ASSESSMENT AND APPLICATION OF IMPORTED BIOMASS FOR THE BIOREMEDIATION OF HEAVY METAL EFFLUENTS

Report to the Water Research Commission

by

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EXECUTIVE SUMMARY

Assessment and Application of Imported Biomass for the Bioremediaton of Heavy Metal Effluents

BACKGROUND AND MOTIVATION

The presence of heavy metal ions in surface water continues to be the most pervasive environmental issue of present time. Heavy metal contamination exists in aqueous streams of many industries such as metal plating facilities, mining operations and tanneries. The release of heavy metal pollutants by the industrial sector into the sewage system has a negative impact on the environment in a number of ways. Water quality is affected, small quantities of metal become incorporated into the food chain and this is toxic to both humans and animals, and surface and groundwater can become contaminated due to leaching of metals (Bux *et al*, 1997).

Metal mining and metal processing activities inevitably increase the presence of toxic heavy metals in the environment. As a result removal of these toxic metals from the environment has become an important priority that is reflected in enforcement and a tightening of environmental regulations. Large volumes of industrial heavy metal-bearing wastewaters require efficient and cost effective treatment (Davis *et al*, 2001).

Treatment processes for metals contaminated waste streams include chemical precipitation, membrane filtration, ion exchange, column adsorption and co-precipitation adsorption. While the removal of toxic heavy metals from industrial wastewaters has been practised for several decades, the effectiveness, and particularly the cost effectiveness, of the most common physical-chemical processes is limited. Conventional treatment technologies such as precipitation and ion-exchange are either too expensive or are not effective in removing metals from the environment. Cost effective alternative technologies or sorbents for treatments of metal contaminated waste streams are needed. Natural materials that are available in large quantities or certain waste products from industrial or agricultural

operations may have potential as inexpensive sorbents. Various bio-materials have been examined for their biosorptive properties and different types of biomass have shown levels of metal uptake high enough to warrant further research (Figueira et al, 2000b). Biosorption is the process whereby metal is sequestered by chemical sites naturally present and even functional groups when the biomass is dead (Volesky, 2001).

This study is divided into three parts. The first part reports on the batch testing of the acquired biomass technologies performance, where the maximum metal uptake and mechanisms were determined. The second part reports on the behaviour of the immobilised biomass in a continuous packed bed laboratory scale reactor and the third part consisted of an on-site pilot scale study to determine field operating conditions and efficiencies.

AIMS

1. To determine whether technologies are available that would serve as biosorbents for the removal of heavy metals from pure solutions and industrial effluents.
2. To compare and quantify the heavy metal removal capacities of the acquired technologies.
3. To determine an affinity series, i.e., if there is any preferential binding for metal ion species by the technologies.
4. To assess any differences in biosorption of metals from synthetic metal effluent and industrial metal effluent.
5. To test technologies exhibiting superior heavy metal removal capacities in pilot-scale processes and determine the economic feasibility of using them.

SUMMARY OF RESULTS

Adams and Adams Patent Company conducted a patent search on our behalf. The research team also carried out literature searches using the Internet, Waterlit, Natal Technikon's Library Catalogue (OPAC), Biotechnology Abstracts, Sabinet and EBSCOhost. Of the seven technologies identified, the following three technologies were selected:

1. Column (~2 litres) of immobilized *Citrobacter* sp cells in hypol polyurethane foam in 0.85% saline from Isis Innovation Ltd- Oxford.
2. *Bacillus subtilis* species free cells isolated from metal bearing industrial effluent fromThapar Centre for Industrial Research & Development – India. The culture was revived in nutrient broth and agar. Gram stain and spore stain was done for confirmation.
3. *Chlamydomonas reinhardtii* for trace metal removal from Ohio State University – Ohio.

Bacillus subtilis and Citrobacter sp were revived in Nutrient agar and *Chlamydomonas reinhardtii in* TAP medium.

Batch experiments were carried out to determine adsorption of metal ions from synthetic single metal and mixed metal solutions and biosorptive capabilities of the three imported technologies were quantified to determine an affinity series of metal to the biomass. The efficiency series for *Bacillus subtilis* was $Cd^{2+} > Pb^{2+} \geq Cu^{2+} \geq Cr^{3+} > Ni^{2+} > Zn^{2+}, Cr^{6+}$ *Citrobacter* sp was $Cr^{3+} > Zn^{2+} > Cu^{2+} = Cd^{2+} = Ni^{2+} = Pb^{2+} > Cr^{6+}$ and for *Chlamydomonas reinhardtii* $Pb^{2+} > Ni^{2+} = Zn^{2+} > Cd^{2+} > Cu^{2+} > Cr^{3+} > Cr^{6+}$.

Mechanisms of metal interaction with the three biomass was also investigated to determine how metal ions were adsorbed onto the surface of the biomass. The simplest theoretical model for monolayer adsorption is the Langmuir Model. At low surface coverage, the Langmuir model reduces to a linear relationship of a calibration of the model by a simple regression of a linear form of the model (Mameri *et al*, 1999). This transformation yields adsorption constants b and q_{max}. The lower the b value the higher the affinity of the sorbent

for the species. *Citrobacter* had a b value of 8.097 for Cu^{2+} biosorption. *C. reinhardtii* showed high affinities for lead and zinc and the b values for these were 2.37 and 3.97 respectively. The shape of the Langmuir model indicates the type of bonding of metal ions to the surface of the biomass. The adsorption isotherms were mainly of the type I, IV and V for single metal solutions. The most common type for all three technologies was the 'L-shaped' or type I adsorption isotherm which indicates monolayer adsorption of metals. Isotherms for multi metal solutions were of the Type IV which indicates multi layer adsorption. The best bioremediating agent for all single metal solutions proved to be *Citrobacter* N14.

Laboratory assessment of the technologies was carried out by immobilising the biomass in polysulfone and packing this immobilised biomass into a stainless steel column. After equilibrium with the metal ions, the metal was desorbed using 0.2 N HCl to determine whether metals biosorbed were recoverable. HCl was found to be efficient at removing metals. *B.subtilis* exhibited high affinities for lead and cadmium, but could not remove chromium. *Citrobacter* removed all metals but could not differentiate between the metals in the first cycle of biosorption. *C. reinhardtii* did not perform well when immobilised in polysulfone and packed into a column. This technology was not used further.

Biosorption may have a potential advantage over other existing technologies for the treatment of effluent from electroplating industries. Once laboratory scale experiments were completed, potential biosorbents, ie *Bacillus subtilis* and *Citrobacter N14* immobilised into a stainless steel column, were transferred to industry in order to treat effluent from Ni^{2+}, Cr, Ag^{2+} and Cu^{2+} drag out baths at Natal Electroplaters. The nickel, chromium, copper and silver concentrations varied in the different baths. *B. subtilis* and *Citrobacter* both showed 5 reproducible cycles for nickel removal before a decrease in uptake capacity was observed. *B. subtilis* did not remove chromium, copper and silver. *Citrobacter* sp was capable of removing chromium in all cycles but uptake decreased from cycle 1 to cycle 5. The bacterium was unable to remove copper and silver.

The cost of current technologies used to treat electroplating effluent and the imported technologies was evaluated to determine which of these is more feasible to use. The cost for treating 1000 L of effluent at Natal Electroplaters is R495.00. The cost to treat the same

quantity of effluent using the imported technologies (including repacking and desorption) is R59 112.50. Start-up costs for the imported technologies is very high as compared to current conventional treatment.

The technologies have not proved themselves cost-efficient and it is not recommended that they be imported and used at this stage for metal plating effluent remediation.

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LIST OF ABBREVIATIONS

G^+ - Gram positive

G^- - Gram negative

AAS - Atomic absorption spectrophotometry

ppm - parts per million

SABS - South African Bureau of Standards

PBS - Phosphate Buffered Saline

BV - Bed Volumes

RBC - Rotating Biological Contractor

CSTR - Continuous flow stirred tank reactor

PUBLICATIONS AND CONFERENCES

Confidentiality Agreements were signed between the project leader and the companies from which the technologies were obtained. It is for this reason that none of the information presented in this report was presented at conferences or published as part of any journal.

CHAPTER 1 - INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL BACKGROUND AND INTRODUCTION

The contamination of surface and ground water by heavy metals as a result of the disposal of industrial and domestic waste has become a serious environmental problem (Nagase *et al*, 1997) Many industries, including mining and electroplating, discharge aqueous effluents containing relatively high levels of heavy metals. Untreated effluent from these manufacturing processes have an adverse impact on the environment (Malekzadeh *et al*, ND). Metallic species released into the environment by the technological activities of humans tend to persist indefinitely, circulating and eventually accumulating throughout the food chain (Volesky and Holan, 1995) , (Figueira *et al.* 2000a).

Important metals, due to their toxicity to the environment, are chromium, copper, lead, zinc, cadmium, nickel, mercury, aluminium, magnesium, cobalt and iron. Heavy metals are not biodegradable and tend to accumulate in living organisms, causing various diseases and disorders (Bailey *et al*, 1999).

Conventional treatment processes for metals include chemical precipitation, membrane filtration, ion exchange and adsorption. These existing technologies for the removal of heavy metals from effluents are hampered by a number of problems. Precipitation produces toxic sludge, activated carbon is associated with reusability problems, electro-winning is expensive to implement and can only be used for valuable metals and ion exchange has specificity problems. The common processes of precipitation, although effective for high concentrations of metal ions, often does not meet stringent effluent quality standards (Williams and Edyvean, 1999). In the City of Durban, no trade effluent is accepted discharged into water streams unless it complies with the bylaws regarding metal concentrations. These bylaws state that for a large operation (>25 Ml/d), the effluent shall not contain concentrations of Cu, Ni, Zn in excess of 50 $mg.L^{-1}$ and Pb, Cd and Cr in excess of 20 $mg.l^{-1}$. For small operations (<25Ml/d), the concentrations of these metals should not exceed 5 $mg.L^{-1}$ (Durban Metro, 2002).
Cost effective alternative technologies or sorbents for treatment of metals contaminated waste streams are needed (Bailey *et al*, 1999). The process of biosorption as a mechanism for

removing metal ions from effluents has shown much potential in the light of environmental legislation and concern over the cumulative toxicity of these pollutants in the environment (Williams and Edyvean, 1999). In recent years, the biosorption process has been studied extensively using microbial biomass as biosorbents for heavy metal removal. These biosorbents include species of bacteria, algae, fungi and yeasts. Unlike physical and chemical treatments, biosorption does not entail high operational costs and many potential sources of suitable biological material are cheaply and readily available (Costley and Wallis, 2001).

Biosorption, in which microbes are directed to accumulate metals from solution, has now evolved into a commercial process. Commercial organisations like B.V. Sorbex Inc., Biorecovery Systems and Advanced Mineral Technologies Inc. have developed biosorbents for a spectrum of heavy metals (Phillip *et al*, 2000). 'MetaGene' and RAHCO 'Bio-Beads' are two commercially available technologies that have undergone extensive laboratory and field trials in both electroplating and mining applications, and are effective at removing heavy metal ions (Bux *et al*, 1997).

1.2 LITERATURE REVIEW AND AIMS

Electroplating is said to be one of the most anti-ecological technologies in current use. It has an annual global production of approximately one cubic kilometre of toxic effluents, containing 5000 tons of heavy metals. Of the wide variety of chemicals used in the process baths, only a small percentage is incorporated in the finished product and the balance is released as waste (Bux, *et al*, 1997).

The appropriate selection of metals for biosorption studies is extremely important since the experimental volume increases exponentially with each additional metallic species. The following metals were chosen due to their occurrence in electroplating wastewater streams.

1.2.1. Lead

Lead is a component of galvanising materials and of many alloys such as solder, brass and bearing metals (International Occupational Safety and Health Information Centre, ND). Lead is extremely toxic; its effect on humans is cumulative. It enters the body either as inorganic lead

(Pb^{2+}) ion or as tetraethyl lead. Ingested or inhaled lead concentrates in the blood, tissues, and bones. Lead (Pb^{2+}) is a general metabolic poison and enzyme inhibitor. It is known to inhibit enzymes that catalyse the reactions for biosynthesis of haemoglobin (Lineone, ND). Earliest symptoms of lead poisoning are usually physical. Young children can suffer mental retardation and semi-permanent brain damage. The most insidious effect of inorganic lead is the ability of lead to replace calcium in the bones and remain there to form a semi-permanent reservoir for long-term release well after the initial absorption (Volesky 1990).

1.2.2 Cadmium

Cadmium is a component of some solder metals and baths used for electroplating (International Occupational Safety and Health Information Centre, ND). Due to its toxicity, cadmium is one of the heavy metals with the greatest potential hazard to humans and the environment. Discharge of cadmium into natural waters is derived partly from electroplating. If cadmium is ingested it accumulates in the kidneys. This causes malfunction of the kidneys and also replaces zinc in some enzymes, thus preventing them from functioning properly (Lee, 1994). Cadmium poisoning causes a disease known as "Itai-Itai" in Japan, which result in multiple fractures arising from osteomacia. Cadmium also tends to accumulate in the human body causing the production of proteinuria and kidney stones (Volesky 1990).

1.2.3 Copper

Copper is relatively soft and mixes with other metals to produce alloys like brass and bronzes (R & S Electroplating, 2001). Copper is one of the most common industrial metals. Absorption of excessive copper in humans results in "Wilson disease" in which copper is deposited in the brain, skin, liver and myocardium (Volesky 1990).

1.2.4 Chromium

Chromium is part of stainless steel and several other alloys, dyes, chrome plating, leather tanning and wood preserving (Lineone, ND) and is used in electroplating various metal parts, from electrical equipment to car parts (International Occupational Safety and Health Information Centre, ND). Hexavalent chromium (Cr^{6+}) is extremely toxic whereas Cr^{3+} is essential in our diet. Extensive use of chromium invariably results in the discharge of

chromium-containing effluent (Volesky, 1990). Long-term exposure to Cr^{6+} can lead to deterioration of the lungs, liver and kidneys that will eventually lead to death (Lineone, ND).

1.2.5 Nickel

Nickel is the most widely used electroplated deposit and its largest single application is a decorative finish in which the nickel deposit is followed by bright chromium (International Occupational Safety and Health Information Centre, ND). With the large number of industries using it, nickel-containing effluents are common. Elevated levels of nickel are harmful to the environment (Volesky, 1990). Nickel affects mostly the lungs, which leads to cancer of the lungs and sinuses (Lineone, ND).

1.2.6 Zinc

Zinc is used in electroplating and is excellent for rust-proofing of iron and steel products including castings, forging and sheet steel pressings (R & S Electroplating, 2001) Zinc is one of the most common elements in the earth's crust. Zinc compounds are widely used in industry to glaze sheet iron, as an ingredient for alloys, in dry cell batteries, etc. (Lineone, ND). Although zinc is an essential element in our diet, too much zinc is harmful. Inhalation of zinc fumes or ingestion of soluble salts could result in throat dryness, cough, weakness, general aching, chills, fever, nausea and vomiting. Long term effects of eating large amounts of zinc result in anaemia, pancreas damage and lower levels of high-density lipoprotein cholesterol (Lineone, ND).

1.3 CONVENTIONAL METHODS FOR INDUSTRIAL EFFLUENT TREATMENT

Traditional methods for the removal of heavy metals from waste water usually comprise physico-chemical processes such as chemical precipitation, ion exchange and activated carbon. They are commonly used but have significant disadvantages, such as incomplete metal removal, high reagent requirement, or the generation of toxic sludge that requires careful disposal (Jeffers *et al.*, 1993, Wilde and Bennemann, 1993).

Conventional treatment technologies like precipitation and coagulation become less effective and more expensive when metal concentrations are in the range of 1 - 100 mg/l. High costs, process complexity and low metal removal efficiency of membrane processes limit their use in

heavy metal removal. Adsorption of heavy metals onto activated carbon is a recognised method for removal, but the cost of the activated carbon limits its use (Kapoor and Viraraghavan, 1995). Activated carbon contains oxygenated surface groups that possess an acidic character and are capable of metal adsorption by ion-exchange. The performance of ion-exchange resins depends largely on pH (DOW Chemical Company, 1992). Ion-exchange is used in more processes in waste water treatment, as an add-on or polishing step in conjunction with traditional methods. The use of ion exchange resins solely for purifying wastewater is in most cases inappropriate because of the high cost of materials (Volesky, 1990). Furthermore, ion exchange resins are not always selective enough to allow an effective recovery of heavy metals present in the waste (Kratochvil *et al,* 1997).

Conventional ion exchange and metal extraction resins developed to date make use of ionic or metal chelating groups bound to the surface of a porous polymer support. Since the metal-binding functional groups are bound at the surface of the polymer, it is necessary for the exchanging ions in solution to diffuse through a stagnant boundary layer at the liquid-solid interface. This diffusion through the stagnant boundary layer is rate-limiting and ion exchange extractions of metals are slow and costly processes (Hammen, 1999). To avoid these limitations, bioremediation of metals should be considered, where adsorption occurs via covalent bond formation.

Heavy metals present at low concentrations cannot be eliminated by conventional treatments and the physico-chemical methods used for the purpose are too expensive (Solisio *et al*, 2000). New pre-treatment minimisation processes are therefore required to reduce heavy metal concentrations to environmentally acceptable levels at affordable costs.

1.4 BIOLOGICAL METAL EFFLUENT TREATMENT METHODS

The search for new technologies has focused on the metal binding capacities of various microorganisms such as bacteria, fungi and algae (Volesky, 1990, Gadd, 1992, Jeffers *et al*., 1993). Various types of inactivated, non-living microbial biomass can be considered potential biosorbent materials either for concentration and recovery of valuable metals from process streams or for the removal of toxic heavy metals from liquid wastes (Brierley, 1990b, Volesky,

1990, Hu and Reeves, 1997). It was demonstrated by Volesky (1990) and Muraleedharan *et al*. (1991) that these non-living types of biomass can retain high quantities of metal ions by passive sorption and/or complexation. This is commonly known as biosorption.

An important feature of biosorption is that it can be responsible for binding and accumulating metallic species even when the cell is no longer metabolically active. When dead and no longer metabolically active, such biomass represents the biosorbent (Volesky, 1990).

Volesky (1990) and Remacle (1990) revealed that microorganisms (biomass) can be highly selective, effective, and cheap, competing with commercial ion-exchange resins and activated carbons in a process arrangement almost identical to that used for these conventional materials. While ion exchange can be considered a 'mature' technology, biosorption is in its early developmental stages and further improvements in both performance and costs can be expected. Biosorptive processes have been shown to have several advantages over existing physical and chemical treatments because they have a high efficiency at low metal concentration and operate successfully over a broad pH and temperature range (Kuyucak and Volesky,1990; Gadd, 1992).

1.5 BIOLOGICAL MECHANISMS OF METAL BIOSORPTION

The biosorption of heavy metals occurs in two phases: a fast (< 4 seconds) surface reaction, which can also occur in dead cells and is a metabolism-independent binding or adsorption to cell walls and other external surfaces, and the second, slower phase of metal uptake (2 hours) which is metabolism-dependent transport across the cell membrane (Gadd, 1992). The first phase is due to surface adsorption, based mainly on anion exchange with the participation of the carboxyl groups of uronic acids. The second phase represents the diffusion of ions into the cell structure (Volesky and Holan, 1995).

1.5.1 Active Immobilisation

Active immobilisation involves the transformation of metals by metal-microbe interactions that are carried out by living, metabolically active microorganisms (Brierley, 1990a). This mode of metal accumulation by living cells is usually referred to as bioaccumulation (Volesky, 1990).

1.5.1.1 Metal precipitation

Metals are actively precipitated when living bacteria produce and excrete a substance that chemically reacts with metals present in the solution to produce an insoluble compound. *Bacillus subtilis*, a sulphate-reducing bacterium produces hydrogen sulphide (H_2S), which reacts with the metals to produce insoluble metal sulphides. Similarly, *Citrobacter* sp., a phosphatase-producing bacterium, precipitates metals from solution by HPO_4^{2-} production (Brierley, 1990b). This leads to undesirable sludge production.

1.5.1.2 Transport across the cell membrane

Veglio and Beolchini, 1997 confirmed Gadd's postulate (1992) that living organisms take up metals in two steps. First, the metal passively binds to the cell wall. Second, the metals are actively transported across the cell membrane into the cell.

1.5.2 Passive Immobilisation

Passive immobilisation of metals occurs when a substance produced and excreted by a microbial cell chelates solubilised metal, or when the metal binds to a cell surface by physical-chemical reactions (Brierley, 1990a). Mechanisms for inactive immobilisation of metals include precipitation (extracellular accumulation or cell surface accumulation) and intracellular accumulation (Brierley, 1990a).

1.5.2.1 Metal precipitation

Precipitation based on a metabolism independent mechanism is a consequence of chemical interaction between the metal and the cell surface (Veligio and Beolchini, 1997).

1.5.2.2 Complexation

Nitrogen and oxygen ligands in the cell wall contribute to the complexation of transition-metal ions and could bind to a single ligand or through chelation (Volesky, 1990).

1.5.2.3 Adsorption

Kuyucak and Volesky (1990) hypothesised that metal biosorption by dead biomass takes place through electrostatic interactions between the ions in solution and the cell wall. Most metal binding occurs after initial metal complexation and neutralisation of the chemically active sites.

Binding to the cell wall may proceed through at least a two-step mechanism, the first step is the interaction of metal with reactive chemical groups followed by a second stage in which the same sites nucleate the deposition of more metal as a chemical precipitate (Urrutia, 1997).

1.5.2.4 Microbial ion exchange

The cell wall contains a mixture of monovalent and divalent cations and displacement of one cation by another is dependant on the strength of the individual ligand complexes (Volesky, 1990).

1.6 SUITABILITY OF MICROBIAL CELL WALLS FOR METAL BINDING

Microbial cells of bacteria, fungi, and algae have a distinct barrier, the cell wall, on the outside of the cytoplasmic membrane (Remacle, 1990). Metal biosorption is understood to occur through a passive mechanism that involves electrostatic interactions between the negatively charged groups in the cell wall and the metal cations (Brierley, 1990a, Remacle, 1990).

Metal elution studies by Volesky, (1990), showed that it was possible to achieve significant concentration of the metallic species sorbed when it was released from the biosorbent in a much smaller volume of eluent solution. This process is know as desorption. Recovery of many metals of value from these concentrates can be easily accomplished using metal-winning technologies.

Living microbial cells have not been successful in the treatment of the solutions containing high concentrations of heavy metals. The microbial cell's metabolism is disturbed thus causing the organism to die. This problem does not exist with the non-living organisms and the biological materials derived from the organisms, which are used to absorb metal ions from the solution (Eccles, 1995).

1.7 THE BACTERIAL CELL WALL AND ITS INVOLVEMENT IN BIOSORPTION

There are two basic types of bacterial cell wall structures, Gram positive (G^+) and Gram negative (G^-). G^+ cells appear purple when Gram stained and G^- cells are red when stained.

1.7.1 Gram positive bacterial cell wall

While most bacteria possess a cell wall structure to bind metals, the Gram positive bacteria, especially members of the genus *Bacillus* have enhanced capacity for metal binding because of a thick network of peptidoglycan with attached macromolecules (Brierley, 1990b). Peptidoglycan is a porous, rigid material that is usually 50 to 150 nm thick (Remacle, 1990).

In the G^+ cell walls, techoic acid and teichuronic acid are attached to the peptidoglycan network and this gives the surfaces a negative charge density. These negatively charged groups in the cell wall interact electrostatically with the metal cations thus immobilising the metal (Brierley, 1990b, Remacle, 1990.) Phosphodiesters of the teichoic acid and the carboxylate groups of the teichuronic acid contribute ion-exchange capacity to the cell wall. This is the primary mechanism for metal binding to the cell wall (Brierley, 1990b).

