

CHROMIUM(VI) BIOSORPTION STUDIES USING  
NON-LIVING MICROORGANISMS

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CHROMIUM(VI) BIOSORPTION STUDIES USING  
NON-LIVING MICROORGANISMS

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A thesis submitted in fulfilment of the  
requirements for the award of the degree of  
Master of Science (Chemistry)

Faculty of Science  
Universiti Teknologi Malaysia

APRIL 2006

*In the name of Allah, the Most Gracious, the Most Merciful,*

Especially for:

Beloved Mum....Fong King Chan,

Beloved elder brother....Lau Kam Hong,

Beloved 1<sup>st</sup> elder sister...Lau Seow Fong and family,

Beloved 2<sup>nd</sup> elder sister....Lau Ling Ling,

Beloved 3<sup>rd</sup> elder sister...Lau Cheng Cheng and family,

Beloved 4<sup>th</sup> elder sister...Lau Siew Sian,

Beloved younger sister...Lau Siew Mung,

The understanding and patient supervisor... Assoc. Prof. Dr Wan Azlina bt Ahmad,

All the beloved ustaz and ustazah in UTM Skudai...

## ACKNOWLEDGEMENTS

*In the name of Allah, the Most Gracious, the Most Merciful,*

All the praise to Allah, for His Mercy has given me patience and strength to complete this work. All the praise to Allah again.

All the praise to Allah for giving me chance to be supervised by Assoc. Prof. Dr Wan Azlina binti Ahmad. I would like to thank her for her sincere advice, assistance, guidance, patience and understanding within the 3 years.

I am also indebted to School of Graduate Studies, for the UTM-PTP Scholarship Scheme, Research Management Centre (RMC), UTM and Ministry of Science, Technology and Innovation for the funds under IRPA votes 74514 and 63280. I also would like to express my gratitude to Dr Farediah Ahmad for her guidance in FTIR analysis, lecturers and staffs in Makmal Sains Bahan, FKM for SEM analysis, as well as all the staffs in Faculty of Science for their assistance and encouragement.

I am also thankful to Berjaya Knitex Sdn Bhd and Baneng Industry in Batu Pahat for the sampling of textile wastewater, SIRIM for the identification of bacteria and Institute for Medical Research (IMR) for TEM analysis.

I am very grateful to all the members in Biotechnology Research Laboratory: Abang Jefri, Abang Zainul, Abang Shahrul, Abang Mior, Abang Azri, Abang Zainal, Abang Amir, Norizan, Bahiyah, Nik, Roobini, Yesotha, Sukhveer, Lim, Lai, Yati, Abu Bakar, Ivy, Voo, Quek, Faisza and Diana for their inspiration, contribution,

support and happiness. I also would like to extend my gratitude to all my elder friends and colleagues: Kak Nab, Kak Mas, Kak Pah, Kak Mai, Kak Linda, Kak S, Raha, Kak Fareh, Kak Aishah, Kak Ju, Ngai Mun Hong, Yen Nee, Kak Marzita, Kak Budi, Kak Hilma, Kak Khairunnisa, Maznah, Norazimar etc. for their inspiration and support as well. Special thanks to Ust Othman and family, Ust Sulaiman, Ust Gani, Ust Ajmain, Ust Abbas and family, Ust Bosran and family, Ust Fadzil, Ustzh Syarifah Fatimah, Ustzh Fatimah (Putrajaya), Ustzh Siti Norlina, Ust Ismail, Ust Selamat, Ust Sabri etc for their advice, guidance and support within the 3 years.

Last but not least, my utmost appreciation to my family for their support and understanding.

## ABSTRACT

Chromium especially chromium (VI) species is a well-known toxic heavy-metal for biological systems and is known to be a human carcinogen. However, hard chromium plating which uses chromium (VI) solutions is still the preferred method due to the higher deposition rates and ability to produce thick coatings. Thus the high concentration of chromium (VI) in chromium electroplating wastewater needs to be removed before being discharged. Due to the disadvantages and limitations of conventional techniques, particular attention is paid to the use of biological systems for heavy-metal removal from industrial wastewater. In this study, indigenous microorganisms in local textile wastewater were isolated and investigated for their chromium (VI) uptake in both simulated and real chromium electroplating wastewater. Preliminary studies showed that among the three bacteria: *Acinetobacter calcoaceticus* genospecies 3, *Clavibacter agropyri* and *Cellulosimicrobium cellulans*, *Acinetobacter calcoaceticus* genospecies 3 showed the highest chromium (VI) uptake at pH 8 with optimum biomass dosage of 0.75% and contact time of 120 hours. Acetic acid-pretreatment of this bacterial biomass was found to reduce chromium (VI) uptake. Chromium (VI) adsorbed on untreated biomass of *Acinetobacter calcoaceticus* genospecies 3 was suggested to occur as a multilayer based on the Brunauer, Emmett and Teller isotherm. The results from Transmission Electron Microscopy and infrared spectroscopy confirmed the involvement of amines, phosphate and carboxylate in surface adsorption of the metal with minor intracellular accumulation. Upon interaction with chromium electroplating wastewater, this bacterial biomass showed chromium (VI) uptake of  $3.82 \pm 0.31$  and  $3.29 \pm 0.67$  mg/g at acidic (3.75) and alkaline (8.08) pH respectively. However, this bacterial biomass could not reduce the chromium (VI) concentration to the legal limits.

