

# **CONVERSION OF Cr (VI) IN WATER AND SOIL USING RHAMNOLIPID**

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# **ABSTRACT**

## **CONVERSION OF Cr (VI) IN WATER AND SOIL USING RHAMNOLIPID**

**Ismat Ara**

Hexavalent chromium is of great concern now as it has highly toxic and carcinogenic properties and also for its potential for the contamination of groundwater due to its greater mobility in soils and in the aquatic environment. The objective of this study is the reduction of hexavalent chromium to trivalent chromium using rhamnolipid both in water and soil media. Rhamnolipid had been used as its toxicity is very low as a commercial surfactant and it is readily biodegradable with a very low environmental impact.

Batch experiments were performed to evaluate the feasibility of using rhamnolipid for the removal and reduction of hexavalent chromium from contaminated soil and water. The initial chromium concentration, rhamnolipid concentration, pH and temperature were evaluated and found to affect the reduction efficiency. The rhamnolipid can reduce 100% of initial Cr (VI) in water at optimum conditions (pH 6, 2% rhamnolipid concentration, 25°C) if the concentration is low (10 ppm). For higher initial concentrations (400 ppm), it takes time to reduce (24.4% in one day). In the case of soil, rhamnolipid only can remove the soluble part of the chromium present in the soil. The extraction increased with the increase of initial concentration in soil but decreased slightly with the increase of temperature above 30°C. The reduction trend of the extracted chromium is the same as in water media. A sequential extraction study was used on soil before and after washing to

determine from what fraction the rhamnolipid removed the chromium. The exchangeable and carbonate fractions accounted for 24% and 10% of the total chromium, respectively. The oxides and hydroxides portion retained 44% of chromium present in the soil. On the other hand, 10% and 12% of the chromium was associated with the organic and residual fractions. Rhamnolipid can remove most of the exchangeable (96%) and carbonate (90%) portions and some of the oxide and hydroxide portion (22%) but cannot remove chromium from the other fractions. This information is important in designing the appropriate conditions for soil washing.

## **Acknowledgements**

I would like to express my sincere gratitude and appreciation to my supervisor Dr. Catherine N. Mulligan for her guidance, suggestions, encouragement and assistance throughout the course of this study.

I also want to extend my thanks to all my colleagues for their advice and helping hands.

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## **List of Abbreviations**

|      |  |
|------|--|
| AA   | Atomic Absorption                          |
| ASTM | American Society for Testing and Materials |
| CEC  | Cation Exchange Capacity                   |
| CMC  | Critical Micelle Concentration             |
| CPC  | Cetylpyridinium Chloride                   |
| CTAB | Cetyltrimethylammonium Bromide             |
| DPC  | Diphenylcarbazide                          |
| EPA  | Environmental Protection Agency (U.S)      |
| MEUF | Micellar- Enhanced Ultrafiltration         |
| NPL  | National Priority List                     |
| ODA  | Octadecylamine Acetate                     |
| SDS  | Sodium Dodecyl Sulfate                     |
| SRB  | Sulfate Reducing Bacteria                  |
| S/S  | Solidification/Stabilization               |
| SSE  | Selective Sequential Extraction            |
| TCE  | Trichloroethylene                          |
| USDA | U.S. Department of Agriculture             |

# **1 Introduction**

## **1.1 Background**

The release of heavy metals into the environment is a serious and ongoing problem. It is becoming a growing concern as the accumulation of heavy metals in the various compartments of the environment could lead to toxic effects on life (Pagilla et al., 1999). The current anthropogenic input is responsible for many soils that are now or soon will become overloaded with toxic metals (Arnfolk et al., 1996). About 63% of the sites of the National Priority List (NPL) are contaminated with toxic heavy metals (Hazardous Waste Consultant, 1996). So, toxic heavy metal contamination of soil and the subsequent impact on groundwater are a major problem in the environment.

The heavy metal pollution of soil results in negative environmental impacts including groundwater contamination. The percolation of rainwater through soil causes slow extraction of metals which may reach aquifers (Arnfolk et al., 1996). Domestic and industrial effluents, the atmosphere, runoff and lithosphere are the sources of metals. Once metals are allowed to pass through the municipal waste treatment facility, the environment receives the heavy metals which are non-biodegradable and persistent and thus follow a number of different pathways. The metals can adsorb onto the soil, runoff into rivers or lakes or leach into the groundwater which is an important source of drinking water. Exposure to heavy metals by taking drinking water and foods can lead to accumulation in animals, plants and humans (Mulligan et al., 2001b).

The improper storage or burial of production residues from different industrial or commercial sites leads to the toxic heavy metal contamination of soil and subsequent groundwater contamination. Sometimes leaking or mishandling in transportation of different hazardous materials cause the contamination. The soil contamination contributes not only to pollution of groundwater but also results in restricted utilization of the site and in some cases, a complete prohibition on cultivation or other potential use of the area (Abumaizar et al., 1999).

Among numerous heavy metals of concern, chromium is of particular interest as both surface waters and groundwater areas are contaminated by the extent of chromium pollution (Pagilla et al., 1999). In the United States, chromium is the second most common inorganic contaminant and at hazardous waste sites it is the third most common pollutant (Xu et al., 2004). The Ministry of Environment in Quebec (2003) published that there were 69 chromium contaminated sites in the province of Quebec which have to be treated or rehabilitated for further development. According to the regulations of the Ministry of Environment in Quebec, the allowable concentration of total chromium for water is 50 µg/L which matches with U.S.EPA requirements. For soil the limits of concentrations are 250 mg/kg and 800 mg/kg for residential and industrial/commercial soils respectively.

Chromium is present in soils principally in the hexavalent chromium [Cr (VI)] or trivalent chromium [Cr (III)] forms. Cr (VI) is of great concern as it has highly toxic and carcinogenic properties and also for its potential for the contamination of groundwater

due to its greater mobility in soils and in the aquatic environment. Therefore, to protect the public health and the environment, the remediation of chromium contaminated sites is a top priority (Reddy et al., 1997).

## **1.2 Problem statement**

Heavy metals at many contaminated sites are a major problem. They continue to be added to air, water and soil in increasing amounts from different sources like industrial, agricultural, military activities and domestic effluents. Therefore, heavy metals are now widely dispersed in the environment in a range of various physicochemical forms. They are one of the worst groups of pollutants of the environment and the release of them into the environment has become an increasing concern due to their potential reactivity, toxicity and mobility in the soil.

To decrease costs, various technologies have been developed and implemented for the remediation of water, soil and sediments contaminated with heavy metals. A few remediation technologies include soil excavation, thermal extraction for volatile metals, electrokinetics, solidification/stabilization, vitrification, chemical oxidation, soil flushing and bioremediation (Mulligan et al., 2001b). The speciation of the contaminants and other site-specific characteristics are among the criteria for a specific technology selected for the treatment of a contaminated site. Another important consideration is that the selected method does not leave toxic residues which must be subsequently removed (Roundhill, 2001).



Bioremediation has the potential for the remediation of heavy metal contaminated sites. It has been established that biosurfactants which are produced from bacteria and yeasts have the potential for environmental remediation of heavy metals from soil and sediments (Mulligan et al., 2001a). As biosurfactants solubilize and disperse contaminants as a soil washing technique, they are a potential solution for heavy metal removal. An attractive characteristic is that they can be produced from readily available and renewable substrates such as sugars and food grade oils. Other advantages include effectiveness at extreme temperatures, pH and salt concentrations, low critical micelle concentration (CMC) values and high degrees of effectiveness in lowering the surface tension (Mulligan and Gibbs, 1993). Therefore, biosurfactants present effective and nontoxic candidates for the remediation of contaminated sites.

This thesis will focus on the investigation of the mechanisms of metal treatment (hexavalent chromium) by selected biosurfactants (rhamnolipid) to understand its role in the enhancement of soil washing.

### **1.3 Objectives**

The objectives of this research are as follows:

- To determine the feasibility of using biosurfactant (Rhamnolipid JBR210) to enhance the removal and reduction of hexavalent chromium in water and soil media.
- To investigate the factors influencing the reduction efficiencies.

## **1.4 Organization of the thesis**

The thesis is divided into five chapters. The contents are as follows:

- Chapter 1 includes the introduction of the subject, description of the problem, the objectives and organization of this particular study.
- Chapter 2 describes the background information for the various concepts covered in the thesis and a literature review of similar studies.
- Chapter 3 includes the materials, methods and procedures used in the experiments.
- Chapter 4 summarizes the results obtained from different experiments and discussion.
- Chapter 5 includes the conclusions, recommendations for further work and contributions.

## 2 Literature Review

### 2.1 Effects of heavy metals

The metals which constitute elements with high toxicity and density ( $\rho > 5 \text{ g/cm}^3$ ) are known as heavy metals. These are elements which lose electrons to form positive ions (Yong et al., 1992). 'Heavy metals' is a loosely defined term but it is recognized world wide and the elements such as arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and zinc (Zn) are the main components of this category which are commonly associated with pollution and toxicity problems. 'Trace metals' is an alternative name for this group of elements but it is not as widely used (Alloway & Ayres, 1997). The toxicity of heavy metals varies from one to another and the order of decreasing toxicity of heavy metals is as follows:  $\text{Hg} > \text{Cd} > \text{Ni} > \text{Pb} > \text{Cr} > \text{Li}$  (Yong et al., 1992).

Heavy metals occur naturally in rock-forming and ore minerals which lead to a range of normal background concentrations of these elements in soils, sediments, waters and living organism. When high concentrations of the metals occur relative to the background levels, it causes pollution. Although the chemical properties of heavy metals differ from one element to another, they are used widely in electronics, machines and the artifacts of everyday life as well as in 'high-tech' applications. As a result, they tend to reach the environment from a vast array of anthropogenic sources as well as natural geochemical process (Alloway & Ayres, 1997). Although heavy metals are ubiquitous in most natural materials, the mining, ore refining, metal producing and electroplating industries are the

main sources of heavy metal contamination and produce them as waste stream materials (Yong, et al., 1992).

### **2.1.1 Chromium**

The heavy metal chromium is included in the VI B group in the periodic table. Its density at 293 K is  $7.19 \text{ g/cm}^3$ . Chromium occurs mainly in three forms. Metallic chromium [Cr (0)] is a steel-gray solid. Chromium metal does not occur naturally but is produced from the chrome ore. Chromium is usually encountered in the environment in the oxidation states of Cr (III) and Cr (VI) which are known as ‘chromite’ and ‘chromate’ respectively. Trivalent chromium occurs naturally in rocks, soil, plants, animals and volcanic emissions. Hexavalent chromium is produced industrially.

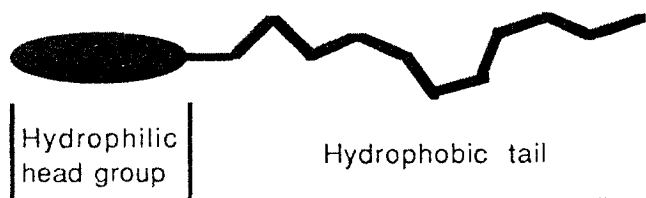
Chromium is one of the most widely used metals in industry which leads to large quantities of discharge of it into the environment and therefore chromium contamination of the environment is extensive (Bartlett, 1991). Chromium (VI), considered to be highly toxic, is often present in industrial wastewaters as chromate ( $\text{CrO}_4^{2-}$ ) and dichromate ( $\text{Cr}_2\text{O}_7^{2-}$ ) (Sen et al., 2005). Wastewaters containing hexavalent chromium are generated by many industries. Major sources of chromium include the metal finishing industry, petroleum refining, leather tanning, iron and steel industries, production of inorganic chemicals, textile manufacturing, dyes, electroplating, metal cleaning, plating, photography, wood treatment and pulp production (Laxman et al., 2002; Xu et al., 2004).

Since hexavalent chromium is a very soluble and mobile species, highly toxic and mutagen and carcinogen, it is considered as a priority pollutant for soils and natural wastes (Erdem et al., 2004). Cr (VI) is toxic to humans, animals, plants and microorganism and is associated with the development of various chronic health disorders including organ damage, dermatitis and respiratory impairment. In the environment, Cr (VI) does not readily precipitate or become bound to components of soil. Therefore, Cr (VI) can move through the sediment into aquifers to contaminate groundwater and other sources of drinking water (Xu et al., 2004).

Cr (VI) compounds are emitted into the air, water and soil by a number of different industries. These compounds are found in the air mainly as fine dust particles which eventually settle over the land and water. They can enter the body when people breathe air, eat food or drink water containing them. Certain compounds of Cr (VI) are carcinogenic in humans but the carcinogenicity is site-specific, i.e., limited to the lung and sinonasal cavity and dependent on high exposures like in an industrial setting (Pellerin & Booker 2000). There are other health effects caused by Cr (VI). The inhalation of relatively high concentrations of Cr (VI) compounds causes the following effects: a runny nose, sneezing, itching, nosebleeds, ulcers and holes in the nasal septum. High level but short-term inhalation has adverse effects at the contact site such as ulcers, irritation of the nasal mucosa and holes in the nasal septum. Kidney and liver damage, nausea, irritation of the gastrointestinal tract, stomach ulcers, convulsions and death might occur after ingestion of very high doses of Cr (VI). Skin ulcers or allergic reactions might be caused by dermal exposures (Pellerin & Booker 2000).

## 2.2 Surfactants

The word surfactant came from the contraction of the descriptive phrase surface-active agent. Surfactants are surface active because they concentrate at interfacial regions (boundaries between at least two immiscible phases). The surface activity of surfactants develops from their amphiphilic structure (containing one soluble/hydrophilic and one insoluble/hydrophobic moiety). In an aqueous system, a surfactant has a polar or ionic hydrophilic portion and a nonpolar hydrophobic portion which are known as the head and tail groups, respectively (West & Harwell 1992).

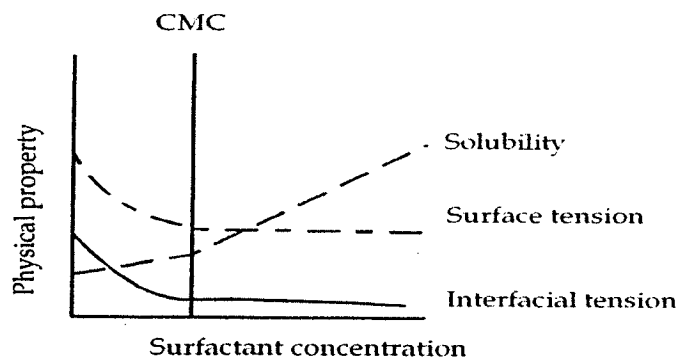


**Figure 2-1: Basic Surfactant Molecular Structure (Myers, 1988)**

Surfactants reduce the free energy of the system by replacing the higher energy bulk phase molecules at an interface. The hydrophobic portion has little affinity for the bulk medium but on the other hand, the hydrophilic group is attracted to the bulk medium (Mulligan et al., 2005). The presence of the hydrophobic group causes a distortion of the solvent (water or organic liquid) liquid structure resulting in an increase of the overall free energy of the system which means less work is required to transport a surfactant molecule to a surface or interface. Since less work is required to bring surfactant

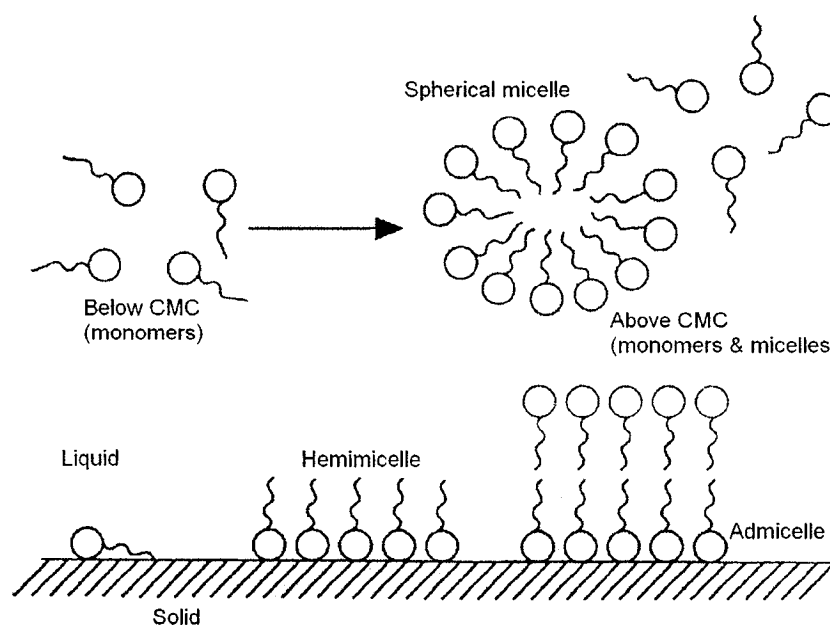
molecules to the available interfaces, the presence of the surfactant decreases the work required to increase the interfacial area. The work per unit area required to form that new interface is known as the surface free energy or surface tension of the system (Myers, 1988). Usually its units are in milliNewtons per meter (mN/m).