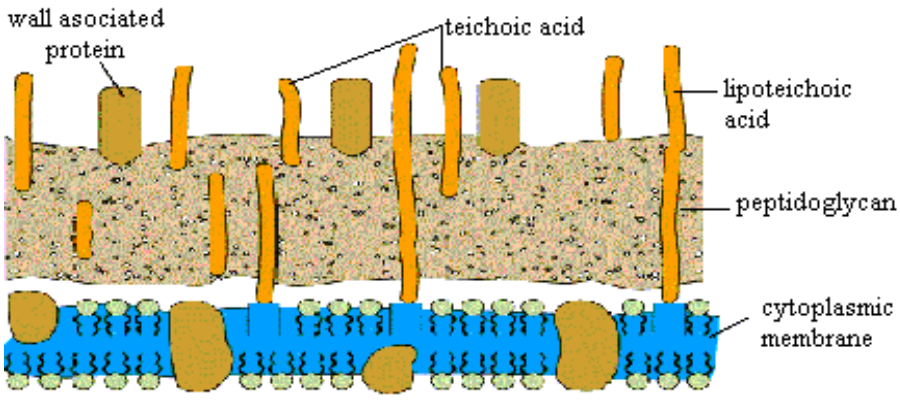


Figure 1.1 Cell wall structure of a Gram positive bacterium.

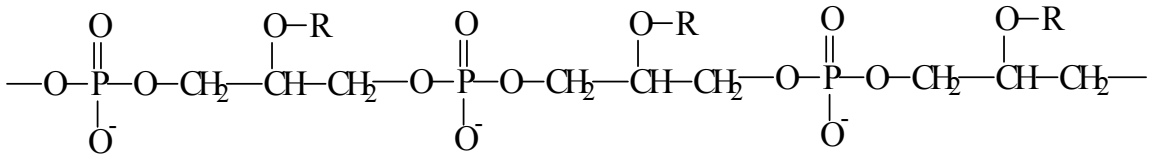


Figure 1.2 Techoic acid is a polymer of glycerol or ribitol joined by phosphorus groups.

1.7.2 Gram negative bacterial cell wall

Gram negative cell walls have a more complicated structure than the Gram positive cells. There are two separate areas with an additional membrane besides the cellular membrane. Techoic acid and teichuronic acid are not attached to the peptidoglycan network so a negative charge density does not exist. Biosorption would be expected to be very low. However, working with a typical *Citrobacter* sp., Macaskie and co-workers (2000) demonstrated that heavy metals were accumulated as cell-bound insoluble metal phosphate. Uptake is contributed to the activity of and acid-type phosphatase that releases phosphate ligand stoichiometrically with the cleavage of a supplied phosphate donor molecule (substrate). This enzyme activity was demonstrated to persist in non-growing immobilised cells and was manipulated to remove metals from waste streams.

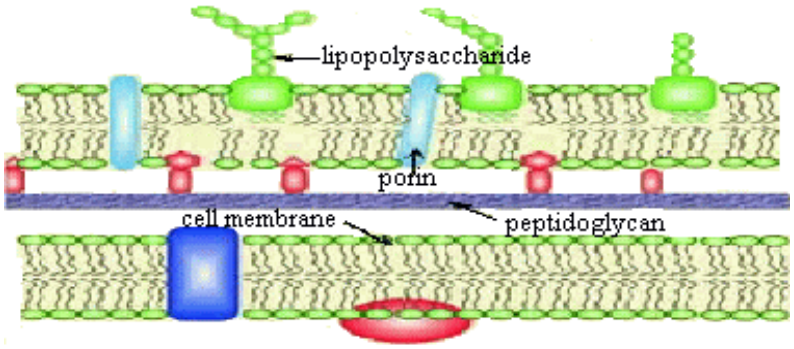


Figure 1.3 Cell wall structure of a Gram negative bacterium.

Binding of metals to the cell walls of *Bacillus subtilis* is a two-step mechanism. The first step is the stoichiometric interaction of the metal with reactive chemical group, followed by a second stage in which those same sites nucleate the deposition of more metal as a chemical precipitate, which results in the development of fine-grained minerals. This initial interaction is the adsorption phase, but metal retention ability by bacterial walls goes further than their adsorption capacity, since bacterial surfaces are favourable interfaces for mineral nucleation (Forster & Wase, 1997).

Bacteria, e.g. *Bacillus* sp. and *Citrobacter* N14, provide a potent biomass for the immobilisation of metals actively or passively. Working with *Bacillus*, Doyle and co-workers (1980) cited by

(Forster and Wase, 1997) showed that Na^{+}, Ca^{2+}, Mn^{2+}, Ni^{+}, Sr^{2+}, Zn^{2+} and Mg^{2+} all bind to the same sites on the walls and that a direct competition for these sites occurs when two or more metals are present. According to Brierley, 1990, the microorganism is not selective in the metal it sorbs; rather, it simultaneously removes several different toxic and heavy metal cations from solution, regardless of their differing concentration. However, Figueira *et al*. (2000b) found that metals, eg. Cd^{2+}, have different affinities for biosorbents tof algae, *Durvillaea, Laminaria* and *Ecklonia,* that results in varying degree of uptake.

Many studies conducted on metal sorption by bacteria have shown that isolated walls from G^{+} organisms bind quantitatively more metal than isolated envelopes of non-capsulated G^{-} bacteria. Typically ten times more metal is bound to G^{+} walls than to G^{-} envelopes (Urrutia, 1997). However, working with whole cells, Mullen and co-workers (1989) did not find significant differences in the ability of two G^{-} and two G^{+} bacteria to bind Cd, Cu, Ag and La. The latter authors claimed that intact cells are much more chemically complex than purified walls or envelopes, and thus may show differences in metal binding capacity, particularly in the case of the G^{-} variety (Forster and Wase, 1997).

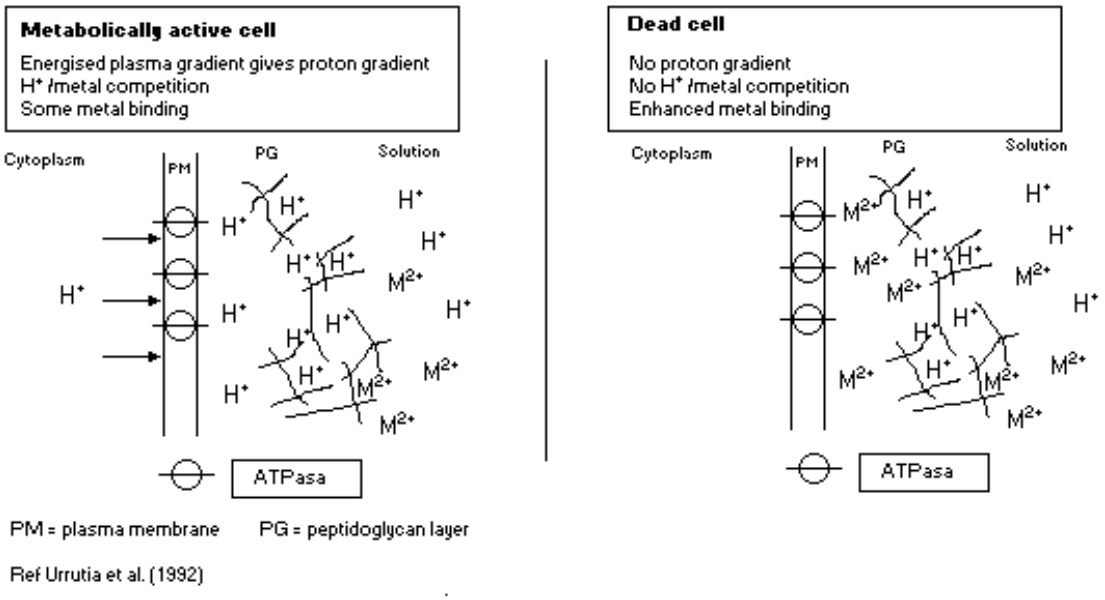


Figure 1.4 Influence of a protonated cell wall on metal binding by *Bacillus subtilis*.

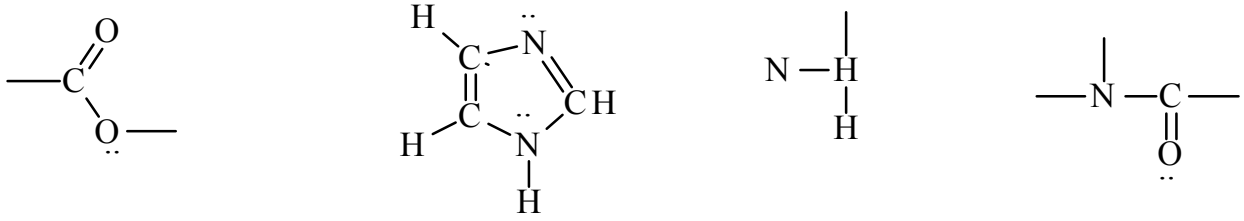
Urrutia (1997) explained that gamma-irradiated or chemically uncoupled cells bind more metal than active metabolising cells with energised plasma membranes because of the competition between the H^+ ions generated by an energised membrane (in metabolising cells) and the metal cations in solution. In a similar way, isolated walls, obtained after several chemical treatments, are probably free of their own counter ions (e.g. protons) and may subsequently bind more metal than intact walls of live cells. Greater metal binding by dead cells in relation to their living counterparts has been observed experimentally on other occasions (Forster and Wase, 1997).

1.8 THE ALGAL CELL WALL

The cell walls of algae are porous and allow for molecules and ions to pass freely through. However, the cell membrane is permeable to neutral molecules but not to ions (Kuyucak and Volesky, 1990). In addition to the porosity of the algal cell wall structure, the constituents can provide an array of chemical ligands and binding functional groups to take up metal ions (Volesky and Holan,1995).

1.8.1 Mechanisms of Algal Biosorption

Biosorption of heavy metals by algal biomass can be considered as rapid metabolism-independent ionic and covalent binding to a particular structure of the cell's surface. Precipitation of the metals may occur within and around cell walls (Garnham, 1997). As in bacterial biosorption, active metal uptake cannot be considered biosorption but rather bioaccumulation.



Carboxyl Imidiazol Amine Amide

Figure 1.5 Protein functional groups involved in biosorption of metals by algae.

Garnham, 1997, using Cobalt accumulation on *Chlorella salina* described the mechanism of metal uptake as consisting of two phases: a rapid metabolism-independent phase were binding occurs on the cell surfaces (biosorption) followed by a slower phase due to active or intercellular uptake by passive diffusion and is dependent on light. Gadd, 1992 and Vegio and Beolchini, 1997, made a similar observation.

The proteins in the cell wall provide functional groups (Figure 1.5) for metal binding. Covalent bonding could be expected with amino and carboxyl groups and ionic bonding with carboxyl and sulphate groups associated with these components (Garnham, 1997).

1.9 pH EFFECTS ON METAL SPECIATION AND MICROBIAL CELL WALLS

An increase in pH increases the negative charge at the surface of the cells until all relevant functional groups are deprotonated, which favour electrochemical attraction and adsorption of cations. Also, increase in metal uptake with an increase in pH may be the result of more efficient competition of cations with H^+ for binding sites on bacteria (Lopez *et al*, 2001).

1.10 SUMMARY

Agricultural runoff, industrial and domestic effluent and acid mine drainage, electroplating metal industries have all greatly contributed to the metal loads in our natural water systems. As a result of their toxicity as well as increasing value of some metals and also a greater awareness of the ecological effects of toxic metals released into the environment (Muraleedharan *et al*, 1991).

Precipitation, ion exchange, electrochemical and/or membrane processes are commonly applied to the treatment of industrial effluents. However, the application of such processes is sometimes restricted because of technical or economic constraints. The search for new technologies for the removal of toxic metals from waste waters has directed attention to biosorption, based on the metal binding capacities of biological material (Veglio and Beolchini, 1997).

The lack of information on biosorption in columns is partially due to the fact that the metal uptake by different biosorbent materials is not fully understood and partially because most biomass have to be immobilised, granulated or processed before their use in a column (Kratochvil *et al*, 1997). This study therefore focuses on bacterial cell masses, *Bacillus subtilis* and *Citrobacter* N14, and an algal biomass, *Chlamydomonas reinhardtii* that have been immobilised in polysulfone resin to form a nonliving granular product and packed into columns for removal of metals from wastewaters. An introduction to the various types of metal uptake by the cells will also be discussed.

1.11 RESEARCH AIMS

1. To identify and acquire technologies with metal remediating capabilities.
2. To compare and quantify the heavy metal removal capacities of the acquired technologies.
3. To determine an affinity series, i.e., if there is any preferential binding for metal

ion species by the technologies.

4. To assess any differences in biosorption of metals from synthetic metal effluent and industrial metal effluent.

5. To select technologies exhibiting superior heavy metal removal capacities for pilot-scale processes and determine the feasibility of using them.

CHAPTER 2 LABORATORY-SCALE ASSESSMENT OF BIOSORBENT TECHNOLOGIES (FREE CELL BATCH BIOSORPTION)

2.1 INTRODUCTION

Biosorption of metals consists of several mechanisms, mainly ion exchange, chelation, adsorption by physical forces and ion entrapment. Also, several chemical groups are involved in attracting and sequestering metals from solution. Despite the simple and well-established foundations of the sorption process, there seems to be a great deal of confusion in evaluating experimental results. Examination of the sorption reactions is based on equilibrium batch tests and continuous flow sorption studies using two very widely used equilibrium adsorption isotherms models, viz. Langmuir and Freundlich Models. The basic evaluation of sorption systems relies on the classical sorption isotherms derived from equilibrium batch contact experiments carried out under controlled environmental conditions (Volesky and Holan, 1995).

2.1.1 Batch Biosorption Studies

Batch biosorption studies are essential for adsorption isotherm modelling. By convention, the quantity of metal bound per unit weight of adsorbent q, is expressed as a function of C, the concentration of solute remaining in solution at equilibrium at a fixed temperature (Bux *et al.*, 1994). The unit q can be expressed in the forms $mg.g^{-1}$, ：$g.g^{-1}$, $mmol.g^{-1}$ and $mequivalent.g^{-1}$. For the purpose of engineering process evaluation, the most common form is $mg.g^{-1}$.

2.1.2 Adsorption Isotherms

Adsorption is often described in terms of isotherms, which show the relationship between the bulk aqueous phase activity of an adsorbate and the amount adsorbed at constant temperature (Mameri *et al*, 1999)

The simplest theoretical model for monolayer adsorption is due to Langmuir. The Langmuir model was originally developed to represent chemisorption on a set of distinct localised sites.

The assumptions on which the model is based are:

i. Molecules are adsorbed at a fixed number of well-defined localised sites.

ii. Each site can hold one adsorbate molecule.

iii. All sites are energetically equivalent.

iv. There is no interaction between molecules adsorbed on neighbouring sites.

The Langmuir model was initially developed for systems in which sorption leads to the deposition of a single layer of solute molecules on the surface of a sorbent. At low surface coverage, the Langmuir isotherm reduces to a linear relationship. Calibration of the model to a set of experimental data can be accomplished by a simple regression of a linear form of the model (Mameri *et al*, 1999). The Langmuir isotherm can be mathematically expressed as:

$$q=qmax\frac{bC_f}{1+bC_f}$$

$$q = \frac{\text{number of adsorption sites occupied}}{\text{number of adsorption sites available}} = \frac{q}{q_{max}}$$

where q_{max} is the maximum sorbate uptake under the given conditions

b is a coefficient related to the affinity between the sorbent and the sorbate

θ is the fractional coverage

The Langmuir relationship can be linearised by plotting either (1/q) vs. ($1/C_f$) or (C_f/q) vs. (C_f). The latter were chosen because it exaggerates deviation from the fitted equations and is a more stringent linearisation.

2.1.2.1 Linearisation of Adsorption Isotherms

2.1.2.1.1. *Langmuir* Isotherm

Fractional coverage, $\theta = \frac{q}{q_{max}} = \frac{bC_f}{1+bC_f}$ … Langmuir (modified hyperbola Type 1)

$$q = \frac{q_{max} bC_f}{1+bC_f}$$

$$q + qbC_f = q_{max} bC_f$$

$$\frac{q}{bC_f} + q = q_{max} \text{ … divide by } bC_f$$

$$q\left(\frac{1}{bC_f} + 1\right) = q_{max}$$

$$q = q_{max}(bC_f + 1)$$

$$\frac{q}{C_f} = \frac{q_{max}(bC_f + 1)}{C_f} \text{ … divide by } C_f$$

$$\frac{q}{C_f} = \frac{q_{max} bC_f}{C_f} + \frac{q_{max}}{C_f}$$

$$\frac{q}{C_f} = q_{max} b + \frac{q_{max}}{C_f}$$

$$\frac{q}{C_f} = q_{max} \frac{1}{C_f} + q_{max} b \text{ … straight line } (y = mx + c)$$

Therefore a plot of $\frac{q}{C_f}$ against $\frac{1}{C_f}$ yields a straight line:

With:

$$y = \frac{q}{C_f}$$

$m = q_{max}$ …….gradient

$$x = \frac{1}{C_f}$$

$c = q_{max}b$ …… y intercept

According to Volesky (1999), the lower the b parameter, the higher the affinity of the sorbent for the sorbed species, i.e. the higher q_{max}, the lower the b value. Generally, 'good' sorbents possess a steep initial sorption isotherm slope and a high q_{max} as indicated by a low Langmuir parameter, b. Langmuir's 'b' relates to affinity between the sorbent and the sorbate (metal). The lower the value of b the higher the affinity (Volesky, 2001).

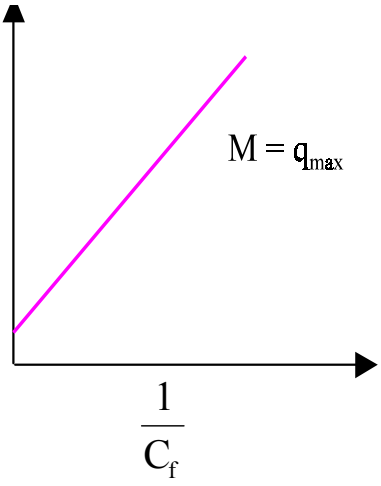
2.1.2.1.2. *Freundlich* Model

$q = kC_f^{1/n}$ … Freundlich

Where k and n are constants.

$\log (q) = \log (kC_f$

$= \frac{1}{n} \log (kC_f)$



$$\log (q) = \frac{1}{n} \log (C_f) + \frac{1}{n} \log (k) \ldots \text{straight line } (y = mx + c)$$

The validity of the *Langmuir* and *Freundlich* adsorption isotherm models was tested for each metal on each biosorbent by plotting their relevant straight-line equations. The Langmuir model is preferred because it contains the two parameters (q_{max} and b) that can be used to characterise the adsorption system.

Brunauer *et al*., 1940 (cited by Ruthven, 1984) have classified the isotherms for physical adsorption into five classes:

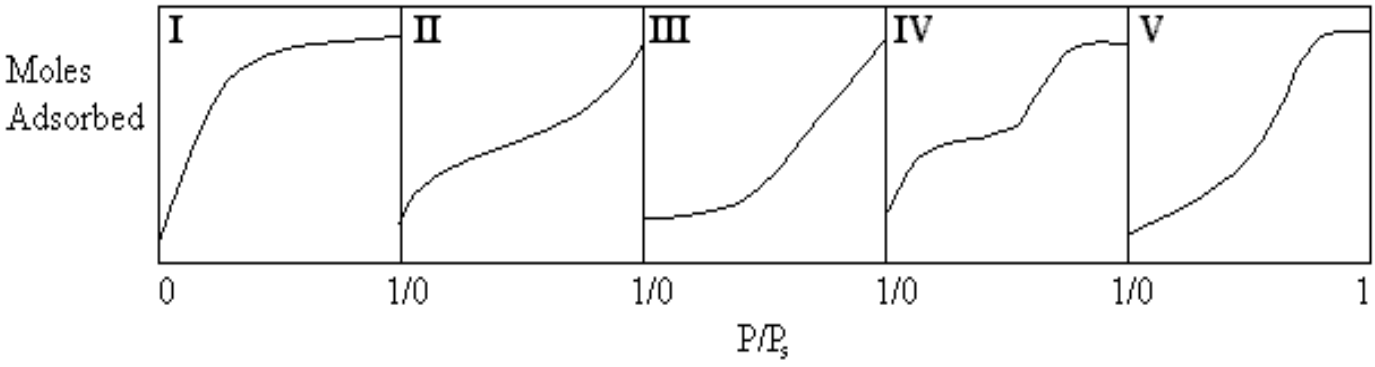


Figure 2.1 Brunauer classification of adsorption isotherms (Ruthven *et al.,* 1984)

Isotherms for true micro porous absorbents in which the pore size is not very much larger than the molecular diameter of the sorbate molecule are normally of type I. This is because for such adsorbents there are definite saturation limits corresponding to complete filling of the micropores. If intermolecular effects are large, an isotherm of type V is observed. An adsorption isotherm of type IV suggests the formation of two surface layers either on a plane surface or on the wall of a pore very much wider than the molecular diameter of the sorbate. Isotherms of type

II and III are only observed in adsorbents containing a wide range of pore sizes (Ruthven, 1984).

The Brunauer Emmet and Teller (BET) model represents isotherms reflecting apparent multilayer adsorption. The BET isotherm is characterised by the equation:

$$\frac{BCq_{max}}{(C_s - C)[1 + (B - 1)(C/C_s)]}$$

where: B = constant expressive of energy of interaction with a surface

C_s= saturation concentrating of solute in solution at given temperature.

This equation can be converted to Langmuir form if:

i. b is equal to $\frac{B}{C_s}$

ii. C is taken to be negligible small compared to C_s

iii. B is greater than one

The extent of removal of heavy metals from wastewater by biosorption depends on the multi-metal competitive interactions in solution with the sorbent material. However, almost all biosorption studies reported so far have been based on one metal solution. Chong and Volesky (1995) have attempted a two-metal quantitative description of multi metal biosorption. In two-metal batch biosorption studies, the results cannot be extrapolated and no predictive conclusions can be made. Also, the effect of the second metal is usually reported based on its starting initial concentration. This is inappropriate because the biosorbent is exposed to the initial concentrations for only a very brief period of time. At equilibrium, which is the predominant state of sorption systems examined, the sorbent material is exposed to the final concentration, not the initial concentration, so the equilibrium (final) concentration has to be

known if equilibrium conditions are being compared (Chong and Volesky, 1995).

2.1.3 Objectives

1. To identify all available bioremediation technologies available via a patent and literature search.
2. To select technologies claiming superior heavy metal removal capacities for batch-scale processes.
3. To determine the metal biosorptive ability of the imported technologies from synthetic metal effluent and industrial metal effluent in batch scale
4. To assess differences in biosorption of metals by plotting the adsorption isotherms and determining related constants q_e and b.

2.2 METHODS

2.2.1 Acquisition of Technologies

Adams and Adams conducted a patent search on our behalf. The research team also carried out literature searches using the Internet, Waterlit, Biotechnology Abstracts, Sabinet and EBSCOhost.

From the search carried out by Adams and Adams, the following technologies were identified:

1. *Citrobacter* from Isis Innovation Ltd, Oxford, USA.
2. *Bacillus subtilis* from Thapar Centre for Industrial Research & Development, Patiala, India.
3. *Chlamydomonas reinhardtii* from Ohio State University, Columbus, USA
4. Sulphate reducing bacteria from Auburn University, USA
5. *Pseudomonas aeruginosa* from Oak Ridge National Lab.
6. Biosorbent granules from Agharkar Research Institute, India
7. Technology based on filter from plant implemented as a resin based ion exchange system from Soreq Development Corporation., Yavne, Israel

The following technologies were selected:

1. Column (~2 L) of immobilized *Citrobacter* cells in hypol polyurethane foam in 0.85% saline from Isis Innovation Ltd- Oxford. Arrived: 10 February 2000.
2. *Bacillus subtilis* species free cells isolated from metal bearing industrial effluent from Thapar Centre for Industrial Research & Development – India. The culture was revived in nutrient broth and agar. Gram stain and spore stain was done for confirmation. Arrived: 23 January 2000.
3. *Chlamydomonas reinhardtii* for trace metal removal from Ohio State University – Ohio. Arrived: 21 August 2000.

