Removal of Heavy Metals from Sediment Using Rhamnolipid

Behnaz Dahrazma

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Abstract

Removal of Heavy Metals from Sediment Using Rhamnolipid

Behnaz Dahrazma, Ph.D.
Concordia University, 2005

The interaction of heavy metals with sediments is complex and means are required to understand this matter more fully. Soil washing is among the methods available to remove heavy metals from sediments. This research was devoted to evaluating the performance of rhamnolipid for the removal of heavy metals, copper, zinc, and nickel, in batch and continuous flow configurations under different conditions, to investigate the mobility and availability of heavy metals by means of selective sequential extraction, and to find the mechanism for removal of heavy metals from the contaminated sediment obtained from the Lachine Canal, Quebec by using a rhamnolipid.

The removal of heavy metals from sediments was up to 37% for Cu which was mostly from the organic, 13% of Zn which was mostly from the carbonate, and 27% of Ni which was mostly from oxide and carbonate fractions when rhamnolipid without additives was applied in the continuous flow configuration. By ion exchange experiments, complexation was found to be the major mechanism of removal for all three metals by rhamnolipid. Adding 1% NaOH elevated the role of ion exchange mechanism up to 67.1% for removal of zinc from the sediment. Rhamnolipid adsorbed to the sediment according to the Freundlich isotherm. The zeta potential of the sediment was measured at –24.3 mV for distilled water, -9.5 mV for 2%, and -29.5 mV for 0.5% rhamnolipid. Scanning electron microscopy showed that washing with rhamnolipid does not change the texture of the sediment. Augmentation of the wetted surface area of the sediment improved the removal for all three metals (up to 3.5 times for copper), which emphasizes the role of this parameter in the removal process. A theory was established to explain the removal process of the metals, including wetting, contact of rhamnolipid to the surface of the sediment and detachment of the metals from the sediment.

Rhamnolipid has proven its ability as a washing agent in heavy metals removal from sediments, but more research is required to improve the performance of the rhamnolipid before scale-up.

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I would like to offer my thanks and appreciations to my family, my mother, my spouse, and my daughter for their deep love, encouragement, and patience they continuously expressed throughout my education. The memories of my father always warmed me up to go further toward my goals.

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Chapter 1

Introduction

1.1. General remarks

Heavy metals, metals with a specific gravity of more than 5, such as copper, zinc, lead, iron have been known and used for centuries. The exponential increase in the use of metals and heavy metals occurred after the Industrial Revolution (World Resource Institute 1999). For centuries, heavy metals along with other metals and minerals have been released from their natural chemical compounds through industrial activities and processes, but there were no codes or regulations to restrict their return to the environment. In the late 1960s politicians started to pay attention to the environmental problems. In 1970, the USEPA, which plays a very important role in environmental issues in the United State was established. In Canada, Environment Canada is the institution responsible to investigate and regulate environmental matters. In 1988, the Canadian government launched the St. Lawrence Action Plan for protection, conservation, and remediation of the river. The study performed by Environment Canada shows that in 1992 the average level of heavy metals in Montreal Harbor was more than 5 times the toxic level (Environment Canada 1993).

Heavy metals have acute effects on human health. For example, mercury affects the nervous, gastric system, and sometimes can cause death. Zn, Cu, Se, Cr, and Ni affect skin, gastric, and respiratory systems. Cadmium may severely damage the gastric and respiratory systems and may lead to death (Governor's Office of Appropriate Technology 1981).

The number of sites contaminated by heavy metals including contaminated soils and sediments, may give a better idea about the dimensions of the problem. According to the Ministry of Environment of Quebec, there are 48 sites contaminated with nickel, 167 sites contaminated with zinc, and 182 sites contaminated with copper, within Montreal Island alone. This number in most cases has an overlap of 2 or 3 metals although the level of toxicity is not the same for all cases (Environment Quebec 2004).

All these facts encourage environmental scientists to seek new methods, materials, and strategies to over come this threat. Among the available methods, biological remediation is becoming more and more important since it is biodegradable and less toxic although at the present less cost effective.

Surface active agents or surfactants are a wide variety of chemical substances with hydrophobic and hydrophilic portions (Friberg and Lindman 1992). Surfactants have been used in soil washing/ soil flushing processes and also for enhancing oil recovery and heavy metal removal (Myers 1992, Mulligan 1998, Holakoo 2001, Dahrazma 2002).

1.2. Objectives of this research

The present research focused on the study of the use of rhamnolipid to remove heavy metals from sediments as well as to develop new techniques that take flow and soil characteristics into account. Using the results of this comprehensive study of heavy metal removal and by performing a variety of experiments under different conditions, the theory of the mechanism of the removal of heavy metal by rhamnolipid is presented.

The objectives of this research can be classified in 4 categories:

- To evaluate the use of rhamnolipid in heavy metal removal from sediment, by using batch and continuous flow tests.
- To determine the speciation of metals in the sediments and the fractions of sediment which respond to rhamnolipid treatment in different conditions using sequential extraction techniques.
- To study the adsorption of rhamnolipid to the sediment.
- To establish a theory of the mechanism of heavy metal removal from sediments.

Among the variety of heavy metals, copper, zinc, and nickel were chosen for this study.

1.3. Organization of this thesis

The present Ph.D. thesis includes 9 chapters as follows:

- Chapter 1 is the introduction to the problem. The definition of the problem, the objectives of this research, and organization of the thesis are the major concepts discussed in this chapter.
- Chapter 2 is devoted to literature review and background information. Most related previous studies are discussed in this chapter.
- Chapter 3 includes the description of the materials, methods, equipments, and procedures used in the experiments. The characteristics of materials used in the present research are reported as well.
- Chapter 4 presents the investigations on the removal of heavy metals by rhamnolipid in both batch and continuous flow tests. Results are presented and followed by discussion.
- Chapter 5 shows the results of selective sequential extraction tests.
- Chapter 6 gives the method and the results of the study of the adsorption of rhamnolipid on the sediment.
- Chapter 7 is devoted to the specific tests and experiments performed to investigate the mechanism of removal. In this chapter results of the previous chapters are reviewed, discussed, and interpreted.
- Chapter 8 includes the economical aspects of the sediment washing with rhamnolipid.
- Chapter 9 includes the conclusions, contribution to knowledge, and recommendations for further studies on the subject.
- References are listed at the end of the thesis.

Chapter 2

Literature Review

2.1 General remarks

Metals are a discovery of the old world. Humans began using metals in about 6000 B.C. Metals are everywhere, from air to water and to the deepest places in Earth. Metals are useful but they can be hazardous and toxic.

Heavy metals are metals with a specific density of more than 5 (Neely 1979). Also, Yong (2000) stated that elements with atomic number greater than 20 are classified as heavy metals. There are 38 heavy metals including cobalt, chromium, copper, iron, manganese, nickel, lead, and zinc (Cameron 1992). Most heavy metals are toxic at levels from 1 to 2 mg/kg tissue. Ores and mining of heavy metals are sources of heavy metals in the environment. They are in the environment for hundreds of years once they are emitted (Truhart 1979).

Industries, urban aerosols, incinerators, ore refineries, and wastes from humans and animals are some sources of metallic wastes (Table 2.1).

Table 2.1 Industrial and domestic sources of nickel, zinc and copper (adapted from EPA 2001, EE 2001, Atkins and Hawley 1978, and Dean et al. 1972).

Metal	Source	Abundance rank in earth's crust
Nickel	Hydrogenation oil processes, rechargeable batteries (nickel-cadmium), ambient air at very low levels as a result of releases from oil and coal combustion, nickel metal refining, sewage sludge incineration, manufacturing facilities.	22
Zinc	Cleaners, cosmetics, fillers, pesticides, inks, oils, medicines, paints, polish, preservatives, water treatment, fertilizers, oil refining, inorganic chemicals.	24
Copper	Pigments, polish, ointments, medicines, fuels, pesticides, inks, cosmetics, cleaners, foundries, paper, electronics, fertilizers, oil refining.	25

2.2 Heavy metals: properties, abundance and their effects

Most heavy metals are too rare or too insoluble to be of biological impact, but eleven elements including vanadium, chromium, manganese, iron, cobalt, nickel, copper, zinc, selenium, molybdenum, and tin are essential for normal functioning of our bodies (Cox 1999 and Jackson 1999).

Determination of the total heavy metal content of the soil is commonly used to indicate the degree of contamination, but the heavy metal concentration in solution most commonly determines the actual environmental exposure or risk. Distribution of heavy metals between soil and solute is the key to evaluating the environmental impact of the metals. Despite the complexity of possible reactions, several important soil factors controlling the distribution of heavy metals between soil and solutes have been identified (Sposito 1989, Temminghoff et al., 1998, Karaca 2004). Environmental risk assessment of heavy metals depends on the mobility of metals based on soil-liquid partitioning coefficients. These coefficients are related to the pH of the soil solution, soil organic matter, and concentration of total soil metal content (Sauvé et al. 2000).

2.2.1 Zinc

Zinc, represented as Zn, is a soft, white, bluish tinged metallic element and is one of the transition elements in the periodic table. It has an atomic number of 30, molar mass of 65.37 g, and density of 7.14 g/cm^3. Zinc has two oxidation states: Zn^0 and Zn^{2+}(O'Conner et al. 1968).

Pure zinc is a crystalline metal. It is insoluble in hot and cold water but soluble in alcohols, acids, and alkalies. Zinc remains unaffected by dry air, but in moist air becomes coated with a carbonate film due to oxidation. This film protects zinc from further corrosion. Zinc is never found free in nature, but occurs as zinc oxide, ZnO, in the mineral zincite, as zinc silicate, $ZnO{\cdot}SiO_2H_2O$, in the mineral hemimorphite, as zinc carbonate, $ZnCO_3$, in the mineral smithsonite, as a mixed oxide of zinc and iron, $Zn(FeO_2)O_2$, in the mineral franklinite, and as zinc sulfide, ZnS, in the mineral sphalerite, or zinc blend. The ores most commonly used as a source of zinc are smithsonite and sphalerite (Encarta Encyclopedia 2001).

Zinc is mainly used as a protective coating, or galvanizer, for iron and steel as well as an ingredient of various alloys, especially brass, for dry electric cells, and for die-castings. Zinc is released to the environment from the same sources: galvanized iron, coal and waste burning, natural ores and municipal waste treatment plants. In wastes, zinc occurs as zinc oxide, zinc chlorides, zinc sulfate, and zinc sulfide (Agency for Toxic Substances and Disease Registry 1994).

Zinc is the second most abundant trace element in the body (after iron), and the brain has its share, with about 10 mg of zinc in a gram of wet tissue (Choi and Koh 1998). The required daily intake for the human body ranges from 10 to 20 mg (Smith et al. 1976).

Zinc is an essential structural element of many proteins. Neural activity releases zinc as a signaling messenger at many central excitatory synapses. New evidence suggests that zinc may also be a parameter in neuronal death associated with transient global ischemia, sustained seizures, and perhaps other neurological disease states (Choi and Koh 1998).

Environment Canada and the Canadian Council of Ministers of the Environment have chosen different criteria for classifying soils and sediments in the sense of contamination (Tables 2.2 and 2.3). Comparison of these two tables shows that the minimum concentrations established by the Canadian Council of Ministers of the Environment (CCME) for heavy metals for sediment are far lower than those used for soils. The zinc concentration allowance in sediment is very strict because of the close interrelation between benthic organic material and its environment. Organisms feeding off this sediment are located at the lower end of the aquatic food chain. Therefore, it is very important to ensure minimum accumulation of toxic substances that could be absorbed by higher organisms (Environment Canada 1993).

Table 2.2 Classification of sediments by their zinc concentration (adapted from Environment Canada 1993).

Effect Level	Concentration of zinc (mg/kg of dry weight sediment)
No effect level	100
Minimal effect level	150
Toxic effect level	550

Table 2.3 Classification of soils by zinc concentration defined by Canadian Council of Ministers of the Environment (adapted from Environment Canada 1993).

Soil use	Maximum concentration of zinc (mg/kg of dry weight sediment allowed)
Residential purposes and parks	500
Agricultural purposes	600
Commercial and industrial purposes	1500

2.2.2 Copper

Copper, represented as Cu, is a brownish-red metallic element that is one of the most widely used of metals. Copper is one of the transition elements of the periodic table with three oxidation states: Cu^0, Cu^{1+}, and Cu^{2+}(Uauy et al. 1998). The physical and chemical characteristics of copper are as follows: atomic number of 29, density of 8.9 g/cm^3, and a molar mass of 63.5 g. Copper was known to prehistoric people and was probably the first metal from which useful articles were made. Copper objects have been found among the remains of many ancient civilizations, including those of Egypt, Asia Minor, China, southeastern Europe, Cyprus (from which the word *copper* is derived), and Crete. It was known to Native Americans, and American ores were found by the European explorers. It also is found in the pure state (Encarta Encyclopedia 2001).

Copper, like silver and gold, is a noble metal. Its properties, including high thermal and electrical conductivity, low corrosion, alloying ability, and malleability, make it very useful for a wide variety of industrial purposes. “Copper is an essential trace element, which is an important catalyst for heme synthesis and iron absorption. The serum copper concentration ranges up to approximately 1.5 mg/L in a healthy person” (Barceloux 1999).

Regulation of copper in the human body is not sufficient with very low amounts of dietary copper (0.38 mg/day) and it appears to be delayed when copper intake is high (Turnlund 1998). Pro-oxidant activity of copper ions can make it extremely toxic at elevated levels. Copper related inherited disorders such as Menkes disease (due to copper deficiency) and Wilson disease (due to copper toxicosis) demonstrate the necessity and potential toxicity of copper. There is a good chance of involving copper induced oxidative damage in other pathogenic or neurodegenerative conditions such as Alzheimer’s disease and prion diseases (Llanos and Mercer 2002).

As indicated in Table 2.1, copper exists in construction materials, pesticides, fertilizers, sprays, and agricultural and municipal wastes as well as industrial wastes. As these materials are exposed to the environment, the level of copper increases. The average content of copper in plants is in the range of 5 to 30 ppm while in soil it varies between 2 to 100 ppm (Cameron 1992).

2.2.3 Nickel

Nickel (Ni) is a silvery white magnetic metallic transition element that mainly is used in making alloys. Nickel is a hard, malleable, ductile metal, capable of obtaining a high polish. The atomic number of nickel is 28, its specific gravity is 8.9, and the atomic weight of nickel is 58.69 (Encarta Encyclopedia 2001). Although it has oxidation states of -1, 0, +1, +2, +3, and +4, the most common valence state in the environment is +2 (Canadian Environmental Protection Act 1994).

Nickel is soluble in dilute nitric acid and becomes passive (nonreactive) in concentrated nitric acid, also, it does not react with alkalies. Nickel is found in minerals such as garnierite, millerite, niccolite, pentlandite, and pyrrhotite combined with other elements. Pentlandite, and pyrrhotite are the principal ores of nickel. Nickel is used widely in coating industries to protect and ornament metals, particularly iron and steel. Nickel steel, containing about 2 to 4 percent nickel is used to make automobile parts such as axles, crankshafts, gears, valves, and rods because of its great strength and corrosion resistance as compared to steel. A fairly new application of nickel is rechargeable nickel-cadmium batteries. Canada is the second largest supplier of nickel in the world. A rich deposit of nickel was discovered in 1957 in northern Québec (Encarta Encyclopedia 2001).

Although the role of nickel in the human body is not known, it is an essential nutrient for other mammalian species and has been suggested to be essential for human nutrition as well. Following data from animals, it is estimated by extrapolation that a 70-kg person would have a daily requirement of 50 µg per kg nickel. Since nickel is an element of the crust of the earth, it exists in food, air, and water. Food is believed to be the major source of nickel exposure. In the US, an average intake for adults of approximately 100 to 300 micrograms per day (μg/d) is estimated. Nickel production, processing, and use, contact with everyday items such as nickel-containing jewelry and stainless steel cooking and eating utensils, and smoking tobacco are major sources of exposure of nickel to humans (U. S. Environment Protection Agency 2001).

Among nickel's properties, its mutagenic, immunotoxic, carcinogenic and teratogenic properties are the most important. Due to efficient phagocytosis into the cells, solid nickel compounds are the most toxic, at least in regard to carcinogenicity and mutagenicity. In addition, water-soluble salts, like $NiCl_2$, are also mutagenic. Nickel salts have been found to be harmful for reproduction of laboratory rodents. Embryonal and fetal mortality have been found to increase when females have been exposed to nickel (Kakela et al. 1999).

Nickel is a relatively mobile heavy metal. In natural waters, nickel is transported in both particulate and dissolved forms. The pH, oxidation-reduction potential, ionic strength, type, and concentration of organic and inorganic ligands (in particular, humic and fulvic acids), and the presence of solid surfaces for adsorption (in particular, hydrous iron and manganese oxides) can all affect the transport, fate, and biological availability of nickel in fresh water and seawater (Semkin, 1975; Callahan *et al.,* 1979; Snodgrass, 1980). In some sediments under reducing conditions and in the presence of sulphur, relatively insoluble nickel sulphide is formed (Ankley *et al.,* 1991). Under aerobic conditions and pH less than 9, nickel forms compounds with hydroxide, carbonate and sulphate. Naturally occurring organic ligands are sufficiently soluble to maintain aqueous Ni^{2+} concentrations above 60 µg/L (Callahan *et al.,* 1979). Most of the nickel in sediments and suspended solids is distributed among organic materials, precipitated and coprecipitated as particle coatings, and crystalline particles (Canadian Environmental Protection Act 1994).

2.3 Surfactants

Surface **act**ive **a**gent or surfactant is the name for a wide variety of chemical substances with the ability to lower the surface tension of solvents. Surfactants also are called surface active substances and surface active compounds (Tsujii 1998). Surfactants form aggregates and micelles in aqueous media (Myers 1999), which is very important as their effectiveness depends on the ability to reduce surface tension (Rosen 1978).

2.3.1. Characteristics of surfactants

Surfactants, amphipathic molecules with both hydrophilic and hydrophobic (generally hydrocarbon) moieties (Fig.2.1), are able to partition between the interfaces of fluid phases with different degrees of polarity. In the case of hydrogen bonding such as at air/water or oil/water interfaces, surfactants concentrate at the interfaces. Positioning at the interface, surfactants are capable of reducing surface and interfacial tensions.

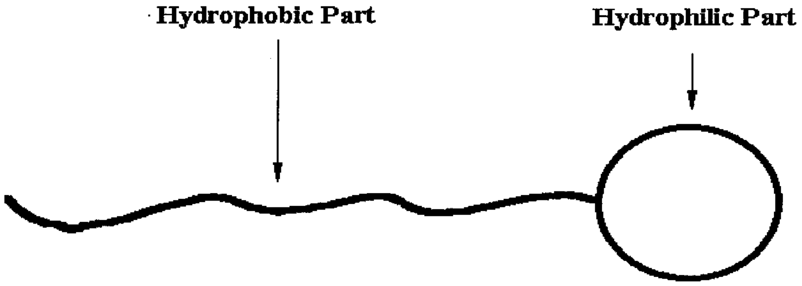


Figure 2-1 Surfactant structure (adapted from Myers 1999).

They form microemulsions where hydrocarbons can solubilize in water or where water can solubilize in hydrocarbons. These characteristics provide surfactants with excellent detergency, emulsifying, foaming, and dispersing traits. In other words, surfactants are some of the most versatile process chemicals (Desai and Banat 1997). Reports indicate that surfactants also are used in other industries as flocculating wetting agents, adhesives, and penetrants (Mulligan and Gibbs 1993). In 2002, only in the USA, a total amount of 7.2 billion kg of surfactants were consumed (McCoy 2004).

Surface tension, hydrophilic-lipophilic balance (HLB), and critical micelle concentration (CMC) are three important characteristics of surfactants. These parameters confer their activity to reduce surface and interfacial tension and enable applications such as detergency (Desai and Banat 1997).

"The attraction between molecules forms an imaginary film capable of resisting tension at the interface between two immiscible liquids or at the interface between a liquid and a gas. The liquid property that creates this capability is known as surface tension" (Daugherty et al 1989). This is the definition of surface tension from a fluid mechanics point of view. With a look to the molecular and atomic arrangement of fluids, we will find the reason for the existence of such tension. In a liquid, each molecule interacts with the interactive forces of surrounding molecules. At the surface, these forces are absent or negligible and thus those molecules have excess energy compared to those in the bulk of the liquid. This excess energy is defined as the surface tension (Tsujii 1998). Most organic compounds have remarkably similar values of surface tension at room temperature, which is in the range of 25 to 40 mN/m (Fox and McDonald 1992). Surfactants can reduce the surface tension of water from 73 to 30 mN/m at room temperature (Desai and Banat 1997).

HLB is another important character of surfactant. HLB is the abbreviated form of hydrophilic-lipophilic balance. HLB is represented by numbers from 1 for the most lipophilic (oleic acid) to 20 for the most hydrophilic (sodium oleate) respectively, and shows the tendency of a surfactant to dissolve preferentially in oil or in water (Kunieda and Sato 1992, Mulligan and Gibbs 1993, Desai and Banat 1997, Tsujii 1998). Emulsifiers with HLB values less than 6 favor stabilization of water-in-oil emulsification, whereas emulsifiers with HLB values between 10 and 18 have the opposite effect and favor oil-in-water emulsification (Desai and Banat 1997).

The critical micelle concentration, CMC, can be defined as the minimum concentration of surfactant at which micelles formation initiates. Figure 2-2 shows the CMC, derived from a typical plot of the surface tension versus logarithmic surfactant concentration (Tsujii 1998).

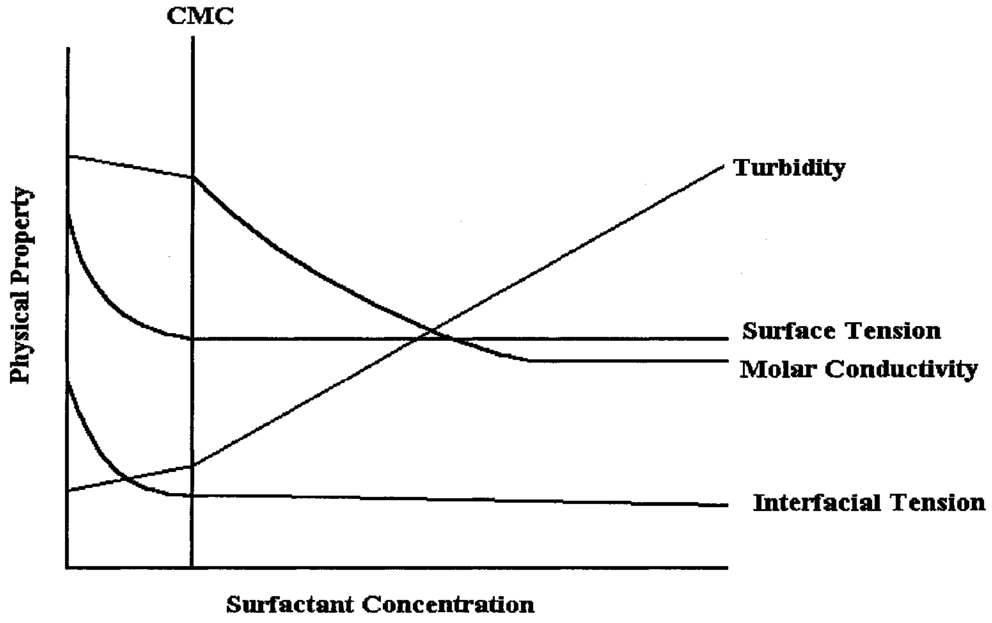


Figure 2-2 Variation of some important physical properties by surfactant concentration (adapted from Myers 1992).

By increasing the surfactant concentration, the surface tension decreases until it reaches the CMC. Beyond this level of surfactant concentration the surface tension remains constant. It is well known and described in the literature that low CMC values represent a more efficient surfactant since less surfactant is needed to decrease the surface tension (Becher 1965 and Mulligan 1998). Figure 2-3 shows the arrangement of surfactant molecules in the two cases: below and above the CMC.

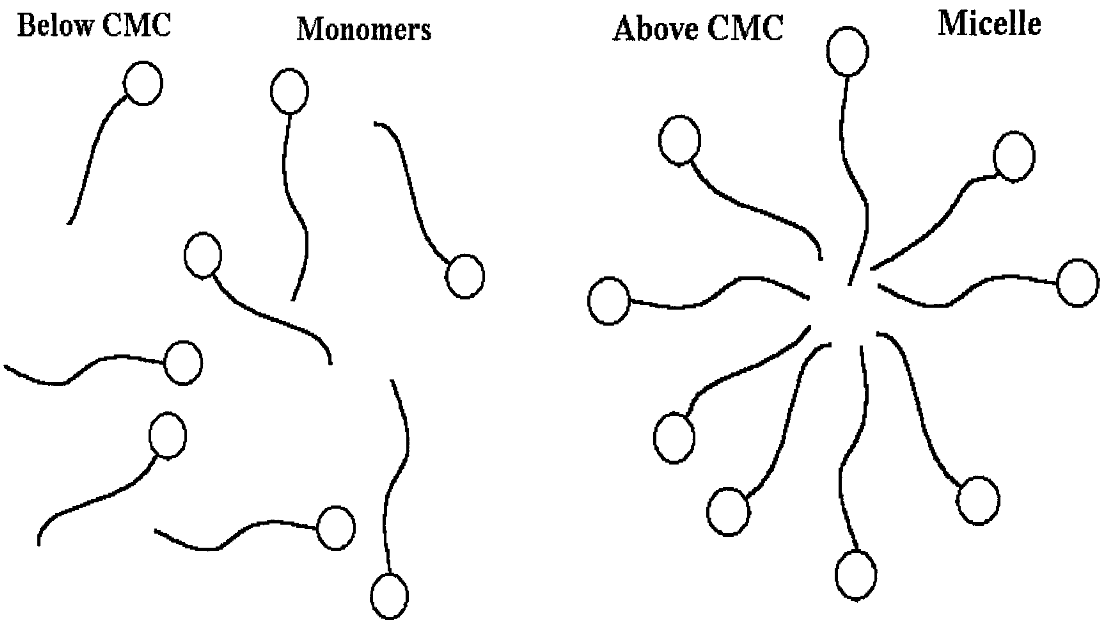


Figure 2-3 **Surfactant Micellization (adapted from Becher 1965).**

2.3.2. Types of surfactants

Surfactants are classified into four groups: anionic, cationic, amphoteric, and nonionic. This classification is based on their hydrophilic groups that contain anionic, cationic, amphoteric, or nonionic portions, respectively (Fig. 2-4). Surfactants in each group are used for particular purposes. In general, anionic surfactants are mainly used in detergents while amphoteric surfactants are used as boosters to enhance detergency and/or foaming. Cationic surfactants are used in fabric softeners and hair conditioners. Nonionic surfactants are the choice for washing oily dirt and also as emulsifiers (Tsujii 1998).

The most important selection criteria for surfactants are the charge type, physicochemical behavior, adsorption behavior, and solubility (Oberbremer et al.

1990). Table 2.4 presents common types of surfactants mentioned above.

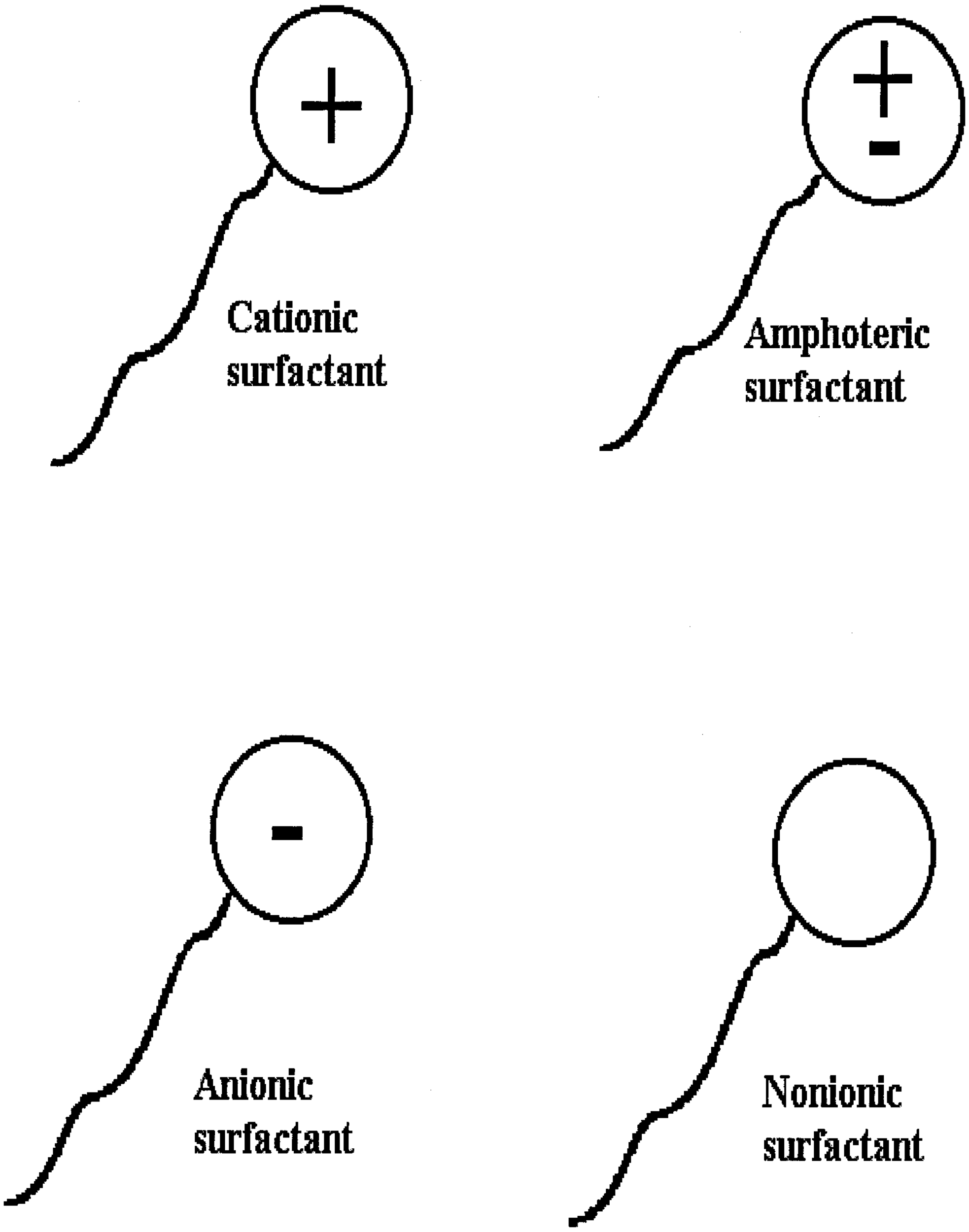


Figure 2-4 **Types of surfactants (adapted from Myers 1992).**

Table 2.4 Chemical types by surfactant class (adapted from Huntsman Surface Sciences 2004).

Type of Surfactant	Chemical types
ANIONIC SURFACTANTS	Alkyl ether phosphates Alkyl ether carboxylic acids and salts Alkyl ether sulphates Alkyl naphthalene sulphonates Alkyl phosphates Alkyl benzene sulphonic acids and salts Alkyl phenol ether phosphates Alkyl phenol ether sulphates Alpha olefin sulphonates Aromatic hydrocarbons
AMPHOTERIC SURFACTANTS	Alkyl ampho(di)acetates Amido betaines Alkyl betaines
CATIONIC SURFACTANTS	Alkyl dimethylamines Alkyl amidopropylamines Alkyl imidazoline derivatives Quaternised amine ethoxylates Quaternary ammonium compounds
NONIONIC SURFACTANTS	Alkyl polysaccharides Amine oxides,block copolymers Castor oil ethoxylates Ceto-oleyl alcohol ethoxylates Ceto-stearyl alcohol ethoxylates Decyl alcohol ethoxylates Dinonyl phenol ethoxylates Dodecyl phenol ethoxylates

2.3.3. Biosurfactants

Surfactants synthesized by microorganisms are called biosurfactants. Biosurfactants are a structurally diverse group of surface active molecules that reduce surface and interfacial tensions in both aqueous solutions and hydrocarbon mixtures. This property makes them applicable to enhance oil recovery and de-emulsification processes (Shennan and Levi 1987).

Currently, most of the surfactants are chemically derived from petroleum. However, interest in microbial surfactants has been steadily increasing in recent years due to their diversity, environmentally friendly nature, the possibility of their production through fermentation, and their potential applications in environmental protection, crude oil recovery, health care, and food-processing industries (Banat 1995(a), Banat 1995(b), and Muller-Hurting et al. 1993). Biosurfactants also have been found useful in various fields such as crude oil drilling, pharmaceuticals, and food processing industries (Ju 1999, Muller-Hurtig et al. 1993, and Fietcher 1992).

Surfactants show some level of toxicity to microorganisms because they can be adsorbed at interfaces. This interaction between the hydrophilic part of a surfactant and the proteins in cell membranes can solubilize components having membranes with a very high protein-lipid ratio. This affinity lowers the cell activity by decreasing the enzyme activity. Such an interaction between surfactant and cell causes a certain level of toxicity. Since the level of toxicity of biosurfactants is much lower, they have been considered as a substitute for synthetic surfactants for the last few decades. New biotechnological methods provide inexpensive ways to produce biosurfactants at large scale and decrease the price of these products to make them more economical to use widely in industry (Hunt et al. 1994).

Biosurfactants have several advantages over chemical surfactants, such as lower toxicity, smaller molecular size (less than 1500), higher biodegradability, better environmental compatibility, higher foaming, high selectivity and specific activity at extreme temperatures, pH, and salinity, and the ability to be synthesized from renewable feed stocks. Earlier work on biosurfactants centered mainly on their properties, biosynthesis, and chemistry and has been reviewed by many researchers. However, in the last two decades, significant work on the fermentative production, genetics, and commercial applications of biosurfactants has been done (Muller-Hurtig et al. 1993, Desai and Banat 1997, Ju 1999, Kim et al. 2000).

2.3.4. Types of biosurfactants

Chemically synthesized surfactants are classified according to the nature of their polar grouping but biosurfactants are categorized mainly by their chemical composition and their microbial origin. The structure of a biosurfactant includes a hydrophilic portion that consists of amino acids or peptides, mono-, di-, or polysaccharides, and a hydrophobic portion that consists of unsaturated and saturated lipids, or fatty acids (Desai and Banat 1997).

Biosurfactants include glycolipids, lipopeptides, phospholipids, fatty acids, and neutra lipids (Tsujii 1998, Biermann et al. 1987). The majority of biosurfactants are anionic or nonionic and only a small group of them are classified as cationic. Their CMC values vary between 1 to 200 mg/L and their molecular weights are between 500 and 1500 (Lang and Wagner 1987). The major types of biosurfactants, with their properties and microbial species of origin, are listed in Table 2-5.

2.3.4.1. Rhamnolipids

Rhamnolipids, used in this study, are biosurfactants from the glycolipid group produced by the bacterium *Pseudomonas aeruginosa*. The relationship between the rhamnolipid and *Pseudomonas aeruginosa* is very special. *Pseudomonas aeruginosa* produces and releases rhamnolipid for emulsification of n-alkanes to transfer them into its cells. Rhamnolipids are active only in the case by assimilation of *Pseudomonas aeruginosa*. Other kinds of glycolipids do not show such a behavior and they are not so specific for the species of microorganisms (Hitsatsuka et al. 1971, Tsujii 1998).

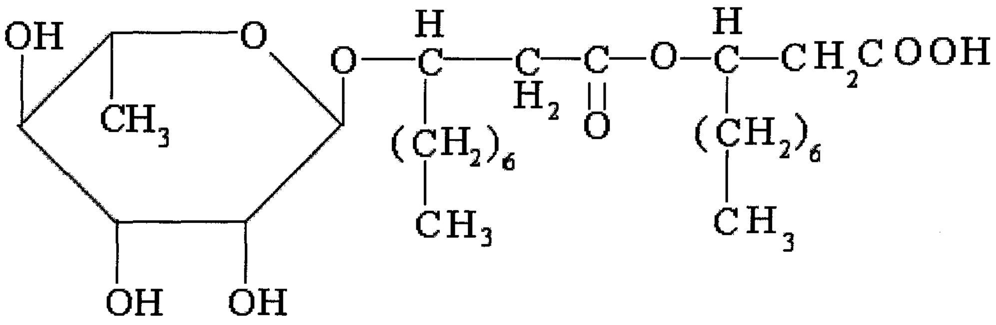
There are four types of rhamnolipids and their chemical structures are shown in Figure 2-5 (Tsujii 1998). Rhamnolipid type I and type II are suitable for soil washing and heavy metal removal while type III is for metal, leather, lubricants andpulp and paper processing. Type IV is usually used in textiles, cleaners, foods, inks, paints, adhesives, personal care products, agricultural adjuvants, and water treatment (Jeneil 2001).

Rhamnolipids like other surfactants can lower the air/water surface tension. For example, for distilled water, the surface tension is lowered from 72 mN/m to around 30 mN/m in the presence of rhamnolipids (Zhang and Miller 1992). Rhamnolipids can also enhance soil flushing efficiency and the rate of removal of heavy metals from soils that makes them suitable to remove these heavy metals from such soil to reduce its toxicity (Miller 1993). Rhamnolipids are very skin compatible and a low irritant. These properties make them suitable to be used even in cosmetic products as an additive by Iwata Co. of Japan (Maier and Soberon-Chavez 2000). Ishigami et al. (1988) patented the use of rhamnolipids in liposomes and emulsions for the cosmetic industry.

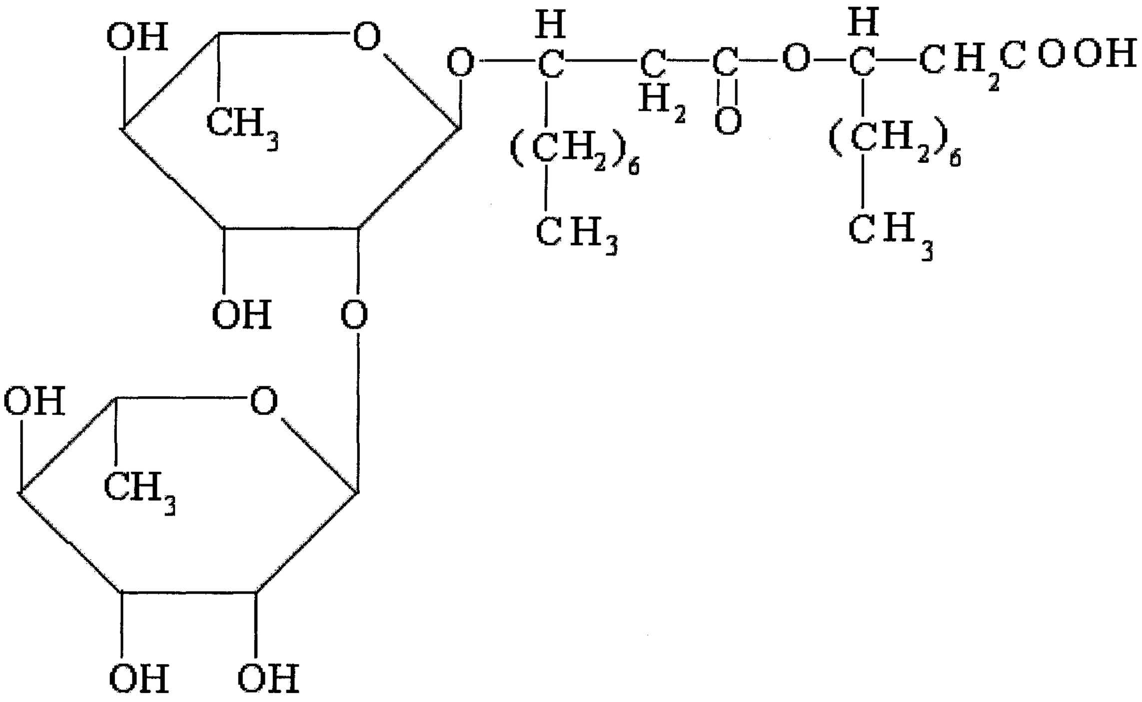
Table 2.5 Microbial source and properties of important types of microbial surfactants (adapted from Desai and Banat 1997, Banat 1995, Zhang and Miller 1995, Thangamani and Shreve 1994, and Mulligan and Gibbs 1993).

Biosurfactant		*Microorganism**	Surface Tension (mN/m)	CMC (mg/L)	Interfacial Tension (mN/m)
Glycolipids	Rhamnolipids	*P. aeruginosa*	29	25-60	0.25
		Pseudomonas sp.	25–30	0.1–10	1
	Trehalolipids	*R. erythropolis*	32–36	4	14–17
		N. erythropolis	30	20	3.5
		Mycobacterium sp.	38	0.3	15
	Sophorolipids	*T. bombicola*	33		1.8
		T. apicola	30		0.9
		T. petrophilum			
	Cellobiolipids	*U. zeae, U. maydis*			
Lipopeptides and lipoproteins	Peptide-lipid	*B. licheniformis*	27	12–20	0.1–0.3
	Serrawettin	*S. marcescens*	28–33		
	Viscosin	*P. fluorescens*	26.5	150	
	Surfactin	*B. subtilis*	27–32	23–160	1
	Subtilisin	*B. subtilis*			
	Gramicidins	*B. brevis*			
	Polymyxins	*B. polymyxa*			
Fatty acids, neutral lipids, and phospholipids	Fatty Acid	*C. lepus*	30	150	2
	Neutral lipids	*N. erythropolis*	32		3
	Phospholipids	*T. thiooxidans*			
Polymeric surfactants	Emulsan	*A. calcoaceticus*			
	Biodispersan	*A. calcoaceticus*			
	Mannan-lipid-protein	*Candida tropicalis*			
	Liposan	*Candida lipolytica*			
	Carbohydrate-protein-lipid	*P. fluorescens*	27	10	
	Protein PA	*P. aeruginosa*			
Particulate biosurfactants	Vesicles and fimbriae	*A. calcoaceticus*			
	Whole cells	*Variety of bacteria*			

* *Note:*
A. = Acinetobacter
B. = Bacillus
C. = Corynebactrerium
N. = Nocardia
P. = Pseudomonas
R. = Rhodococcus
S. = Serratia
T. = Thiobacillus
U. = Ustilago

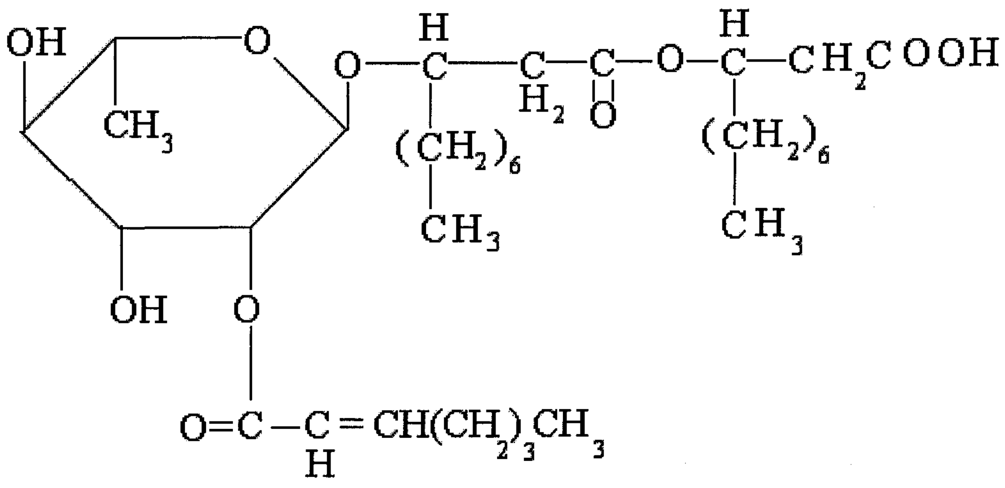


Rhamnolipid Type I

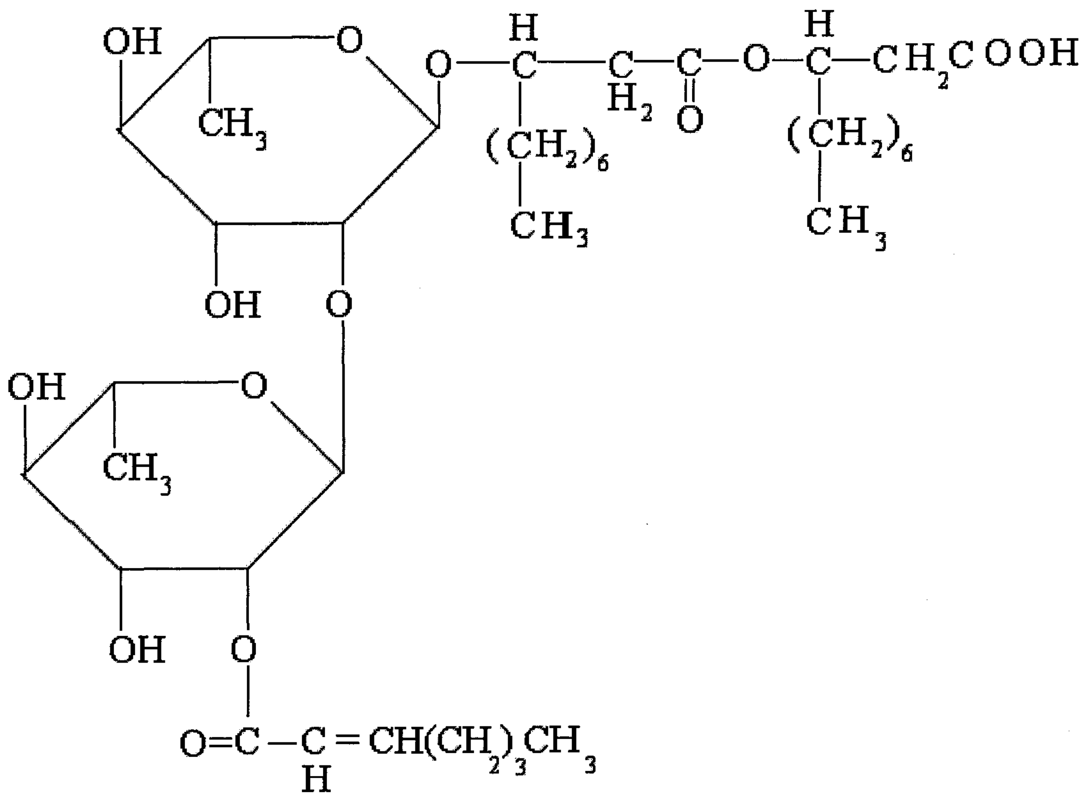


Rhamnolipid Type II

Figure 2-5 Different types of rhamnolipids (adapted from Tsujii 1998)



Rhamnolipid Type III



Rhamnolipid Type IV

Figure 2-5-cont'd Different types of rhamnolipids (adapted from Tsujii 1998)

2.4 Characteristics of soils and sediments

“Soils are formed by the natural process of disintegration of rock and decomposition of organic matter. The minerals derived from parent rock material constitute the primary minerals of soils. The term ”soils” bears different connotations depending upon the discipline in which the term is used. In its most general usage soil may be defined as finely divided rock material. This can range from colloidal particles to boulders up to a few feet in diameter” (Yong and Warkentin 1975).