The ability of a surfactant to lower the surface tension determines its effectiveness. A good surfactant can lower the surface tension of water from 72 to 35 mN/m. The surface tension correlates with the concentration of the surface active compound until the critical micelle concentration (CMC) is reached. Efficient surfactants have a low critical micelle concentration which means less surfactant is necessary to decrease the surface tension (Mulligan et al., 2005). Figure 2-2 shows how different parameters vary as a function of surfactant concentration.



**Figure 2-2: Surface tension, interfacial tension and solubilization as a function of surfactant concentration (Mulligan et al., 2005)**

The CMC is the minimum concentration of surfactant at which micelle formation initiates. A phenomenon unique to surfactants is the self-assembly of molecules into dynamic clusters called micelles. The micelle is a 3-4 nm diameter structure with an ionic or polar exterior. The CMC is different for every surfactant. In a micelle, the individual monomers get oriented with their hydrophilic portions in contact with the liquid phase and their hydrophobic portions tucked into the interior of the aggregate (West & Harwell 1992). Figure 2-3 shows the arrangement of surfactant molecules.



**Figure 2-3: Surfactant micellization (West and Harwell, 1992)**

In the selection process of surfactant, economic consideration and chemical questions often play an important role. Unless the cost of the surfactant is insignificant as compared to the rest of the system, the material which is least expensive but produce the desired effect usually will be chosen. But economics cannot be the only factor for the selection of



surfactant, since the final performance of the system is the crucial importance (Myers, 1988). Charge-type, physicochemical behavior, solubility and adsorption behavior are some of the most important selection criteria for surfactants (Mulligan et al., 2005).

### **2.2.1 Types of surfactants**

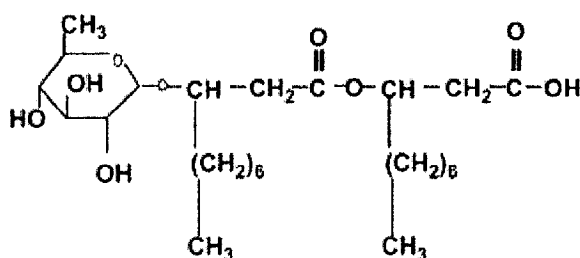
Surfactants are classified according to the nature of the hydrophilic portion of the molecule. The head group may carry a negative charge (anionic), a positive charge (cationic), both negative and positive charges (zwitterionic) or no charge (nonionic). The chemistry of surfactants differs due to the nature of the hydrophobic tails (West & Harwell 1992). Surfactants in each group are used for particular purposes. Cationic, anionic and nonionic surfactants are particularly used for soil washing or flushing while zwitterionic surfactants are used as boosters to enhance the detergency.

### **2.2.2 Biosurfactants**

Bacteria, yeasts and fungi have the capability to synthesize surfactants, commonly referred to as biosurfactants. Most of the biosurfactants are anionic or nonionic and only a small group of them are classified as cationic. The CMC of biosurfactants varies from 1 to 200 mg/L. Biosurfactants can be potentially effective with some distinct advantages over the highly used synthetic surfactants. They are useful due to their anionic nature, low toxicity, biodegradability and excellent surface active properties.

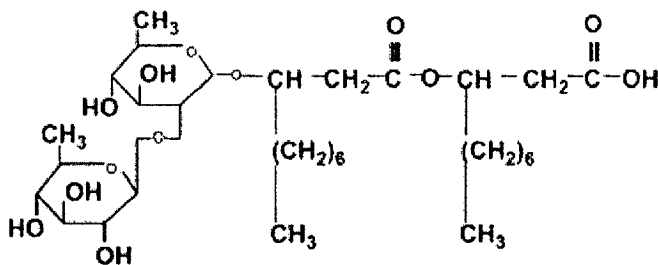
The biosurfactant used in this study is called rhamnolipids which is from the glycolipid group and made by *Pseudomonas aeruginosa*. There are four types of rhamnolipids.

Rhamnolipids type I and type II are suitable for soil washing and heavy metal removal which is the concern of this study. Type III is appropriate for metal processing, leather processing, lubricants, pulp and paper processing while type IV is used in textiles, cleaners, foods, inks, paints, adhesives, personal care products, agriculture adjuvants and water treatment. Figure 2-4 shows the chemical structure of rhamnolipids type I and type II.



**Formula: R1 or RLL:  $C_{26}H_{48}O_9$**

**Molecular Weight: 504**



**Formula: R2 or RRLL:  $C_{32}H_{58}O_{13}$**

**Molecular Weight: 650**

**Figure 2-4: Structure of Rhamnolipids (Adapted from Jeneil Biosurfactant Co., LLC)**

### **2.2.2.1 Rhamnolipid (JBR210)**

The biosurfactant JBR210 is an aqueous solution of 10% rhamnolipid containing two major rhamnolipids, RLL (R1) and RRLL (R2).

In aqueous solutions, JBR210 has a very low critical micelle concentration which indicates the strong surface activity at low concentrations, characterized by low surface tension for water and electrolyte solutions with very low interfacial tensions for water/hydrocarbon systems. It is an excellent emulsifier for a wide range of organic solvents producing emulsions of greatly enhanced stability. It also has corrosion inhibition properties in aqueous solutions particularly for ferrous metals. The general chemical and physical characteristics of JBR210, with the high degree of surface activity produced at very low concentrations, indicates the use of JBR210 as a performance enhancing additive in a wide range of application areas. Various surfactant-based applications of JBR210 are agriculture, building and construction, environmental remediation, metal processing, paper processing and many others.

JBR210 is a readily biodegradable surfactant with a very low environmental impact. Its toxicity is very low as a commercial surfactant and also it has low skin irritation at use. Several tests were done in accordance with U.S.EPA guidelines by an independent laboratory which indicates the degree of biodegradability and toxicity of JBR210 match the EPA requirements.

## 2.3 Soil environment

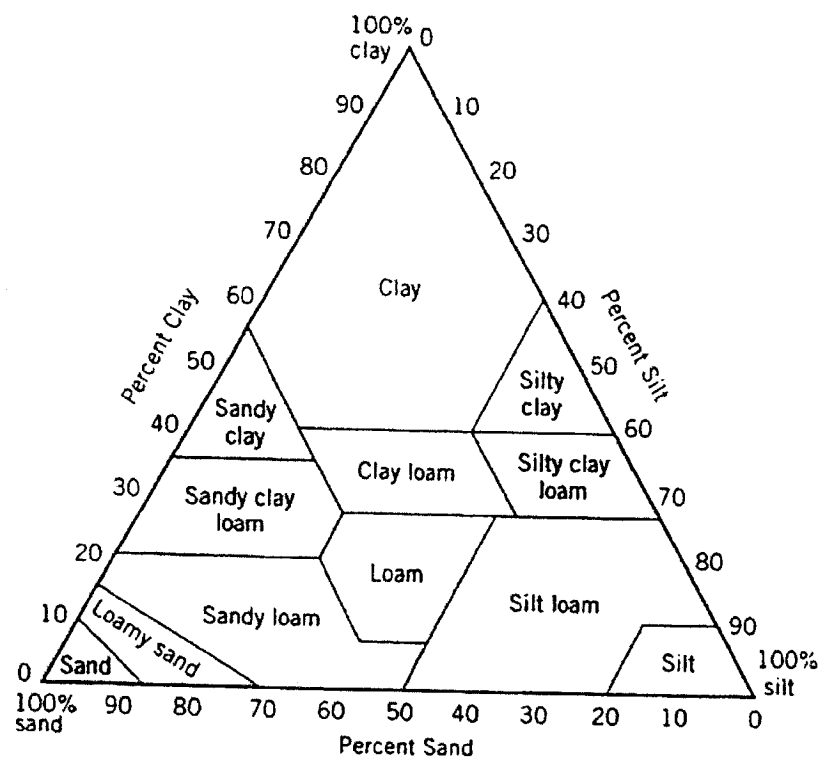
The physical structure and chemical composition of surface and subsurface soils are highly variable. Soils are heterogeneous and complex media composed of solids, liquid and gaseous phases. Soils, whether saturated or unsaturated, are not completely mixed as most aquatic systems are, so soil and groundwater contamination tends to be heterogeneous. Important soil properties that relate to hazardous waste management include the soil textural class, soil mineralogy, bulk density, porosity and organic carbon content (Watts, 1998).

Soils are composed of four major components: an inorganic or mineral fraction, organic matter, water and air. Approximately half of the soil is pore space which consists of air and water. The remaining 50% is solid—the mineral fraction (45% to 49.9%) and organic matter (0.1% to 5%). These numbers are highly variable and depend on site-specific conditions. A system was developed by the U.S. Department of Agriculture (USDA) to classify soils by particle size. The size fractions are classified as gravel, sand, silt and clay.

**Table 2-1: USDA Soil Particle Size Classifications (Watts, 1998)**

| Soil Type | Particle Size Range |
|-----------|---------------------|
| Gravel    | 2.0 – 15 mm         |
| Sand      | 0.075 – 2.0 mm      |
| Silt      | 0.002 – 0.075 mm    |
| Clay      | < 0.002 mm          |

Classification of natural material by such a simple size fractionation is impossible because almost all natural soils contain a percentage of each of the size fractions. Therefore, three general textural classes have been developed—sands, clays and loams. Sands are soils that contain at least 70% of the sand separate. Soils classified as clays consist of at least 35% clay. A loam soil is a general classification that contains equal weights of sand, silt and clay separates (Watts, 1998). The USDA has developed a relationship between the percentage of soil and its textural classes which is shown in Figure 2-5.



**Figure 2-5: Relationship between soil class and particle size distribution (Watts, 1998)**

### 2.3.1 Soil components

The inorganic components of soils represent more than 90% of the solid components which include both primary and secondary minerals. They range in size from clay sized colloids (0.002 mm) to gravel (>2mm) and rocks. A primary mineral is one that has not been altered chemically since its deposition and crystallization from molten lava. Primary minerals occur primarily in the sand and silt fractions of the soils but may be found in slightly weathered clay-sized fractions. Examples of common primary minerals in soils include quartz and feldspar. Other primary minerals found in soils in smaller quantities include pyroxenes, micas, amphiboles and olivines (Sparks, 2003). Primary minerals do not have a very great influence on contaminant retention as they have large particle sizes and low specific surface areas (Yong et al., 1992).

On the other hand, secondary minerals result from the weathering of a primary mineral either by an alteration in the structure or from reprecipitation of the products of weathering of a primary mineral. Common secondary minerals in soils are aluminosilicate minerals such as kaolinite and montmorillonite, oxides such as gibbsite, goethite and birnessite, amorphous materials such as imogolite and allophone, and sulfur and carbonate minerals. The secondary minerals are primarily found in the clay fraction of the soil but can also be located in the silt fraction (Sparks, 2003).

**Clay minerals:** Clay minerals refer to specific minerals that mainly occur in the clay-sized fraction of the soil. They are formed as a result of weathering and are essential components of most soils. The clay minerals in soils play a profound role in affecting

numerous soil chemical reactions and processes (Sparks, 2003). Examples of clay minerals are kaolinite, illite, smectite, vermiculite, mica, chlorite etc. The clay minerals dominate the content in soils and play a dominant role in the retention of contaminants.

**Oxides, hydroxides and oxyhydroxides:** The general term 'oxides' refers to metal hydroxides, oxyhydroxides and hydrous oxides. Aluminum, iron and manganese oxides have significant effects on many soil chemical processes such as sorption and redox because of their high specific surface areas and reactivity. Although these oxides may not be found in large quantities, they play an important role in the chemistry of soils (Sparks, 2003).

**Carbonate and sulfate minerals:** The major carbonates found in soils are calcite, magnesite, dolomite, ankerite and siderite. The major sulfate mineral is gypsum (Sparks, 2003). The carbonate and sulfate minerals are highly soluble and are found in arid and semiarid regions. Carbonates can significantly increase the pH of the soil and can form precipitates with metal contaminants.

**Organic matter:** The content of organic matter in soils is usually small, ranging from 0.5 to 5%. It plays an important role in the retention of contaminants. Soil organic matter is a mixture of plant and animal residues in different stages of decomposition, substances synthesized microbiologically or chemically from the breakdown products and the bodies of live and dead microorganisms and their decomposing remains. It improves soil structure, water-holding capacity, aeration and aggregation. It also contains large

quantities of carbon. Soil organic matter has a high specific surface area and CEC (cation exchange capacity) which causes retention of contaminants (Sparks, 2003).

### **2.3.2 Interactions of soils with contaminants**

The transport of contaminants in the soil by external and internal forces will result in reactions occurring between contaminants and soil constituents. These include chemical, physical and biological processes and are generally identified as contaminant-soil interactions. Contaminant transport is an accumulative process, i.e., physical and chemical processes of contaminant-soil interaction which result in accumulation of contaminants in soil (Yong et al., 1992). Because of the involvement of various factors, the interaction of contaminants, organic or inorganic, in their liquid phase with soil is a very complex matter. Contaminant retention is controlled by the physicochemical and physical properties of the soil solid phase, by the properties of the contaminants themselves and by environmental factors such as temperature and soil moisture content. The composition of the soil is one of the most important factors influencing the retention of contaminants (Yong et al., 1992). The proportion of each of the components affects the retention of contaminants because of their cation exchange capacity, surface area and functional groups. Also, various types of interaction including ionic exchange, adsorption, precipitation, complexation and mechanical trapping of the contaminants in the solid-phase pores are related to contaminant retention by different soil compositions.

**Cation exchange capacity (CEC):** It is the exchange of counter-ions at the solid surface with the ions in solution (Brown, 1954). CEC increases as surface area increases. For the



retention of cations in clay minerals, ion exchange is an important mechanism due to the particle size, crystallinity and the nature of the broken bonds at the surface of the clay minerals (Fitzpatrick, 1980). The cation exchange capacity of a soil also varies with pH. Organic matter has an extensive contribution to the cation exchange capacity of the soil (Foth, 1984). For organic matter, the cation exchange rates are highest, the rate is intermediate for expanding clays and allophanes and lowest for nonexpanding clays and hydrous oxides (Hausenbuiller, 1985).

**Sorption:** The general term sorption is used to indicate the process in which the solutes (ions, molecules and compounds) are partitioned between the liquid phase and the soil particle interface. Of the various phenomena that can contribute to sorption, chemical interactions constitute the major subject of interest in contaminant-soil interactions. In regard to contaminant-soil interaction, the adsorption reactions which occur are processes by which contaminant solutes in solution become attached to the surface of soil particles through mechanisms which seek to satisfy the forces of attraction from the soil solid surfaces. These processes are governed by the surface properties of the soil solids (inorganic and organic) and the chemistry and physical-chemistry of the contaminant leachate and its constituents, e.g., cations, anions and nonionic molecules. The net energy of the interaction due to adsorption of a solute ion or molecule onto soil constituent surface is the result of both short range chemical forces such as covalent bonding and long range forces such as electrostatic forces (Yong et al., 1992).

**Complexation:** Complexation occurs when a metallic cation reacts with an anion that functions as an inorganic ligand. The metallic ions which can be complexed by inorganic ligands include the transitional metals and alkaline earth metals. The inorganic ligands which will complex with the metallic ions include most of the common anions such as  $\text{OH}^-$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{CO}_3^{2-}$  etc. The complexes formed between the metal ions and inorganic ligands are much weaker than those complexes formed with organic ligands. Organic ligands include amines, phenols etc. Complexation occurs when a central metallic cation becomes attached to two or more inorganic or organic groups by coordinate covalent bonds. The attached groups are generally identified as ligands and since the electrons for the covalent bond are furnished only by the ligand, the bond is called a coordinate covalent bond and the compound is referred as a coordinate compound (Yong et al., 1992).