The following technologies were not selected:

1. Sulphate reducing bacteria from Auburn University was not appropriate for the metals of interest.
2. The start up cost of *Pseudomonas aeruginosa* from Oak Ridge National Lab was too expensive (US$ 50 000- 80 000) as production had been stopped previously.
3. Biosorbent granules from Agharkar Research Institute were too expensive (US$ 20000).
4. Soreq Development Corporation wanted to collaborate on a separate project and be paid as a project consultant and was not interested in releasing the technology to us on any other basis.

2.2.2 Preparation of Synthetic Effluents

A 1 000 mg.L^{-1} metal solution was prepared by weighing out the appropriate weight of the metal salt, and dissolving this in 1 000 mL of deionised water. Appropriate quantities were prepared before use for each experiment. Metal salts that were used during experimentation included K_2CrO_4 (Potassium chromate), (Merck, South Africa), $Zn(NO_3)_2$ (Zinc nitrate), (Associated Chemical Enterprises, South Africa), $Cu(NO_3)_2.3H_2O$ (Copper nitrate), (Associated Chemical Enterprises, South Africa), $Ni(NO_3)_2.6H_20$ (Nickel nitrate), (Associated Chemical Enterprises, South Africa), $Pb(NO_3)_2$ (Lead nitrate), (Associated Chemical Enterprises, $Cd(NO_3)_2$ (Cadmium nitrate), (Associated Chemical Enterprises, South Africa) and $Cr(NO_3)_2.9H_2O$ (Chromium nitrate), (Saarchem, South Africa).

2.2.3 Collection of Synthetic Effluents and Analysis of Metal Content

Effluent was obtained from Saayman Danks Electroplaters and Natal Electroplating in 20 L plastic containers and stored at 20 ^{0}C. Effluents were brought to room temperature before use. Heavy metals analysed for included Zn^{2+}, Cd^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}, Cr^{3+}, and Cr^{6+}. Results were expressed as the mean of the experiment done in triplicate and the standard deviations determined.

Total Chromium content was determined from AAS analysis. Hexavalent chromium content was determined spectrophotometrically using the SABS 206 method and an Ultraspec 2000 spectrophotometer (Pharmacia Biotech). The difference between total and hexavalent chromium gave the amount of trivalent chromium.

2.2.4 Biomass Preparation

The bacteria, *Bacillus subtilis* and *Citrobacter* were grown in nutrient broth (Appendix1)(Merck, SA) and the alga, *Chlamydomonas reinhardtii* was grown in Tris Acetate Phosphate medium (TAP) . (Appendix 2). The culture was incubated for 24-48 hrs at 29°C in 50 mL nutrient broths (Merck, SA) and TAP medium. This represented the starter culture. Five L Erlenmeyer flasks containing three L of nutrient broth were innoculated with the starter culture. Incubation was on a rotary shaker at room temperature for one week. Cells were harvested by centrifugation at 25 000 x g for 15 mins at 25°C. The above process was repeated three times to remove traces of growth media. After resuspension of biomass in PBS, five mL of suspension was dispensed into pre-weighted bijou bottles and dried overnight in an oven at 105°C. The mass of cells in five ml aliquots of culture was the difference between the weighted bijou bottles and biomass and mass of empty bijou bottles.

2.2.5 Batch Free Cell Biosorption

For practical and engineering process evaluation purposes that are eventually concerned with process mass balances, it is customary to use weight (dry) per weight [e.g., mg of metal sorbed per gram of dry sorbent material].

Since sorption processes tend to be exothermic and since the sorption performance may vary with temperature, constant temperature during the sorption process was a basic requirement.

i. 20, 80, 140, 200, 260, 320, 380 $mg.L^{-1}$ nitrate salts of single metal solutions (Cr^{3+},Cr^{6+},Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}) were prepared from 1 000 $mg.L^{-1}$ stock solutions.

ii. 20 mL of a single metal solution was added to 50 mg of sorbent in a polypropylene test

tube and agitated for 3 hrs at 100 rpm in a 25°C incubator.

iii. Metal analysis was done on a GBC 933 flame atomic absorbance spectrophotometer except for hexavalent chromium which was analysed using Ultraspec 2000 Spectrophotometer (Pharmacia Biotech)

iv. From a plot of amount of metal uptake against final concentration of solute, the maximum uptake, q_{max} of the sorbent was determined.

v. All experiments were performed in triplicate.

2.2.6 Adsorption Isotherms

The degree of sorption was determined by measuring the concentration of the metal ion in the aqueous phase before and after contact with the biomass and expressed according to the Langmuir and Freundlich equations.

The amount of metal biosorbed by the sorbent (q, mg/g) was calculated as follows:

$$q = \frac{(C_o - C_f)V}{w}$$

where: C_o = initial metal concentration (mg/L)

C_f = final concentration after contact with the sorbent (mg/L)

V = solution volume (L)

w = sorbent weight in dry form (g)

2.3 RESULTS

2.3.1 Batch Free-cell Biosorption for Single Metal Solutions

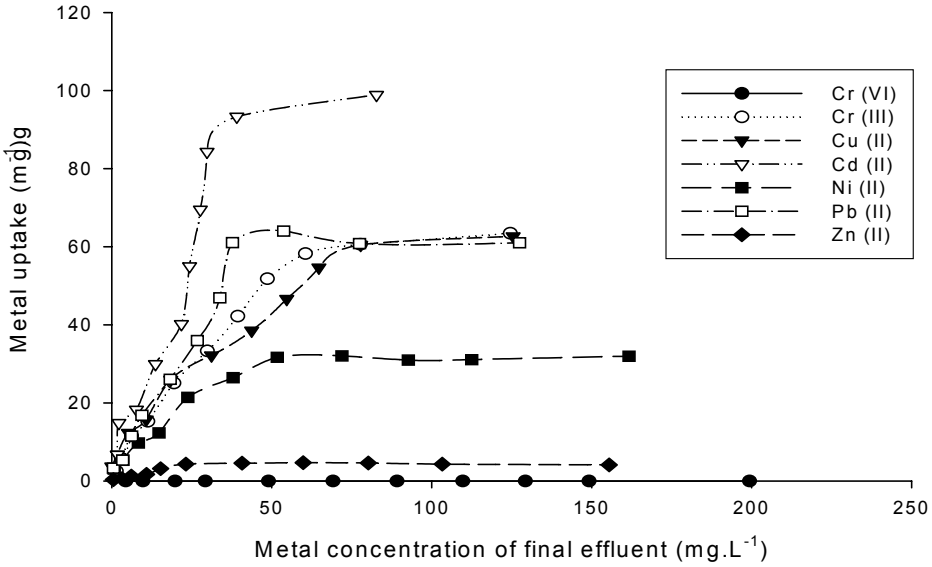


Figure 2.2 Langmuir adsorption isotherm for six metals biosorbed by *Bacillus subtilis* in free-cell suspensions at 25°C.

Bacillus subtilis had a maximum metal uptake (q'_{max}) of 62 to 65 mg.g^{-1} for Cr^{3+}, Pb^{2+} and Cu^{2+}. Cd^{2+} showed the highest affinity for the gram positive biomass. Cr^{6+} was not biosorbed. Cd^{2+} and Pb^{2+} showed Type V adsorption isotherms, which indicate larger intermolecular attraction effects. Cu^{2+} was of the type IV adsorption isotherm showing the formation of two surface layers and , Ni^{2+} type 1, which had a definite saturation limit. The effectiveness for metal biosorption was establish as $Cd^{2+} > Pb^{2+} \geq Cu^{2+} \geq Cr^{3+} > Ni^{2+} > Zn^{2+} > Cr^{6+}$.

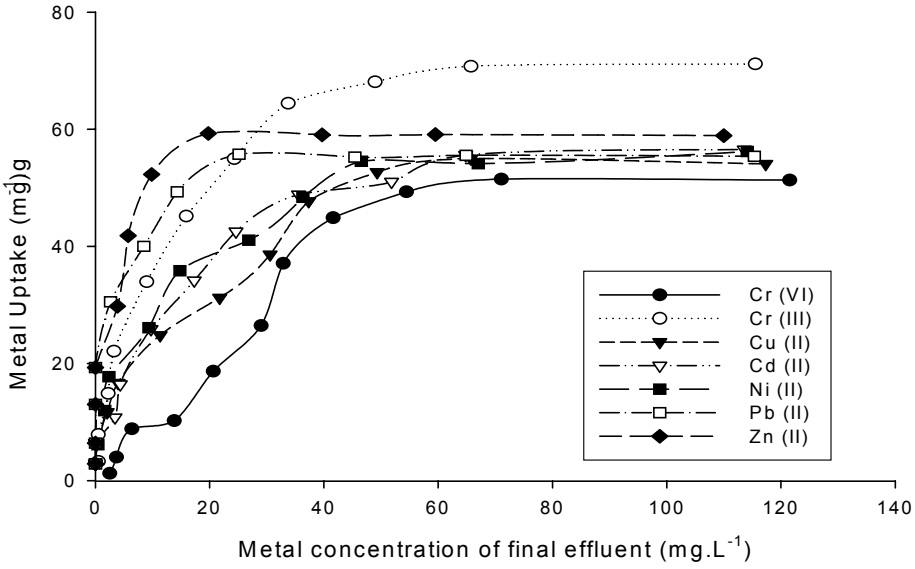


Figure 2.3 Langmuir adsorption isotherm for six metals biosorbed by *Citrobacter* N14 in free cell suspensions at 25^{O}C.

Citrobacter N14 precipitated 77 mg.g^{-1} Cr^{3+}, 60 mg.g^{-1} Zn^{2+} and 57 mg.g^{-1} Cr^{6+}. Cu^{2+}, Cd^{2+} and Pb^{2+} had similar uptake in the 57 mg.g^{-1} regions. Shapes of the adsorption isotherms for Cd^{2+} ,Ni^{2+}, Pb^{2+} and Zn^{2+} were of the type I isotherm. Cr^{3+} and Cu^{2+} biosorption isotherms were type IV. The efficiency for biosorption was $Cr^{3+} > Zn^{2+} > Cu^{2+} = Cd^{2+} = Ni^{2+} = Pb^{2+} > Cr^{6+}$.

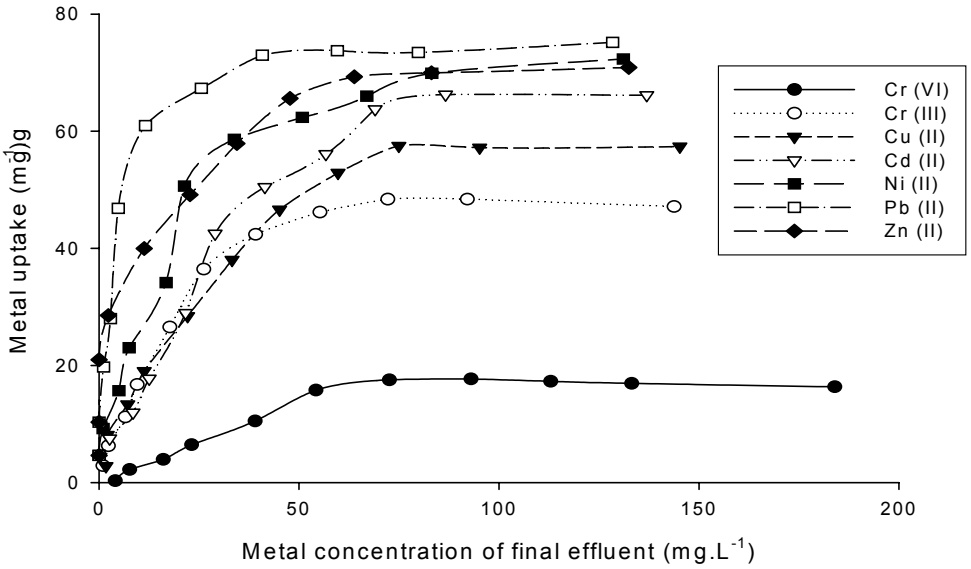


Figure 2.4 Langmuir adsorption isotherm of six metals biosorbed by *Chlamydomonas reinhardtii* in free-cell suspensions at 25°C.

Chlamydomonas reinhardtii biosorption was the best for Pb^{2+}, 75 $mg.g^{-1}$ and worst for Cr^{6+}, 17 $mg.g^{-1}$. Cr^{3+}, Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+} data for adsorption isotherms fitted the type I isotherms, indicating a definite saturation limit. The efficiency for biosorption was $Pb^{2+} > Ni^{2+} = Zn^{2+} > Cd^{2+} > Cu^{2+} > Cr^{3+} > Cr^{6+}$.

Table 2.1 Comparison of regression coefficients for *Freundlich* and *Langmiur* adsorption isotherms.

Metal	**r^2**					
	Bacillus subtilis		***Citrobacter* N14**		***Chlamydomonas reinhardtii***	
	Freundlich	***Langmuir***	***Freundlich***	***Langmuir***	***Freundlich***	***Langmuir***
Cr(VI)	-	-	0.93	0.5205	0.88	0.84
Cd(II)	0.93	0.76	0.92	0.98	0.95	0.92
Cu(II)	0.99	0.91	0.96	0.97	0.94	0.98
Ni(II)	0.9	0.99	0.95	0.99	0.95	0.98
Pb(II)	0.97	0.41	0.83	1	0.86	1
Zn(II)	-	-	0.68	1	0.97	1

- No biosorption

Table 2.1 compares the regression coefficients for Freundlich and Langmuir adsorption isotherms. Data presented here shows that the Freundlich model is better suited for the biosorption of metals by *Bacillus subtilis.* The Langmuir model fits the data for bio-precipitation by *Citrobacter* N14 and *C. reinhardtii*.

Table 2.2 Summary of adsorption isotherms for single metal biosorption by *Bacillus subtilis*.

Metal	Adsorption type	Description	q' max ($mg.g^{-1}$)	q max ($mg.g^{-1}$)	b (1/mM)	r^2
Chromium (VI)	N/A	No biosorption	0	0	0	N/A
Copper	IV	Two surface layers	74.36	86.96	25.39	0.91
Cadmium	V	Large intermolecular attraction effects	117.19	169.5	34.05	0.76
Nickel	I	Definite saturation limit	38.31	43.1	15.63	0.99
Lead	V	Large intermolecular attraction effects	112.53	238.1	91.48	0.41
Zinc	N/A	No biosorption	0	0	0	N/A

q'_{max} - the maximum adsorption from the graph of uptake against residual concentration,

q_{max} - maximum theoretical adsorption,

b - Langmuir constant and

r^2 - regression coefficient.

Table 2.2 is the summary of adsorption isotherms by *B. subtilis.* Type V isotherms, which indicate large intermolecular attractions, were the most common for Cd^{2+} and Pb^{2+} biosorption. Adsorption isotherms for Cu^{2+} was of the IV type, which shows the formation of two distinct surface layers. Cr^{6+} and Zn^{2+} were not biosorbed by the biomass. *Bacillus subtilis* biosorbed the most amount of Pb^{2+} (238.1 $mg.g^{-1}$) and the b value was 91.476.

Table 2.3 Summary of adsorption isotherms for single metal bios-precipitation by *Citrobacter* N14.

Metal	Adsorption type	Description	q' max ($mg.g^{-1}$)	q max ($mg.g^{-1}$)	b (1/mM)	r^2
Chromium (VI)	IV	Two surface layers	79.02	166.67	95	0.52
Copper	IV	Two surface layers	85.67	88.5	8.1	0.97
Cadmium	I	Definite saturation limit	85.67	92.59	8.68	0.98
Nickel	I	Definite saturation limit	85.67	90.09	6.4	0.99
Lead	I	Definite saturation limit	85.67	85.47	0.86	0.99
Zinc	I	Definite saturation limit	90	90.91	0.87	1

q'_{max} - maximum adsorption from the graph of uptake against residual concentration,

q_{max} - maximum theoretical adsorption

b - Langmuir constant and

r^2 - regression coefficient.

Bio-precipitation of Cd^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} by *Citrobacter* was mainly of type I adsorption isotherms, which indicate a saturation limit. Q'_{max} values were between 85 and 90 $mg.g^{-1}$. These type I isotherms had regression values of between 0.97 and 0.99. Cr^{3+} and Zn^{2+} were not biosorbed by the biomass. 166.67 $mg.g^{-1}$ Cr^{6+} was the most amount of metal that was removed by *Citrobacter* N14. The b value was 95.

Table 2.4: Summary of adsorption isotherms for single metal biosorption by *Chlamydomonas reinhardtii*.

Metal	Adsorption type	Description	q' max ($mg.g^{-1}$)	q max ($mg.g^{-1}$)	b (1/mM)	r^2
Chromium	I	Definite saturation limit	17.387	25.063	64.385	0.8358
Copper	I	Definite saturation limit	56.705	68.027	26.469	0.9787
Cadmium	I	Definite saturation limit	63.942	80	29.752	0.9161
Nickel	IV	Two surface layers	68.887	75.188	12.684	0.9803
Lead	I	Definite saturation limit	71.578	72.464	2.369	0
Zinc	I	Definite saturation limit	67.226	68.966	3.9724	0.9925

q'_{max} - maximum adsorption from the graph of uptake against residual concentration,

q_{max} - maximum theoretical adsorption,

b - Langmuir constant and

r^2 - regression coefficient.

Biosorption by *Chlamydomonas reinhardtii* was mainly of the type I isotherm, the only exception being Ni^{2+} biosorption which was type IV. q'max values varied between 17.387 and 71.578 $mg.g^{-1}$. The maximum amount of metal biosorbed was Pb^{2+} (71.578 $mg.g^{-1}$) and the b value for this was 2.369. The least amount of metal removed was Cr^{2+}. The alga also showed a high affinity for Ni^{2+} (68.887 $mg.g^{-1}$) and Zn^{2+} (67.226 $mg.g^{-1}$).

2.3.1.2 Multi component adsorption isotherms for mixed metal solution biosorption

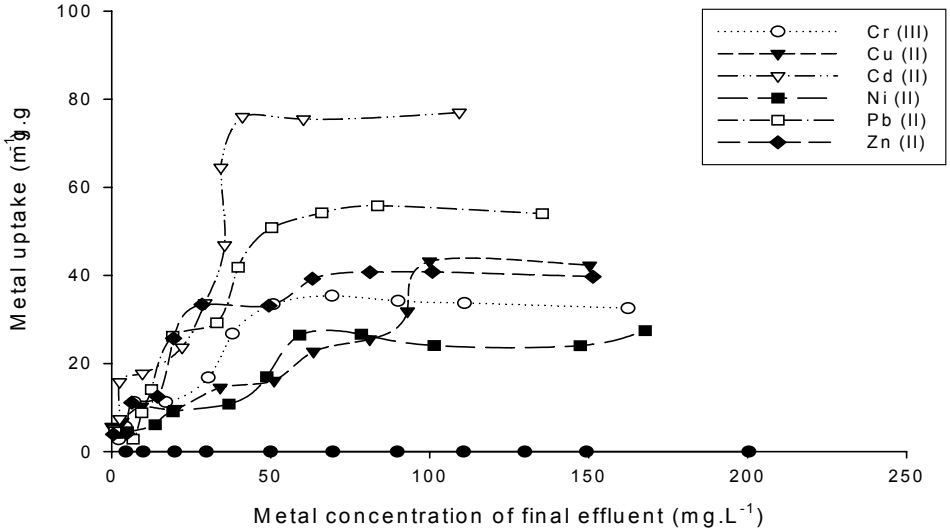


Figure 2.5 Multicomponent Langmuir adsorption Isotherm for six metals biosorbed b *Bacillus subtilis* in free cell suspensions at 25°C.

Figure 2.5 represents the uptake of mixed metals by *B. subtilis*. Adsorption isotherms were mainly of the IV type. These isotherms indicate the formation of two surface layers. All metals biosorbed displayed this type of isotherm. q' max values ranged from 27.46 $mg.g^{-1}$ for Ni^{2+} to 76.98 $mg.g^{-1}$ for Cd^{2+}.

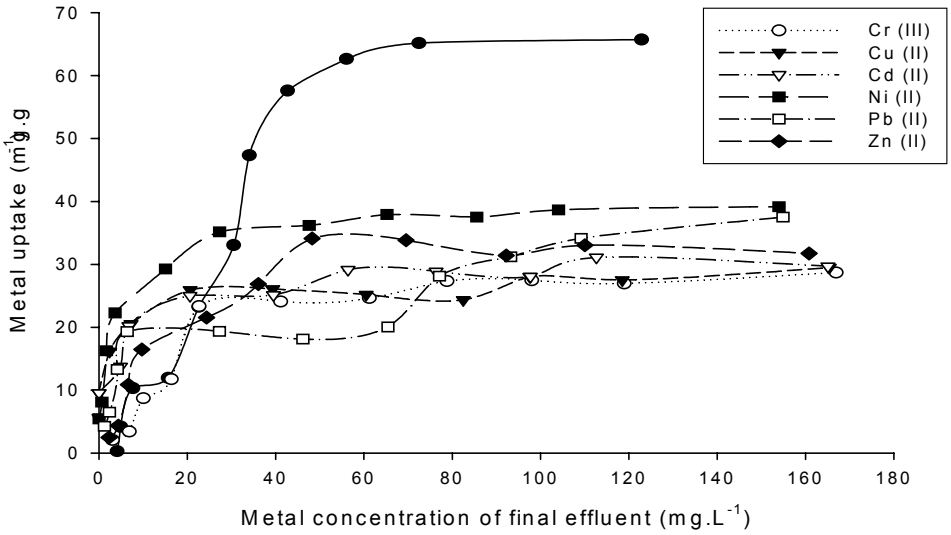


Figure 2.6 Multicomponent Langmuir adsorption isotherm for six metals biosorbed b
Citrobacter N14 in free-cell suspensions at 25^0C.

Figure 2.6 represents the multi component adsorption isotherm for *Citrobacter* N14. Metals analysed for displayed the type IV adsorption isotherm, except for Ni^{2+}, which was type I. q' max values ranged from 28.69 $mg.g^{-1}$ for Cr^{3+} to 65.7 $mg.g^{-1}$ for Cr^{6+}.

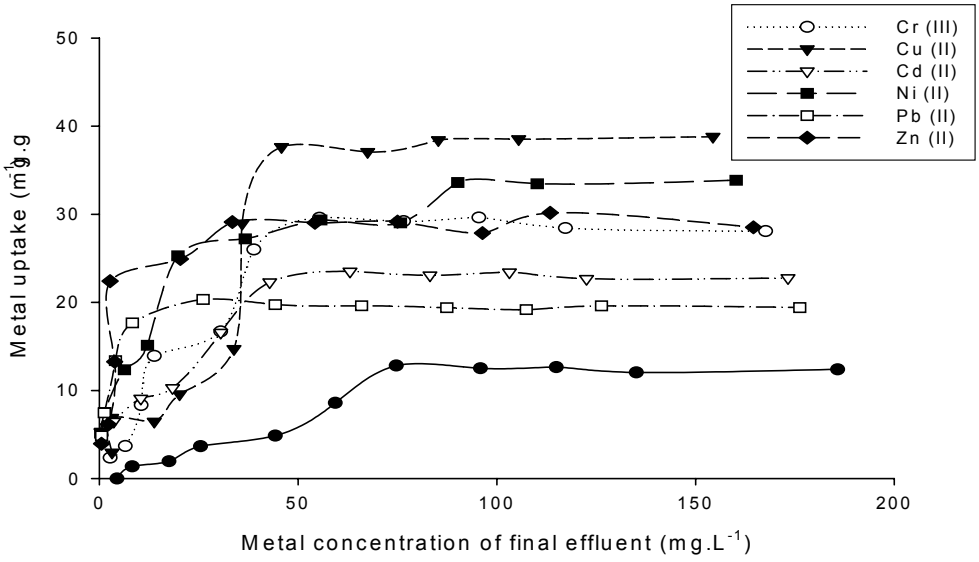


Figure 2.7 Multicomponent adsorption isotherm for six metals biosorbed by *Chlamydomonas reinhardtii* in free-cell suspensions at 25^0C.