## ABSTRAK

Kromium terutamanya spesis kromium (VI) adalah terkenal sebagai logam berat yang toksik kepada sistem biologi and diketahui sebagai karsinogen kepada manusia. Namun demikian, penggunaan kromium (VI) untuk penyaduran kromium keras masih digunakan kerana kadar penyaduran yang lebih tinggi serta keupayaannya untuk menghasilkan penyaduran yang tebal. Maka, kepekatan kromium (VI) yang tinggi di dalam air sisa industri penyaduran kromium perlu disingkirkan sebelum dibuang. Kelemahan dan kekangan teknik konvensional telah menyebabkan perhatian dialihkan kepada penggunaan sistem biologi untuk penyingkiran logam berat daripada air sisa kilang. Kajian ini melibatkan pemencilan mikroorganisma setempat daripada air sisa kilang tekstil dan penyelidikan keupayaannya untuk menjerap kromium (VI) daripada air sisa industri penyaduran kromium (buatan dan sebenar). Kajian awal mendapati daripada ketiga-tiga bakteria: *Acinetobacter calcoaceticus* genospesies 3, *Clavibacter agropyri* and *Cellulosimicrobium cellulans*, didapati *Acinetobacter calcoaceticus* genospesies 3 menunjukkan keupayaan penjerapan kromium (VI) yang tertinggi pada pH 8 dengan dos biomas optimumnya 0.75% dan masa interaksi selama 120 jam. Pra-rawatan ke atas biomas bakteria ini dengan asid asetik telah menurunkan keupayaan penjerapan kromium (VI). Penjerapan kromium (VI) pada biomas *Acinetobacter calcoaceticus* genospesies 3 adalah secara pelbagai lapisan berdasarkan isoterma Brunauer, Emmett and Teller. Keputusan daripada mikroskopi elektron transmisi dan spektroskopi infra-merah mengesahkan penglibatan kumpulan berfungsi amina, fosfat dan ion karbositat dalam penjerapan logam ini pada permukaan di samping sedikit pengumpulan dalam sel. Biomas bakteria menunjukkan keupayaan penjerapan kromium (VI) sebanyak  $3.82 \pm 0.31$  and  $3.29 \pm 0.67$  mg/g masing-masing dalam keadaan berasid (3.75) dan alkali (8.08) daripada air sisa penyaduran kromium. Namun begitu, biomas bakteria ini tidak berupaya menurunkan kepekatan kromium (VI) ke paras yang dibenarkan.

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## LIST OF SYMBOLS / ABBREVIATIONS

<i>A. calcoaceticus</i>	-	<i>Acinetobacter calcoaceticus</i>
AAS	-	Atomic Absorption Spectroscopy
asym.	-	asymmetrical
ATR	-	Attenuated Total Reflection
B	-	BET isotherm constant
B. C.	-	Before Christ
BET	-	Brunauer, Emmett and Teller
<i>C. agropyri</i>	-	<i>Clavibacter agropyri</i>
<i>C. cellulans</i>	-	<i>Cellulosimicrobium cellulans</i>
C <sub>eq</sub>	-	final or residual (equilibrium) metal concentration (mg/L)
CEW	-	chromium electroplating wastewater
C <sub>i</sub>	-	known initial metal concentration (mg/L)
Cr	-	chromium
Cr(III)	-	chromium(III)
Cr(VI)	-	chromium(VI)
C <sub>s</sub>	-	saturation metal concentration
DDW	-	Double Distilled Water
def.	-	deformation
DMSO	-	dimethylsulfoxide
DNA	-	deoxyribonucleic acid
DPC	-	diphenylcarbazine
e.g.	-	for example
EDAX	-	Energy-Dispersive X-ray
EDTA	-	ethylenediamine tetracetic acid

EELS	-	Electron Energy Loss Spectroscopy
<i>et al.</i>	-	et alii (and others)
etc	-	et cetera
FAAS	-	Flame Atomic Absorption Spectroscopy
FADH <sub>2</sub>	-	flavin adenine dinucleotide
FTIR	-	Fourier-transform Infrared
gcm <sup>-3</sup>	-	gram per centimeter cubic
GGM	-	Glycerol-Glycerophosphate Medium
H <sub>2</sub> O <sub>2</sub>	-	hydrogen peroxide
H <sub>2</sub> SO <sub>4</sub>	-	sulfuric acid
HCl	-	hydrochloric acid
i.e.	-	id est (that is to say)
ICPMS	-	Inductively Coupled Plasma Mass Spectrometry
IMR	-	Institute for Medical Research
IR	-	Infrared
k	-	constant related to the maximum binding capacity
KBr	-	Potassium Bromide
kPA	-	kiloPascal
LB	-	Luria Broth
M	-	known amount of biomass (g)
m	-	medium intensity
M	-	Molar
m/g	-	milligram per gram
mg/L	-	milligram per Liter
mM	-	milliMolar
MOPS-NaOH	-	Morpholinopropane sulfonic acid, sodium salt
n	-	constant related to the affinity or binding strength
NA	-	Nutrient Agar
NAD(P)H	-	phosphorylated nicotinamide adenine dinucleotide
NaOH	-	sodium hydroxide