**Precipitation:** Precipitation occurs when the transfer of solutes from the aqueous phase to the interface results in accumulation of a new substance in the form a new soluble solid phase. It can occur on the surfaces of the soil solids or in the pore water. Since both adsorption and precipitation are concerned with the removal of substances from the aqueous phase—as net accumulation at the soil-water interface for adsorption and the formation of a new solid phase for the case of precipitation—distinction between the two processes is not always easy to obtain. This is because the chemical bonds formed in both processes can be similar. The pH of both the soil and the soil pore water and the concentration of the solutes are important factors which control precipitation. Much higher concentrations of solutes are needed for precipitation to occur in the pore water

since the process requires the ionic activity of the solutes to exceed the solubility product. Precipitation is a major factor in the retention of heavy metals in soils (Yong et al., 1992).

**Trapping:** Trapping is an additional form of non-adsorptive retention of pollutants in soils and occurs in the case of pollutants adsorbed on suspended particles. The trapped contaminant remains behind as small, immobilized, no longer connected to the main body of the contaminant. This condition is usually referred to as the residual fraction of the contaminant. Pollutants which are retained in the soil pores by trapping create a source of future contamination of the soil medium (Yaron et al., 1996).

### **2.3.3 Sorption by soil constituents**

Determination of sorption of contaminants by soil constituents is most often accomplished through selective sequential extraction (SSE) of the contaminants from soil samples. This method of extraction and analysis is most often applied to the study of heavy metal retention in soils and sediments (Yong et al., 1992). The basic utility of SSE is its use of appropriate chemical reagents in a manner that releases the different heavy metal fractions from the soil solids by destroying the binding agent between the metals and the soil solids, thus permitting the individual metal species to be detected through appropriate analytical procedures. This method of analysis however is not precise—it does not allow one to determine exactly the concentration of the different metal fractions bound with the different soil solids. However, it does provide one with a qualitative appreciation of the capability of the various soil constituents to accumulate the heavy

metals and to speculate on their potential for release into the local environment. The five different metal-soil associations are as follows:

**Exchangeable:** Metals in this group are considered to be non-specifically adsorbed and ion exchangeable, i.e., they can be replaced by competing cations.

**Associated with Carbonates:** Metals precipitated or co-precipitated as natural carbonates can be released by application of an acid. The generally used extractant is acidified acetate.

**Associated with Metal Oxides:** Metals considered here are those metals which are attached to amorphous or poorly crystallized Fe, Al and Mn oxides.

**Associated with Organic Matter:** The binding mechanisms for metals in association with organic matter include complexation, adsorption and chelation.

**Residual Fraction:** This metal fraction is generally considered within the lattice of silicate minerals and can become available only after digestion with strong acids at elevated temperatures. The residual material consists of silicates and other resistant materials and determination of the metal associated with this fraction which is not considered to be significantly large is important in completing mass balance calculations.

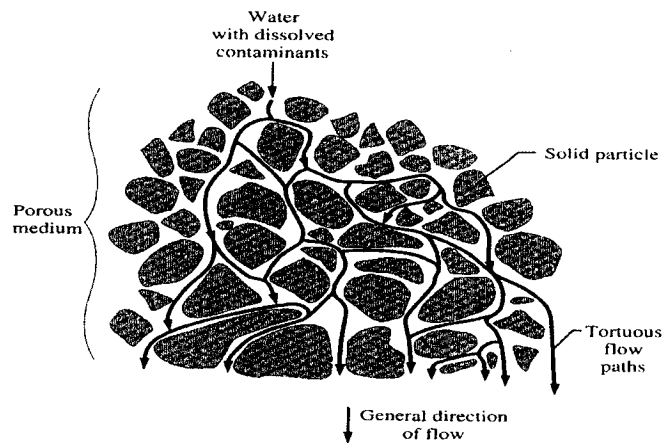
In applying selective sequential extraction to soil samples, the sequence of extraction is most important. The heavy metals first extracted by potassium nitrate ( $\text{KNO}_3$ ) were those retained by cation exchange mechanisms (Yong, et al. 1992). The second extraction sequence used a reagent consisting of sodium acetate ( $\text{NaOAc}$ ) buffered to pH 5 by acetic acid ( $\text{HOAc}$ ). The heavy metals released were those retained by the carbonates, i.e., precipitated with the carbonates in the soil. The third step in the selective sequential extraction used hydroxylamine hydrochloride ( $\text{NH}_2\text{OH} \cdot \text{HCl}$ ) to extract heavy metals that were retained by precipitation as hydroxides and adsorbed on the oxides or the amorphous hydroxides of the soil. The next reagent used in the SSE procedure was  $\text{H}_2\text{O}_2$ , which extracted the heavy metals retained by the organic constituents of the soil. The final step in the extraction procedure included digestion of soil with strong acids. The heavy metals that were obtained from this method of extraction were the residual metals.

#### **2.3.4 Contaminant transport mechanisms**

Contaminants exist in the subsurface environment in various forms like solutes, water-liquid immiscible, in gaseous form and adsorbed on colloids and other fine particles. The extent of this distribution and the kinetics of distribution depend on the soil and contaminant properties, environmental conditions and the management of the contaminated land.

Contaminants that are dissolved in water are transported with water as the water flows. This process of contaminant transport is called advection which is defined as the transport of solutes (contaminants that are dissolved in water) along stream lines at the

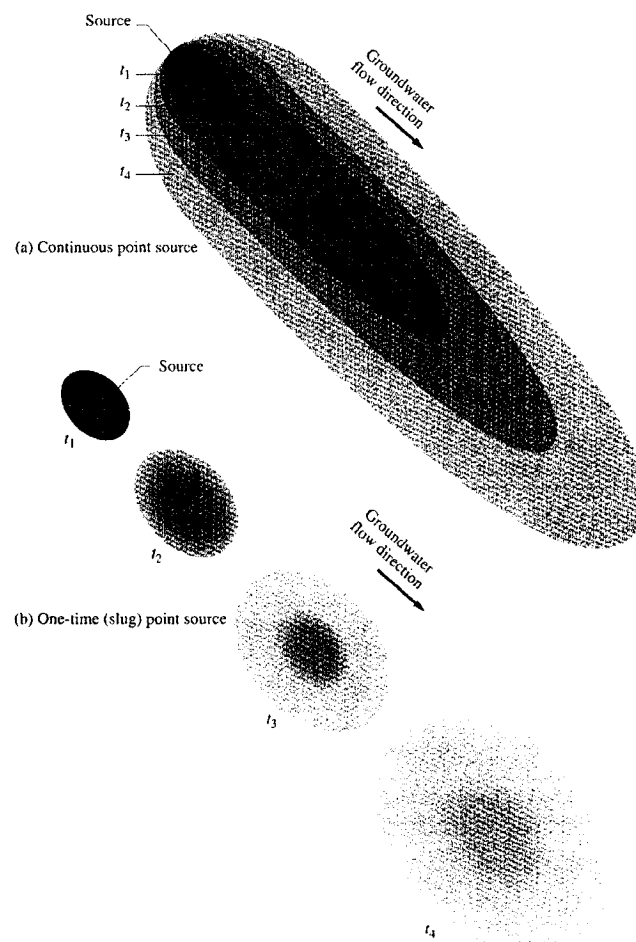
average seepage flow velocity. Water flows not through the solid particles but around them through the interconnected pore space. Flowing water alters its direction by flowing around the solids, either to the right or left of the solid or by spreading and migrating to both sides. This process is repeated millions of times by millions of water particles which results in a mixing of the flowing water by mechanical means termed mechanical dispersion or hydrodynamic dispersion or just dispersion. The most important effect of dispersion is to spread the contaminant mass beyond the region (LaGrega, 2001). Dissolved contaminants flowing in a porous medium are shown in Figure 2-6.



**Figure 2-6: Schematic diagram of dissolved contaminants flowing in soil (LaGrega, 2001)**

Sometimes contaminants can enter the porous medium instantaneously at a discrete location with known concentration of contaminants which is known as point source contamination. This category includes accidental spills and leaks from underground storage tanks. There are two types of point source contamination. One is a one-time point

source and the other one is continuous point source contamination. The contaminant concentration decreases as it migrates as a result of mechanical dispersion spreading the mass of contaminants over a larger and larger volume and mixing with water without the contaminants. This results in a dilution or reduction in contaminant concentration. Therefore, contaminants are transported primarily by advection and their concentration changes as a result of dispersion. The distribution and extent of contaminants migrating in the subsurface is termed as a plume (LaGrega, 2001). The migration of single and continuous point source contamination is shown in Figure 2-7.



**Figure 2-7: Plume migration affected by dispersion and source type (LaGrega, 2001)**

Contaminants also move in response to their chemical kinetic activity. The movement is from areas of higher concentration to areas of lower concentration which is termed as diffusion. The rate of transport of contaminants is proportional to the concentration gradient normal to the direction of movement. As solutes (dissolved contaminant) are not distributed uniformly through the soil, solution concentration gradients will exist and solutes will tend to diffuse from higher concentration to lower concentration (Yaron et al., 1996).

## **2.4 Soil and water remediation**

The remediation methods for soil and groundwater depend on the concentration and types of pollutants to be removed. Heavy metals can occur in several forms in water and soils (Mulligan et al., 2001b). The accurate assessment of the types, extents and forms of contamination in the subsurface increases the likelihood of achieving treatment goals.

### **2.4.1 Various remediation techniques**

**Isolation and Containment:** The isolation and containment of the contaminants can be done to reduce the permeability of the waste to prevent further movement. During site assessment and site remediation of the contaminated sites, it can be isolated temporarily to limit the transport of contaminants. To isolate the contaminated soil and water of a contaminated site, subsurface barriers are designed by controlling the movement of groundwater (Rumer & Ryan, 1995). Physical barriers made of steel, cement, bentonite and grout walls can be used for capping, vertical and horizontal containment. Capping is



a site-specific proven technology to reduce water infiltration and synthetic membranes can be used for this purpose (Mulligan et al., 2001b).

Vertical barriers reduce the movement of contaminated or uncontaminated groundwater through a contaminated area. The barrier has to be extended to a clay or bedrock layer of low permeability to prevent the transport of contaminants past the barrier. This type of barrier is mainly installed upstream, downstream or completely surrounding the site to prevent the lateral flow of groundwater. Slurry walls, grout or geomembrane curtains and sheet pile walls are used for this type of barriers among which slurry walls are the least expensive and most common. Horizontal barriers on the other hand are not as effective but are potentially useful in restricting downward movement of metal contaminants. The main types of horizontal barriers are grout injection by vertical or horizontal boring and block displacement. Vertical boreholes can increase the likelihood of the migration of contaminant (Mulligan et al., 2001b).

Solidification/stabilization (S/S) technologies are very common as they contain the contaminants not the contaminated area. Solidification is the physical encapsulation of the contaminants in a solid matrix while stabilization includes chemical reactions to reduce contaminant mobility. Bitumen, fly ash and cement are injected to encapsulate the soils. Soils can be treated in situ or after excavation. In situ techniques are preferred because of low labor and energy costs but this process is most suitable for shallow contamination (Mulligan et al., 2001b). The in situ process is also preferred if volatile or semi volatile organics are present, as the excavation may cause the exposure of those

contaminants in the air. This type of treatment is not suitable for some metals like arsenic, chromium (VI) and mercury.

Vitrification is a solidification/stabilization process requiring thermal energy which involves insertion of electrodes into the soil which must be able to carry a current, and then to solidify, as it cools. Toxic gases can be produced during this process. Full-scale applications exist for arsenic, lead and chromium contaminated soils. Mixed wastes can also be treated in this way. High clay and moisture contents and debris can affect the efficiency of the process. This process is suitable for contamination in shallow depths and of large volume (Mulligan et al., 2001b).

**Mechanical Separation:** This process is used to remove the larger and cleaner particles from the smaller and more polluted ones. The particle size and the contamination level in each fraction is the main parameter in determining the suitability of this process. Several processes such as hydrocyclones (separates larger particles by centrifugal force), fluidized bed separation (removes smaller particles at the top) and flotation are used to accomplish this method. Magnetic separation which is based on the magnetic properties of metals can also be used. Physical separation processes are becoming more common and their application is increasing as they have the potential to remove metal contamination in a particular form or in combination with other processes. This helps to reduce the volume of soil to be treated by other methods (Mulligan et al., 2001b).

**Pyrometallurgical Separation:** High temperatures like 200-700 °C are used to volatilize the metals in contaminated soil in this process. Metals are recovered or immobilized after volatilization. This method is most suitable for mercury as it is easily converted to its metallic form at high temperatures. Other metals may require pretreatment. This type of method is usually performed off site because of lack of a mobile unit and is most applicable to highly contaminated soils where metal recovery is profitable. Pretreatment is often necessary to reduce the volume of soil to be treated (Mulligan et al., 2001b).

**Chemical Treatment:** In order to alter the form of metal contaminants, chemical processes can be used to detoxify or decrease the mobility of metal contaminants. This process converts hazardous contaminants to non-hazardous or less toxic compounds which are more stable and less mobile. Reductive and oxidative mechanisms are used in this method. This method is commonly used for wastewater treatment. Sometimes this process is used as pretreatment of the soil for solidification or other treatments. Chemical treatment can be performed in situ by injection into the groundwater but has the potential to introduce further contamination (Mulligan et al., 2001b).

**Permeable Treatment Walls:** Permeable barriers are composed of a permeable reactive material that passively removes contaminants from flowing groundwater. These barriers are installed in the subsurface allowing continued use of the land. On-going maintenance or energy input is not required for this method. Above ground treatment and disposal of groundwater is not required either. In order to remediate a contaminant plume successfully, the reactive wall must be large enough that the entire groundwater plume

passes through it. The advantages of this technique are that it is in situ, a wide variety of contaminants can be treated and flow control can be used (Mulligan et al., 2001b). A permeable barrier composed of granular iron was applied as an innovative groundwater remediation technology to treat both dissolved Cr (VI) and TCE (trichloroethylene) in groundwater at the U.S. Coast Guard Support Center, Elizabeth City, NC. This site was contaminated by Cr (VI) and TCE derived from historical electroplating and degreasing operations. Site investigations showed maximum groundwater concentrations of greater than 10 mg/L Cr and 19000 µg/L TCE which exceed the maximum contaminant level. The chosen dimensions for the reactive barrier were 46 m long x 7.3 m deep x 0.6 m thick. The 46 m length and 7.3 m depth of the barrier were thought to be sufficient to intercept the Cr (VI) plume, which was approximately 35 m wide and 6.5 m deep. For a slight safety factor for residence time, a width of 0.6 m was used. Peerless granular iron was chosen as the reactive medium. In the column test, an input concentration of 11-12 mg/L Cr was depleted to < 0.02 mg/L over less than 10 cm travel distance in the column and TCE concentrations of 1500-2000 µg/L were reduced to non-detectable concentrations before reaching the half way point (30 cm) in the column. The total cost of the barrier installation, including initial design work, soil treatment and follow-up work was approximately \$985000 U.S. But this reactive barrier led to a cost savings of \$4000000 U.S. over a 20 year span compared to a traditional pump-and-treat system (USEPA, 1999).

**Electrokinetics:** Electrokinetic remediation consists of applying a low level DC current or a low voltage gradient across electrodes which are inserted in the soil. Electrolysis of

water occurs at the electrodes as a result of inducing electric potential. Water, ions and small charged particles are transported between the electrodes. Anions and cations move towards positive and negative electrodes, respectively. The main contaminant migration mechanisms that occur during the electrokinetic process are electroosmosis (movement of pore water), electromigration (movement of ions in the pore fluid), diffusion and electrophoresis (transport of charged particles). Electrokinetic remediation is an in situ technology that has significant potential for effectively and economically removing chromium from soils. But the soil composition plays an important role in the removal of contaminants from the soil. The feasibility and efficiency of this technology depends on the geologic, hydraulic, chemical and electrical conditions (Reddy et al., 1997).