Soil is a mixture of solid particles, solution of salts in water as well as air and water vapor. The variation in these three phases and also variation in specific interactions of the three phases of soil result in different soils with various compositions, characteristics and properties (Yong and Warkentin 1975).

Sediment is a common name for the soil particles that are carried by a river or flood and then settle when the flow velocity slows. These particles are mobile and, therefore, they may be contaminated in one place and then move to another place. The quality of sediment is important to the environment since the particles are in immediate contact with fish and their food. Usually sediments have higher organic matter and water contents in comparison with natural soils but generally soils and sediments have the same components and all the rules for soils are applicable to sediments (Yong and Warkentin 1975).

Some of the common properties of soils which result in different behaviors regarding the contaminant retention mechanisms, contact with contaminants, mobility, transportation and transformation of contaminants, and also in remediation techniques are as follows: size of particles, porosity and compactibility, clay content, cation exchange capacity (CEC), surface area of soil particles, the degree of wetness, wetted surface area, density, pH, organic matter content, oxide and carbonate content, and permeability .

2.4.1 Properties of soil

Size of particles: Particles are made of minerals and organic matter of various sizes and distribution. The size of the particles represents the physical nature of soil and is the main criterion to distinguish one soil from another. A typical particle size distribution diagram is shown in Figure 2-6.

Soil particles, in terms of effective particle diameter, fall into four classes and each represents a specific range of soil particles. These ranges and names are shown in Table 2.6 (Kashef 1987, Yong and Warkentin 1975).

The Department of Agriculture of the United States, based on the percentage of clay, silt, and sand in natural soils, classified soils into 12 groups such as clay, silty clay, sandy clay loam, etc. Sediment particle sizes vary from clay to silt and sands.

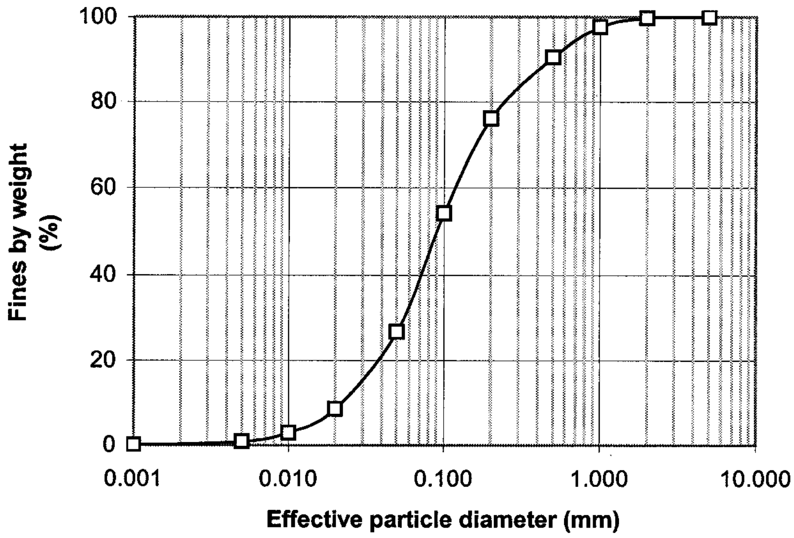


Figure 2-6 Typical particle size distribution of a soil. The mean diameter is in mm and the percent of the particles which are finer than that size by weight is on the Y-axis.

Table 2.6 Particle size distribution of a soil and the names associated with each ranges (Kashef 1987, Yong and Warkentin 1975).

Name	Clay	Silt	Sand	Gravel
Size d (mm)	d < 0.002	0.002 < d< 0.02	0.02 <d< 2	2<d

Continuous monitoring by the Water and Atmospheric Resources Monitoring Program (WARM) at the University of Illinois shows that nearly 90% of particles found in the rivers in Illinois are less than 0.0625 mm in diameter. Although the size distribution diagram gives a good idea about the mean diameter of particles of a soil, it does not provide information on the composition of the soil, which is necessary to predict the behavior of a soil under certain conditions.

Clay contents: Clay is the general name for particles with diameter lower than 0.002 mm and a family of minerals. Clay minerals change due to weathering and therefore the surface may have a different chemical composition than the inner part (Yong and Warkentin 1975).

Clay minerals are made of oxides of aluminum and oxides of silicon, alumino-silicates, with a limited amount of metal ions placed on the crystal. The basic structural units are made of a combination of aluminum-oxygen that forms a sheet. Different minerals result from the different shapes of layers, different bonding between layers and also substitution of various ions for aluminum and silicon. Each unit is constructed from silicon, aluminum, magnesium atoms, etc. surrounded by a number of oxygen atoms. A silica unit has an atom of silicon and four oxygen atoms that form a tetrahedron as shown in Figure 2-7. In the case of alumina, one atom of aluminum with six oxygens or hydroxyls form an octahedron. Every oxygen is shared by two aluminum ions. It results in the formation of sheets of two layers of oxygen atoms or hydroxyl groups in close packing (Figure 2-8).

The classification of clays is based on the composition of layers. Each composition includes some groups of clays and each group may include one or more minerals.

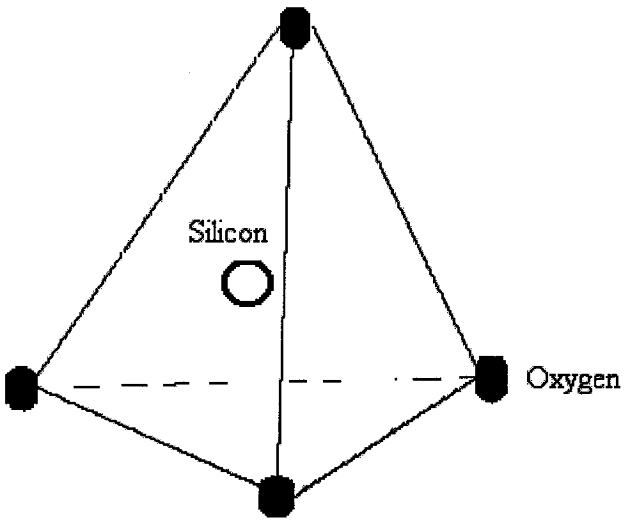


Figure 2-7 **Schematic map of single silica tetrahedral (adapted from Yong and Warkentin 1975).**

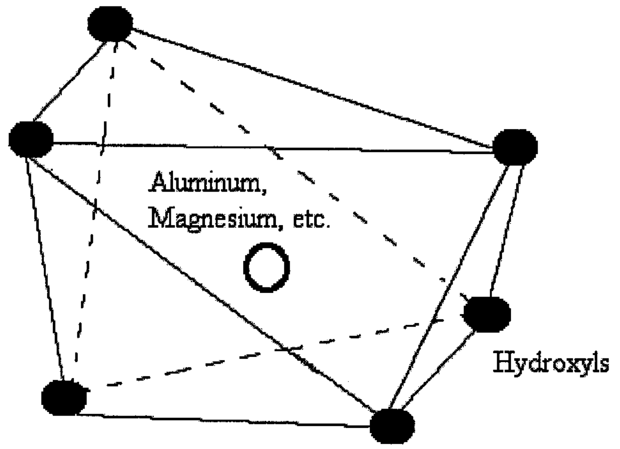


Figure 2-8 **Schematic map of single aluminum octahedral unit (adapted from Yong and Warkentin 1975).**

The classification of clays is based on their layer composition as follows:

- 1:1 composition (one silica to one alumina sheet) such as kaolinites and serpentine minerals.
- 2:2 composition (two silica to two alumina sheets) such as chlorites.

- 2:1 composition (two silica to one alumina sheet) such as micas, montmorillonites, and vermiculites.
- 1:1 and 2:1 or mixed such as interstratified minerals.

Some clays have a layer arrangement of particles and usually are able to keep a remarkable amount of water between these layers. The water is called adsorbed water. Adsorbed water has different properties from free water and changes as the number of layers change. Clay particles with an adsorbed water layer and hydrated cations and anions of a diffused ion layer are modeled in Figure 2-9 (Yong and Mulligan 2004).

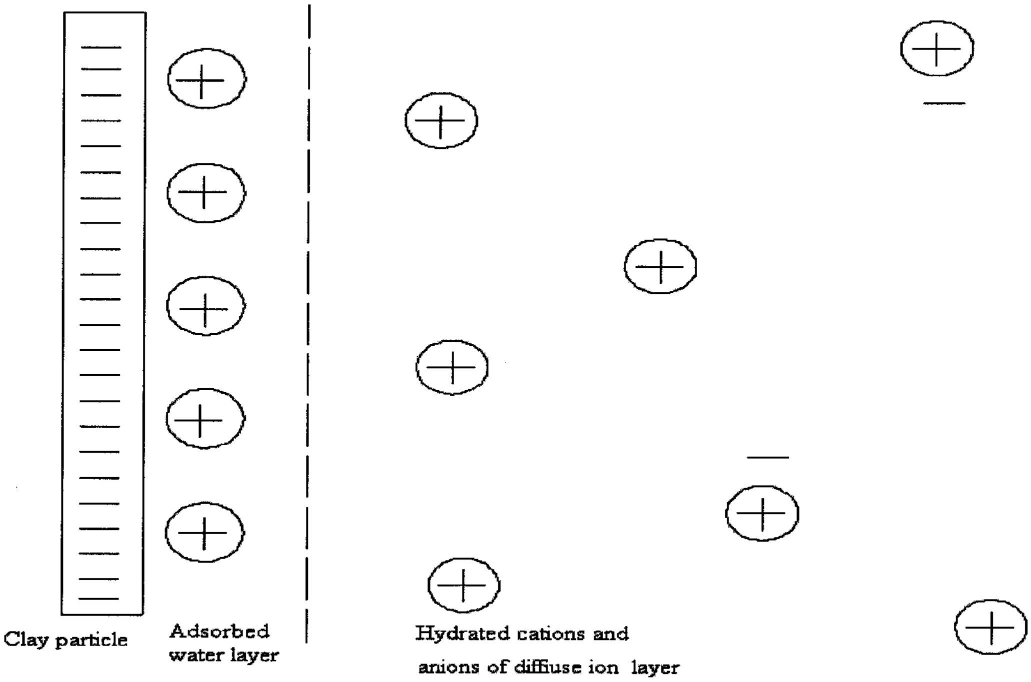


Figure 2-9 A diagram of a clay particle with negative charge covered by an adsorbed water layer. A fraction of available cations are at the surface, and the remaining exchangeable cations are in the diffuse ion layer (adapted from Yong and Mulligan 2004).

The ion distribution that interacts with clay particles is of interest in interpreting soil properties and behavior. Some of the ions, which surround the clay particle exist in the layer near the surface and the remainder are in the diffuse layer. For example, most of the sodium ions exist in the diffuse layer while calcium ions are held mostly in a layer close to the surface of the clay particle (Yong and Mulligan 2004).

Porosity, compaction, and hydraulic conductivity: Soils contain solid particles as well as liquid and some free space between particles occupied by gases. The grain size and distribution vary from one soil to another but, generally, there is always a part of the soil volume that is left for liquid and gaseous phases. The arrangement of the phases is different in granular soils and clays. In granular soils, the shape of particles can be approximated by spheres while clays are usually compounds of layered flat particles. In general, a decrease in void volume results in a larger density and also less space for the liquid phase to flow. This condition can be obtained when smaller particles fill the space between bigger particles. This situation is called the ideal particle size distribution (Fig. 2-10). The arrangements of granular particles were classified by Deresiewicz (1958) into five groups, assuming that the particles have a spherical shape with radius R. These groups are shown in Table 2.7.

The ratio of this void volume to the total volume of a soil is called porosity and is one of the most important parameters in soil engineering since it may represent the stress the soil can bear as well as the potential for liquid transmissibility.

Table 2.7 **Packing of particles with spherical shape of radius R (adapted from Deresiewicz 1958).**

Type of Packing	Coordination number	Spacing of layers	Volume of unit prism	Density	Porosity (%)
Simple cubic	6	2R	$8R^3$	$\pi/6$	47.64
Cubic – tetrahedral	8	2R	$4\sqrt{3}R^3$	$\pi/(3\sqrt{3})$	39.54
Tetragonal–spheroidal	10	$R\sqrt{3}$	$6R^3$	$2\pi/9$	30.19
Pyramidal	12	$R\sqrt{2}$	$4\sqrt{3}R^3$	$\pi/(3\sqrt{2})$	25.95
Tetrahedral	12	$2R\sqrt{2/3}$	$4\sqrt{3}R^3$	$\pi/(3\sqrt{2})$	25.95

For most soils, the void volume can be reduced by compaction. This property can be measured and by comparison with a sample with a standard compaction, the degree of compaction can be determined. This characteristic of soil is widely used in highway engineering and so may result in different values for the same sample from one country to another (Yong and Warkentin 1975).

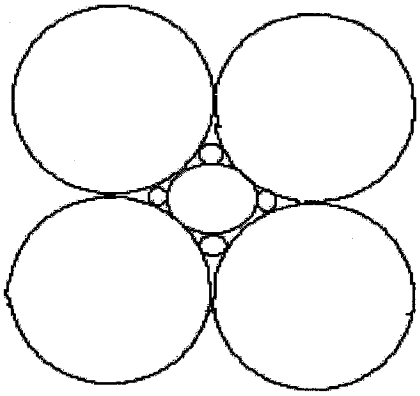


Figure 2-10 **Ideal particle size distribution (adapted from Yong and Warkentin 1975).**

As previously discussed, soil is a porous medium and is able to let the liquid phase flow due to hydrostatic pressure. This ability can be measured and the index is called the hydraulic conductivity. Hydraulic conductivity is affected by two groups of parameters: parameters related to the soil particles and soil structure and parameters related to the liquid phase or if the soil is not saturated the liquid and gaseous phases of the soil. The smaller diameter of the particle decreases the hydraulic conductivity while a more uniform distribution of the particles size results in a higher porosity. At the same time, the higher hydraulic conductivity usually refers to a larger porosity. This property of the soil also is called permeability (Yong and Mulligan 2004, Kashef 1987, Yong and Warkentin 1975). According to Bouma (1992), both static and dynamic parameters of the soil structure such as soil bulk density, porosity, and equivalent pore size can be determined by finding the hydraulic conductivity.

Surface properties of soil: Reactive surfaces of soil fractions, surface charge density, specific surface area (SSA), and wetted surface area are some of the most important surface properties of soil. *Reactive surface*s are the surfaces of soil that are capable of reacting chemically and physically with solutions in the pore water. *Surface functional groups* are chemically reactive groups that are associated with the surfaces of different soil fractions in the reactive surface. Layer silicates, hydrous oxides, soil organics, sulfates, and carbonates are the soil fractions with reactive surfaces. Functional groups are able to protonate or deprotonate at specific pHs of the ambient environment. The charges due to the functional groups depend on pH, pK_a, and pK_b of soil. The two latter parameters represent the logarithms of acidity and basicity constants (Yong and Mulligan 2004 and Yong and Warkentin 1975).

Surface charge density is the ratio of the total number of electrostatic charges on the soil particle to the total surface area of the involved particles. Concentrations of electrolytes and pH in the pore water and functional groups are the effective parameters in surface charge density (Yong and Warkentin 1975).

Specific surface area of the soil represents the total surface of all particles in a unit mass of soil. Obviously, this area is not totally exposed to the water around the particle. *Wetted surface area, Sw,* is defined as the total area of all particles that get wetted by flowing fluid through the pore channels in the unit volume of soil. The wetted surface area ranges from a small fraction of the specific surface area to the total specific surface area in a very well distributed soil in the solution. The larger value for this parameter means a higher degree of exposure to the contaminants i.e. Sw represents the availability of soil particle surfaces for interaction with the contaminant and permeate directly, which is a very important parameter in soil behavior. Specific surface areas of clays are usually larger than silts and sands and vary from 800 m^2/g for montmorillonite to 15-20 m^2/g for kaolinite (Table 2.8) (Yong and Mulligan 2004).

Table 2.8 Some characteristics of common clays (adapted from Yong and Mulligan 2004).

Soil Fraction	Cation Exchange Capacity (meq/100g)	Surface area (m^2/g)	Source of Charges
Kaolinite	5-15	10-15	Surface silanol, edge silanol and aluminol group
Clay Mica and Chlorite	10-40	70-90	Silanol groups, isomorphous substitution and some broken bonds at edges
Illite	20-30	80-120	Isomorphous substitution, silanol groups, and some edges contribution
Montmorillonite	80-100	800	Primarily isomorphous substitution with very little edge contribution
Vermiculite	100-150	700	Primarily isomorphous substitution with very little edge contribution

Cation Exchange Capacity

Clay particles have negative electrical charges that attract cations from the water that exists in the soil body. This electrical charge depends on the pH. This helps the particles to remain neutral and if they are expressed as negative charge per unit weight, it is called cation exchange capacity (CEC). Cation exchange capacity is usually expressed in milliequivalent per gram (meq/g) or per 100 grams (meq/100g). Exchangeable cations come from soluble salts that are attracted to the negative charges of the clay surface. These cations can be replaced with other equivalently charged cation, for example, two atoms of sodium in clay may be exchanged with one atom of calcium. In other words, the amount of exchangeable cations that are held by the soil is equal to the amount of the negative charge and this is called cation exchange capacity. Calcium and magnesium are the most frequent exchangeable cations in soils. The next places are given to potassium and sodium in terms of availability in the soils (Yong and Warkentin 1975, Yong and Mulligan 2004). The experiments by Appelo and Postma (1993) showed that CEC is directly related to the presence and quantity of clay and organic carbon in the soil. They proposed an equation to determine CEC directly from the measurement of clay (less than 2mm) and organic carbon. Equation 2-1 shows this relation:

$$\text{CEC (meq/100g soil)} = 0.7\ \text{Clay (\%)} + 3.5\ \text{Organic Carbon (\%)} \qquad [2\text{-}1]$$

Organic matter content: All living organisms and the remains of living organisms in soil are considered as soil organic matter regardless of the degree of their decomposition (DPIWE 2004). Organic matter content varies from 0.5% to 5% of typical soil mass but its effect on the soil should not be underestimated by this scale. The organic matter plays an important role in soil stability, processes associated with contaminant attenuation, formation and maintenance of good soil structure, and improved water retention (Yong and Mulligan 2004). The following classification (Fig. 2-11) is a schematic view of soil organic matter (Weber 2004).

In Figure 2-11:

- Unaltered materials are fresh and non-transformed components of older debris.

- Transformed products are morphologically changed and disconnected from the original substances.
    - Humic substances have high molecular weights and their color varies from brown to black. Humic acids (soluble in bases, precipitate in acids), fulvic acids (soluble in acids and bases), and humins (insoluble in acids and bases) are in this group of organic matter (Yong 2000).
    - Nonhumic substances are other biochemical substances including carbohydrates, lipids, and amino acids.

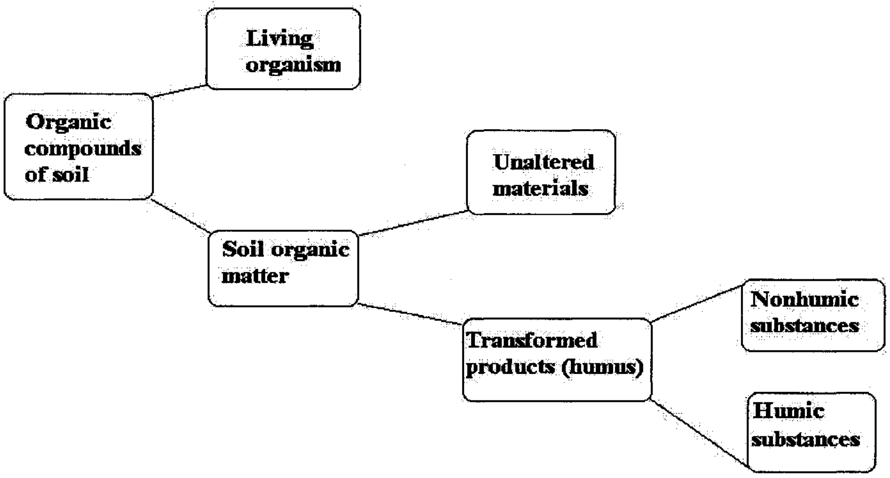


Figure 2-11 Schematic classification of organic compounds of soil (Weber 2004).

Organic matter in soil exists in two manners: strongly bound with the mineral molecules or mixed with mineral particles. Organic matter affects the functionality of soils. It elevates the degree of porosity that subsequently increases the infiltration and water retention capacities of soil. The latter is responsible for decreasing runoff, when runoff potentially increases contaminant mobility (Yong and Warkentin 1975). The cation exchange capacity of organic matter is 100-300 meq/100g. The organic fraction of soil takes part in 50 to 90% of the CEC of mineral soils. Important cations such as K^+, Ca^{2+}, and Mg^{2+} remain in exchangeable form in soil because of the CEC. Another importance of organic matter is the improvement in pH buffering capacity of soil (Ecosystem Restoration 2004). It should be noted that organic matter does not have an anion exchange capacity and is not able to bind anions like phosphate and sulphate (DPIWE 2004). A summary of the functions of soil organic matter is shown in Table 2.9.

Table 2.9 Functions of organic matter in soils (adapted from DPIWE 2004).

Physical functions	• Bind soil particles together in stable aggregates • Influence water holding and aeration • Influence soil temperature
Chemical functions	• Major source of cation exchange capacity • Source of pH buffering • Binding sites for heavy metals and pesticides
Biological functions	• Food source for microbes and small animals • Major reservoir of plant nutrients

Sediments usually have more organic matter content than soils. Moreover, sediments are distinct from soils since they can be transported (Yong and Mulligan 2004).

Hydroxyls, carboxyls, phenolics, with negative charges, and methoxyls, amines and carbonyls with positive charges are the common functional groups in organic matter. The most important groups for CEC and chelating abilities are carboxyl and phenolic groups (Yong et al., 1992).

Oxides, hydroxides, and oxyhydroxides: Weathering on parent rock materials results in the formation of oxides, hydroxides and oxyhydroxides. As an example, iron oxides and iron hydroxides are products of weathering of iron-bearing silicates. Hydrolytic and oxidative reactions release iron bound to the silicates and iron precipitates as an iron oxide or iron hydroxide since Fe^{3+} has a low solubility in the environment with normal pH (Schwertmann and Taylor 1977). Oxides, hydrous oxides of iron and aluminum and oxides of silicon are called amorphous materials in soils. Amorphous materials do not have any crystalline structure. The surfaces of oxides of iron, aluminum, manganese, titanium, and silicon have broken bonds that differ from layered silicate minerals. The most common oxides in natural soils are the oxides of aluminum, iron, and manganese, which have low solubilities in the neutral pH range. The amorphous oxides, like most other oxides, cover surround particles whose surfaces have net negative charges. This is because of their surface charge characteristics that are pH dependent. This change in the charge properties of the soil particles results in changes of the physical, chemical, and interaction properties of the soil (Yong and Mulligan 2004).

Carbonate contents: Carbonate minerals found in soil and groundwater are as follows: calcite ($CaCO_3$), magnesite ($MgCO_3$), siderite ($FeCO_3$), dolomite ($CaMg(CO_3)_2$), trona ($NaCO_3HCO_3.H_2O$), nahcolite ($NaHCO_3$) and soda

($Na_2CO_3.10H_2O$). Calcite is the most frequently found carbonate in nature. In the presence of CO_2, calcite with the pore water forms the following reaction:

$$CaCO_3 + CO_2 + H_2O \leftrightarrow Ca^{2+} + 2HCO_3^-$$

The ion, HCO_3^-, increases pH and can cause precipitation of contaminants such as metals in soil (Yong and Mulligan 2004). It also can make a remarkable increase in the retention capability of the soil (Yong and Phandungchewit 1993).

2.4.2 Selective sequential extraction techniques

Measurement of the mobility and availability of metals is required to predict and interpret their behavior. Trace metals can be found in numerous sediment and soil components in different ways (Krishnamurthy et al. 1995). Metals in river sediments can be bound to different compartments: adsorbed on to clay surfaces or iron and manganese oxyhydroxides; present in the lattice of secondary minerals like carbonates, sulphates or oxides; attached to amorphous materials such as iron and manganese oxiyhydroxides; and complexed with organic matter or in the lattice of primary minerals such as silicates (Tessier et al. 1979, Schramel et al. 2000, and Gismera et al. 2004).

To determine the speciation of metals in soils, various methods are used. One such method uses specific extractants. By sequentially extracting with solutions of increasing strength, a more precise evaluation of the different fractions can be obtained (Tessier et al. 1982). A soil or sediment sample is shaken over time with a weak extractant, centrifuged and the supernatant is removed by decantation.

The pellet is washed in water and the supernatant removed and combined with the previous supernatant. A sequence of reagents is used following the same procedure until finally, mineral acid is used to extract the residual fraction. Heavy metal concentrations then are determined in the various extracts by atomic absorption or other means. Numerous techniques and reagents have been developed and have been applied to soils (Shuman 1985), sediments (Tessier et al. 1982), sludge-treated soils (Petrozelli et al. 1983) and sludges.

These methods are not standardized and even the results can vary with the same reagents, pH, temperature, extractant strength and solid to volume of extractant ratio. None of the extractions is completely specific. However, the extractants are chosen in an attempt to minimize solubilization of other fractions and provide a distribution of the partitioning of the heavy metals. The chemical agents are used to destroy the bonds of heavy metals to the various sediment components. The extracting agents increase in strength throughout the sequence (Yong 2000). As an example, Koeckritz et al.(2001) proposed an equivalent step to simplify the sequential extraction procedure designed by Zeien and Brummer (1989). They reduced four initial steps in the procedure to one with no significant change in the results.

To extract the exchangeable fraction, ammonium acetate, barium chloride or magnesium chloride at pH 7.0 is generally used (Lake 1987). They cause the displacement of the ions in the soil or sediment matrix bound by electrostatic attraction. Pickering (1986) showed that magnesium chloride extracted low quantities of other sulfides, organic matter, aluminum and silicon. Other agents such as calcium chloride, potassium nitrate and sodium nitrate also can be used (Yong 2000).

The reducible fraction (iron and manganese oxides) is extracted with hydroxylamine hydrochloride with acetic acid at pH 2.0. The hydroxylamine hydrochloride reduces the ferrous and manganese hydroxides to soluble forms. Other components such as organic matter and clay components are not solubilized to any great extent (Tessier et al. 1979).

The carbonate phase (calcite and dolomite) is extracted at pH 5.0 with sodium acetate acidified with acetic acid. This solubilizes the carbonates, releasing carbonate-entrapped metals. Organic matter, oxides or clay components are not solubilized (Yong and Mulligan 2004).

Hot hydrogen peroxide in nitric acid is used to oxidize the organic matter. The oxidized organic matter then releases metals that are complexed, adsorbed and chelated. These agents are used so that the silicates are not affected by this treatment (Yong 2000).

In the final step, the silicates and other materials are dissolved by strong acids at high temperatures. This residual fraction is usually used to complete the mass balances for the metals.

Yong et al. (1999) reported that through selective sequential extraction techniques (SSE), they found that precipitation of heavy metal with carbonates and amorphous materials (oxides and hydroxides) is higher than heavy metal retention by exchangeable mechanisms. The study indicates that the strength of retention mechanisms of heavy metals by the phases of soil solids decreases in the following order: Carbonates > Amorphous > Organics > Exchangeable

Ho and Evans (2000) investigated the mobility of heavy metals through SSE methods with assessment of readsorption effects. The study showed that

1) Cd was highly mobile.
2) Cu and Pb were associated with oxidizable organic matter (primarily)
3) Zn was found in all fractions.

Chartier et al. (2001) pointed out that 18-42% of Pb, Zn, and Cd exist in the carbonate bound fraction while 39-60% of these metals are associated with the iron and manganese oxide bound fraction. The study also showed that 65-72% of total copper present in sediments is found in the organic matter and sulfide bound fractions. 50-80% of Ni and Cr in the sediment exist in the residual fraction.

Another important conclusion related to SSE is the evaluation of a proposed removal technique for specific heavy metals. Peters (1999) reported that SSE results show that Cu, Pb, Zn, and Cr exist mostly in the amenable fractions (i.e. exchangeable + carbonate + reducible oxide) to soil washing while Cd, Mn, and Fe are less amenable to soil washing.

2.5 Contamination of soil and sediment by heavy metals and their removal

Contamination with heavy metals among other contaminants has been the subject of numerous studies. The liquid phase of the contaminants interacts with soil and sediment. This is a very complex matter and various parameters are involved in the process of sorption and retention of contaminants in soils (Yong et al. 1992). The sources of heavy metals in soil are greater than for sediments but since sediments are a habitant for living species, it needs more attention and stricter regulation is required. In the case of the Saint Lawrence River, industries and municipalities discharging wastewater, drainage contaminated soil, and naval vehicles are the prime sources of contamination for the sediments. Among them 50 industries received the most media attention in 1998 when the Government of Canada launched the St. Lawrence Action Plan (EC1993).

2.5.1 Sorption

Sorption is the primary mechanism for partitioning by the soil solids and categorized in physical, chemical (chemisorption), and precipitation. One of the main processes for sorption of contaminants is based on the development of bonds due to interaction among electrically charged soil particles and also between the charged contaminants with them. The interaction between oppositely charged atoms and molecules is responsible for the development of inter-atomic bonds namely ionic, covalent, and coordinate covalent bonds. The bond between clay particles and organic matter is a hydrogen bond. The bond between oxygen on the clay particle surface and the hydrogen from a molecule of water is very strong thus keeping the clay layers together as well as holding water on the surface of clay particle and bonding organic molecules to the clay surface. Chemical adsorption or chemisorption is a high-affinity adsorption that occurs in the inner Helmholtz layer by a covalent bond. This covalent bond occurs between ions that penetrate the coordination shell of the structural atoms and structural cotions through oxygen (O) and hydroxide (OH) groups. The shape and energy of chemical compounds are the results of this valence (Yong and Mulligan 2004).

More common adsorption isotherms are classified as high-affinity-type, constant-type, Freundlich-type, and Langmuir type isotherms. By determining the equilibrium concentration of the solutes in the aqueous solution and concentration of the solutes adsorbed, a curve can be drawn for any specific isotherm sorption at different concentrations (Yong and Mulligan 2004). Figure 2-12 shows the schematic representation of these types of adsorption.

The constants $K_{1,..,4}$ and m are found through common methods of fitting curve to the data from the experiments. Unlike the high-affinity-type and Langmuir-types which predict a limit for the adsorption, the Freundlich-type and constant-type show limitless adsorption and measures should be taken based on experimental information to avoid unrealistic results at higher concentrations (Yong and Mulligan 2004).

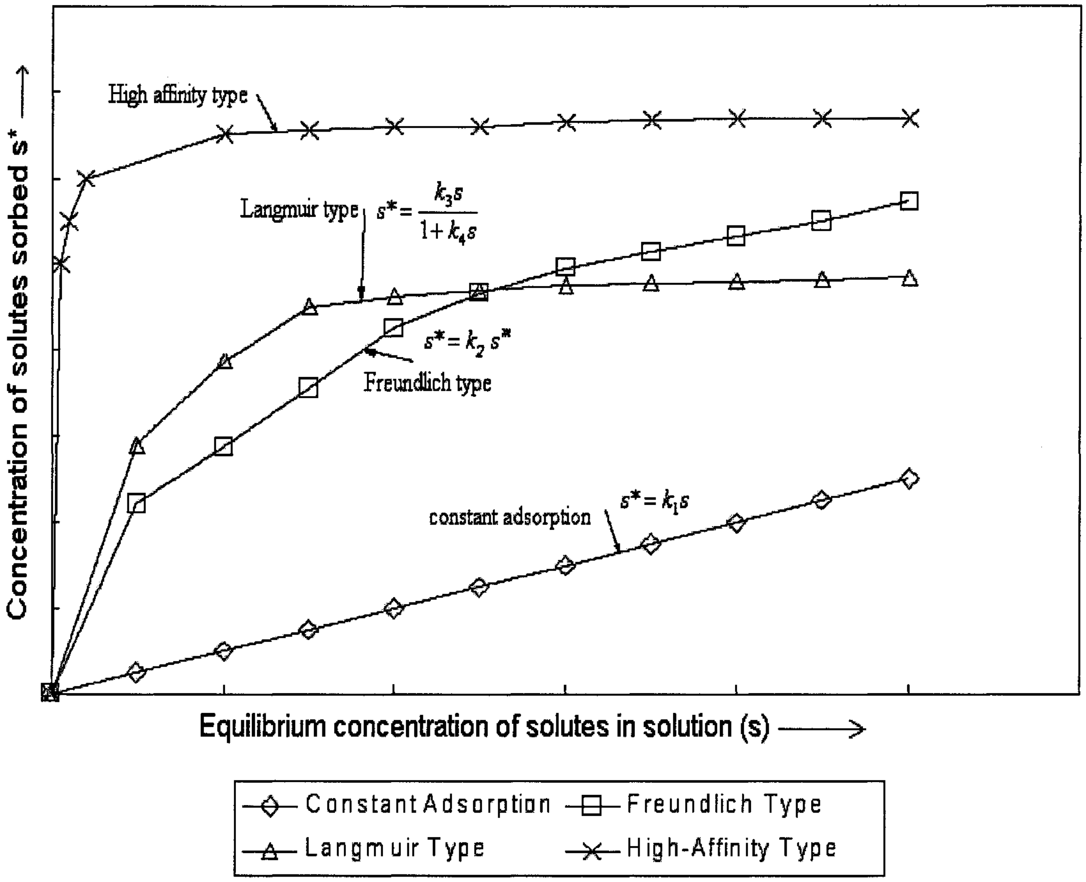


Figure 2-12 Schematic representation adsorption isotherms (adapted from Yong and Mulligan 2004).

The slope of the adsorption isotherm curve shows the distribution coefficient K_d. K_d can be determined as follows (Yong and Mulligan 2004):

- K_1 for constant-type
- The tangent of the curve for Freundlich-type and Langmuir-type (K_2, K_3, and K_4.) The tangent is usually measured at the initial part of the curve (Figure 2-12).

2.5.1.1. Adsorption of surface active materials onto a solid surface

The adsorption of surface active materials onto a solid surface from solution is an important process, scientifically and technologically, in various cases: *detergency* where removal of unwanted materials from a system is desired, *dispersion stabilization* in which a finely divided solid system is to be stabilized in a liquid material, and *waterproofing* that is the change in wetting characteristics of a surface. The adsorption facility and strength depends on three factors (Myers 1988):

- Chemical characteristics of surfactant including the charge of the head group, the length, the chain, and degree of branching of the hydrophobic portion.
- The characteristics of the solid surface where the surfactant is adsorbed such as the highly charged and non-polar surface.
- The liquid environment parameters such as pH, electrolyte contents, additives, and temperature.

One of the major forces to form micelles in aqueous solution and also to surfactant adsorption at interfaces is the property of hydrophobic groups to "escape" from the aqueous. In the presence of the solid surface, the adsorption process may be supported by the "push" to remove the molecules of surfactants from the structure of water. Adjunction of these two mechanisms (escape and push), results in the aggregation of surfactant molecules on the solid surface (Myers 1988).

To study the adsorption of a surfactant onto a solid surface some qualitative and quantitative concepts are desired (Myers 1988):

- The quantity of the adsorbed surfactant per unit mass or area of the solid.
- The concentration of surfactant needed to cover the given surface.
- The concentration of surfactant for surface saturation.
- The effects that adsorption may cause on the properties of the solid.
- The position of the adsorbed molecules in relation with the surface and solution.

It is generally found that adsorption of surfactants increases with the increment of molecular weight of the hydrophobic portion of surfactant. It means a larger hydrocarbon chain will result in a larger amount of surfactant adsorbed at saturation. In this case a lower surfactant concentration is needed to reach to saturation adsorption (Myers 1988).

2.5.2. Methods of heavy metal removal

Generally speaking there are several classifications for heavy metal removal from soil and sediment; depending on the place: *in situ*, *ex situ*; or depending on the equipment, materials and type of contaminant: physical, chemical, and biological methods. The common remediation techniques are: soil washing, excavation, land farming, phytoremediation, solidification, reactive barriers, bioremediation, thermal treatment, electro-kinetic, and mechanical separation (CEPA 1994).

Since sediments have large quantities of water, dewatering is necessary before using treatment techniques. Sediments have different characteristics than soils, such as higher clay and organic matter contents. Therefore processes that are effective for soil treatment may not be as efficient for sediment remediation. Solidification/ stabilization and soil washing are the two main treatment methods for metal-contaminated sediments (Mulligan et al. 2001).

Remediation methods in different soils and sediments depend on the types, concentrations of pollutants and their characteristics such as solubility, biodegradability, and volatility and also on the characteristics of the soil. CEC and wetted surface area are major factors in the attenuation process (Yong 2000).

Several methods and techniques have been developed for soil and sediment removal during the past three decades. Here, some of the more common methods are presented.

Excavation

Perhaps the oldest remediation method for contaminated soil is physical removal of the soil and excavation, which is still in use at many locations, including the United States. Advantages of excavation include the complete removal of the contaminants and the relatively rapid cleanup of a contaminated site (Wood 1997), but the problem is that the contaminants are simply moved to a different place untreated. The removed soil must be monitored and the risk of spreading contaminated soil and dust particles during removal and transport of contaminated soil exist. It is also a relatively expensive method. Excavation can be the most expensive option when large amounts of soil must be removed or disposal as hazardous or toxic waste is required. The cost may reach up to $400 per cubic meter (Lambert et al. 2000).

Stabilization of metals in soil

In this method, heavy metals are left on site and treated in a way that reduces or eliminates their ability to adversely effect human health and the environment. Basically, using this method, the bioavailability of heavy metals on site is eliminated or remarkably reduced. Adding some chemicals such as phosphate to the contaminated soil causes the formation of minerals, which, are not easily absorbed by plants, animals, or people. This method is an *in situ* treatment and so the costs of excavation and transportation are eliminated. From an economical point of view, the cost of treating the soil by *in situ* stabilization is considerably less than the cost of excavation and disposal of heavy metal contaminated soil, $200 per cubic meter. Based on the equipment used and the soil properties, this method can be as rapid as excavation (Lambert et al. 2000).

An example of *in situ* stabilization of heavy metal is the process involving addition of phosphate fertilizer to a highly lead contaminated soil. Chemical reactions between the phosphate and the lead cause lead pyromorphite formation, a heavy metal phosphate that is extremely insoluble (Lambert et al. 1997).

Electro-kinetics

One of the new techniques of soil remediation is electrokinetics (Figure 2-13). “Electrokinetic remediation is an *in situ* process in which an electrical field is created in a soil matrix by applying a low-voltage direct current (DC) to electrodes placed in the soil. As a result of the application of this electric field, heavy metal contaminants may be mobilized, concentrated at the electrodes, and extracted from the soil” (USAEC 2004). The heavy metal extraction rate and efficiency is dependent upon many subsurface characteristics such as soil type and grain size, contaminant concentration, ionic mobility, total ionic concentration, types of contaminant species and their solubility (USAEC 2004).

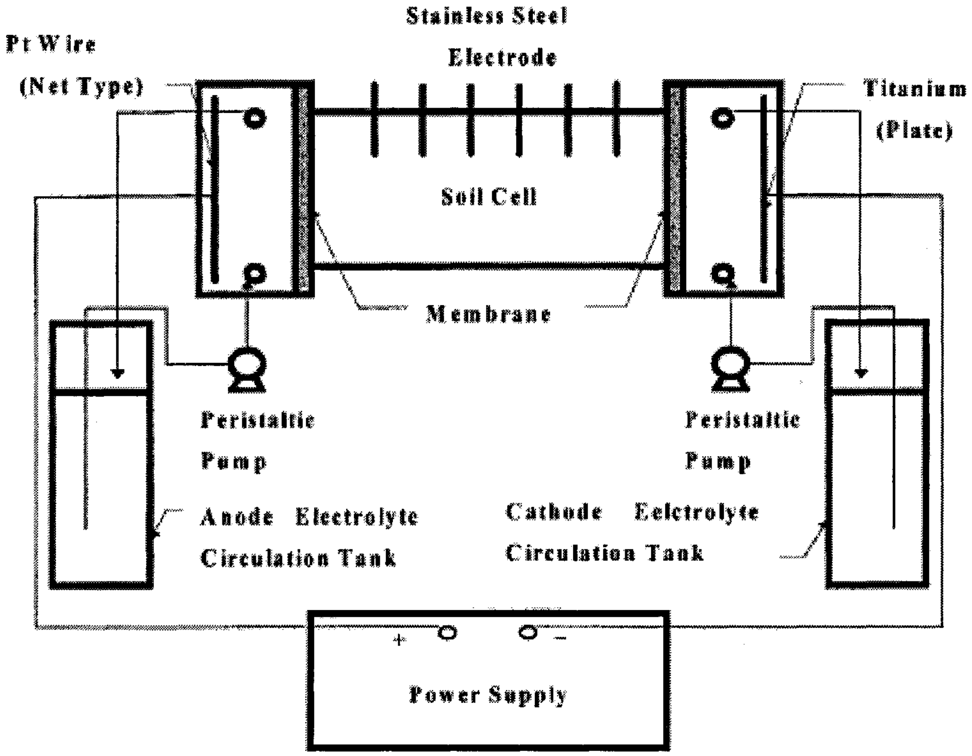


Figure 2-13 Schematic diagram of electrokinetic remediation technology (adapted from Kim et al. 2001).

The conductivity of the soil cell is the main factor in energy consumption. The higher concentration of heavy metals in soil results in a higher performance for the system. As the adsorption and desorption mechanisms were predominant for removing heavy metals from soils, the conductivity of the soil cell decreased and the efficiency decreases (Kim et al. 2001).

In some cases, the available data on cost efficiency is not accurate since, only the costs for electricity and chemicals were considered, thus giving the impression that this is a low-cost technology. Equipment costs, installation, maintenance, removal, and contaminant disposal significantly increase the total cost of metal remediation by electrokinetics. Treatment costs may vary from $150 to $1,200 per cubic yard depending on the site conditions and system design (USAEC 2004).

Soil washing

Soil washing is the common name for various techniques that are based on washing the contaminated soil *in situ* or *ex situ*, with water, inorganic acids such as sulfuric acid and hydrochloric acid, organic acids such as acetic acid and citric acid, chelating agents such as ethylenediaminetetraacetic acid (EDTA) or any other liquid agent (EPA 1991). The techniques include spreading the solvent into the soil and collecting the solution containing heavy metals from the soil. The pH, soil type and horizon, cation exchange capacity, natural organic matter, presence of other inorganic contaminants, age of contamination, and metal mobility are the factors that can affect heavy metal retention by soils. Soils with low concentrations of fluoride, sulfide and cyanide, particle sizes of 0.25 to 2 mm, CEC of 50 to 100 meq/kg, and a contaminant solubility of greater than 1000 mg/L are able to be cleaned up most effectively by these techniques. Soil washing is used to treat soil contaminated with fuel hydrocarbons, semi-volatile organic compounds (SVOCs), and inorganic compounds such as heavy metals. It is less effective for volatile organic compounds (VOCs) and pesticides. Batch chelant extraction studies with EDTA, citric acid, and nitrilotriacetic acid (NTA) show a good degree of effectiveness in simultaneous removal of copper, lead, and zinc in soil washing. The new advances in soil washing and soil flushing techniques have opened new horizons in soil decontamination (Peters 1999).

Surfactants with cationic, anionic, or nonionic charge can be used as washing agents in soil washing. Surfactants can solubilize, disperse, and desorb contaminants from excavated soils or sediments in a washing unit (Abriola et al. 1998).

Bioremediation techniques

"Bioremediation involves stimulation of microbially mediated processes applied exclusively or in combination with other physicochemical processes for the clean up of diverse waste matrices including vapor, liquid, and solid contaminants. Bioremedial technologies for soils, sludge, and dredged materials are applicable *in situ* or *ex situ* depending on whether the waste matrix involved is in its natural setting or is removed and transported into a reactor" (Lewandowski and DeFilippi 1998). The main methods include bioleaching, phytoremediation, and soil washing with biological products.

- *Bioleaching*

The basis of this method is very simple: an organism or a group of organisms are used in leaching and the method is called bioleaching (Rossi 1990). A recent study shows that *Aspergillus niger* exhibits a remarkable potential in production of organic acids which are effective for metal solubilization in mining residues with dissolution of 68% of the copper (Mulligan et al. 2003).

Microbial activities are sensitive to some chemical factors including pH, toxicity, heavy metals, molecular structure and co-metabolism. A pH of 6.5 to 9 is preferred by most microbes. In soil or water addition of lime, calcium hydroxide, calcium carbonate, and magnesium carbonate can be used to increase the pH. Sulfur compounds such as sulfuric acid lower soil pH (Yong and Mulligan 2004). *Thiobacillus sp.* bacteria in a specific type of bioleaching showed the ability to reduce sulfur compounds under aerobic and acidic conditions at pH 4, when the temperature remains in the range of 15 to 55°C. Bioleaching is generally performed in three modes: heap leaching, bioslurry and *in situ* (Mulligan 1998).

- *Phytoremediation*

Phytoremediation is a method of growing plants to contain or reduce heavy metal pollution (USEPA, 1998). It has the advantage of relatively low cost and wide public acceptance (Schnoor, 1997). The cost for phytoremediation can be a quarter of that of excavation or *in situ* stabilization. This method costs as little as \$15 to \$40 per cubic meter of soil (Lambert et al. 1997).

There are three common processes for phytoremediation: phytostabilization, rhizofiltration, and phytoextraction. Phytostabilization is used to reduce wind and water erosion that is transported materials containing heavy metals. For example, at a contaminated site in Galena, Kansas, grass or tree buffers reduced 18% to 25% of sediment loss from the chat piles (Green et al. 1997). Phytoextraction is based on the fact that some plant species can take up heavy metals from soil and concentrate them in their tissue. Although there are some reports that in the case of revegetation of all the ground sediment, loss could be cut by approximately 70%, it would be necessary to find plants that could tolerate high levels of heavy metals (Lambert et al. 2000). Some types of plants used for this purpose are: Indian mustard, alfalfa, cabbage, tall fescue, juniper, thlaspi, urtica, polygonum sachalase, and poplar trees. These plants have the capability to collect copper, cadmium, nickel, and lead (Baker et al. 1991). Rhizofiltration is a process in which heavy metals are removed directly from water by plant roots. The plants are grown directly in water or in water rich materials such as sand, using aquatic species or hydroponic methods. Some field tests show a good level of success for this method. For example at Chernobyl, sunflowers on floating rafts have removed radioactive metals from water in ponds. Also it is reported that other plants removed metals from mine drainage flowing through diversion troughs (EPA 2000). The contaminated plants have to be disposed of in an appropriate way to prevent re-contamination of the soil. Some techniques are drying, incineration, gasification, acid extraction, and anaerobic digestion (Bolenz et al. 1990).

- *Soil washing with biological products*

Generally the method of soil washing with biological products follows the same principles as chemical soil washing but the washing agents are produced through biological activities. Although the method is new, the results are promising. The advantages of these products are numerous namely they can be used in both *in situ* and *ex situ* soil washing processes. It is possible to add these products to washing water that results in improvement of solubilization, dispersal, and desorption of pollutants for washing the excavated soil as well as to add them during the pump and treat process (Ellis et al. 1985, Nash et al. 1987).