NB	-	Nutrient Broth
nm	-	nanometer
OD	-	optical density
PBS	-	Phosphate Buffered Saline
ppm	-	part per million
q	-	specific uptake of metal (mg of metal / g of biomass)
$q_{\max}$	-	maximum uptake (mg/g)
$Q^{\circ}$	-	metal uptake when a complete monolayer on the surface is formed
$R^2$	-	correlation coefficient
RNA	-	ribonucleic acid
rpm	-	rotation per minute
s	-	strong intensity
sec.	-	secondary
SEM	-	Scanning Electron Microscopy
str.	-	stretching
sym	-	symmetrical
TEM	-	Transmission Electron Microscopy
tert.	-	tertiary
V	-	volume of metal solution (L)
v/v	-	volume per volume
vib.	-	vibration
w	-	weak intensity
w/v	-	weight per volume
XPS	-	X-ray Photoelectron Spectroscopy
YUM	-	Yeast Universal Medium
$\mu\text{m}$	-	micrometer
$^{\circ}\text{C}$	-	degree Celsius

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## **CHAPTER 1**

### **INTRODUCTION**

#### **1.1 Electroplating Industry**

##### **1.1.1 Basic of Electroplating**

Electroplating or electrodeposition may be defined as the production of metal coatings on solid substrates by the action of an electric current. In contrast to various other process of applying coatings, electroplated coatings are applied to improve appearance, corrosion resistance and physicochemical properties of the surfaces (hardness, electrical and thermal conductivity, solderability, reflectivity etc.). Some of the advantages of electroplated coatings over the other methods of applying coatings are absence of an intermediate layer between the coatings and the substrate metal as in the case of hot dip and diffusion processes, fine structure and often very valuable physical properties mentioned above and easy control of the coating thickness to fractions of a micrometer. Besides these, it is the most convenient method of applying coatings of metals with high melting points such as copper, nickel, chromium, iron, silver, gold and platinum (Noor Hisham, 1994).

### 1.1.2 Processes of Electroplating

In general, electroplating process is divided into three stages i.e. pretreatment, electroplating and post treatment. The nature of finish and design of the product dictate the procedures to be adapted in order to produce quality finish. Table 1.1 shows the activities involved in the different stages (Noor Hisham, 1994).

**Table 1.1** : Activities involved in different stages of electroplating process.

Stage	Activities
<b>Product</b>	Semi finish, finish
<b>Pretreatment</b>	Mechanical/chemical surface preparation.
<b>Plating</b>	Alkaline cyanide, alkaline non-cyanide, acid
<b>Post treatment</b>	Chromation, passivation, blackening and antiquing, heat treatment, hot air blow drying, centrifugal drying etc.

### 1.1.3 Chromium Plating

There are two principal types of chromium plating i.e. decorative and hard. Conventional chromium plating solutions contain chromic acid and a small amount of sulfuric acid or a mixture of sulfuric acid and fluosilicate or fluoride ions. The ratio of the concentration of chromic acid to the catalyst acid radicals or anions ranges from about 50:1 to 250:1 and preferably should be about 100:1 (Sittig, 1978).

### **1.1.3.1 Decorative chromium plating**

In decorative plating, a thin chromium coating serves as a protective, nontarnishing, durable surface finish. It is difficult to obtain dense pore-free chromium deposits, and therefore chromium is generally applied over coatings of copper plus nickel or nickel alone. These metals have greater ductility and good corrosion resistance (Sittig, 1978).

Typical parts coated with decorative chromium include: exterior and interior automotive parts; boat hardware, household appliances; home, office and school furniture; plumbing fixtures; bicycle hardware and cabinet hardware (Sittig, 1978).

The trivalent baths are used for thin, decorative chromium, since the process is self-limiting and the deposition rate tends to be low. Hexavalent solutions offer higher deposition rates, thus making them cheaper for decorative coatings (Legg *et al.*, 1996).

### **1.1.3.2 Hard chromium plating**

In hard (also known as industrial or engineering) chromium plating, heavier coatings are used to take advantage of the special properties of chromium plating, such as the ability to withstand heat and corrosion (Sittig, 1978). Besides these, hard chromium is used because of its ease of application, wear resistance and ability to provide a smooth finish (Legg *et al.*, 1996). Unlike decorative chromium plating, hard chromium is generally applied to the base metal without an intermediate coating (Sittig, 1978).

Representative applications for hard chromium plating include: restoration to original dimensions of worn, mismachined or undersized parts; coating of tools, dies and gauges and other parts to minimize wear and to reduce galling, friction and corrosion; coating of electrotypes, engraving plates and other items intended for prolonged runs (Sittig, 1978).

Hexavalent solutions have higher deposition rates and can be used to produce thick coatings. For this reason, hexavalent solutions are at present the only method used for commercial hard chromium plating (Legg *et al.*, 1996).

Electrolytic hard chromium plating as currently practiced is a source of several types of environmental hazard. Direct human exposure occurs as a result of air emissions (bath mist results from the bursting of gas bubbles) and skin contact to the plating solution. The toxic wastes can consist of spent plating solution, bath drag-out (solution pulled from the bath on the parts), spent acids and bases used in cleaning and stripping operations, lead sulfate sludge from anode decomposition, waste rinse water (very high volumes of water are required for rinsing both parts and scrubbing filters) and contaminated mask material (typically wax and paint) (Legg *et al.*, 1996).

The primary problems with hard chromium plating are that it uses a hexavalent chromium solution and produces large volumes of chromium - contaminated toxic waste. While chromium metal and trivalent chromium are fairly benign, hexavalent chromium in solution is a known human carcinogen and creates other health problems such as skin and lung irritation (Legg *et al.*, 1996).

In order to dispose off its large volumes of spent solutions and contaminated water, the plating operation must remove all toxic materials by precipitation and evaporation, discharge the water to the sewer system and ship the solids to a toxic waste dump. The shipping and disposal of toxic wastes are becoming increasingly

expensive and pose a threat to companies by exposing them to potentially ruinous future liability suits. Furthermore, mistakes in waste processing frequently result in fines for illegal sewer discharges (Legg *et al.*, 1996).

