biomass. The adsorption increases at pH range (4-6), since Cr(III) species are cationic and predominant interactions in adsorption process must have been electrostatic. Yun et al., 2001 reported that below pH 2.0 almost all the binding sites were occupied by protons and the metal binding could not be expected. As the pH increased, Cr(III) species began to bind to the functional groups, and the maximum binding of this species occurred at pH 4.3. At lower pH, the $CrOH^{2+}$ binding remained at a level lower than that of Cr(III). However, it gradually increased with the pH, eventually exceeding the level of Cr(III) binding at pH > 4.5. While the concentrations of Cr(III) and $CrOH^{2+}$ in the aqueous phase were identical at pH 3.55, the uptake of the two species were same at pH 4.5. Studies were not performed beyond 6.0 due to possibility of Cr(III) precipitation (Leyva-Ramos et al., 1995; Li et al., 2004). The increase of chromium removal was 2.0-2.6 times (from 20-30% to 50-60%) at pH 2 and of 1.6-2.0 at pH 3.2.

These findings suggested that, in the pH (pH of 2-3.2) range, the adsorbent surface might have different affinities to the different species of chromium existing in the solution, and their affinity towards chromium ions are strongly affected by the solution pH (Fahim et al., 2006). The adsorption at pH 4 was found to be rapid and almost accomplished (Bishnoi et al., 2007; Blázquez et al., 2009). Similar observations were made for biowaste generated as a by-product of large-scale industrial fermentation (Dias et al., 2000; Fahim et al., 2006).

**Effect of Cr(III) concentration**

The chromium removal using microbial waste biomass corresponding to different initial metal concentrations (5-50 mg/L) was done. This was because at high initial concentrations the number of moles of Cr(III) available to the surface area were high, so functional adsorption becomes dependent on initial concentration. This sorption characteristic indicated that surface saturation was dependent on the initial metal ion concentrations (Bhatti et al., 2007). The initial concentration provides an important driving force to overcome all mass transfer resistance of metal ion between the aqueous and solid phase. The increase in initial concentration of chromium results in the increased uptake capacity (q) and decreased percent removal since at high initial concentrations, the number of moles of chromium available to the surface area are high and adsorption depends upon initial concentration (Zubair et al., 2008). Previous reports also suggests that removal of Cr(III) is dependent on concentration of chromium because the increase in the initial chromium concentration (50-300 mg/L) increased the amount of Cr(III) adsorbed (Fahim et al., 2006).

**Effect of contact time**

Adsorption was rapid and ultimate adsorption (40-45%) occurs within first hour of contact and saturation is reached within next 48 h. The equilibrium time is one of the important parameters for an economical wastewater treatment system. The equilibrium time for chromium removal was 4 h at constant values of pH. The chromium occurred in two steps: an initial fast step which lasts for 0.5 h (shorter time measured) followed by the slower second phase which continued until the equilibrium was reached within 4 h. Further increase in contact time did not show an increase in biosorption. Lower adsorption rate in the latter stage was due to the difficulty faced by chromium ions to occupy the remaining vacant surface sites because of forces between the solute molecules of the solid and bulk phase (Ucun et al., 2002; Chand et al., 1994; Saravanane et al., 2002; Bishnoi and Garima, 2004). The diminishing removal with increasing time may also be due to intraparticle diffusion process dominating over adsorption (Deo and Ali, 1992; Volesky 2001). Bai and Abraham (1998) reported 11mg/g of chromium uptake capacity by dead fungal biomass of *Rhizopus nigricans* in the

pH range of 2.0-7.0. Both were studied in batch mode with continuous agitation at 120 rpm showed maximum removal of chromium up to 95% *Aspergillus* sp. (MB1) followed by *Streptomyces* sp. (MB2) respectively at equilibrium.

Srinath et al., 2002 had also observed the similar trend in *Bacillus coagulans* and *Bacillus megaterium*. The accumulation of the metal appeared to be biphasic with initial surface binding or biosorption followed by a slower phase of uptake, presumably intracellular. On the other hand adsorption with non living fungal biomass involves physico-chemical or ion exchange interaction (de Rome 1991; Volesky and Schiewer 2000). Increase in contact time from 0.08 to 4 h led to an increase in Cr(III) removal from 40-70% and 11.5-57% from aqueous solution and tannery effluent respectively, whereas 65% and 57.34% removal from aqueous solution and tannery effluent respectively were observed within first 2 h by *Streptomyces* sp. (MB2), which represents the time at which equilibrium of chromium biosorption is presumed to have been attained. This suggests that adsorption is rapid and typically 40-50% of the ultimate adsorption occurs within the first hour of contact and saturation is reached within next 48 h (Chandrasekhar et al., 1998; Mohan et al., 2006).

The chromium removal occurred in two steps: an initial fast step which lasts for 30 min (shorter time) followed by the slower second phase which continued until the equilibrium was reached within 240 min and further increase in contact time did not show an increase in removal (Bishnoi et al., 2007; Zubair et al., 2008). A contact time of 2-4 h was enough to achieve significant Cr(III) removal at pH higher than 3.5 and lower than 2.0, however an elapsed contact time was necessary to establish equilibrium. In previous work, using *Streptomyces* biomass as adsorbent, it was observed that at pH 2 and 3.2 the chromium removal were initially rapid for all the adsorbents studied. As the contact time increases, the rate of adsorption decreases depending on the chemical characteristics of the surface (Chergui et al., 2007; Ashwini et al., 2009).