Biosurfactants were primarily used in washing soils polluted with oil and grease. Mulligan et al. (1999) studied the use of rhamnolipid and sophorolipid for washing a soil with 12.6% of grease and oil containing 890 mg/kg of zinc and 420 mg/kg of copper in batch tests. 12% rhamnolipid removed 19.5% of the zinc while 4% sophorolipid with 0.7% HCl removed 15.8% of the zinc. In the case of copper, using 2% rhamnolipid and 1% NaOH or 25% surfactin with 1% NaOH resulted in metal removal up to approximately 25%.

Rhamnolipids also were used to extract copper from mining ores. The result of using 2% rhamnolipids showed an extraction of 28% copper from a mining residue. Higher concentrations of rhamnolipids improved the extraction but due to its higher viscosity, a lower concentration was used (Dahrazma and Mulligan 2004).

Rhamnolipids have found applications in the removal of oil from soil as well. Harvey et al. (1990) reported an improvement of 300% in removal of oil from Alaska beaches after the oil spill from the Exxon Valdez in 1989 comparing with water alone. These results should be treated cautiously since the degree of removal depends on the type of hydrocarbons and the concentration of the biosurfactant (Scheibenbogen et al. 1994).

2.6 Mechanism of heavy metal removal by biosurfactants

Few investigations related to the mechanism of removal of heavy metals from soil and sediments by rhamnolipid were performed during the last decade with different approaches. There are studies that have documented the removal of heavy metals by biosurfactants and specially by rhamnolipid since these techniques are quite new. Herman et al. (1995) investigated the removal of cadmium, lead, and zinc singly and in a mixture from a prepared contaminated soil. They pointed out that complexation and ion exchange could be responsible for the removal. They reported that 2% of soil-bound Pb^{2+} was dissolved from soil by 80 mM rhamnolipid through ion exchange which was less than 4% of total removal of Pb^{2+}.

Mulligan (1998) investigated the feasibility of removing hydrocarbons and heavy metals from soil and sediments by different biosurfactants, including rhamnolipid. Ultrafiltration techniques were used to investigate if the removed metals were associated with micelles or monomers in the surfactant solution. These studies showed that as the concentration of rhamnolipid and surfactin increased above the CMC, more metals were retained in the retentate and less in the permeate. This finding showed that the metals should be incorporated into the micelles. Determining the octanol-water coefficient, K_{ow}, Mulligan (1998) also pointed out that rhamnolipid and surfactin can be powerful solubilizers for metals into the micelles since they have significant K_{ow} values.

Ocha-Loza et al. (2001) have studied the conditional stability constants of heavy metals in solution by rhamnolipid. They determined these constants by ion-exchange technique for the most common cations in soil and sediment. According to their research the order of the conditional stability constant has the following order:

$$Al^{3+} > Cu^{2+} > Pb^{2+} > Cd^{2+} > Zn^{2+} > Fe^{3+} > Hg^{2+} > Ca^{2+} > Co^{2+} > Ni^{2+} > Mn^{2+} > Mg^{2+} > K^{+}$$

This order indicates that rhamnolipids prefer to make complexes with metals such as cadmium, mercury, and lead in the presence of common water or soil cations like potassium.

According to the available literature and the necessity of moving toward the use of environmentally friendly products for treatment of soil and sediments, development of new techniques to enhance heavy metal removal using rhamnolipid is a valid alternative. Also, the continuous flow of the washing agent through the soil placed in a column gives a better simulation of *in situ* soil washing or so called soil flushing. *In situ* treatment is preferable to *ex situ* washing since the cost and environmental damage due to excavation and transportation of the soil and sediments are omitted.

Chapter 3

Methods and Materials

3.1 General remarks

As mentioned in section 1.1, the goals of this research are to study the use of rhamnolipid for treatment of contaminated sediment with heavy metals in batch tests and to develop new techniques using continuous flow tests and also to determine the effect of different parameters on the removal of the heavy metals from the sediment. The study also included the use of sequential extraction techniques, ion exchange techniques, and other tests and techniques to determine the mechanism of heavy metal removal from sediment and also to investigate the mechanism of interaction of metals with the rhamnolipids. To achieve these goals, a combination of materials, procedures and methods were used. This chapter is devoted to specifying the materials, experimental configuration, and describing those procedures. The commonly used materials such as 70% laboratory grade nitric acid and laboratory made distilled water are not discussed in this chapter.

3.2 Sediment

3.2.1 Origin of the sediment

The metal-contaminated sediment sample was obtained from the Lachine Canal area that was surrounded by metal and steel industries. The canal passes through residential and industrial areas and the area was defined a contaminated site by Environment Canada (EC 1993). To proceed with the experiment, the sample was air-dried and mixed fully to obtain a homogenous sample.

3.2.2 Particle size distribution

Using the standard method for particle size distribution for soils and sediment ASTM D422-63 (ASTM 1998) 300 g of the sample were taken. After drying the sample for 24 hours at 100°C, the sample was sieved. The sieves were arranged from Sieve No.5 (2 mm) to 200 (0.075 mm). After shaking the sample in the sieve column for 15 minutes, the weight of sediment remaining in each sieve was measured. The results were plotted in a semi-log graph (Figure 3-1). According to the graph and textural classification system by ASTM (1998), the sample gradients were 15% sand, 65% silt and 20% clay. Based on textural classes defined by ASTM D-2487 (1997), the sediment is classified as silty clay with sand.

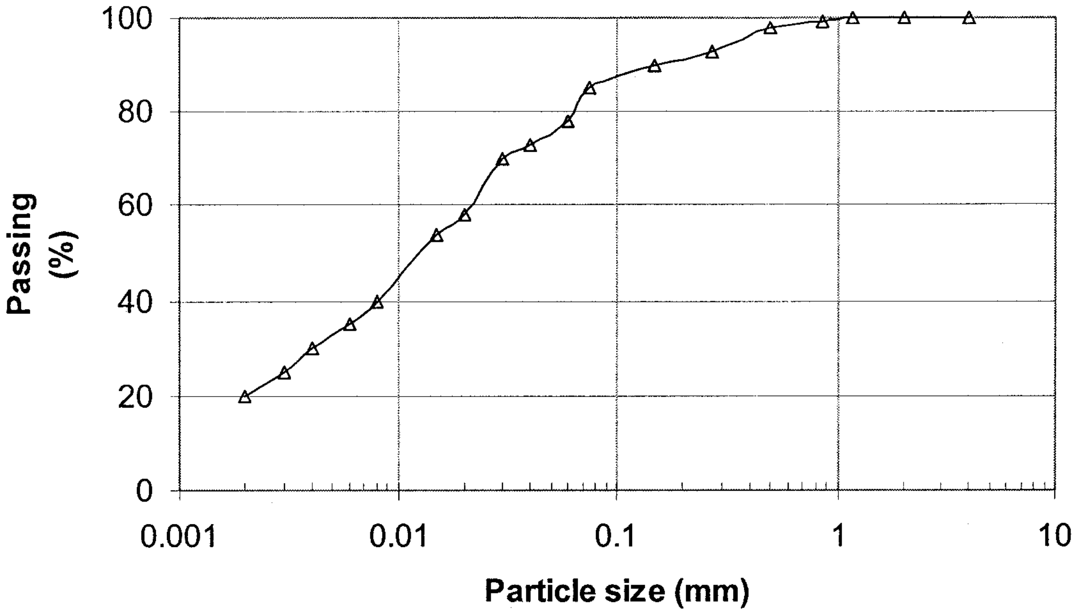


Figure 3-1 Particle size distribution of sediment sample.

3.2.3 Organic matter content

The organic matter content was measured based on the method involving digestion by hydrogen peroxide (H_2O_2). The procedure is as follows:

I. 2 g of air-dried, pulverized sediment were weighed and placed in a dry pre-weighed 600 mL beaker.

II. 10 mL of hydrogen peroxide (H_2O_2 30%) were added to the sediment. Bubbling started.

III. When the bubbling died down, more H_2O_2 was added to wash down the sides of beaker.

IV. Adding H_2O_2 was continued periodically until no further reaction was observed.

V. The sample was left overnight, then the clear liquid was pipetted off. Then, by adding more H_2O_2, the remaining material in the beaker was checked to see if the reaction has been completed.

VI. 150 mL distilled water was added and after mixing well, the sample was washed into 4 oven-dried pre-weighed glass centrifuge tubes.

VII. By centrifuging at 3000 rpm for 30 minutes, the supernatant was separated from the sediment particle. The supernatant was collected and this step was repeated 3 times.

VIII. Tubes and the beaker were air-dried and then all of them were weighed again.

IX. The percentage of organic matter was calculated by the following equation [Eq. 3-1].

$$\%\,Organic\ matter = \frac{Total\ weight\ before\ test - Total\ weight\ after\ test}{Total\ weight\ before\ test} \times 100\,\% \quad [3\text{-}1]$$

By performing the above procedure on the sediment sample, the total organic matter was measured at 20% (w/w).

3.2.4 Heavy metal content

To find the concentration of heavy metals in the sediment, it was digested by the method recommended by Environment Canada (1990) as follows:

i. 1.00 g of the sediment was placed in a 1L beaker.

ii. 100 mL of 16N nitric acid were added to the beaker over a minimum period of 2 minutes.

iii. 40 mL of H_2O_2 (30%) were added to the beaker. The beaker was left for 5 minutes to react.

iv. The beaker was placed on a hot plate (Fisher Stirring Hotplate) until it boiled. Then, it was removed from the heat and cooled to room temperature.

v. A solution of 1000 mL aqua regia was made through the following process:

    a. 200 mL of concentrated nitric acid were added to a 1000 mL flask.

    b. 500 mL of distilled water were added to the flask.

    c. 50 mL of concentrated hydrochloric acid was added to the flask.

    d. The flask was filled to the 1000 mL mark with distilled water.

vi. 200 mL of aqua regia were added to the beaker.

vii. The beaker was filled to the 500 mL level with distilled water.

viii. The solution was prepared for analysis by the atomic absorption spectrophotometer.

ix. The amount of the metal concentration was measured using a Perkin Elmer Atomic Absorption Analyst 100 Spectrophotometer.

x. The process was repeated four times and the average was calculated.

The results of the sediment digestion are summarized in Table 3.1.

Table 3.1 Characterization of sediment for total heavy metal content. The last two columns are adapted from Canadian Environmental Quality Guidance (2003) to compare with the level of contamination in the sample.

Parameter	Concentration (mg/kg)	Allowed concentration by Interium Sediment Quality Guidance (ISQG) (mg/kg)	
		Freshwater	Marine
Chromium	145	37.3	52.3
Copper	140	35.7	18.7
Nickel	76	N/A	N/A
Lead	572	35	30.2
Zinc	4854	123	124

3.2.5 Cation exchange capacity

Cation exchange capacity (CEC) can be determined by a variety of methods and procedures. In this research, the method proposed by Chapman (1965) was used.

The procedure is as follows:

1. 5 g of sediment were weighed into a centrifuge tube.
2. 20 mL of 1 M potassium acetate were added to the tube which was shaken for 5 minutes to replace the sediment cations with K^+ cations.
3. The sides of the tubes were rinsed with distilled water and the remaining sediment particles were washed down into the solution.
4. The tube was placed in the centrifuge at 1000 rpm for 15 min and the clear supernatant was taken out. Through this step, the displaced cations were removed.

5. Steps 2 through 4 were repeated three times to ensure that the sediment cations were replaced.
6. 20 mL methyl alcohol was added and the tube was shaken until the suspension of all sediment particles was certain.
7. The cation K^+ was removed in Step 6. To do so, the sample was centrifuged (1000 rpm for 15 min) and Steps 6 and 7 were repeated several times to ensure that the cation was totally washed out.
8. 25 mL of 1 M ammonium acetate were added to the tube and shaken until all the soil particles became suspended in the solution.
9. The tube walls were washed down with ammonium acetate and the sample was centrifuged (1000 rpm - 15 min).
10. Steps 8 through 10 were repeated several times to replace adsorbed K^+ on the soil surface with NH_4^+.
11. The concentration of the displaced K^+ ions in the solution was measured to find the initial cation concentrations in the sediment.

After performing the test and the related calculations, the CEC of the soil was determined as 14.55 meq/100g.

3.2.6 Soil pH

Referring to EPA SW-846 Method 9045 to measure the pH of soil, 20 g of soil was placed in a beaker and 20 mL distilled water was added. The mixture was stirred for 30 minutes and the beaker was left for an hour. After clearing the solution, the pH was measured with an AR25, dual channel pH/Ion meter from

Fisher Scientific. The pH of the sediment in room temperature was measured at 6.4.

3.2.7 Carbonate content

To measure the carbonate content, the following procedure was followed:

1- 10 grams of oven-dried soil were weighed and placed in a glass beaker.

2- Dilute hydrochloric acid was added carefully to the soil.

3- The mix was stirred carefully and the step was repeated until no reaction from the soil was observed.

4- The residue was poured through a filter paper (No. 40 ashless circles, 110 mm diameter x 100 circles) in a funnel and the extract was kept.

5- The material was air-dried.

Collecting all remaining residue from the filter paper and the beaker and repeating the measurement, the weight of the residue was calculated. The difference between the initial weight and final weight is the carbonate content which was equal to 55 g/kg or 5.5% of the soil.

3.2.8 Amorphous content

The method used for determining the amorphous content (Fe_2O_3, Al_2O_3 and SiO_2) consists of alternate acid and base washings of the sediment (Segalen 1968). In this method two reagents A (8N HCl or 680 mL of concentrated HCl in 1 liter) and B (0.5N NaOH or 20.0 g NaOH in 1 liter), were used. The method includes six steps as explained in the following:

I. 0.5 g of sediment was weighed into a 40 mL plastic centrifuge tube.
II. 40 mL reagent A was added and filled the tube to the line and then was put on a mechanical shaker for 20 min. After centrifuging (1000 rpm for 3-4 min), supernatant was decanted into a 100 mL volumetric flask.
III. The tube was filled with distilled water, shaken well, centrifuged (1000 rpm for 5 min). and decanted into the same volumetric flask from step II. It was made up to the mark with distilled water and then was mixed and ~40 mL were poured off into a small plastic bottle and saved for analysis.
IV. A centrifuge tube was filled with reagent B and was placed in a steaming water bath for exactly 5 min. It was immediately centrifuged (1000 rpm for 5 min) and then the supernatant was decanted into a 100 mL plastic volumetric flask, made up to the100 mL mark, mixed, and poured ~40 mL into a small plastic volumetric flask and was saved for analysis.
V. Steps 2, 3 and 4 were repeated for a total of 8 times, resulting in 8 acid supernatants and 8 base supernatants for analysis for each sample.

In the above process, iron and aluminum contents were determined by acid washings while using base washing, determination of silica and aluminum contents became possible.

In addition to the above determinations on all 8 washings, the silica content was measured in the first 2 acid washings and the iron in the first 2 basic washings to detect any carry over.

The total contents of each washing were determined (combining acid + base results) and the cumulative amounts of each (Fe_2O_3, Al_2O_3 and SiO_2) were calculated. A schematic plot of these cumulative amounts versus time is given in Figure 3-2.

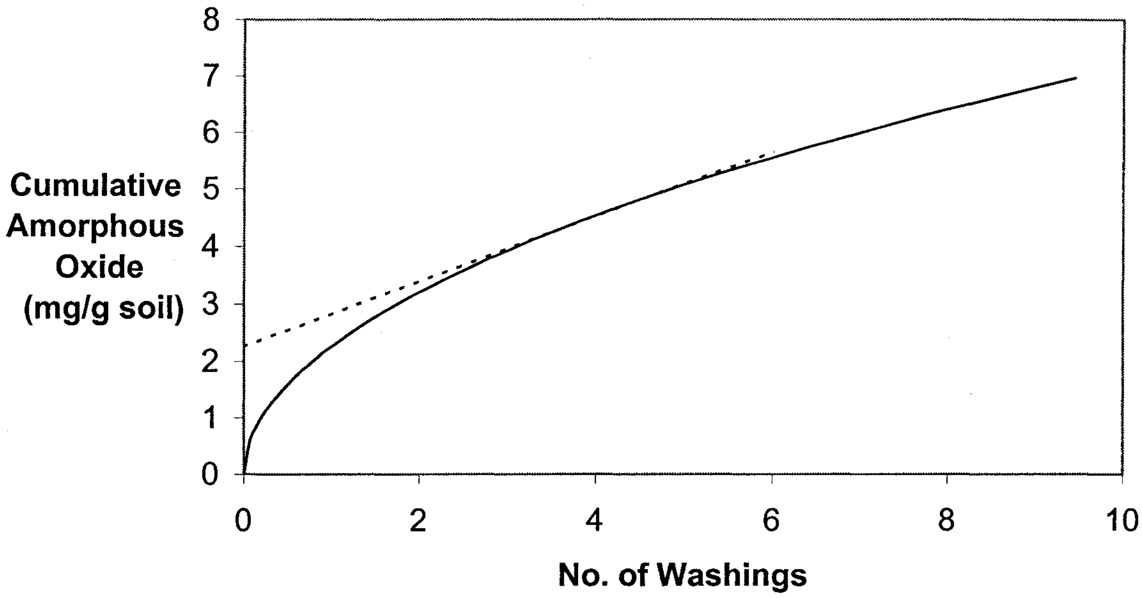


Figure 3-2 Schematic graph of cumulative amorphous amount by time.

The amorphous material was removed quickly while the crystalline material was dissolved at a slow steady rate. Therefore the amorphous content was determined by extrapolating the straight portion of the graph back to the y-axis. The intercept gives the amorphous content. The results are shown in Table 3.2.

Table 3.2 **Concentration of amorphous material in the sediment.**

Amorphous material	Concentration (mg/kg soil)
Fe_2O_3	17800
Al_2O_3	11200
SiO_2	23400

3.2.9 Hydraulic conductivity

Hydraulic conductivity tests were performed to determine the effect of bulk density on hydraulic conductivity. The method was adopted from Kashef (1987), which performed the conductivity tests under a constant head. The sample was placed in a column with a constant flow. A combination of glass beads and glass wools at each end was used to ensure a uniform velocity through the soil section. The test was repeated for different densities (600, 900, 1200, and 1500 kg/m^3) of sediment in the same column and under the same head and the same thickness. The results are presented in the next chapters.

3.2.10 Soil density

The absolute density of soil was measured using the basic definition of the mass in unit volume. Soil (10 g) was placed in a graduated cylinder and then 20 mL of distilled water were added. Then the cylinder was shaken to ensure that there were no air bubbles in the sample. The volume of the mixture was determined and the difference between the new volume of the mixture and the original 20 mL of water was calculated as the volume of soil. The density was determined to be 2500 kg/m^3.

3.2.11 Mineralogical analysis by X-ray diffraction

To identify the crystalline components of the sediment, an X-ray diffraction procedure was used. Minerals have distinct inter-atomic distances within their planes. Using X-rays with wavelengths of 0.01 to 100 Å, the diffraction of crystalline structures of each mineral can be detected. The experimental setup included a Siemens D-500 X-ray diffractometer, a Hewlett Packard D-500 AT system, and a DACO-MD interface system. To analyse the obtained data, the procedure of Starkey et al. (1984) was used. Approximately 15 g of sediment after washing with distilled water and drying in room temperature was used for the analysis. A 20 to 30% sediment suspension was prepared and 6 ml were added to a glass slide. Using Cu-K alpha radiation the samples were scanned at 0 to 65 2θ degrees with 40 kV and 20 mÅ on 1° beam slit and 0.5° detector slit at room temperature. These scans were obtained in the rate of 0.02 2θ degrees/min. The rate meter was set to 2×10^3 cps while the time constant was set to 1.2 sec. The peaks were counted and a semi-quantitive analysis was calculated over the total counts obtained for the scan.

The X-ray diffraction results are summarized in Table 3.3. The scan for the sediments is shown in Figure 3.3. Quartz ($Si0_2$) and feldspar ($NaAlSi_3O_8$, $KAlSi_3O_8$) were the main primary minerals in the scans. Quartz was indicated at 4.25 and 3.34 Å and feldspar at 3.29, 3.24, 3.2 and 2.99 Å. Illite $(Na,K)Al,2(Si_3AlO_{10})$ was also found in the sediment at 9.99, 4.99 and 3.48 Å. Small amounts of chlorite ($(Mg,Fe)_6\ (Al,Si)_4O_{10}(OH)_8$) were seen in the sample.

Also, amphibole ($Ca_2(Mg, Fe, Al)_5 (Al, Si)_8 O_{22}(OH)_2$) was also detected at 8.44 3.46, and 2.71 Å.

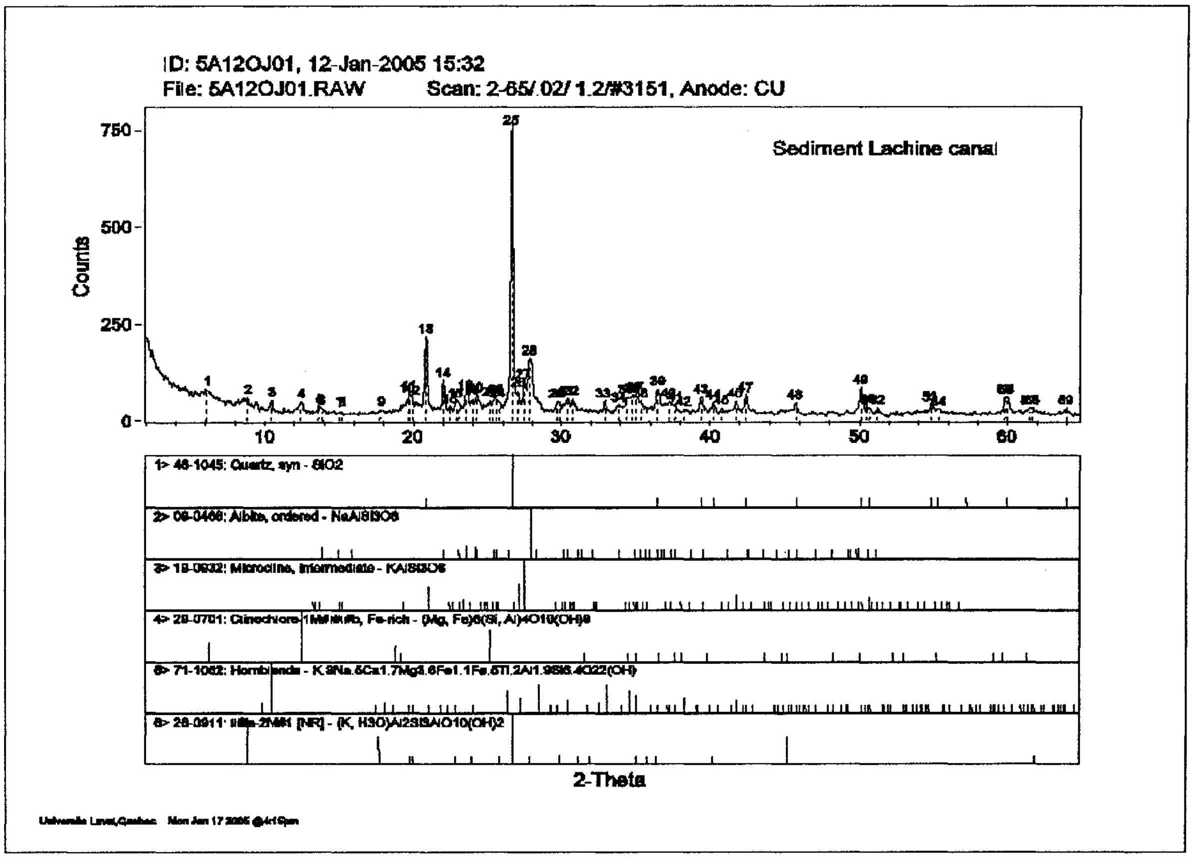


Figure 3-3 X-ray diffraction for sediments.

Table 3.3 Mineralogical composition of sediments.

Component	Concentration (%)
Quartz	33.5
Feldspar	38.0
Chlorite	5.4
Amphibole	18.5
Illite	4.48

3.2.12 Zeta potential

The zeta potential of the sediment particles was measured using a Zeta meter Model System 3.0 from Zeta Meter Incorporated. A sample of 0.12g was taken from the particles passing sieve No. 200. A volume of 150 mL of distilled water with pH 6.5 was added to the sediment before measurement. The zeta potential (ζ) is a function of electrophoretic mobility or Be. Electrophoretic mobility is the ratio of the migration velocity u_r to the field intensity E of the test ($Be=u_r/E$). The zeta potential then was calculated from the equation: $\zeta = \frac{u_r}{E} \times \frac{\eta}{\varepsilon_0 \varepsilon_r}$, where η is the viscosity, and ε_0 and ε_r are absolute dielectric constant and influence constant respectively. The above equations are built into the zeta potential meter used and the zeta potential was measured at –24.3 mV with a standard deviation of 1.859 for 20 samples.

3.2.13 Specific surface area

The method proposed by Carter et al. (1965) was used to determine the specific surface area of sediment (SSA). The procedure is given as follows:

I. The soil (1.1 g) was weighed in an aluminum weighing dish. The sample was dried to aconstant weight in an evacuated dessicator over P_2O_5. The constant weight was obtained by periodic measurement.

II. Reagent grade EGME (ethylene glycol monoethyl ether) was added to cover the dried sample and left to equilibrate for 1 hour.

III. The P_2O_5 in the bottom of the dessicator was removed (with care and avoiding the use of water in contact with P_2O_5). The dessicator was filled with approximately 130 g of $CaCl_2$ EGME solvate.

120 g of $CaCl_2$ (40 mesh) was placed into a one-liter beaker and was dried in oven for 1 hour and 30 g of EGME in a 400 mL beaker was weighed. 100g of the hot, dry $CaCl_2$ was added to the EGME, and mixed thoroughly with a spatula. After cooling, it was spread at the bottom of the dessicator.

IV. The sample-EGME slurry was put in its aluminum dish in a Petri dish and covered over and was placed in a dessicator. Then an evacuation was applied with high vacuum pump for 45 min. Dessicator was left closed for 1 hour, and then the sample was weighed.

V. Evacuation was repeated for 45 min and then the sample was reweighed at successively longer intervals until a constant weight was reached.

VI. According to the calculation:

$$2.86\times10^{-4}\, g\ EGME = monolayer\ of\ 1\,m^2\ surface\ area.$$

$$\text{Surface area } (m^2/g) = \frac{\text{weight of EGME retained (mg)}}{\text{weight of dry soil (g)}} \times \frac{1m^2}{0.286mg\ EGME}$$

By using the method on the sediment, the specific surface area (SSA) was determined to be equal to 174.82 m^2/g.

3.3 Resin

The ion exchange resin used to investigate the mechanism of heavy metal removal with rhamnolipid was a strongly acidic cation exchange resin (Amberlite 200C Na^+ form; 20-45). The cation exchange capacity of the resin was reported as 4.2 meq/g as reported by the supplier, Sigma Aldrich Canada Ltd.

3.4 Rhamnolipid (JBR215)

The biosurfactants used were rhamnolipids with the trademark JBR215 from "JENEIL BIOSURFACTANT CO., LLC". JBR215 is an aqueous solution of rhamnolipid at 15% concentration. It is produced from sterilized and centrifuged fermentation broth. Two major types of rhamnolipids, RLL (R1) and RRLL (R2), are present in the solution.

Chemically, rhamnolipids are glycosides of rhamnose (6-deoxyrhamnose) and β–hydroxydecanoic acid. The molecular weight of RLL ($C_{26}H_{48}O_9$, rhamnolipid type I) is 504 and of RRLL ($C_{32}H_{58}O_{13}$, rhamnolipid type II) is 650 as determined by the supplier. The chemical structures of both types are given in Chapter 2 (Figure 2-5). Several tests done by the manufacturer and independent laboratories show that the degree of biodegradability and toxicity of JBR215 match the EPA requirements (Jeneil 2001). Some physical and chemical properties of these types of JBR215 are shown in Table 3.4.

The biosurfactant is supplied as a 15% concentration solution. Different concentrations of rhamnolipid were chosen to perform preliminary tests. Therefore, each 1mL of biosurfactant was diluted to 15 mL with distilled water to obtain a solution of 1% concentration or to 7.5 mL for a solution of 2%, etc.

Table 3.4 Physical and chemical properties of JBR215 (adapted from Jeneil Biosurfactant Co. Ltd. 2001).

Physical/ Chemical property	Description
Appearance	Dark reddish-brown solution
Concentrations	Viscous suspension
Odor	Soapy
Specific Gravity	1.03 – 1.05
pH	6.5 – 7.5
Solubility in water	Soluble at neutral pH
Suitable diluents	Water and most common alcohols
Suggested starting	Active rhamnolipid Ingredient: 0.01, 0.1, and 1.0%
Surface tension	29 mN/m
Interfacial tension	0.3 mN/m

3.4.1 CMC measurement

The CMC can be defined as the minimum concentration of surfactant at which micelle formation initiates. By increasing the surfactant concentration, the surface tension decreases until it reaches CMC. Beyond this level of surfactant concentration the surface tension remains approximately constant. There are several methods to determine the CMC for biosurfactants. The CMC can be found by graphing conductivity, turbidity, surface tension, detergency and interfacial tension versus concentration (Myers 1992).

The CMC was found by determining the variation of conductivity and surface tension with biosurfactant concentration. Conductivity was measured with a

Fisher Scientific digital conductivity meter. In this case, the crossing point of the two tangents of the curve is the CMC (Figure 3-4). The CMC was also measured by surface tension with Fisher Scientific Surface tensiometer. The result is shown in a graph of surface tension versus rhamnolipid concentration (Figure 3-5). In a combined graph, the crossing point shows the CMC since the production of tangents of these two curves is negative. In other words these two curves have only one crossing point that represents the CMC. The graph is shown in Figure 3-6. The value of CMC was determined at about 0.035 g/L that is equivalent to 0.003% rhamnolipid. Therefore, for the rest of the experiments, concentrations above the CMC were used in the testing program to ensure the formation of micelles.

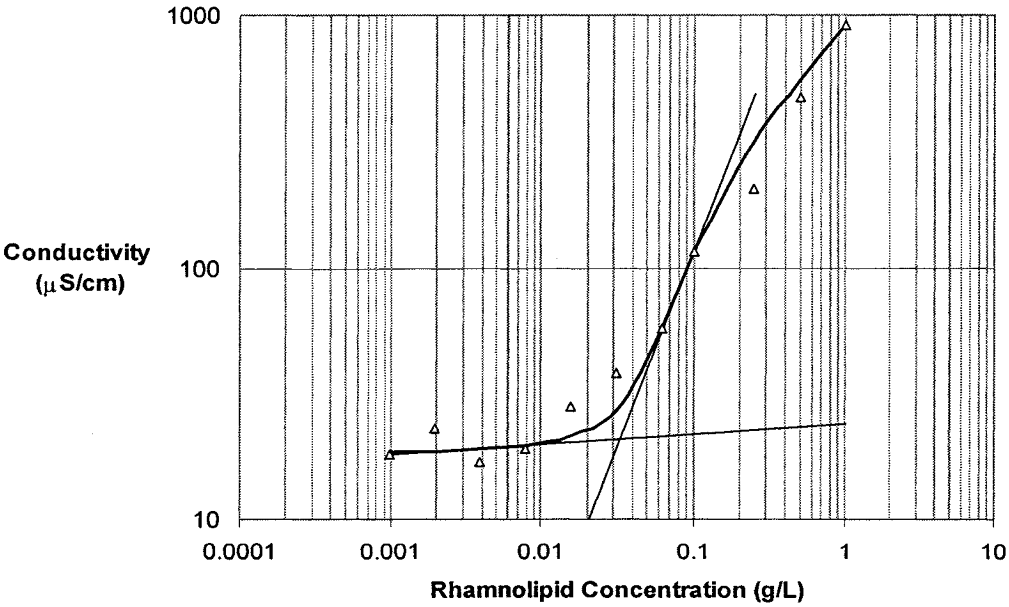


Figure 3-4 Conductivity versus concentration of rhamnolipid to find CMC.

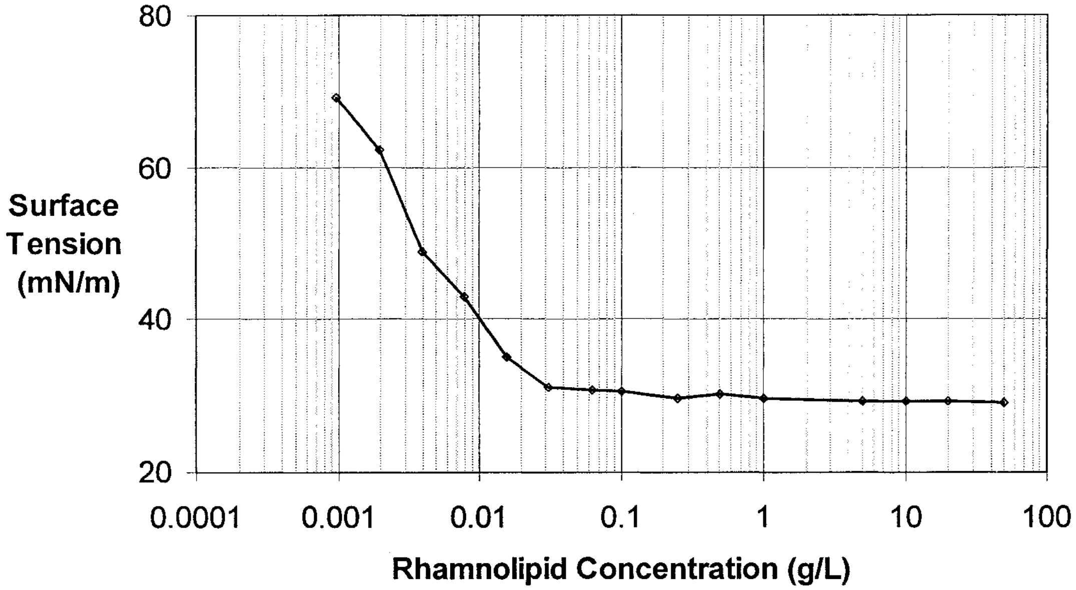


Figure 3-5 CMC measurement by surface tension.

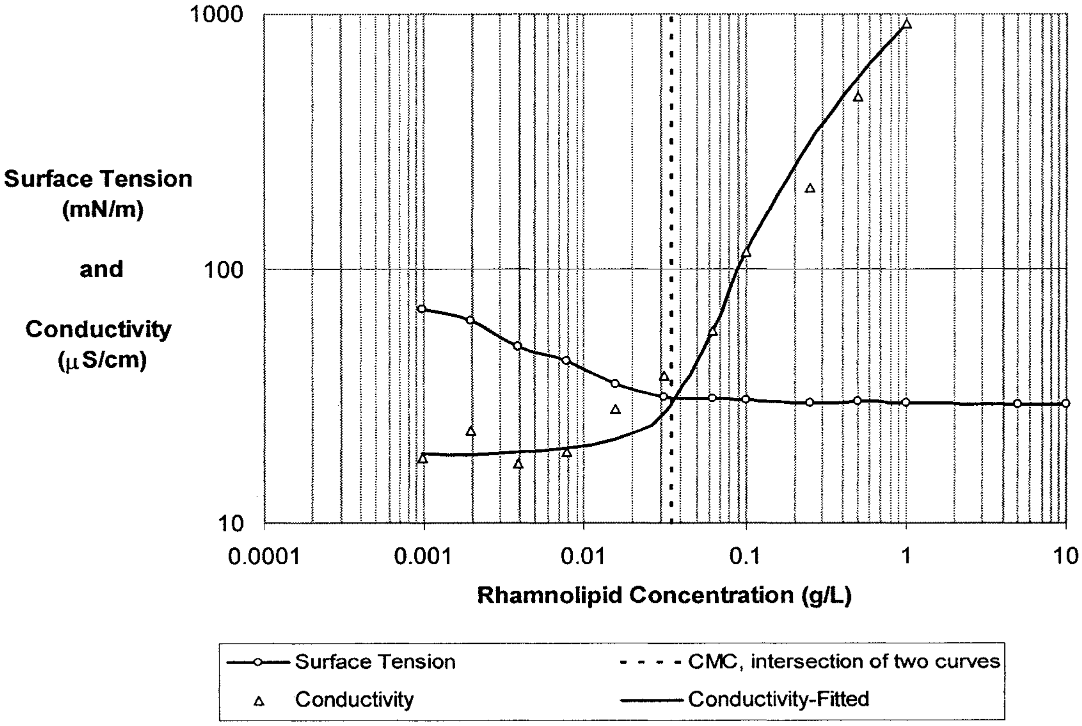


Figure 3-6 CMC measurement by surface tension and conductivity.

3.5 Procedure for the experiments

In this study, a set of tests was designed to determine the effects of the parameters involved in the removal process. This section presents all the tests performed in chronological order. The equipment used for each test is specified in the description of the test.

3.5.1 Metal removal

Removal of metals from the sediment was performed using two methods: batch tests and continuous flow tests. A detail explanation of each method is as follows:

3.5.1.1 Batch washing tests

The biosurfactant solution was diluted with distilled water to make different concentrations of rhamnolipid. A quantity of 1.50 g from the sediment was placed in each 50 mL vial (Fig. 3-7). 15.0 mL of biosurfactant solution was added to each vial and the pH was set at 6.5 using a ±0.002 Fisher Scientific Dual Channel pH/Ion meter. The samples were kept at room temperature on a Thermolyne/Barnstead adjustable orbital shaker Model M66025 that was set at 100 rpm at the desired times. Supernatants and sediment particles were separated by using a centrifuge IEC HN-SII (3000 rpm, 30 min) and samples from supernatants were taken. All tests were performed in triplicates. The samples were digested in order to release the heavy metals trapped in the biosurfactant micelles and then the concentrations of copper and other metals in each sample were measured by the Atomic Absorption Spectrophotometer.

Organic metal colloids were oxidized by adding 30% H_2O_2 slowly until no reaction was observed. The method chosen for biosurfactant digestion was 3030E, which is recommended by APHA (1995) and approved by the EPA. The steps of the method are:

i. 50 mL of the mixed sample were transferred to a 125 mL beaker.

ii. 25 mL of concentrated HNO_3 and a few boiling chips were added to the beaker's contents.

iii. The beaker was heated over a hot plate and brought to a slow boil.

iv. The digestion was considered done when the solution in the beaker became a clear light-colored liquid.

v. The solution was cooled to room temperature, filtered through filter paper (40 ashless circles, 110 mm diameter x 100 circles) and the volume returned to the initial volume (50 mL).

The percentage of metal removal was calculated using the following equations:

$$\text{metal in sediment}_{(\text{initial})}\,(\text{mg}) = \text{Total metal content}\left(\frac{\text{mg}}{\text{kg}}\right)\times\left(\frac{1\,\text{kg}}{1000\,\text{g}}\right)\times \text{m}_{\substack{\text{Sediment}\\ \text{before}\\ \text{washing}}}(\text{g}) \qquad [3\text{-}2]$$

$$\text{metal in solution}_{\text{After washing}}\,(\text{mg}) = \text{metal concentration}_{\substack{\text{in soluton}\\ \text{after washing}}}\left(\frac{\text{mg}}{\text{L}}\right)\times\left(\frac{1\,\text{L}}{1000\,\text{mL}}\right)\times \text{V}_{\substack{\text{Solvent}\\ \text{before}\\ \text{washinhg}}}\,(\text{mL}) \qquad [3\text{-}3]$$

$$\text{metal removal}\,\% = \frac{\text{metal in solution}_{\text{After washing}}}{\text{metal in sediment}_{\text{initial}}}\left(\frac{\text{mg}}{\text{mg}}\right)\times 100 \qquad [3\text{-}4]$$

For verification of the procedure, a mass balance was also performed occasionally using the following equations:

$$\text{metal in sediment}_{\text{Before washing}}\ (\text{mg}) = \text{metal in solution}_{\text{After washing}}\ (\text{mg}) + \text{metal in sediment}_{\text{After washing}}\ (\text{mg}) \quad [3\text{-}5]$$

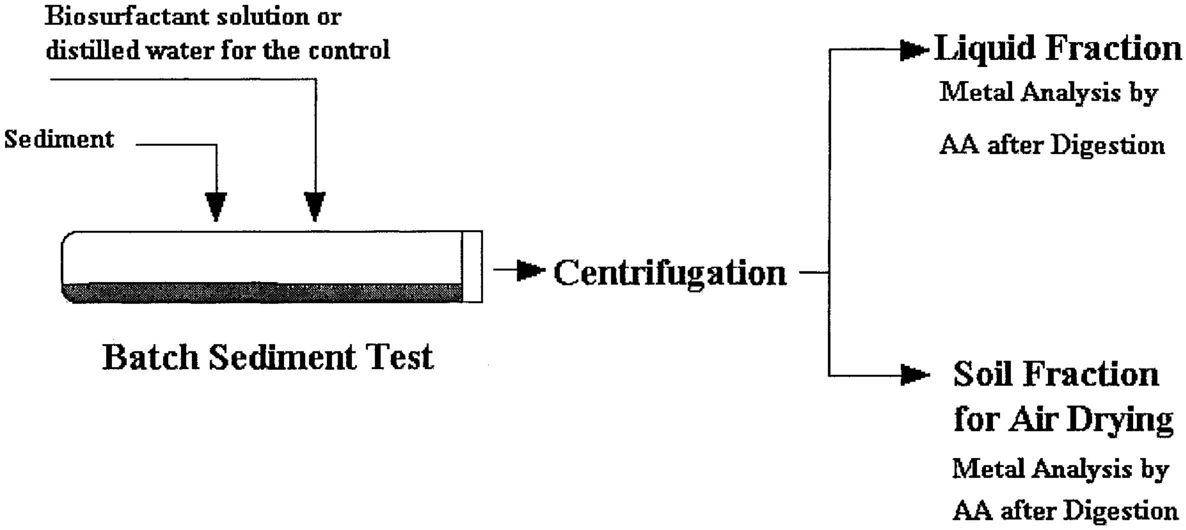


Figure 3-7 **Schematic of batch metal removal procedure.**

3.5.1.2 Continuous flow tests

Previous studies show that several washing steps improved the performance of soil washing and metal extraction by biosurfactants (Mulligan 1998 and Dahr Azma 2002). The idea of using continuous flow for sediment and soil washing is based on the batch tests. Since the batch tests show that the metal removal from sediments is possible, another configuration was used to study the effect of flow on the removal process to simulate *in situ* soil treatment. Beneficial results by this method will open a new horizon in in-situ sediment and soil remediation. A full range of possibilities and information that the continuous flow soil washing may bring is discussed in Chapters 4 and 5.

To perform the continuous flow tests, a 47 mm diameter Teflon PFA Holder cylindrical test section (column) was used. Both inlet and outlet tubing had the same diameters. The connecting tubing are Masterflex PharMed Pump Tubing and PTFE Tubing with outer diameter (OD) equal to 6.35 mm and inner diameter (ID) equal to 4.76 mm. A cartridge pump (6 Masterflex model 7519-15) was used to provide the dynamic head for the flow.

The biosurfactant solution was placed in a tank and the suction tubing was placed close to the bottom to prevent air entry. The flow was pumped and entered the column from the bottom. This ensured the exhausting of air from the column. The column was filled from bottom to top by: 0.2 g Pyrex Brand Wool – Filtering Fiber, 22 g of 4-mm Pyrex Brand solid glass beads, 0.2 g of glass wool, sediment and 0.5 g of glass wool (Figure 3-8).

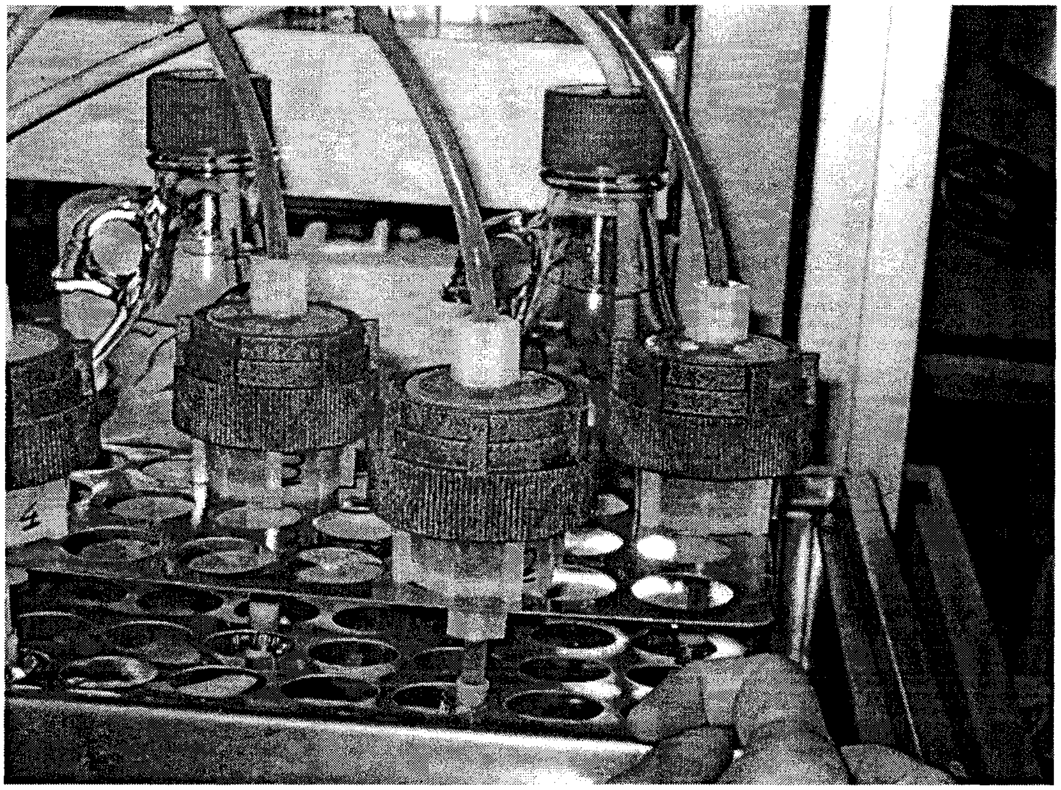


Figure 3-8 Column used in the continuous flow tests.

The flow rate (ranged between 0.5 to 20 mL/min) was controlled by a valve downstream of the column section. A 3-way polypropylene stopcock with Teflon TFE 4 mm plug was used to measure the flow rate through counting of drops. The washing solution (720 mL) was collected in another tank (Figures 3-9). Each 24 hours, the feeding and collecting tanks were switched. Samples from the supernatant were taken and in order to release the heavy metals trapped in the biosurfactant micelle samples were digested. The concentrations of copper and other metals in each sample were measured by the Atomic Absorption Spectrophotometer. The method of digestion and related calculations are explained in section 3.5.1.1. Figure 3-10 shows a schematic view of the continuous flow configuration.