**Biological Treatment:** Microbial activity strongly influences metal speciation and transport in the environment. Different organisms exhibit diverse responses to toxic ions which confer upon them a certain range of metal tolerance. Sulfate-reducing bacteria (SBR) show a certain level of metal tolerance. They are anaerobes that produce sulfide and immobilize toxic ions as metal sulfides (Valls & Lorenzo, 2002). Microorganisms are also known to oxidize and reduce metal contaminants. Cr (VI) can be oxidized to the less mobile and toxic state of Cr (III) by *Bacillus subtilis* bacteria and sulfur-reducing bacteria in the presence of sulfur (Mulligan et al., 2001b).

Biosorption is a biological treatment method to perform metal recovery through the use of biomass such as algal or bacterial cells that can be dead or alive. Sometimes a strong base is used in this process to enhance the metal binding ability and remove metal species

from aqueous solutions. This method is principally applicable for the removal of low concentrations of metals in water (Mulligan et al., 2001b).

**Phytoremediation:** This method consists of decontaminating the soil or water by using plants which are able to contain or degrade the metals. This process is suitable for shallow depths of contamination. Rhizofiltration, in which pollutants are adsorbed by the plant roots, can be used to remove metal from contaminated groundwater. Phytoextraction eliminates contamination by taking up the substances into the root biomass. Phytostabilization reduces the mobility of the substances in the environment. Phytotransformation involves the chemical modification of the environmental substances. Phytostimulation enhances the soil microbial activity to degrade the contaminants. When using this method, the main things that have to be considered are the climatic conditions and the bioavailability of the contaminants. The plants will have to be disposed of properly when they are contaminated. This process is an attractive alternative to current clean up technologies that are energy intensive and very expensive. The main disadvantage of this method is it requires a longer time as compared to other methods. The possibility of using phytoremediation with weed plant species in Thailand to remove chromium from soil was investigated. Six plant species, *Cynodon dactylon*, *Pluchea indica*, *Phyllanthus reticulatus*, *Echinochloa colonum*, *Vetiveria nemoralis* and *Amaranthus viridis* were chosen for their abilities to accumulate total chromium at tanning industry sites. The results suggested that chromium was removed mainly via phytoaccumulation and *Pluchea indica* is more suitable than *Cynodon dactylon* for the phytoremediation of chromium-contaminated soil (Sampanpanish et al., 2006).

**In situ treatment (soil flushing):** This is the extraction of contaminants from the soil with water or other suitable aqueous solution and is accomplished by passing the solution into the soil using the injection or infiltration process. The main theme of this technology is to flood the contaminated soils with a solution that moves the contaminants to an area from where it can be removed. Extraction fluids have to be recovered from the underlying aquifer and are recycled when possible. Water with or without additives is applied to solubilize the contaminants. The hydraulic conductivity of the soil controls the efficiency of the extraction process. High permeability gives better results.

Since water solubility is the controlling mechanism, additives are used to enhance the efficiencies. Chemical enhancements can be applied for the recovery of contaminants with low water solubility and sorbed contaminants. Chemical agents such as a cosolvent, when mixed with water, increase the solubility of some compounds and surfactants, which may cause contaminants to desorb and may increase the mobility of the contaminants.

This technology is mostly applied for the removal of organic compounds and is limited for use with metals. For the removal of Cr (VI) from United Chrome, a chrome plating plant at Corvallis, Oregon, full-scale treatment of this technology was performed and significant removal of chromium was achieved (USEPA, 1997). In situ soil flushing works best at sites where wash solution can move through the soil. If the soil contains a high percentage of silt or clay, the flushing solution cannot move through the soil easily and also can not make contact with the contaminants. This limits the overall effectiveness

of this process. Furthermore, some additives can create new groundwater contamination if they are not removed completely.

#### **2.4.2 Surfactant soil flushing and washing**

Because of the limitations of others technology, attention is now focused on the feasibility of surfactant use to increase soil flushing efficiency. Surfactants have been studied for use in soil washing and enhanced oil recovery (West & Harwell, 1992). The solubility of many hydrophobic organic compounds and chlorinated hydrocarbons increases due to the addition of surfactant. Moreover, the combination of surfactants or surfactant with other complexing agents has a more effective extracting capability. Various types of surfactants such as cationic, anionic and nonionic surfactants can be used for soil washing. Previous studies on heavy metal remediation using surfactant/biosurfactant are discussed here.

Surfactants have been used in the ultrafiltration process. Yang et al. (2005) used ultrafiltration as a part of a surfactant-enhanced aquifer remediation process to remove heavy metal and organic pollutants from contaminated groundwater and wastewater. Four anionic and seven nonionic surfactants were tested.

Shin and Barrington (2005) investigated the effectiveness of an iodide ligand along with two surfactants on desorbing heavy metals from soil. They used SDS, an anionic surfactant and Triton X-100, a nonionic surfactant. The ligand iodide was evaluated as a washing agent for the desorption of cadmium (Cd) from naturally and artificially



contaminated soils. Increasing amounts of ligand with a surfactant removes higher levels of Cd but not Cu, Zn and Pb. After seven washings, the ligand iodide with nonionic surfactant, Triton X-100, removed 65 and 90% of Cd from soil I and II, which contains 15 and 1275 mg/kg of Cd. Ligand iodide, on the other hand, with anionic surfactant, SDS, removed 35 and 70% of the Cd from soil I and II, respectively.

Chang et al. (2005) used a biodegradable chelating agent, LED3A and two surfactants, SDS and Triton X-100 to evaluate a potential alternative to remediate metal contaminated soil. LED3A alone removed only 40% of Cd but the addition of surfactants significantly enhanced its Cd removal capacity up to 80% for a wide range of pH. The enhancement increased with both the surfactant concentrations and LED3A concentration.

Wang and Mulligan (2004) conducted research to evaluate the feasibility of using rhamnolipid foam to remove Cd and Ni from a sandy soil. The contamination levels were 1706 mg/L and 2010 mg/L for Cd and Ni, respectively. The rhamnolipid foam removed 73.2% of Cd and 68.1% Ni. A chemical surfactant, Triton X-100 was also investigated for comparison. It removed 65.5% of Cd and 57.3% of Ni. Therefore, this study established that rhamnolipid foam technology can be an effective way for the remediation of cadmium and nickel contaminated soil.

Dantas Neto et al. (2004) used a surfactant, which was derived from vegetable oil, to remove chromium from a tannery effluent by microemulsion in the Morris extractor. Simultaneous removal of ferric cyanide and chromate by MEUF (micellar-enhanced

ultrafiltration) with octadecylamine acetate (ODA) as a cationic surfactant was investigated by Beak et al. (2003). The removal of ferric cyanide and chromate in the ferric cyanide/ODA and chromate/ODA system were 98 and 99.9% respectively with the 5 molar ratio of ODA. MEUF of chromate anions from aqueous streams has been studied using two cationic surfactants (cetyltrimethylammonium bromide (CTAB) and cetylpyridinium chloride (CPC) by Gzara and Dhahbi (2001).

Surface-active compounds (surfactant) are available in the subsurface. They are present as a result of indigenous biological activity and disposal of waste products and effluents and are now considered for use in the remediation process. An important issue in the use of surfactants in the remediation process is the toxicity of the surfactants in the subsurface environment and possible interference in active biodegradation of contaminants. Biosurfactants may have advantages over synthetic chemical surfactants. Biosurfactants are readily biodegradable, many are tolerant to wide variations in temperature, pH and salt concentrations. They may be produced in situ and in some cases they are cheaper to produce than synthetic surfactants (West & Harwell, 1992).

The use of a surfactant-based remediation process depends on several factors. These are the selection of surfactants for optimum efficiency (minimizing losses to sorption, precipitation and phase change), environmental acceptability and balanced biological degradation. It must be ascertained that the use of surfactants in subsurface remediation will not add further contamination to the environment.

Massara et al. (2007) used rhamnolipid to determine its effects on chromium-contaminated kaolinite. Results showed that rhamnolipid can extract 25% of Cr (III) from the kaolinite under optimum condition. Rhamnolipids remove Cr (III) mainly from carbonate and oxide/hydroxide portions of the kaolinite which was obtained using sequential extraction procedure. The rhamnolipids also enhance the removal of Cr (VI) by a factor of 2 compared to water. They also have the capability of reducing almost 100% of the extracted Cr (VI) to Cr (III) over a period of 24 days. Therefore, rhamnolipids could be beneficial for the removal or long term conversion of Cr (VI) to Cr (III).

## **3 Materials and Methods**

### **3.1 Experimental approach**

The purpose of this study is to convert Cr (VI) to Cr (III) both in water and soil media using the biosurfactant, rhamnolipid. Batch tests were carried out to determine the effect of various parameters on the reduction of Cr (VI) both in water and soil media. The study also included the use of the sequential extraction technique to determine the mechanism of Cr (VI) reduction in soil and also to investigate the mechanism of interaction of metals with rhamnolipid. The following experimental procedures were carried out.

Distilled water was prepared in the environmental engineering laboratory at Concordia University.

Natural soil was used and it was collected from a park. The soil was free from contamination. It was contaminated artificially and then used for the experiments.

Rhamnolipid, a commercially available biosurfactant, was used with trademark JBR 210 from 'Jeneil Biosurfactant Co Ltd'. JBR210 is an aqueous solution containing 10% rhamnolipids. It was diluted with various amounts of distilled water to obtain different concentrations.

**Table 3-1: Physical and chemical properties of JBR210 (Jeneil Biosurfactant Co. LLC, 2001)**

| Physical/chemical property        | Description                                    |
|-----------------------------------|--|
| Appearance                        | Dark brown viscous suspension                  |
| Odor                              | Mild cooked odor                               |
| Specific Gravity                  | 1.12 – 1.14                                    |
| pH                                | 6.5 – 7.5                                      |
| Solubility in water               | Soluble at neutral pH                          |
| Suitable diluents                 | Water, most common alcohols                    |
| Suggested starting concentrations | Active rhamnolipid ingredient: 1.0, 0.1, 0.01% |
| Surface tension                   | 29 mN/m  |
| Interfacial tension               | 0.3 mN/m                                       |

### 3.2 CMC measurement

One of the most widely used indices for evaluating surfactant activity is the critical micelle concentration (CMC). CMC is the minimum surfactant concentration required for reaching the lowest interfacial or surface tension values. At concentrations above the CMC, amphiphilic molecules associate readily to form molecular structure micelles (Lin, 1996). The surface tension remains approximately constant beyond the CMC level because all additional surfactant molecules form micellar structures. CMC for biosurfactant can be measured by several methods. It can be found by plotting conductivity, turbidity, surface tension, detergency and interfacial tension versus surfactant concentration.

The CMC was found by determining the variation of surface tension with biosurfactant concentration. The surface tension of rhamnolipid solution at different concentrations was measured with a Fisher Scientific Surface tensiometer. The duNouy ring method was used, where the ring was pulled through the rhamnolipid solution until it broke through the surface. The value of the surface tension at that point was recorded. A graph of surface tension versus rhamnolipid concentration was plotted and the crossing point of the two tangents of the curve is the CMC. The obtained value of CMC was about 32 mg/L which is equivalent to 0.0032 % rhamnolipid. Hence, concentrations above the CMC were used for the experiments in order to ensure the formation of micelles.

### **3.3 Procedure for batch test: Water part**

Potassium dichromate ( $K_2Cr_2O_7$ ) purchased from Fisher Scientific was used as a source of hexavalent chromium. A stock solution of 2000 ppm of Cr (VI) was prepared by dissolving  $K_2Cr_2O_7$  in distilled water. The working solutions were prepared by diluting the stock solution with distilled water. Batch studies were performed by varying pH, surfactant concentrations, Cr (VI) concentrations, temperatures and contact time. Samples were taken after 24 h shaking to ensure that equilibrium has been reached and then centrifuged. The supernatant was then analyzed by Perkin Elmer 'Lambda 40' UV/VIS spectrometer for Cr (VI) concentration by a colorimetric method. Purple color was generated with 1,5-diphenylcarbazide complexation agent (DPC) at a visible wavelength of 540 nm according to the procedure of U.S.EPA (1992). The initial Cr (VI) concentration was also measured the same way. Then the percentage of Cr (VI) reduction was determined by the equation:

$$\% Cr (VI)_{reduction} = \frac{[Cr (VI)_{initial} - Cr (VI)_{final}]}{Cr (VI)_{initial}} \times 100 \% \quad \dots\dots\dots(3-1)$$

### 3.3.1 Optimization of pH

As pH is a very important parameter for the removal/reduction of heavy metal, this test was designed to investigate the effect of pH on the conversion of Cr (VI) to Cr (III) in water. Since rhamnolipid precipitates at pH 5.5, pH values of 6, 7, 8, 9 and 10 were selected. For each pH, three samples were taken each of which contains 10 ppm of Cr (VI) solution and 0.5% rhamnolipid solution. The final volume was 50 mL. The pH was adjusted using 1N hydrochloric acid (HCl) and 1N sodium hydroxide (NaOH). pH was measured using Oakton pH meter (Acorn series). The samples were placed on AROS 160 adjustable reciprocating orbital shaker (at 60 rpm) in an incubator at temperature 25°C. The samples were taken after 24 h shaking and then centrifuged and analyzed for Cr (VI) concentration. The pH of the sample with maximum reduction of Cr (VI) was considered the best pH.

### 3.3.2 Optimization of concentration of rhamnolipid

To find the most efficient concentration of biosurfactant, the following test was done. 10 ppm of Cr (VI) solution and various concentrations of rhamnolipid with an optimized pH of 6 were placed in the vials. The final volume of the solution in each vial was 50 mL. The various concentrations of rhamnolipid were 0.05%, 0.1%, 0.2%, 0.4%, 0.5%, 1%, 2%, 4% and 5%. The samples were placed on the shaker at 25°C and were analyzed after

24 h. The rhamnolipid concentration at which maximum reduction was obtained was the optimum concentration of rhamnolipid.

### **3.3.3 Study of Cr (VI) concentrations**

To see the effect of the initial concentration of Cr (VI) on the reduction process, this test was performed. Different initial concentrations of Cr (VI) solutions such as 10, 50, 100, 200 and 400 ppm with optimum rhamnolipid concentration (2%) and optimum pH (6) were taken in final volume of 50 mL. The samples were placed on the shaker at 25°C and analyzed after 24 h for Cr (VI) levels.

### **3.3.4 Temperature study**

This test was established to investigate the effect of temperature on the conversion of Cr (VI) to Cr (III). This test was done at temperatures of 10, 20, 25, 30, 40 and 50°C. At each temperature, 10 ppm of Cr (VI) solution with optimum rhamnolipid concentration (2%) and optimum pH of 6 were placed in a final volume of 50 mL. The samples were placed on the shaker and analyzed after 24 h.

### **3.3.5 Time study**

This test was performed to investigate the effect of contact time on the reduction process. Samples were taken at optimum conditions (2% rhamnolipid concentration and pH 6). The Cr (VI) solution was 10 ppm and the final volume was 50 mL. The samples were placed on the shaker and analyzed every hour to determine the percentage of reduction of Cr (VI).



### **3.4 Characterization of soil**

The soil was collected from a park in Montreal. Before contaminating the soil, tests for its characterization were done.