### 5.3.3 Adsorption isotherms

Langmuir model assumes that all sites are initially free and does not consider any reverse reaction of displaced ion with the site, whereas Freundlich model assumes that all sites to which metal ions are sorbed are initially occupied i.e. the number of free sites remain constant. Both Langmuir and Freundlich model fitted well throughout the concentration range (5-50 mg/L) with the correlation coefficient of 0.9, which indicates that both physico-chemical adsorption and ion exchange interactions are involved in metal binding.

Equilibrium sorption isotherms of Ni, Cd, Pb and Zn by non living biomass of *R. arrhizus* with no pH control followed typically Langmuir adsorption pattern (Fourest and Roux, 1992) and *Rhizopus arrhizus* (Zhou and Kiff, 1991) followed Langmuir and Freundlich adsorption isotherm for single layer adsorption. Langmuir and Freundlich adsorption isotherms were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by microbial biomass. Correlation coefficient ($r^2$) values indicate that the adsorption pattern for chromium removal by microbial biomass followed both the Langmuir ($r^2 > 0.98$) and Freundlich ($r^2 > 0.98$) isotherms. Isotherm can accurately indicate the mechanism involved in microbe-metal interaction such as electrostatic, physical or chemical (Bux et al., 1994). In our studies both the Langmuir and Freundlich adsorption isotherm for Cr(III), the correlation coefficient ($r^2$) was greater than 0.9 indicating that both physico-chemical and ion-exchange interaction takes place by both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2). The waste biomass which is organic in nature, where $r^2$ values of Langmuir model was 0.99 and Freundlich model was greater than 0.99, showing that ion exchange interaction dominates over physico-chemical interaction due to inorganic nature. Mattuschka and Strube (1993) reported that adsorption equilibrium of Cu, Pb, Cr and Ag by waste biomass of *Streptomyces noursei* at low concentration can be described by Freundlich and Langmuir adsorption models. Desirable isotherm should be steep from the point of origin, indicating a high affinity for the sorbate at low concentration. Like wise a high saturation plateau ensures low residual concentration of a particular metal species in the contaminated effluent once equilibrium is obtained (Volesky 1986). Decrease in the concentration resulted in decrease in the adsorption capacity in both models. Typical values found in earlier literature were $0.6 < K_f < 3.8$ mg/g and $1.4 < Q_L < 119$mg/g for Cr(III) (Dias et al., 2000; Yun et al., 2001; Machado et al., 2002; Mohan and Pittman Jr, 2006; Romero-Gonález et al., 2006).

### 5.3.4 Adsorption Kinetices

Kinetic modelling of Cr(III) and Cr(VI) study describes the solute uptake rate and this rate controls the residence time of adsorbate uptake at the solid-solution interface by using Lagergren, Ho and McKay, Elovich and Morris-Weber equation (Demirbas et al., 2004). A comparison of kinetic models applied to the biomass indicates that adsorption of Cr(III) follows best of Ho and McKay pseudo second-order rate equation and correlation coefficient ($r^2$) correlated with the experimental data.

A relatively high $r^2$ value indicates that the model successfully described the kinetics of Cr(III) adsorption.

**Lagergren Equation** (pseudo-first-order)

The sorption data of Cr(III) and Cr(VI) uptake by *Citrus reticulata* waste biomass was fitted using Lagergren pseudo-first-order model (Zubair et al., 2008).

**Ho and McKay equation** (pseudo-second-order)

Experimental points shown together with theoretically generated values reflect extremely high correlation coefficients. The pseudo-second order model is based on the assumption that the rate-limiting step may be a chemical sorption involving valence forces through sharing or exchange of electrons between adsorbent and adsorbate and provides the best correlation of the data. These two models do not provide a definite mechanism; therefore another simplified model was tested in which the kinetic data is better fitted by second-order rate equation. Kinetic studies of microbial biomass were conducted at varying initial concentrations (5-50 mg/L) of Cr(III). The experimental points shown together with the theoretically generated values reflect extremely high correlation coefficients The data showed good compliance with the pseudo second-order kinetic model ($r^2 > 0.99$).

Data showed good compliance with pseudo-second order kinetic model ($r^2 > 0.99$). However, these two models do not provide a definite mechanism. Therefore, another simplified model Ho et al., 1996; Ho and Mckay 1999 was tested, in which kinetic data is better fitted by second-order rate equation. Experimental $q_e$ values were compared with $q_e$ value determined by pseudo-first and second-order rate kinetic models, which suggests that sorption system is not a pseudo first-order reaction and that a pseudo second- order model can be considered.

**5.3.5 Removal of Cr(III) from aqueous solution by pretreated microbial biomass**

Different methods of pretreatment of biomass have been discovered to cause certain changes which enhance or change the performance of the biomass in metal biosorption. Metal affinity to the biomass can be manipulated by pre-treating the biomass with alkali, acids, detergents and heat, which may increase the amount of the metal sorbed (Kapoor and Viraraghavan, 1998; Ilhan et al., 2004). Various chemical treatments were applied to enhance the removal of Cr(III) from aqueous solution in batch sorption experiments using native and chemically treated biomass.