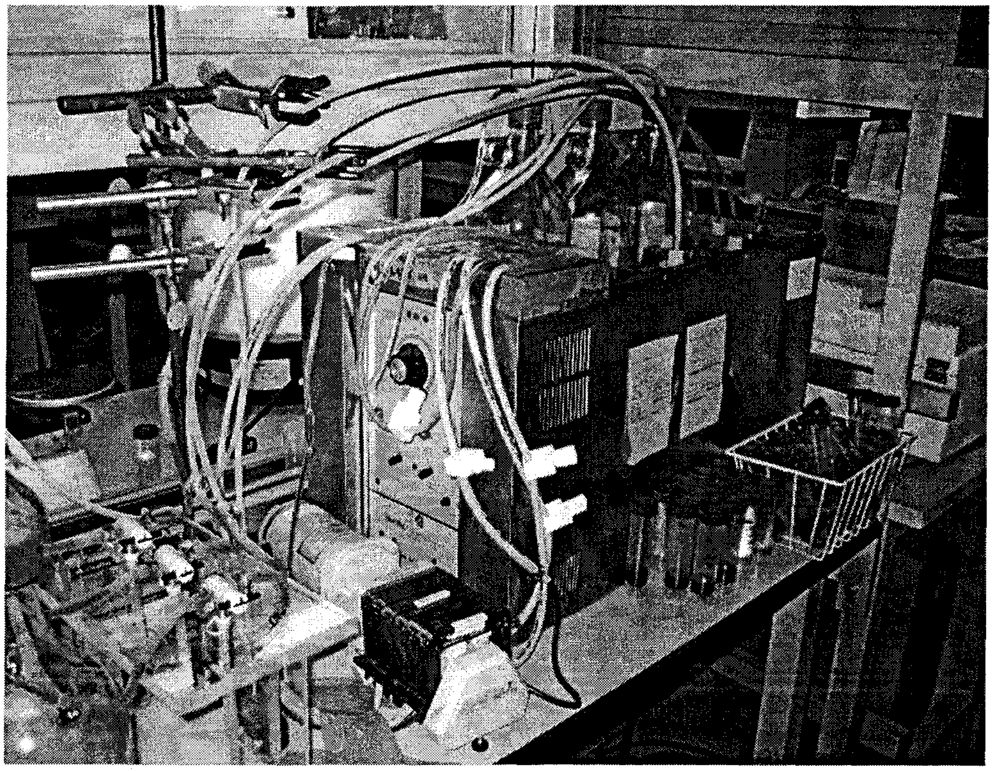


Figure 3-9 A general view of the test set-up for the continuous flow tests.

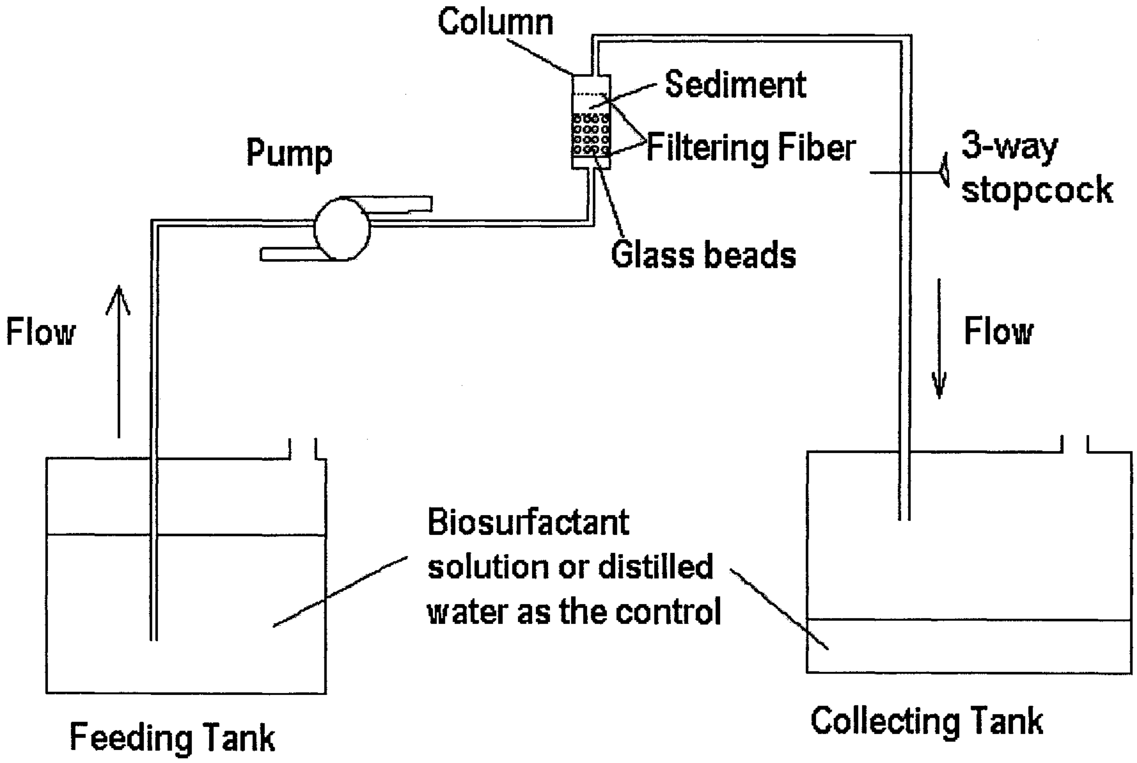


Figure 3-10 Schematic of continuous flow configuration.

3.5.2 Selective sequential extraction tests

The procedure used for the batch sequential extraction was similar to that of Yong et al. (1993) shown in Table 3-5. Sediment samples (1.5 g) were washed with the biosurfactant solutions and controls. Each of the fractions was collected and the concentrations of heavy metals were determined by atomic absorption spectrometry (Perkin Elmer Atomic Absorption Aanalyst 100 Spectrophotometer). Samples were washed with distilled water after each step to prepare samples for the next step. The amounts of copper, zinc and nickel extracted from each of the extractants were then calculated.

Table 3.5 Sequential extraction process (adapted from Yong et al. 1993).

Sequence	Chemical Reagents	Fraction
1	Overnight extraction of metals by surfactants and controls with 15 mL of solution	Soluble
2	Extraction of metals with 8 mL of 1 M $MgCl_2$ (pH 7) for 1 h	Exchangeable
3	Extraction of metals with 8 mL of 1 M NaOAc(1) adjusted to pH 5 with acetic acid for 5 h	Carbonates
4	Extraction of metals with 20 mL of 0.04 M $NH_2OH.HCl$ in 25% (v/v) acetic acid (pH 2.5) at 96°C for 6 h	Oxides and hydroxides
5	Extraction with 3 mL of 0.02M HNO_3 and 5 mL of 30% H_2O_2 (pH 2) for 2 h at 85°C, followed by 3 mL of 30% H_2O_2 (pH 2) at 85°C for 3 h and then 5 mL of 3.2 M NH_4OAc^1 in 20% (v/v) 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 HCl, 200 mL HNO_3 and 750 mL water) for 3 h	Residual fraction

(1) Ac denotes acetate.

3.6 Adsorption of rhamnolipid to the sediment

To investigate the isotherm of the adsorption of rhamnolipid to the sediment, a procedure was designed and a series of tests was performed. Various concentrations (0.5, 1, 2, and 5 percent) of the biosurfactant, rhamnolipid, were applied to 10 g of sediment samples in a continuous flow configuration with a rate of 0.5 mL/min. The concentration of rhamnolipid before and after the short term (3 days) washing process was measured using surface tension analysis. The method is based on the proportionality of the rhamnolipid concentration and the surface tension of the solution. Figure 3-5 was used to find the concentration of rhamnolipid from the measured surface tension. The difference between the concentrations of the rhamnolipid before and after washing is considered to be the adsorbed rhamnolipid on the sediment surface. This value (S) also is called sorbed rhamnolipid. If the sorbed rhamnolipid for each concentration is plotted versus the amount of rhamnolipid remaining in the supernatant at equilibrium (C), the type of the isotherm can be identified. The results are presented in Chapter 6 of this thesis.

3.7 Methods relevant for the investigation of the mechanism of metal removal

3.7.1 Ion exchange experiments

An ion exchange experiment also was performed. The supernatants from soil washing experiments using 2% rhamnolipid with and without 1% NaOH in continuous flow configuratrion were used for ion exchange experiments. The concentration of surfactant also was measured. The ion exchange experiments included eight steps as follows:

1. The cationic resin was put in distilled water according to the manufacturer's instructions to make the resin wet. The water was drained over 2 hours.
2. The resin was placed in 4M NaCl for 1 hour on the shaker (100 rpm) and then the NaCl solution was drained. This step was performed to saturate the resin with Na^+ ions.
3. The resin was washed with water several times to remove the unabsorbed NaCl from the surface of the resin.
4. Supernatant samples taken from soil washing tests were added to the resin and shake for 1 hour on the shaker (100 rpm). During this step all of the metals that were removed through an ion exchange process from the sediment, were re-exchanged with Na^+ in the resin.
5. The samples from the latter solution were taken to measure the concentration of the heavy metals using AA. The concentration of rhamnolipid also was measured by surface tension analysis.
6. The resin was washed with distilled water for several times to remove the unabsorbed metals from the surface of the resin.
7. 4M NaCl was added to the resin and was agitated for 1 hour. This step was performed to replace absorbed metals with Na^+.
8. The solutions from step 7 were analyzed for metal concentration by AA. This step is to verify the quantity of the metals that were removed by ion exchange mechanism from sediment.

9. The resin was washed with water several times and was stored for further use.

To determine the proportion of metal removed by each mechanism (ion exchange and complexation) from the sediments during the washing process with the rhamnolipid, calculations were performed on the measured concentration of each metal (Eqs. 3-6, 3-7, and 3-8).

Removed metal by complexation (%) =

$$\frac{C_{\text{metal in supernatant after contact with resin}}}{C_{\text{metal in supernatant before contact with resin}}} \times 100 \qquad [3\text{-}6]$$

Removed metal by ion exchange (%) =

$$\frac{C_{\text{metal in NaCl solution after regeneration (Step8)}}}{C_{\text{metal in supernatant before contact with resin}}} \times 100 \qquad [3\text{-}7]$$

or:

Removed metal by ion exchange (%)= 100- Removed metal by complexation (%) [3-8]

3.7.2 Scanning electron microscopy of the sediment

A scanning electron microscope (SEM) (JEOL JSM-840A) was used to take microscopic pictures of the sediment. The apparatus works in a way similar to reflected light microscope with difference that the reflected light microscope forms an image from light reflected from a sample surface while the SEM uses electrons for image formation. Due to their shorter wavelength, electrons have a much better capability of generating higher-resolution information. The high resolution provides higher magnification without a loss of image information. Pictures were taken by SEM micrographs to preserve their three-dimensional appearance of textured surfaces. Although the magnification ranged between 10 to 300000, the pictures were taken with 1000X zoom for a better view of the sediment texture rather than the details of a fraction of a particle.

Samples were sprinkled over a carbon tape that was fixed over an aluminum stub. This is a mandatory step since the particles should maintain their position during photography. To prevent adsorption of electrons by the sediment, it was then coated by a thin gold and palladium film. This metallic thin film was prepared using the sputtering system Hummer 6 using Au and Pd to make the thin film. Gold and palladium were used because they can form very thin films without suppressing fine surface features. The results are presented in Chapter 7.

Chapter 4

Experimental Results for Soil Washing Experiments

4.1 General remarks

Sediment remediation is a complex matter in the sense of choosing the right techniques and reliable washing agents. To employ environmentally friendly techniques and materials for remediation of soil, several strategies were developed but more techniques and strategies are needed. New advances in biological methods including the use of microorganisms and/or biological products have opened new horizons in soil and sediment remediation. Biosurfactants are among those products that are newly found for heavy metal removal.

This research was performed to investigate the functionality of rhamnolipids in heavy metal removal. In this chapter, the results of soil washing experiments using rhamnolipids both in batch and continuous flow configurations are presented. Various parameters were studied and the effect of each on the removal of heavy metal from sediment is illustrated in the appropriate figures, tables, and graphs.

4.2 Batch tests

The batch tests were performed to investigate the efficiency of using rhamnolipids in heavy metal removal and the effect of rhamnolipid concentration, time, addition of NaOH, pH, and the ratio of sediment mass to the volume of rhamnolipid. The general configuration of the batch tests was explained in detail in Chapter 3.

In these tests, the pH of the initial solution was set at 6.5 (except for the pH study) because the rhamnolipid used for this study has a pH close to 6.5. Moreover, rhamnolipid precipitates at a pH lower than 5.5. All samples were placed on the orbital shaker set at 100 rpm, based on the reports from other researchers, to provide a full contact between the sediment particles and the washing solutions (Herman et al. 1995, Szulczewssski et al. 1997, McCray et al. 2001, and Mulligan et al. 1999). The rhamnolipid concentration (except for the concentration study tests) was chosen to be 2%. The preliminary tests showed that although a higher concentration may provide a better removal, the viscosity of the rhamnolipid solution is impractically high at a 5% concentration. On the other hand, to have enough rhamnolipid molecules for interaction with the sediments a 2% concentration was chosen. According to the available literature, the ratio of soil mass to the washing solvent is usually chosen as 1 g in 10 mL (Szulczewssski et al. 1997, Mulligan et al. 1999, and Dahrazma and Mulligan 2004). In the present study the same ratio was respected although further investigation showed that better results are achievable. All samples were analyzed after three days except for the time study based on the preliminary tests.

4.2.1 Concentration study

Three heavy metals found in the sediment were of interest for this research namely copper, zinc and nickel. By performing several triplicate tests, the percentage of removal for each metal was determined from analysis of the supernatant and graphed in an appropriate figure. A series of washings was performed on the sediments using 0.5%, 1%, 2% and 5% rhamnolipid solutions with and without 1% NaOH. The controls were distilled water and 1% NaOH when NaOH was added to rhamnolipid.

The results for removal of copper, zinc and nickel after washing the sediment with a broad range of rhamnolipid concentrations with and without NaOH are shown in Figures 4-1, 4-2, and 4-3. Removal rates for all of the three metals increased by increasing the concentration of rhamnolipid in an almost linear fashion without NaOH. However, in the case of copper, highest removal rates, 14.5%, occurred for 0.5% rhamnolipid with 1% NaOH. Increasing the biosurfactant concentration in the presence NaOH did not enhance the removal rates.

Maximal removals were 34.0% of nickel, 10.7% of zinc, and 12.9% of copper without NaOH. However, since zinc has the highest concentration in the sediment, this metal was relatively removed the most. For example, for a 5% rhamnolipid concentration, approximately 521 mg/kg of zinc were removed compared to 18.1 mg/kg of copper.

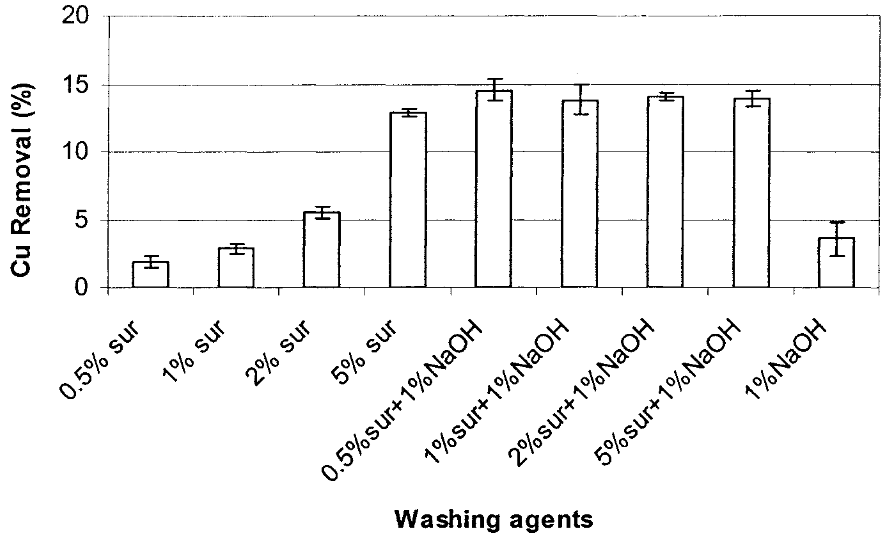


Figure 4-1 **Removal of copper from sediment by rhamnolipids with and without NaOH at different concentrations in the batch tests (sur is used for the rhamnolipid).**

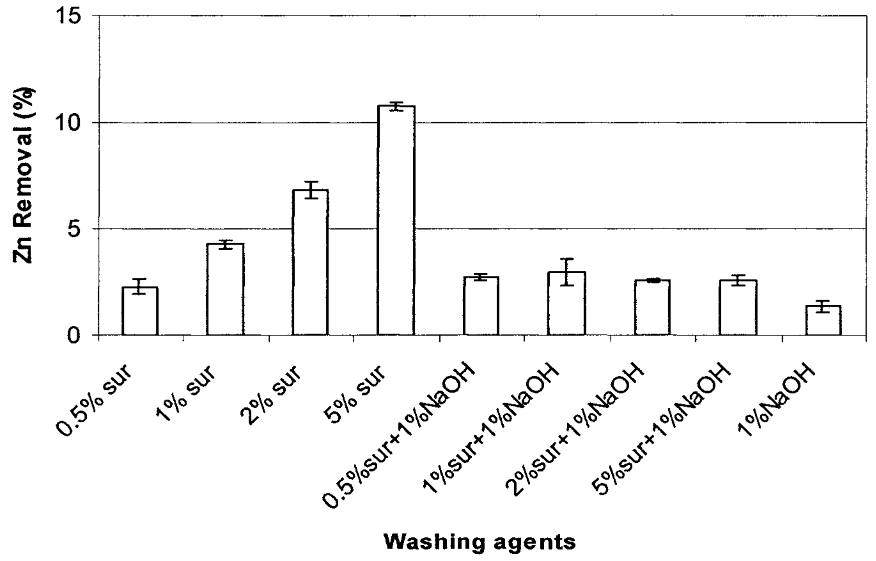


Figure 4-2 **Removal of zinc from sediment by rhamnolipids with and without NaOH at different concentrations in the batch tests (sur is used for the rhamnolipid).**

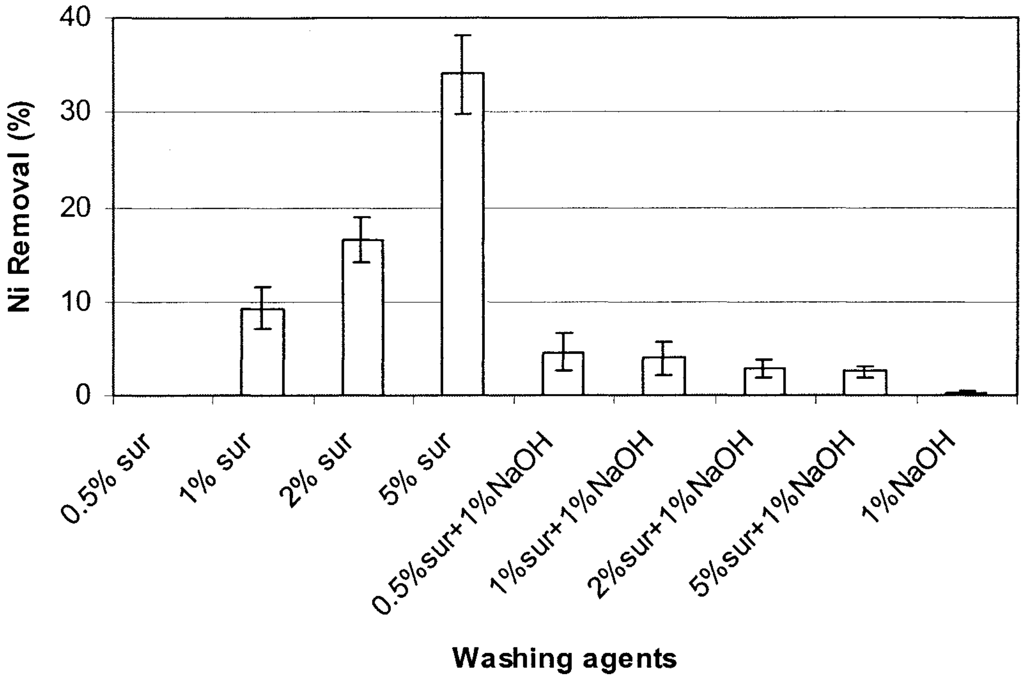


Figure 4-3 **Removal of nickel from sediment by rhamnolipids with and without NaOH at different concentrations in the batch tests (sur is used for the rhamnolipid).**

4.2.2 Importance of pH

The behavior of the removal of copper, zinc, and nickel was studied in batch configuration using a washing agent of 2% rhamnolipid at different pH values. The results are plotted in Figures 4-4, 4-5, and 4-6. The pH was adjusted with a solution of 1M NaOH and 10% HNO_3. The same pattern of removal is repeated for all three metals: starting from a higher value of removal at pH 5.5, declining and then passing a minimum and increasing with pH. The minimum removal for copper occurred at pH 9, for zinc at pH 9, and for nickel at pH 10.

These results can be compared with the solubility curves of the metal hydroxides versus pH shown in Figure 4-7. These curves show that the variation of removal with pH follows the pattern as the variation of solubility of metal hydroxide with pH. The minimal removal of the studied metals occurs where the solubility of dissolved metal is at its minimum point.

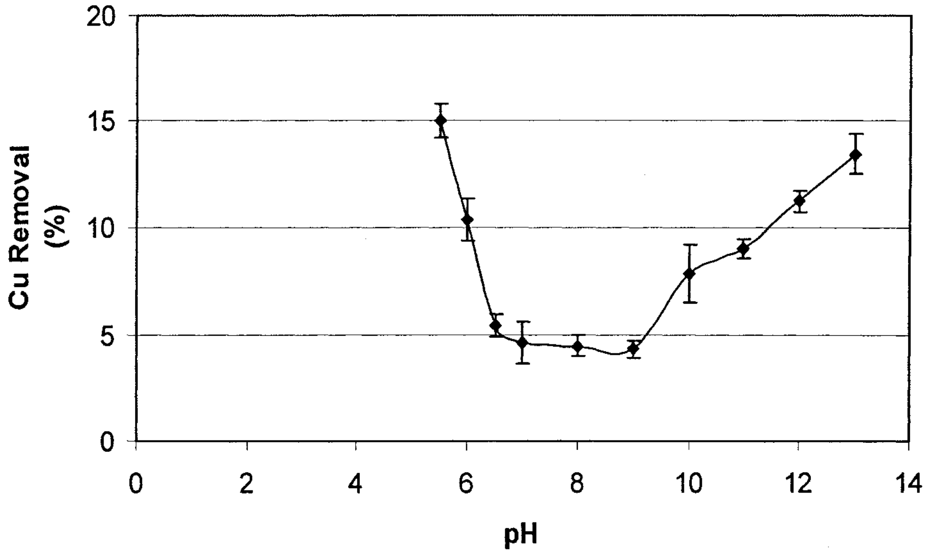


Figure 4-4 **Variation of copper removal from sediment with pH for the batch test with 2% rhamnolipid.**

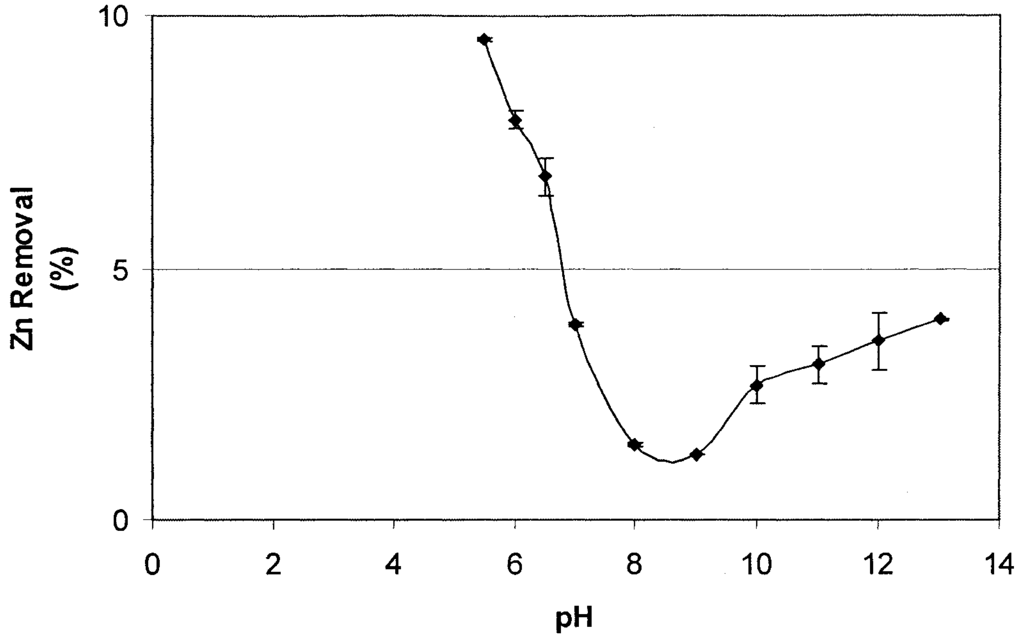


Figure 4-5 Variation of zinc removal from sediment with pH for the batch test with 2% rhamnolipid.

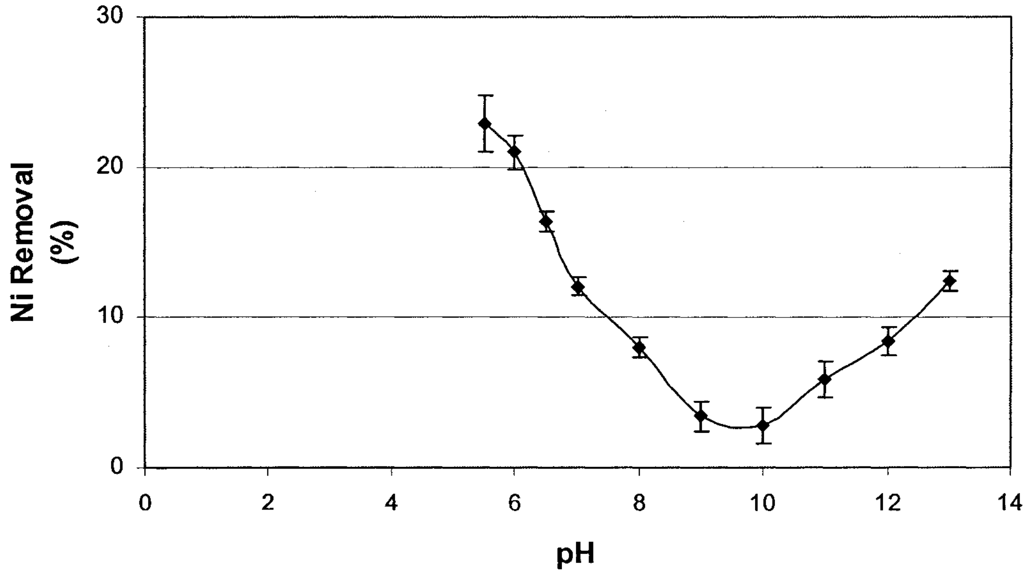


Figure 4-6 Variation of nickel removal from sediment with pH for the batch test with 2% rhamnolipid.

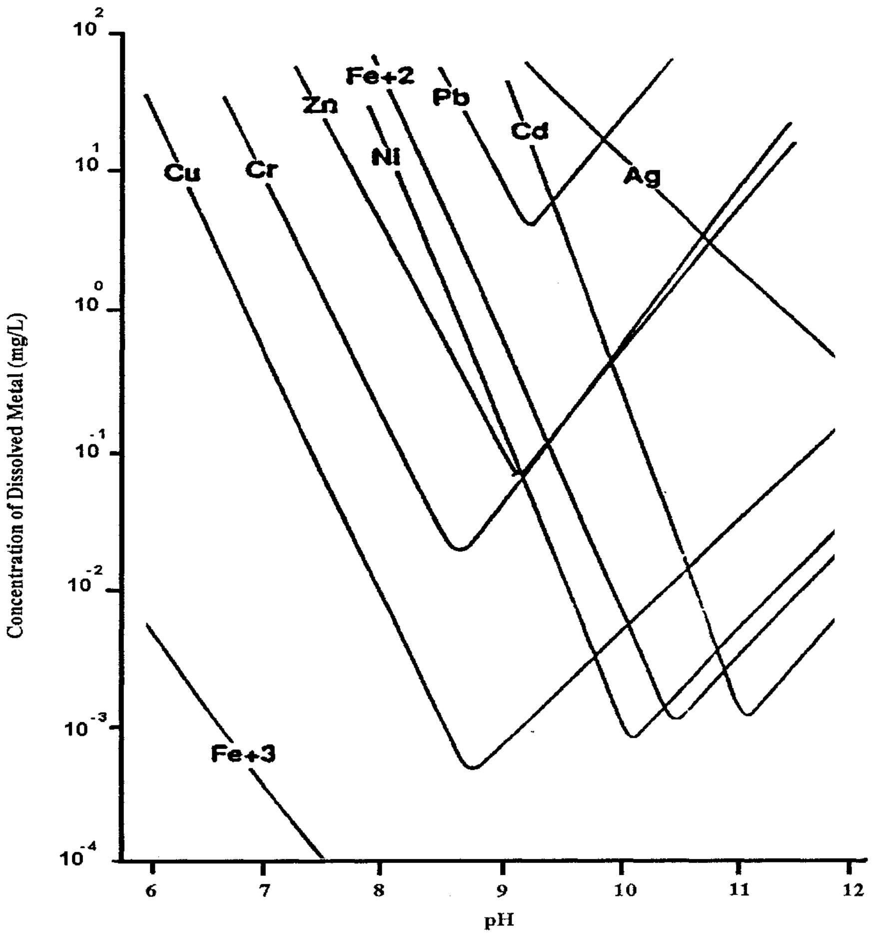


Figure 4-7 Solubility of metal hydroxides with pH (adapted from Radha Krishnan 1993).

4.2.3 Time course tests

A series of washings was performed on the sediment using 2% rhamnolipid solution with and without 1% NaOH and the removal of metals was measured with respect to time. The control was distilled water and 1% NaOH when NaOH was added to rhamnolipid. The pH of the rhamnolipid was adjusted to 6.5 in all tests. The results of the test for the target metals are shown in this section.

4.2.3.1 Time study using rhamnolipid without 1% NaOH

The removal of copper, zinc, and nickel in time series tests is shown in Figures 4-8, 4-9, and 4-10, respectively. The batch copper removal tests show that a major portion of removal (5.5%) occurs in the first day of the experiment (almost 90% of the total metal removed after day 16). In other words, although copper removal was enhanced with time up to 6.1% in day 16, the improvement was not remarkable (less than 0.6% of total copper in the sediment). The removal time graph shows that the process was time progressive. The immediate result of this trend is that copper remains soluble with the biosurfactants, at least for the first 16 days. Maximum removal was 8.5 mg copper from one kilogram of sediment on day 16 (Figure 4-8).

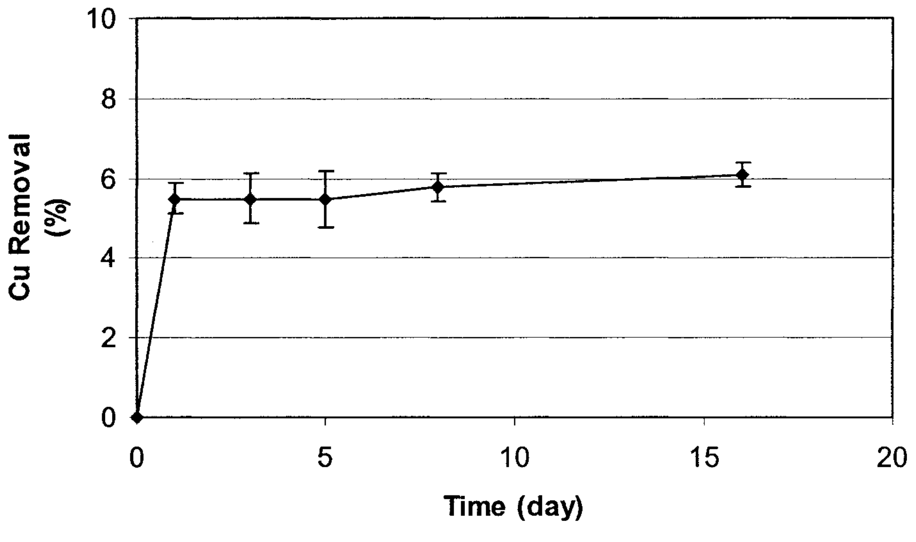


Figure 4-8 Washing of copper from sediment in progressive time intervals for the batch test using 2% rhamnolipid at pH 6.5.

Figure 4-9 shows that in the first 24 hours, the biosurfactant released 5% of the zinc which is similar to the removal of copper. This similarity did not stay long since in successive days the removal improved up to 10.7% after 16 days. In other words, as the concentration of zinc in the sediment was much higher than copper, the removal of zinc also was higher. The removal improved up to 519.3 mg/kg of sediment after 16 days.

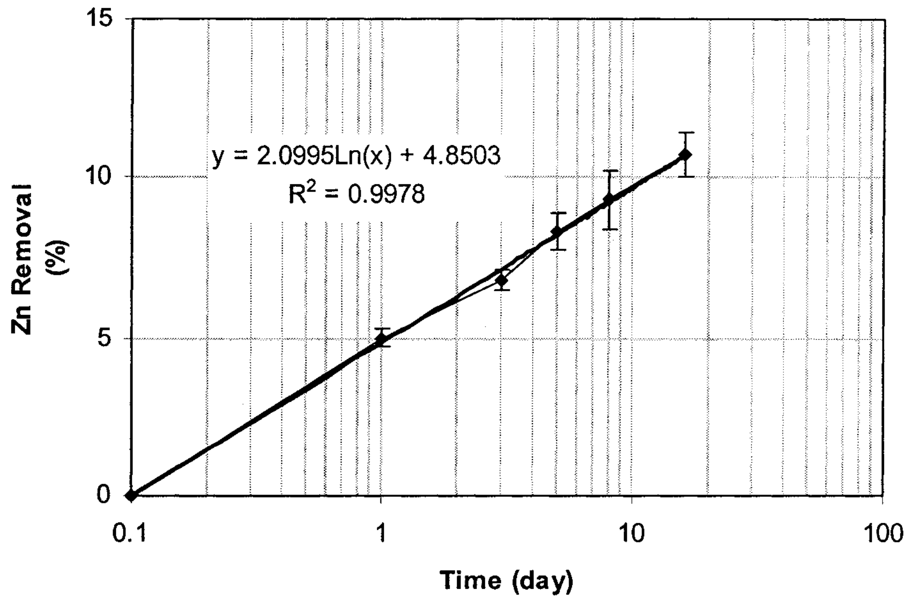


Figure 4-9 Washing of zinc from sediment in progressive time intervals for the batch test using 2% rhamnolipid at pH 6.5.

Figure 4-10 shows the nickel removal over time. Clearly nickel showed a different trend than copper and zinc in the washing process using the rhamnolipid. The soluble Ni in the supernatant declined after day 8, when the removal reached the inflection point. The maximum removal was recorded at 24 mg/kg or 31.6%. Between day 8 and day 16, a decrease in total nickel removal occurred.

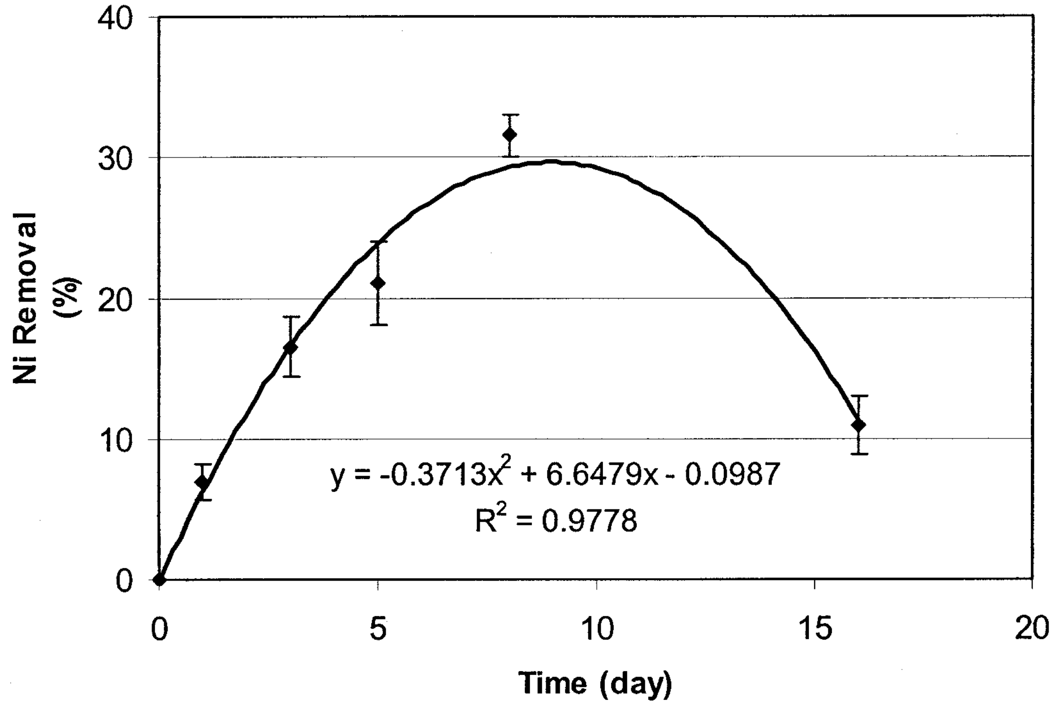


Figure 4-10 Washing of nickel from sediment in progressive time intervals for the batch test using 2% rhamnolipid at pH 6.5.

4.2.3.2 Time study using rhamnolipid with 1% NaOH

Adding 1% NaOH to the washing agent, 2% rhamnolipid, changes the removal process of the heavy metal from the sediment significantly during the time study tests. The results of these tests are shown in Figures 4-11, 4-12, and 4-13.

For copper, the amount of removal when 1% NaOH was added to the solution of

2% rhamnolipids, increased drastically up to 2.6 times more in comparison with the removal using 2% rhamnolipid in the first three days (14.1%). The increaset lasts for 3 days and from that day the concentration of copper in the supernatant dropped to 71% of its maximum on day 5 (10%). The rate of decrease became smaller after day 5 and on day 16 the detected soluble copper (6.6%) was almost 47% of the first day of the experiment. Another notable point of this test was the removal of copper with the control, 1% NaOH, that shows a growing removal rate up to 5.4% on day 16. The concentration of copper in the control became almost constant after day five.

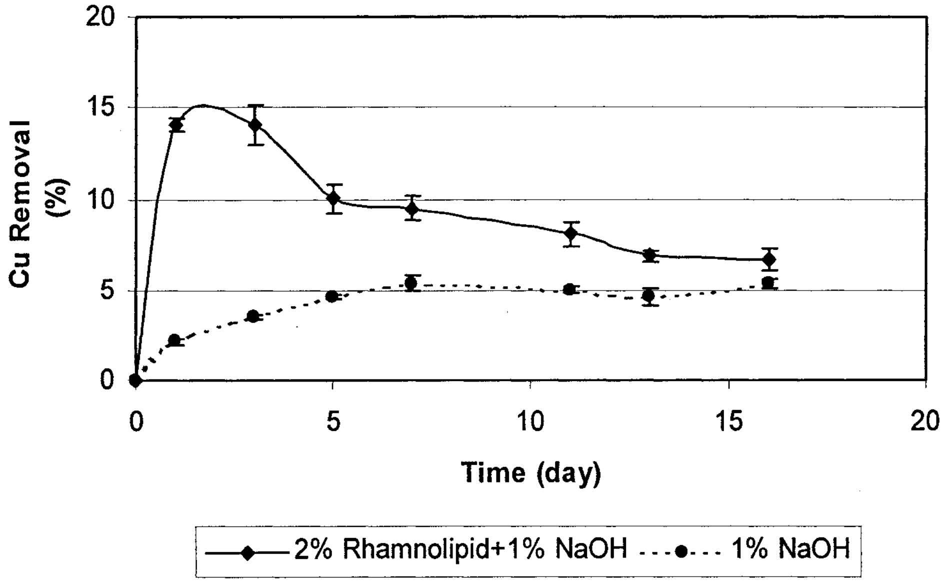


Figure 4-11 **Washing of copper from sediment in progressive time intervals for the batch test with 2% rhamnolipid and 1% NaOH. 1% NaOH was used as the control.**

Figure 4-12 shows the removal of zinc by 2% rhamnolipid with 1% NaOH. The effect of adding 1% NaOH to 2% rhamnolipid did not change the pattern of removal and the removal rates increased by time up to 3.2% or 157 mg/kg of sediment. The percentage of the removed zinc by this washing agent showed a remarkable decrease compared with the removal by 2% rhamnolipid on day 16 (approximately one third). The same removal pattern is seen for the removal by the control with a smaller concentration of zinc in the supernatant (1.4% or 67.4 mg/kg of sediment).

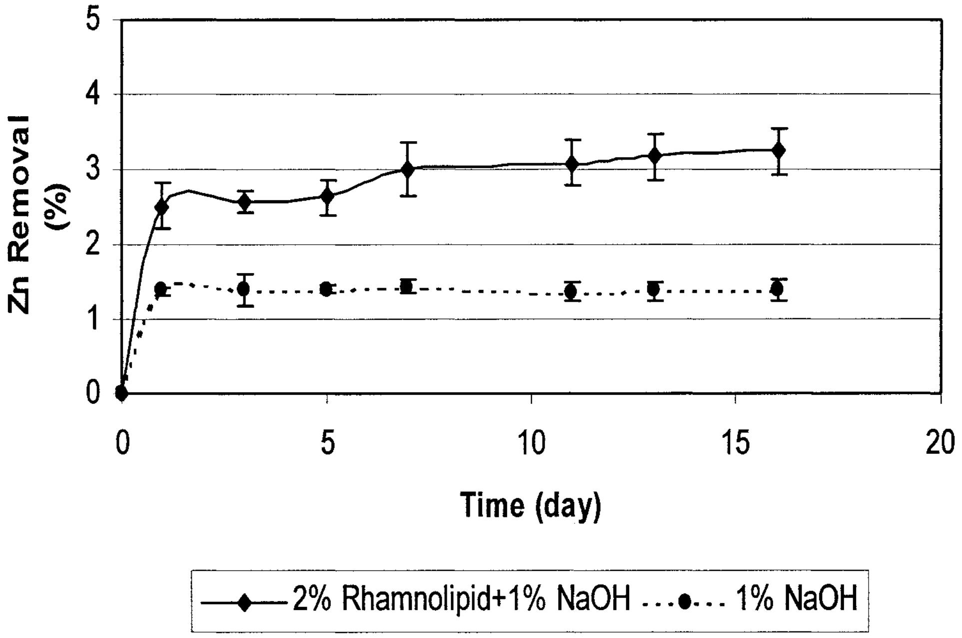


Figure 4-12 Washing of zinc from sediment in progressive time intervals for the batch test with 2% rhamnolipid and 1% NaOH. 1%NaOH was used as the control.

Washing the sediment with the mixture of 2% rhamnolipid and 1% NaOH resulted in the removal of 2.9% of nickel after three days (Figure 4-13) which is about 6 times less compared with the washing of the sediment by 2% rhamnolipid.

Although the rate of nickel removal increased over time up to 3.3 during the first five days of the experiment the concentration of nickel in the supernatant after 16 days dropped to the same concentration as day one. The removal by the control showed some step increments of nickel concentration in the supernatant for the first ten days and remained steady after day ten.

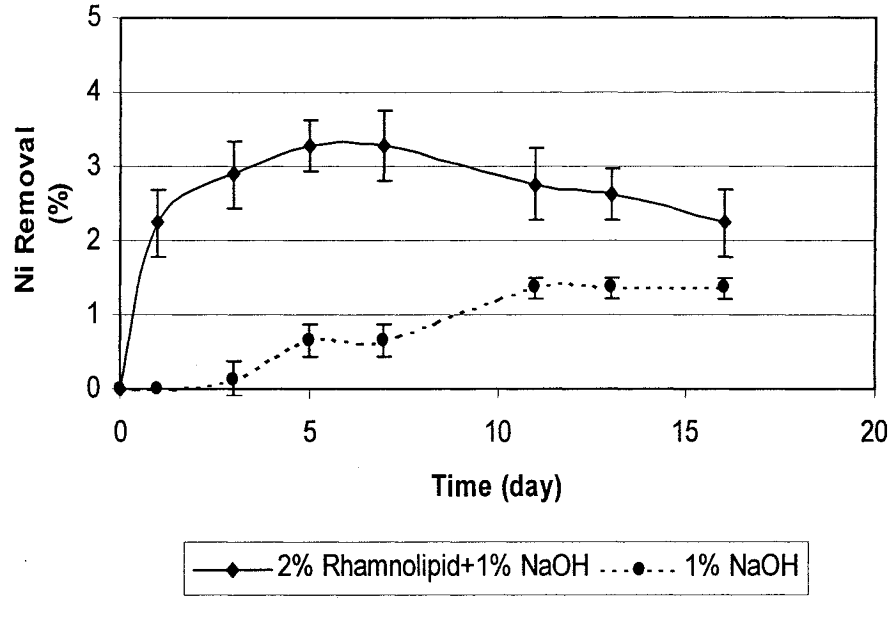


Figure 4-13 Washing of nickel from sediment in progressive time intervals for the batch test with 2% rhamnolipid and 1% NaOH. 1% NaOH was used as the control.

4.2.4 Study of the ratio of sediment mass to rhamnolipid volume

The effect of the ratio of mass of the sediment to volume of the rhamnolipid solution was investigated. Different volumes (5, 10, 15, 20, 30, 40, and 50 mL) of 2% rhamnolipid solution at pH 6.5 were used to wash 1 g of the sediment in batch tests and the concentrations of copper, zinc, and nickel in the supernatants were measured after three days. The results of this investigation are plotted in Figures 4-14, 4-15, and 4-16.

The percentages of removal for all three metals (Cu, Zn, and Ni) increased with the volume of rhamnolipid until it reached a ratio of 1 g of the sediment per 20 mL of solution. The maximum removal at this point was 15.4% for copper, 11.7% for zinc, and 21.6% for nickel. After this point, increasing the volume of rhamnolipid did not affect the amount of removal of metals and the graphs show constant values for the removal of metals in increments of the volume of rhamnolipid. In other words, in 20 mL of rhamnolipid, there were enough rhamnolipid molecules to reach the maximum possible removal of the metals from 1 g of the sediment, as well as enough contact between the molecules of rhamnolipid and sediment surface.

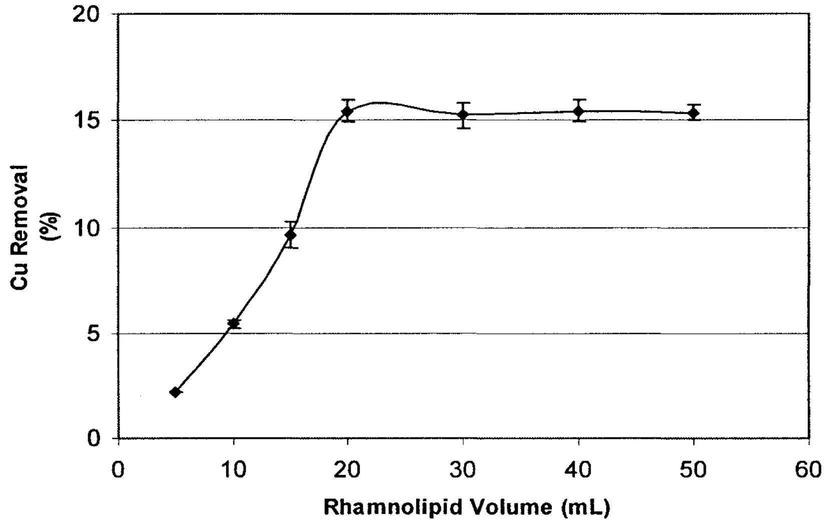


Figure 4-14 Variation of copper removal from sediments with different ratios of sediment mass to the volume of 2% rhamnolipid solution in the batch tests with 1g of the sediment (pH 6.5).