Figure 2.7 represents the adsorption isotherm for mixed metal uptake by *C. reinhardtii*. Once again adsorption isotherms were of the type IV, except for Pb^{2+} which displayed a Type I adsorption isotherm. q' max values ranged from 12.83 $mg.g^{-1}$ for Cr^{6+} to 38.8 $mg.g^{-1}$ for Cu^{2+}.

Table 2.5 Summary of adsorption isotherms for mixed metal bioosrption by *Bacillus subtilis* in batch free-cell systems.

Metal	**Adsorption type**	**Description**	**q' max** **($mg.g^{-1}$)**
Chromium VI	IV	Formation of two surface layers	0
Chromium III	IV	Formation of two surface layers	35.386
Copper	IV	Formation of two surface layers	43.228
Cadmium	IV	Formation of two surface layers	76.976
Nickel	IV	Formation of two surface layers	27.463
Lead	IV	Formation of two surface layers	55.826
Zinc	IV	Formation of two surface layers	40.807

Table 2.5 represents the summary of the adsorption isotherms for mixed metals biosorbed by *B. subtilis.* Uptake of metals by this biomass resulted in the distinct formation of two surface layers. This is indicated by the Type IV adsorption isotherms. q'_{max} represents the maximum adsorption from the graph of uptake against residual concentration and the value for *B. subtilis* biosorption is 76.976 $mg.g^{-1}$ Cd^{2+}.

Table 2.6 Summary of adsorption isotherms for mixed metal bio-precipitation by *Citrobacter* N14 in batch free-cell biosorption.

Metal	**Adsorption type**	**Description**	**q' max ($mg.g^{-1}$)**
Chromium VI	IV	Formation of two surface layers	65.713
Chromium III	IV	Formation of two surface layers	28.693
Copper	IV	Formation of two surface layers	29.481
Cadmium	IV	Formation of two surface layers	31.111
Nickel	I	Definite saturation limit	39.162
Lead	IV	Formation of two surface layers	37.508
Zinc	IV	Formation of two surface layers	34.105

All metals biosorbed by *Citrobacter*, except for Ni^{2+}, resulted in the formation of type IV isotherms. Ni^{2+} was of the type I isotherm. q' max values ranged from 28.69 $mg.g^{-1}$ for Cu^{2+} to 65.71 $mg.g^{-1}$ for Cr^{6+}. q'_{max} represents the maximum adsorption from the graph of uptake against residual concentration and the value for *Citrobacter* N14 biosorption is 65.713 $mg.g^{-1}$ of Cr^{6+}.

Table 2.7 Summary of adsorption isotherms for mixed metal biosorption by *Chlamydomonas reinhardtii* in batch free-cell biosorption.

Metal	Adsorption Type	Description	q' max ($mg.g^{-1}$)
Chromium VI	IV	Formation of two surface layers	12.828
Chromium III	IV	Formation of two surface layers	29.62
Copper	IV	Formation of two surface layers	38.807
Cadmium	IV	Formation of two surface layers	23.51
Nickel	IV	Formation of two surface layers	33.862
Lead	I	Definite saturation limit	20.333
Zinc	IV	Formation of two surface layers	30.164

Table 2.7 represents the summary of the adsorption isotherms for mixed metals biosorbed by *C. reinhardtii.* Uptake of metals by this biomass resulted in the distinct formation of two surface layers. This is indicated by the type IV adsorption isotherms. Pb^{2+} biosorption was of the type I isotherm, indicating a definite saturation limit. q'_{max} represents the maximum adsorption from the graph of uptake against residual concentration and this value for *C. reinhardtii* biosorption is 38.807 $mg.g^{-1}$ Cu^{2+}.

2.4 DISCUSSION

The ability of biological material to sequester heavy metal ions from aqueous solution has been well established (Williams and Edyvean, 1999). Only those materials with a high metal binding capacity and selectivity for heavy metals are suitable for use in full scale biosorption processes. The first major challenge was to determine the biosorptive capability of the three biosorbents being used in the laboratory.

Experimental C_f and q data were used to evaluate the Langmuir q_{max} and b constants. Figures 2.2, 2.3 and 2.4 represent the curves generated from the Langmuir equation, for metal uptake by the three technologies based on *Bacillus subtilis, Citrobacter N14* and *Chlamydomonas reinhardtii* biomass. Mechanisms of adsorption were ascertained from shapes of adsorption isotherms classified according to Brunauer *et al*, (1940), (cited by Bux *et al*. 1994). First order regression coefficients of greater than 0.91 indicated that the data fitted the Langmuir model. The uptake of the metals was initially evaluated in batch experiments using both single and multiple metal solutions.

Cr(VI) was not biosorbed by *B. subtilis* (Figure 2.2). The amount of Cr(VI) biosorbed by *Citrobacter* N14 was 79.023 $mg.g^{-1}$ compared to the theoretical value of 166.67 $mg.g^{-1}$ The adsorption isotherm is a type IV isotherm which indicates the formation of two surface layers (Figure 2.3). *Citrobacter* N14 biosorbed more Cr^{6+} than *C. reinhardtii,* which only biosorbed 17 $mg.g^{-1}$. A low b value indicates a better biosorbent, however the b value for *Citrobacter* N14 was much higher (95). (Table 2.3). Regression values indicated that the data did not fit the Langmuir model very well, 0.52 for *Citrobacter* N14 and 0.836 for *C. reinhardtii*.

The amount of Cu^{2+} biosorbed by the three technologies varied between 74.356, 85.671 and 56.705 $mg.g^{-1}$ for *B. subtilis, Citrobacter* N14 and *C. reinhardtii* respectively (Figures 2.2, 2.3 and 2.4). Beveridge *et al* (1985) cited by Mullen *et al* (1989) showed that the cell walls of gram positive bacteria like *B. subtilis* bind a larger quantity of metal than cell envelopes of gram negative bacteria. Cu^{2+} biosorption by *B. subtilis* and *Citrobacter* N14 was of the type IV

isotherms which indicates the formation of two surface layers, and Cu^{2+} biosorption by *C. reinhardtii* was of the type I which indicates a definite saturation limit (Figures 2.2, 2.3 and 2.4). Studies carried out by Whitten (1970) cited by Wilde and Benemann (1993) show that *C. reinhardtii* was copper resistant. It was concluded that Cu^{2+} bound to algal cell surfaces and to the ligands produced and excreted by *C. reinhardtii.* This could explain the high uptake of Cu^{2+} by the green alga (56 $mg.g^{-1}$) (Table 2.4). Cu^{2+} was best biosorbed by *Citrobacter* which removed 85 $mg.g^{-1}$ from solution. This value is very close to the theoretical q_{max} which indicates that almost ideal conditions for biosorption were obtained. The b value for *Citrobacter* N14 bio-precipitation was 8.097 (Table 2.3) and a low b value implies a high affinity of the metal for the sorbent.

B. subtilis biosorbed 117.19 $mg.g^{-1}$ of Cd^{2+}, however the regression data did not fit the Langmuir model (Table 2.1). The calculated q_{max} for Cd^{2+} was 169.5 $mg.g^{-1}$. The adsorption isotherms for Cd(II) removal by this biomass was of the type V which indicates large intermolecular attraction effects (Figure 2.2). 85.675 and 63.942 $mg.g^{-1}$ of Cd^{2+} was removed by *Citrobacter* N14 and *C, reinhardtii* (Figures 2.3 and 2.4). Matheickal *et al* (1999b) showed that the maximum cadmium adsorption capacity and affinity constant of marine alga *Durvillaea potatorum* was 29.22 $mg.g^{-1}$ at a pH of 2 up to 125.89 $mg.g^{-1}$ at a pH of 5.4. At higher pH, the amount removed by this alga is far superior to the three technologies used. It was shown that a calcium pre-treatment step prevented alginate from leaking out of the alga, and this improved adsorption quality of the biomass. The adsorption isotherms for *Citrobacter* N14 and *C. reinhardtii* biomass were of the type I isotherms which indicate a definite saturation limit (Figures 2.3 and 2.4) . Only the data for these two biomass fit the Langmuir model (Tables 2.3 and 2.4).

Citrobacter N14 removed 85.671 $mg.g^{-1}$ of Ni^{2+} from a single metal solution (Figure 2.3). A low b value of 6.39 was obtained (Table 2.3). A low b value indicates a good biosorbent. The data had an r^2 value of 0.99 and fit the selected model very well. The adsorption isotherms was of the type I which indicates a definite saturation limit (Figure 2.3). Values of 38.313 $mg.g^{-1}$ and 68.88 $mg.g^{-1}$ of Ni^{2+} was removed from solution by *B. subtilis* and *C. reinhardtii*

respectively. Ni^{2+} biosorption by *B. subtilis* was of the type I and Ni^{2+} biosorption by *C reinhardtii* was of the type IV isotherm (Figures 2.3 and 2.4).

B. subtilis showed the highest affinity for Pb^{2+}, and the amount removed was 112.53 $mg.g^{-1}$ (Figure 2.2). However *Citrobacter* N14 had a much lower b value (0.85) compared to that of *B. subtilis* (91.47) (Table 2.3 and Table 2.1). This correlates with Volesky's observation (1999) that the lower the b value the higher the affinity of the sorbate for the metal. *C. reinhardtii* removed 71 $mg.g^{-1}$ of Pb^{2+} (Figure 2.3). The theoretical q_{max} value for Pb^{2+} biosorption was determined to be 72.464 $mg.g^{-1}$, indicating that experimental maximum and theoretical maximum were close and almost ideal conditions for biosorption were obtained. The macro fungus, *Phellinus badius* (Matheickal and Yu, 1997) removed 58.016 $mg.g^{-1}$ of Pb^{2+} at a pH of 3.5. The b value for this data was 6.38 and this is much higher than that obtained for *Citrobacter* N14 and *C. reinhardtii* (0.85 and 2.369 respectively) indicating that this fungus is a much poorer biosorbent than *Citrobacter* N14 and *C. reinhardtii* The data for these two technologies fit the Langmuir model better than that observed for the fungus. It was concluded that these two technologies were much better at biosorbing Pb^{2+} than the fungus. Adsorption isotherms for *B. subtilis* was of the Type V and for *Citrobacter* and *C. reinhardtii* it was type I isotherms (Figures 2.2, 2.3 and 2.4). Pb^{2+} bio-precipitation by *Citrobacter* N14 and *C. reinhardtii* fit the Langmuir model very well.

B. subtilis did not biosorb Zn^{2+}. *Citrobacter* N14 bio-precipitated 90 $mg.g^{-1}$ of Zn^{2+} from single metal solutions (Figure 2.2). The calculated q_{max} for zinc was also 90.909 $mg.g^{-1}$. The amount removed was equal to the theoretical value obtained for Zn^{2+} removal. A low b value of 0.864 and an r^2 value of 0.99 was obtained for Zn^{2+} bio-precipitation by *Citrobacter* N14 (Table 2.3). Mameri *et al* (1999), when working with *Streptomyces rimosus* showed that a limiting biosorption capacity was attained at equilibrium concentrations of 200 $mg.l^{-1}$ and that the curves obtained from fitting the data to the Langmuir model was the type I isotherm, which indicates adsorption on active sites up to their saturation. Adsorption isotherms for Zn^{2+} biosorption by *Citrobacter* and C. *reinhardtii* was of the type I isotherms (Figures 2.3 and 2.4). C. *reinhardtii* removed 67. 226 $mg.g^{-1}$ of Zn^{2+} from solution (Figure 2.4). A low b value of 3.97 was obtained. The data fitted the Langmuir model very well (Table 2.4).

All the isotherms are steep at lower concentrations of the metal solution. Matheickal and Yu (1999a) indicated that this steepness in the isotherms, implies that the biosorbent is suitable to treat dilute heavy metal solutions. They also observed that the typical 'L' shape of the isotherms indicates a reduction in the number of active sites on the adsorbent at a high residual heavy metal concentration in the solution..

Very often there is more than one metal in the solution and in this case, the assessment of the sorption performance becomes more complicated. Figures 2.5, 2.6 and 2.7 represent the multi metal uptake by the three technologies. *Bacillus subtilis* biosorbed 35.386 $mg.g^{-1}$ Cr^{3+}, 43.228 $mg.g^{-1}$ Cu^{2+}, 76.976 $mg.g^{-1}$ Cd^{2+}, 27.463 $mg.g^{-1}$ Ni^{2+}, 55.826 $mg.g^{-1}$ Pb^{2+} 40.807 $mg.g^{-1}$ Zn^{2+} but did not adsorb Cr^{6+} (Figure 2.5). *B. subtilis* was able to remove a larger quantity of these metals from a single metal solution rather than from the multi metal solution. All the curves obtained from the data were of the type IV adsorption isotherm (Figure 2.5). The affinity series for metal biosorption was establish as $Cd^{2+} > Pb^{2+} > Cu^{2+} > Zn^{2+} > Ni^{2+} > Cr^{3+}$ (Table 2.5). The efficiency of biosorption established for single metals was similar for Cd^{2+}, Pb^{2+} and Cu^{2+}, with the biomass showing a greater affinity for these metals from both single and multi metal solutions. Chromium was not absorbed from both single and multi metal solutions. Zn^{2+}, which was not biosorbed by *B. subtilis* from single metal solutions was taken up from mixed metals solutions, but in very small quantities (40.807 $mg.g^{-1}$) (Figure 2.5).

Citrobacter N14 precipitated 65.713 $mg.g^{-1}$ Cr^{6+}, 28.693 $mg.g^{-1}$ Cr^{3+}, 29.481 $mg.g^{-1}$ Cu^{2+}, 31.111 $mg.g^{-1}$ Cd^{2+}, 39.162 $mg.g^{-1}$ Ni^{2+}, 37.508 $mg.g^{-1}$ Pb^{2+} and 34.105 $mg.g^{-1}$ Zn^{2+} (Figure 2.6). Although all metals from the mixed metal solution were taken up by *Citrobacter*, the values obtained were very low compared to the uptake of these metals from single metal solutions (Figure 2.2). Cr^{6+} was best precipitated by *Citrobacter* N14. Precipitation of all metals except for Ni^{2+} were of the type IV, indicating the formation of two surface layers (Table 2.6). Ni^{2+} uptake displayed the typical 'L' shaped isotherm. The affinity series for metal biosorption was $Cr^{6+} > Cd^{2+} > Ni^{2+} > Pb^{2+} > Zn^{2+} > Cu^{2+} > Cr^{3+}$, represented in Table 2.6. For single metal solutions, *Citrobacter* had the least affinity for Cr^{6+} ions and the most for Cr^{3+}.

Chlamydomonas reinhardtii biosorbed 12.828 mg.g^{-1} Cr^{6+}, 29.620 mg.g^{-1} Cr^{3+}, 38.807 mg.g^{-1} Cu^{2+}, 23.510 mg.g^{-1} Cd^{2+}, 33.862 mg.g^{-1} Ni^{2+}, 20.333 mg.g^{-1} Pb^{2+} and 30.165 mg.g^{-1} Zn^{2+} (Figure 2.7). Although all metals were removed from the mixed metal solution in different quantities, the values of uptake were much lower than that for single metal solutions. The biomass took up the most amount of Cu^{2+} (38.807 mg.g^{-1}) (Table 2.7). 56.705 mg.g^{-1} of the same metal was removed from the single metal solution (Table 2.4). All metals were biosorbed, except for Pb^{2+} were of the type IV isotherms indicating the formation of two surface layers (Table 2.7). The affinity series for biosorption was $Cu^{2+} > Ni^{2+} > Zn^{2+} > Cr^{3+} > Cd^{2+} > Pb^{2+} > Cr^{6+}$ shown in Table 2.7. *Chlamydomonas reinhardtii* had a low affinity for Cr^{6+} in both single and multi metal solutions.

2.5 CONCLUSIONS

According the regression data presented in Table 2.1, biosorption by *B. subtilis* fits the Freundlich model better whereas biosorption by *Chlamydomonas reinhardtii* and precipitation by *Citrobacter N14* and fits the Langmuir model better. First order regression coefficients of greater than 0.91 indicated that the data fitted the Langmuir model.

B. subtilis, *Citrobacter N14* and *Chlamydomonas reinhardtii* are all capable of removing metals from both single and multi-metal solutions. *B. subtilis* has a greater affinity for Cd^{2+} in single and multi metal solutions, *Citrobacter* N14 for Cr^{3+} and *Chlamydomonas reinhardtii* for Pb^{2+} in single metal solutions and for Cu^{2+} in multi metal solutions.

The efficiency for metal biosorption by *B. subtilis* was established as $Cd^{2+} > Pb^{2+} > Cu^{2+} > Ni^{2+}$ (Table 2.2), for precipitation by *Citrobacter* N14, $Cr^{3+} > Zn^{2+} > Cu^{2+} = Cd^{2+} = Ni^{2+} = Pb^{2+} > Cr^{6+}$ (Table 2.3), for biosorption by *C. reinhardtii*, $Pb^{2+} > Ni^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} > Cr^{6+}$ (Table 2.4).

The best remediating agent for single metal solutions proved to be *Citrobacter* N14. This biomass is capable of removing large amounts of Cu^{2+}, Cd^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+} from solution.

The b values obtained for *Citrobacter* N14were also very low and ranged from 0.85 to 8.09. The data obtained for *Citrobacter* N14 precipitation fit the Langmuir model very well.

Adsorption isotherms were of the type I, V and IV for single metal solutions. The most common type was the 'L' shaped isotherm indicating monolayer adsorption of metals. Isotherms for the three biosorbents for multi metal adsorption were of the type IV which is synonymous with multilayer adsorption. Ni^{2+} precipitation by *Citrobacter N14* and Pb^{2+} biosorption by *C. reinhardtii* were of the type I isotherm..

CHAPTER 3 LABORATORY-SCALE ASSESSMENT OF BIOSORBENT TECHNOLOGIES (COLUMN BIOSORPTION)

3.1 INTRODUCTION

Most separation and purification processes that employ sorption technology use continuous-flow columns. Packed column operation, as a continuous process, is often considered to be more efficient and economical than batch operation, therefore adsorption of heavy metal ions is more commonly carried out in a packed bed column (Volesky, 1990). Starting at the inlet, the saturated solid sorbent zone gradually extends throughout the column, with the sorbate eventually breaking through the column. The record of the breakthrough usually gives a typical s-shaped breakthrough curve whose shape and slope is the result of the equilibrium sorption isotherm relationship, the mass transfer to and throughout the sorbent in the column, and operation of macroscopic fluid-flow parameters, such as axial mixing which affect the deviation from the ideal plug flow (Volesky, 2001).

Columns packed with microorganisms or plant material have already been studied by many researchers. Volesky and Prasetyo (1994) used a column packed with brown marine algae, *Ascophyllum nodosum*, to remove Cd^{2+} from solution. The biosorption of Cu, Cd and Zn from multi-component mixtures was studied in equilibrium systems in a column packed with potassium saturated *Sargassum* seaweed by Figueira *et al* (2000a). The length of this column was 50 cm with a diameter of 2.5 cm and an approximate packing density of 100 $g.L^{-1}$.

Biosorption of Cu(II) by dry, protonated *Sargassum fluitans* packed in chromatography column of 2.5 cm in internal diameter and 50 cm length was used by Kratochvil *et al* (1997). This column had a packing density of 190 $g.L^{-1}$ and a flow rate of 7.5 $ml.min^{-1}$. Chang *et al* (1998) have shown that biomass of *Pseudomonas aeruginosa* PU21 can effectively adsorb heavy metals, including mercury, lead, copper and cadmium. In their study, cells of *P. aeruginosa* were immobilised with calcium alginate and polyacrylamide and packed into glass columns, 2.2 cm in diameter with a bed length of 5 to 21 cm.

The main parameters that influence the performance of the column are the length of the column, the flow rate and initial concentration of the influent. These parameters are important in the design of the column (Volesky and Prasetyo, 1994).

The breakthrough time represents the duration of ongoing sorption until a pre-defined exit threshold sorbate concentration is reached. Any optimized column system is based on the accurate prediction of the breakthrough time under given specific operating conditions. When the sorbate concentration in the effluent stream reaches a pre-defined level, the column operation is terminated. At this point the regeneration process may begin before activation for the next cycle of operation.

3.1.1 Bioreactor Types

Several bioreactor configurations can be used to interact metal ions with biosorbent. These configurations can range from batch, semi-continuous to continuous flow and can be arranged in either stirred tank reactors, fixed packed bed contractors and packed bed contractors. In stirred tank reactors, the biosorbent is suspended in the effluent that is contaminated with metal. The metal is recovered and the biosorbent is reused (Bux, *et al*, 1997). In fixed packed bed contactors the metal bearing solution percolates through a bed of active biosorbent. Equilibrium is not attained since the upper layers become saturated before the lower ones. Once all the biomass is saturated, the bed becomes inactive and this is indicated by a sharp rise in metal concentration, called the 'breakthrough point' (Bux *et al*, 1994).

The rotating biological contractor (RBC) represents a viable means for the secondary treatment of both municipal and industrial wastewaters by exploiting the advantages of both fixed film and suspended growth systems. Although RBC's entail high initial capital costs, their low costs of operation and maintenance as well as their simple process control, increase their economic viability (Costley and Wallis, 2001).

When compared to the sorption fixed bed column reactor, the continuous flow stirred tank reactor (CSTR) is on the opposite end of the reactor spectrum. While the flow in the theoretical

fixed bed reactor is considered as non-mixed plug flow, the basic assumption for the CSTR is complete and instantaneous mixing. Its contents is considered homogenous, so its outflow will reflect exactly what is inside (Volesky, 2001).

3.1.2 Immobilisation Agents

Raw, non living cells tend to clump together in a column and exhibit very poor flow characteristics. Therefore excessive pressure drops are required to maintain an acceptable flow rate through the column. To circumvent this problem the microorganism must be immobilised into some type of matrix so that the cells will not stick together and better column flow characteristics will result (Greene and Bedell, 1990). Polyacrylamide, calcium alginate and silica are among the more common porous matrices that have been used to immobilise non viable microorganisms and to permit their use in packed columns for metal ion recovery (Bedell and Darnall, 1990).

Pelletised and immobilised yeast and fungal biomass in alginate have been tested for heavy metal and radionuclide recovery in various types of small batch and column reactors (Hu and Reeves, 1997). Alginate is a linear copolymer of D-mannuronic and L-guluronic acid residues aranged in a non-regular and blockwise order (Davis *et al*, 2000). Alginate immobilisation of cells occurs by mixing an aqueous slurry of algal cells with sodium alginate after selecting the desired microorganism-to-alginate ratio, and adding the slurry to calcium chloride to form beads containing the cells (Bedell and Darnall, 1990).

Another matrix for immobilising nonviable cells for use in metal recovery is polyacrylamide. The procedure is based on the free-cell radical polymerisation of acrylamide in an aqueous solution containing the microbial cells. Polyacrylamide gels have been the most extensively used immobilisation material for laboratory research studies, but the application of these materials without appropriate hardening agents is limited due to their low mechanical strength.

Silica gel is the material on which a proprietary, nonliving immobilised algal biomass is based. The biologically derived resin is termed AlgaSORB® (Hu and Reeves, 1997). It offers advantages, particularly regarding both re-cyclability and stability.

Polysulfone beads are prepared from commercially available reagents, chemically stable and resistant to attack by strong alkali and acid solutions and can be easily handled (Jeffers *et al*., 1993, Banks, 1997). Previous research carried out by the Bureau of Mines demonstrated that porous polysulfone beads containing thermally killed *Sphagnum* moss biomass removed metal contaminants from a variety of mining and mineral processing waste waters the beads designated BIOFIX beads, successfully removed cadmium, maganese and zinc from a contaminated waste water (Jeffers *et al*, 1990). The above two technologies have since been discontinued and were not considered for this research.