#### **1.1.4 Sources of Contaminants to Electroplating Wastewater**

Contaminants in the wastewater from electroplating shops 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).

Discarded process solutions are another source of wastewater contaminant. These solutions are primarily spent alkaline and acid cleaners used for surface preparation of parts before electroplating. The solutions are not usually made up of metals; however a few cleaners contain cyanide. Plating baths and other process solutions containing high metal concentrations, such as chromate solutions are rarely discarded. However, some shops do discard such solutions on a regular basis (Cushnie, 1985).

Accidental spills, leaks and drips of process solutions also can contribute significantly to wastewater contamination. In some shops, the dripping of plated parts is a significant source of pollution. Process solution tanks and rinse tanks are

often separated by several feet. Carrying the racks of parts between tanks will cause plating solution or drag-out to drip on the floor and enter the drain system (Cushnie, 1985).

Other sources of contaminants include sludges from the bottom of plating baths generated during chemical purification, backwash from plating tank filter systems and stripping solutions. These sources, however are not as common as those described earlier (Cushnie, 1985).

#### **1.1.5 Regulation of Electroplating Wastewater**

The high concentrations of metals in wastewater discharges from electroplating operations will cause severe effects on the environment and public health unless being removed before discharge. In Malaysia, the electroplating industry has been reported as one of the major polluter to Straits of Malacca. This industry generates pollutants such as heavy or trace metals including chromium (Mohd Nizam, 1995). This heavy metal especially the Cr(VI) species has been known as toxic heavy-metal and being carcinogenic. The chemistry of chromium and the health issues arise from Cr(VI) are as described in section 1.3.1.5 and 1.3.1.6 respectively. Due to their high toxicity, the industrial wastewaters containing heavy metals are strictly regulated and must be treated before being discharged in the environment. The industrial wastewaters must meet the parameter limits as stated in the Environmental Quality (Sewage and Industrial Effluents) Regulations 1978 (Table 1.2) (Malaysia, 1986).

Table 1.2: Environmental Quality Act 1974.

**THIRD SCHEDULE**  
**ENVIRONMENTAL QUALITY ACT 1974**  
**ENVIRONMENTAL QUALITY (SEWAGE AND INDUSTRIAL EFFLUENTS)**  
**REGULATIONS 1978**  
 [Regulation 8 (1), 8 (2), 8 (3)]  
**PARAMETER LIMITS OF EFFLUENT OF STANDARDS A AND B**

Parameter	Unit	Standard	
		A	B
(1)	(2)	(3)	(4)
(i) Temperature .. .. .	C	40	40
(ii) pH Value .. .. .	—	6.0-9.0	5.5-9.0
(iii) BOD <sub>5</sub> at 20°C .. .. .	mg/l	20	50
(iv) COD .. .. .	mg/l	50	100
(v) Suspended Solids .. .. .	mg/l	50	100
(vi) Mercury .. .. .	mg/l	0.005	0.05
(vii) Cadmium .. .. .	mg/l	0.01	0.02
(viii) Chromium, Hexavalent .. .. .	mg/l	0.05	0.05
(ix) Arsenic .. .. .	mg/l	0.05	0.10
(x) Cyanide .. .. .	mg/l	0.05	0.10
(xi) Lead .. .. .	mg/l	0.10	0.5
(xii) Chromium, Trivalent .. .. .	mg/l	0.20	1.0
(xiii) Copper .. .. .	mg/l	0.20	1.0
(xiv) Manganese .. .. .	mg/l	0.20	1.0
(xv) Nickel .. .. .	mg/l	0.20	1.0
(xvi) Tin .. .. .	mg/l	0.20	1.0
(xvii) Zinc .. .. .	mg/l	1.0	1.0
(xviii) Boron .. .. .	mg/l	1.0	4.0
(xix) Iron (Fe) .. .. .	mg/l	1.0	5.0
(xx) Phenol .. .. .	mg/l	0.001	1.0
(xxi) Free Chlorine .. .. .	mg/l	1.0	2.0
(xxii) Sulphide .. .. .	mg/l	0.50	0.50
(xxiii) Oil and Grease .. .. .	mg/l	Not Detectable	10.0

**FOURTH SCHEDULE**  
**ENVIRONMENTAL QUALITY ACT 1974**  
**ENVIRONMENTAL QUALITY (SEWAGE AND INDUSTRIAL EFFLUENTS)**  
**REGULATIONS 1978**  
 [Regulation 8 (1)]

**LIST OF CATCHMENT AREAS WHERE STANDARD A APPLIES**

1. The catchment areas referred to in this regulation shall be the areas upstream of surface or above sub-surface water supply intakes, for the purpose of human consumption including drinking.
2. For the purpose of this regulation, the water supply intakes shall include the public water supply intakes specified below;

## 1.2 Heavy-metals and Their Toxicity

### 1.2.1 Heavy-metals

Heavy-metals are metals with a density above  $5 \text{ gcm}^{-3}$ , which is five times higher than water. Of the 90 naturally occurring elements, 21 are non-metals, 16 are light-metals and the remaining 53 (with As included) are heavy-metals. Most heavy-metals are transition elements with incompletely filled d orbitals. These d orbitals provide heavy-metal cations with the ability to form complex compounds which may or may not be redox-active. Thus, heavy-metal cations play an important role as trace elements in sophisticated biochemical reactions (Nies, 1999).