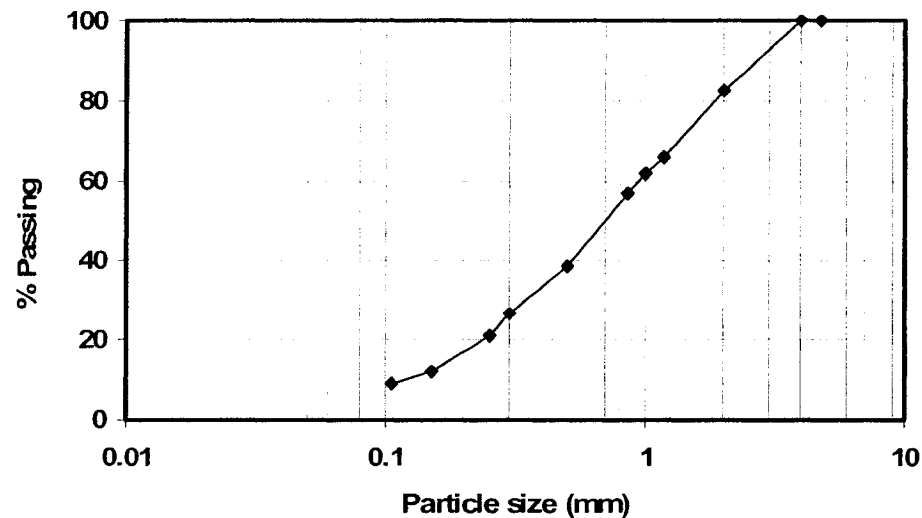
#### **3.4.1 Cation exchange capacity**

Cation exchange capacity (CEC) was determined using the method proposed by Chapman (1965). Initially, 20 mL of 1M potassium acetate was added to 5 g of soil in a centrifuge tube and shaken for 5 minutes and the supernatant discarded. This step was repeated several times. Then 20 mL of methyl alcohol were added to the tube, shaken and the supernatant discarded. This step was repeated several times. Finally, 25 mL of 1M ammonium acetate were added, shaken and the supernatant collected. This step was repeated and each time the supernatant was collected in the same beaker. The  $K^+$  concentration in the supernatant was measured using an atomic absorption spectrophotometer. The cation exchange capacity was then calculated to be 11.2 cmoles/kg.

#### **3.4.2 Particle size distribution**

Particle size distribution was performed according to the standard method for soil ASTM D422-63 (ASTM, 1998). Approximately 700 g of air dried soil was taken and sieved using standard brass sieves with mesh numbers from 5 (4 mm) to 200 (0.075 mm). After placing the sieve column in the shaker for 15 minutes, the weight retained by each sieve was determined. The results were plotted in a semi-log graph (Figure 3-1). The grain size

distribution of the soil indicates a sandy soil. Approximately 80.8% was finer than 2 mm and 1.8% passed through a 0.075 mm sieve.



**Figure 3-1: Particle size distribution for soil samples**

### **3.4.3 Organic matter content**

Organic matter content was determined by the ignition method (Mulligan, 1998). Approximately 5 g of oven dried soil were weighed in pre-weighed porcelain dishes. The samples were then heated to 550°C for 1.5 hour. After cooling the samples overnight in a desiccator, they were weighed. The difference in weight before and after ignition divided by the initial weight and multiplied by 100% gave the organic matter %. The obtained organic matter content was 5.5%.

### **3.4.4 Heavy metal content**

To check the heavy metals content in the soil, it was digested by the method recommended by Environment Canada (1990). Soil samples (1 g) were digested with 100

mL of concentrated nitric acid. After approximately 2 minutes, 40 mL of H<sub>2</sub>O<sub>2</sub> (30%) were added and left for 5 minutes to react. Then it was boiled on a hot plate and removed to cool to room temperature. Subsequently 200 mL of aqua regia (200 mL concentrated HNO<sub>3</sub> + 50 mL concentrated HCl + 750 mL distilled water) were added to the solution and the beaker was filled to the 500 mL level with distilled water. Analyses were performed using a Perkin Elmer Atomic Absorption Analyst 100 Spectrophotometer. Chromium (357.9 nm), lead (244.8 nm), zinc (213.9 nm), copper (324.8 nm) and nickel (232.0 nm) were analyzed after preparing the standards, blanks and samples according to standard methods (APHA-AWWA-WPCF, 1980). There was a negligible amount of chromium in the soil but Pb (5 mg/kg), Zn (70 mg/kg), Cu (70 mg/kg) and Ni (45mg/kg) were found.

### **3.4.5 Soil pH**

Soil pH was determined by using a 1:10 soil to water ratio (Mulligan, 1998) using an Oakton pH meter Acorn series. The mixture was shaken for an hour and was left for another 30 minutes. After clearing the solution, the pH was measured and the value obtained was 7.3.

### **3.4.6 Soil moisture content**

Approximately 5 g of soil was placed in a pre-weighed porcelain dish and dried at 105°C for 24 hours. It was then placed in a desiccator for cooling and weighed. The difference in the sample weight before and after drying divided by the original weight x 100% gave the moisture content. The obtained moisture content was 9.2%.

### **3.4.7 Carbonate content**

Carbonate content was measured by following the procedure of Dahrazma (2005). In brief, 10 g of oven-dried soil were placed in a glass beaker and to which was added diluted hydrochloric acid. The mixture was stirred carefully and the step was repeated until no reaction from the soil was observed. The residue was filtered and the material was air dried. The weight of the residue was calculated by collecting all of the residue from the filter paper and the beaker. The difference between the initial weight and final weight divided by the initial weight x 100% gave the carbonate content. The obtained carbonate content was 3.5%.

### **3.4.8 Specific gravity**

Soil specific gravity was determined by using the standard method ASTM D854 – 98. Approximately 20 g of oven-dried soil was taken in a volumetric flask. The flask was filled with distilled water to a level slightly above that required to cover the soil and soak the specimen for at least 12 hours. To remove the entrapped air, the specimen was boiled gently for at least 10 minutes while agitating the flask occasionally to assist in the removal of air. Then the heated specimen was cooled to room temperature. The flask was then filled just below the calibration mark with distilled water at room temperature. Distilled water was added slowly and carefully to avoid the entrapment of air bubbles in the specimen and the flask was left to obtain a uniform water temperature. The flask was then filled with distilled water at the same temperature to the mark and cleaned and dried the outside with clean dry cloth and determined the record the mass of the flask filled

with soil and water. A thermometer was inserted into the water and determined its temperature. Then the specific gravity was measured using the following formula:

$$G_{T_b} = \frac{M_0}{[M_0 + (M_a - M_b)]} \dots\dots\dots(3-2)$$

Where,  $M_0$  = mass of sample of oven-dry soil, g

$M_a$  = mass of volumetric flask filled with water at temperature  $T_b$ , g

$M_b$  = mass of volumetric flask filled with water and soil at temperature  $T_b$ , g

$T_b$  = temperature of the contents of the volumetric flask when mass  $M_b$  was determined, °C.

### 3.5 Soil spiking procedure

The required amount of predissolved potassium dichromate ( $K_2Cr_2O_7$ ) was added to the air dried uncontaminated soil. A ratio of 1 g of soil per 10 mL of solution (2000 mg/L) was used. The soil was left in the solution over one week. The soil was shaken on a reciprocating orbital shaker for 24 hours and then removed by centrifugation (3000 rpm, 20 min) and oven dried. For different concentrations in soil, the soils were left in the solutions (2000 mg/L, 4000 mg/L) for 3, 7 and 10 days. For most experiments, the soil was used with a contamination level of 1040 mg/kg (of soil) which was obtained by keeping 2000 mg/L solution in soil for one week. Other concentrations were 880 mg/kg, 1480 mg/kg, 1820 mg/kg and 2040 mg/kg. The experiments were done 2.5 months after contaminating the soil.

### **3.6 Initial contamination measurement**

Contaminated dry soil samples were digested with concentrated nitric acid and the samples were prepared for Atomic Absorption Spectrophotometer analysis. Then initial Cr concentrations in the soil were measured.

### **3.7 Procedure for batch soil washing studies**

Batch soil washing studies were performed by varying pH, soil solution ratios, surfactant concentration, initial Cr (VI) concentration, temperatures and contact time. Samples were taken after 24 hours shaking to ensure that equilibrium has been reached and then centrifuged. The supernatant was then analyzed for total Cr concentration by atomic adsorption spectrophotometer and for Cr (VI) by a UV/VIS spectrometer.

The rhamnolipid biosurfactant was used to determine its capability in removing Cr (VI) from the soil. Distilled water alone was used as a control to account for the removal of contaminants by physical mixing. All results are the average of duplicate experiments and are presented as % Cr removal.

#### **3.7.1 Optimization of pH**

This test was performed to investigate the effect of pH on the extraction process. Various pHs from 6 to 10 were checked as rhamnolipids precipitate below pH 5.5. Each sample contained 1 g of contaminated soil and 10 mL of 0.5% rhamnolipids solution. The pH was adjusted using HCl and NaOH. All of the samples were placed on an orbital shaker at 25°C. The samples were collected after 24 hours and both total Cr and Cr (VI) were

measured. The pH at which the maximum removal of Cr was found was considered as optimum pH. The optimum pH was 6 and all other experiments were done at this pH.

### **3.7.2 Evaluation of soil to surfactant ratio**

To find the best soil to biosurfactant ratio, 1 g of soil and 0.5% rhamnolipid at the optimized pH were placed in the vials. The volumes of the surfactant solution in the vials were 10, 20, 30, 40 and 50 mL. The samples were kept on the shaker at 25°C. The samples were collected after 24 hours and then the total Cr and Cr (VI) concentrations were measured in the supernatant. The ratio at which maximum removal was obtained was considered as the optimum ratio and other experiments were done using this ratio.

### **3.7.3 Optimization of biosurfactant concentration**

This test was performed to establish the most efficient concentration of biosurfactant for the extraction process. One gram of soil at optimum pH and solution ratio was taken. The selected biosurfactant concentrations were 0.05%, 0.1%, 0.2%, 0.4%, 0.5%, 1.0%, 2.0%, 4.0% and 5.0%. All samples were placed on the shaker and the temperature was 25°C. After 24 hours shaking, the samples were collected for the measurement of total Cr and Cr (VI) concentrations in the supernatant. The surfactant concentration which removed maximum Cr was selected as the optimum surfactant concentration.

### **3.7.4 Evaluation of initial contaminant level**

An experiment was done to see how the initial concentration of contaminant affects the extraction process. One gram of contaminated soil of various contamination levels was

taken at optimum pH, surfactant ratio and surfactant concentration. The samples were placed on the shaker and the temperature was 25°C. The samples were collected after 24 hours shaking and total Cr and Cr (VI) concentrations were measured.

### **3.7.5 Temperature study**

To investigate the effect of temperature on the removal process, this test was performed. One gram of soil at optimum pH, surfactant ratio and concentration were placed in the vials. Temperature was varied from 10 to 50°C. For each temperature, the samples were kept on the shaker. Then the samples were collected after 24 hours shaking and total Cr and Cr (VI) concentrations were measured.

### **3.7.6 Time study**

This experiment was performed to see the effect of contact time on the extraction process. One gram of soil at optimum condition was taken and this experiment was set for one week on the shaker. Every day samples were collected and total Cr and Cr (VI) concentrations were measured.

## **3.8 Sequential extraction procedure**

Sequential extraction of heavy metals includes several steps to determine each portion of the metal component in the soil (Mulligan, 1998)). Sequential extractions of soils were done before and after the soil washing. Soil samples of one gram were washed with the biosurfactant solution and control and then dried prior to sequential extraction. Each of the fractions was collected and the concentration of Cr was determined in each of the



fractions by AA analysis. The amounts of Cr extracted from the soil by each of the extractants were then calculated.

**Table 3-2: Sequential extraction procedure (Mulligan 1998)**

| <b>Order of sequence</b> | <b>Chemical reagents</b>  | <b>Fractions</b>      |
|--------------------------|---|-----------------------|
| 1                        | Extraction of metal by rhamnolipid and control (distilled water) overnight with 20 mL of solution   | Soluble               |
| 2                        | Extraction of metal with 8 mL of 1M $\text{MgCl}_2$ (pH 7) for 1 hour   | Exchangeable          |
| 3                        | Extraction of metal with 8 mL of 1M $\text{NaOAc}$ adjusted to pH 5 with acetic acid for 5 hours  | Carbonates            |
| 4                        | Extraction of metal with 20 mL of 0.04M $\text{NH}_2\text{OH}.\text{HCl}$ in 25% (v/v) acetic acid (pH 2.5) at 96° C for 6 hours  | Oxides and hydroxides |
| 5                        | Extraction with 3 mL of 0.02M $\text{HNO}_3$ and 5 mL of 30% $\text{H}_2\text{O}_2$ (pH 2) for 2 hours at 85° C, followed by 3 mL of 30% $\text{H}_2\text{O}_2$ (pH 2) at 85° C for 3 hours and then 5 mL of 3.2M $\text{NH}_4\text{OAc}$ in 20% (v/v) $\text{HNO}_3$ diluted to 20 mL at room temperature for 30 min | Organic matter        |
| 6                        | Digestion at 90° C with 25 mL of dilute aqua regia (50 mL $\text{HCl}$ , 200 mL $\text{HNO}_3$ and 750 mL water) for 3 hours  | Residual fraction     |

## **4 Results and Discussion**

### **4.1 Introduction**

The results of the experiments are discussed in this chapter. As mentioned earlier in this thesis, experiments were done both in water and soil. Therefore, results are presented in two parts where part1 contains the results of water media and part2 contains results and discussion on soil. In both media rhamnolipid JBR210 was used for the reduction of Cr (VI) to Cr (III). The studied parameters were chosen based on the related literature review.

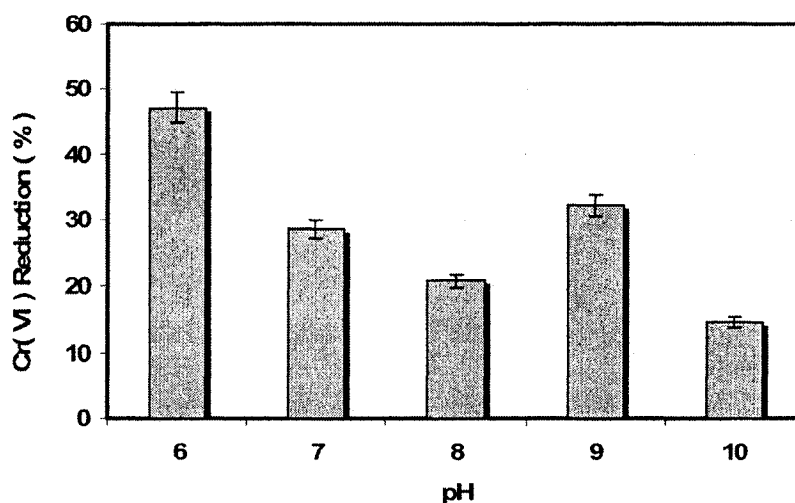
### **4.2 Part1: Results of experiments in water**

The results of batch experiments in water are presented here with some discussion. The objective of this work was to determine what parameters affect the reduction of Cr (VI) and also to find out the optimum conditions for the reduction using rhamnolipid. The considered parameters were pH, different concentrations of rhamnolipid, different concentrations of Cr (VI), temperatures and contact times.

#### **4.2.1 Effect of pH**

As pH is an important factor for metal removal, the first experiment was done varying the pH to see its effect on the reduction of Cr (VI). The rhamnolipid precipitates below pH 5.5 (Dahrazma, 2005) and therefore, pH values of 6 to 10 were studied. The effect of pH was investigated by treating 10 ppm of Cr (VI) solution with 0.5% rhamnolipid at 25°C for 24h. Figure 4.1 shows the reduction of Cr (VI) at different pH values studied. The

reduction of Cr (VI) appeared to decrease with the increase of pH. The result was in accordance with previous research performed on reduction of hexavalent chromium by ascorbic acid in aqueous solutions (Xu et al., 2004) where increasing the pH resulted in more Cr (VI) remaining in aqueous solutions.

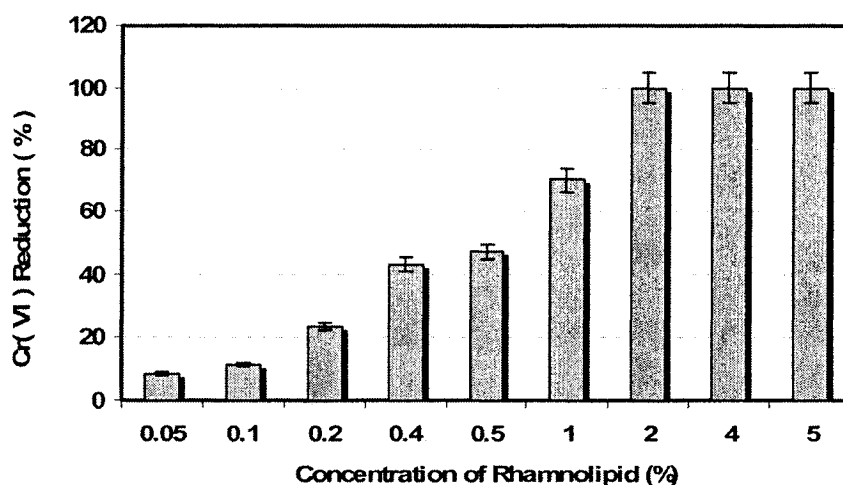


**Figure 4-1: Effect of pH on the reduction of Cr (VI) using 10 ppm Cr (VI) solution & 0.5% rhamnolipid solution**

The maximum reduction efficiency (47.1%) was obtained at pH 6 and this result agrees with the research performed on reduction of hexavalent chromium by *Streptomyces griseus* (Laxman & More, 2002) where maximum conversion of hexavalent to trivalent form was observed in the pH range of 6-7. At pH 10, a small amount of Cr (VI) was reduced. The pH value of groundwater generally varies between 5 and 9. Therefore, rhamnolipid could be used in remediation of Cr (VI) contaminated groundwater at its natural range of pH.