Immobilisation serves to improve the physical characteristics of the biomass for use in reactors, permits reusability of the substance and tends to make the microorganism biomass more inert to microbial degradation (Brierley, 1990a). Therefore, immobilised biomass have a great potential for use in packed or fluidised-bed reactors, with benefits including control of particle size, more efficient regeneration of biomass, easy separation of the biomass from the effluent, high biomass loading, and minimal clogging under continuous flow conditions (Hu and Reeves, 1997).

Modified biomass must have a particle size similar to other commercial adsorbents (0.5 to 1.5 mm) and possess particle strength, high porosity, hydrophilicity and resistance to aggressive chemicals. These characteristics can be achieved through immobilisation technology (Jeffers *et al.*, 1993, Brierley, 1990a). It is therefore necessary to convert biomass into a form whereby it can by employed in modes similar to that of ion-exchange resins and activated carbon (Hu and Reeves, 1997).

3.1.3 Desorption of heavy metals

If the biosorption process were to be used as an alternative in the wastewater treatment scheme, the regeneration of the biosorbent may be important for keeping the cost of the process down and to open the possibility of metal recovery. Desorption of metal from metal loaded biosorbents may provide some insight into the extent of metal ion penetration into the cell structure. The desorption of the process should:

a. Yield the metals in a concentrated form,

b. Restore the biosorbent to close to the original condition for effective reuse with undiminished metal uptake and no physical changes or damage (Volesky, 2001).

In the case of non-viable cells, elution of total accumulated metal can be achieved by acid digestion (Chang *et al*, 1997). While the regeneration of the biosorbent may be accomplished by washing the metal laden one with an appropriate solution, the type and strength of this solution would depend on how the metal has been bound. The first step would be to screen for an appropriate solution (Volesky, 2001). Chang *et al* (1997) showed that hydrochloric acid (HCl) has the best desorption efficiency among other chemicals (Sulphuric acid (H_2SO_4), Sodium carbonate (Na_2CO_3), EDTA and $-mercapto-ethanol) that they tested. Repeated desorption are performed in order to examine the reusability and metal recovery efficiency of the biomass. Bux *et al*, (1995) showed that although both acetic acid and HCl displayed high overall desorption efficiencies, HCl was the better desorbing agent. Cd desorption from various sludge samples by HCl ranged from 94 to 98%. Thus this reagent was selected for the desorption agent in the study.

3.1.4 Objectives

1. To assess technologies claiming superior heavy metal removal capacities in laboratory scale biosorption processes by obtaining experimental data on the biosorption of several heavy metals in laboratory- scale column experiments using the three imported technologies immobilised onto polysulfone beads, and exposing them to heavy metals, Cr^{3+},Cr^{6+}, Cu^{2+}, Cd^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+}.

3. To determine the reusability of the biosorbent by carrying out desorption studies using 0.2 N HCl as the desorbing agent.

3.2 METHODS

3.2.1 Growth of Microorganisms

The bacteria, *Bacillus subtilis* and *Citrobacter* were grown in nutrient broth (Merck, SA) and the alga, *Chlamydomonas reinhardtii* was grown in TAP medium. The culture was incubated for 24-48 hrs at 29°C in 50 mL Nutrient broths and TAP medium. This represented the starter culture. Five-L Erlenmeyer flasks containing three L of nutrient broth were innoculated with the starter culture. Incubation was on a rotary shaker at room temperature for 1 week. Cells were harvested by centrifugation at 25 000 x g for 15 mins at 25°C. The above process was repeated three times to remove traces of growth media. After resuspension of biomass in PBS, 5 mL of suspension was dispensed into pre-weighted bijou bottles and dried overnight in an oven at 105°C. The mass of cells in 5 mL aliquots of culture was the difference between weighted bijou bottles and biomass and mass of empty bijou bottle.

3.2.2 Method of immobilisation

Each of the biomass, *B. subtillus*, *Citrobacter* N14 and *C. reinhardtii* was dried at 100^{o}C then ground into a powder. Biomass was immobilised by entrapment in polysulfone resin that was dissolved in dimethyl formamide (DMF) to yield a final concentration of 100 g.L^{-1} of the immobilising agent. Dry biomass (5 g) was mixed with 100 ml of polysulfone-DMF solution and extruded drop-wise, through a 16-gauge surgical needle attached to a peristaltic pump via silicone tubing (ID = 4 mm), into a beaker containing ice cold deionised water, to form spherical beads 2-3 mm in diameter. Bead size provided sufficient strength of beads while maintaining void volumes in packed bed configuration. Beads were stored overnight at 4^{o}C to ensure complete solidification. Beads were rinsed in deionised water prior to the biosorption procedure.

3.2.3 Column Biosorption

Columns were chosen for their simplicity in design and could either be pump or gravity driven. No movable parts meant a less chance of mechanical failure. The columns were constructed from grade 316 stainless steel (250 mm X 27 mm) and contained 5 g of biomass. The upflow feed was maintained at 10 mL.min^{-1} by peristaltic pump. Dry *B. subtillus*, *Citrobacter* N14 and

C. reinhardtii biomass immobilised onto polysulfone were each packed into stainless steel columns (25 cm X 2.7 cm), yielding an approximate packing density of 40 $g.L^{-1}$. The column's bed volume was determined by measuring the amount of solute in the column at any give time. The metals, Cr^{6+}, Cr^{3+}, $Cu2^{2+}$, Cd^{2+}, Pb^{2+}, Ni^{2+} and Zn^{2+}were mixed together to yield a final volume of 25 L and a concentration of 250 $mg.L^{-1}$ for each metal. Two L of this mixed metal solution was fed into the column from the top, at a rate of 10 $mL.min^{-1}$. Effluent was collected. No compaction of the column was noted as the polysulfone beads maintained their integrity in the column.

3.2.4 Desorption studies

Desorption studies were conducted by back-washing metal laden biomass with 500 mL of 0.2 N HCl in the direction opposite to that of adsorption. The change in direction of the column for the desorption process prevents the high concentration of metal accumulated at the top from being washed back into the column. Instead, the reversed column pushes out the high metal concentration. Eluents were collected in 20 mL aliquots. These were then analysed using atomic absorption spectrophotometer. After acid desorption columns were reconditioned with 500 mL 0.2 M NaOH. This was incubated for 20 mins to reverse the acidity of the biomass. Two bed volumes of distilled water were added to the column to condition it.

3.3 RESULTS

3.3.1 *Bacillus subtilis* Column Biosorption

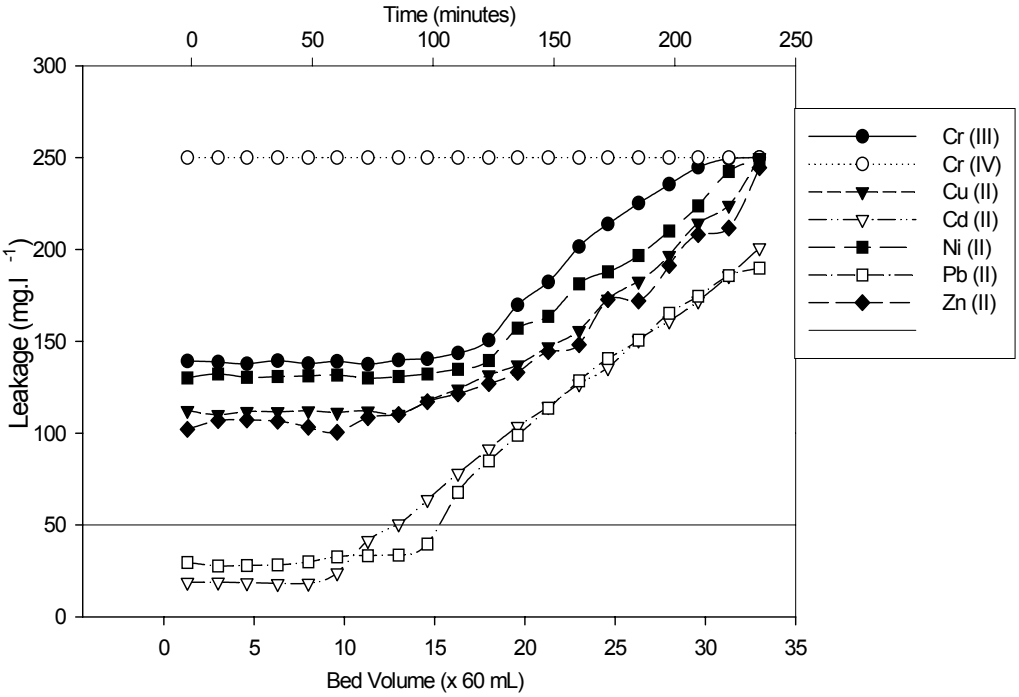


Figure 3.1 Column breakthrough curves for *Bacillus subtilis* cycle 1, with line drawn at 50 $mg.l^{-1}$ indicating minimum breakthrough concentration.

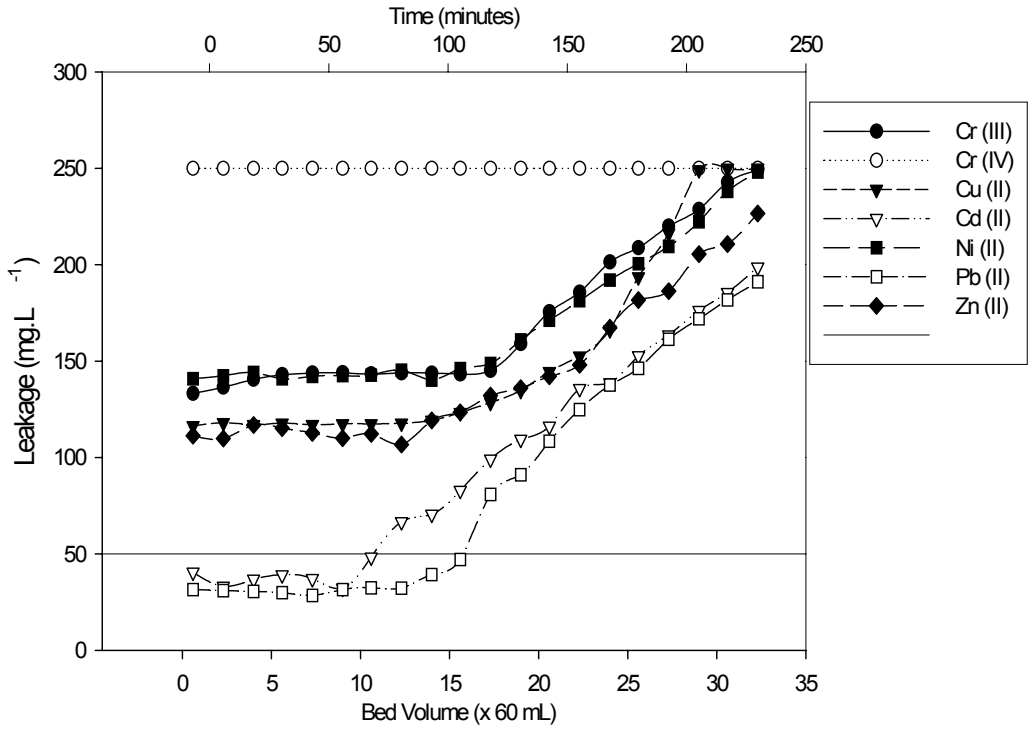


Figure 3.2 Column breakthrough curves for *Bacillus subtilis* cycle 2, with line drawn at 50 $mg.l^{-1}$ indicating minimum breakthrough concentration.

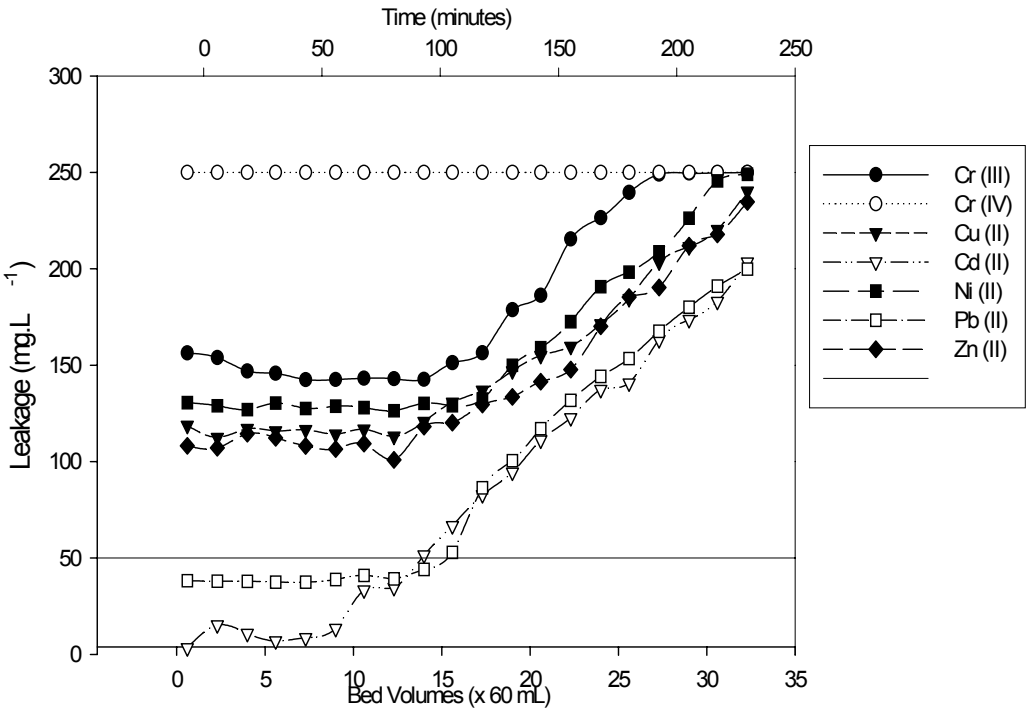


Figure 3.3 Column breakthrough curves for *Bacillus subtilis* cycle 3, with line drawn at 50 mg.l-1indicating minimum breakthrough concentration.
(Column length: 250 x 27, flow rate:10 ml.min^{-1})

Table 3.1 Percent metal removal by *Bacillus subtilis* immobilised onto polysulfone from a 2 L mixed metal solution, with each metal having a concentration of 250 mg.L**.**

	Cr^{3+}	Cr^{3+}	Cu^{2+}	Cd^{2}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	**30**	**0**	**42**	**65**	**35**	**65**	**44**
Cycle 2	**26**	**0**	**39**	**66**	**35**	**62**	**42**
Cycle 3	**30**	**0**	**38**	**60**	**32**	**64**	**41**

Table 3.2 Percent metal (of adsorbed values) desorbed from *Bacillus subtilis* immobilised onto polysulfone from a 2 L mixed metal solution, with each metal having a concentration of 250 mg.L.

	Cr^{3+}	Cu^{2+}	Cd^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	93	93	74	59	59	92
Cycle 2	77	75	68	39	44	77
Cycle 3	79	99	76	99	57	101

3.3.2 *Citrobacter* N14 Column Precipitation

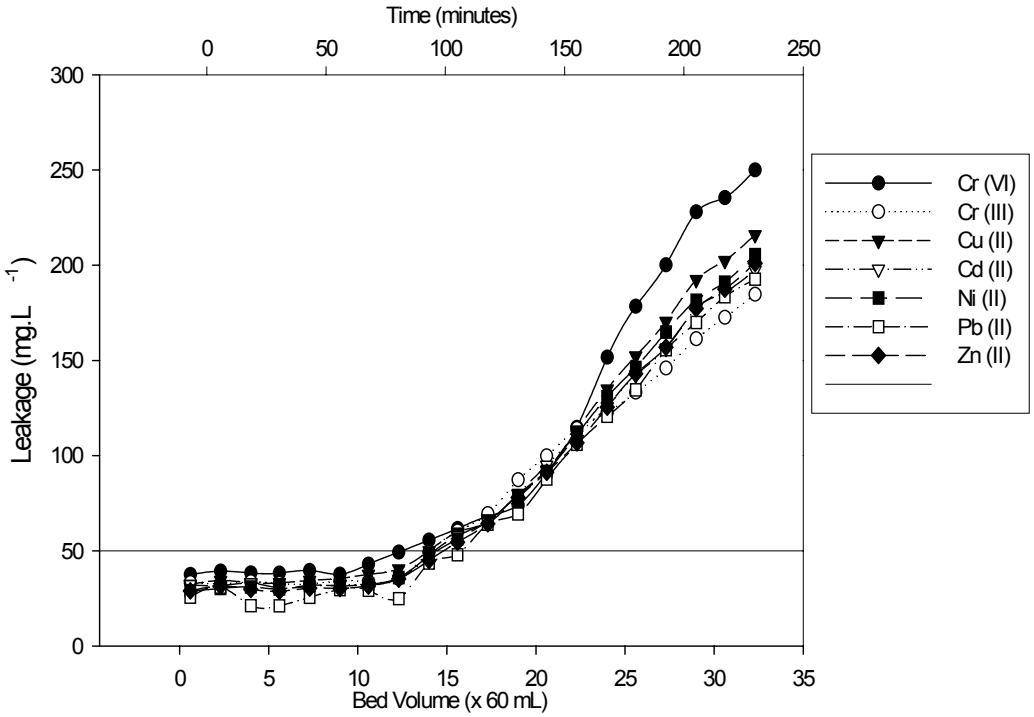


Figure 3.4 Column breakthrough curves for *Citrobacter* N14 cycle 1, with line drawn at 50 mg.l-1 indicating minimum breakthrough concentration.
(Column length: 250 x27, flow rate:10 ml.min^{-1})

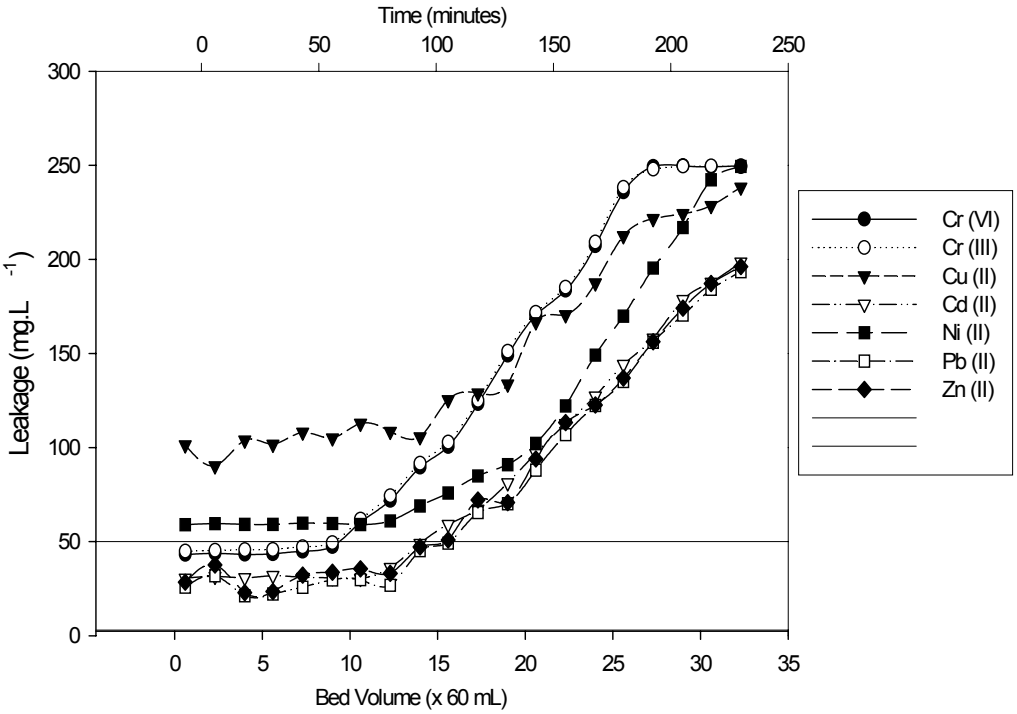


Figure 3.5 Column breakthrough curves for *Citrobacter* N14 cycle 2, with line drawn at 50 mg.l^{-1} indicating minimum breakthrough concentration.

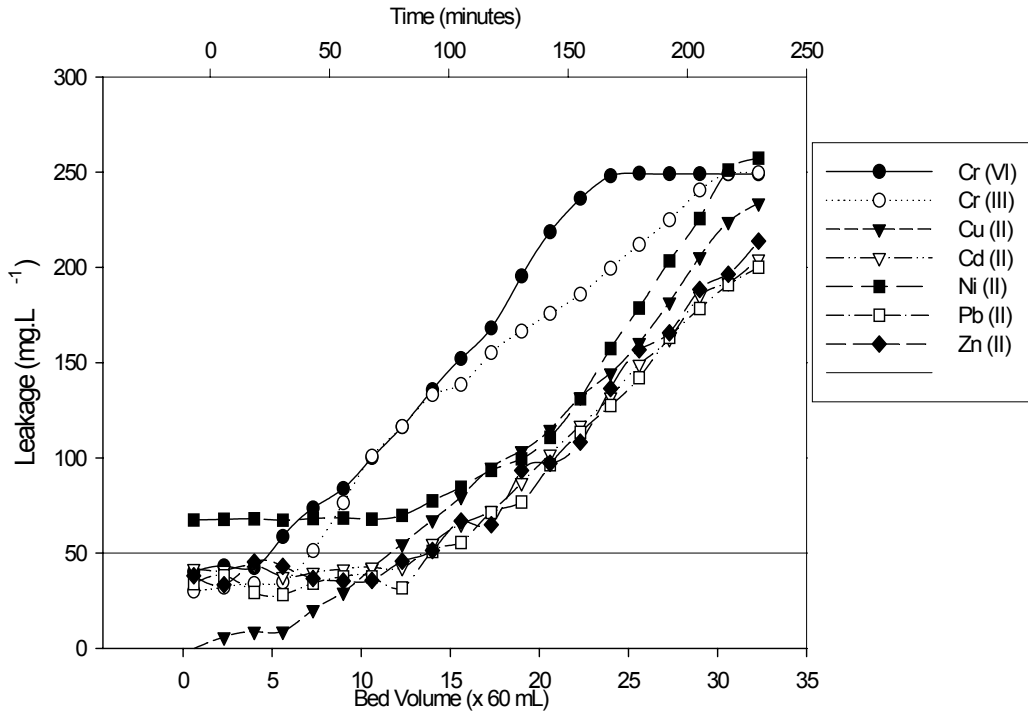


Figure 3.6 Column breakthrough curves for *Citrobacter* N14 cycle 3, with line drawn at 50 mg.l^{-1} indicating minimum breakthrough concentration.
(column length: 250 x 27, flow rate: 10 ml.min^{-1})

Table 3.3 Percentage metal removal by *Citrobacter* N14 immobilised onto polysulfone from a 2 L mixed metal solution, with each metal having a concentration of 250 $mg.L^{-1}$.

	Cr^{3+}	Cr^{6+}	Cu^{2+}	Cd^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	58	66	63	65	65	68	66
Cycle 2	36	43	61	62	51	64	61
Cycle 3	46	46	40	65	54	67	65

Table 3.4 Percent metal (of adsorbed values) desorbed by *Citrobacter* N14 immobilised onto polysulfone from a 2 L mixed metal solution, with each metal having a concentration of 250 $mg.L^{-1}$.