**Effect of alkali and acid pretreatment**

Alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites (Muraleedharan and Venkobachar 1990 a, b). Removal of impurities from surface and after rupturing of cell-membrane is the reason behind the increase in metal uptake capacity of biomass after basic pretreatment (Mittleman and Geesey 1985; Dow and Rubery, 1977; Sar et al., 1999). However, Cr(VI) bioadsorption capacity of biomass was significantly decreased as a result of alkali pretreatment. The net decrease may be due to the fact that, after certain concentration of an alkali used for pretreatment of biomass, the number of protein amino groups that can be engaged in metallic ion binding markedly decreased. Deproteination should, theoretically, reduce metal retention (Loaec et al., 1997; Kapoor and Viraraghvan, 1998; Ahmet et al., 2005). Alkali treatment causes breakage of cellulose polymer (which could may involve in significant Cr(VI) uptake), thereby hindering the operational stability of biomass, resulting in decrease in Cr(VI) sorption capacity. Treatment of *Aspergillus* sp. (MB1) biomass with NaOH showed 96.21% removal of Cr(III) removal from aqueous solution. Cr(III) uptake and removal by alkali-treated MB1 and MB2 biomass were 2.75 (96.2%) and 1.72 (76.7%) mg/g respectively. Among all the pretreatment methods alkali treatment improved the biosorption capacities by both the biomasses. The increase biosorption after alkali treatment may be due to an exposure of active metal binding sites embedded in the cell wall or chemical modifications of the cell wall components or may be due to removal of surface impurities, rupture of cell membrane and exposure of available binding sites for chromium (Huang and Huang, 1996). Tunali et al., (2005) suggested that the increase of metal uptake rate after alkali pretreatment could be due to the release of certain biopolymers having high affinities towards heavy metal ions from the cell wall by autolytic enzymes Similar reports by *Penicillium digitatum* (Galun et al., 1987) and *P. lanosa coeruleum* (İlhan et al., 2004) suggested that increase in metal biosorption after pre-treating the biomass could be due to exposure of available binding sites for metal biosorption. Luef et al., 1991 tried to explain the increase in zinc uptake by *A. niger* after sodium hydroxide treatment and they suggested that the removal of certain polysaccharides from the cell wall by alkali treatment generates more accessible space within the ß-glucane chitin skeleton, thus allowing more zinc ions to be sequestered by this structure. Ashkenazy et al., (1997) reported enhanced adsorption of Pb by *Saccharomyces uvarum* biomass after boiling with NaOH. Therefore difference in the adsorption observed after a specific pre-treatment is mainly attributed to the type of treatment and microorganism. Liu et al., (2004) explained that the alkali-pretreated biomass showed higher metal adsorption capacity could be due to an increase in the availability of binding sites or to the removal of polysaccharides that presumably block the access of metals to the binding sites. Another

researcher relates the improvement of metal binding by alkali-treated biomass to the release of proteins, making additional binding-sites available for metals (Baik et al., 2002). Rao and Viraraghavan (2002) suggested that alkali pretreatment has been shown to be a probable cause for destroying autolytic enzymes that cause putrefaction of biomass and removing lipids and proteins that mask reactive sites. Mameri et al., (1999) offered two possible explanations on the increased performance of NaOH-pretreated biomass. One was explained by the fact that in the sodium form, the ion-exchange sites are more easily able to exchange this cation with metallic cations than when the ion-exchange sites are protonated. In the case of alkali pre-treatment, bioadsorption capacity of *Mucor rouxii* biomass was significantly enhanced in comparison with autoclaving (Yan and Viraraghavan, 2000). In a study by Galun et al., (1987), NaOH treated *Pencillium digitatum* also showed enhancement of cadmium, nickel and zinc biosorption. Removal of surface impurities, rupture of cell-membrane and exposure of available binding sites for metal bioadsorption after pretreatment may be the reason for the increase in metal bioadsorption. Muraleedharan and Venkobachar (1990) showed that alkali treatment of biomass may destroy autolytic enzymes that cause putrefaction of biomass and remove lipids and proteins that mask reactive sites. The cell wall of *Mucor rouxii* was ruptured by NaOH treatment besides, the pre-treatment could release polymers such as polysaccharides that have a high affinity towards certain metal ions (Loaec et al., 1997).

Among the two acids $H_2SO_4$ (0.1M) and HCl (0.1M) pretreatment of MB2 only showed enhancement in Cr(III) removal from aqueous solution by 75.8 and 77.3 % as compared to native biomass (68.5%). There was an increase in case of *Streptomyces* sp. (MB2) biomass, which rather showed decline in *Aspergillus* sp. (MB1). The net increase or decrease after and acidic pretreatment can be related to polymeric structure of biomass and strength/concentration of an acid. The polymeric surface exhibits a negative charge due to the ionization of organic and inorganic groups. Biomass having the higher electro negativity and removal capacity of biomass will decrease due to the increasing H+ ions (Bhattacharyya and Sharma, 2004; Kapoor and Viraraghvan 1998). At a certain strength/concentration acid can enhance uptake capacity of biomass by increasing the surface area and porosity of original sample (Moreno-Castilla et al., 1997; Benguella and Benaissa 2002; Bhatti et al., 2007; Zubair et al., 2008; Batista et al., 2009). Solisio et al., 2000 reported the effect of acid pretreatment on biosorption of Cr(III) by *Sphaerotilus natans* from industrial wastewater. Acid pretreatment significantly decreased biosorption of lead and nickel 66% and 46% respectively in comparison with living biomass, which is in agreement with the observation made by Kapoor and Viraraghavan 1998 in *A. niger* where, pretreatment insufficiently increased copper biosorption capacity of *P. lanosa coeruleum* in comparison with living biomass (5%). The polymeric structure of