#### 4.2.2 Effect of the concentration of biosurfactant

This experiment was performed taking 10 ppm of Cr (VI) solution with various rhamnolipid concentrations such as 0.05%, 0.1%, 0.2%, 0.4%, 0.5%, 1%, 2%, 4% and 5%. The pH was 6, temperature was 25°C and time was 24h. Figure 4-2 shows the results which indicate that the reduction efficiency increases with the increase of the concentration of rhamnolipid. The maximum reduction (100%) was achieved by a 2% of rhamnolipid concentration which was chosen as the optimum concentration.

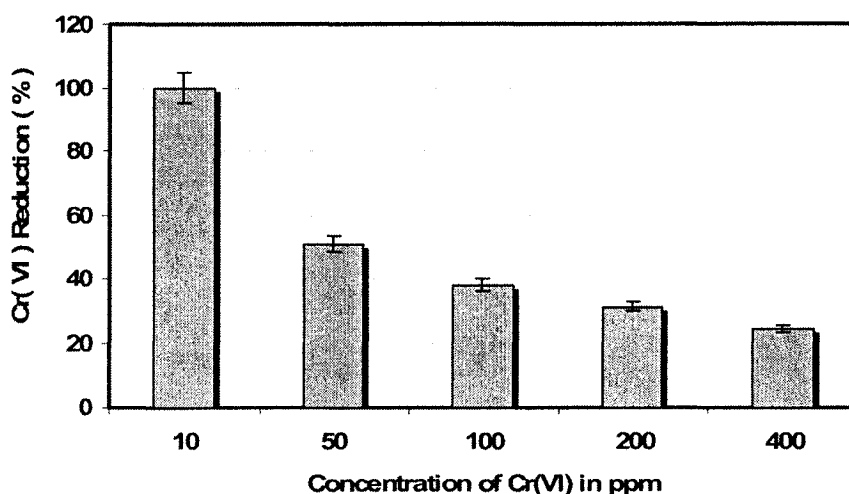


**Figure 4-2: Effect of concentration of rhamnolipid on Cr (VI) reduction using 10 ppm Cr (VI) solution**

With the increase of concentration of rhamnolipid, the concentration of Cr (VI) decreased when the concentrations of rhamnolipid were in the range of 0.05 to 2% (20000 mg/L). At a 2% rhamnolipid concentration and higher, the concentrations of Cr (VI) in the solution were negligible. Therefore, the amount of rhamnolipid for the reduction of 10 ppm hexavalent chromium is 20000 mg/L. Hence, the molar ratio of rhamnolipid required for the reduction of Cr (VI) was 1:180.

### 4.2.3 Effect of initial concentration of Cr (VI)

To see the effect of different initial metal ion concentrations on the reduction of Cr (VI), this experiment was performed. The experiment was done using various initial concentrations of Cr (VI) at optimum pH (6) and rhamnolipid concentration (2%) at 25°C for 24 h. Figure 4-3 shows the percentage reduction of Cr (VI) at different initial metal ion concentrations ranging from 10 to 400 ppm. An increase in Cr (VI) concentration from 10 to 400 ppm resulted in a decreased reduction of Cr (VI).



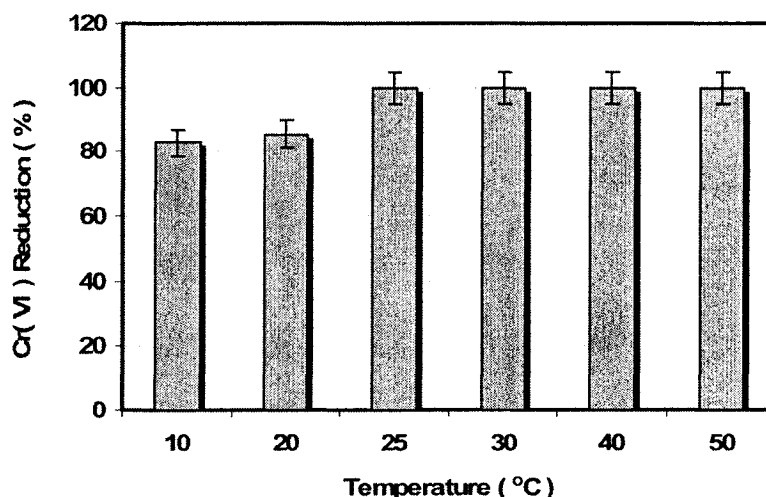
**Figure 4-3: Effect of different initial concentration of Cr (VI) on the reduction process using 2% rhamnolipid solution**

The percent reduction of Cr (VI) decreased by increasing initial Cr (VI) concentrations in the solutions with complete reduction at 10 ppm and only 25% reduction of 400 ppm. But the amount of Cr (VI) reduced was increased with an initial Cr (VI) concentration and maximum reduction value of 100 ppm was observed at 400 ppm. Erdem et al. (2005) also showed that the amount of the Cr (VI) reduced increases with increasing the initial

chromium concentration. The 100% reduction of 10 ppm initial Cr (VI) at 2% rhamnolipid concentration was also obtained from previous experiments (Figure 4-3).

#### 4.2.4 Effect of temperature

The temperature dependence of Cr (VI) reduction by rhamnolipid was studied in the range of 10-50°C taking 10 ppm Cr (VI) solution at pH 6 and 2% rhamnolipids concentration. Figure 4-4 shows the percentage of the reduction of Cr (VI) as a function of temperature which indicates that reduction percentage of the Cr (VI) is enhanced with the increase of temperature. This may be a result of increase in the solubility and mobility of Cr (VI) ions with temperature (Malkoc and Nuhoglu, 2007). The solubility of rhamnolipid also increases with temperature and consequently so does the reduction capacity.



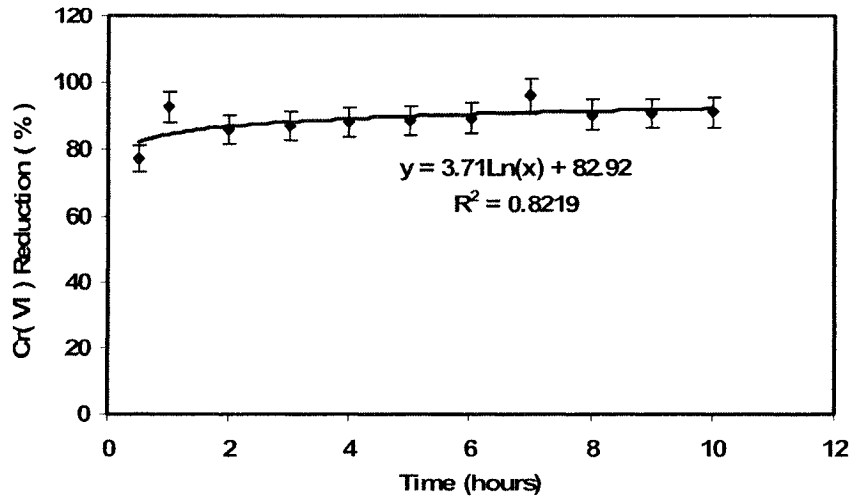
**Figure 4-4: Effect of temperature on Cr (VI) reduction using 10 ppm Cr (VI) solution & 2% rhamnolipid solution**

Xu et al. (2004) and Erdem et al. (2004) also showed that reduction percentage of the Cr (VI) increases by increasing the temperature of the solution, where the former one used ascorbic acid and the latter used siderite for the reduction of Cr (VI) in aqueous solutions.

The maximum reduction (100%) was obtained at 25°C and after that temperature, the reduction was the same. At low temperatures, reduction decreases because the temperature might be below the Krafft point (the temperature at which the solubility of an ionic surfactant becomes equal to the CMC of the surfactant) of the surfactant components which can cause the surfactant concentration to drop below the CMC, thereby rendering the surfactant useless (West & Harwell, 1992).

#### **4.2.5 Time optimization**

The results of the reduction of Cr (VI) by rhamnolipid over time are presented in Figure 4-5. This experiment was performed taking 10 ppm of Cr (VI) solution at optimum condition (pH 6 and 2% rhamnolipid concentration) at 25°C. The reduction rate of Cr (VI) was very fast initially and about 77% of the starting Cr (VI) was reduced within the first 30 minutes and about 93% reduction happened within the first hour but after that the reduction rate decreased over the reaction time.



**Figure 4-5: Effect of time on Cr (VI) reduction using 10 ppm Cr (VI) solution & 2% rhannolipid solution**

As the curve is not showing any local maximum, a time at which the slope of the curve becomes less than 1% reduction per hour can be considered as optimum time.

$$\text{Slope} = y' = \frac{3.71}{x} = 1\% \text{ reduction/hour}$$

$$x = \text{time} = 3.71 \approx 4 \text{ hour}$$

$$\text{And, } y'' = \frac{-3.71}{x^2} < 0$$

Which indicates the slope of the curve always decreases.

If 4 hours is considered as optimum time, the difference in reduction from the next hour will be:

$$\Delta y = \frac{88.89 - 88.06}{88.06} \times 100 = 0.9\%$$

This is negligible.

Therefore, 4 hours can be considered as the optimum time for the reduction of Cr (VI) in water media.



### 4.3 Summary and discussion of water medium

Distilled water was used to determine the behavior of hexavalent chromium in water media.  $K_2Cr_2O_7$  was mixed with distilled water to prepare the Cr (VI) solution. The objective of this work was to evaluate the capability of biosurfactant to reduce or convert the Cr (VI) and to determine what parameters affect the reduction of Cr (VI).

The pH is an important factor for the reduction of Cr (VI). Maximum reduction was observed at pH 6 (Figure 4-1) since both Cr (VI) and rhamnolipid are more soluble at this pH compared to other pHs at which the experiment was performed. Higher concentrations of rhamnolipid showed higher reduction rates than did lower concentrations (Figure 4-2). Although the percentage reduction of Cr (VI) decreases with the increase of initial Cr (VI) concentration (Figure 4-3), the amount of Cr (VI) reduced increased with the increase of initial Cr (VI) concentrations in the solution.. Even the temperature has a positive effect on the reduction of Cr (VI). Below 25°C the reduction is a little bit less but still can reduce the major percentage of Cr (VI) (Figure 4-4). The temperature of groundwater usually varies from 10-20°C. Maybe in that case, more time is needed to get the complete reduction of Cr (VI) or a higher concentration of rhamnolipid is needed. The optimum time for the reduction of Cr (VI) from water was obtained by 4 hours (Figure 4-5) but for higher concentrations of Cr (VI), more time is needed.

The pH of groundwater varies from 5 to 9. As rhamnolipid works well at pH 6 and at 2% concentration, rhamnolipid can be used to clean up the groundwater at optimum conditions to obtain effective results.

#### 4.4 Part 2: Results of experiments in soil media

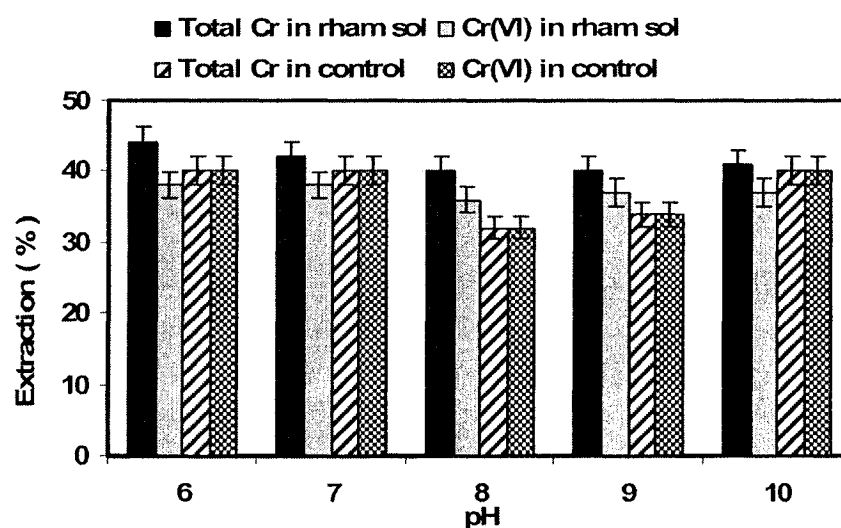
The results of soil washing experiments are presented in this section. The objective of this work was to evaluate the capability of rhamnolipid in the removal of Cr (VI) and to determine what parameters affect its behavior. The considered parameters were pH, soil and rhamnolipid solution ratio, concentration of rhamnolipid, initial Cr concentration of soil, temperature and contact time. The soil washing efficiency is presented as percent Cr removal which is as follows:

$$\% Cr\ removal = \frac{mg\ Cr\ in\ supernatant}{mg\ Cr\ in\ original\ soil} \times 100\% \dots\dots\dots(4-1)$$

$$\% Cr\ (VI)\ reduction = \frac{Total\ extracted\ Cr - Cr(VI)}{Total\ extracted\ Cr} \times 100\% \dots\dots\dots(4-2)$$

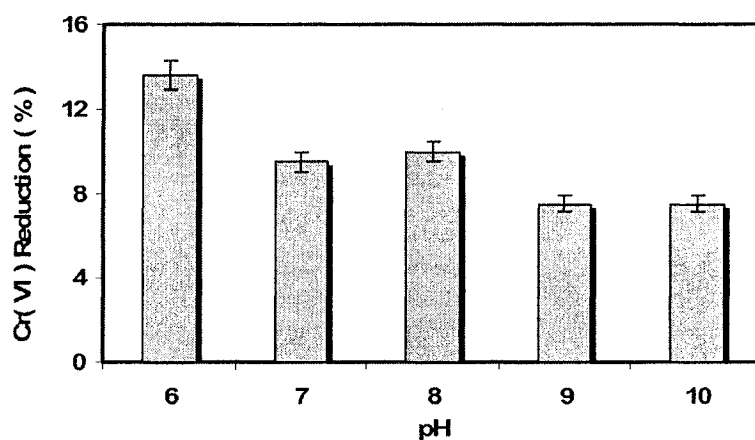
##### 4.4.1 Effect of pH

Soil washing experiments were performed at various pH values to see their effects on Cr extraction and also on the reduction of Cr (VI). One mg Cr per gram soil, i.e., the initial contamination level of soil was 1000 ppm for this experiment. The experiment was done taking one gram of soil and 10 mL of 0.5% rhamnolipid solution at 25°C.



**Figure 4-6: Extraction of Cr (VI) at different pH using 1000 mg/kg contaminated soil & 0.5% rhamnolipid solution**

The extraction of total Cr (VI) at pHs 6 to 10 is almost the same but the maximum Cr (VI) was removed at pH 6 which is 44% of initial Cr (VI) concentration in soil (Figure 4-6). Even the control (distilled water) can extract a good percentage of Cr (VI) which is 40% as Cr (VI) is very soluble in water. But the control cannot reduce the Cr (VI) where the reduction of Cr (VI) was the objective of this work. The decrease of Cr (VI) in rhamnolipid solution indicates the reduction of Cr (VI) to trivalent form.