	Cr^{3+}	Cu^{2+}	Cd^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	81	66	99	100	98	99
Cycle 2	119	70	81	96	81	82
Cycle 3	78	86	63	77	66	64

3.3.3 *Chlamydomonas reinhardtii* Biosorption

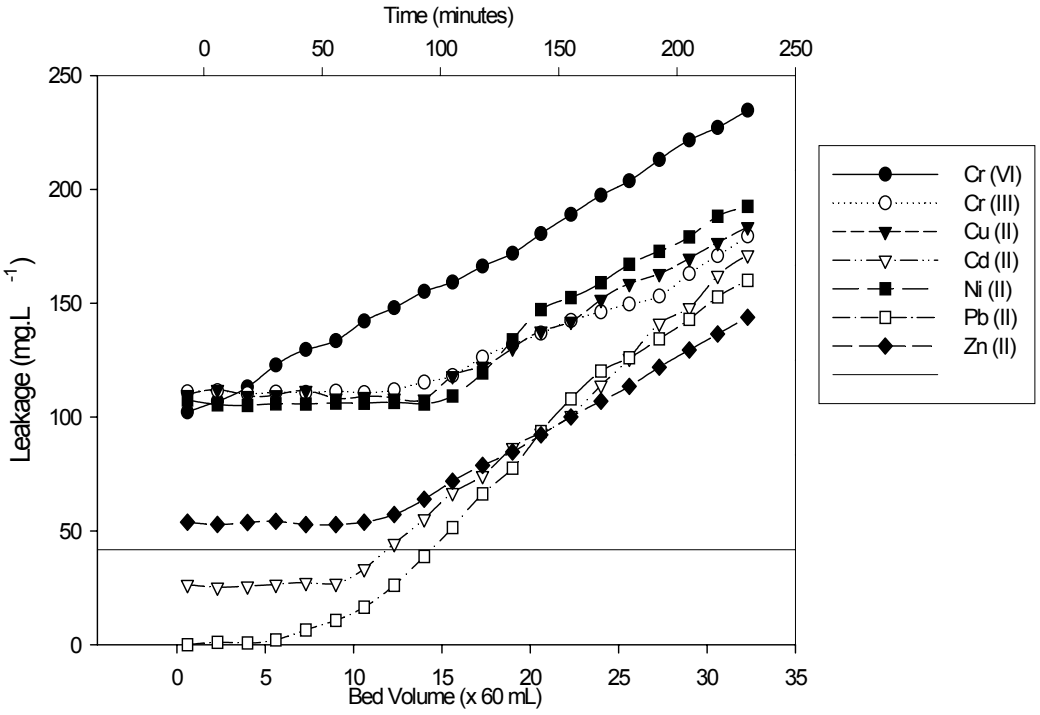


Figure 3.7 Column breakthrough curves for *Chlamydomonas reinhardtii* cycle 1, with line drawn at 50 $mg.l^{-1}$ indicating minimum breakthrough concentration.
(Column length 250 x 27, flow rate: 10 $ml.min^{-1}$)

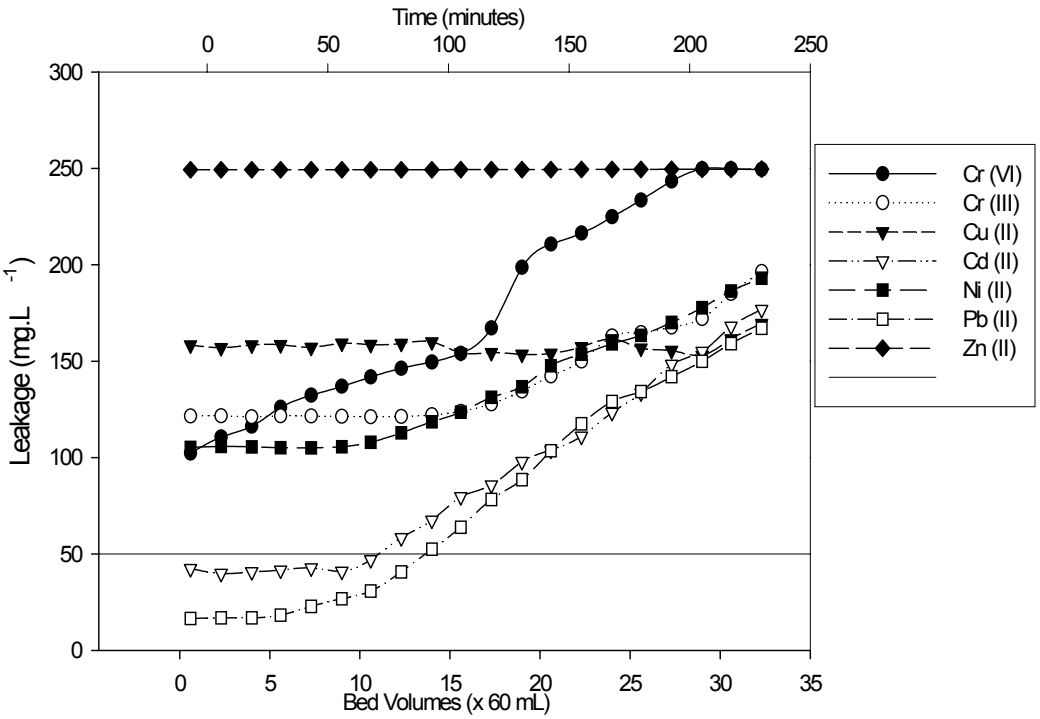


Figure 3.8 Column breakthrough curves for *Chlamydomonas reinhardtii* cycle 2, with line drawn at 50 mg.l^{-1} indicating minimum breakthrough concentration.
(Column length: 250 x 27, flow rate: 10 ml.min^{-1})

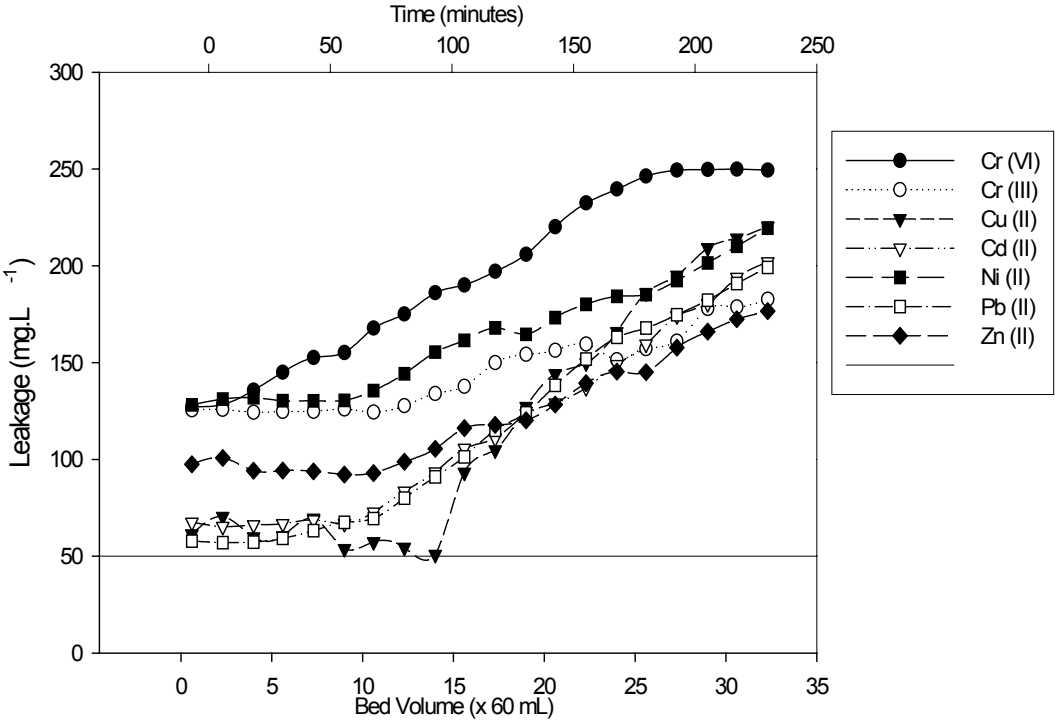


Figure 3.9 Column breakthrough curves for *Chlamydomonas reinhardtii* cycle 3, with line drawn at 50 mg.l^{-1} indicating the minimum breakthrough concentration.
(Column length: 250 x 27, flow rate: 10 ml.min^{-1})

Table 3.5 Percent metal removal by *C. reinhardtii* immobilised onto polysulfone from 2 L mixed metal solution, with each metal having a concentration of 250 $mg.L^{-1}$.

	Cr^{3+}	Cr^{6+}	Cu^{2+}	Cd^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	33	47	47	68	46	73	66
Cycle 2	21	41	51	52	34	67	66
Cycle 3	28	43	36	62	45	67	62

Table 3.6 Percentage metal (of adsorbed values) desorbed from *C. reinhardtii* immobilised onto polysulfone from a 2 L mixed metal solution, with each metal having a concentration of 250 $mg.L^{-1}$

	Cr^{3+}	Cr^{6+}	Cu^{2+}	Cd^{2+}	Ni^{2+}	Pb^{2+}	Zn^{2+}
Cycle 1	103	99	109	84	95	92	100
Cycle 2	86	48	64	58	53	74	65
Cycle 3	29	17	17	13	29	14	27

3.4 DISCUSSION

Initially the intention was to use polyurethane foam as the immobilising agent because of its high porosity. However the foam exhibited low mechanical strength and was compressed in the column experiments. Polysulfone with its superior mechanical stability was chosen as the immobilising agent.

Sewage disposal bylaws state that the heavy metal concentration present in effluent must not exceed 50 $mg.L^{-1}$ for each of the heavy metal solutions (Durban Metro, 2002). This was the basis of determining if the operation would be feasible or not in treating electroplating effluent. If the elluent (leakage) for a particular metal was greater than 50 $mg.L^{-1}$, then the biomass was not efficient in removing that metal.

The column breakthrough curves for the various metals by *B. subtillis* are indicated in Figures 3.1, 3.2 and 3.3. *Bacillus subtilis* exhibited greatest efficiency for the removal of Pb^{2+} and Cd^{2+} ions with Cd^{2+} breaking through at 8 bed volumes (BV) and Pb^{2+} ions at 14.5 BV (Figure 3.1). Cd^{2+} breakthrough was observed after 50 minutes of exposure of the effluent to the biosorbent and Pb^{2+} breakthrough after 100 minutes (Figure 3.1). This pattern was observed in all three cycles of *B. subtilis* biosorption (Figures 3.1, 3.2 and 3.3). Cd^{2+}, being one of the 'big three' toxic metals, is of profound concern as toxic waste and contaminant of surface waters becomes concentrated throughout the food chain. Volesky and Prasetyo, (1994) showed that *Ascophyllum nodosum* in packed bed flow through columns was able to reduce 10 $mg.L^{-1}$ Cd^{2+} concentration of wastewater to 0.003 $mg.L^{-1}$. Leakage of Cd and Pb^{2+} was well below 50 $mg.L^{-1}$. An average of 64% Pb^{2+} and Cd^{2+} was removed in the three cycles (Table 3.1). Cr^{6+}, which exists as the chromate ion (CrO_4^{2-}) had a negative charge and was not removed by the gram positive biosorbent (Table 3.1). Cr^{3+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions produced similar breakthrough curves and were insufficiently removed from solution. Low to average metal removal was achieved. The column produced reproducible results for all three cycles, therefore it can be regenerated and reused to reduce the cost of the operation.

The chromate ion (Cr^{6+}/CrO_4^{2-}) (orange) is reduced to chromium ion (green) and could not be recovered by desorption using acid. In cycle 1, large amounts of Cr^{3+}, Cu^{2+} and Zn^{2+} were desorbed (Table 3.2). Desorption using 0.2 N nitric acid was inadequate for Pb^{2+} removal. The average amount of Pb^{2+} desorbed for the three cycles was 53.33%.

Citrobacter N14 immobilised onto polysulfone beads was capable of removing all metals from the mixed metal solution fairly well. The breakthrough curves indicate the typical 'S' shaped curves. In the first cycle, *Citrobacter* N14 did not differentiate the metals that were adsorbed (Figure 3.4). Removal for all the metals for cycle 1 ranged from 58% for Cr^{3+} to 68% for Pb^{2+} (Table 3.1). Saturation of the biomass column occurred at 12 BV (Figure 3.4).

The second cycle shows *Citrobacter* N14 losing its affinity for Cu^{2+} ions and Cr^{3+} ions. 61% of Cu^{2+} was removed (Table 3.2). Cu(II) breakthrough occurred at 10 BV (Figure 3.5). Cr^{3+} and Cr^{6+} breakthrough simultaneously at 7 bed volumes (Figure 3.5). This observable fact is further illustrated in the third cycle with Cr^{3+} and Cu^{2+} breaking through almost immediately at 360 ml (6 BV). (Figure 3.6).

Cr^{3+} and Cu^{2+} did not produce reproducible results. Adsorption and desorption affects these active sites. Metal recovery was easily obtained with 0.2 N nitric acid but an overall decrease in desorption was observed as the cycles increased (Table 3.4).

Pb^{2+} and Cd^{2+} were effectively removed from solution for all three cycles (Figures 3.7, 3.8 and 3.9), with a reproducible breakthrough at 8 BV(480 ml). Leakage was below 50 $mg.L^{-1}$. Moderate biosorption was obtained for Ni^{2+} and breakthrough decreased through the cycles. In cycle 2, Zn^{2+} was not biosorbed (Figure 3.8), but in cycles 1 and 3, Zn^{2+} breakthrough occurred at 15 BV (900 ml) (Figures 3.7 and 3.9). Cu^{2+} biosorption improved from cycle 1 to cycle 3 and exhibited better removal than Cu^{2+} or Cd^{2+} in the final cycle. Cr^{6+} exhibited a similar phenomenon.

Although *Chlamydomonas reinhardtii* produced fairly reproducible biosorption cycles, metal

recovery (desorption) got worse. During cycle 1 of the desorption process, percentage metal remove varied from 84 to 109%. At the end of the 3rd cycle, these values had decreased to between 13 and 29% (Table 3.6). This biomass cannot be reused to reduce its cost.

3.5 CONCLUSIONS

Chlamydomonas reinhardtii was the best biosorbent for Cr^{3+} (97.872 $mg.g^{-1}$) and *Citrobacter* N14 was the best remediating agent for Zn^{2+} (124.837 $mg.g^{-1}$), Pb^{2+} (75.006 $mg.g^{-1}$), Cu^{2+} (73.051 $mg.g^{-1}$), Cd^{2+} (72.252 $mg.g^{-1}$), Cr^{6+} (74.540 $mg.g^{-1}$) and Ni^{2+} (71.411 $mg.g^{-1}$). *Bacillus subtilis* exhibited better Ni^{2+} removal (55.063 $mg.g^{-1}$) than *Chlamydomonas reinhardtii* (46.604 $mg.g^{-1}$) in column biosorption. Similar biosorption occurred for Pb^{2+} and Zn^{2+} by *Chlamydomonas reinhardtii* and *Bacillus subtilis*. *Bacillus subtilis,* however, was unable to remove Cr^{6+}.

Batch free-cell biosorption removed 14% Cd^{2+}, 22% Cr^{6+}, 15% Ni^{2+}, 36% Pb^{2+}, 23% Cu (II) more than column biosorption. Column biosorption removed 24% more Zn^{2+} than batch free-cell biosorption.

68% of Cd^{2+} and Pb^{2+} was removed by *C. reinhardtii* and *Citrobacter* respectively. This was the highest percentage of metal removed by these biomass in the first cycle.

C. reinhardtii produced fairly reproducible results, but the desorption decreased from cycle 1 to cycle 3. Also, the alga took far too long to cultivate and required light during the growth phase. Since this process was considered too time consuming, only the remaining two technologies were considered for the pilot scale studies.

CHAPTER 4 BIOSORBENT TECHNOLOGY TRANSFER TO INDUSTRY (PILOT SCALE)

4.1 INTRODUCTION

Tightened laws and statutory orders for prevention of water pollution and waste disposal impose stricter limits and make greater demands on the treatment and discharge of especially toxic waste. Industrial enterprises have to treat wastewaters with dangerous contents, to the most progressive and efficient methods to limit pollution of the natural waters (Volesky, 1990). The laws set by the Durban Wastewater Department governing effluent discharge dictate that no trade effluent will be accepted for discharge into the sewage disposal system if:

1. the concentration of Cu^{2+}, Ni^{2+} and Zn^{2+} exceeds 50 $mg.l^{-1}$ for large sewage works,
2. the concentration of Pb^{2+}, Cd^{2+} and Cr^{6+} exceeds 20 $mg.l^{-1}$ for large sewage works
3. Cu^{2+}, Ni^{2+}, Pb^{2+}, Cd^{2+}, Cr^{6+} and Zn^{2+} exceed 5 $mg.l^{-1}$ for small works (Durban Metro, 2002).

Biosorption may have a potential marketing advantage over other treatment techniques, in that it can be perceived as environmentally friendly and, particularly for use of waste biomass, a natural and maximal use of gathered material (Wase and Forster, 1997). Laboratory-scale experiments showing the affinity that a biosorbent material exhibits for a specific metal cation will dictate the practicality of its implementation for remediation of a particular waste stream. Once laboratory trials are complete and a potential biosorbent has shown the ability to adsorb and sequester the required metal from solution, several questions need to be resolved. These include the type and volume of effluent, the metal species present, the biomass to be used, the process equipment as well as desorption costs (Atkinson *et al*, 1998).

4.1.1 Electroplating Industries

Electroplating is a process whereby a thin coating of metal possessing certain desirable properties is deposited onto a component of cheaper base metal by means of an electric current (Binnie and Partners, 1987). Two electroplaters situated in the Durban region were contacted and one of them, Natal Electroplaters was willing to comply with having the imported biomass treat the effluent that the plant produces.

4.1.1.1 Effluent characterisation and production

Each process stage within the electroplating sequence is followed by a rinse stage, removing traces of process solution which adhere to the article being plated. These rinse flows contain quantities of the various process solutions which are then carried to the drain. Acidic electrolyte solutions are used for Cu^{2+}, Ni^{2+}, Cr^{6+} and $Zinc^{2+}$, whilst alkaline cyanide complexes are used for Cu^{2+}, Zn^{2+}, Cr^{6+}, Au^{2+} and Ag^{2+}. The rinse flows therefore contain traces of each of these process solutions in concentrations depending on the rinse flow rates and solution carry-over (Binnie and Partners, 1987). The effluent contains all the metals of interest (that is Cr, Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+} and Zn^{2+}). The concentrations of these metals vary continuously from trace amounts to several hundred parts per million ($mg.L^{-1}$). After each stage in the electroplating process, the article being plated is rinsed in a water bath, the drag-out bath. Each stage has its own drag-out bath. The contents of these baths flow into a stream that leads to a reservoir which contributes to the effluent produced. (Binnie and Partners, 1987).

4.1.1.2 Current treatment

The most common methods of removal of metals from industrial effluents include chemical precipitation, solvent extraction, dialysis or electrodialysis, electrolytic extraction, cementation, reverse osmosis, evaporative methods, ion-exchange resins, carbon sorption and dilution. Conventional physical and chemical treatment of low concentration, large volumes of waste tends to be costly. Chemical precipitation processes entail large capital and operating costs (Wase and Forster, 1997). Currently, the metal effluents from all waste streams are collected in a reservoir. This mixed metal solution is then subjected to alkaline precipitation. Although this is a cheap and simple method, it leads to an undesirable, highly toxic sludge formation that is a problem to dispose even in landfills. The mechanisms of accumulation of heavy metal by the

imported technologies have been studied extensively and is important from the industrial point of view since removal of heavy metals from industrial effluents is the aim of biosorption research. All three technologies have advantages of high metal binding capacity, and two show low cost maintenance. The aim of this chapter was to examine the possible industrial usage of column immobilised *Bacillus subtilis* and *Citrobacter* N14 to remove heavy metals from electroplating effluent.

4.1.2 Objectives

1. To select technologies showing superior biosorption in laboratory trials.
2. To transfer these technologies to an electroplating industry to treat the effluent produced.
3. To assess feasibility of implementation after pilot-scale trials

4.2 METHODS

4.2.1 Growth of Microorganisms

The bacteria, *Bacillus subtilis* and *Citrobacter* were grown in Nutrient broth (Merck, SA), and incubated for 24-48 hrs at 29°C . This represented the starter culture. Five L erlenmeyer flasks containing three L of nutrient broth were innoculated with the starter culture. Incubation was on a rotary shaker at room temperature for one week. Cells were harvested by centrifugation at 25 000 x g for 15 mins at 25°C. The above process was repeated three times to remove traces of growth media. After resuspension of biomass in Phosphate buffered saline, five ml of suspension was dispensed into pre-weighted bijou bottles and dried overnight in an oven at 105°C. The mass of cells in five ml aliquots of culture was the difference between weighted bijou bottles and biomass and mass of empty bijou bottle.

4.2.2 Method of Immobilisation

Each of the two biomasses, *B. subtillus* and *Citrobacter* N14 was dried at 100ºC then ground into a powder. Biomass was immobilised by entrapment in polysulfone resin that was dissolved in dimethyl formamide (DMF) to yield a final concentration of 100 $g.L^{-1}$ of the immobilising agent. Dry biomass (5 g) was mixed with 100 ml of polysulfone-DMF solution and extruded drop-wise, through a 16-gauge surgical needle attached to a peristaltic pump via silicone tubing (ID = 4 mm), into a beaker containing ice cold deionised water, to form spherical beads 2-3 mm in diameter. This was the smallest size that could be produced with the above-mentioned set up without clogging. Beads were stored overnight at 4ºC to ensure complete solidification. Beads were rinsed in deionised water prior to the biosorption procedure.

4.2.3 Column Biosorption

The columns were constructed from grade 316 stainless steel (250 mm X 270 mm) and contained five g of biomass, yielding an approximate packing density of 40 $g.l^{-1}$. The upflow feed was maintained at 10 $mL.min^{-1}$ by peristaltic pump. A single column, packed with approximately five g of biomass (immobilised in 25 g polysulfone resin) was connected to the outlets of the drag-out baths for Ni^{2+}, Cr^{6+}, Ag^{2+} and Cu^{2+}. The effluent in each of the drag-out

baths contains only single metals. After two L (approximately 40 bed volumes) of metal solution was treated, the column was desorbed with 500 mL 0.2 N HCl and then neutralised with 500 mL 0.2 N NaOH. The column was reused in this manner until a decrease in biosorption was observed. Breakthrough was set at 20 $mg.l^{-1}$.

4.2.4 Desorption Studies

Desorption studies were conducted by back-washing metal laden biomass with 500 ml of 0.2 N HCl in the direction opposite to that of adsorption. The change in direction of the column for the desorption process prevents the high concentration of metal accumulated at the top from being washed back into the column. Instead, the reversed column pushes out the high metal concentration. Eluents were collected in 20 ml aliquots. These were then analysed using flame atomic absorption spectrophotometry. After acid desorption, columns were reconditioned with 500 ml 0.2 M NaOH. This was incubated for 20 mins to reverse the acidity of the biomass. Two bed volumes of distilled water were added to the column to condition it.

From the previous chapter it was noted that although *C. reinhardtii* produced fairly reproducible results, the desorption decreased from cycle 1 to cycle 3. Also, *C. reinhadtii* took far too long to cultivate and required light during the growth process. This process was considered to consume too much time and electricity, hence not practical. For this reason only the bacteria *Bacillus subtilis* and *Citrobacter* N14 were used in pilot scale studies.

4.3 RESULTS

4.3.1 *Bacillus subtilis* Column Biosorption

Figure 4.1 Column containing immobilised *Bacillus subtilis* biomass connected to the Ni^{2+}drag out bath at Natal Electroplaters, Durban, South Africa..