biomass surface exhibits a negative charge due to the ionization of organic and inorganic groups. The higher the biomass electro-negativity the greater is the attraction and adsorption of heavy metal cations. Thus, the remaining $H^+$ ions on the acidic pretreated *P. lanosa coeruleum* biomass may change the biomass electro-negativity, resulting in a reduction in bioadsorption capacity. On the contrary, Huang and Huang, (1996) reported that acid pretreatment can strongly enhance the adsorption capacity of *A. oryzae* biomass. Generally, acid treatment has been used for cleaning the cell wall and replacing the natural mix of ionic species bound on the cell wall with protons and sulfates (Yun et al., 2001; Yun, 2004). Another explanation could be the extraction of the organic constituent and thus the improvement of the accessibility of the solute to the internal sites of the biomaterial. Both Huang and Huang (1996) and Arica et al., (2005) explained that acid pretreatment results in physical cleaning or washing-out of the biomass surface from impurities, thus produces additional available binding sites. Rao and Viraraghavan (2002) suggested that acid pretreatment could generate positively charged sites in its surface and this will help in the binding of anions. Acid pretreatment of *Mucor rouxii* significantly decreased the bioadsorption of heavy metals (Yan and Viraraghavan, 2000), which is in agreement with the observation of Kapoor and Viraraghvan (1998) in the case of *A. niger*. This is attributed to the binding of $H^+$ ions to the biomass after acid treatment may be responsible for the reduction in adsorption of heavy metals. The polymeric structure of biomass surface exhibits a negative charge due to the ionization of organic and inorganic groups (Hughes and Poole, 1989). Bux and Kasan (1994) suggested that the higher the biomass electro negativity, the greater the attraction and adsorption of heavy metal cations. Thus the remaining $H^+$ ions on the acidic pretreated *M. rouxii* biomass may change the biomass electronegativity, resulting in a reduction in bioadsorption capacity. However, Huang and Huang (1996) reported that acid pretreatment can strongly enhance the adsorption capacity of *Aspergillus oryzae* mycelia. In case of *A. oryzae,* live biomass after acid pre-treatment was directly used in bioadsorption of heavy metals instead of being autoclaved and dried. The difference in results after a specific pretreatment may be attributed to the different strains of fungi used and whether the biomass was live or dead when it is used in biosorption of metal ions.

**Effect of organic solvent pretreatment**

Pretreatment by organic solvents, such as acetone, chloroform, diethylether and methanol affected the removal rate of Cr(III). The removal percentage of *Aspergillus* sp. (MB1) after treatment with organic solvent was in the order of acetone (68%) > methanol (64.12%) > chloroform (63.01%) > diethylether (54.28%) which was lower than the native biomass (73.98%). However pretreatment of *Streptomyces* sp. (MB2) with organic solvent was in the order of Cr(III) removal diethylether

(74.49%) > acetone (71.59%) > chloroform (69.06%) which were higher than native biomass (68.513%). All the organic solvents showed the improvement of Cr(III) removal efficiency accept methanol (58.97%). Treatment with organic solvents removes the protein and lipid fractions of the biomass surface (Ashkenazy et al., 1997). Adsorption capacities of organic solvent treated microbial biomass were found to be 1.95 to 1.80 and 1.76 to 1.48 mg/L respectively. Components of microbial biomass are more dissolve in organic solvents than water. Dissolving effect is affected by the intermolecular forces such as, hydrogen bonding, ion-dipole force, dipole-dipole force, dipole-induced dipole forces, electron pair donor- electron pair acceptor interactions. Thus, this treatment might expose more metal binding sites and improve the adsorption property of the biomass Park (2005). Göksungur et al., (2005) suggested that higher metal uptake by ethanol pretreated cells was due to the increase in the availability of binding sites.

Park et al., (2005) also studied the methylation of the amino groups strongly affected the Cr(VI) removal. The removal rate of Cr(III) and Cr(VI) by the methylated biomass significantly decreased as compared with the raw biomass. The methylation reaction reduces the number of positively charged sites on the biomass surface. According to Drake et al., (1996), treatment of biosorbent with methanol results in esterification of carboxylic acids present on the cell wall of biosorbent. Same is also true in case of pretreatment with ethanol (Beveridge and Murray 1980; Cohen-Shoel et al. 2002).

The methylation of amine groups prevents their participation in metal biosorption and hence reduces the biosorption efficiency. Therefore, the reduction in biosorption efficiency observed when amine-methylated biosorbent was used. It reveals that the amino group present in the cell wall of *Citrus reticulata* contributes towards biosorption of Cr(III) and Cr(VI) (Zubair et al., 2008). Similar results were also discussed by Akhtar et al., (1996) and Drake et al., (1996).