A trace element is considered essential if it meets the following criteria: it is present in all healthy tissues of living things; its concentration from one animal to the next animal is fairly constant; its withdrawal from the body induces, reproducibly the same physiological and structural abnormalities regardless of the species studied; its addition either reverses or prevents these abnormalities; the abnormalities induced by deficiency are always accompanied by pertinent, significant biochemical changes and these biochemical changes can be prevented or cured when the deficiency is corrected. A total of 30 elements are now believed to be essential to life (Figure 1.1). They can be divided into the 6 structural elements, 5 macrominerals and 19 trace elements (Florence, 1989).

Bulk Elements

Essential Trace Elements

Essential Trace Elements (Proposed)

H																				He
Li	Be													B	C	N	O	F	Ne	
Na	Mg													Al	Si	P	S	Cl	Ar	
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr			
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe			
Cs	Ba		Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn			
Fr	Ra		<div> <div>Lanthanides</div> <div>Actinides</div> </div>																	

**Figure 1.1** : Essential elements and the periodic table.

Virtually, all metals whether essential or inessential can exhibit toxicity above certain threshold concentrations which for highly toxic metal species may be extremely low. The toxicity caused by heavy-metals is generally a result of strong coordinating abilities (Gadd, 1992). Certain metals have been known to be toxic for centuries. For example, Theophrastus of Erebus (370-287 B.C.) and Pliny the Elder (23-79) both described poisonings that resulted from arsenic and mercury. Other heavy-metals, such as cadmium were not recognized as poisonous until the early nineteenth century (Young, 2000).

### 1.2.2 Biogeochemistry of Heavy-metals

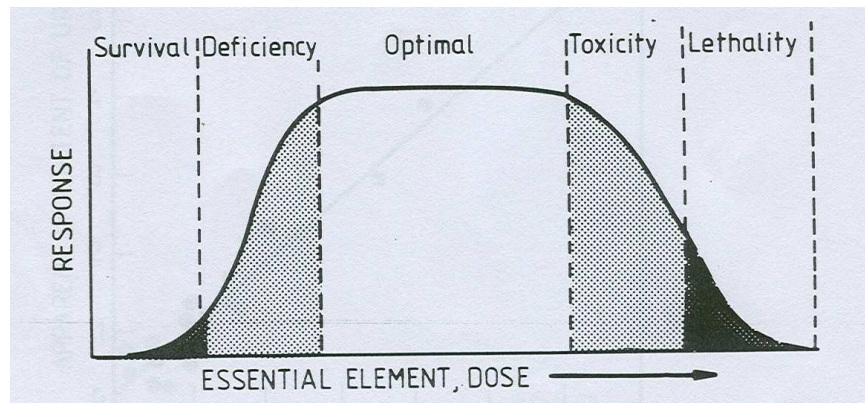
Heavy-metals occur naturally in the environment in rocks and ores and cycle through the environment by geological and biological means. The geological cycle begins when water slowly wears away rocks and dissolves the heavy-metals. The heavy-metals are carried into streams, rivers, lakes and oceans and may be deposited in sediments at the bottom of the water body or they may evaporate and be carried elsewhere as rainwater. The biological cycle includes accumulation in plants and animals and entry into the food web (Young, 2000). Some heavy-metals are not

available to the living cell in the usual ecosystems. They may be present in the earth's crust only in very low amounts or the ion of the particular heavy-metal may not be soluble (Nies, 1999).

### **1.2.3 Heavy-metal Contamination and Toxicity**

It has been realized that sometimes the natural cycles can pose a hazard to human health because the level of heavy-metals exceed the body's ability to cope with them. The situation becomes worst by the addition of heavy-metals to the environment as a result of both the rapidly expanding industrial and domestic activities. The metals are introduced into the environment during mining, refining of ores, combustion of fossil fuels, industrial processes and the disposal of industrial and domestic wastes (Xie *et al.*, 1996). Human activities also create situations in which the heavy-metals are incorporated into new compounds and may be spread worldwide (Young, 2000).

Many aquatic environments face metal concentrations that exceed water criteria designed to protect the environment, animals and humans. Every essential element is toxic if taken in excess and there is a safe window for essential dose between deficiency and toxicity (Figure 1.2). Some elements such as Ca and Mg have wide window whereas others such as Se and F have narrow window whereby an excess will rapidly lead to toxicity and death. Metal toxicity can be divided into three categories i.e. blocking the essential biological functional groups of molecules, displacing the essential metal ion in biomolecules and modifying the active conformation of biomolecules (Florence, 1989). The toxicity effects greatly depend on the bioavailability of the toxicant meaning the proportion of the contaminant present in the environment in the form(s) that can be assimilated by organism (Petänen, 2001).



**Figure 1.2 :** Dose response curve for an essential element.

The health hazards presented by heavy-metals depend on the level of exposure and the length of exposure. In general, exposures are divided into two classes: acute exposure and chronic exposure. Acute exposure refers to contact with a large amount of the heavy-metal in a short period of time. In some cases the health effects are immediately apparent; in others the effects are delayed. Chronic exposure refers to contact with low levels of heavy-metal over a long period of time (Young, 2000).

## **1.3 Chromium And Biosorption**

### **1.3.1 Chromium**

#### **1.3.1.1 General description**

Chromium is a transition metal. It is a hard, steel gray, shiny metal that breaks easily. It has a melting point of 1 900°C, a boiling point of 2 642°C and a density of 7.1 gcm<sup>-3</sup>. 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<sub>2</sub>O<sub>3</sub>). The chromium oxide formed acts as a protective layer, preventing the metal from reacting further with oxygen (Young, 2000).