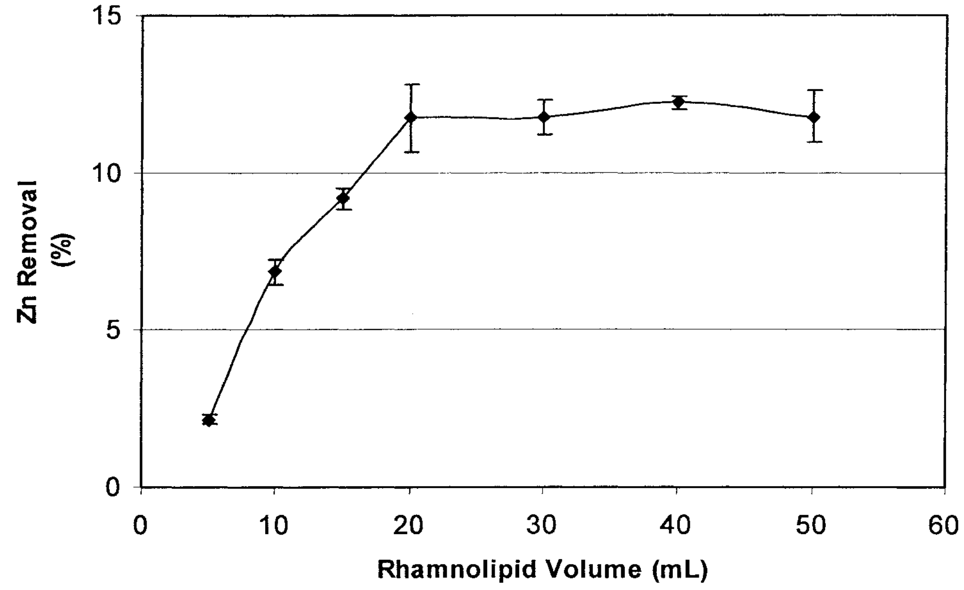


Figure 4-15 Variation of zinc removal from sediments with different ratios of sediment mass to the volume of 2% rhamnolipid solution in the batch tests with 1g of the sediment (pH 6.5).

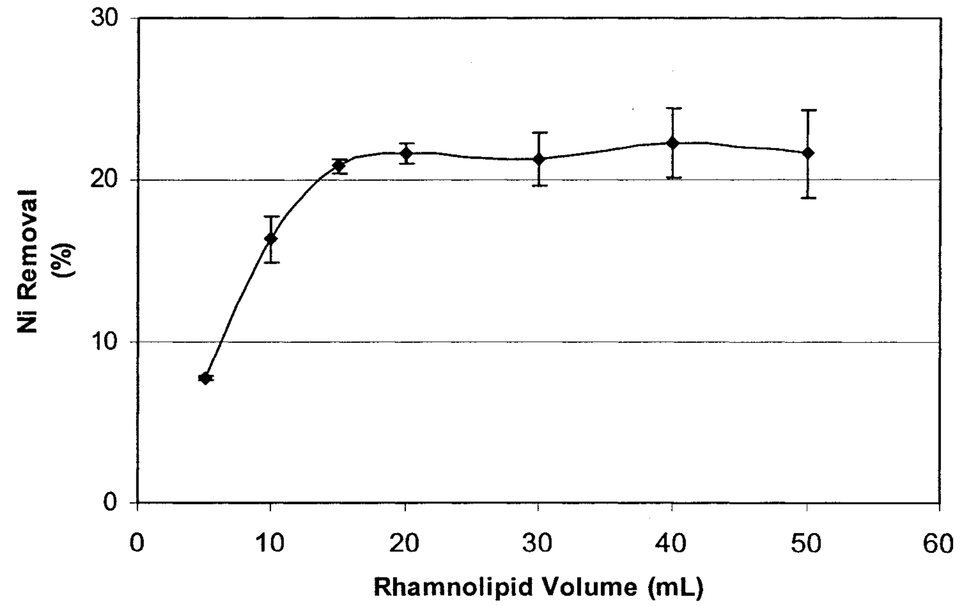


Figure 4-16 Variation of nickel removal from sediments with different ratios of sediment mass to the volume of 2% rhamnolipid solution in the batch tests with 1g of the sediment (pH 6.5).

4.3 Column tests

Another important aspect of this research was to study the performance of rhamnolipid solution flowing through the sediment for heavy metal removal. To do so, a solution of 2% rhamnolipid at pH 6.5 (except for the concentration study) with a constant flow rate of 0.5 mL/min (except for the flow rate study) continuously passed a test section holding 10 g of sediment (except in the permeability studies). Each 24 hours, the collected solution was used to refill the feeding tank. This recycling helped the method to be more economic. All samples were collected after undergoing the related tests for 3 days (72 hours) except for the time study (Figures 3-8, 3-9, and 3-10).

4.3.1 Concentration study

By performing numerous triplicate assays, the percentage of removal for each metal was measured and graphed. The sediment was washed with solutions of 0.5%, 1% and 2% rhamnolipid with and without 1% NaOH. The control was distilled water for rhamnolipid and 1% NaOH when NaOH was added to the rhamnolipid solutions. The concentration of copper, zinc, and nickel were analyzed in the supernatants. These results are shown in Figures 4-17, 4-18, and 4-19. The results of removal by distilled water were eliminated from the graphs since no removal was detected in this series of tests.

Removal rates for all of the three metals increased in a linear manner by increasing the concentration of rhamnolipid in the samples without NaOH. Using

5% rhamnolipid removed copper up to 37%, zinc up to 7.5% and nickel almost 33.2%. As an example for zinc this was equal to 363.3 mg/kg of sediment.

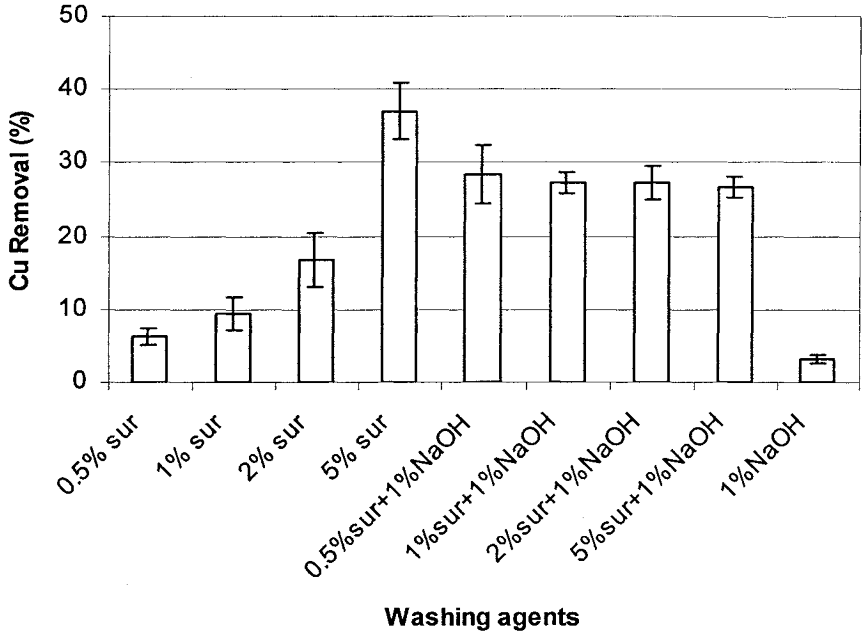


Figure 4-17 **Removal of copper from sediments by rhamnolipids with and without NaOH at different concentrations in column tests (sur is used for rhamnolipid). The flow rate was kept at 0.5 mL/min.**

Adding NaOH to the solutions of 0.5% rhamnolipid enhanced the removal of copper (up to 28.3%) and nickel (up to 11.5%) from sediments. These removals were 9, 4, and 6 times more for copper, zinc, and nickel respectively compared with the removals by 1% NaOH as the control. It is remarkable that increasing the concentration of rhamnolipid in the presence of NaOH did not affect the metal removal rates as drastically as the increasing concentration in the absence of NaOH. In other words, at a glance one can conclude that the removal of copper, zinc, and nickel with solution of 1% NaOH with 0.5%, 1%, 2%, and 5%

concentrations of rhamnolipid were almost the same.

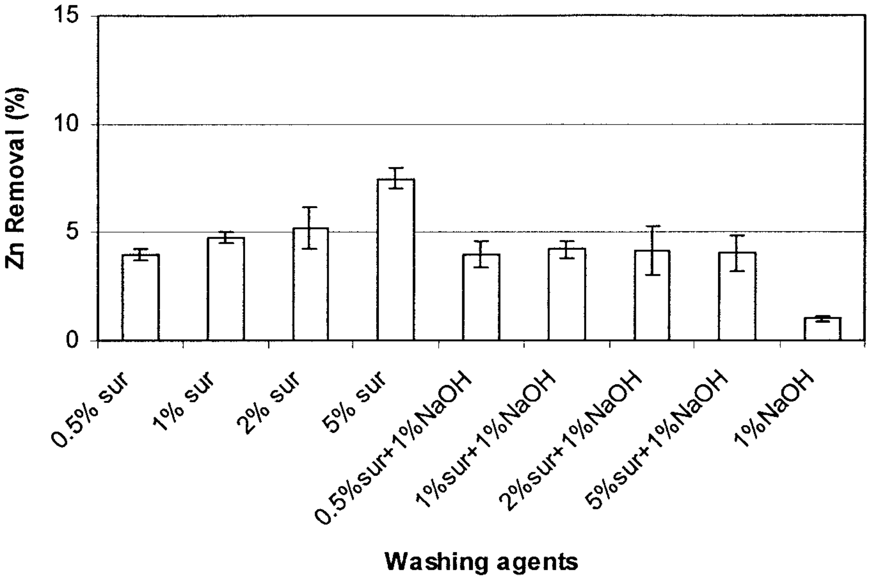


Figure 4-18 **Removal of zinc from sediments by rhamnolipids with and without NaOH at different concentrations in column tests (sur is used for rhamnolipid). The flow rate was kept at 0.5 mL/min.**

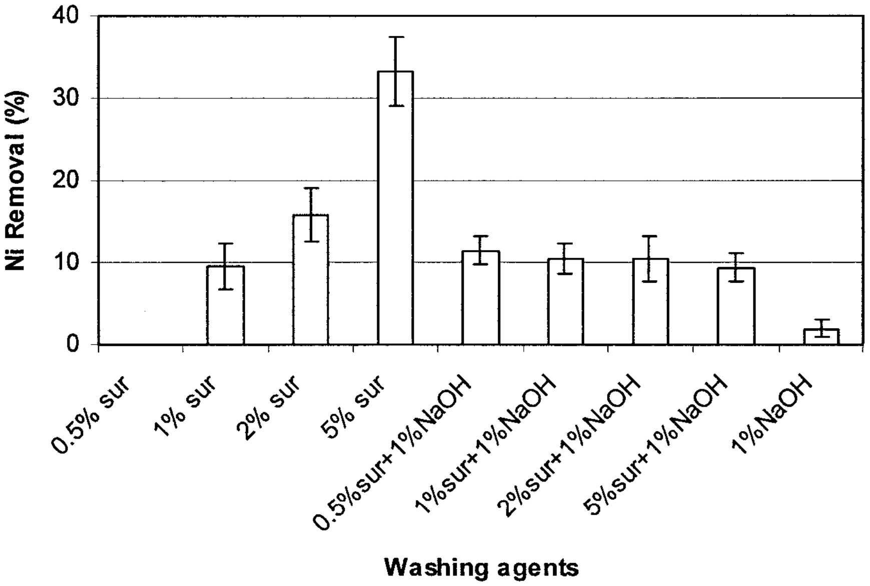


Figure 4-19 **Removal of nickel from sediments by rhamnolipids with and without NaOH at different concentrations in column tests (sur is used for rhamnolipid). The flow rate was kept at 0.5 mL/min.**

4.3.2 Investigation of the effect of additives

NaOH has been shown to be an effective additive in the removal process of metals. To investigate whether this effect comes from Na^+, OH^-, or both of them, another series of tests was performed. In these tests, the sediment was washed with solutions of:

- 0.5% rhamnolipid and 1% NaCl to study the effects of Na^+. 1% NaCl was used as the control.
- 0.5% rhamnolipid and 1% KOH to track the effects OH^- can have. 1% KOH was used as the control.
- The results of these tests are shown in Figure 4-20. The results for 0.5% rhamnolipid with and without NaOH are shown as well.

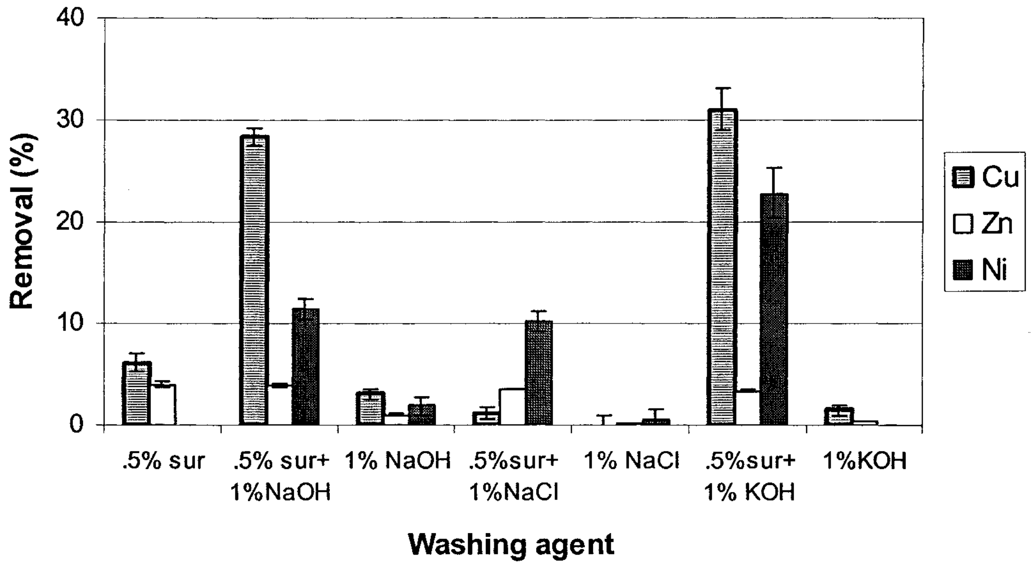


Figure 4-20 Removal of heavy metals from sediments by 0.5% rhamnolipids without and with additives in column tests at a flow rate of 0.5 mL/min (sur is used for rhamnolipid). Additives were 1% NaOH, 1% NaCl and 1% KOH which were used as controls as well.

According to these experiments, adding basic additives to low concentrations of rhamnolipid (0.5%) can improve the removal of copper and nickel in comparison with adding salt additives with the same M^+ ion. The results clearly show that the OH^- not Na^+ is responsible for improvement of copper removal. On the other hand, improvement of removal using base additives with different M^+ (M=metal) will not be the same. For example in this case, K^+ is more effective in comparison to Na^+. This result is backed up by the comments of Myers (1988) who states that the effectiveness of an additive ion is related to the radius of hydration of the added ion. The smaller radius of hydration of the ion causes a smaller CMC for the surfactant. The effectiveness of the cations are in the following order:

$$NH_4^+ > K^+ > Na^+ > La^+ > 1/2\ Ca^{2+}$$

As explained in detail in Chapter 2, the smaller CMC means a more effective surfactant, i.e., less surfactant is needed to remove the metals. In this case, it explains the cosistent removal of the metals after adding NaOH to different concentrations of rhamnolipid.

4.3.3 Time course experiments

Sediment (10 g) was placed in a column where a solution of 2% rhamnolipid with or without NaOH was passed at a rate of 0.5 mL/min. Samples from the supernatants were taken on days 1, 3, 5, 7, 9, 11, and 13. All samples were treated in the same way as the batch tests to measure the removal of the metals.

Since the results from the concentration study indicated a different behavior of heavy metal removal from sediment by rhamnolipid in the absence or presence of

1% NaOH, the effect of NaOH in the time course study was considered.

4.3.3.1 Time study using rhamnolipid without 1%NaOH

The removals of copper, zinc, and nickel by 2% rhamnolipid in a time series are shown in Figures 4-21, 4-22, and 4-23 respectively. In the case of copper, the removal in the first 3 days is progressive but after 3 days the rate of removal declined to zero (Figure 4-21). The solution of 2% rhamnolipid removed approximately 24.4 mg/kg or 17.4% of the copper. It should be noted that the removal after 24 hours was determined at 14% which is more than 80% of the highest detected removal on day 7.

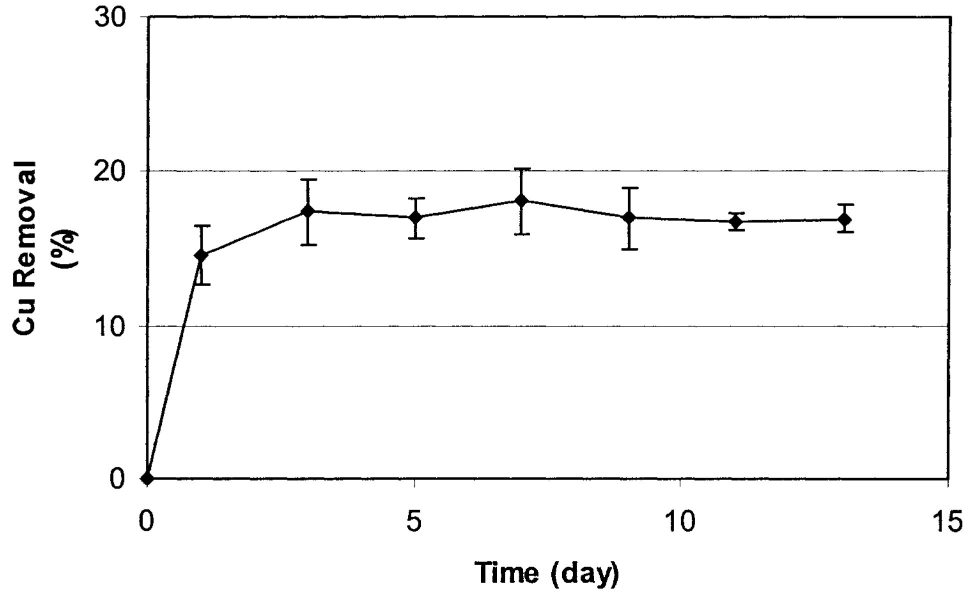


Figure 4-21 Removal of copper by a solution of 2% rhamnolipid over time. The continuous flow rate was 0.5 mL/min (pH 6.5).

The removal of zinc has a behavior different from copper. The removal of zinc was close to linear over time. The removal rate declined slightly after day 7 and on day 13 it became 0.8% per day. This indicates an almost constant rate for zinc removal. A 2% rhamnolipid solution was able to remove a total amount of 610.5 mg/kg zinc after 13 days (Figure 4-22).

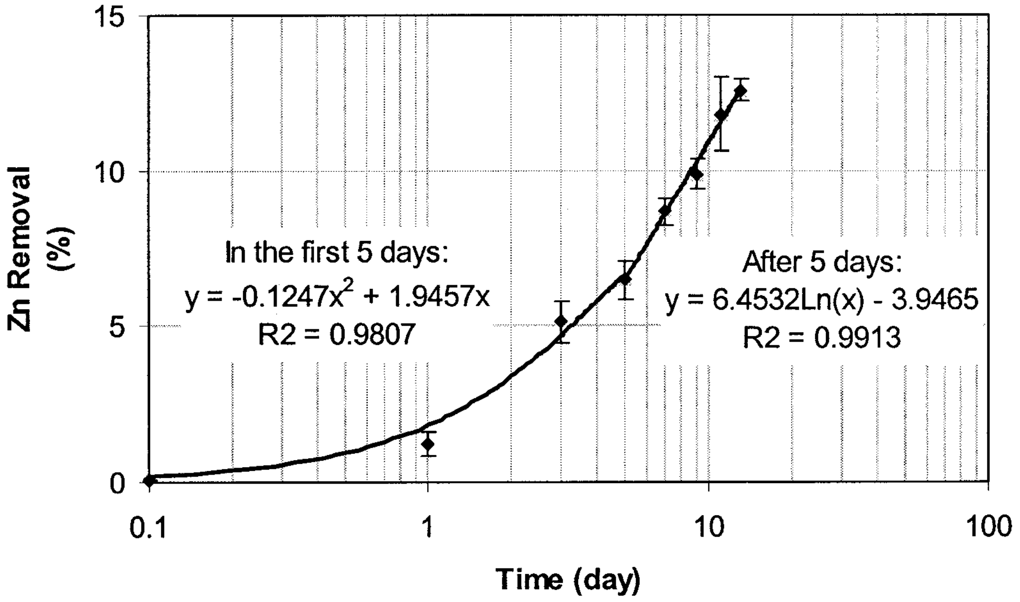


Figure 4-22 Removal of zinc by a solution of 2% rhamnolipid over time. The continuous flow rate was 0.5 mL/min (pH 6.5).

Figure 4-23 shows the nickel removal at different times. Removal of nickel through continuous flow tests showed almost the same trend as the batch test. A maximum concentration of soluble nickel in the supernatant was detected on day 9 (20.2 mg/kg or 26.8% removed) that is close to the quantity of nickel removed by the same solution in the batch test.

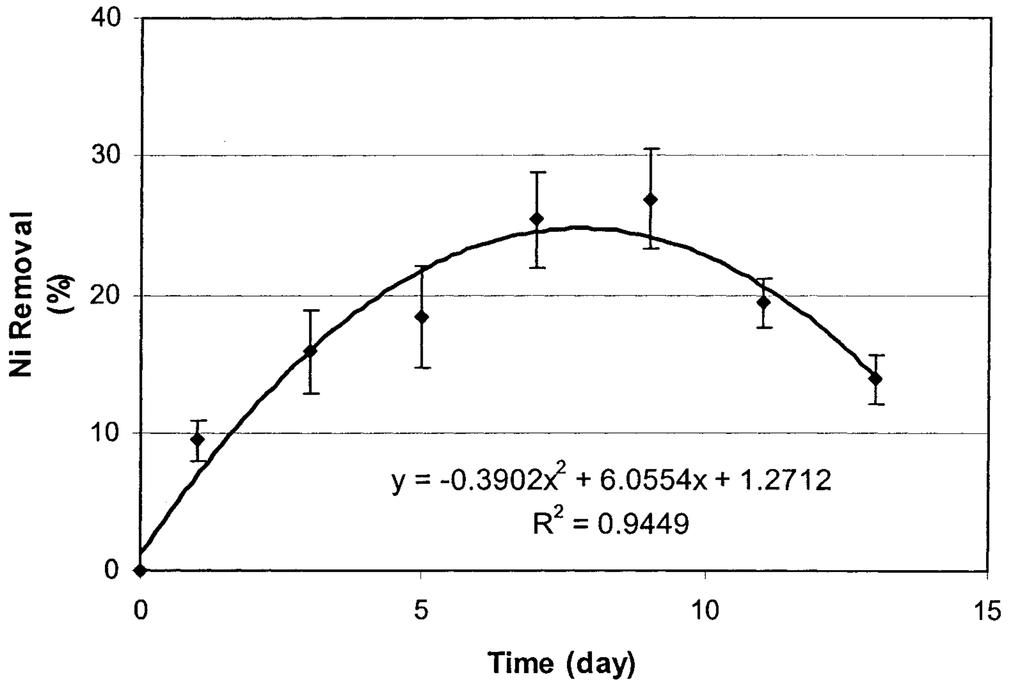


Figure 4-23 Removal of nickel by a solution of 2% rhamnolipid over time. The continuous flow rate was 0.5 mL/min (pH 6.5).

4.3.3.2 Time study using rhamnolipid with 1%NaOH

The time study was performed on the sediment samples using a mixture of 2% rhamnolipid and 1% NaOH flowing through the samples in a column. The flow rate was kept constant at 0.5 mL/min and samples from the supernatant were taken at days 1, 3, 5, 7, 9, 11, and 13. All other conditions were the same as the time study using 2% rhamnolipid without NaOH. Results of these tests are presented in Figures 4-24, 4-25 and 4-26 for removal of copper, zinc, and nickel respectively.

Looking over the results, one can realize that adding NaOH has the most significant effect on removal of copper from sediments in the continuous flow configurations. Under continuous flow of the washing agent, the 2% rhamnolipid with 1% NaOH released 26.9% of copper in the sediment after 3 days while the solution of 2% rhamnolipid alone can remove 17.4% of copper from the same sediment during the same period.

Regardless of the amount of removal, batch and continuous flow configurations with 1% NaOH show the same patterns of copper removal: a maximum during the first three days, a sharp drop in the concentration of soluble copper between days 3 and 5, a milder slope of decline between days 5 and 11, and a constant concentration after day 11. In both cases the removal by the control (1% NaOH) smoothly grows during the testing period until it reaches a constant value (Figure 4-24).

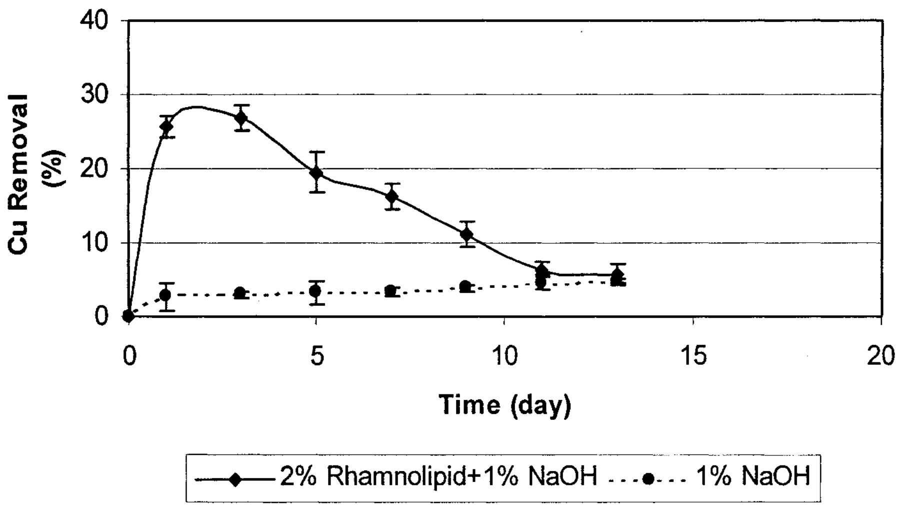


Figure 4-24 Removal of copper by a solution of 2% rhamnolipid and 1% NaOH over time with a continuous flow rate of 0.5 mL/min.

The removal of zinc with 2% rhamnolipid and 1% NaOH in a continuous flow set up has a progressive pattern for the first 11 days with a decreasing growth rate and a constant value after day 11. In comparison with the removal by the solution of 2% rhamnolipid, both washing agents release the same amount of zinc in the first seven days. The solution of 2% rhamnolipid acts more effectively after day 7 while the removal of zinc by the 2% rhamnolipid with NaOH remained at a maximum 9.3% of the total zinc in the sediments (Figure 4-25).

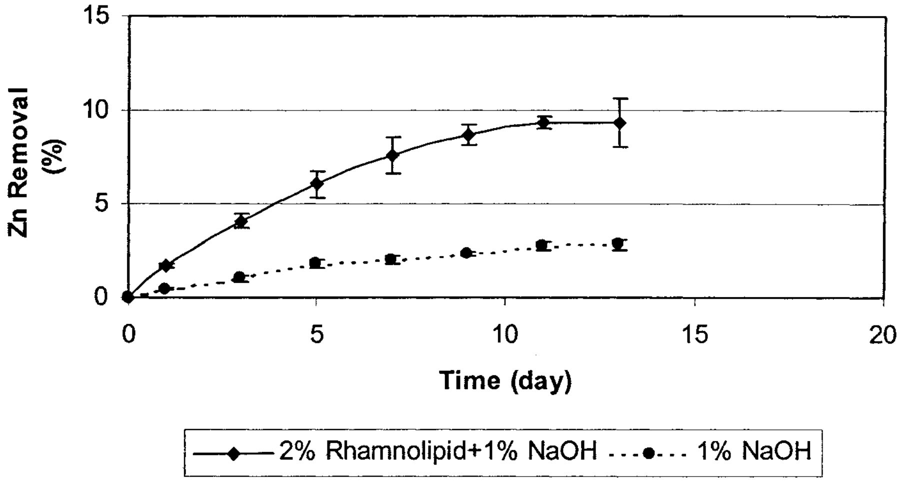


Figure 4-25 Removal of zinc by a solution of 2% rhamnolipid and 1% NaOH over time with a continuous flow rate of 0.5 mL/min.

Figure 4-26 shows the results of removal of nickel during a 13-day experiment. The removal curve reached its maximum (10.3%) in the first three days and then decreased from days 3 to 9 after which it remained constant (5.3%). The presence of NaOH in the solution of 2% rhamnolipid changes the pattern of removal in the sense of positions of maxima (10.3% on day 3 with NaOH compared with 26.8% on day 9 without NaOH) and the behavior of the removal curve in the last days of the experiments. The control solution (1% NaOH) removed the nickel continuously until the removal curve reached a maximum (approximately 5%) where it remained constant for the rest of the experiment.

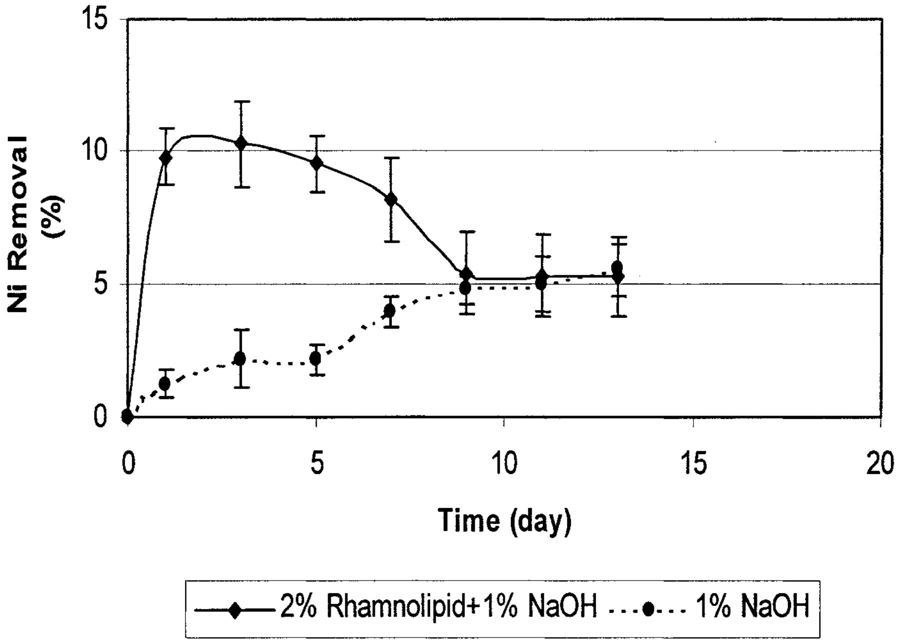


Figure 4-26 Removal of nickel by a solution of 2% rhamnolipid and 1% NaOH over time with a continuous flow rate of 0.5 mL/min.

4.3.4 Effect of flow rate on removal

This research was devoted to studying the effect of the flow rate of the washing solution, which passes continuously through the column section, on the removal of copper, zinc, and nickel from sediments. The washing agent was a solution of 2% rhamnolipid with flow rates of 0.5, 1, 5, 10, and 20 mL/min. The results of this study are presented in Figures 4-27, 4-28, and 4-29.

In all three cases, a maximum removal was followed by a declining curve that approaches a very low removal. Copper and nickel removals had absolute maximum (29.5% and 19.3% respectively) at a flow rate of 1 mL/min (Figures 4-27 and 4-29). Zinc had a relative maximum removal with 0.5 mL/min equal to 5.2% (Figure 4-28).

To explain this behavior, it should be noted that when a chemical reaction occurs some bonds are broken enabling new bonds to form. This process needs time and energy. In this experiment energy exchange was not studied but the change in the flow rate consequently causes a change in contact time between washing solution and sediment particles containing the heavy metals. The contact time was determined as 8.6, 4.3, 0.9, 0.4, and 0.2 minutes for flow rates of 0.5, 1, 5, 10, and 20 mL/min respectively. This explains why the curves decline by increasing the flow rate. Increasing the flow rate beyond a certain value, decreased the contact time and consequently decreased the removal.

At the same time, one important point must be mentioned to interpret the increase in removal from a very low flow rate to the maximum. Rhamnolipid should leave the contact area after the production of the new bonds to free the space for non-bonded rhamnolipid in the solution. Thus, increasing the flow rate in some domain also can enhance the rate of removal in the case of copper and nickel where flow rates changed from 0.5 to 1 mL/min. The same pattern is expected for zinc for flow rates less than 0.5 mL/min. These flow rates could not be applied to the system due to technical difficulties.

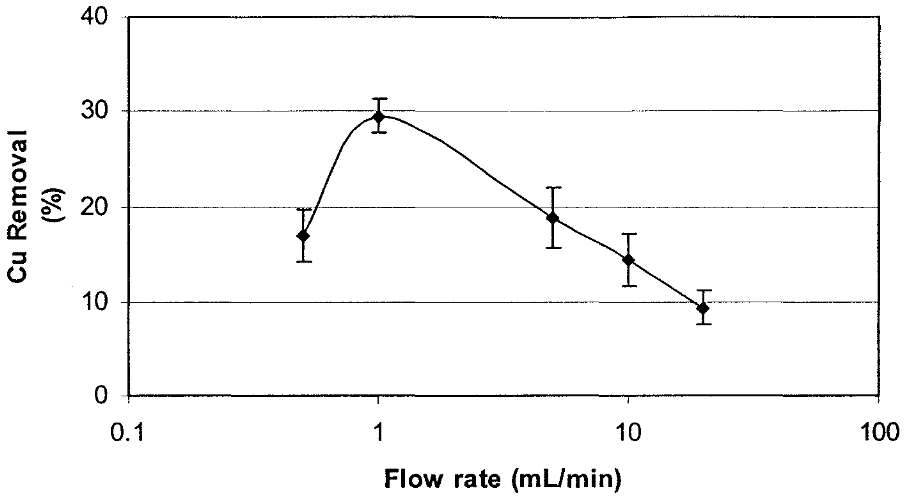


Figure 4-27 Removal of copper under various continuous flow rates in the column tests with a washing agent of 2% rhamnolipid (pH 6.5).

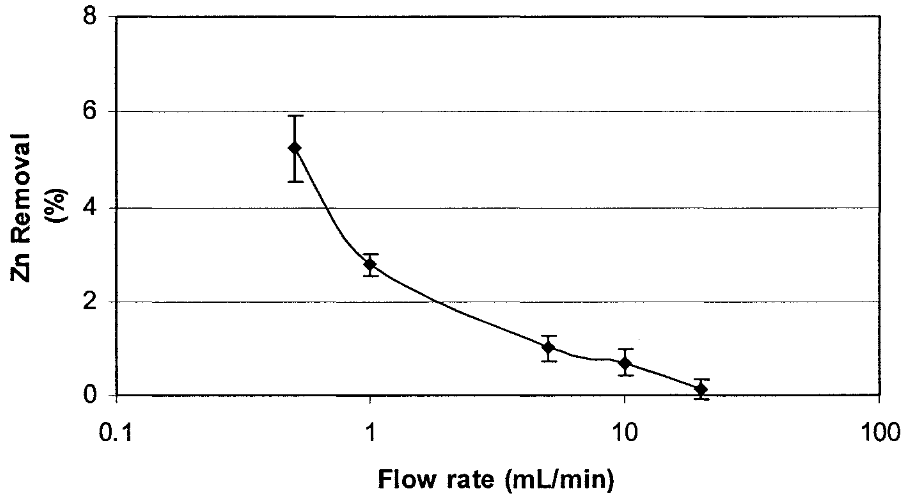


Figure 4-28 Removal of zinc under various continuous flow rates in the column tests with a washing agent of 2% rhamnolipid (pH 6.5).

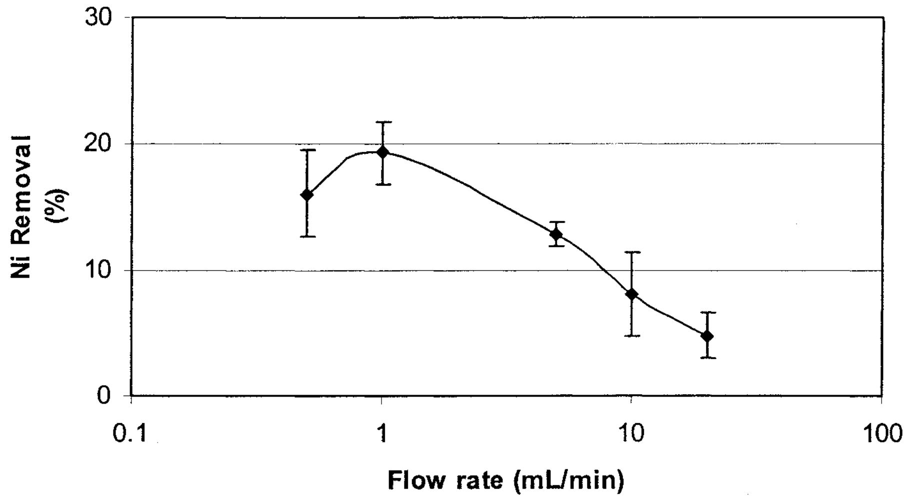


Figure 4-29 Removal of nickel under various continuous flow rates in the column tests with a washing agent of 2% rhamnolipid (pH 6.5).

4.3.5 Porosity and removal

Porosity was varied in this set of tests and the removals were determined. Porosity was determined using the standard relation of $n = 1 - \frac{\rho}{\rho_s}$ where n is the porosity, ρ and ρ_S are bulk density and real density of soil, respectively. The variation of porosity was obtained by changing the mass of sediment in a constant volume of test column. The ratio of the mass of sediment to the volume of the biosurfactant remained constant in all tests. The removal of the metals was measured for porosities of 0.42, 0.52, 0.64, and 0.76. The results are shown in Figures 4-30, 4-31, and 4-32 for copper, zinc, and nickel, respectively.

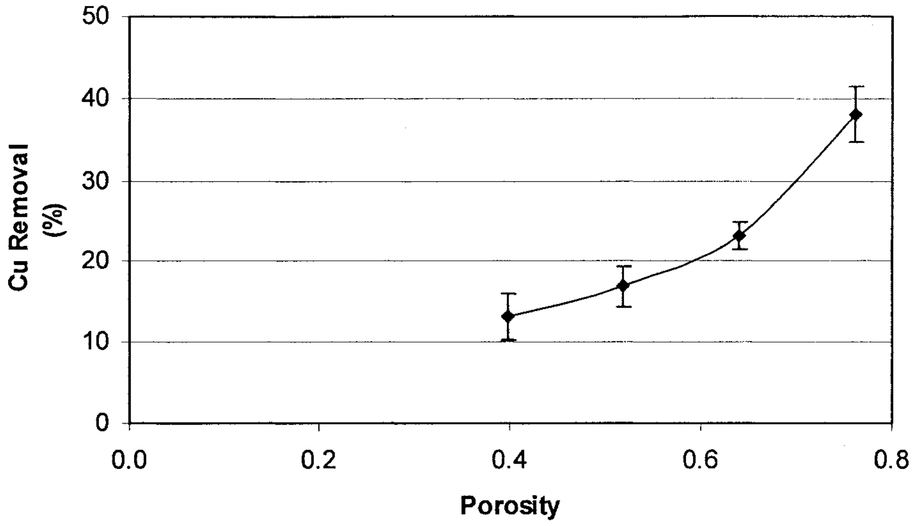


Figure 4-30 Variation of copper removal from sediments at various porosities. The washing agent was 2% rhamnolipid and a flow rate of 0.5 mL/min (pH 6.5).

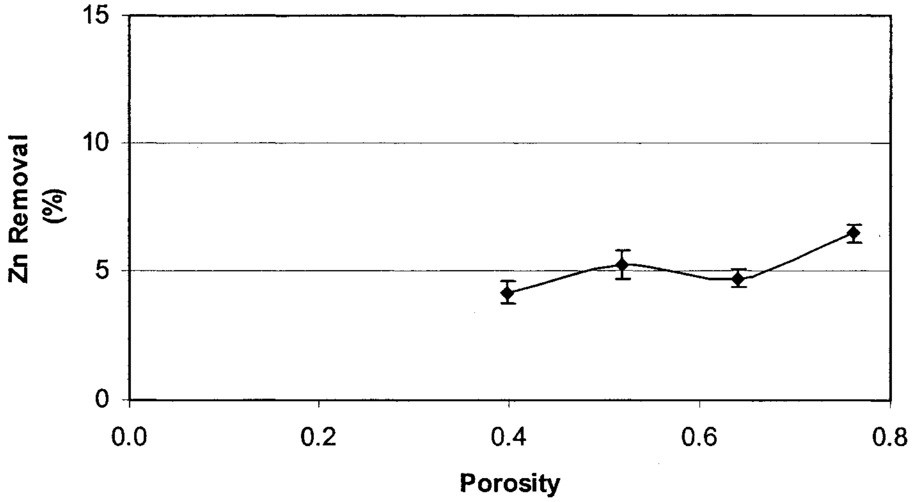


Figure 4-31 Variation of zinc removal from sediments at various porosities. The washing agent was 2% rhamnolipid and a flow rate of 0.5 mL/min (pH 6.5).

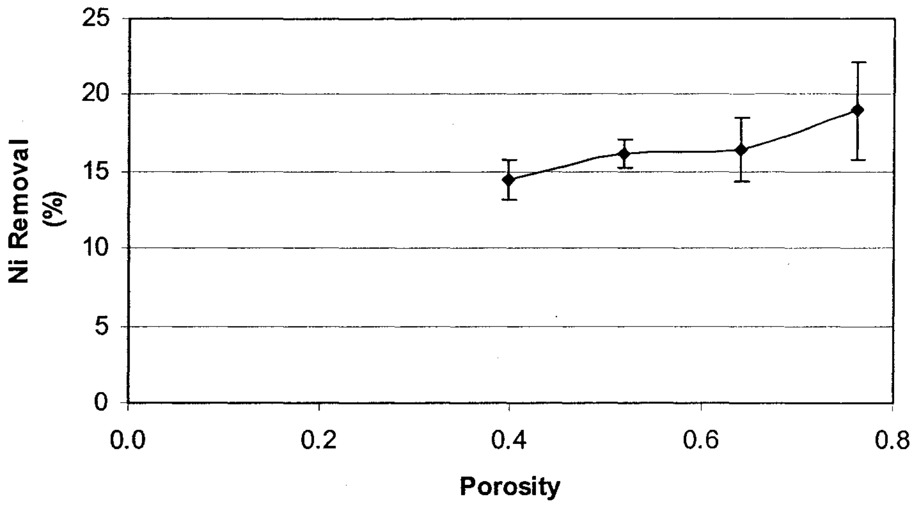


Figure 4-32 Variation of nickel removal from sediments at various porosities. The washing agent was 2% rhamnolipid and a flow rate of 0.5 mL/min (pH 6.5).

For all three metals, the removals decreased as the compaction of sediment samples increased, i.e as porosities became lower. This can be interpreted as the decline in the porosity, or augmentation in compaction, decreases the wetted contact surface area between sediment particles and rhamnolipid solution, which is the first step in the removal of the metals from the sediment.

Copper removal is sensitive to the change in porosity and the percentage of copper removal is non-linearly proportional to porosity. For example, the removal at the porosity of 0.76 is 38.1% or 2.25 times more than the removal at a porosity of 0.52 (17.0%). The latter is the porosity that was used in the rest of the experiments. In another words for the range used in this study, an incremental of

1.5 times in porosity led to 2.25 times more removal.

Zinc shows a different behavior than copper and the removal has a milder decline after a steep slope in the removal curve when porosity decreased. The change in the removal was much less compared to copper. This may be caused by the higher concentration of zinc in the sediment that subsequently provided a higher availability of the metal for possible removal by the rhamnolipid.

The curve of nickel removal by porosity showed a pattern similar to the one of copper and zinc but the variation was much more limited compared to copper removal (from 19% for a porosity of 0.76 to 16.1% for a porosity of 0.52).

4.3.6 Effect of hydraulic conductivity in removal of metals

The hydraulic conductivity for the samples used in the porosity study were determined. The variation of metal removal versus hydraulic conductivity for each metal was determined and shown in Figure 4-33, 4-34, and 4-35. For any particular type of sediment, the hydraulic conductivity varies proportionally to the porosity. Therefore, increasing the porosity increased the hydraulic conductivity for the same mass of the sediment. In other words, the similarity in the patterns of removals of the heavy metals from sediments versus porosity and hydraulic conductivity were expected as seen in Figures 4-33 through 4-35.

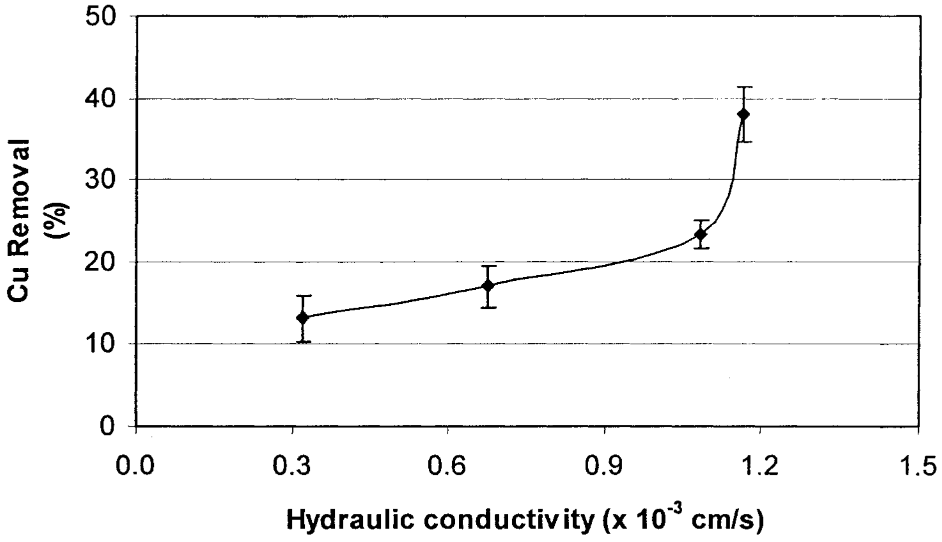


Figure 4-33 Variation of copper removal from sediments with hydraulic conductivity using a washing agent of 2% rhamnolipid with a flow rate of 0.5 mL/min (pH 6.5).