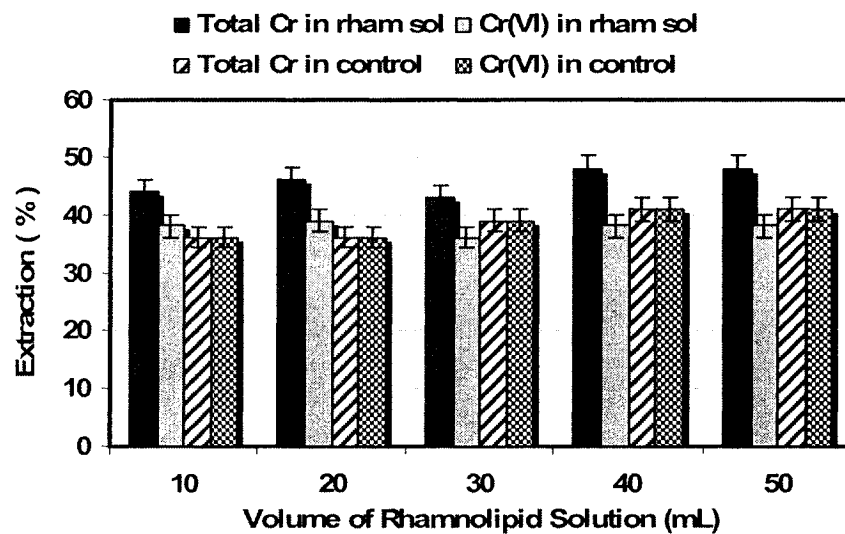


**Figure 4-7: Effect of pH on Cr (VI) reduction**

Figure 4-7 shows the reduction of Cr (VI) at different pH values. Maximum reduction was obtained at pH 6 which is 13.6% of extracted Cr. As maximum reduction was obtained at pH 6, this pH was selected as the optimum pH and all other experiments were performed at this pH.

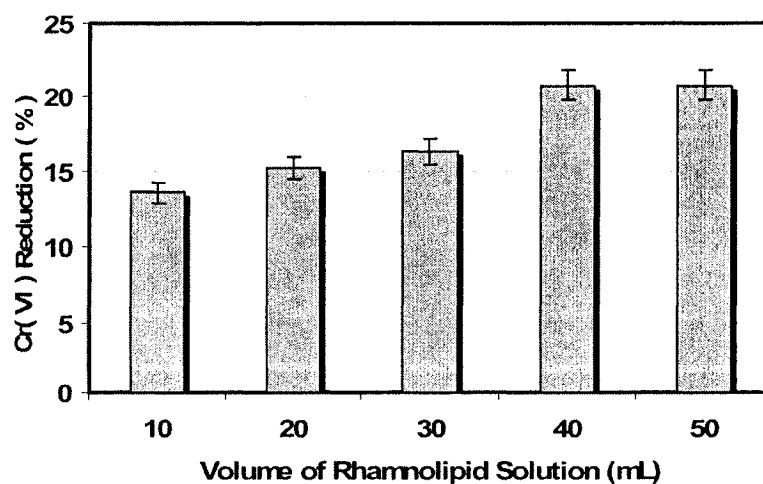
#### **4.4.2 Effect of surfactants to soil ratio**

This experiment was performed by taking one gram of contaminated soil (1000 ppm) with various volumes of 0.5% rhamnolipid solutions at pH 6 and 25°C. The rhamnolipid volumes were 10, 20, 30, 40 and 50 mL. Figure 4-8 shows the extraction of Cr at various rhamnolipid volumes.



**Figure 4-8: Extraction of Cr (VI) at various surfactants to soil ratios using 1000 mg/kg contaminated soil & 0.5% rhamnolipid solution**

The extraction increases with the increased volumes of rhamnolipid solutions but not that much. As using more surfactant solutions will not be economic, 20 mL was chosen for all other experiments.

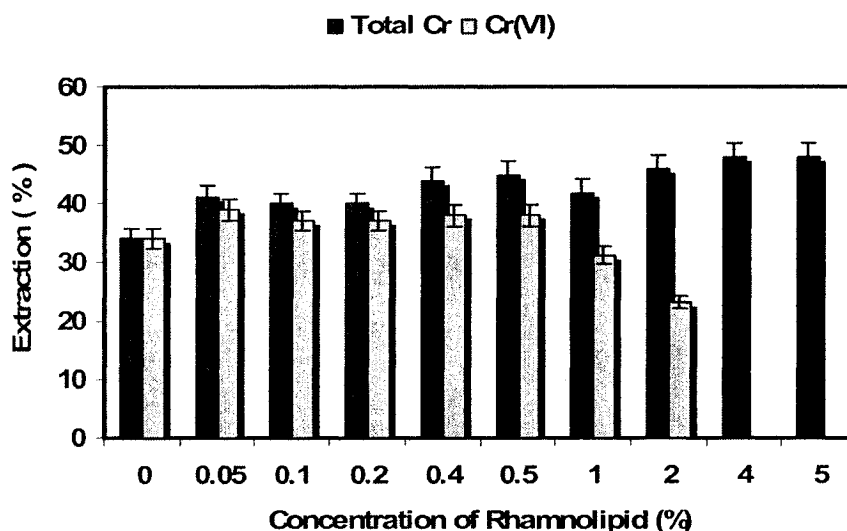


**Figure 4-9: Effect of various rhamnolipid to soil ratios on Cr (VI) reduction**

The reduction of Cr (VI) also increases with the increase of rhamnolipid solutions. Figure 4-9 shows the reduction of Cr (VI) at various surfactant volumes. The maximum reduction is 20.8% of extracted Cr which was obtained at 40 and 50 mL of rhamnolipid solutions. Around 15% reduction was obtained at 20 mL solution which is not that different from the maximum reduction obtained.

#### 4.4.3 Effect of various rhamnolipid concentrations

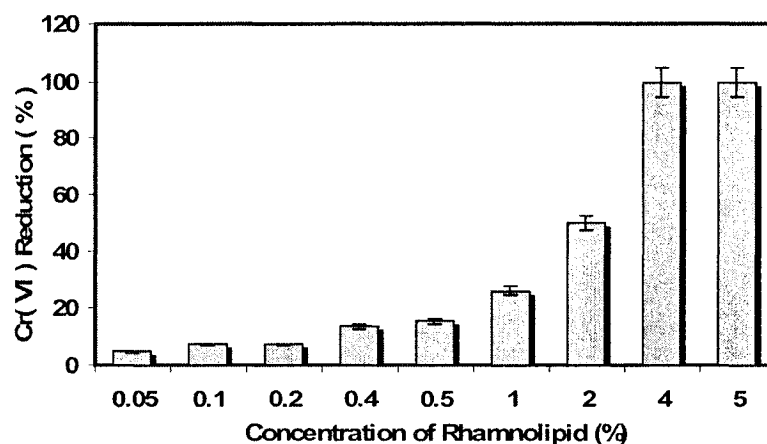
This washing experiment was performed taking one gram of contaminated soil (1000 ppm) and using different percentages of rhamnolipid concentrations at pH 6 and 25°C.



**Figure 4-10: Extraction of Cr (VI) at various rhamnolipid concentrations using 1000 mg/kg contaminated soil**

Both the extraction of Cr and the reduction of Cr (VI) increases with an increase in the rhamnolipid concentration. Figure 4-10 shows the extraction of Cr at various rhamnolipid concentrations. The maximum extraction is 48% of initial concentration which was obtained at 4% and 5% rhamnolipid concentrations. However, the 4% and 5%

concentrations are viscous and hard to work with. On the other hand, 2% rhamnolipid concentration gave a 46% extraction which was chosen as the optimum rhamnolipid concentration.

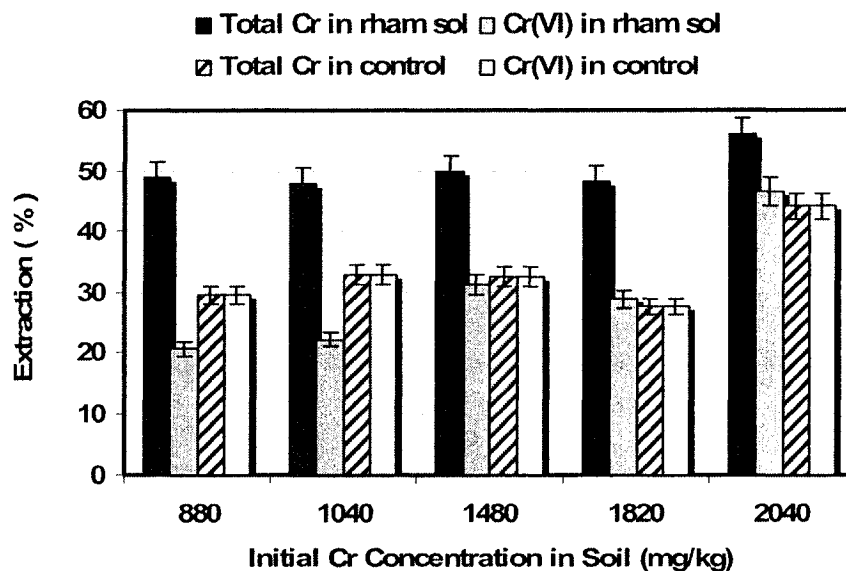


**Figure 4-11: Effect of various rhamnolipid concentrations on Cr (VI) reduction**

Figure 4-11 shows the reduction of Cr (VI) at various rhamnolipid concentrations. The extracted Cr (VI) was fully reduced at 4% and 5% concentration of rhamnolipid whereas a 2% rhamnolipid concentration reduced 50% of the extracted Cr.

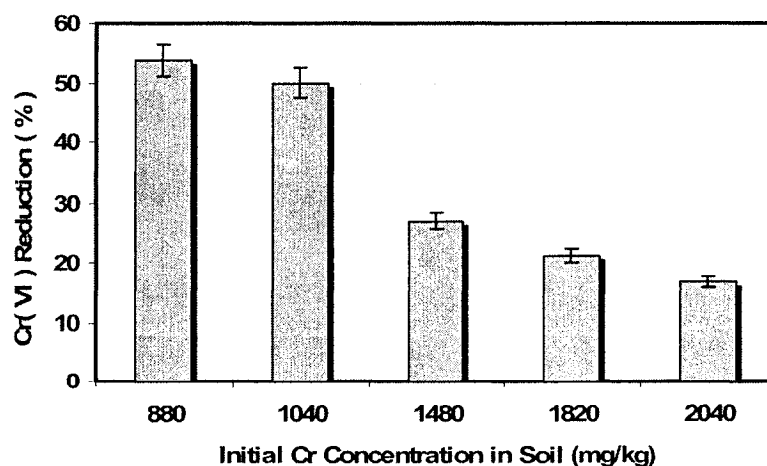
#### **4.4.4 Effect of initial Cr concentration in soil**

The effect of initial Cr concentration was also investigated to see how it affects removal efficiency. The concentrations were 880, 1040, 1480, 1820 and 2040 ppm.



**Figure 4-12: Extraction of Cr (VI) at various initial concentrations in soil using 2% rhamnolipid solution**

The experiment was performed using 2% rhamnolipid at pH 6 and 25°C. Figure 4-12 shows that the extraction of Cr increases with the increase of initial Cr concentration in soil. The maximum removal value of 56% was observed at an initial concentration of 2040 mg/kg of soil.



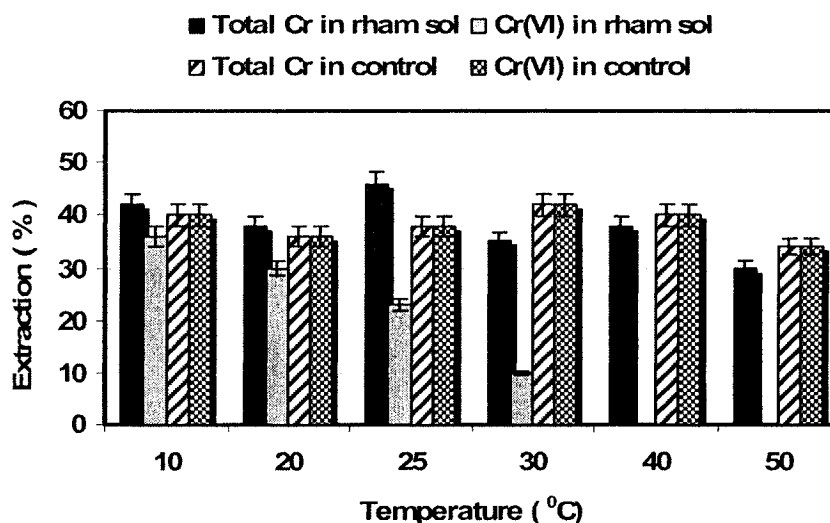
**Figure 4-13: Effect of different initial Cr (VI) concentrations on reduction of Cr(VI)**



On the other hand, Figure 4-13 shows that the percentage reduction of Cr (VI) decreases with the increase of extraction of Cr. In other words, the percentage reduction decreases with higher initial Cr concentration in soil. The maximum reduction percentage is 53.8% of extracted Cr which corresponds to an initial concentration of 880 mg/kg of soil. The minimum reduction percentage was observed for an initial concentration of 2040 mg/kg of soil which is 16.8% of extracted Cr.

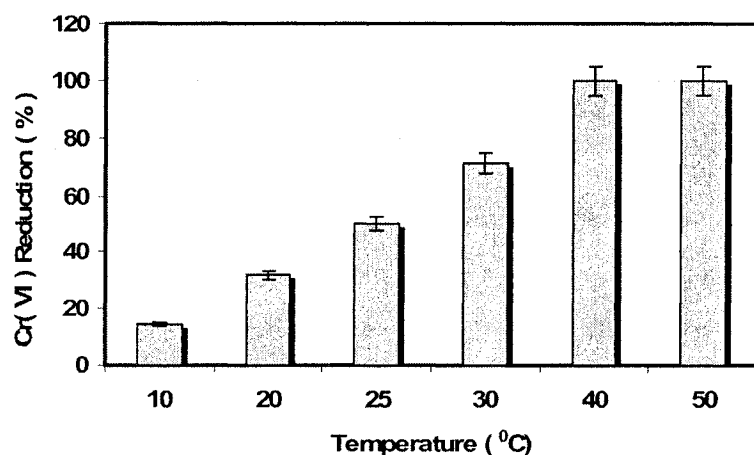
#### 4.4.5 Effect of temperature

This experiment was performed to see the effect of temperature on the extraction and also on the reduction of Cr (VI). One gram of soil (1000 ppm) was washed using 2% rhamnolipid solutions at pH 6 at different temperatures.



**Figure 4-14: Extraction of Cr (VI) at different temperatures using 1000 mg/kg contaminated soil and 2% rhamnolipid solution**

The considered temperatures were 10, 20, 25, 30, 40 and 50°C. Figure 4-14 shows that the extraction of Cr fluctuates with temperatures without any trend with temperatures. The maximum extraction was observed at 25°C which is 46% of initial concentration. The extraction decreases at temperatures of 30 to 50°C.

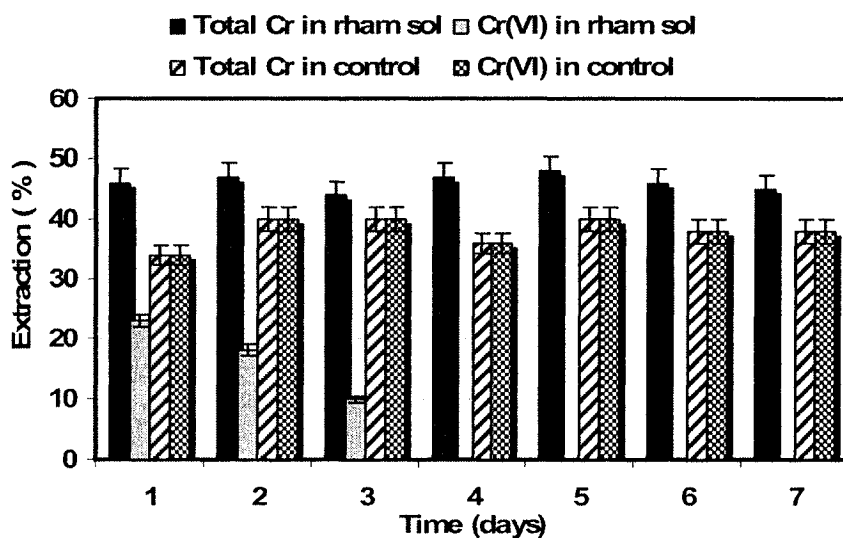


**Figure 4-15: Effect of temperature on Cr (VI) reduction**

On the other hand, Figure 4-15 shows that reduction of Cr (VI) increases with temperatures. Maximum extraction was at 25°C but at this temperature Cr (VI) was reduced 50% of extracted Cr. But in water media maximum reduction (100%) was obtained at 25° C (Figure 4-4) at the same conditions. Rhamnolipid gets adsorbed or precipitated onto soil surface for which the effective concentration of rhamnolipid gets reduced and consequently it reduces the removal efficiency. The complete reduction of extracted Cr was observed at 40 and 50°C. The reason is the solubility of both Cr (VI) and rhamnolipid increases with temperature which was discussed earlier.