### 5.3.6 Fourier transform infrared (FTIR) analysis

Microorganisms are complex mixtures of many different types of organic molecules which result in a broad band of overlapping IR resonance frequencies. However, the resonance frequencies associated with similar repeating or replicated structures in various biomolecules accumulate in certain regions of the IR spectral range, giving the bacterial spectrum discernable features which can be identified as originating from particular classes of biopolymer. The three major main groups of biomolecules which can be distinguished in an IR spectrum of a bacterium are proteins, RNA/DNA and polysaccharides. Aliphatic chains of lipids can also be identified as well as the carbonyl stretch associated with an ester linkage or the salt of a carboxylic acid (Suci et al., 1998).

The FTIR spectrum of Aspergillus sp. (MB1) and *Streptomyces* sp. (MB2) shows peak at 3428.7 $cm^{-1}$ which is indicative of the existence of −OH and −NH (O−H stretching) and presence of hydroxyl and amine groups on the biomass surface. The spectra of the biomass displayed a number of absorption peaks at 2927.8 $cm^{-1}$ (C−H stretch), 1631.7 $cm^{-1}$, 1663 $cm^{-1}$ (C=C stretch), 1550.3, 1531.7 $cm^{-1}$ (C=C stretch), 1450.2 $cm^{-1}$, 1400.1 $cm^{-1}$ (C−N stretch), 1062.1 $cm^{-1}$ (C−O stretch), 898.41 $cm^{-1}$ (C−N stretch) and 698.86 $cm^{-1}$, 667.56 $cm^{-1}$ (C−O−H) (Mohan et al., 2005; Mohan and Pittman Jr, 2006). On comparing the spectra of Cr(III) containing tannery effluent treated biomass and native biomass, some of the peaks such as 3428.7 $cm^{-1}$ (O–H stretch), 1631.7 $cm^{-1}$, 1550.37 $cm^{-1}$ (C=O, C=C stretch) are increased and becomes sharp and peak 1400.1 $cm^{-1}$ (C−N stretch) is disappeared whereas peak 1531.7 $cm^{-1}$ (C=C stretch) is reduced as a result of metal uptake by the biomass. Changes observed in the spectrum indicate possible involvement of these C=C, C=O and O–H, functional groups in sorption process. The 1800-1540 $cm^{-1}$ band is associated with C=O stretching mode in carbonyls, carboxylic acids and lactones, while 1440-1000 $cm^{-1}$ band was assigned to the C–O stretching and O–H bending modes such as phenols and carboxylic acids (Gardea-Torresdey et al*.*, 2002; Mohan and Pittman Jr, 2006) The maximum uptake ($q$ max) increased with increasing equilibrium pH. Since the carboxyl group has (p*K*H) 4.6, it can be easily expected that at a low pH these sites are occupied by protons, whereby chromium cannot become easily bound to the sites. Furthermore, the biosorption of trivalent chromium is qualitatively shown to be a cationic-exchange process between chromium ions ($Cr^{3+}$ and/or $CrOH^{2+}$) and protons of the biomass carboxyl groups, as previously indicated (Yun et al., 2001). Absorption band shifting may be caused by various factors such as intra-molecular and intermolecular hydrogen bonding, stearic range and position of C=O stretching band (common to carbonyls, carboxylic acids and lactones) is determined by many factors. These include physical state, electronic and mass effects, conjugation, hydrogen bonding and ring strain. Surface area is not the only criteria and other factors such as precipitation, surface complexation and ion exchange also play an important role in the adsorption of Cr(III) on the adsorbents (Mohan and Pittman Jr, 2006; Blázquez et al., 2009). Kapoor and Viraraghavan (1997) reported the involvement of various functional groups such as carboxyl and amine groups on the cell wall of *Aspergillus niger* ATCC 11414 in biosorption of lead, cadmium and copper. Beveridge and Murray 1980 also observed that modification of amine and carboxyl group resulted in significant drop in copper biosorption by *Bacillus subtilis*. Bayramoglu et al., (2005) reported that the peak at 576 $cm^{-1}$ and 542 $cm^{-1}$ representing O-C-O scissoring and C=O bending vibrations were not observed for the treated fungus, *Lentinus sajor-caju* and these was due to the removal of lipid compounds after physical and/or chemical treatments. Arica et al., (2005)