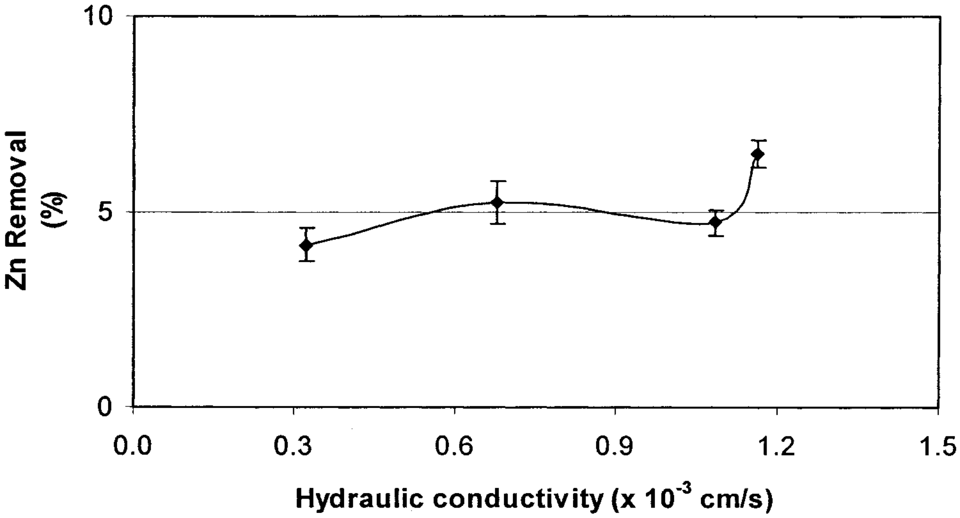


Figure 4-34 Variation of zinc removal from sediments with hydraulic conductivity using a washing agent of 2% rhamnolipid with a flow rate of 0.5 mL/min (pH 6.5).

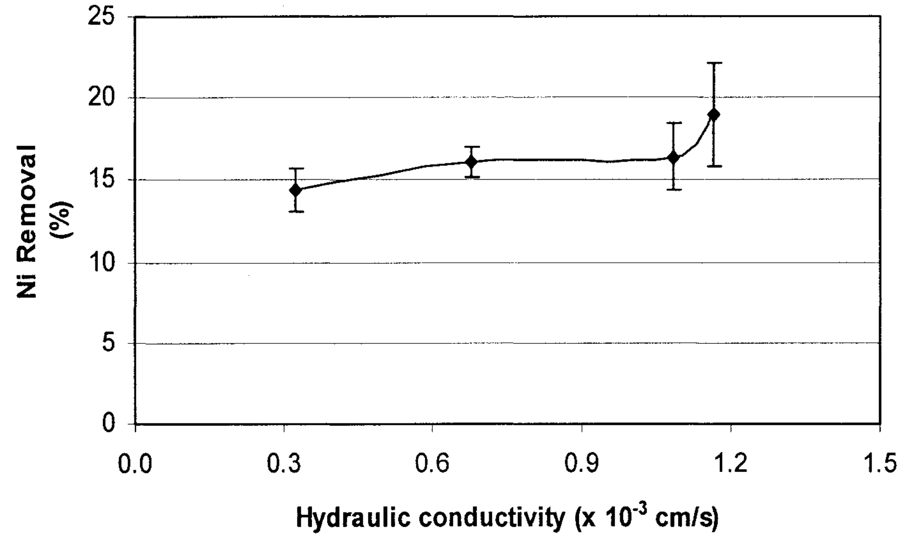


Figure 4-35 Variation of nickel removal from sediments with hydraulic conductivity using a washing agent of 2% rhamnolipid with a flow rate of 0.5 mL/min (pH 6.5).

4.4 Discussion of results

In this research the importance of the configuration (batch and continuous flow) and of a wide range of parameters on the same sediment were studied. There is some available literature on using rhamnolipid for washing of heavy metals from soil in batch tests, but using continuous flow of rhamnolipid solution to wash sediment has not been investigated (Tan et al. 1994, Herman et al. 1995, Ochoa-Loza 1998, Mulligan et al. 1999, Ochoa-Loza et al. 2001, and Mulligan et al. 2001). The advantages of a comprehensive study of various parameters that affect the removal of the heavy metal in different configurations enables one to determine the degree of importance of each parameter or configuration for engineering applications in *in situ* soil washing techniques.

After performing several experiments, it was shown that the washing of sediments needs washing agents more effective than water. Among a wide variety of choices, the rhamnolipid was chosen to be studied since this biological product has shown its capabilities for removal of heavy metals from soil. There are other studies that support this idea. For example Miller (1995) reported that 92% of Cd^{2+} in a 0.5 mM solution of $Cd(NO_3)_2$ was complexed by a 5 mM solution of rhamnolipid. Dahrazma and Mulligan (2004) have employed 2% rhamnolipid for the extraction of 28% of copper from a mining residue. Mulligan et al. (2001) indicated that a 0.5% rhamnolipid solution is able to remove up to 18% of zinc from sediments in a batch test. A summary of the results of the present study on the removal of heavy metals from the sediments by rhamnolipid are presented in Tables 4.1 and 4.2 for batch and continuous flow configurations respectively.

Table 4.1 Summary of results for metal removal from the sediments by rhamnolipid in batch configurations.

Metal	Parameter			
	Concentration (%)	Volume of 2% rhamnolipid to1 g sediment (mL)	pH	Time (day)
Cu	5 *(R=12.9)*	20 *(R= 16)*	5.5 *(R=15)*	3 *(R= 5.5)*
Zn	5 *(R=10.7)*	20 *(R=12.5)*	5.5 *(R=9)*	16 *(R=10.7)*
Ni	5 *(R=34)*	40 *(R=23)*	5.5 *(R=23)*	8 *(R=31.6)*

R= Removal (%)

Table 4.2 Summary of results for metal removal from the sediments by rhamnolipid in continuous flow configuration.

Metal	Parameter				
	Concentration (%)	Flow rate (mL/min)	Porosity	Time (day)	Hydraulic Conductivity (cm/s)
Cu	5 *(R=37)*	1 *(R=29.5)*	0.75 *(R=38.1)*	3 *(R=17.4)*	1.2 x 10^{-3} *(R=38)*
Zn	5 *(R=7.5)*	0.5 *(R=5.2)*	0.75 *(R=7)*	13 *(R=12..6)*	1.2 x 10^{-3} *(R=7)*
Ni	5 *(R=33.2)*	1 *(R=19.3)*	0.75 *(R=19)*	9 *(R=26.8)*	1.2 x 10^{-3} *(R=19)*

R= Removal (%)

In both configurations, batch and continuous flow, the removal of the heavy metals (Cu, Zn, Ni) improves as the concentration of rhamnolipid increases. These results were expected since at higher concentrations, more molecules of rhamnolipid are available to participate in the washing process. The same trend also was reported by Herman et al. (1995) for Pb^{2+} and Cd^{2+}. 2.2% Cd^{2+} was removed from soil with 12.5 mM rhamnolipid while 55.9% Cd^{2+} was removed with 80 mM rhamnolipid.

The addition of 1% NaOH changes the behavior of the washing. Any combination of rhamnolipid and NaOH in the range of this study showed the same degree of removal for all three metals (Cu, Zn, Ni). It can be concluded that adding NaOH to the rhamnolipid solutions improves the copper removal significantly while the most deficit in removal is seen for nickel when 2% rhamnolipid and 1% NaOH was used (Figure 4-19). Results from the experiments show that OH^- not Na^+ is responsible for improvement of copper removal (Figure 4-20). Removal of nickel in batch tests was more sensitive to NaOH than in continuous flow. Further discussion on the effects of NaOH on the metal removal is presented in the next chapters.

The pH was shown to be an important parameter in heavy metal removal. Solutions of different pH with the same conditions were used to wash the sediment. In extreme cases, the maximum removal of nickel was six times that of the minimum removal. This variation of pH includes only the initial pH at the beginning of each test. Variation of pH over time for a particular rhamnolipid solution also was studied. The aspect will be presented in detail in the study of mechanism of removal in this thesis (Section 7-4).

Although the metals responded to the changes in pH and the concentration of rhamnolipid in the same manner, they responded differently over time. Copper rapidly reached its maximum removal and then the concentration of copper in solution remained constant over time, while the concentration of zinc increased in solution over time. Nickel showed different behavior in comparison with copper and zinc. The removal rate reached a maximum point and then the concentration of nickel in the supernatant dropped over time. This behavior of removal for the metals over time can result from the differences between affinities of rhamnolipid to bond with different metals. Ochoa-Loza et al. (2001) showed that the affinity of the metals (Cu, Zn, Ni) to bond with rhamnolipid has the following order:

$$Al^{3+}>Cu^{2+}>Pb^{2+}>Cd^{2+}>Zn^{2+}>Fe^{3+}>Hg^{2+}>Ca^{2+}>Co^{2+}>Ni^{2+}>Mn^{2+}>Mg^{2+}>K^{+}$$

They suggested that the Mg^{2+} and K^{+} ions, which are the common soil and water cations, do not enter into competition with contaminant metals for rhamnolipid complexation sites. Based on this information, rhamnolipid in contact with the sediment prefers removing copper from the sediment rather than the two other metals. This explains the rapid increment of copper concentration in the supernatant until the available copper for rhamnolipid is completely removed. When no more copper is available for rhamnolipid, zinc finds more space to make bonds with rhamnolipid. This can be a reason for the increasing removal of zinc in the next few days. Nickel concentration in the supernatant drops after a certain period of time. Readsorption is suspected to be responsible for this behavior since the drop occurs more rapidly in batch rather than continuous flow test. This interpretation is in agreement with Kurosaki et al. (2002) who explained that readsorption is a known limitation of the batch method.

The time study of metal removal with rhamnolipid and NaOH showed that NaOH not only had a short time effect on the removal of the metal as stated earlier in the study of effect of concentration, but also in the case of copper removal, it had a significant effect if the tests continued over time. In another words, over a short time, adding NaOH improves the removal of copper while in the long term the concentration of copper in the supernatant decreases in the presence of NaOH.

This can be a result of the effects of the change in pH over time. This matter will be studied in Chapter 7 by monitoring pH during the experiment. Cameron (1992) stated that zinc and cadmium have more mobility in pH less than 7.0. Adding NaOH increases the pH that causes the reduction of the mobility and consequently dicrease of removal.

As expected, if more volume of rhamnolipid is available for a certain mass of sediment, removal of all three metals will increase. This increment has a limit since a certain amount of metals is available for removal under that specific condition (Figures 4-14 to 4-16). These results can explain that one of the reasons for less metal removal in batch tests in comparison with continuous flow tests where the ratio of one gram of sediment to 10 mL of rhamnolipid solution that was used for study in the batch configuration.

Flow rate has been shown to be an effective parameter in the removal of the metals. A brief explanation for the pattern of removal of the metals using different flow rates is given in section 4-3. Further comments can be made to support the idea of using a continuous flow for heavy metal removal with rhamnolipid from sediment. The available data from this research showed that in all cases removal in continuous flow configuration is higher than in the proportional test in batch configuration. Variation of flow rate changes the contact time between rhamnolipid and the sediment. Increasing the contact time from 0.2 minutes (flow rate of 20 mL/min) to 4.3 minutes (flow rate of 1 mL/min) drastically increased the removal of the metals, 3 times for copper, 10 times for zinc, and 4 times for nickel. Shin and Kim (2004) had observed the same pattern and reported that the efficiency of removal of phenanthrene and diesel in sand decreases as the flow rate of rhamnolipid increases.

When rhamnolipid bonds with metal on the surface of the sediment, it should be returned to the bulk solution to provide free space for further reactions between unbonded rhamnolipid with the surface of the sediment. This mechanical act needs energy that can be provided by the kinetic energy of the surrounding solution. Obviously, providing this kinetic energy in continuous flow configuration is much easier and more economic than in a batch configuration that needs agitation.

Another reason for more metal removal in continuous flow configuration in comparison with a batch set up is the limitation of the possibility of readsorption of the metals to the sediment. This idea is supported with the results in Figure 4-23. In a continuous flow configuration, flowing a solution containing rhamnolipid through the sediment changes the balance between adsorption and readsorption of the heavy metals in any moment in favor of adsorption (removal) since the incoming flow contains less metal concentration than the outgoing flow. In batch configuration the removed heavy metals always remain in the vicinity of the sediment particles. Kurosaki et al. (2002) stated that one of the known limitations of the batch method is the possibility of readsorption of contaminant to the soil and sediment.

As the physical and chemical properties of washing agents have shown some degrees of importance in heavy metal removal, the characteristics of sediments can play an important role in that phenomenon too. Investigations of the effects of some chemical characteristics of sediment such as distribution of the heavy metals in each fraction of soil are presented in the next chapters. Among the physical characteristics of sediment, porosity and hydraulic conductivity were chosen since these parameters are important in the flow of solution in porous media. A detailed explanation of the effects of these two parameters in metal removal was given in section 4-3. In all cases, larger hydraulic conductivity and porosity resulted in better removal of metal from the sediment. Anderson (1993) stated that if hydraulic conductivity is low, *in situ* remediation cannot be employed since the washing agents cannot make contact with all soil particles, seepage lines form and only a part of soil is exposed to the washing agent.

Chapter 5

Selective Sequential Extraction (SSE)

5.1 General remarks

The procedure used for the sequential extraction was similar to that of Yong et al. (1993) shown in Chapter 3. Sediment samples were washed with the surfactant solutions and controls under various configurations and conditions. Subsequently, samples were dried prior to sequential extraction. Each of the fractions was collected and the concentrations of heavy metals were determined by atomic absorption spectrometry. The amounts of copper, zinc, and nickel extracted from each of the extractants then were calculated.

5.2 SSE of the sediment before washing

Sequential extraction procedures were used on the sediments to determine the speciation of the heavy metals (Cu, Zn, and Ni) before surfactant washing. The exchangeable fractions of all metals were small. The carbonate and oxide fractions accounted for over 60% of the zinc present in the sediments (Table 5.1). The organic fraction constituted over 80% of the copper. A major proportion of nickel was found in the residual fraction. Similar to the results of the present study, Chartier et al. (2001) reported that in their sediment 65% of copper was found in organic matter and 50% of nickel was in the residual fraction. Jenne (1968) stated that the fraction of soil in which zinc exists is dependent to the Fe and Mn oxide.

Table 5.1 Sequential extraction characterization of metal contaminants in sediment before washing with the biosurfactant (Mulligan and Dahrazma 2003).

Metal	Fraction (% of total)				
	Exchangeable	Carbonate	Oxide	Organic	Residual
Copper	1	1	4	86	12
Nickel	0	9	23	29	39
Zinc	4	18	46	22	10

5.3 SSE of the sediment after washing with different agents in batch configuration

Sequential extraction of the sediments was performed after washing with the biosurfactant. The results of these experiments for copper, zinc and nickel are shown in Figures 5-1, 5-2 and 5-3 respectively. The rhamnolipid could remove the organically bound copper when NaOH was added and the oxide fraction when no NaOH was used since the pH was 6.5 and more appropriate for copper removal associated with the oxide fraction. For zinc, there was a major decrease in the carbonate fraction when no NaOH was added with the biosurfactant, since this was removed more easily than the oxide fraction. In the presence of NaOH and the biosurfactant, there was removal from the organic and exchangeable fractions of zinc. Almost all of the zinc that exists in the exchangeable fraction was removed by adding NaOH to the rhamnolipid solution. For nickel, the oxide and carbonate fractions decreased as the biosurfactant concentration increased. There were also significant reductions in the organic fraction, upon addition of NaOH with 0.5 and 1.0% surfactant.

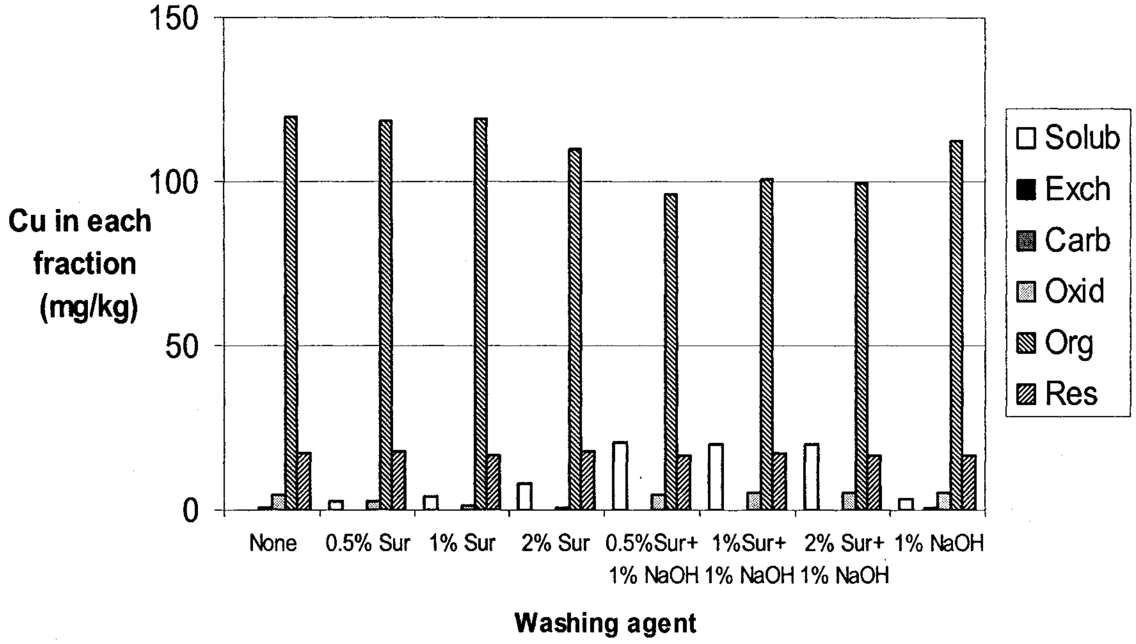


Figure 5-1 **Sequential extraction of copper after washing sediments with each agent, 0.5%, 1% and 2% rhamnolipid, with and without 1% NaOH and 1% NaOH in batch configuration. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

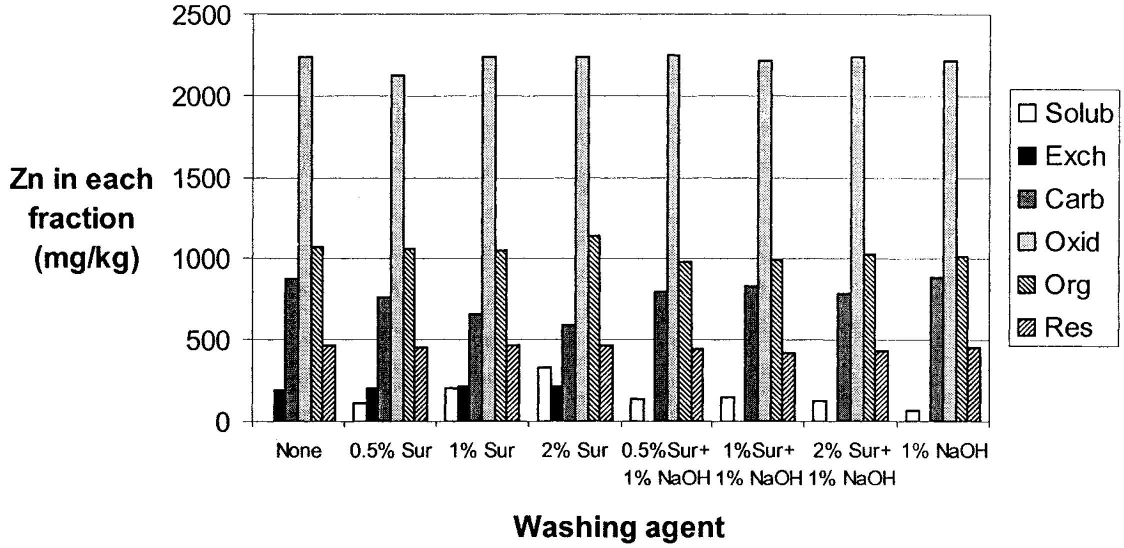


Figure 5-2 Sequential extraction of zinc after washing sediments with each agent, 0.5%, 1% and 2% rhamnolipid (Sur), with and without 1% NaOH and 1% NaOH in batch configuration. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.

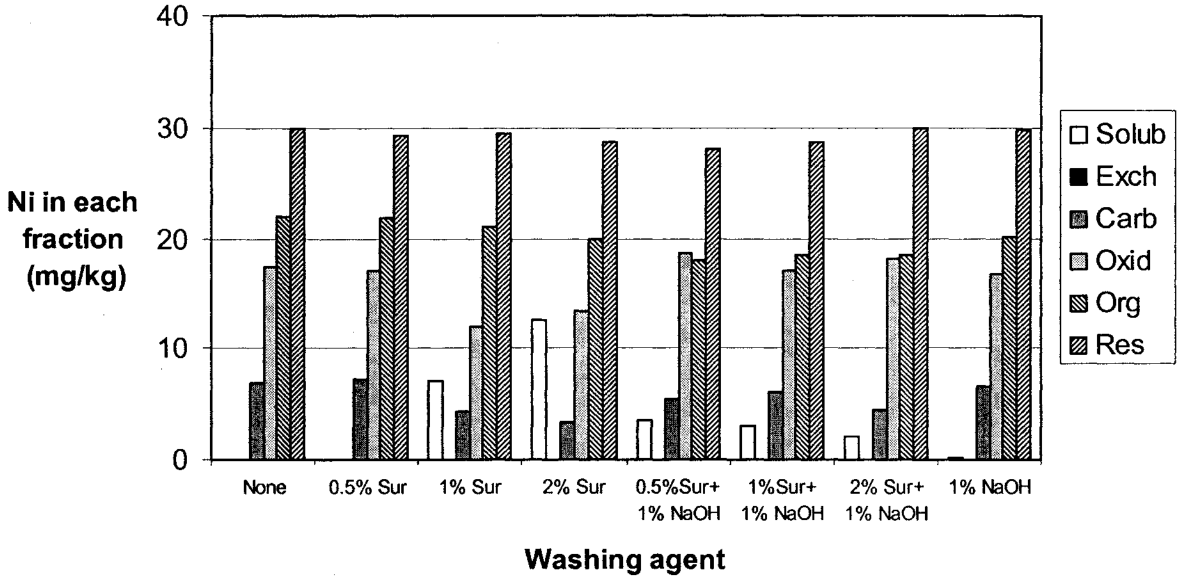


Figure 5-3 Sequential extraction of nickel after washing sediments with each agent, 0.5%, 1% and 2% rhamnolipid (Sur), with and without 1% NaOH and 1% NaOH in batch configuration. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.

5.4 SSE of the sediment after washing with different agents in continuous flow configuration

Sequential extraction of the sediments was performed after washing with the biosurfactant in a column configuration. The flow rate was kept at 0.5 mL/min for 3 days. The results of these experiments for copper, zinc and nickel are shown in Figures 5-4, 5-5 and 5-6, respectively.

Rhamnolipid, like in the batch configuration, removes copper from the organic fraction when NaOH was added while with no NaOH in the solution, the oxide fraction is the most affected fraction in copper removal. Comparing the SSE results for copper washed by 1% NaOH with the other solutions of rhamnolipid and NaOH, indicates the proficiency of NaOH in association with the rhamnolipid for solubilizing the organic bonded copper in the sediment since the removal of copper from the organic fraction by 1% NaOH is not as significant as by the others (Figure 5-4).

For zinc, a major decrease in the carbonate fraction for the solution with no NaOH was detected. A smaller decrease of zinc in the exchangeable fraction of sediment also was observed. Although in the presence of NaOH and the biosurfactant, there was removal from the organic and oxide fractions of zinc, the most influenced fraction was the exchangeable fraction where the zinc was almost completely removed. This shows that for this sediment, the ion exchange mechanism can play a major role of removal for zinc in the presence of NaOH

(Figure 5-5).

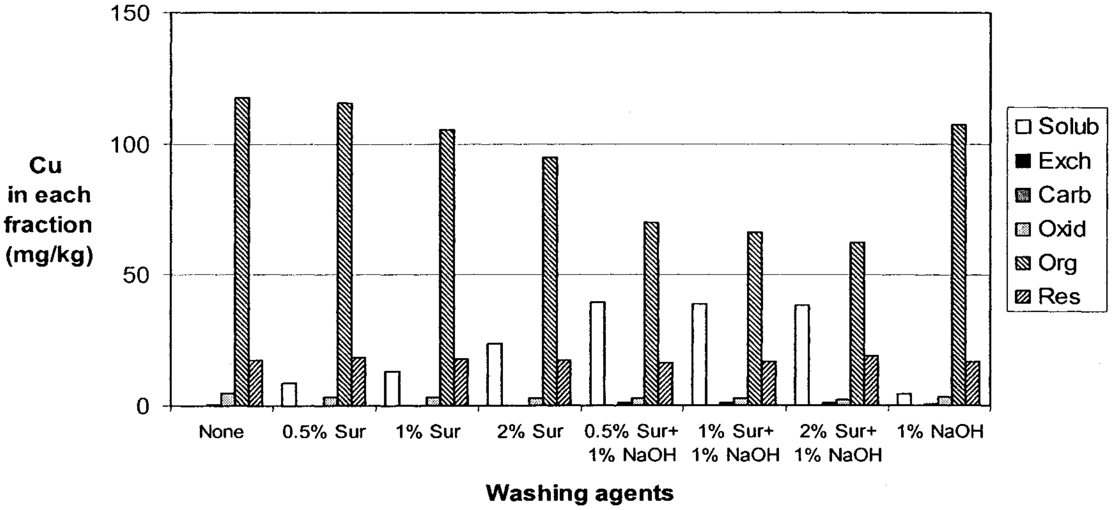


Figure 5-4 **Sequential extraction of copper after washing sediments with each agent, 0.5%, 1% and 2% rhamnolipid, with and without 1% NaOH and 1% NaOH in a continuous flow configuration of 0.5 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

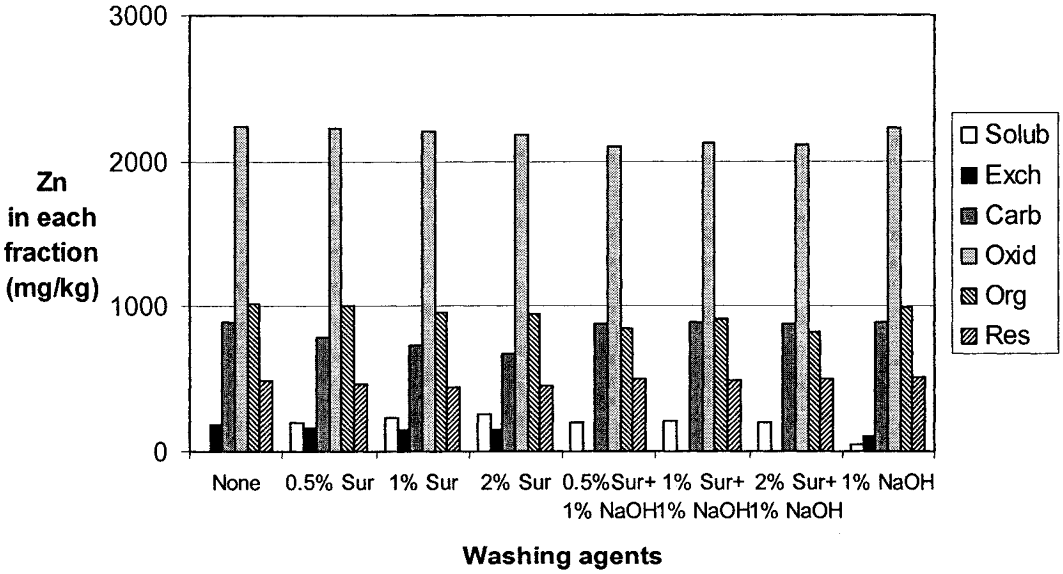


Figure 5-5 **Sequential extraction of zinc after washing sediments with Each Agent, 0.5%, 1% and 2% rhamnolipid (Surf), with and without 1% NaOH and 1% NaOH in a continuous flow configuration of 0.5 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

In the case of nickel, the reduction of the metal in the oxide and carbonate fractions is directly proportional to the concentration of the biosurfactant (Figure 5-6). There also were significant reductions in the organic fraction, upon addition of 1% NaOH to 0.5% surfactant solution. The same result was obtained from the SSE tests for the two other metals in presence of 1% NaOH.

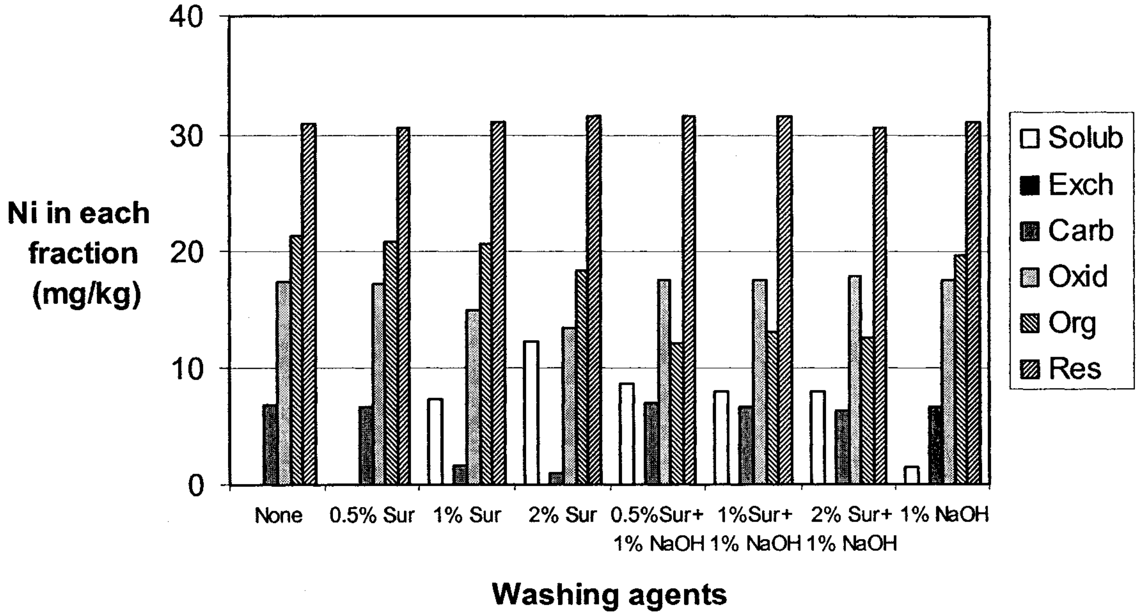


Figure 5-6 **Sequential extraction of nickel after washing sediments with each agent, 0.5%, 1% and 2% rhamnolipid (Surf), with and without 1% NaOH and 1% NaOH in a continuous flow configuration of 0.5 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

5.5 SSE of the sediment after washing by different flow rates in continuous flow configuration

Since the flow rate was shown to be an effective parameter in removal, the SSE test was performed to investigate the sensitivity of each fraction to the change of this parameter. Flow rates of 0.5, 1, 5, 10, and 20 mL/min were applied to the system for 3 days to measure the soluble fraction of sediment while the total volume of the solution, 2% rhamnolipid, was kept constant. The results are shown in Figures 5-7, 5-8, and 5-9 for copper, zinc and nickel, respectively.

The flow rate affects the quantity of copper removal as explained in Chapter 4. The SSE tests confirmed those results and also show that carbonate, oxide, and organic fractions of the sediment are the most affected as a function of the flow rate in copper removal. Rhamnolipid solutions at low flow rates (0.5, 1, and 5 mL/min) removed almost all copper that exists in the carbonate fraction while at higher flow rates the rate of copper removal from carbonate fraction decreased. By increasing the flow rate up to 1 mL/min, copper removal from organic and oxide fractions increased. Increasing the flow rate causes a decrease in the removal of copper in these two fractions (Figure 5-7).

In the case of zinc, removal declined as the flow rate increased (Figure 4-28). Confirming the results of the removal tests, the SSE tests showed that the removal from the carbonate, organic and exchangeable fractions was affected by the change in flow rate (Figure 5-8 and Table 5.3). Reaction time is suspected to be responsible for these variations.

Figure 5-9 indicates that the flow rate changed the removal of nickel from the carbonate fraction significantly while the change of concentration of nickel in the organic and oxide fractions also was significant.

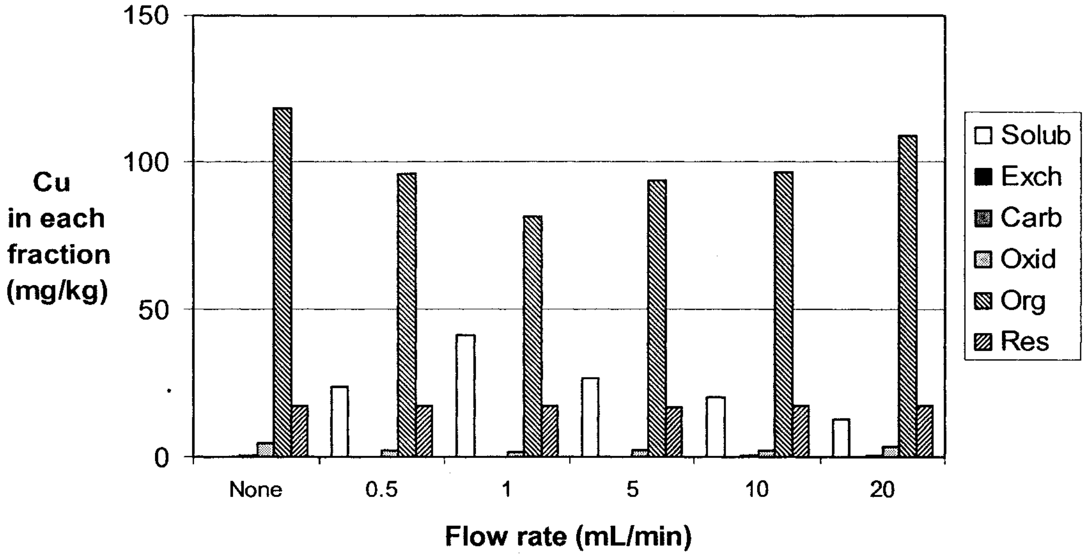


Figure 5-7 Sequential extraction of copper after washing sediments with different flow rates of 2% rhamnolipid in a continuous flow configuration. The flow rates were 0.5, 1, 5, 10, and 20 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.

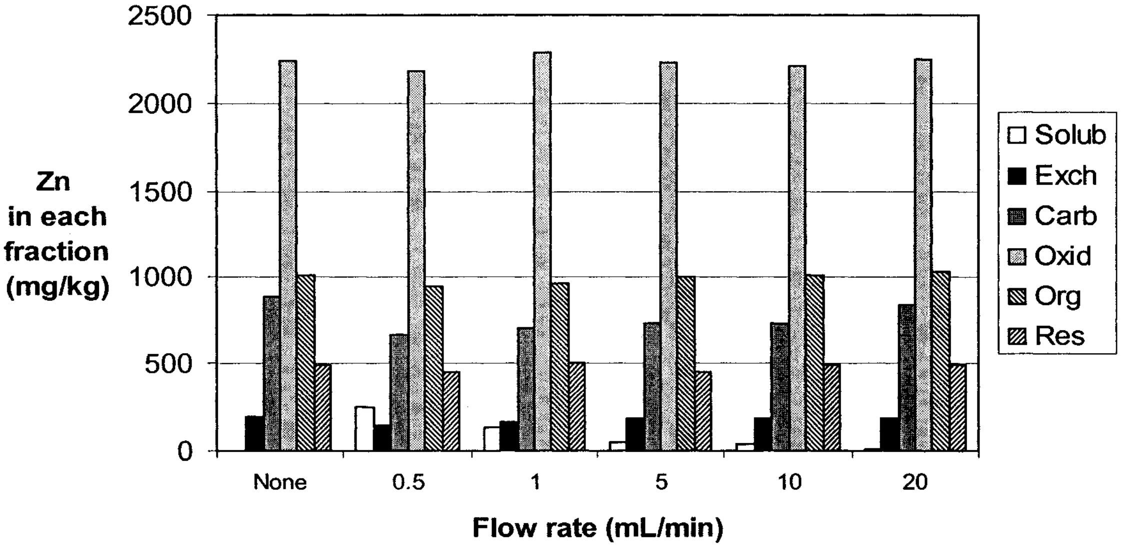


Figure 5-8 Sequential extraction of zinc after washing sediments with different flow rates of 2% rhamnolipid in a continuous flow configuration. The flow rates were 0.5, 1, 5, 10, and 20 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.

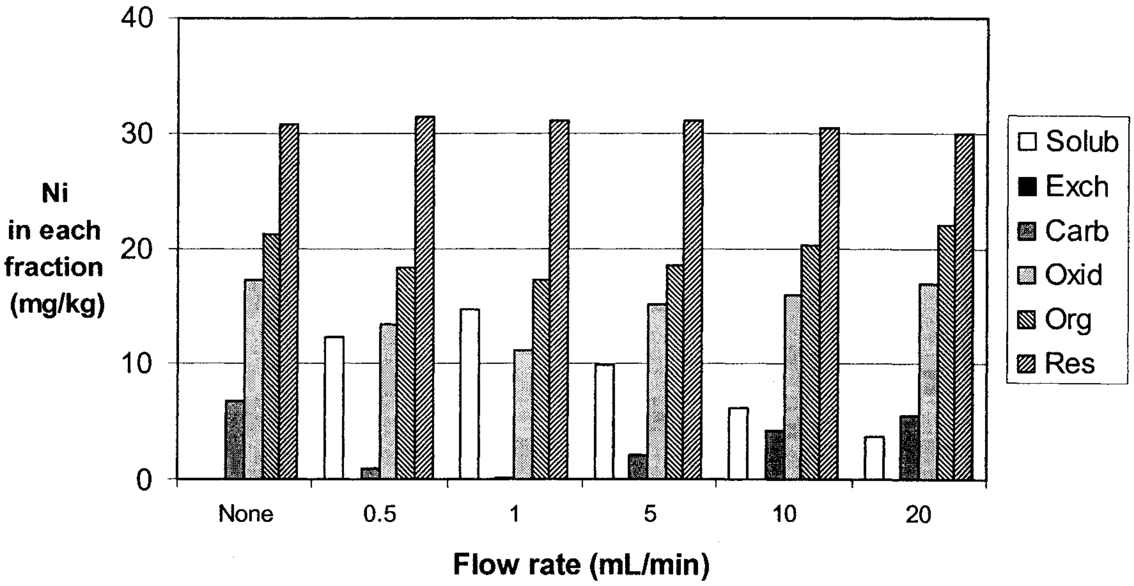


Figure 5-9 **Sequential extraction of nickel after washing sediments with different flow rates of 2% rhamnolipid in continuous flow configuration. The flow rates were 0.5, 1, 5, 10, and 20 mL/min. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

5.6 SSE of the sediment after washing over time in continuous flow configuration

The removal of metals over time showed that this is a time sensitive phenomenon (Figures 4-21, 4-22, and 4-23). SSE tests were performed to study the sensitivity of the removal of each fraction to time. Samples underwent the SSE tests before (day 0) as well as after washing with a solution of 2% rhamnolipid for 3 days and 13 days. Figures 5-10, 5-11, and 5-12 show the results of these tests for Cu, Zn, and Ni respectively.

The results of the SSE tests for copper after 3 days and 13 days support the results of the test of removal of copper from the sediment which showed that the removal of copper remained constant after 3 days. It indicates that not only the total removal of copper from the sediment after 3 days remains constant, but also that the concentration of copper in each fraction will be the same after 13 days in comparison with 3 days (Figure 5-10).

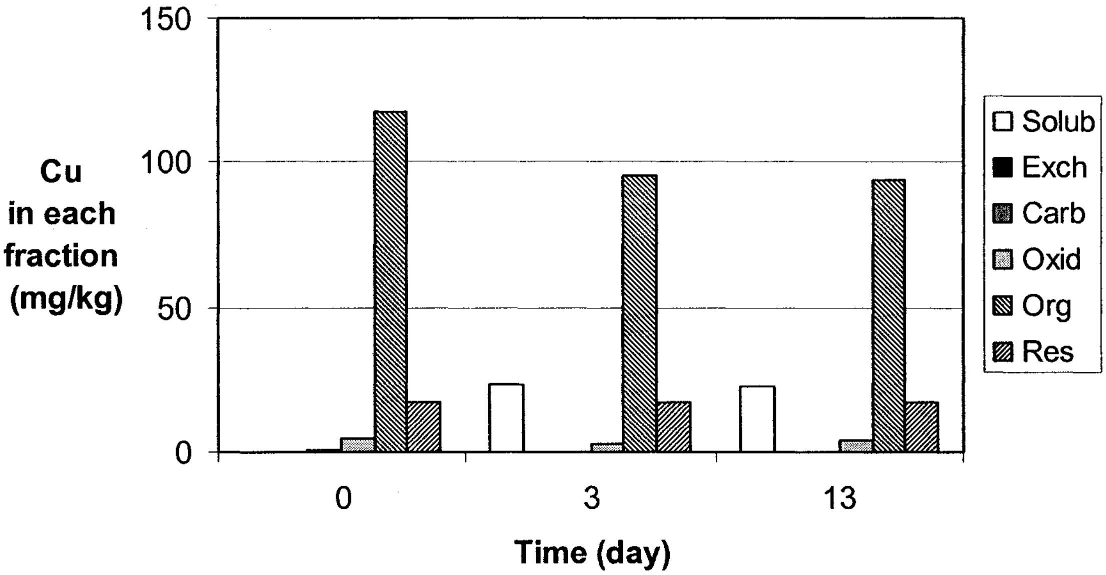


Figure 5-10 **Sequential extraction of copper after washing sediments for different periods of time with the solution of 2% rhamnolipid in a continuous flow configuration. The flow rate was 0.5 mL/min and samples were taken on days 0, 3, and 13. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

Figure 5-11 shows the outcome of the SSE tests for zinc removal over time. The removal is progressive over time as indicated in Figure 4-22 as well. The noticeable result of the SSE test for zinc in this part of the investigation is the behavior of the exchangeable fraction interacting with the rhamnolipid. The concentration of the metal in the exchangeable fraction remained constant over time while the concentration of zinc in the carbonate and organic fractions continuously decreased during the period of the test. The major effect was seen on the organic fraction, where the concentration of zinc decreased remarkably over time.

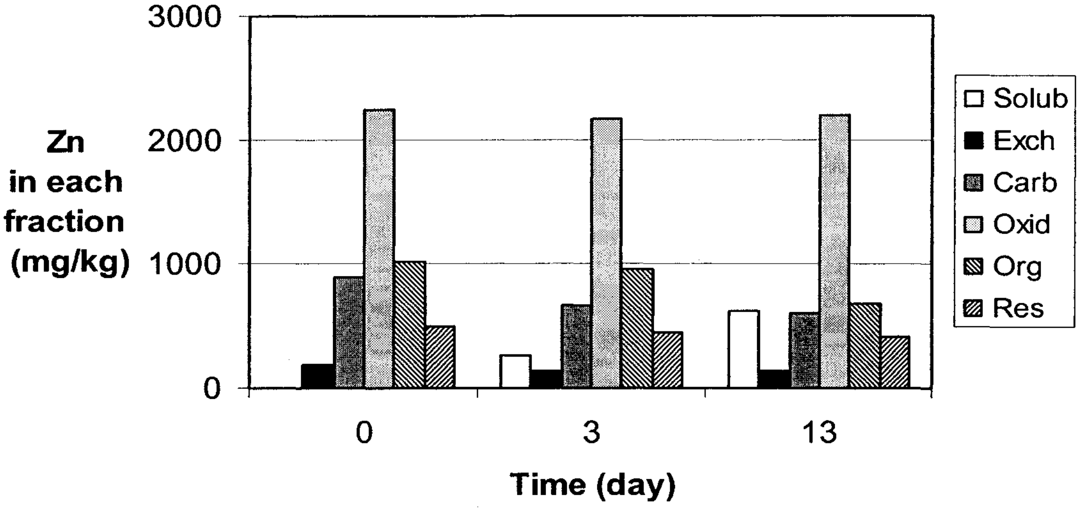


Figure 5-11 **Sequential extraction of zinc after washing sediments for different periods of time with the solution of 2% rhamnolipid in a continuous flow configuration. The flow rate was 0.5 mL/min and samples were taken on days 0, 3, and 13. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

Nickel showed a different behavior over time (Figure 4-23). The concentration of nickel in the soluble fraction increased until 9 days and then declined over time, which means the removed nickel returned to the sediment when the removal rate was lower than the readsorption rate. By using the results of the SSE tests this behavior can be interpreted. The returning nickel to the sediment, the readsorbed nickel, was not evenly distributed over the fractions of sediment. As shown in Figure 5-12, the concentration of nickel in carbonate and organic fractions declined over time although the concentration of nickel decreased in the soluble fraction at the same point in time (after 9 days). The reason for this decrease can be explained by the accumulation of the readsorbed nickel in the oxide and hydroxide fraction, which was at a higher rate than the rate of nickel removal from the other fractions after 9 days. According to Yong and Mulligan (2004), carbonate minerals are more soluble than aluminum/iron oxides, hydroxides and oxyhydroxides; thus nickel that was removed from oxide and carbonate fractions of the sediments, preferentially reabsorbs to the oxide fraction which is less soluble.

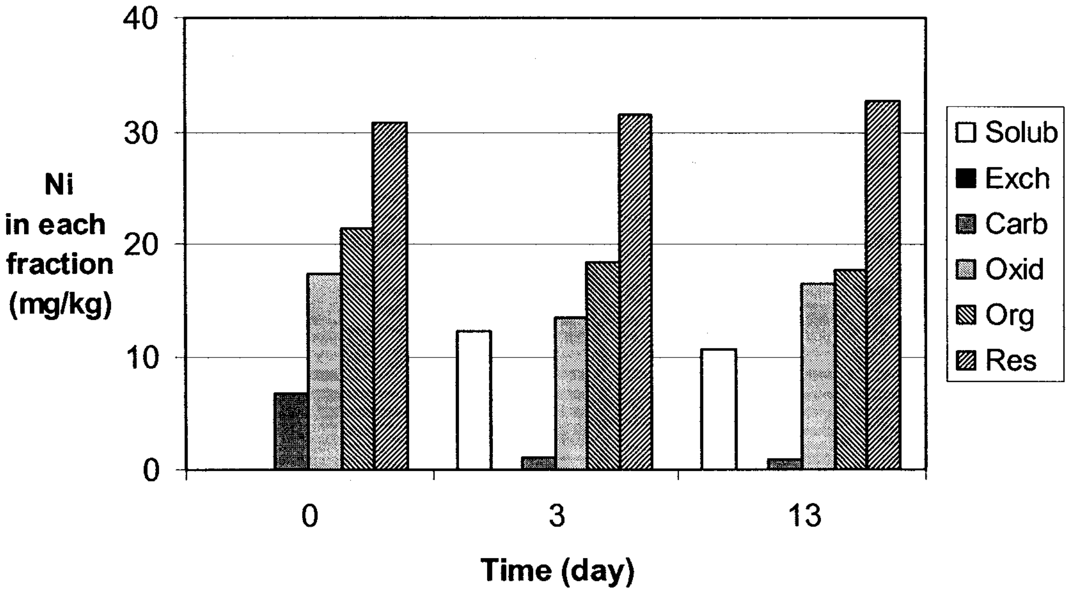


Figure 5-12 **Sequential extraction of nickel after washing sediments for different periods of time with the solution of 2% rhamnolipid in a continuous flow configuration. The flow rate was 0.5 mL/min and samples were taken on days 0, 3, and 13. The fractions are indicated as Solub for Soluble, Exch for Exchangeable, Carb for Carbonate, Oxid for Oxide, Org for Organic and Res for Residual.**

5.7 Discussion

The interaction of contaminants with sediments is a very complex phenomenon and means are required to understand this matter more fully. As Kabala and Singh (2001) stated, the mobility and availability of heavy metals depends on the ways that metals are associated with different soil components. Krishnamurti et al. (1995) and Gismera et al. (2004) also have indicated that the measurement of mobility and availability of heavy metals is needed to predict the behavior of heavy metals in the soil. To determine the speciation of metals in sediments (the distribution of elements among chemical forms or species), specific extracts are used in a process called selective sequential extraction. This method can be used to determine if heavy metals are removable by remediation techniques or to predict removal efficiencies. Sequential extraction can be employed for the evaluation of the most appropriate sediment remediation technology and for monitoring remediation procedures. Exchangeable, carbonate, reducible oxide and organic fractions are amenable to washing techniques, and residually bound contaminants are not economical or feasible to remove. This information is not only important in designing the most appropriate conditions for sediment washing, but also, as Gismera et al. (2004) mentioned, even a slight variation in metal availability may make trace metals toxic to animals and plants. With due attention to the importance of the SSE, these tests were performed in a variety of manners by different researchers (Tessier et al. 1979, Yong et al. 1993, Ho and Evans 2000, Chartier et al. 2001, Koeckritz et al. 2001, and Elass et al. 2004).