#### **1.3.1.2 Discovery and naming**

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).

### 1.3.1.3 Occurance

Chromium ranks about 20<sup>th</sup> among the elements present in the earth's crust with an abundance of about 100-300 ppm. It never occurs as a free element, but is found primarily in the form of chromite, a chromium iron ore ( $\text{FeCr}_2\text{O}_4$ ) (Young, 2000).

### 1.3.1.4 Industrial uses

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 is also added to cobalt and nickel-base alloys for the same purpose. Chromium coatings are applied on the surface of other metals for decorative purposes, to enhance resistance and to lower the coefficient of friction. Radioactive chromium-51 is used as a tracer in the diagnosis of blood volume (Patnaik, 2003).

Chromium(II) chloride is used as a 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).

Chromium(III) chloride is used for chromium plating, as a textile mordant, in tanning, as a waterproofing agent and as a catalyst for polymerization of olefins. Chromium(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

textile industry, in leather tanning, to dissolve gelatin, to impart green color to paints, varnishes, inks and ceramic glazes and as a catalyst. Chromium(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. Chromium(III) fluoride is used in printing and dyeing wools, mothproofing woolen materials, metal polishing and coloring marbles. Chromium(III) hydroxide trihydrate is used as green pigment, as mordant, as a tanning agent and as a catalyst (Patnaik, 2003).

Chromium(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).

#### **1.3.1.5 The chemistry of chromium**

Chromium can exist in several chemical forms displaying oxidation numbers from 0 to VI. Only two of them, Cr(III) and Cr(VI) are stable enough to occur in the environment. Cr(IV) and Cr(V) form only unstable intermediates in reactions of trivalent and hexavalent oxidation states with oxidizing and reducing agents, respectively (Kotaś and Stasicka, 2000).

The Cr(III) oxidation state is the most stable and considerable energy would be required to convert it to lower or higher states. Cr(III) presence, concentration and forms in a given compartment of the environment depend on different chemical and physical processes i.e. hydrolysis, complexation, redox reactions and adsorption. Cr(III) is a hard acid which exhibits a strong tendency to form hexacoordinate octahedral complexes with a variety of ligands such as water, ammonia, urea,

ethylenediamine and other organic ligands containing oxygen, nitrogen or sulphur donor atoms. The complexation of Cr(III) by ligands other than  $\text{OH}^-$  increases its solubility when the ligands are in discrete molecules or ions forms. When, however, donor atoms are bound in a macromolecular system, as humic acids, then the Cr(III) complex is more or less immobile. If the complexation from these ligands can be neglected, under redox and pH conditions normally found in natural systems, Cr is removed from the solution as  $\text{Cr}(\text{OH})_3$  or in the presence of Fe(III), in the form of  $(\text{Cr}_x, \text{Fe}_{1-x})(\text{OH})_3$  (where  $x$  is the mole fraction of Cr). The redox potential of the Cr(VI)/Cr(III) couple is high enough, thus only a few oxidants are present in natural systems capable of oxidizing Cr(III) to Cr(VI). Oxidation of Cr(III) by dissolved oxygen without any mediate species has been reported to be negligible, whereas mediation by manganese oxides was found to be the effective oxidation pathway in environmental systems (Kotaś and Stasicka, 2000).

Cr(VI) forms several species, the relative proportions of which depend on both pH and total Cr(VI) concentration.  $\text{H}_2\text{CrO}_4$  belongs to the strong acids and at  $\text{pH} > 1$ , its deprotonated forms are prevailing while above pH 7 only  $\text{CrO}_4^{2-}$  ions exist in solution throughout the concentration range. In the pH between 1 and 6,  $\text{HCrO}_4^-$  is the predominant form up to the Cr(VI) concentration  $10^{-2}$  M when it starts to condense yielding the orange-red  $\text{Cr}_2\text{O}_7^{2-}$ . Within the normal pH range in natural waters, the  $\text{CrO}_4^{2-}$ ,  $\text{HCrO}_4^-$  and  $\text{Cr}_2\text{O}_7^{2-}$  ions are the forms expected. They constitute a lot of Cr(VI) compounds which are quite soluble and thus mobile in the environment. However, Cr(VI) oxyanions are readily reduced to trivalent forms by electron donors such as organic matter or reduced inorganics species, which are ubiquitous in soil, water and atmospheric systems (Kotaś and Stasicka, 2000).

### 1.3.1.6 Health issues

Chromium is unique among regulated toxic elements in the environment in that different species of chromium, specifically Cr(III) and Cr(VI), are regulated in different ways based on their differing toxicities. All other toxic elements such as lead, cadmium and arsenic are regulated based on their total concentrations, irrespective of their oxidation state (Kimbrough *et al.*, 1999). The reduction/oxidation reactions between Cr(VI) and Cr(III) are thermodynamically possible under physiological conditions, thus chromate and Cr(III) are both biologically important ions. Chromate is more toxic than Cr(III), so beneficial functions of chromium can only be performed by Cr(III) (Nies, 1999).

Cr(III) is relatively immobile in the aquatic system due to its low solubility in water. The low solubility retains Cr(III) in the solid phase as colloids or precipitates (Lin, 2002). It is known that Cr(III) is essential for the maintenance of an effective glucose, lipid and protein metabolism in mammals (Marqués *et al.*, 2000). Cr(III) salts such as chromium polynicotinate and chromium picolinate are used as micronutrients and dietary supplements (Bagchi *et al.*, 2001). 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).