4.3.1.1. Pilot-scale column biosorption for Ni^{2+} bath

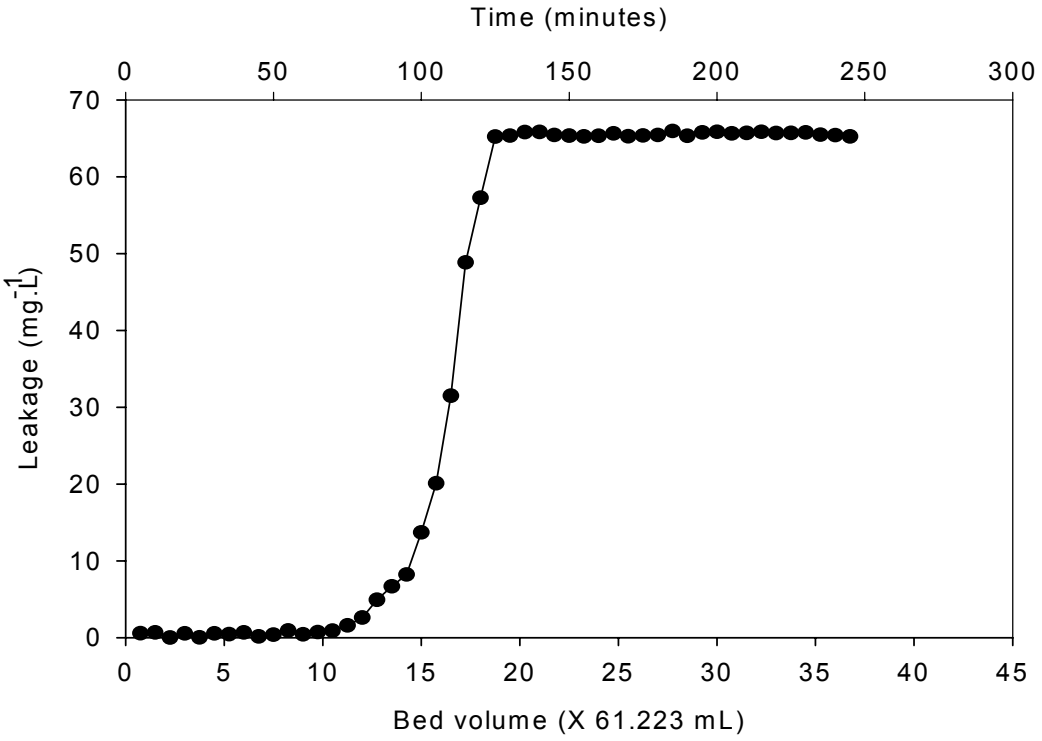


Figure 4.2 Column breakthrough curve for *Bacillus subtilis* cycle 1 used to treat Ni^{2+} from outlet of drag out bath at Natal Electroplaters

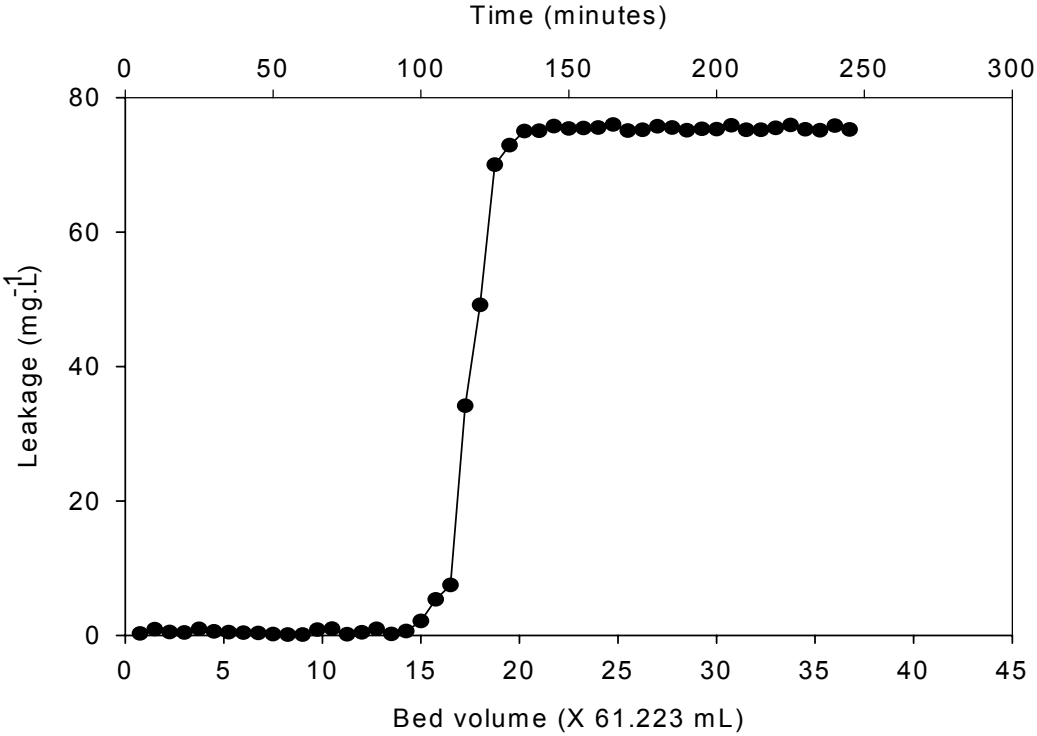


Figure 4.3 Column breakthrough curve for *Bacillus subtilis* Cycle 2 treating Ni^{2+} from outlet of drag-out bath at Natal Electroplaters

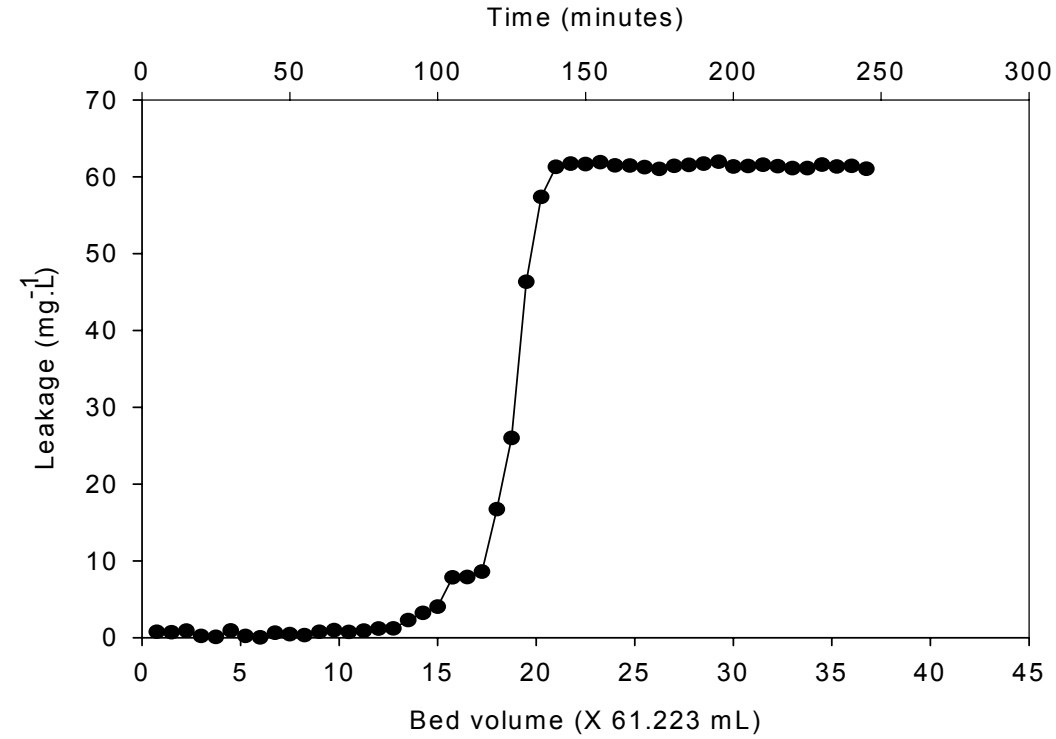


Figure 4.4 Column breakthrough curve for Bacillus subtilis cycle 3 used to treat Ni^{2+} from drag-out bath at Natal Electroplaters

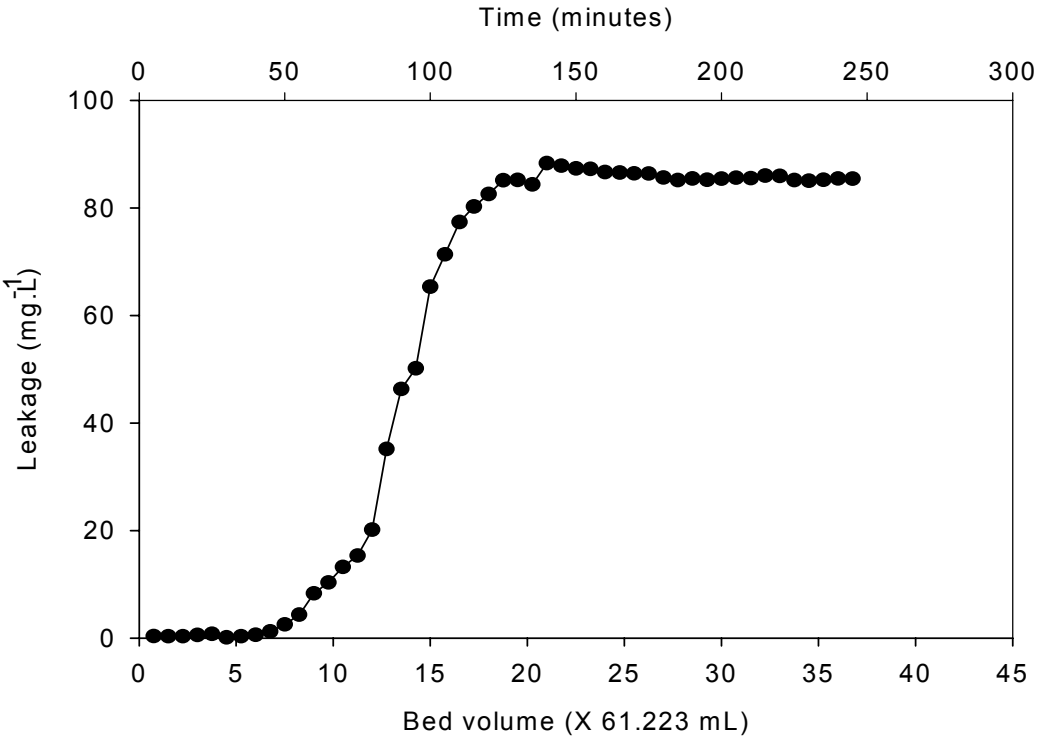


Figure 4.5 Column breakthrough curve for *Bacillus subtilis* cycle 4 used to treat Ni^{2+} from the drag-out bath at Natal Electroplaters

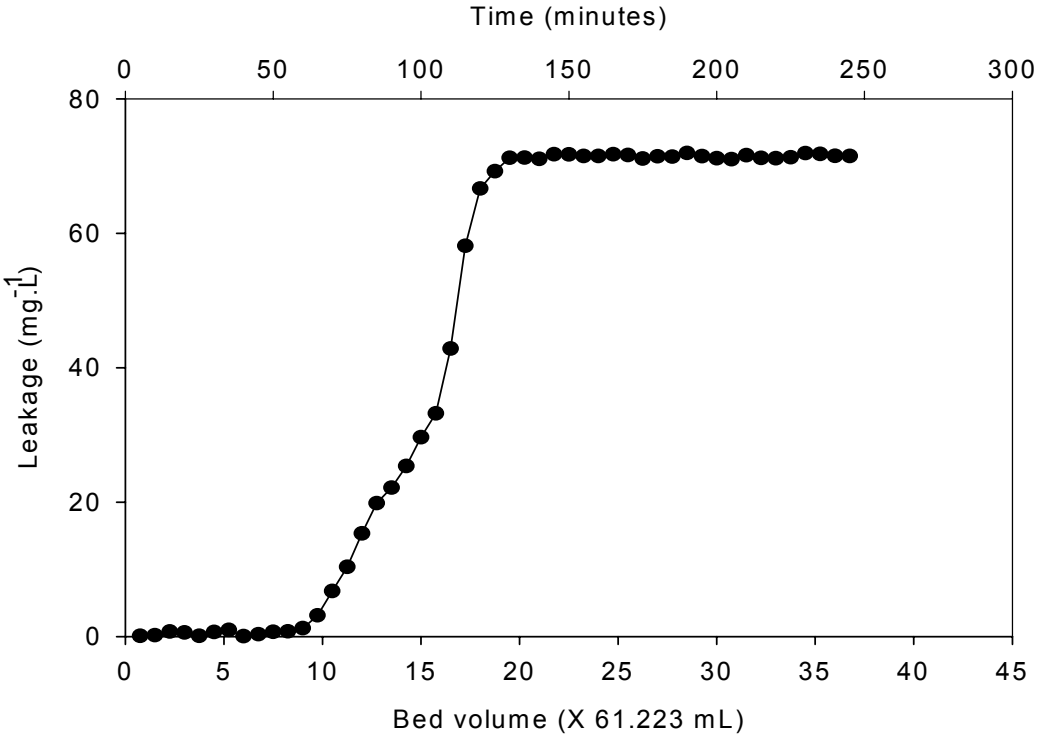


Figure 4.6 Column breakthrough curve for *Bacillus subtilis* cycle 5 used to treat Ni^{2+} from the drag-out bath at Natal Electroplaters

Figures 4.2 to 4.6 show the quantity of Ni^{2+} removed by *Bacillus subtilis* for five cycles. The effluent had a pH of 5 and an approximate concentration of between 55 and 75 $mg.l^{-1}$. Breakthrough occurred at 20 $mg.l^{-1}$ of effluent. The maximum amount of Ni^{2+} removed was during cycle 4 (87.892 mg). Cycle two removed 81.812 mg, and 98% of this metal was recovered by desorption. The least amount of Ni^{2+} was removed in cycle five (58.5 mg).

Table 4.1 Summary of biosorption and desorption by immobilised *Bacillus subtilis* column for Ni^{2+} from outlet of drag-out bath at Natal Electroplaters

Cycle	Breakthrough (BV)	Mass adsorbed at breakthrough (mg)	Mass adsorbed at 40 BV (mg)	Percentage recovered by desorption
1	16	72.84	77.11	92.29
2	18	81.81	85.81	98
3	20	71.95	76.1	91.29
4	13	87.89	113.15	52.03
5	14	58.5	71.35	61

4.3.1.2 Pilot-scale column biosorption for Cr^{6+} bath

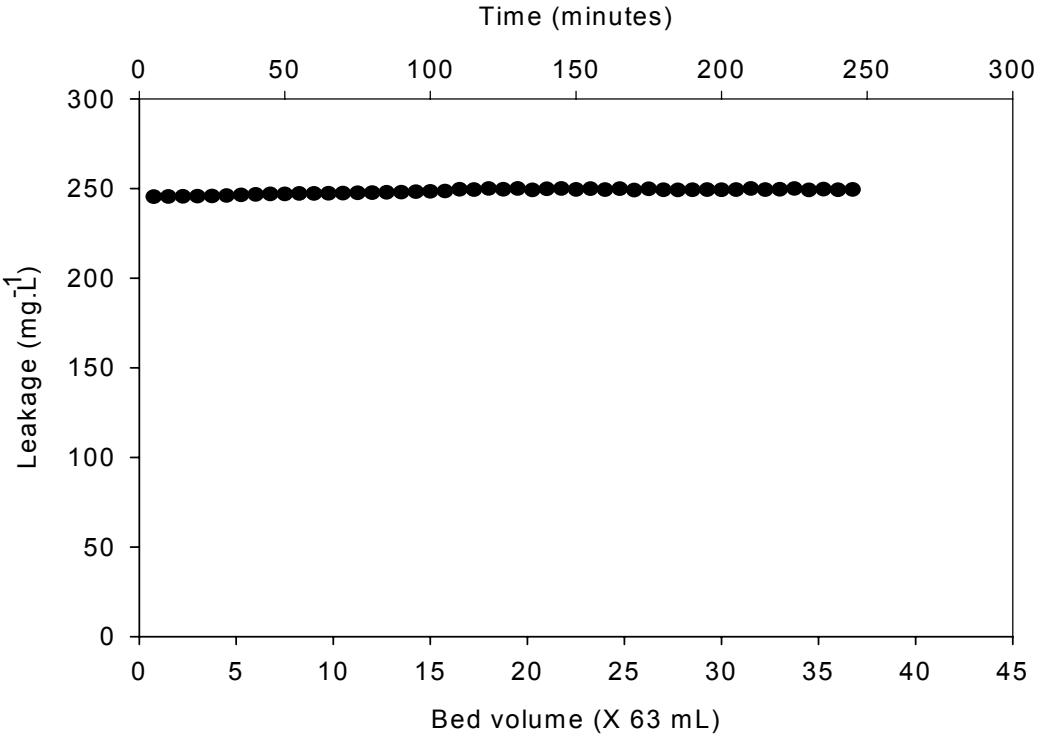


Figure 4.7 Column breakthroug curve for *Bacillus subtilis* cycle 1 used to treat Cr^{6+} from the drag-out bath at Natal Electroplaters

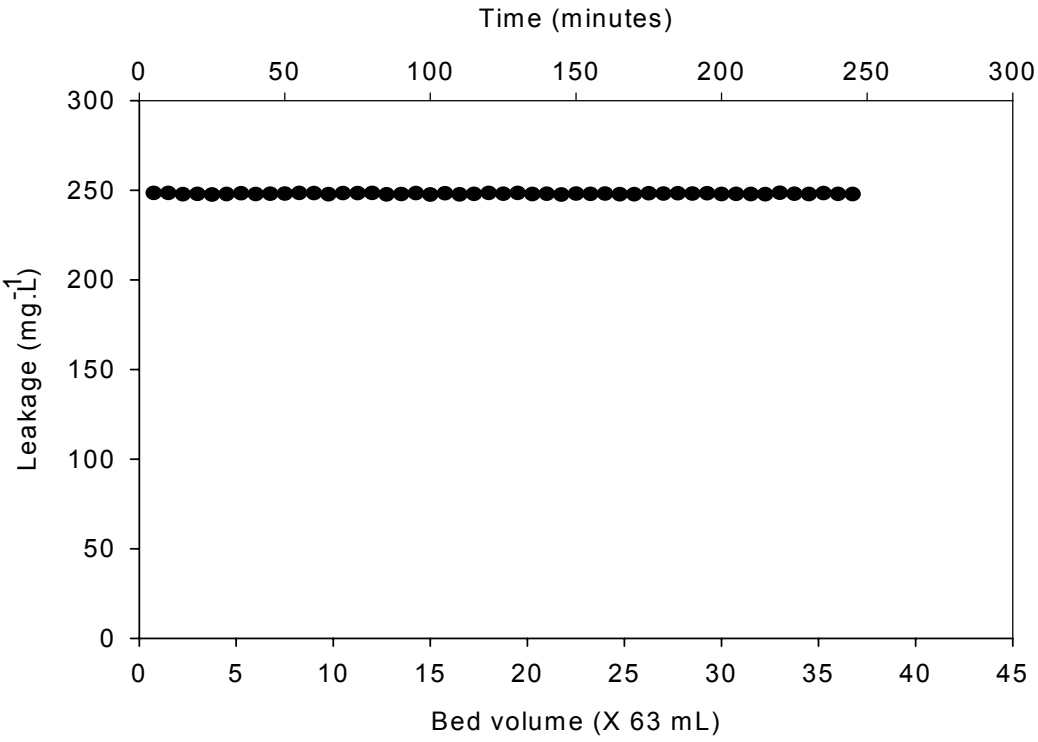


Figure 4.8 Column breakthrough curve for *Bacillus subtilis* cycle 2 used to treat Cr^{6+} from drag-out bath at Natal Electroplaters

Figures 4.7 and 4.8 represent the column biosorption of Cr^{6+} by *Bacillus subtilis*. The chromate influent varied form 250 to 500 $mg.l^{-1}$ at a pH of approximately 6.5. *Bacillus subtilis* was unable to remove Cr^{6+} form the Cr bath.

4.3.1.3 Pilot-scale column biosorption for Ag^{2+} bath

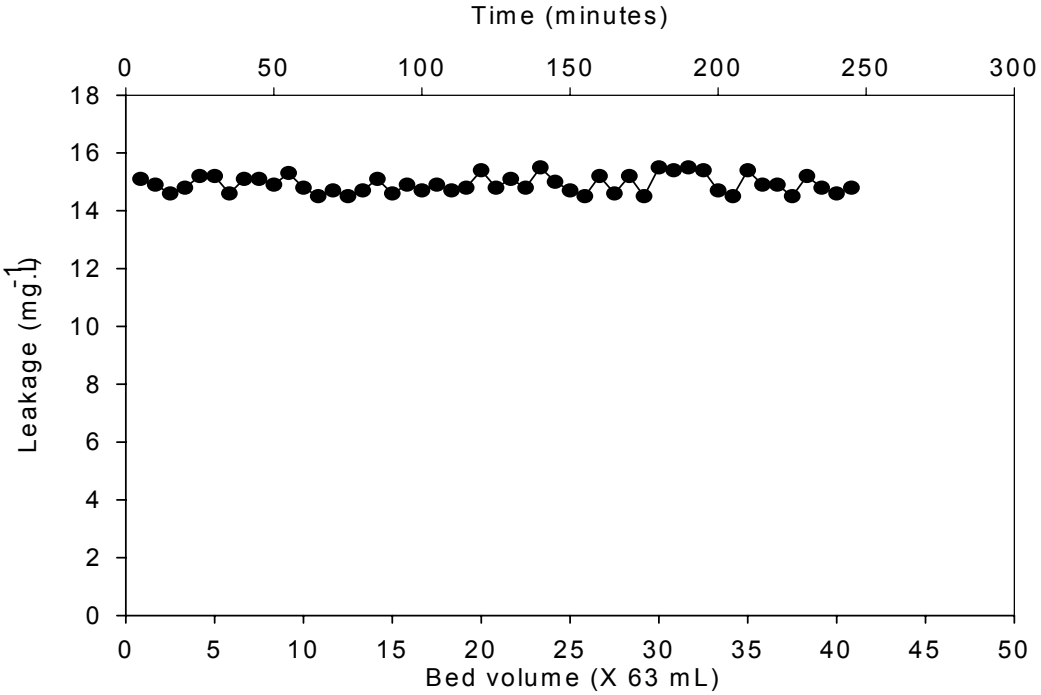


Figure 4.9 Column breakthrough curve for *Bacillus subtilis* cycle 1 used to treat Ag^{2+} from drag-out bath at Natal Electroplaters

4.3.1.4 Pilot-scale column biosorption for Cu^{2+} bath

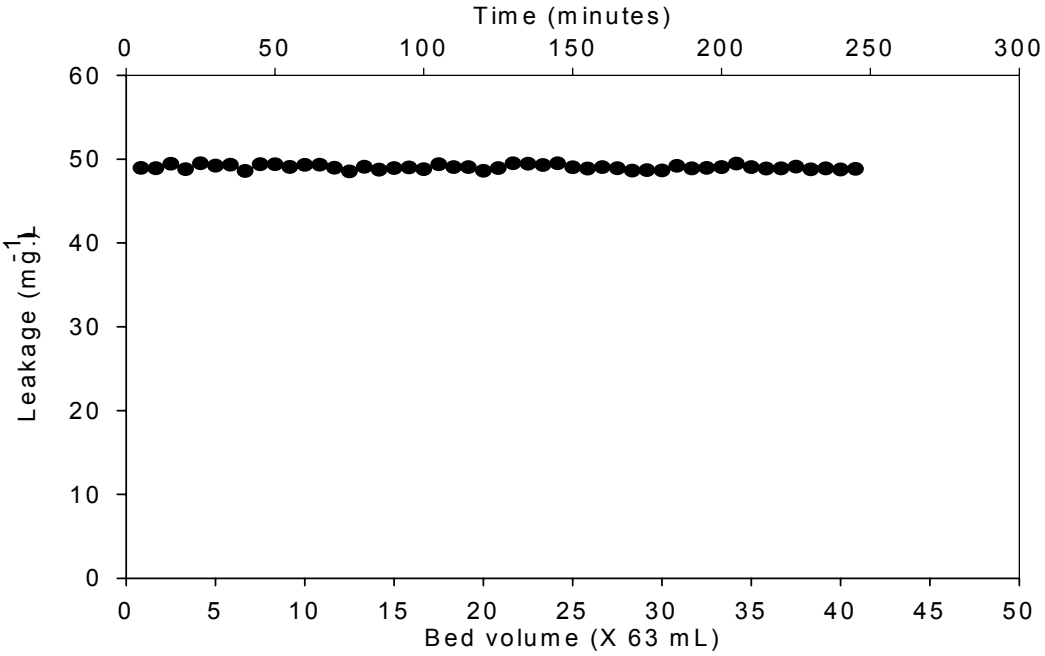


Figure 4.10 Column breakthrough curve for *Bacillus subtilis* cycle 1 used to treat Cu^{2+} from drag-out bath at Natal Electroplaters

Figures 4.9 and 4.10 represent the breakthrough curves for *Bacillus subtilis* used to treat Ag^{2+} and Cu^{2+} respectively. The figures show that the imported biomass was unable to remove these metals from solution.