In this chapter, SSE tests were performed for different samples of sediment with regards to the procedure proposed by Yong et al. (1993). The sediment samples were subjected to SSE before and after washing with the agents during a short time (3 days) in both batch and continuous flow configurations. The purpose of this part of the present research was to investigate if the configuration can change the contribution of each fraction in the heavy metal removal process. To have a comprehensive study of the phenomenon of heavy metal removal through the continuous flow configuration, which is the main goal of this research, and to be able to predict and interpret this soil washing technique (continuous flow configuration), SSE tests were performed for different samples that were washed with a variety of agents, flow rates, and also over time.

The affinity of the heavy metals for various fractions in the sediment was determined by SSE tests for the sediment before applying any washing agent (Table 5.2). Similar to these results, Chartier et al. (2001) reported that 65% and 73% of total copper existed in the sediments detected in the organic matter and sulfide fractions respectively while 50 and 80% of nickel and chromium was found in the residual fractions. Ho and Evans (2000) reported that copper and lead mostly existed in oxidizable organic matter and zinc was found in all soil fractions. Jenne (1968) stated that Fe and Mn oxides determine the fractions of soil in which Zn exists. The present study also has found that 46.2% of Zn exists in oxide fraction of the sediments. Karam et al. (2003) stated that humic substances (HS) have a high affinity to form stable complexes with copper. As a result, copper is usually immobile in organic soil. Kabata Pendias (2001) indicated that copper and HS interaction can be a result of complexation, ion exchange, precipitation, and dissolution.

Table 5.2 Heavy metal affinity for various fractions in the sediments (Mulligan and Dahrazma 2003).

Heavy metal	Affinity for each fraction
Copper	Organic>residual> oxide > carbonate, exchangeable
Nickel	Residual>organic> oxide>carbonate> exchangeable
Zinc	Oxide> organic> carbonate>residual >exchangeable

Comparing the results of SSE after washing the sediment with different washing agents in batch and continuous flow setups, one can conclude that regardless of the quantity of metal removal, each fraction follows the same pattern in interaction with an individual agent. It also indicates that both configurations have the same principal mechanisms for heavy metal removal from sediments.

The results of the SSE for the samples that were washed with different agents in batch and continuous flow column configurations show that the organic phase-associated metals can be removed mostly by rhamnolipid with sodium hydroxide. Conditions without NaOH addition were effective for removing the zinc and nickel in the oxide and carbonate phases. Residual fractions, the most difficult to remove, were not affected during the surfactant washing studies. It was shown that copper could be removed from the organic-bound fractions and zinc could be removed from the oxide-bound fractions during washing with rhamnolipid biosurfactants.

This information is important in designing the appropriate conditions for soil washing. A combination of acidic and basic conditions, therefore, would increase the amounts of metals removed in the case of carbonate, oxide and organic-associated metals. Sequential extraction procedures can provide indications but not specific information on the chemical binding of metals. This information can then be used to determine remediation procedures and to monitor the procedures during the treatment phase. Knowing the fraction(s) in which heavy metals exist, the engineer is able to conclude the degree of mobility of heavy metals and consequently the environmental impact of the contaminated soil. In the case, that removal becomes necessary, the SSE guides the selection of the right treatment techniques based on the fractions from which heavy metals should be removed.

Selective sequential extraction tests were employed to investigate the effect of flow rate on the share of each fraction in heavy metal removal from sediment. The results show that for each metal an individual pattern governs, which means each fraction of the sediment responded differently to the change of flow rate. The different responses to the change of flow rate were resulted from different required contact times for various sediment fractions in the removal processes using rhamnolipid. These patterns can be summarized in Table 5.3.

It has also become known that copper removal from each fraction remained constant throughout the tests over time (between 3 to 13 days). Zinc removal from each fraction is time dependant. Exchangeable Zn is available for removal in the short time (3 days), while the organic fraction releases more Zn as time goes. Nickel shows a high degree of dependency with time. According to the SSE and removal tests, the removable nickel was available in carbonate, oxide, and organic fractions. If the removal was applied over a short time (3 days), all of these fractions participated in the release of metal to the solution but over an extended time (13 days) the oxide fraction acted differently. Accumulation of readsorbed nickel can be observed in the oxide and hydroxide fractions as illustrated in Figure 5-12.

Table 5.3 Affinity of various fractions of metals in the sediments with regards to the flow rate.

Heavy metal	Affinity of each fraction
Copper	organic> carbonate >oxide>exchangeable> residual
Zinc	carbonate> organic , exchangeable > oxide> residual
Nickel	carbonate> oxide> organic> exchangeable, residual

By summarizing the results of selective sequential extraction tests, it becomes more reasonable to employ such tests before designing any treatability protocol. Ho et al. (2000) mentioned that SSE results help in understanding the feasibility of remediation and the ecological significance of contamination by metals. Flow rate, time, and the type of washing agent can be optimized according to the type of contaminant and the fraction in which the heavy metal exists. To illustrate the above statement, one can say that for a zinc-contaminated sediment, an extended time period will not be helpful in removing the zinc if the metal exists mostly in the exchangeable fraction (Figure 5-11). Also, it can be added that if the contamination is by nickel in the carbonate fraction in the presence of the oxide fraction, removal shows a remarkable deficiency because of readsorption if the time is extended (Figure 5-12). With the same analogy, it can be said that copper removal is more sensitive to the washing agent than time (Figure 5-10).

As indicated earlier, SSE can be used as a basic to design a sediment washing protocol depending on the metal, the fraction in which it mostly exists, and the washing agent to which it responded effectively as well as other physical and chemical conditions. Such a washing protocol was designed for the sediment used in this research to remove Cu, Zn, Ni based on the results of SSE and removal experiments. The sample protocol is presented for each metal individually and shown in Figure 5-13.

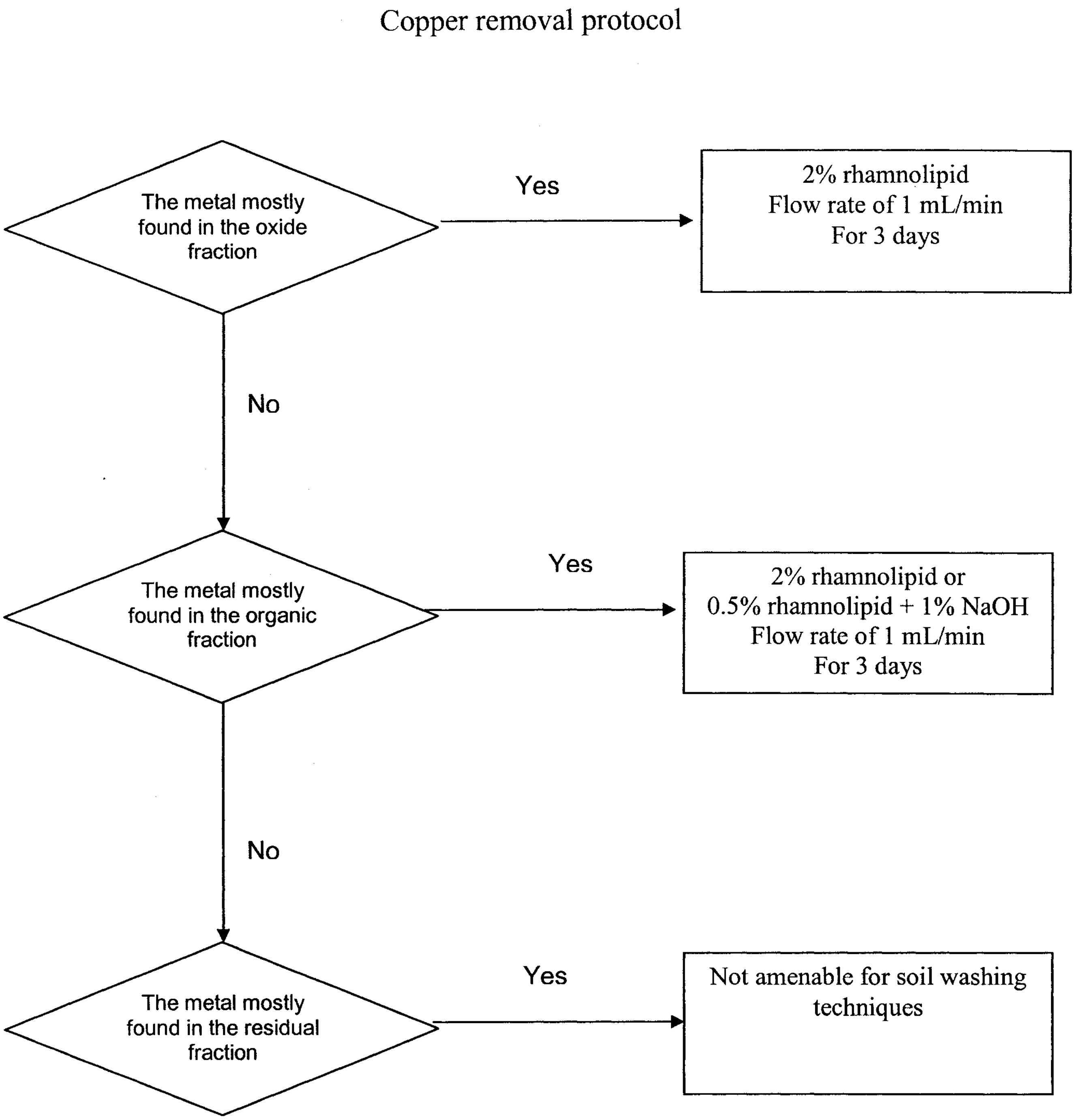


Figure 5-13 **A sample protocol for sediment washing based on the results of SSE and removal tests.**

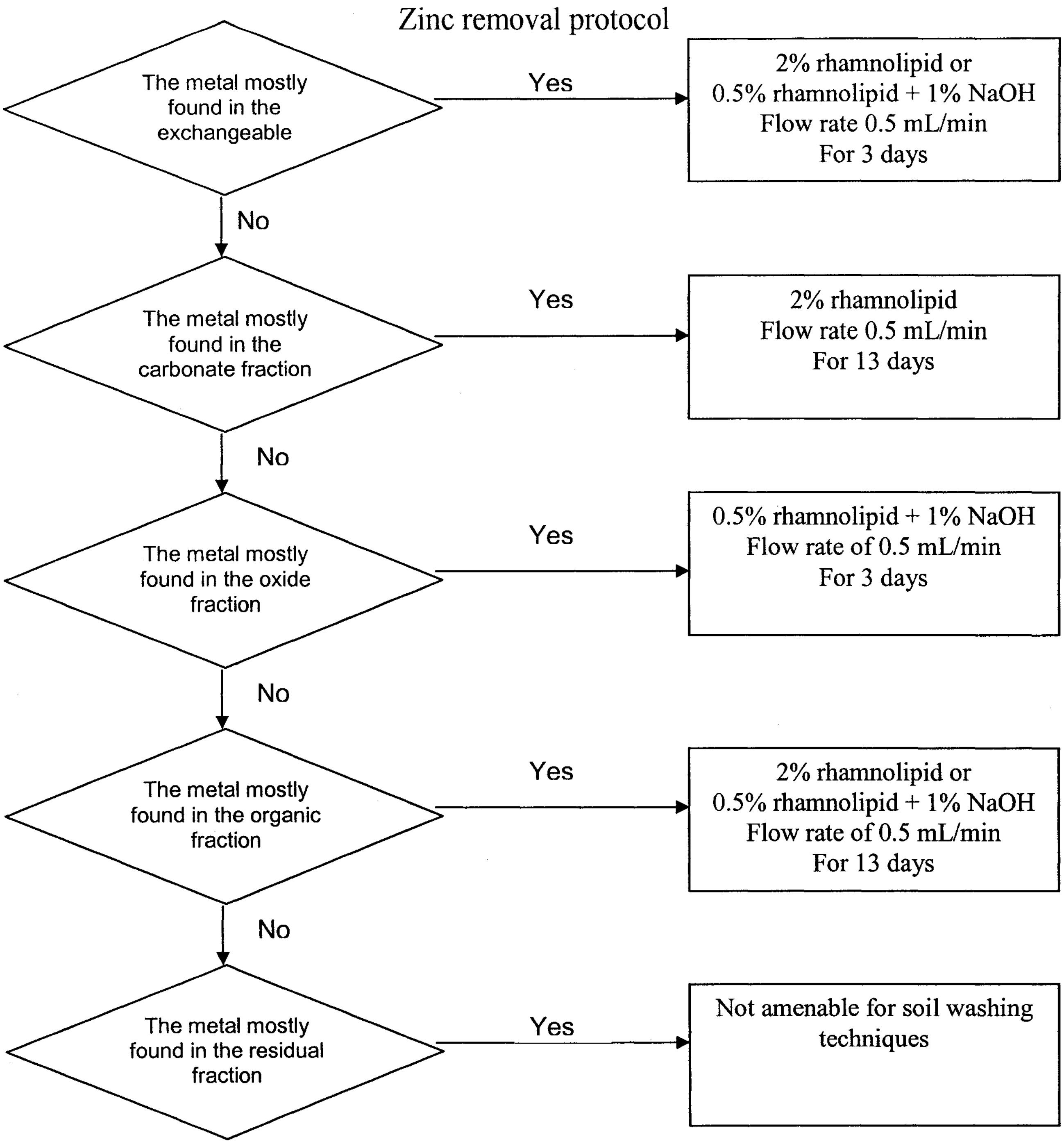


Figure 5-13 **A sample protocol for sediment washing based on the results of SSE and removal tests (Continued).**

Nickel removal protocol

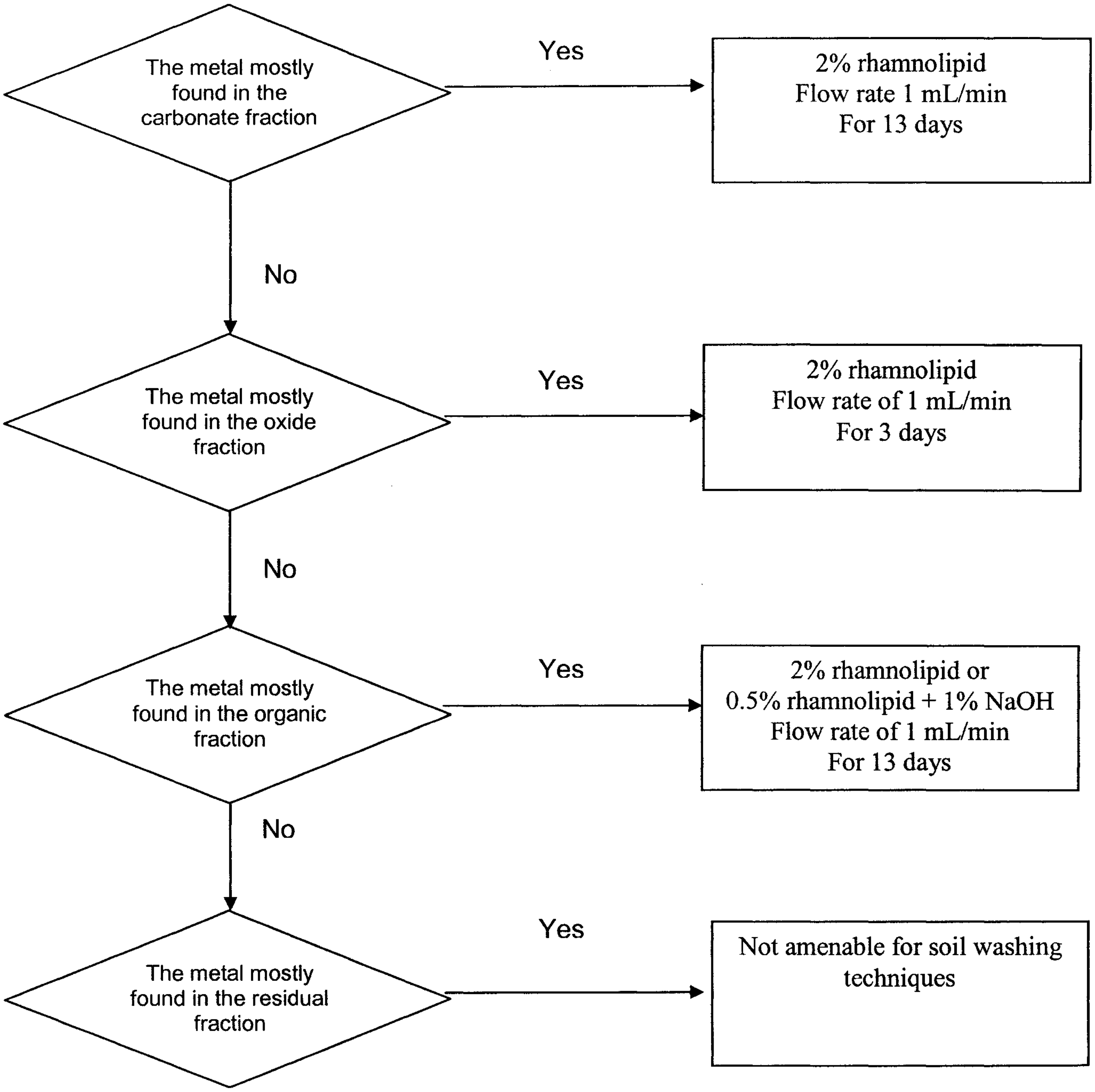


Figure 5-13 A sample protocol for sediment washing based on the results of SSE and removal tests (Continued).

Chapter 6

Biosurfactant Adsorption

6.1 General remarks

In all soil washing techniques that involve a solution for removal of contaminant from the solid phase, an understanding of the interaction of solid and liquid phases on the surface of contact is required for a better view of the process. Investigation on adsorption is helpful in understanding this interaction and consequently to develop a better knowledge of the mechanisms of the removal. It is also important in terms of the economy of the removal process. Adsorption of rhamnolipid to the sediment was studied by means of continuous flow configuration as part of the comprehensive investigation on the heavy metal removal by rhamnolipid. This chapter is devoted to the results of a brief study on adsorption of rhamnolipid on the sediment particles.

6.2 Adsorption of rhamnolipid to the sediment

The tests were performed with the procedure explained in Chapter 3. Various concentrations (0.5, 1, 2, and 5%) of the biosurfactant, rhamnolipid, were applied to the sediment samples in a continuous flow configuration. The results are plotted in Figure 6-1 and a nonlinear sorption isotherm was obtained.

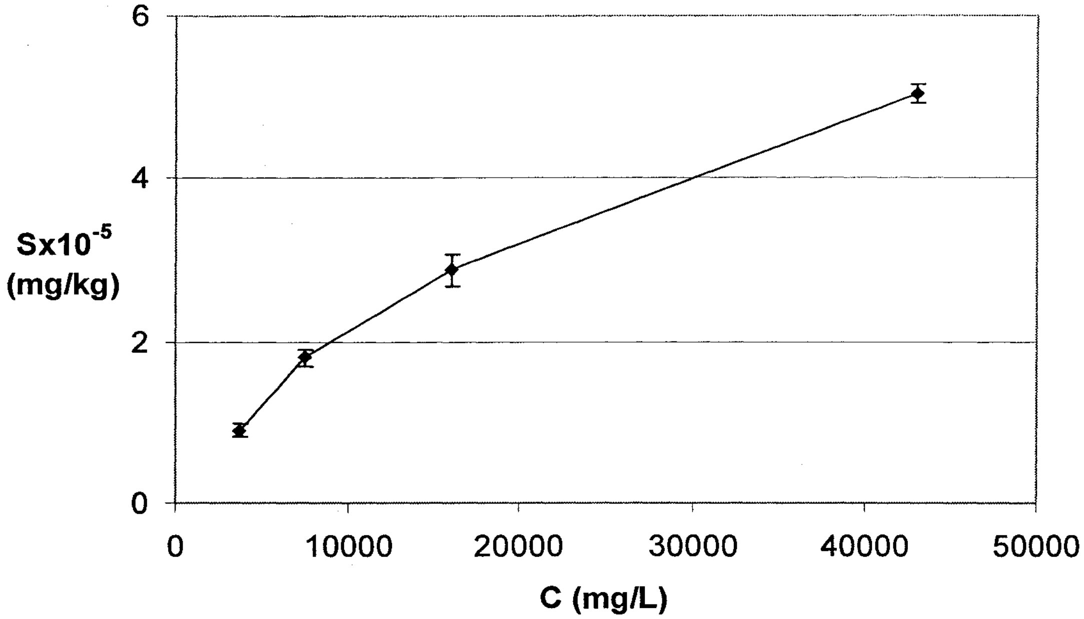


Figure 6-1 Sorption of rhamnolipid to the sediment at different concentrations in continuous flow configuration at a flow rate of 0.5 mL/min. S is the sorbed rhamnolipid and C is the equilibrium concentration of rhamnolipid in the solution.

The pattern of the sorption of rhamnolipid was compared with different types of isotherms and it was determined that this type of sorption can be best fitted to a Freundlich equation with a correlation coefficient of 0.98 (Figure 6-2). The Freundlich isotherm indicates that the adsorption follows a different slope at various concentrations. Since the isotherm of the adsorption is Freundlich, a maximum value for adsorption cannot be determined. It also can be concluded that in higher concentrations the adsorption is higher but it is not linearly proportional to the concentration (Sparks 2003). In other words, the slope of the adsorption curve declines as concentration increases as shown in Figure 6-1.

The Freundlich isotherm equation is given as following:

$$s=k_fC^n \qquad [6\text{-}1]$$

where

s: the rhamnolipid adsorbed onto the soil (mg/kg)

C: the rhamnolipid remaining in the solution at equilibrium (mg/L)

k_f: the Freundlich constant in this case equals to 341.7

n: the index of nonlinearity in this case equals to 0.69

The correlation coefficient for this fit surpassed 0.98, which is an indication of very good agreement between the data and the fitted curve.

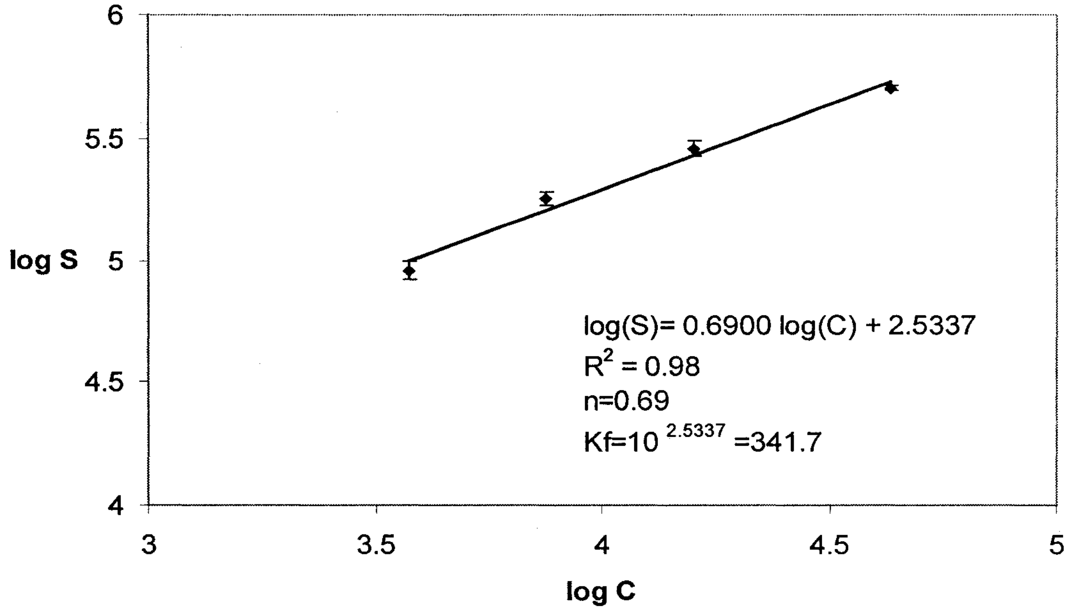


Figure 6-2 **Sorption of rhamnolipid to the sediment at different concentrations in a continuous flow configuration at a flow rate of 0.5 mL/min in logarithmic scale. S is the sorbed rhamnolipid and C is the equilibrium concentration of rhamnolipid in the solution.**

6.3 Discussion

In the application of the rhamnolipid to wash metal contaminated soil, the efficiency of the rhamnolipid should be improved. A study of the sorption leads treatable protocol designers to have a rough estimation of the quantity of washing agents.

In this study, adsorption of rhamnolipid on to the solid surface of sediments has a Freundlich isotherm, which is valid only over a limited range of solute concentrations (Yong and Mulligan 2004). This finding is supported also by the results of the experiments performed by Herman et al. (1995) on Hayhook soil. In that study, they found that the adsorption isotherm of rhamnolipid to the soil could be fitted to the Freundlich equation with K= 6761 and n=0.17. Webber (1972) has found that in continuous flow and batch operations in water or wastewater treatment, film diffusion and pore diffusion limit the rate of adsorption.

Herman et al. (1995) have pointed out that the sorption of rhamnolipid to the soil depends on the concentration of metals in the soil. The presence of Cd^{2+} (1.46 mmol/kg) in the soil decreased the sorption of rhamnolipid to the soil. A mixture of metals decreased sorption even further. Noordman et al. (2000) also stated that the adsorption of rhamnolipid may provide new sites of adsorption for hydrophobic compounds.

Understanding the adsorption of the biosurfactant has an economical importance too, as mentioned by Hermann et al. (1995) who stated that improvements should be made to reduce the total adsorption of rhamnolipid to the soil to gain a better economical performance. Figure 6-3 shows the loss of different concentrations of rhamnolipids due to adsorption to the sediments used In the present study. As seen in this figure using a higher concentration of rhamnolipid causes a higher adsorption but it should be noted that the increment in the loss is much less than the increment in rhamnolipid concentration. For example, if the concentration of rhamnolipid becomes 5 times higher (from 10000 to 50000 mg/L) the loss of rhamnolipid becomes less than three times more (from 2500 to 7000 mg/L). The loss of rhamnolipid due to sorption also is presented in Table 6.1.

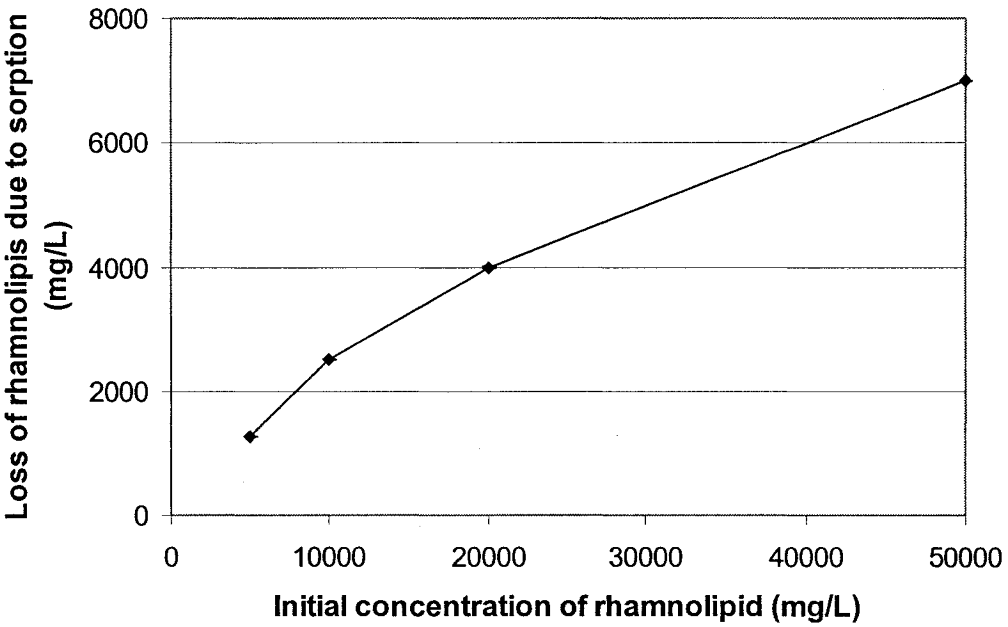


Figure 6-3 **Relation between initial concentration and the loss of rhamnolipid due to adsorption to the sediments in continuous flow configuration. Flow rate was 0.5 mL/min with pH 6.5.**

Table 6.1 Loss of rhamnolipid due to sorption to the sediment.

Concentration of rhamnolipid (%)	Loss of rhamnolipid due to sorption (%)
0.5	25.1
1	12.9
2	20.1
5	14.2

The possible mechanisms for sorption of anionic surfactants are the following (Myers 1988 and Herman et al. 1995):

- Cation bridging between sorbed cations in the soil and anionic head of surfactant.
- Hydrophobic interactions between the organic matter in soil and hydrophobic portion of surfactant.

According to Herman et al. (1995), it can be stated that the two mechanisms can be responsible for adsorption of rhamnolipid to the sediment, since the percentage of organic matter in the sediment is high (20%), and on the other hand, the sediment was contaminated with heavy metals. The share of these two mechanisms in rhamnolipid adsorption under different conditions cannot be the same and varies by changing parameters such as concentration of rhamnolipid solution. In addition, Noordman et al. (2000) explained that the adsorption of rhamnolipid to a soil is an interfacial adsorption process that resulted from hydrophobic interactions between the rhamnolipid components. They also recommended that for soil remediation a concentration higher than CMC should be used.

Summarizing all of the above, one can come to this point that sorption is a very important and effective phenomena in soil washing techniques since the adsorption reduces the efficiency of the surfactant. Results in this research showed that the percentage of loss of rhamnolipid decreases as the concentration of rhamnolipid increases (Table 6.1). Similar to the conclusion from this research, Hermann (1995) stated that minimizing the adsorption of rhamnolipid on to the soil minimizes loss of effective surfactant in the treatment of metal contaminated sediments with rhamnolipid. This opens more room for further investigation on the mechanism of the adsorption.

Chapter 7

Mechanism of heavy metal removal

7.1 General remarks

By collecting information from the removal tests, SSE experiments, and the study of adsorption, the final step in this research was to find the possible mechanisms of metal removal from the sediment. Understanding the mechanisms of removal can be helpful in the prediction and explanation of the behavior of rhamnolipid in contact with soils and sediments and helps to choose the right strategy for metal removal, and to provide a guide to choose the supplementary removal method to complete the removal process.

The mechanism of removal was partially determined by SSE and sorption experiments in the previous chapters. In addition, in this chapter, the mechanism of metal removal from sediment using rhamnolipid was studied by means of ion exchange techniques, zeta potential, scanning electron microscopy, wetted surface area, and pH monitoring over time.

7.2 Ion exchange experiments

Two possible mechanisms for metal removal are ion exchange and complexation (Herman et al. 1995). To determine the importance of each mechanism, the ion exchange experiment was performed. Washing tests were performed in continuous flow configurations using 2% rhamnolipid with and without 1% NaOH and 1% NaOH alone as control. The flow rate was kept at 0.5 mL/min. The samples of supernatants that contained removed heavy metals from all tests were taken and the concentrations of heavy metals were measured. These solutions were used for ion exchange experiments as explained in detail in Section 3.7.1.

The concentration of rhamnolipid before and after exposure to the resin was measured by surface tension and no difference was observed. This indicates that rhamnolipid does not adsorb to the resin.

The results of the ion exchange tests are shown in the Table 7.1. As the results indicate, there is no ion exchange for copper and nickel. This is supported by the SSE tests which have shown already that exchangeable fraction of sediment contains a very limited amount of Cu (1%) and does not contain any Ni. With these results and the results from the SSE for this sediment, all of the copper and nickel were removed by other mechanisms. In the case of zinc, 17.8% of removal by 2% rhamnolipid was by ion exchange but adding NaOH increased the share of ion exchange mechanism for removal up to 67.1%. Complexation was the main mechanism of removal of zinc in the absence of NaOH (77.9%).

Table 7.1 Results for ion exchange tests.

Washing Agent	Mechanism of removal	Metal (%)		
		Cu	Zn	Ni
2% Rhamnolipid	Complexation	93.9	77.9	96.6
	Ion Exchange	0.0	17.8	0.0
	Error	6.1	4.2	3.4
2% Rhamnolipid +1%NaOH	Complexation	91.7	27.9	91.8
	Ion Exchange	0.0	67.1	0.0
	Error	8.3	5.0	8.2
1% NaOH	Complexation	90.3	28.6	93.3
	Ion Exchange	0.0	78.6	0.0
	Error	9.7	7.1	6.7

7.3 Zeta potential measurements

Zeta potential is an important property of a solid-liquid interface. The shear plane is an imaginary surface separating the thin layer of liquid bound to the solid surface from the rest of liquid. This surface shows some elastic behavior while the liquid by itself in the absence of solid particles or surface has normal viscous behavior. The electrical potential at the shear plane that is called the zeta potential. The stability of hydrophobic colloids depends on the zeta potential. Zeta potential is an electrical potential and has the unit of volts. Since the

magnitude of this potential is low, usually mV are used instead. If the absolute value of the zeta potential is above 50 mV, dispersion is very stable due to mutual electrostatic repulsion. If the zeta potential approaches zero, it causes a faster coagulation and consequently fast sedimentation (Kosmulski 2002).
Since the washing agents and sediment make a liquid-solid interface, the zeta potential was measured for a variety of supernatants. In this section, the investigation on the connection between zeta potential and metal removal is presented. Zeta potential for sediment in water and rhamnolipid solution above CMC was measured. The results are shown in Table 7.2.

The measured data show that the addition of 0.5% rhamnolipid can increase the magnitude of the zeta potential. Addition of more rhamnolipid causes a radical decline in the magnitude of the zeta potential. This behavior can be explained by the mean of the potential and electrostatic field. Rhamnolipid is an ionic surfactant and if a small amount of it is added to the distilled water, the potential at the shear plane increases. In other words, by increasing the negative charge surrounding the surface, the electrostatic attraction increases and removal of the metals detected. Additional rhamnolipid, as indicated in Chapter 6, causes more adsorption on the surface. The accumulated rhamnolipid molecules over the surface form a shear plane at a further distance relative to the actual surface. Since the potential is inversely proportional to the square of the distance, the zeta potential decrease is observed. These results and by analogy the discussion is also supported by the explanation of the adsorption process by Shaw (1980) who stated that when a layer of macromolecules, polyelectrolyte or uncharged polymer, is adsorbed on the surface, the shear plane shapes in a distance that is proportional to the size of the ions and molecules involved in the process. Consequently, the zeta potential will be altered according to the size of these ions and molecules. This accumulated rhamnolipid molecules increase the removal rates at a higher concentration of rhamnolipid.

Table 7.2 Zeta potential measurement of the sediment in the presence of different washing agents.

Washing agent	Zeta potential of the sediment (mV)
Distilled water (pH 6.5)	-24.3
0.5% rhamnolipid (pH 6.5)	-29.5
1% rhamnolipid (pH 6.5)	-13.4
2% rhamnolipid (pH 6.5)	-9.5

7.4 Monitoring of pH

Since pH variation causes a change in the removal of metals, the monitoring of the pH was performed to verify if the removal of metals from sediments changes the pH during the process. Figures 7-1 and 7-2 show the variation of pH over time for samples washed in batch and column configuration at a flow rate of 0.5 mL/min with solutions of 2% rhamnolipid with and without 1% NaOH.

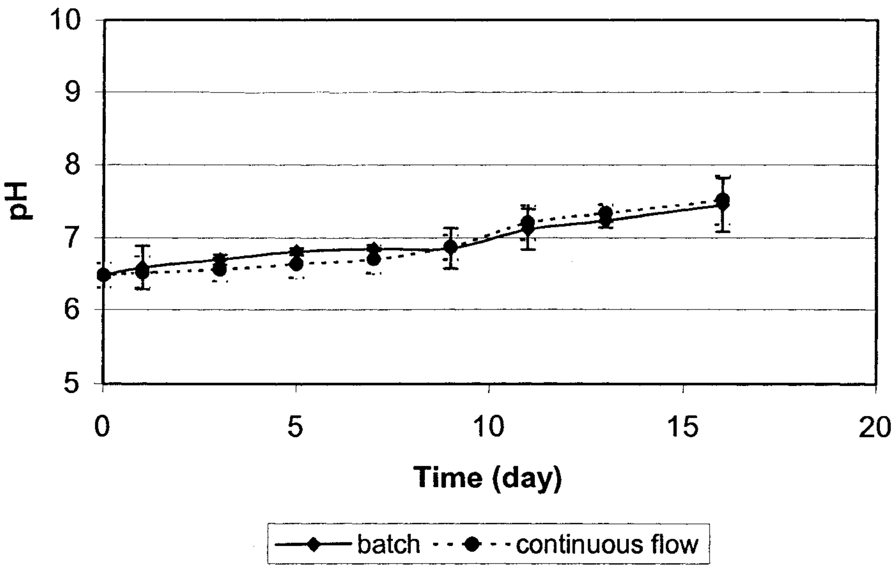


Figure 7-1 Variation of pH over time during the washing of the sediment by a solution of 2% rhamnolipid in batch and continuous flow configurations.

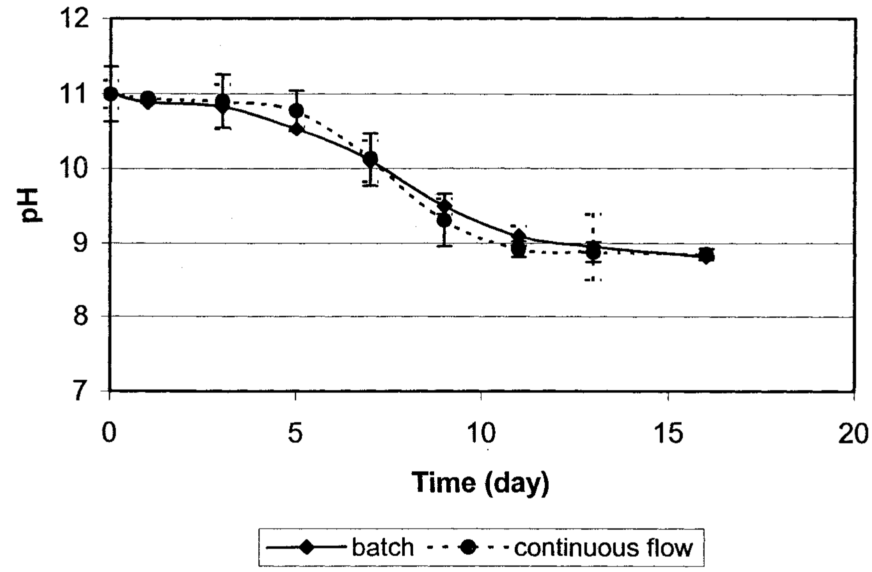


Figure 7-2 Variation of pH over time during the washing of the sediment by a solution of 2% rhamnolipid with 1% NaOH in batch and continuous flow configurations.

At a first glance it can be clearly observed that in a solution of rhamnolipid with and without NaOH, pH changes over time showed the same pattern in batch or continuous flow configurations. One can easily conclude that the similarity in pattern is a result of similarity in the mechanism of removal for both configurations.

For the solution of 2% rhamnolipid without NaOH, pH, which was adjusted to 6.5 at the beginning of the test, gradually increased to 7.4 for batch and 7.5 for continuous flow configuration. As stated in the ion exchange experiments, complexation is the main mechanism of removal with rhamnolipid for all three metals. Ion exchange is responsible for a portion of the total removal of zinc. The H^+ ion exchanges with the exchangeable fraction of the metals such as Zn. Therefore the concentration of H^+ decreases in the solution over time and pH increases gradually. Since the quantities of the H^+ ion and the exchangeable metal are limited, the pH does not widely vary.

In the presence of 1% NaOH in the rhamnolipid solution, pH increases to 11. In the first few days (3-5 days), the pH remained almost at the same level. After this period of time, in days 11 to 13, pH declines to 9 where it remained for the rest of the testing period. The decrease of pH can be the result of involvement of OH^- in solubilization of organic matter in the sediment, which causes a decrease of OH^- in the solution over time. Sparks (2003) stated that "Coiling was not prevalent and humic substances dispersed into small aggregates, less than 0.1 μm, in solutions of pH > 8”. The decline in pH causes a decrease in the solubility of metals in solution. Since the final pH in solution is close to the minimum solubility of these three metals (Figure 4-7) the decline in the concentration of copper and nickel in solution over time in presence of NaOH is justified (Figures 4-24 and 4-26). In the case of zinc, (Figure 4-25) the removal was progressive over time in the presence of NaOH since ion exchange was shown to be an important mechanism in the removal process. Yong and Mulligan (2004) stated that the CEC depends on the pH. Also, the proportion of pH-dependent surface charges in the oxides is high. According to the SSE tests in the present study more than 45% of zinc in sediments exists in the oxide fraction. Therefore increasing the pH of the system increased the sediment CEC and consequently higher removal of zinc by ion exchange mechanism was occurred. It should be noted that NaOH releases Na^+ into the solution and pushes the mechanism of removal toward ion exchange. Also, as the pH declined over time, the ultimate removal for zinc was limited by its solubility.

7.5 Wetted surface area study

The specific surface area of a soil is the total surface area of all particles in a specific volume usually a unit volume. “Wetted surface area is the surface area of the pore channels in the soil through which fluid flow occurs (Yong and Mulligan 2004)”. Since the first step in the removal of contaminants from the soil and sediment is wetting the sediment particle surface with the washing agents, for the study of the removal mechanisms, the relationship between the removal and wetted surface area needs to be investigated.

The governing equation for the velocity of water in the pore media is based on the Darcy Equation:

$$v = ki = k\frac{\Delta\Psi}{\Delta l} \tag{7-1}$$

where v is the velocity, k is the hydraulic conductivity coefficient, and i is the hydraulic gradient. $\Delta\Psi$ and Δl are the difference in the potential and the length of the column respectively. Since the experimental value of k does not represent the properties and the influence of the permeant, the Kozeny-Carman Equation is an approach to take those properties into account (Yong and Mulligan 2004). The K-C equation has several terms but it is usually reduced to the form of (Hanson 2004):

$$k = C\frac{g}{v_w}\left(\frac{e}{\rho_s S_s}\right)^2 n \tag{7-2}$$

where:

C= the sphere factor, usually between 0.33 to 0.56.

g= acceleration of gravity 9.81 (m/s^2).

v_w= kinematic viscosity of the fluid passing through the medium (m^2/s).

e= void ratio of soil.

ρ_s= density of the soil (kg/m^3).

S_s= Specific surface area per unit mass of soil particles (m^2/kg).

n= porosity of the unit soil mass = $\frac{e}{1+e}$.

Yong and Mulligan (2004) made some modifications to Equation 7-2 and proposed the following equation for k:

$$k = \frac{Cn^3\gamma}{\eta T^2 S_w^2} \qquad [7\text{-}3]$$

in which:

γ= specific gravity of rhamnolipid (N/m^3)

η= viscosity of the permeating fluid (N.s/m^2).

T= tortuosity, the ratio of effective flow path to the thickness of test sample, usually taken as T^2=2.

S_w= wetted surface area per unit volume of soil particles (m^2/m^3).

the other parameters are defined earlier. Yong and Warkentin (1975) suggested C= 0.4.

Equation 7-4 was recommended by U.S. Corps of Engineers (1993) to consider the viscosity and density of the fluid in calculation of the coefficient of permeability of a soil for different liquids.

$$k = k_0 \frac{\gamma}{\mu} \qquad [7\text{-}4]$$

in which:

k_0 = intrinsic or specific hydraulic conductivity (m^2)

γ= unit weight of the fluid (N/m^3)

μ= viscosity of the liquid (N.s/m^2).

The viscosity of the 2% rhamnolipid solution was measured at 2.167 centipoise by the method proposed by Shaw (1980) while viscosity of water was obtained from the engineering tables equal to 1.0 centipoise (Viessman and Hammer 1998). The removal tests were performed for the sediment with different hydraulic conductivities at a flow rate of 0.5 mL/min with 2% rhamnolipid for 3 days. The relationship between hydraulic conductivity and removal for the metals is shown in Figures 4- 33, 4-34, and 4-35. By means of Equation 7-3, for a known fluid and specific soil sample, S_w can be determined if the k is known for any specific configuration. The removal versus wetted surface area is plotted in Figures 7-3, 7-4, and 7-5 for copper, zinc, and nickel, respectively. In all cases, the reduction of surface area causes a decline in the removal of the metal from the sediment. This shows that the wetted surface area is among the parameters that control the mechanism of metal removal. It should be noted that this is an important issue in continuous flow configuration. In batch configuration the assumption is that specific surface area and wetted surface area are the same and the surfaces of all soil particles are wetted.

The removal of copper is more sensitive to wetted surface area. To explain this phenomenon, looking back over the results of selective sequential extraction is helpful. The majority of copper in this sediment exists in the organic fraction. Organic materials have the largest surface area among all the fractions in the sediment. A decrease in the wetted surface area affects this fraction more than the others and consequently reduces the copper removal from the sediment more effectively.