On the other hand, Cr(VI) compounds can be toxic for biological systems (Marqués *et al.*, 2000). These compounds have been considered to be a group 'A' human carcinogen (Bai and Abraham, 2001). Cr toxicity is related to the process of reduction of Cr(VI) to lower oxidation states, not necessarily to Cr(III), in which free radicals are generated. Reduction of Cr(VI) to Cr(III) has been reported in many biological systems; transient formation of Cr(V) is the most likely mechanism involved in Cr toxicity. Cr(V) complexes are formed from Cr(VI) by physiological reducing agents such as NAD(P)H, FADH<sub>2</sub>, several pentoses and glutathione. These complexes react with H<sub>2</sub>O<sub>2</sub> to generate significant amounts of ·OH radicals with no associated generation of O<sub>2</sub><sup>-</sup>. The ·OH radicals may trigger directly DNA alterations

as well as other toxic effects. Additional intracellular chromate-reducing agents are vitamins C and B<sub>12</sub>, cytochrome P-450 and the mitochondrial respiratory chain. Intracellular Cr(III) may be sequestered by DNA phosphate groups affecting replication, transcription and causing mutagenesis. Oxidative damage on DNA is considered the basis of the genotoxic effects produced by Cr. Cr(III) may also react with carboxyl and sulfhydryl groups of enzymes causing alterations in their structure and activities. Modification of the DNA polymerase and other enzyme activities may be caused by the displacement of magnesium ions by Cr(III) (Cervantes *et al.*, 2001).

Meanwhile, chromium is a non-essential metal for plants and microorganisms (Viti and Giovannetti, 2001). 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 (Brady and Duncan, 1994).

## **1.4 Removal of Heavy Metals from Aqueous Waste Streams**

### **1.4.1 Conventional Treatments and the Disadvantages**

Many procedures have been applied in order to remove heavy-metals from aqueous streams. Among the most commonly used techniques are chemical precipitation, chemical oxidation and reduction, ion-exchange, filtration, electrochemical treatment, reverse osmosis (membrane technologies), evaporative recovery and solvent extraction (Xia and Liyuan, 2002).

These classical or conventional techniques give rise to several problems such as unpredictable metal ions removal and generation of toxic sludges 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 from 1 to 100 mg/L of dissolved metal(s) (Cossich *et al.*, 2002). Another disadvantage of using these classical techniques for heavy-metal removal is the extremely expensive cost due to the high 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. Section 1.4.2 discusses the different interactions between microorganism with metal which enable metal removal.

#### **1.4.2 Metal-microorganism interactions**

Microorganisms cannot destroy metals but they can alter their chemical properties via a surprising array of mechanisms. The microbiological processes or mechanisms for the removal of metals from solution have been observed in the laboratory and in natural environments where conditions are suitable for specific types of biological activity. The mechanism can be divided into three categories: the adsorption of metal ions onto the surface of microorganism; the intracellular uptake of metals and the chemical transformation of metals by biological agents (Brierley, 1982).

Most microorganisms have a negative charge owing to the presence of negatively charged groups of atoms on the cell membrane and cell wall. The charged groups or ligands are responsible for the adsorption of positively charged metal ions in solution. The adsorption is typically rapid, reversible and independent of temperature and energy metabolism. The deposition of insoluble metals has been observed at the surface of some microorganisms (Brierley, 1982). Recent comparisons have suggested that biosorbents may be cheaper to implement than other commercially available ion-exchange resins (Lloyd, 2002).

Microorganisms ordinarily take up some ions that are necessary for cellular activity. The transport systems for the ions are dependent on both temperature and energy. Although the mechanism by which the cells assimilate the ions are highly selective, substitutions are possible. The phenomenon sometimes cause intracellular accumulation of very high concentrations of toxic metals (Brierley, 1982).

Microorganisms have evolved a wide range of biochemical tricks to protect themselves from potentially toxic metals and these natural activities can be used for bioremediation applications. Some metal ions can be reduced to a less toxic oxidation state. To be detoxified by reduction, the redox potential of a given metal should be between that of the hydrogen/proton couple and that of the oxygen/hydrogen couple, which is the physiological redox range of most aerobic cells. A metal compound that can be reduced should be able to diffuse out of the cell or it might re-oxidize itself. Thus, if the cell chooses to detoxify such a compound by reduction, an efflux system should be present to export the reduced products (Nies, 1999). The efflux or exclusion of metal ions from the cell sometime can result in high local concentrations of metals at the cell surface where they may react with biogenic ligands and precipitate (Lloyd, 2002).

Many microorganisms synthesize specific chelation compounds that immobilize heavy-metals by precipitating them in chelates (Brierley, 1982). Bacteria can involve in metal precipitation reactions either directly as catalysts of aqueous

chemical reactions or indirectly as geochemically reactive solids. In the first case, metabolic activity of the organism is important in developing supersaturated conditions that allow precipitation to occur, through the production of reactive ligands such as sulfide or carbonate. The local concentrations of these products of metabolic processes alone is sufficient to lower the energy barrier for both homogenous and heterogenous nucleation reactions to occur. In the latter case, adsorption of metal ions to reactive sites on bacterial cell surfaces encourages heterogenous nucleation and precipitation (Webb, 2001).