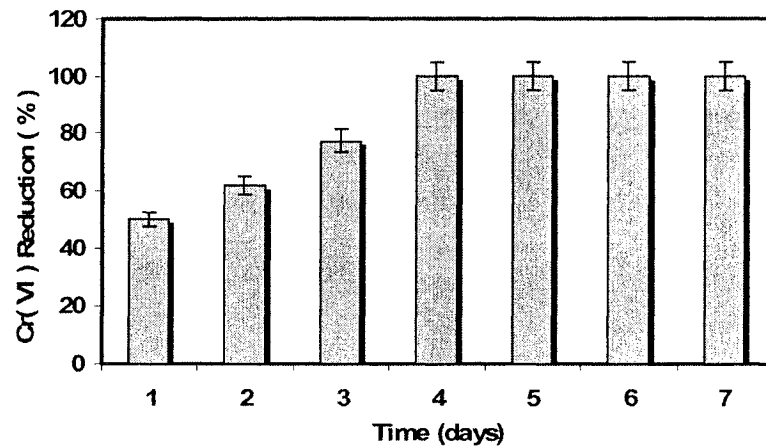
#### 4.4.6 Effect of time

This experiment was set to see how time affects the extraction of Cr and on the reduction of Cr (VI). One gram of soil (1000 ppm) was washed with 2% rhamnolipid solution at pH 6 and 25°C.



**Figure 4-16: Extraction of Cr (VI) with time using 1000 mg/kg contaminated soil & 2% rhamnolipid solution**

The experiment was carried out for 7 days and no significant changes in Cr extraction were observed. The extraction was almost the same for each day which varies from 45 to 48% of the initial Cr concentration.

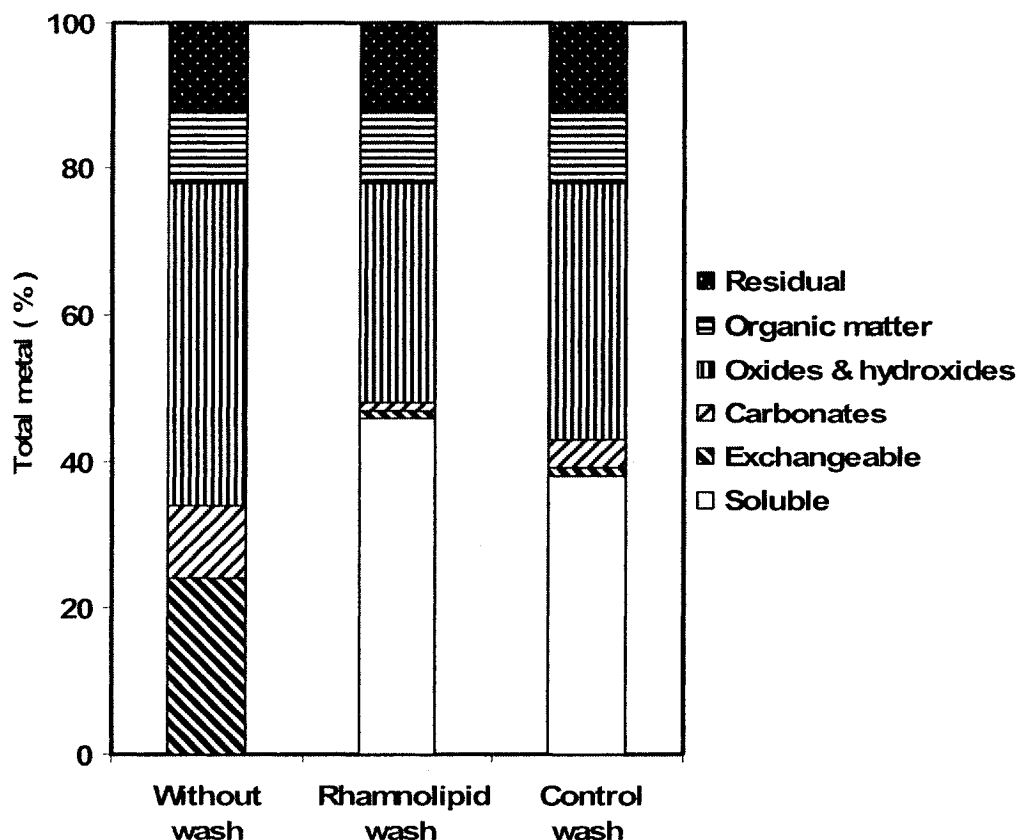


**Figure 4-17: Effect of contact time on Cr (VI) reduction**

Although the extraction was the same each day, the reduction of Cr (VI) increased with time. Figure 4-17 shows the reduction of Cr (VI) with time. After one day the reduction of Cr (VI) was 50% of the extracted Cr. After 4 days Cr (VI) was completely reduced. Massara et al. (2007) also showed that hexavalent chromium was reduced by the rhamnolipid over time where they used chromium contaminated kaolinite. They found that rhamnolipid has the capability of reducing almost 100% of the extracted Cr (VI) to Cr (III) over a period of 24 days and the reduction of Cr (VI) to Cr (III) was initiated after a three-day period.

#### **4.5 Sequential extraction experiments**

Sequential extraction experiments were performed on the soil without soil washing or pH adjustment prior to the procedure and also following soil washing to determine which fractions were removed by the surfactants. The soil was washed using 2% rhamnolipid solution at pH 6 and distilled water was used as control.



**Figure 4-18: Sequential extraction of Cr (VI) contaminated soil**

The fraction removed by the rhamnolipid or control is designated as the soluble fraction. Figure 4-18 shows the sequential extraction of Cr. It can be seen from the figure that the exchangeable and carbonate fractions of Cr are 24% and 10% respectively whereas the oxide fraction accounted for 44% of Cr present in the soil. The organic fraction made up about 10% of the Cr. The residual amount is about 12% of the Cr present in the soil.

#### **4.6 Summary and discussion of soil media**

Soil contaminated with hexavalent chromium was used in this part. The objective of this work was to evaluate the capability of rhamnolipid in enhancing the removal of Cr (VI)

from the soil and in reducing the Cr (VI). The objective also included determining what parameters affect the extraction and reduction of Cr (VI). Here the same parameters were considered as in the water part but in this case one extra parameter (surfactant to soil ratio) was evaluated since this was not relevant for water media.

From the soil washing experiments at different conditions, it has been observed that the amount of extracted Cr was almost the same for all conditions. The extracted Cr ranges from 40-48% of the initial Cr concentration. Although Cr (VI) is very soluble in water not all Cr (VI) is removed when rhamnolipid or water is added to the contaminated soil. Rhamnolipid can remove only the soluble part (40-48%). Even water can sometimes extract 40% as Cr (VI) is very soluble. The other part of Cr (VI) in soil may be converted to another form. This can be explained as follows. Jardine et al. (1999) suggested that the organic matter in the soil speculated to have reduced the Cr (VI) to Cr (III). They found that the presence of organic matter significantly retarded the transport of Cr (VI). When Cr (VI) was reduced to Cr(III) , negatively charged functional groups associated with organic constituents adsorb cationic chromium (Banks et al., 2006) resulting in lower concentrations of Cr(VI) in the soil.

The presence of Fe (II) in the soil can also reduce Cr (VI) to Cr (III) (Eary and Rai, 1991). On the other hand, chromate ion, a form of Cr (VI) may have formed insoluble precipitates with minerals such as Al, Ba and Fe (Palmer and Wittbrodt, 1991).

The extraction of chromium is almost the same in all conditions as rhamnolipid can extract only the soluble part present in the soil. The extraction increases with the increase of initial Cr concentration in the soil (Figure 4-12). The reason is that the soluble part is more there. But the extraction decreased a little bit at a temperature of 30-50°C (Figure 4-14). This might occur as rhamnolipid can reduce Cr (VI) to Cr (III) quickly above 30°C. Therefore, some soluble part of Cr (VI) in the soil might be converted to Cr (III) before it is extracted by the rhamnolipid solution.

Although the extraction of Cr doesn't vary much with different parameters, the reduction of Cr (VI) to Cr (III), which is the target of this work, is the same as in water media.

The purpose of the sequential extraction on soil which has been previously washed with rhamnolipid was to determine from what fraction rhamnolipid removed the metals. This information can then be used to determine if soil washing by rhamnolipid is useful or effective. The study shows the exchangeable portion in the soil is 24% and rhamnolipid can remove 96% of this portion. Therefore, ion exchange plays an important role in the chromium extraction process by rhamnolipid. The carbonate portion is lower (10%), but rhamnolipid can remove 90% of the carbonate portion. The study shows that Cr is mainly retained in oxides and hydroxides portion (44%). The organic and residual fractions retained 10% and 12% of chromium. Rhamnolipid can remove some (22%) of the oxides and hydroxides portion but it cannot remove the organic and residual portions.

Under acidic conditions, the oxide phase containing chromium could be released but rhamnolipid precipitates under acidic conditions (pH below 5). So, it would be difficult to remove chromium from the oxide part. The chromium associated with the organic part can be removed by rhamnolipid under basic conditions. The residual fraction is difficult to remove. The sequential extraction study gives important information to design the appropriate conditions for soil washing.

#### **4.7 Comparison of results of water and soil media**

The reduction trend of Cr (VI) both in water and soil media was the same but the reduction efficiency was better in water than soil. The maximum reduction was obtained at pH 6 both in water and soil but the reduction was 47.1% of initial Cr (VI) concentration in water and 13.6% of extracted Cr in the soil (Figure 4-1 & Figure 4-7). For both cases, 0.5% rhamnolipid was used. In water the initial Cr (VI) concentration was 10 mg/L and in soil the initial concentration was 1040 mg/kg. The initial Cr (VI) in water was 0.5 mg (10 mg/L, 50 mL) and the extracted Cr (VI) in the soil was 0.44 mg (Figure 4-6) which seems the initial amount Cr (VI) in the liquid solution was almost the same in both cases but the reduction was more in water than in soil. Rhamnolipid adsorbed onto the soil surface and the composition of the surfactant mixture remaining in the aqueous phase changed due to adsorption. The changes in composition of the surfactant due to adsorption affect the CMC of the surfactant mixture and potentially also the solubilizing or emulsifying properties (Noordman et al., 2000) for which the reduction efficiency gets decreased.



In the case of Cr (VI) reduction versus the percentage of rhamnolipid concentration, the maximum reduction was obtained (100%) in water media at 2% rhamnolipid concentration (Figure 4-2). On the other hand, in soil media the 100% reduction of extracted Cr was obtained at 4% rhamnolipid concentration (Figure 4-11). In both cases the reduction increases with the increase of rhamnolipid concentration.

To see the effect of initial Cr (VI) concentration on its reduction 10, 50, 100, 200 and 400 ppm of initial Cr (VI) concentrations in water were used. In soil media the initial concentrations were 880, 1040, 1480, 1820 and 2040 mg/kg. In both cases all other conditions were the same (2% rhamnolipid at pH 6 and 25°C). The reduction trend was similar and reduction decreases with the increase of initial Cr (VI) concentration (Figure 4-3 and Figure 4-13).

Temperature is an important factor for the reduction of Cr (VI). The reduction increases with the increase of temperature both in water and soil media. In water the maximum reduction was obtained at 25°C and above this temperature the level of reduction was the same (Figure 4-4). On the other hand, in soil media the maximum reduction was obtained at 40°C (Figure 4-15). The optimum time for the reduction of Cr (VI) in water media was around 4 hours whereas in soil media the complete reduction of extracted Cr was obtained after 4 days (Figure 4-17). From the above discussion it is clear that the reduction efficiency in soil media was less than that of water media although the experimental conditions were the same in both cases. The main reason for this is that soil adsorbs rhamnolipid thereby lowering the reduction capacity of rhamnolipid.

The following table shows a comparison of the results of water and soil media for maximum reduction and extraction.

**Table 4-1: Maximum reduction and extraction of Cr (VI) in water and soil**

| Experimental Conditions  | Water Media                       | Soil Media                          |  |
|--|-----------------------------------|-------------------------------------|--|
|  | Maximum Cr (VI) Reduction (%)     | Maximum Cr (VI) Extraction (%)      | Maximum Reduction (%) of Extracted Cr (VI) |
| pH 6-10, 0.5% rhamnolipid, 25° C                                 | 47.1 (at pH 6)                    | 44 (at pH 6)                        | 13.6 (at pH 6)                             |
| pH 6, rhamnolipid conc. (0.05- 5%), 25° C                        | 100 (at 2% rhamnolipid conc.)     | 48 (at 4% rhamnolipid conc.)        | 100 (at 4% rhamnolipid conc.)              |
| pH 6, various initial Cr (VI) conc., 2% rhamnolipid conc., 25° C | 100 (at initial conc. of 10 mg/L) | 56 (at initial conc. of 2040 mg/kg) | 53.8 (at initial conc. of 880 mg/kg)       |
| pH 6, 2% rhamnolipid conc., different temperatures               | 100 (at 25° C)                    | 46 (at 25° C)                       | 100 (at 40° C)                             |
| pH 6, 2% rhamnolipid conc., 25° C, different time                | 100 (after 1 day)                 | 46-48 (All most same at any day)    | 100 (after 4 days)                         |

## 5 Conclusions

### 5.1 Conclusions and summary

This research was performed to evaluate the feasibility of using rhamnolipid for the reduction of hexavalent chromium both in water and soil media. The study dealt with several factors involved in the extraction and reduction of Cr (VI) to find the optimum conditions. The parameters were pH, surfactant to soil ratio, concentration of biosurfactant, initial Cr concentration, temperature and contact time, pH, initial Cr concentration and rhamnolipid concentration have a significant affect on the reduction of Cr (VI).

The trend of reduction of Cr (VI) was the same in both water and soil media. But the extraction of Cr (VI) from the soil media did not vary as rhamnolipid removes mainly the soluble part. As Cr (VI) is very soluble, it comes into contact with liquid, it comes out from soil to solution.

The benefit of sequential extraction is that it provides important information which can be used to design the appropriate conditions for soil washing. The sequential extraction was used to determine the effect of rhamnolipid on Cr (VI) removal. This study also shows that rhamnolipid can remove the soluble part of Cr (VI) present in the soil (Figure 4-18). The exchangeable and carbonate portions contain good amounts of Cr (VI) which can be removed easily by rhamnolipid. The oxides and hydroxides portions retain significant amounts of Cr for which an additive might be needed to remove chromium from that portion. The residual fraction is the most difficult to remove.

According to the regulation of the ministry of Environment in Quebec (2003), the maximum concentration of total chromium allowable in water is 50 µg/L and for commercial/industrial soils is 800 mg/kg. So, the target of any technology should be to reach the contamination level at those points or below that. The experimental results show that rhamnolipid works effectively for the reduction of Cr (VI) in water media. It also works well in soil media to extract chromium from soil but it might be more effective if some additives are used with rhamnolipid.

## **5.2 Suggestions for future studies**

- Investigation of the effect of additives with rhamnolipid on the extraction and reduction efficiency of hexavalent chromium.
- Investigation of the effect of mixtures of surfactants or other surfactants on the extraction of Cr (VI) from the soil.
- Column studies to simulate in situ flushing with surfactants for the extraction and reduction of Cr (VI).
- Investigation of the effect of rhamnolipid on the extraction and reduction of Cr (VI) from actual contaminated soil.

## **5.3 Contribution to knowledge**

- Evaluation of the potential of low toxicity biosurfactants to extract hexavalent chromium from soil and to reduce Cr (VI) to Cr (III) in water and soil media.
- Correlation of soil washing procedure with sequential extraction study to find which fractions are involved in the extraction of hexavalent chromium.

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