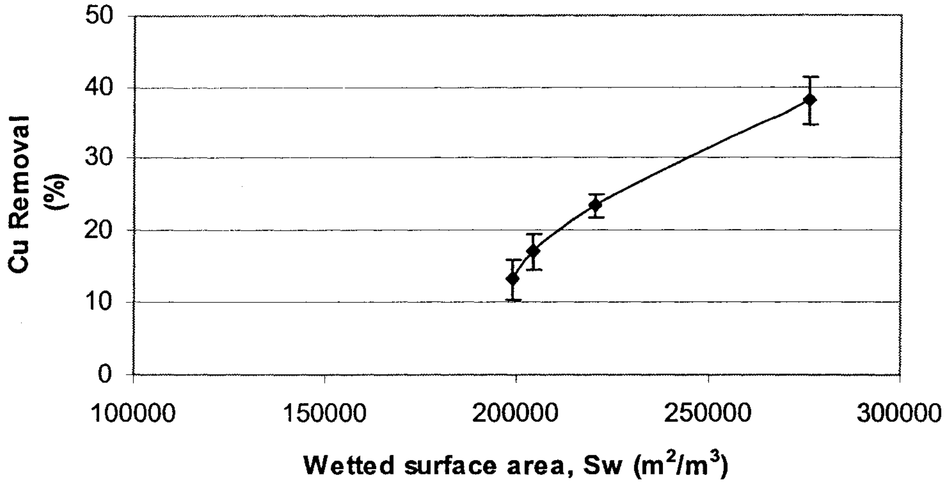


Figure 7-3 Copper removal and wetted surface area. 2% rhamnolipid with 0.5 mL/min in a continuous flow configuration was used.

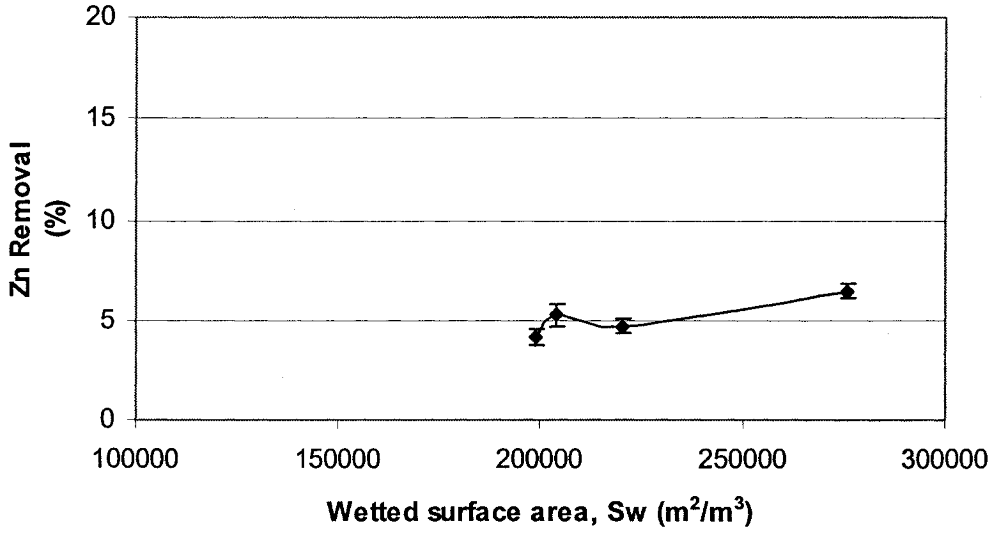


Figure 7-4 Zinc removal and wetted surface area. 2% rhamnolipid with 0.5 mL/min in a continuous flow configuration was used.

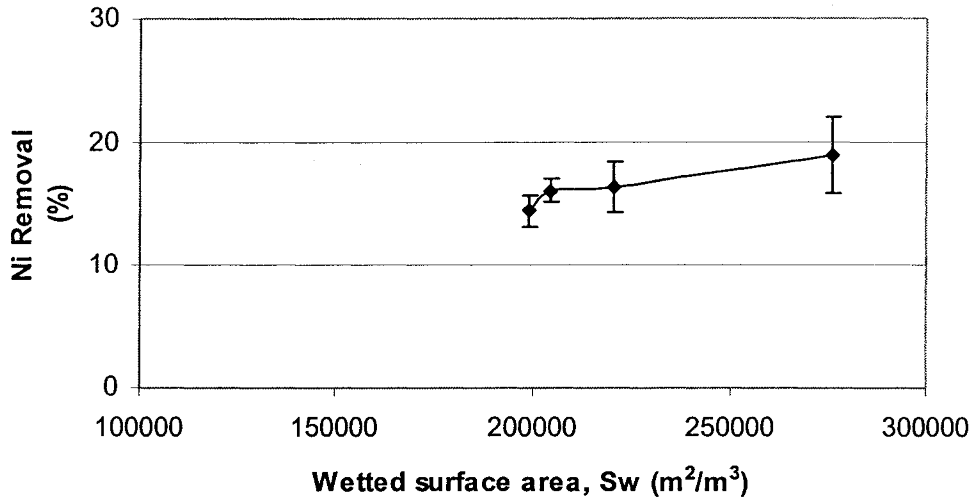


Figure 7-5 Nickel removal and wetted surface area. 2% rhamnolipid with 0.5 mL/min in a continuous flow configuration was used.

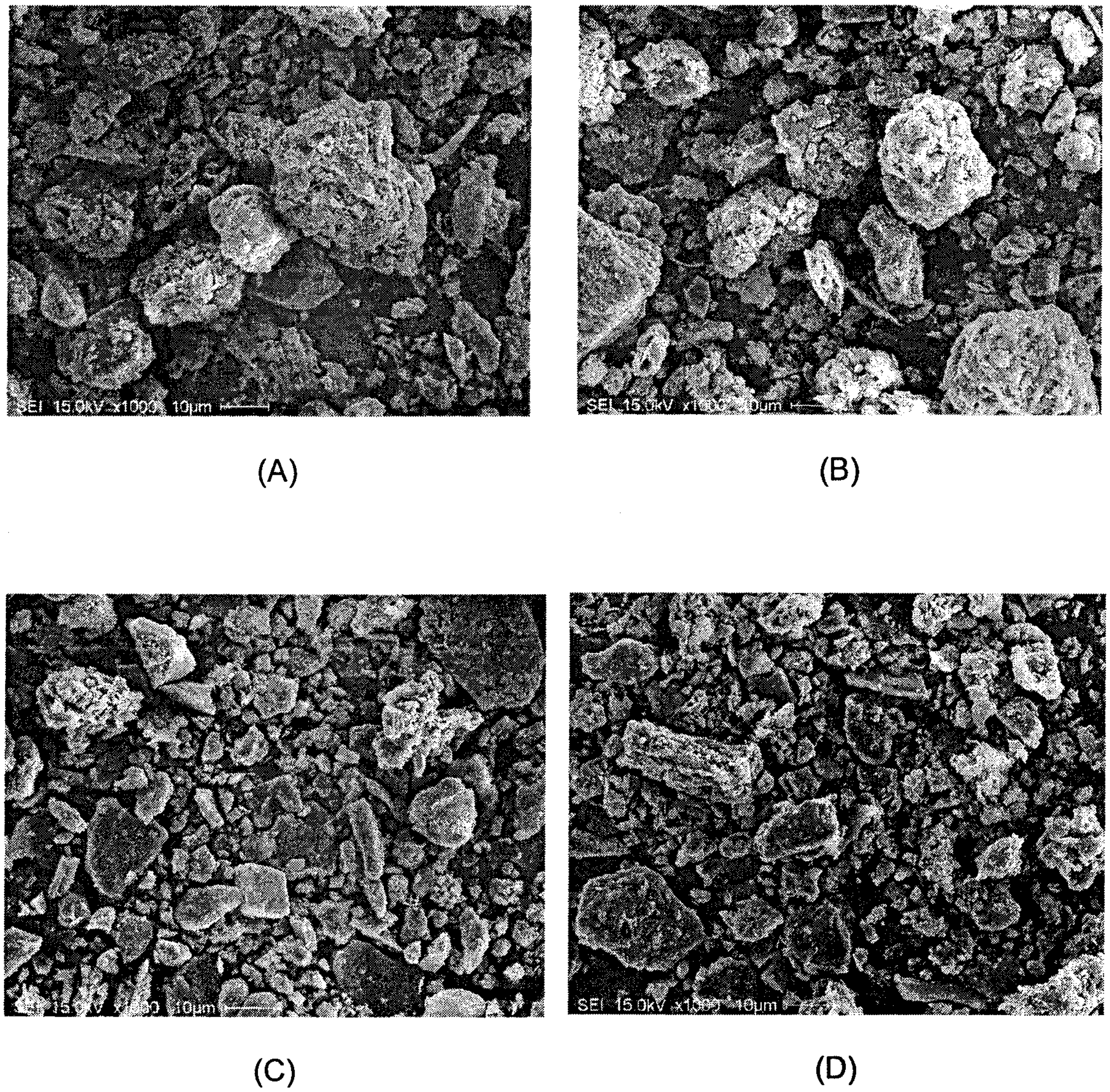
7.6 Scanning electromicroscopy of the sediment

A scanning electron microscope (SEM) (JEOL JSM-840A) was used to take microscopic pictures of the sediment. The technical information about the equipment and the method was presented in Chapter 3. The washing tests were performed in a continuous flow configuration with a flow rate of 0.5 mL/min. SEM was performed for four samples. The samples were:

- Sediment before washing;
- Sediment after washing with 2% rhamnolipid;
- Sediment after washing with 1% NaOH;
- Sediment after washing with 2% rhamnolipid and 1% NaOH.

Pictures were taken with the SEM and shown in Figure 7-6. These pictures provide a general view of the fabric structure of the sediment. Comparing the pictures of the sediment samples before and after washing with 2% rhamnolipid (Figures 7-6.A and 7-6.B) shows that the textural structure of the sediment remains the same during and after the washing processes. In other words, the use of the rhamnolipid does not affect the natural size distribution of the soil. This can be named as an advantage of rhamnolipid as a washing agent. It also can be added that removal by rhamnolipid is an environmentally safe soil treatment technique in both *ex situ* and *in situ* soil remediation. The sediment after washing can be returned to the environment with minimal damage to its natural texture in *ex situ* remediation while the soil can remain in its natural place and texture for *in situ* soil treatment.

Adding 1% NaOH, according to Figures 7-6.C and 7-6.D , causes a remarkable change in the fabric structure of the sediment and decreases the particle size of the sediment. One can also conclude that the removal by the rhamnolipid in the absence of NaOH is mostly from the surface of the particles. Since the rhamnolipid has a limited penetration into the particles, the concentration of rhamnolipid is very important in the removal of copper which mostly exists in the organic fraction. Adding NaOH dissolves the organic matter of the particles, which is larger than the carbonate and oxide fractions in the sediment. These smaller particles provide more available copper for removal by rhamnolipid.



(A) (B)

(C) (D)

Figure 7-6 **Scanning electron micrographs of sediment at 1000X magnification. Washing tests were performed in a continuous flow configuration at the flow rate of 0.5 mL/min. (A) Sediments before washing, (B) Sediments after washing with 2% rhamnolipid, (C) Sediments after washing with 1% NaOH, and (D) Sediment after washing with 2% surfactant and 1% NaOH.**

7.7 Discussion

7.7.1 Introduction

As the last step in this research, the mechanism of heavy metal removal from the sediments by rhamnolipid was investigated. This chapter is the closure to the study and thus, all data from removal tests, selective sequential extraction (SSE) tests, and adsorption experiments are taken into account in addition to the results of individual experiments explained in this chapter including ion exchange experiments, zeta potential analysis, monitoring pH over time, study of wetted surface area, and scanning electron microscopy of the sediment.

7.7.2 Mechanisms of the metal removal by rhamnolipid solution

To explain the mechanisms of the removal in this section, the mechanisms are divided into several steps. These steps are discussed in order and in more detail here as follows:

- ***Step 1: Wetting***

When the solution comes in contact with sediment, the wetting process starts. The process includes coverage of the particle surface by the solution. Since the sediment was air dried, according to Osterhuf and Bartell (1943), the wetting is categorized as adhesional wetting. Adhesional wetting represents a wetting of a solid with a liquid, when the solid previously was in contact with the vapor phase. During this process, liquid replaces the vapor over the surface as the gaseous phase leaves the surface of the solid. The surface area of this exchange remains constant. The free energy change for the process is derived by Dupré (1969) as:

$$-\Delta G = A\,(\gamma_{SA}+\gamma_{LA}-\gamma_{SL}) \qquad [7\text{-}5]$$

where:

ΔG = the free energy change for the process (J)

A = the area of the exchange = 2x area of leaving air (m^2)

γ_{SA} = interfacial energy of solid-air (J/m^2)

γ_{LA} = interfacial energy of liquid-air (J/m^2)

γ_{SL} = interfacial energy of solid-liquid (J/m^2)

This equation indicates that the lower interfacial energy of solid liquid consequently helps the wetting process. Since the rhamnolipid decreases the interfacial tension between solid and liquid, the wetting process is improved by adding the rhamnolipid compared to water alone (Tsujii 1998 and Myers 1992).

In this research, several tests were conducted through which a relationship between wetted surface area and removal of metals was shown (Section 7.5). In addition, the wetted surface interacts with the properties of the soil such as porosity, hydraulic conductivity and of the washing agents including viscosity. It was made clear that the augmentation of wetted surface area provides a higher rate of heavy metal removal from the sediment (Figures 7-3, 7-4, and 7-5). This finding is in agreement with the statement made by Yong and Mulligan (2004) that a higher wetted surface area provides a larger contact space for permeant with the soil particles. The wetted surface area, Sw, itself changes inversely proportionally to the hydraulic conductivity coefficient and the viscosity of the flowing fluid and proportional to the porosity of the soil (Equation 7.3).

- ***Step 2: Contact of rhamnolipid to the surface of the sediment***

When the surface of the sediment becomes wet, contact between rhamnolipid and sediments is initiated. The adsorption tests (Chapter 6) were studied and through measurement of rhamnolipid concentration, the isotherm of adsorption was investigated. The adsorption isotherm is clearly a type of Freundlich isotherm that is limitless in the range of this study, i.e., more adsorbed rhamnolipid was observed at higher concentrations. It should be noted that in the case of soil with low organic matter content, the adsorption isotherm of rhamnolipid to the soil maybe fitted to the Freundlich isotherm (Herman et al. 1995) or to a multi-stage isotherm (Noordman et al. 2000). The adsorption isotherm explains the behavior of adsorption in relation to the concentration, but it does not give an answer to the question of how the rhamnolipid accumulates on the surface of the sediments.

To answer this question, the zeta potential analysis was performed using a range of rhamnolipid concentrations in the solution used in the present research, from 0 (distilled water) to 2%. The variation of zeta potential with rhamnolipid concentration (Table 7-2) can be explained by meaning of the diffused double layer theory, DDL (Figure 7-7).

The sediment is contaminated with heavy metals. This leads to a positive charge over the surface of the sediment. Rhamnolipid is an anionic surfactant with negative charge and is attracted to positively charged metals by electrostatic forces. From the other side, due to the organic matter content, the rhamnolipid can attach to the sediment by a hydrophobic bonds. At this stage, the concentration of rhamnolipid plays a critical role in the magnitude of the zeta potential. As stated earlier in this chapter, increasing the concentration of rhamnolipid to 2% increases the thickness of the stern layer and consequently decreases the magnitude of the zeta potential from 24 mV to 9 mV. The supplier of the zeta potential meter, Zeta-Meter Inc. (2004), has performed a series of zeta potential measurements with a a variety of electrolytes and reported that inorganic electrolytes can have a remarkable effect on zeta potential. Adding potassium sulfate electrolyte at lower concentrations increases the magnitude of the zeta potential up to a certain level. Increasing the concentration of the electrolyte does not change the zeta potential for a range that varies from one electrolyte to another. After this plateau, the magnitude of zeta potential declines with an increase in the concentration of the electrolyte because the double layer becomes compressed by the ions. Zeta potential tests showed that addition of 0.5% rhamnolipid increased the zeta potential from –24 mV to –28 mV and consequently increased the negative charge and electrostatic attraction over the surface. This attraction leads to an increase in removal of the metals in the presence of 0.5% rhamnolipid in comparison with water. Zeta potential measurement also showed that an increment of the concentration of rhamnolipid causes a decline in magnitude of the zeta potential due to the accumulation of rhamnolipid molecules over the surface of the sediment. This accumulation increases the removal rate of the metals with higher concentrations of rhamnolipid molecules.

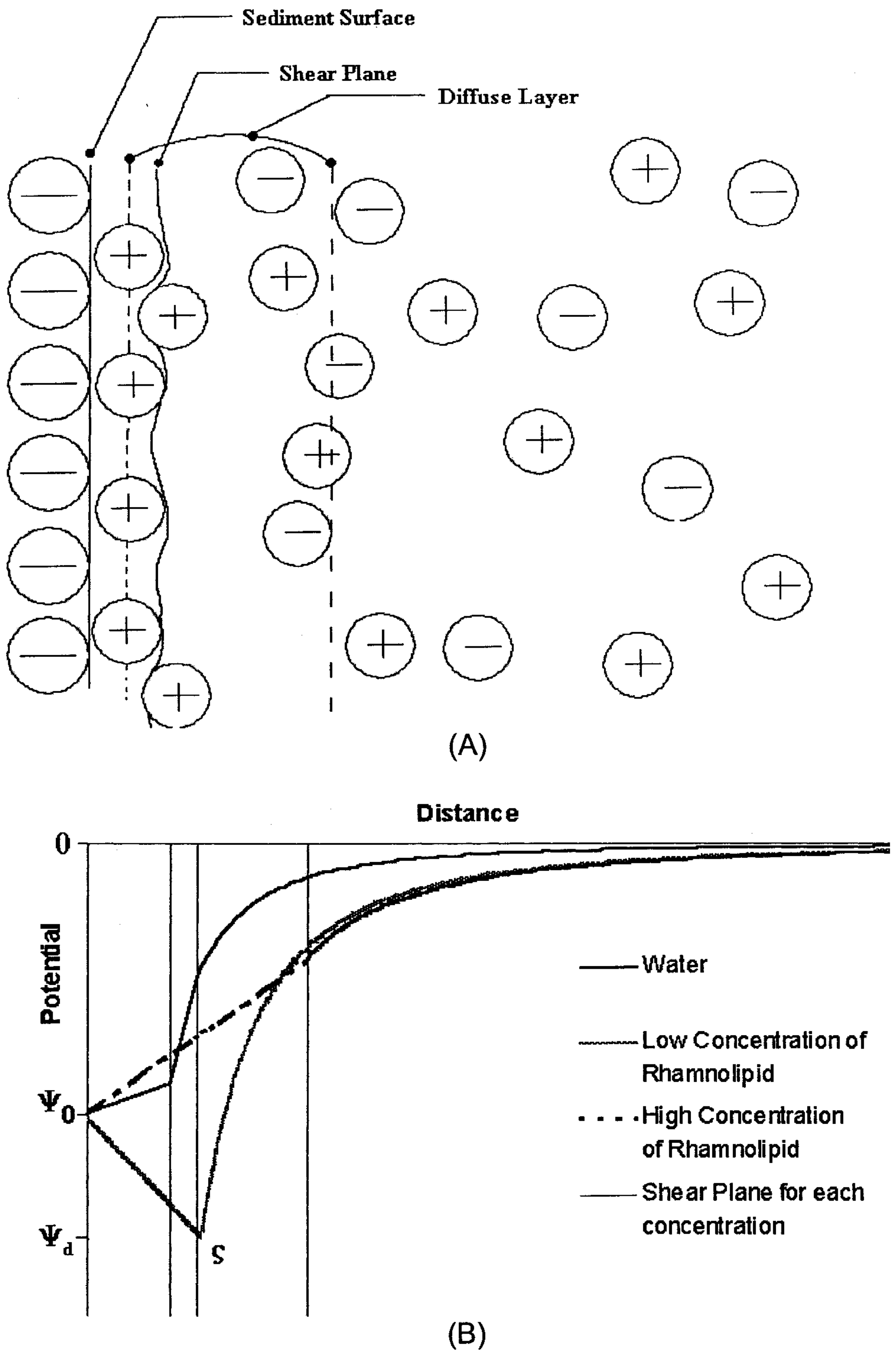


Figure 7-7 Schematic representation of the structure of the electric double layer(A) and reversal of charge due to adsorption (B) (adapted from Shaw 1980).

- ***Step 3: Detachment of the metals from the sediment***

The next stage after contact of rhamnolipid to the sediment is detaching the heavy metals from the sediment and displacing them into the bulk solution. The effective parameters in the removal were investigated and discussed in detail in Chapter 4. The fractions from which removal occurs also were studied through SSE tests (Chapter 5). Having the information from the removal and SSE tests with the aid of the results of ion exchange tests and pH monitoring over time, now it is possible to explain the mechanism of detachment of the heavy metals from the sediment.

Ion exchange experiments clearly showed that ion exchange is not the main mechanism of removal of the metals (Cu, Zn, and Ni) from the sediment. Ion exchange was responsible for 17.8% of the zinc removal.

By monitoring pH over time, it also was observed that the pH does not change significantly during the removal (from 6.5 to 7.4 in 16 days). Increasing pH indicates a decrease of H^+ in solution. The H^+ was initially produced through ionization of the carboxylic group (-COOH) into COO^- and H^+. The H^+ in solution exchanges with cations in the sediment over time which consequently increased the pH. This exchange process is responsible for removal of Zn from the exchangeable fraction. Since the available exchangeable fraction for removal is limited to CEC, which is 14.55 meq/100g, the variation of pH is limited too.

The pK_a of the carboxylic group is 4.8 (Morrison and Boyd 1992). The pH of the rhamnolipid solution was adjusted to 6.5 at the beginning of the experiment. Thus hydrophilic portion of the rhamnolipid (COOH) is negatively charged. The closeness of rhamnolipid to the sediment surface leads to the establishment contact between the rhamnolipid and metals on the sediments surface. This contact aids the formation of complexes between rhamnolipid and cations on the surface of the sediment. After contact between rhamnolipid and the metals occurs, a competition among the metals for complexation with rhamnolipid initiates. According to Ochoa-Loza et al. (2001) the stability constants for the metals in solution for rhamnolipid have the following order:

$$Al^{3+}>Cu^{2+}>Pb^{2+}>Cd^{2+}>Zn^{2+}>Fe^{3+}>Hg^{2+}>Ca^{2+}>Co^{2+}>Ni^{2+}>Mn^{2+}>Mg^{2+}>K^{+}$$

The finding from the removal tests in this research indicates that the metals subjected to study compete in the removal process with the same order ($Cu^{2+}>Zn^{2+}>Ni^{2+}$) as shown in Figures 4-21, 4-22, and 4-23.

This complex breaks the bond between sediment and metal and causes the metal to be lifted from the surface. From a mechanical point of view, the work for this movement is $W_1 = W_{lift} = mg \times L$, mg is the weight of the metal and L is the distance of secondary position of metal from the initial position. Although the metal is disconnected from the sediment surface, it is still attracted to the negatively charged sediment surface. As a result, additional work is needed to move out the complex from the vicinity of the sediment. This work, W_2, is provided by either agitation in the batch configuration or the flow momentum in the continuous flow configuration. Derjaguin, Landau, Verway, and Overbeck (DLVO) theory states that attractive forces between particles are inversely proportional to the distance between them. The sequence of the work is very important i.e. W_2 has to be applied in a way to give the system the chance to complete the first work, W_1. In other words, enough time and energy should be given to the rhamnolipid to detach the metal before it is washed out. This was investigated and presented in Chapter 4 as the effect of flow rate and residence time on removal. Micelles of rhamnolipid form before and after the entrance of the complex to the solution. The new micelles include the monomers with and without the metals.

According to Stokes and Evan (1997) the equilibrium of work and energy for this process is:

$$W_t=W_1+W_2-\Sigma\Delta\gamma \qquad [7-\,]$$

where:

W_t= total energy required to overcome the adhesion of the metal to the sediment surface

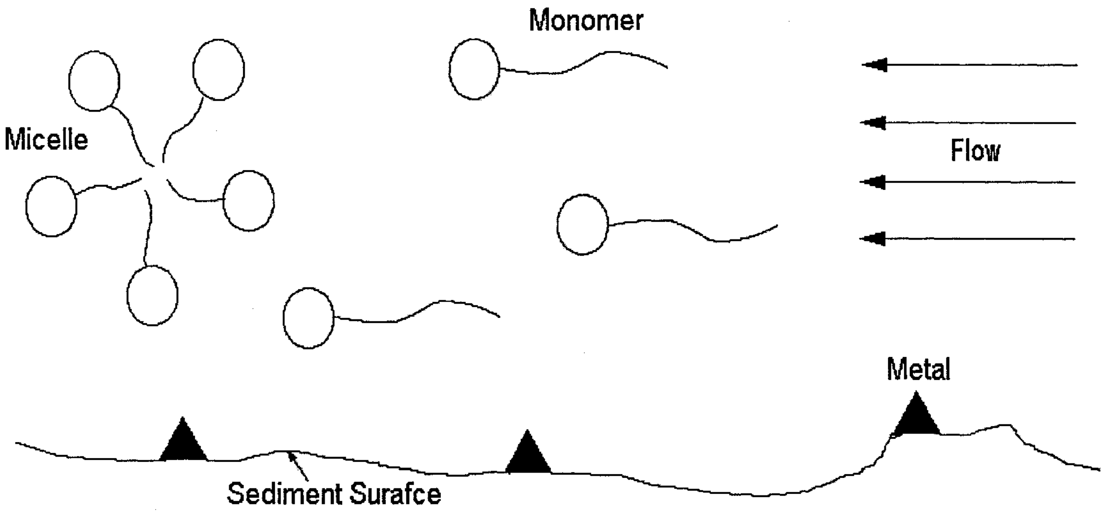
$\Sigma\Delta\gamma$ = change in surface interfacial energy

W_1, and W_2 were defined previously.

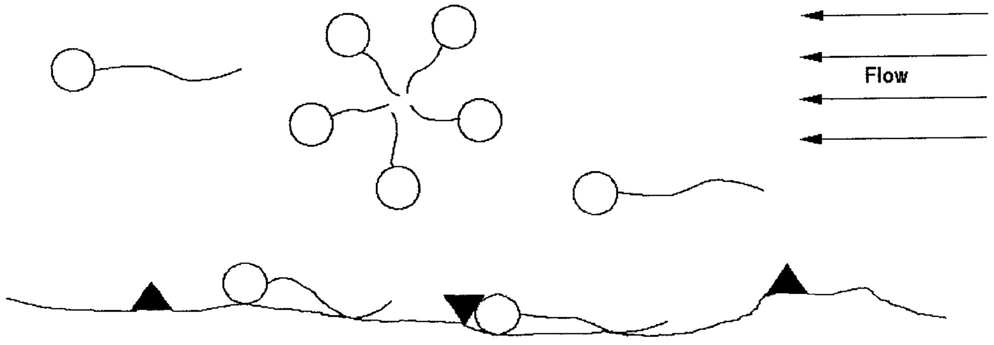
The rhamnolipid as a surfactant, reduces the interfacial tension between the metal and water (γ_{MW}) and soil and water (γ_{SW}) and consequently reduces the total work required for the removal of the metal from the surface of the sediment. Since this reduction occurs during the first work (W_1), it is more efficient.

SEM was also performed and the pictures of the surface clearly show that the removal by rhamnolipid does not affect the texture of the sediment. This indicates another advantage of rhamnolipid as a washing agent.

The mechanism of metal removal by rhamnolipid is illustrated in Figure 7-8.

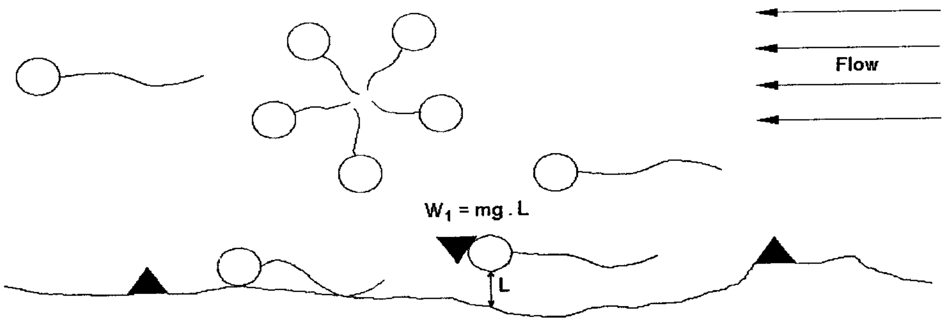


a. Introduction of rhamnolipid to the sediment.

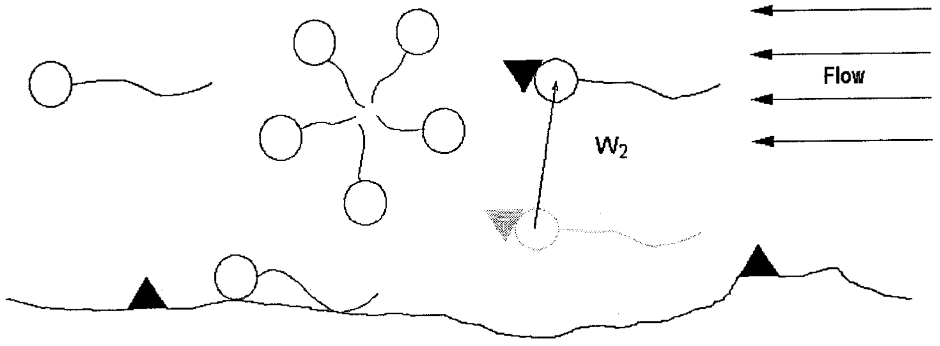


b. Wetting and initialization of the contact between rhamnolipid with metal and sediment.

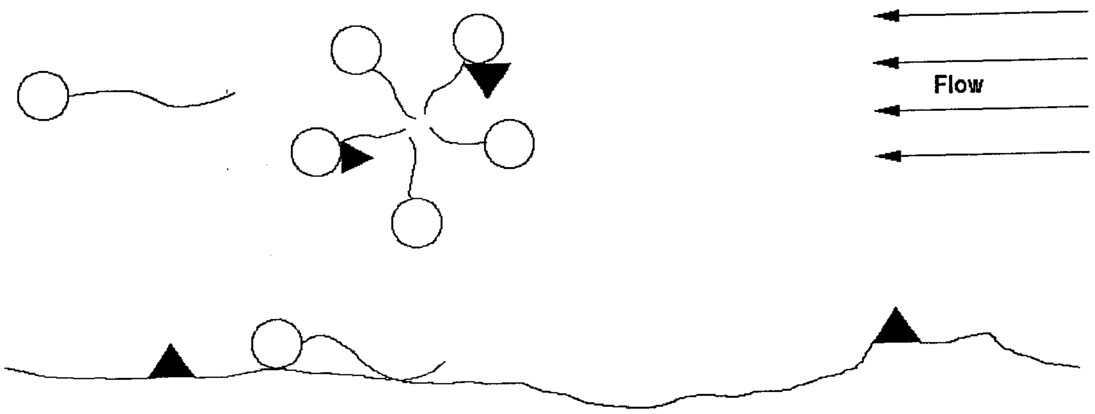
Figures 7-8 Proposed mechanism of removal of metal by rhamnolipid.



c. Detachment of metal (W_1) from the sediment after forming new bonds with rhamnolipid



d. Rhamnolipid monomer containing metal joins the bulk solution (W_2).



e. Formation of new micelles including the rhamnolipid monomer containing metal.

Figures 7-8 Proposed mechanism of removal of metal by rhamnolipid (cont'd).

7.7.3 Effect of NaOH in removal

The effect of NaOH was investigated through removal tests, SSE tests, ion exchange experiments and SEM. Removal tests showed that copper removal with rhamnolipid and NaOH has a higher performance compared with rhamnolipid or 1% NaOH alone. According to SSE, copper is mostly released from the organic fraction. SEM showed that NaOH can decrease the particle size. This finding confirms the statement by Sparks (2003) that in a solution of pH>8, humic substances dispersed to small aggregates less than 0.1 μm. This may explain the additional removal of copper with NaOH. In the case of zinc, the ion exchange experiment showed that the mechanism of removal is shifted to ion exchange from 17.8% to 67.1%. It also should be considered that the removal of zinc and nickel at pH 11, which the pH of 2% rhamnolipid and 1% NaOH, is much less than their removal in pH 6.5 which the pH of the solution of 2% rhamnolipid (Figures 4-5 and 4-6). The increment of pH justifies the decrease of total removal of Zn and Ni in the presence of NaOH in the rhamnolipid solution. The solubility of copper at pH 11 was higher than its solubility at pH 6.5 and the same trend was seen for the removal of copper in those solutions (Figure 4-4). All of this confirms the dependency between pH and removal of metals from the sediment.

NaOH contains two ions, Na^+ and OH^-. The effectiveness of NaOH on organic matter is due to OH^- that solubilizes the organic matter while Na^+ reduces the CMC of rhamnolipid solution which improves the effectiveness of surfactant (Myers 1988). This subject was investigated and detailed information is presented in Section 4-3-2. The comparison of the effects of Na^+ and K^+ on the removal of the heavy metals showed that K^+ has a better capability for enhancement of removal.

Chapter 8

Economical perspective of heavy metal removal by rhamnolipid

8.1 General remarks

Engineering projects, regardless of their nature, follow the same pattern of economic analysis. This pattern can be broken down into eight steps including problem definition, data collection, analysis, development of technically feasible alternatives, evaluation of alternatives, implementation, monitoring and adjustment (Bilodeau 2003). If unlimited funds are available, comparison between variables could be easily done but since one of the first limitations of each project is the cost, the economy of each variant should be analyzed precisely (Linsley and Franzini 1979). As the final step to study the feasibility of heavy metal removal from sediment using rhamnolipid, the subject was analyzed from an economical point of view. Based on the available literature (Anderson 1993) the comparison can be made with the available methods of soil washing and soil flushing currently used in the industry.

8.2 Economical aspects of using rhamnolipid as a soil washing agent

As indicated previously in this thesis, rhamnolipid has proven its abilities in removal of heavy metals from natural sediment. To study the economy of using rhamnolipid as a washing agent, a real project in which a contaminated land has undergone a soil washing process, Toronto Harbour Commission's (THC) soil recycling Demonstration Project was chosen for comparison (Anderson 1993 and EPA 1993). In this project, 4000 tonnes of Toronto Harbour Commission's (THC) soil was treated for 9 months with the total cost of $8,000,000. The characterization of heavy metal contaminated soil was different than the sediment used in this research. For example, the level of copper is higher (550 mg/kg) while the level of zinc is much lower (600 mg/kg) than the sediment (EPA 1993). To be able to compare this project with sediment washing using rhamnolipid as washing agent, it is assumed that all costs of treatment of the sediment excluding the washing agent are the same for the same quantity of sediment (4000 tonnes) with the THC soil treatment project.

The effect of inflation was taken into account based on Price Index of year 1992 when the project was started and completed (Anderson 1993, Bilodeau 2003). The Price Index of year 1992 was assumed to be 100 and based on the inflation rate reported by Statistics Canada; the Price Index for year 2004 was 125.4 (Statistic Canada 2005). According to Bergman USA (EPA 1995), the price of the surfactant is about 5.7% of the overall cost of the project. Since the total cost of project is about $8,000,000, the price of surfactant was limited to about $456,000 for treatment of 4000 tonnes of THC soil (Figure 8-1). The excavation and transportation cost were considered in this report. The costs are equal to the current price (year 2004) of $10,032,000 and $571,824 for the total cost and washing agent price, respectively.

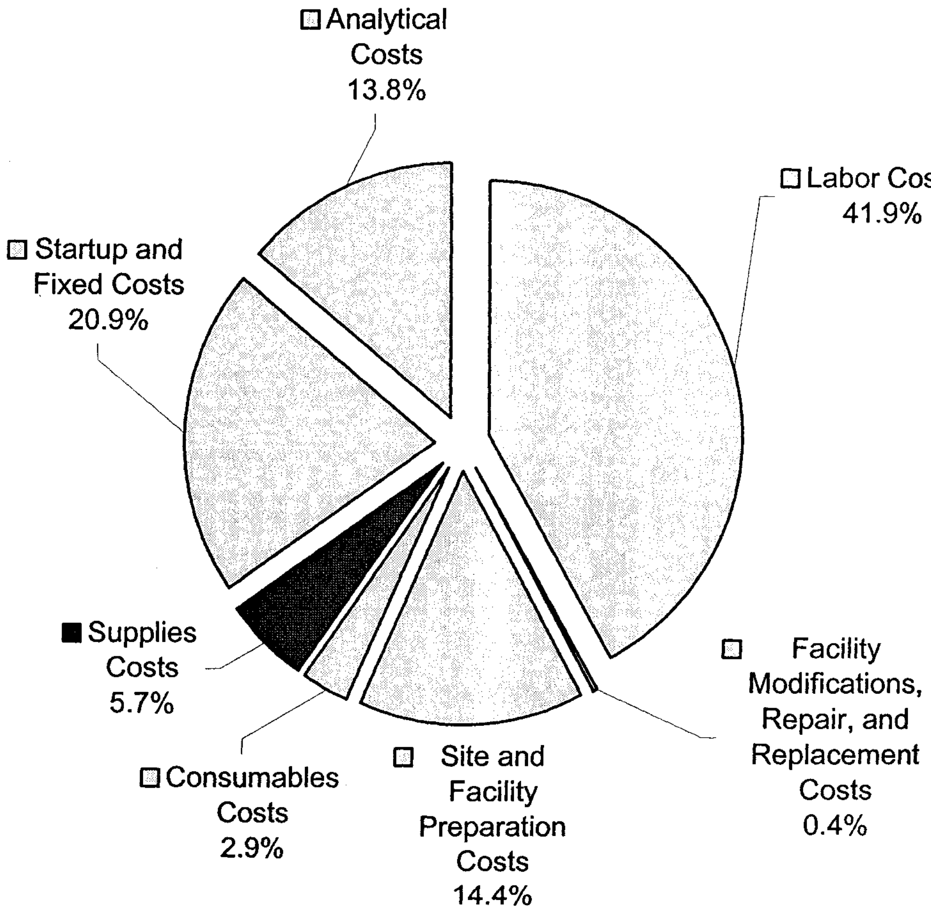


Figure 8-1 **Cost breakdown to elements of importance. Adapted from Bergman USA (EPA 1993).**

Among all the available data from different tests to study the removal of the heavy metals from sediment in batch and continuous flow configurations, since the concentration of 2% rhamnolipid in continuous flow configuration with flow rate of 0.5 mL/min was widely used in this research, the same concentration and configuration were selected to compare with the THC soil treatment project to perform the economical analysis. The results of the experiments for the study of adsorption also were used to determine the loss of effective surfactant due to adsorption.

According to the results of the tests on adsorption of rhamnolipid on the sediment (Figures 6-1 and 6-2), $2.88\text{x}10^5$ mg of 2% rhamnolipid in one period of experiment (3 days) were adsorbed to 1 kg of sediments. For a total amount of 4000 tonnes, the consumption of 2% rhamnolipid would be $1152\text{x}10^3$ kg or 57600 m^3. Using JBR215 this quantity will be 7680 m^3. This value should be added to the volume of surfactant needed for washing and can be considered as the loss of effective surfactant since adsorbed rhamnolipid cannot be reused.

According to the results from the removal tests, the ratio of washing agent to the sediment equal to 20 mL for 1g of soil was used, therefore for 1 tonne of soil, 20 m^3 of washing agent is needed. The rest of the calculations are as follows:

Total sediment weight =4,000 tonne= $4\text{x}10^6$ kg

Duration of project = 9 months x 30.5 days/month = 274.5 days

No. of days for each period of washing =3 days

No. of period of washings = 274.5/3 = 91.5 ~ 91 periods

Weight of washed soil in each period = $4x10^6/ 91$ = 43,956 kg/period= 43.956 tonne/period

Volume of 2% rhamnolipid used in one period of washing = 43.956(tonne)x 20 m^3

= 879.1 m^3 =879121 L

Volume of JBR 215= $7680x10^3$ (Adsorbed) + (879121 x (2/15))=$7.797x10^6$L

Price of rhamnolipid (JBR 215) per liter = \$4.86 US/L = \$6 CAN/L

Price of rhamnolipid (JBR 215) = $7.797x10^6$ (L) x 6 (\$/L)= M\$46.782

Total cost of project if rhamnolipid was used = $46.782x10^6$ + 10,032,000 - 571,824

=\$$56.243x10^6$

The increment of the price of washing agent: $\frac{46.782x10^6}{571824} = 81.8$ times

The increment of total price of the project: $\frac{56.243x10^6}{10{,}032{,}000} = 5.6$ times

This calculation may not be considered as an economical analysis for a real project, but it gives a rough estimation of the cost of the project if the rhamnolipid was used. According to these calculations, if the rhamnolipid is able to reduce the level of heavy metals down to an acceptable level for any specific purpose, the cost does increase drastically. The difference between the cases using rhamnolipid as a washing agent versus conventional washing agents for the total price of the project is not negligible (5.6 times more) and the difference between the price of washing agents is about 82 times more.

This economic study shows that to be able to use rhamnolipid for sediment washing in industrial scale, it needs more improvement in production and minimizing loss of effective surfactant, which opens new horizons in research and development of soil washing methods.

8.3 Discussion

Rhamnolipid is a safe product even used in the food industry (EPA 2004). The use of rhamnolipid for heavy metal removal from soil was investigated through batch tests (Mulligan et al. 1999, Herman et al. 1995, Dahrazma and Mulligan 2004) and in a column configuration in the present research. The economical analysis based on common methods used in engineering economy (Bilodeau 2003) was performed on an actual project, the THC soil. Based on the Price Index for Years 1992 and 2004 (Statistic Canada 2005), the new procedure using rhamnolipid was evaluated 5.6 times more expensive than the conventional materials for total cost. With the available data, it is not possible to make a quantitative comparison between the removals but it is clear that if the price of rhamnolipid decreases due to improvement in the cost of production and the recovery of rhamnolipid in industrial scale and also minimizing the loss of effective rhamnolipid, it can be a promising washing agent in the near future.

Chapter 9

Conclusions

9.1 Conclusions of the results of this research

The goal of this research was to perform a comprehensive study of the removal of copper, zinc, and nickel using rhamnolipid from a sediment sample taken from the Lachine Canal, Montreal, Canada.

The metal-contaminated sediment sample was obtained from the Lachine Canal area. To proceed with the experiment, the sample was air-dried and mixed fully to obtain a homogenous sample. X-ray analysis indicated the presence of 33.5% quartz, 38% feldspar, 5.4% chlorite, 18.5% amphibole, and 4.5% illite. The grain size distribution was as follows: 15% sand, 65% silt, and 20% clay. At the first step, the characterization of the sediment including organic matter and heavy metal contents, cation exchange capacity, pH, amorphous and carbonate contents, density, and specific surface area was determined. The results are summarized in Table 9.1. The CMC of the rhamnolipid, determined by the means of conductivity and surface tension measurement, is 0.035 g/L (Chapter 3).

Table 9.1 Characterization of the sediment sample

Parameter	Content
Chromium	145 mg/kg
Copper	140 mg/kg
Nickel	76 mg/kg
Lead	572 mg/kg
Zinc	4,854 mg/kg
Organic matter	20 %
Cation exchange capacity	14.55 meq / 100g
pH	6.4
Fe_2O_3	17800 mg/kg
Al_2O_3	11200 mg/kg
SiO_2	23400 mg/kg
Specific surface area	174.82 m^2/g

Sediment was washed with rhamnolipid in batch and continuous flow configurations although the focus of this research was on the tests with the continuous flow configuration since it simulates the *in situ* remediation of sediment. The effect of concentration of rhamnolipid, additives, pH, time, and ratio of mass of the sediment to the volume of rhamnolipid were studied in batch and continuous flow configurations. For continuous flow configuration, additional parameters including the effect of flow rate, porosity and hydraulic conductivity also were investigated. Increasing the concentration of the rhamnolipid enhanced the removal rate while adding 1% NaOH has a major improvement on removal of copper. Zinc and copper had a higher rate of removal over time, but nickel showed a parabolic shape of removal with a maximum. Removal of all metals was pH dependent. Increasing the flow rate generally reduced the removal in continuous flow configuration due to the reduction of the contact time between rhamnolipid and the sediment. Porosity and hydraulic conductivity also were found to be important parameters in the removal process since these parameters are related to the space between particles where the washing agent moves (Chapter 4).

To find the mobility and availability of metals in different fractions of the sediments, selective sequential extraction (SSE) tests were performed. By repeating these tests, after washing with rhamnolipid, the fractions of the sediment responsible for releasing the heavy metals under different conditions were identified. According to these tests, copper mostly existed in organic matter, nickel in the residual fraction and zinc was mostly found in the oxide fraction. SSE tests also showed that adding NaOH to rhamnolipid can radically enhance the removal of zinc from the exchangeable fraction and copper from the organic fraction while it had a negative effect on the removal of nickel from the carbonate fraction. Through SSE experiments, it also was determined that each metal has its own individual pattern of removal from the different fractions at various flow rates. Zn was released in a short time from the exchangeable fraction and gradually from the organic fraction. Nickel was released from all fractions but readsorbed onto the oxide and hydroxide fraction in the long term (Chapter 5).

Adsorption of the rhamnolipid on the sediment surface also was studied and a Freundlich isotherm was identified for the adsorption (Chapter 6).

The mechanism of removal of the heavy metals from the sediment using rhamnolipid was investigated through ion exchange tests, wetted surface area measurement, pH monitoring over time, zeta potential measurement, and scanning electron microscopy. Ion exchange experiments showed that complexation was the main mechanism of removal for Cu, Zn, and Ni. The ion exchange mechanism was responsible for removal of about 1/5 of the zinc by the rhamnolipid, while adding NaOH increased the share of this mechanism to 2/3. Monitoring pH over time illustrated a limited variation of pH. Wetted surface area showed to be an effective parameter in the removal process. Scanning electron microscopy indicated that washing the sediment with rhamnolipid does not change the texture of the soil but adding NaOH made a noticeable change on the texture of the sediment. Wetting, contact between the rhamnolipid and sediment surface, forming the new bonds between rhamnolipid and the metals, detachment of the metals from the sediment and displacement of metal into the bulk solution with the aid of mechanical means were found to be the major steps on the metal removal process (Chapter 7).

The economics of using rhamnolipid as a washing agent was analyzed and a rough estimation of the cost for a sample project was also determined. The total cost of the project, based on the assumptions of the analysis, showed a major increase of 5.6 times, while the price of washing agent was about 82 times more than that of conventional washing agents (Chapter 8).

9.2 Contribution to knowledge

The present research has made the following contributions to scientific knowledge:

- Applying rhamnolipid for sediment washing in continuous flow configuration to simulate *in situ* sediment remediation techniques.
- Determination of the fractions responsible for releasing the metals from sediments under different conditions in continuous flow configuration.
- Study of the adsorption of rhamnolipid to the sediment.
- Investigation of the mechanism of the heavy metal removal from the sediment by rhamnolipid using different approaches such as ion exchange experiments, zeta potential measurement and scanning electron microscopy.
- Pre-evaluation of the economic aspects of using rhamnolipid as a washing agent in sediment washing.

9.3 Suggestions for future studies

The author suggests the following topics for future research:

- Investigation of the effective parameters in micellization of rhamnolipid.
- Study of the interaction of metals with rhamnolipid micelles using small angle neutron scattering (SANS).
- Investigation of the feasibility of using a mixture of anionic biosurfactants in metal removal from soil and sediments.

- Study of the effect of the presence of oil and grease in the sediment on heavy metal removal process and mechanisms.
- Investigation on the adsorption of the rhamnolipid on soil and sediments to minimize this phenomena and consequently enhance the economics of the removal process.
- Develop a model for the transport of the metals by the rhamnolipid.

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