observed a clear increase in the intensity of the C–N–C band at 540-470 $cm^{-1}$ of *Chlamydomonas reinhardtii* representing scissoring found in polypeptide structures which are due to the modification of cell wall compounds after acid treatment. On the other hand Adhiya et al., (2002) reported that the reaction of *Chlamydomonas reinhardtii* biomass with $H^+$ ions resulted in a significant growing in of bands at 1398 $cm^{-1}$ and 1344 $cm^{-1}$, which were attributed to carboxylate stretching. Tunali et al., (2005) has reported that the band indicating the $COO^-$ and –CO group was shifted from 1408 $cm^{-1}$ to 1379 $cm^{-1}$ in chromium loaded *Neurospora crassa* biomass. The peak at 1232.4 $cm^{-1}$ appears to be shifted to 1240.1 $cm^{-1}$ after interaction with Cr(VI). This could be assigned to secondary amides or C-O of acids. Besides these, the peak at 1166.8 / 1167.8 $cm^{-1}$ becomes less prominent after Cr(VI) biosorption. This could be caused by the interaction of Cr(VI) anion with the P-O-ethyl, aliphatic amines, aromatic methine or primary and secondary amines. The involvement of phosphate group in Cr(VI) biosorption by *Neurospora crassa* biomass has been reported by Tunali et al., (2005) as a clear decrease in band intensity was observed at 1151 $cm^{-1}$ and 1035 $cm^{-1}$ (P=O and P-OH stretching). Pethkar et al., (2001) has reported the same phenomenon in the interaction of *Cladosporium cladosporioides* with gold and this indicated that chemical reaction might not be involved in the biosorption of gold and probably gold anions were bound to protonated carbonyl and carboxyl groups by electrostatic interactions. Thus this might explain the low Cr(VI) uptake shown by acetic acid pretreated *C. agropyri* in the biosorption studies. Fourier Transform Infrared spectral (FTIR) analysis studies of metal loaded and unloaded biomass was done to study the involvement of functional groups in metal binding and mode of interaction between metal and the biomass. A variety of ligands (carboxyl, amine, hydroxyl, phosphate and sulfhydryl) located on the biomass surface due to the presence of polysaccharides and glycoproteins (glucans, chitin, mannans and phosphomannans) are known to be involved in metal chelation (Beveridge and Murray, 1980). Several different kinds of mechanism could be involved in the accumulation of metals by microorganisms e.g. adsorption precipitation, complexation and transport (Gadd, 1992). Functional groups such as C=O, NH2, C−Cl, C=S, C=H, OH, C−O, CS−NH, $CH_2$, C−Cl, C=S and C=C-H, C≡C, C−N, COOH and CH=CH on the microbial surface interact with metals. Among both the biomass in *Aspergilus* sp. C−O, C=O, C=C groups were involved in chromium binding. In other biomass functional groups such as amine, C=N, C−Cl groups were found to play a role in chromium binding.

The major chemical group on the surface of biomass, involved in sorption and chelation of a number of bivalent metal cations are polar or anionic in nature such as carboxylate, amine, phosphate and hydroxyl groups of polysaccharide materials of the cell wall (Kapoor and Viraraghavan 1997). During interaction of chromium with microbial biomass surface some absorption peaks were shortened or enlarged or disappeared from spectra, which further depends upon the surface properties of microbial biomass. It could be assumed that carboxylic groups are the primary sites, but other functional groups can also act as potential binding sites. Microbial metal accumulation can be influenced by (a) surface properties, such as charge and orientation of the metal binding functional groups on the cell surface and (b) metal speciation and chemistry in aqueous solution (Akhtar et al., 1996; Ledin 2000).

### 5.3.7 X-ray diffraction (XRD) analysis

The XRD data of treated adsorbent provided evidence of decrease in the peak intensity which shows the adsorption of Cr(III) on the surface of the adsorbent changing its structure from crystalline to amorphous. The X-ray diffraction analysis of *Aspergillus* sp. (MB1) biomass showed peaks that indicated its crystalline structure while *Streptomyces* sp. (MB2) showed amorphous nature. After Cr(III) adsorption by both microbial biomass the surface structures were changed to amorphous nature. The X-ray diffraction analysis of activated carbon did not show any peak indicating amorphous nature of the carbon prepared from coconut shell fibers (Mohan and Pittman Jr, 2006). The Cr-loaded maghemite particles were characterized by XRD, XPS, and Raman spectroscopy techniques after Cr(VI) adsorption at different pHs. XRD showed that Cr-adsorbed particles matched well with standard y gama $Fe_2O_3$. Other crystalline phases did not appears after adsorption. The adsorption mechanism for Cr(VI) onto modified MnFe2O4 nanoparticles were a combination of electrostatic interactions and ion exchange. This was determined by X-ray diffraction and X-ray photoelectron spectroscopy (Mohan et al., 2007). This is in agreement with the previous studies on adsorption (Mohan et al., 2006; Gopal and Elango, 2007; Karthikeyan and Ilango, 2008). XRD diffractogram pattern of rice husk ash (RHA) reveals that RHA was completely amorphous indicated by the featureless pattern and the absence of significant peaks and appearance of diffusion maximum at 23° typical for amorphous silica (Hamdan et al., 1997). The diffractogram exhibits many significant peaks from 5° to 50° indicating that the samples are in the crystalline form (Malek and Yusof 2007).

## 5.4 Process optimization in continuous flow biosorbent column

Batch studies with microbial biomass of *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were conducted at varying initial concentrations (5-50 mg/ L) of tannery effluent. Removal of Cr(III) from tannery effluent containing 25 mg/L Cr(III) by MB1 and MB2 biomass were 64.48 and 88.28 %, respectively. The pseudo second-order dynamics model was found to correlate with the experimental data.

Both *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were used as an adsorbent for chromium removal from aqueous solution as well as tannery effluent in continuous flow sorption system. Chromium sorption capacity of microbial biomass (MB1) was found to be 96% whereas of MB2 it was 82%. Breakthrough curves concluded that microbial biomass could be used for Cr(III) and Cr(VI) removal from aqueous solution and tannery effluent. Column studies revealed that both biomass efficiently transforms Cr(VI) to Cr(III) at the rate of 99%. Microbial biomass could be desorbed with dilute HCl and regenerated for its subsequent reuse as metal sorbent and there was no change in chromium removal efficiency up to 5 sorption cycles. The present study significantly implies that microbial biomass is more effective for removal of chromium in successively cycles which is applicable for treatment of tannery effluent at large scale.