#### **1.4.3 Potential of Biosorption for the Removal of Chromium from Wastewater**

The attempts to remove Cr compounds from wastewater with biological methods have been performed especially using phytoremediation techniques, biosorption, bioaccumulation and bioprecipitation as well as bacterial activated sludge treatment (Ksheminska *et al.*, 2003). Biosorption which is one of the emerging biological methods poses several advantages over the conventional method among which are: the process does not produce chemical sludge, hence non-polluting, it is easy to operate and very efficient for removal of pollutants from very 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). Besides that, the use of microorganisms, particularly bacteria as sorbents in wastewater treatment plants may be competitive compared to conventional technologies whereby the very small size of bacterial particles (0.2 to 0.5  $\mu\text{m}$ ), which permits close contact between the biosorbent and the solution (Vecchio *et al.*, 1998). 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, 1990). 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 1.3). Though none of the biosorbent technologies have been reported to replace the conventional treatment methods, bioremediation could be considered as an eco-friendly complementary device to the existing high cost technologies (Bai and Abraham, 2002).

**Table 1.3 :** Laboratory studies involving biosorption of several solutes using several biological materials.

Biosorbent	Solutes	Reference
Brown, green and red seaweeds	Metal	Hashim and Chu (2004)
Cyanobacterium ( <i>Microcystis aeruginosa</i> )	Uranium	Li <i>et al.</i> (2004)
Marine algae	Heavy-metals	Sheng <i>et al.</i> (2004)
Seaweed	Metal	Volesky <i>et al.</i> (2003)
<i>Bacillus firmus</i>	Heavy-metals	Salehizadeh and Shojasodati (2003)
Marine miroalga ( <i>Tetraselmis suecica</i> )	Heavy-metal	Rama <i>et al.</i> (2002)
Fungi ( <i>Cladosporium cladosporioides</i> )	Metal	Pethkar <i>et al.</i> (2001)
Brown seaweed	Heavy-metal	Yun <i>et al.</i> (2001)
<i>Pseudomonas aeruginosa</i> and <i>Bacillus thuringiensis</i>	Heavy-metals	Hassen <i>et al.</i> (1998)

Living cells have been used and pretreatment using physical or chemical means with the objective to increase the metal biosorption capacity has been suggested (Kapoor and Viraraghavan, 1998). 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).

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 the performance of different biosorbents (Davis *et al.*, 2003). Among the isotherms, the Langmuir and Freundlich models are the most frequently used to describe metal biosorption (Ledin, 2000). Table 1.4 shows agreement with Ledin (2000) for Cr(VI) biosorption studies using different types of biosorbents.

**Table 1.4 :** Cr(VI) sorption isotherm studies using different types of biosorbents.

Biosorbent	Sorption isotherm used	Reference
<i>C. reinhardtii</i>	Langmuir	Arica <i>et al.</i> (2005)
<i>Lentinus sajor-caju</i> mycelia	Langmuir	Bayramoğlu <i>et al.</i> (2005)
<i>Mucor hiemalis</i>	Langmuir	Tewari <i>et al.</i> (2005)
<i>Pantoea</i> sp. TEM 18	Langmuir	Ozdemir <i>et al.</i> (2004)
<i>Aeromonas caviae</i>	Langmuir	Loukidou <i>et al.</i> (2004)
Cationic surfactant-modified yeast	Langmuir	Bingol <i>et al.</i> (2004)
Seeds of <i>Ocimum basilicum</i>	Langmuir	Melo and D'Souza (2004)
<i>Bacillus licheniformis</i> , <i>Bacillus laterosporus</i>	Langmuir	Zouboulis <i>et al.</i> (2004)
Low cost sorbents	Langmuir.	Fiol <i>et al.</i> (2003)
<i>Saccharomyces cerevisiae</i>	Langmuir	Ozer and Ozer (2003)
<i>Dunaliella</i> sp 1 and <i>Dunaliella</i> sp 2	Langmuir and Freundlich	Donmez and Aksu (2002)
Cone biomass	Freundlich	Ucun <i>et al.</i> (2002)
<i>Rhizopus nigricans</i>	Freundlich.	Bai and Abraham (2001)
<i>Rhizopus arrhizus</i>	Freundlich	Prakasham <i>et al.</i> (1999)

## 1.5 Objective of Thesis

The aim of this research is to screen and characterize new biosorbents to be used for removal of toxic heavy-metal, i.e. Cr(VI) from chromium electroplating wastewater. Many types of microorganisms have been studied for their adsorption capabilities of heavy-metal. In this particular research, indigenous microorganisms in local textile wastewater will be isolated and investigated for their Cr(VI) uptake capacity in both simulated and real electroplating wastewater.

## 1.6 Scope of Thesis

In order to achieve the objective of this thesis, this research was designed with initial effort of isolation and characterization of microorganisms from local textile wastewater. Both Cr(III) and Cr(VI) toxicity tests were carried out on the isolated microorganisms in defined and complex media using repli-plate technique. This was followed by metal uptake studies in simulated wastewater i.e. Cr(VI) solution. Preliminary metal uptake studies were carried out to investigate the effect of operational parameters on metal uptake by the non-living biomass. The effect of pH ranging from 2 to 12 on metal uptake was studied. The experiments on effect of contact time, biomass dosage and acetic acid pretreatment were conducted at an initial pH of 8. The biosorption isotherm for the native of the best biosorbent was studied at pH 8 using the optimum operational parameters. Eventually, the best biosorbent was studied for Cr(VI) uptake from real chromium-bearing wastewater. It is worth mentioning here that initially textile wastewater was chosen but as Cr(VI) concentration in the wastewater fluctuated with time, chromium electroplating wastewater was used in the study. The study was conducted at both original pH (acidic) and pH 8. Last but not least, the mechanism of Cr(VI) biosorption by the best biosorbent was determined using FTIR analysis, SEM and TEM.

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