### 5.4.1 Continuous flow sorption column

**Removal of chromium by microbial biomass in continuous flow sorption system**

Removal rate of the Cr(III) was rapid during the first hour of influent flow. This type of biosorption is typically for sorption of metals involving metabolically inert biomass, where metal removal from solution is purely due to the physico-chemical interaction between biomass and the metal in solution.

Batch experimental data are often difficult to apply directly to fixed bed column adsorber because isotherms are unable to give accurate data for scale up since a flow column is not at equilibrium. Fixed bed column adsorption experiments were carried out the study the adsorption kinetics. The fixed bed column operation allows more efficient utilization of the adsorptive capacity than batch studies (Sarin et al., 2006). The design of up flow fixed bed column adsorber includes estimation of shape of breakthrough curves and the appearance of breakpoint. The effect of fixed bed column parameters such as sorbent bed height, flow rate and initial chromium concentration on the

performance of breakthrough curves were investigated. All the fexed bed column experiments were carried out at ambient conditions at pH 4 and having Cr(III) concentration as 25 mg/L. Flow rate of the effluent through the column was maintained at 10 to 15 mL.The total chromium adsorbed in the column for a given feed concentration is equal to the area under the breakthrough curve (Sarin et al., 2006). The gradient of breakthrough curve was a strong function of fixed bed column parameters.

Among both the biomass MB1 showed maximum removal of chromium from aqueous solution and nearly complete removal of chromium in continuous flow sorption system within first 6 h. The overall performance of flow through column is strongly related to the length and shape of ion-exchange zone that develops during sorption. This zone develops between the section of the column that saturated with heavy metals and the section that still contains fresh biosorbent. As the loading of the biosorbent progresses, the zone moves along with the column in the direction of the liquid flow. When the sorbent saturation zone approaches the end of the column, the metal concentration in the outlet stream increase sharply and the useful life of the column is over, which is the breakthrough point and the time before breakthrough point is the service time of the column. Clearly, the shorter the dynamic ion exchange zone in the column, longer the column service time and larger the fully utilized sorbent portion inside the column. Therefore, a favorable breakthrough curve is steep and sharp showing the effective utilization of the biosorbent materials inside the column, whereas unfavorable breakthrough curve on the other hand is flat and trailing indicating a wasteful long transfer zone inside the column (Kratochvil and Volesky 1998). The choice of an optimal reactor for removal of metal ions from solution by biomass depends on the biomass characteristics.

Continnuous packed bed column system are the most suitable and economic ways to remove heavy metals, offering an alternative treatment for the removal and recovery of heavy metals in aqueous solution (Gin et al., 2002; Sag et al., 2001). Breakthrough curves are necessary for the adsorption column design, due to the information about the dynamic behavior of the metal concentration of the effluent in time (Sag et al., 2001; Guangyu and Viraraghavan, 2001). Another important factor for the column design is the maximum capability of adsorption of the metallic ion with a specific amount of biomass this process was studied with the help of sorption isotherms (Guangyu and Viraraghavan, 2001). *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were studied in continuous flow sorption column system. A flow-through biosorption column demonstrated a high overall column Cr(III) sorption capacity of 238 mg/g by *Aspergillus* sp. (MB1) at pH 5 followed by 74 mg/g by *Streptomyces* sp. (MB2). On the other hand a flow-through biosorption column packed with *Aspergillus* sp. (MB1) had Cr(III) sorption capacity of 12.6 mg/g at pH 5. Whereas *Streptomyces* sp.

(MB2) show 28 mg/g of Cr(III) from aqueous solution. Puranik and Paknikar (1999) reported the loading capacity of 57.7 mg/g for lead and 21.3 mg/g for zinc by using waste biomass from fermentation industry comprising of *Streptoverticillium cinnamoneum* pretreatment with boiling water. In our studies loading capacity of the *Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) were 33 mg/g for Cr(III) and 12.6 mg/g for Cr(III) without any pretreatment of the *Aspergillus* sp. (MB1) biomass. This is of significant since biowaste can be procured as a waste byproduct from one industry and using it for the treatment of tannery effluent from another industry becomes cost effective and eco-friendly. Which otherwise involves usage of salts and acids for its treatment generating huge quantity of toxic chrome sludge.

### 5.4.2 Regeneration and reused of microbial biomass

The objective of desorption is to weaken the biomass metal binding, the conditions that are least favorable for biosorption are ideally suited for desorption. The eluent used for desorption must have a higher binding affinity for the metal than biomass metal binding. Studies with adsorption of Cr(III) by *Aspergillus* sp. (MB1) in continuous flow sorption mode revealed that *Aspegillus* sp. (MB1) can be desorbed/regenerated with dilute HCl with no change in adsorption of Cr(III) upto 5 cycles. Whereas in *Streptomyces* sp. (MB2) biomass the adsorption was decresead drastically after first adsorption. Norberg and Persson (1984) have reported that acid treatment does not affect the metal uptake values of the biomass when it exposed to a metal solution after regeneration. Hung and Huang (1996) used the mineral acid for the regeneration of Cu laden mycelia and reported that metal uptake capacity of the regenerated mycelia was higher than the untreated and acid washed mycelia. Further, Cu binding capacity of the biomass remains constant up to $12^{th}$ regeneration cycles. Moreover there was no mechanical damage. The suitability of biowaste as a reusable metal biosorbent is worthwhile as it can be procured from the fermentation industries in large amount and is available through out the year.

The breakthrough curves with normalized concentration defined as the mesursed concentration divided by the inlet concentration was plotted against time and shows the amount of chromium present in solution was removed with biomass inside a continuous packed bed column system. The S shape and the appearance of the breakthrough point describe a typical behavior of biosorption inside fixed bed column systems. The sorption process for Cr(III) did not happen very fast: this can be concluded from the behavior of the curve that did not show a linear vertical behavior between the breakthrough point and the saturation point. According to Treybal and Aksu et al., 2002 the flow rate is an important factor Involved in the biosorption process. Breakthrough point observed for Cr(III)

by *Aspergillus* sp. (MB1) was early than *Streptomyces* sp. (MB2) possibly the chemical properties of biomass are different. Cr(III) in the form of oxides, hydroxides or sulfates, is much less mobile. Most cells are impermeable to Cr(III) probably because it forms water insoluble compounds in non acidic aqueous solution (Cervantes et al., 2001; Travis et al., 2002).

The high efficiency of the biosorption and elution, low biomass damage and stability over a prolonged operation time makes the new biosorption process an effective alternative for chromium pollution control.

# Conclusions

1. Tannery effluent collected from A.V. Tanneries, Kapurthala, Punjab, was acidic, greenish in colour and contained highest concentration of TS, TDS, TSS, BOD, COD and nitrogen. Chromium concentration in the effluent was 1700 mg/L besides this effluent also contained other heavy metals.

2. Microbial waste biomass (*Aspergillus* sp. (MB1) and *Streptomyces* sp. (MB2) generated as a byproduct of pharmaceutical fermentation industry had acidic pH with ash content ranging from 5-6%. The CHN analysis showed that *Streptomyces* sp. (MB2) had high nitrogen content than *Aspergillus* sp. (MB1).

3. Bacterial consortium developed from chrome sludge (CS) was more efficient than tannery effluent consortium (CT) in transforming Cr(VI) to Cr(III). Growth inhibition by Cr(VI) was evident in all the bacterial culture, although the Cr(VI) did not completely arrest bacterial growth. The percent decrease in the growth of bacterial consortium (CT) was 11%, 19% and 21% whereas in bacterial consortium (CS) it was 30%, 34% and 36% at 3.6, 7.9 and 14.18 mg/L of Cr(VI) respectively. Biotransformation of Cr(VI) by resting cells of tannery consortium (CT) was 30% more than by chrome sludge consortium (CS). Among the three suspension media saline was best suited for Cr(VI) transformation.

4. Four isolates showing highest Cr(VI) removal were selected and identified on the basis of 16S rDNA sequencing as *Raoultella* sp. (CT4) and *Citrobacter* sp. (CT5) from tannery effluent and *Bacillus cereus* (CS7) and *Citrobacter freundii* (CS8) from chrome sludge.

5. High Cr(VI) transformation or reduction efficiency was observed for all the bacterial isolates at 4 and 8 mg/L of Cr(VI) which was nearly 90% whereas CT4 (*Raoultella* sp. IS1) showed complete reduction after 22 h of incubation. The time for reduction of Cr(VI) increased with increased in incubation time at high concentration (~ 18 mg/L).

6. Removal of Cr(III) by microbial biomass *Aspergillus* sp. (MB1) obtained from pharmaceutical fermentation industry was higher than *Streptomyces* sp. (MB2). Maximum Cr(III) removal capacity of *Aspergillus* sp. (MB1) was 94.6% and 64.48% whereas by *Streptomyces* sp. (MB2) biomass was 68.2% and 73.9% from aqueous solution and tannery effluent respectively.

7. Pretreatment of microbial biomass with alkali improved removal of Cr(III). Cr(III) removal by *Aspergillus* sp. (MB1) biomass was 96.21% after treatment with alkali and by *Streptomyces* sp. (MB2) it was 77.73% after treatment with acid.

8. Adsorption isotherms and adsorption kinetices were predicted from equilibrium sorption data, which indicated that physico-chemical and ion exchange interactions play a role in binding of chromium by microbial biomass. A comparison of kinetic models (Lagergren, Ho & McKay, Elovich & Morris-Weber) applied to microbial biomass (MB1 and MB2) indicates that adsorption of Cr(III) on biomass follows best Ho & McKay pseudo second-order rate equation and correlation coefficient ($R^2$) values correlated with experimental data.

**9.** Fourier transform infrared spectral analysis of microbial biomass revealed that C=N, C=C, C–H and C–O functional groups are involved in chromium binding which was additionally supported by XRD analysis that showed change in the surface of microbial biomass after biosorption.

10. Removal of Cr(III) from tannery effluent by microbial biomass from fermentation industry implies that it is a cost effective and eco-friendly process for bioremediation of chromium from industrial effluent.

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