4.3.2 *Citrobacter* N14 Studies

4.3.2.1 Pilot-scale column biosorption for Ni^{2+} bath

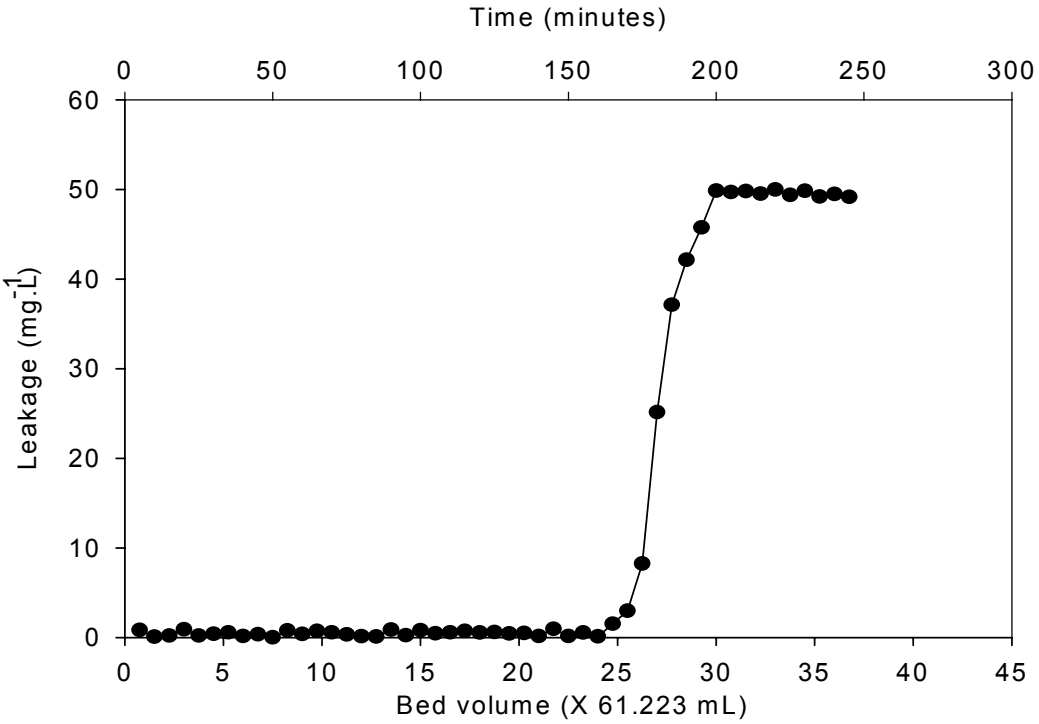


Figure 4.11 Column breakthrough curve for *Citrobacter* N14 cycle 1 used to treat Ni^{2+} from outlet of drag out bath at Natal Electroplaters

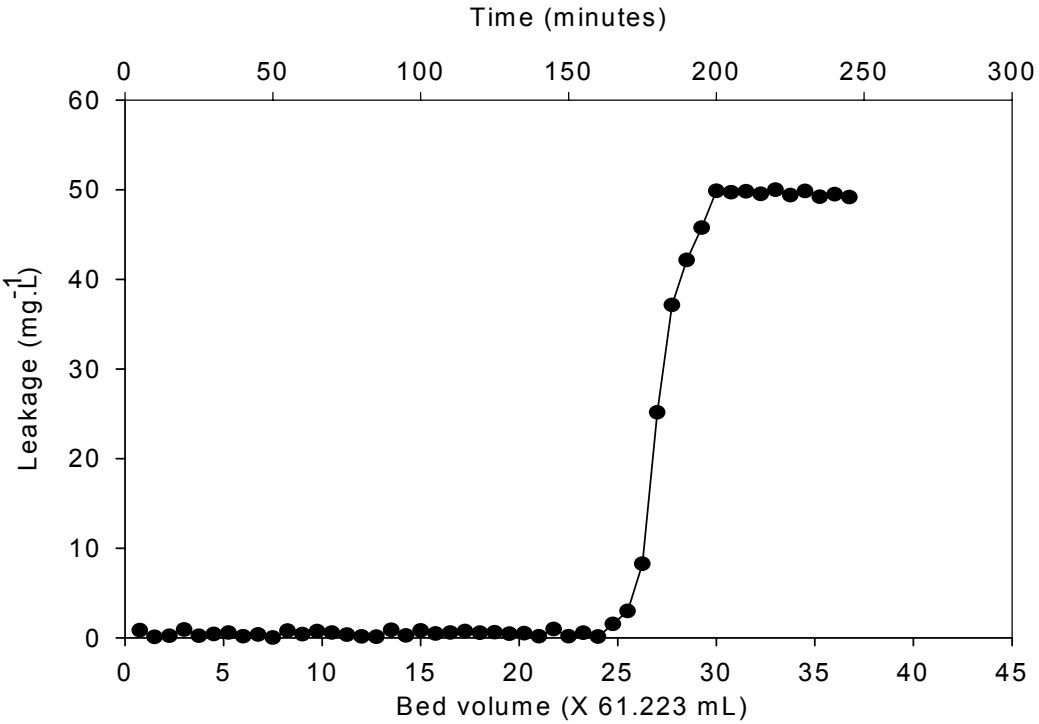


Figure 4.12 Column breakthrough curve for *Citrobacter* N14 cycle 2 used to treat Ni^{2+} from outlet of drag out bath at Natal Electroplaters

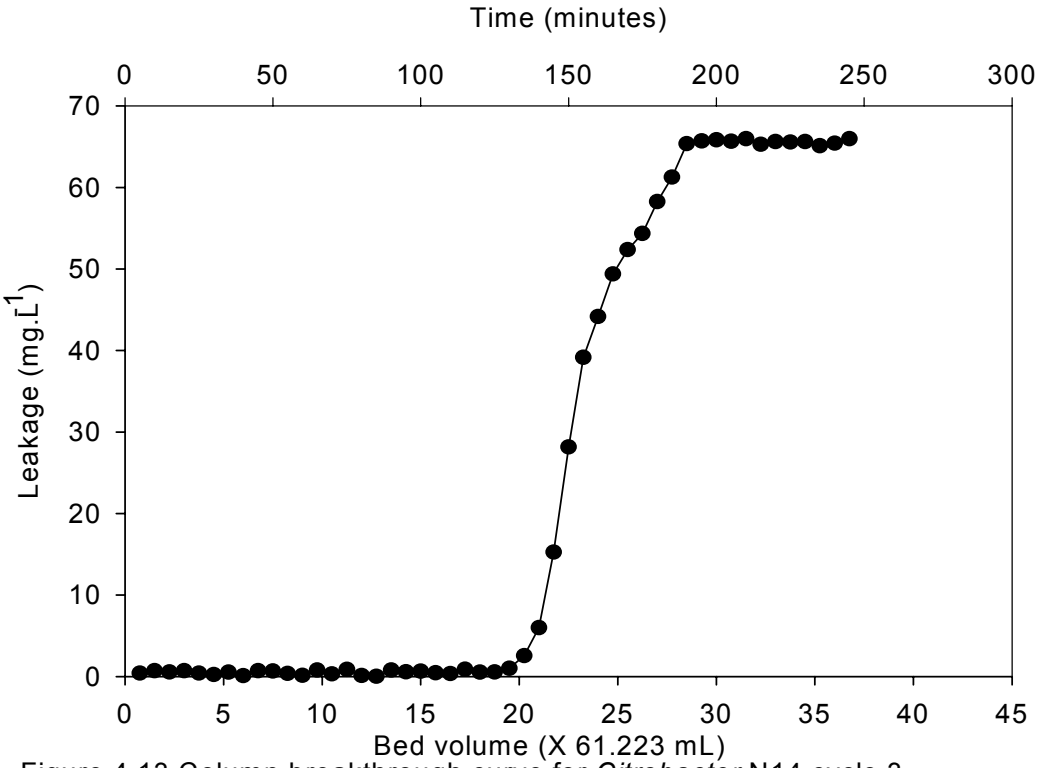


Figure 4.13 Column breakthrough curve for *Citrobacter* N14 cycle 3 used to treat Ni^{2+} from outlet of drag-out bath at Natal Electroplaters

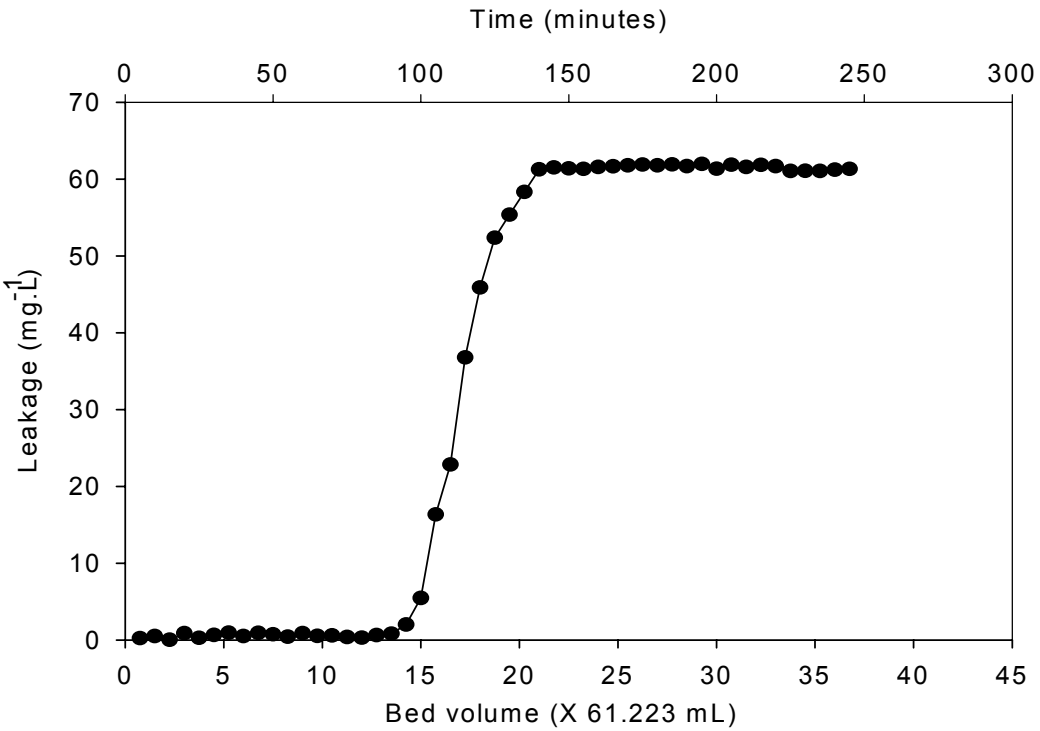


Figure 4.14 Column breakthrough curve for *Citrobacter* N14 cycle 4 used to treat Ni^{2+} from outlet of darg-out bath at Natal Electroplater

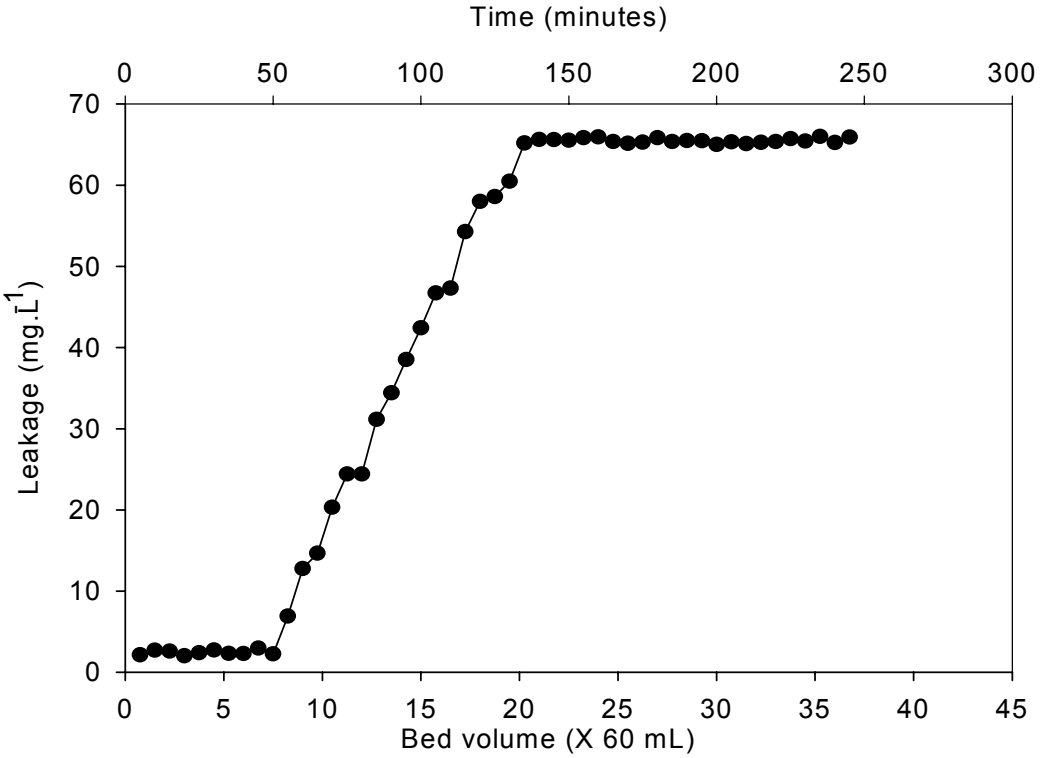


Figure 4.15 Column breakthrough curve for *Citrobacter* N14 cycle 5 used to treat Ni^{2+} from outlet of drag-out bath at Natal Electroplaters

Figures 4.11 - 4.15 represent the quantity of Ni^{2+} precipitated by *Citrobacter* N14 for five cycles. The effluent pH was 5 and the concentration varied between 55 and 75 $mg.l^{-1}$. Breakthrough of the column occurred between 12 and 29 BV. The largest amount of Ni^{2+} precipitated by *Citrobacter* at breakthrough was 95.559 mg during cycle 4. Best biosorption was obtained in the second and third cycles. The fifth cycle produced the lowest biosorption. Breakthrough decreased through the cycles except cycle two, were a slight increase was observed. Metal recovery was reasonably reproducible and fluctuated between 77.748 and 96.62%.

Table 4.2 Summary of biosorption and desorption by immobilised *Citrobacter* N14 column for Ni^{2+} from outlet of drag-out bath at Natal Electroplaters

Cycle	**Breakthrough (BV)**	**Mass adsorbed at breakthrough (mg)**	**Mass adsorbed at 40 BV (mg)**	**Percentage recovered by desorption**
1	26	68.6	86.97	92.28
2	29	83.67	92.48	94.6
3	24	95.56	102.88	87.04
4	17	63.95	70.21	77.75
5	12	41.21	55	96.62

4.3.2.2 Pilot-scale column biosorption for Cr bath

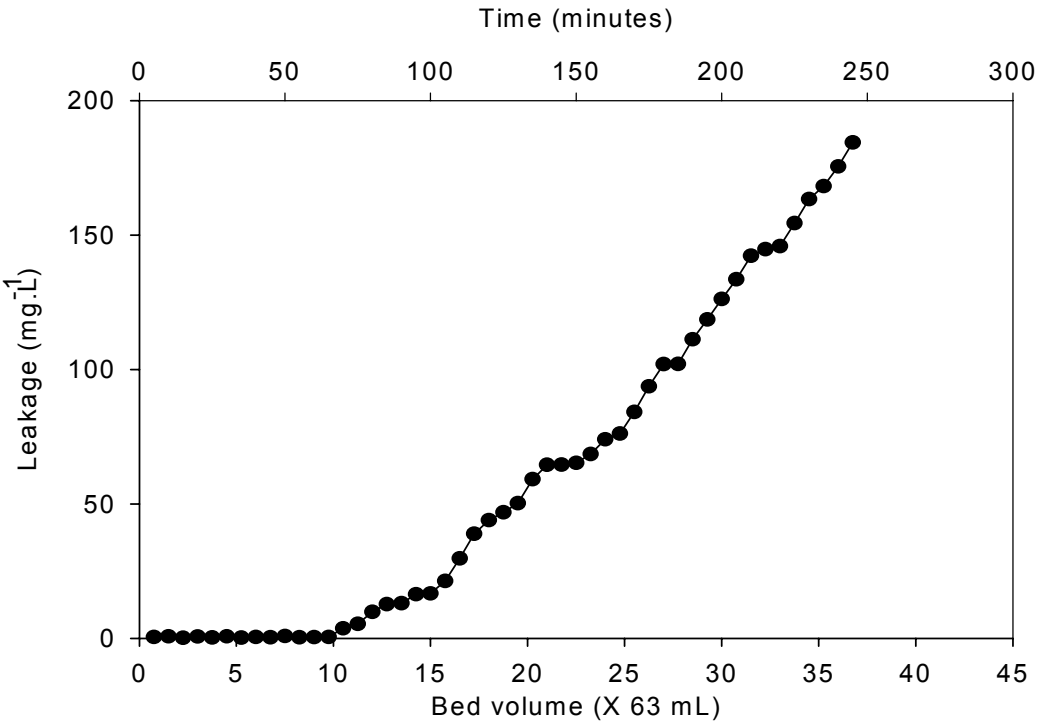


Figure 4.16 Column breakthrough curve for *Citrobacter* N14 cycle 1 used to treat Cr^{6+} from outlet of drag-out bath at Natal Electroplater

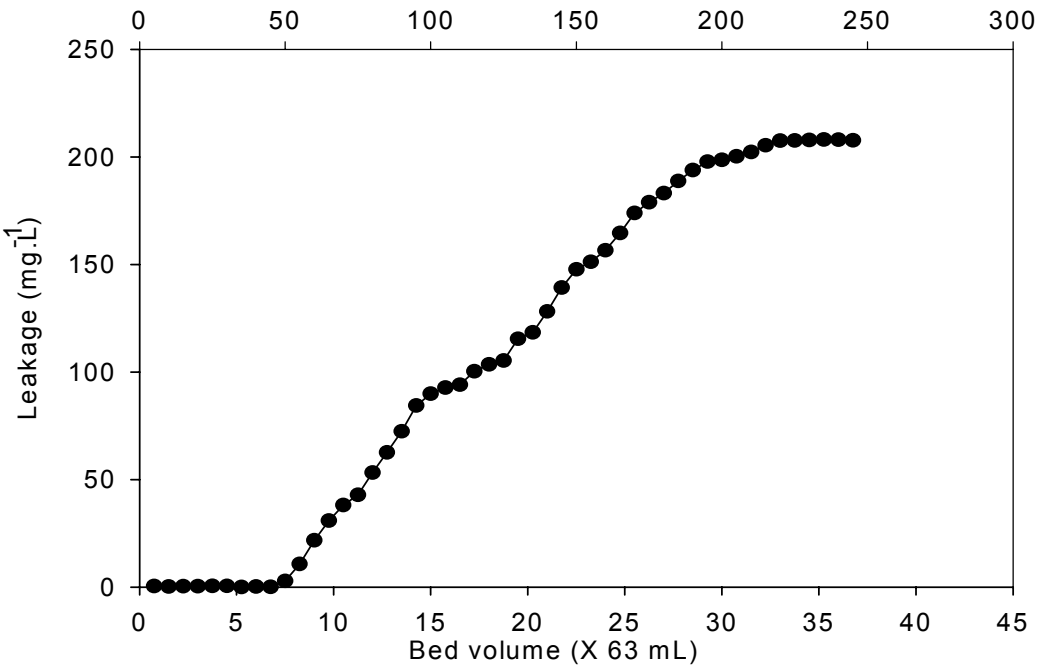


Figure 4.17 Column breakthrough curve for *Citrobacter* N14 cycle 2 used to treat Cr^{6+} from outlet of drag-out bath at Natal Electroplater

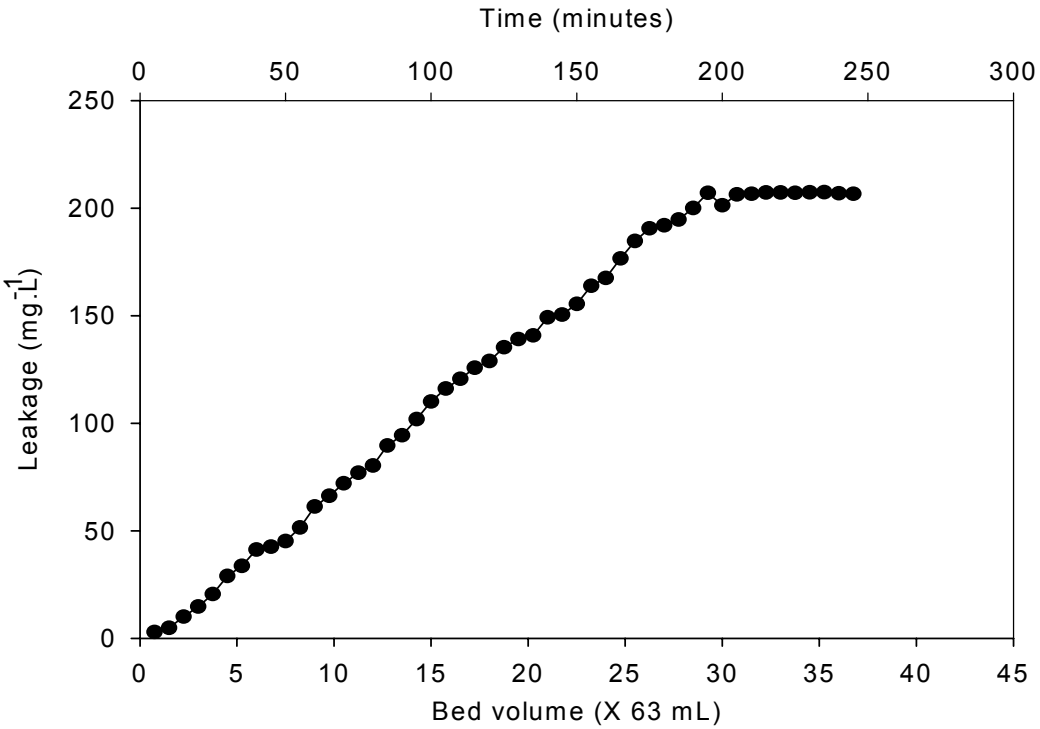


Figure 4.18 Column breakthrough curve for *Citrobacter* N14 cycle 3 used to treat Cr^{6+} from outlet of drag-out bath at Natal Elecroplaters

Figures 4.16, 4.17, and 4.18 represent the breakthrough curves *Citrobacter* N14 and Cr^{6+} removal from the electroplating effluent tank.. The influent concentration was 250 to 500 $mg.l^{-1}$ with a pH of 6.5. Mass of Cr^{6+} adsorbed by *Citrobacter* N14 and desorbed from the biomass decreased from cycle 1 to cycle 3.

Table 4.3 Summary of biosorption and desorption by immobilised *Citrobacter* N14 column for Cr from outlet of drag-out bath at Natal Electroplaters

Cycle	Breakthrough 20 ppm (BV)	Mass adsorbed at breakthrough (mg)	Mass adsorbed at 40 BV (mg)	Percentage recovered by desorption
1	16	212.14	360.22	97.54
2	9	122.85	244.36	89.09
3	4	49.15	200.17	26.6

4.3.2.3 Pilot-scale column biosorption for Ag^{2+} bath

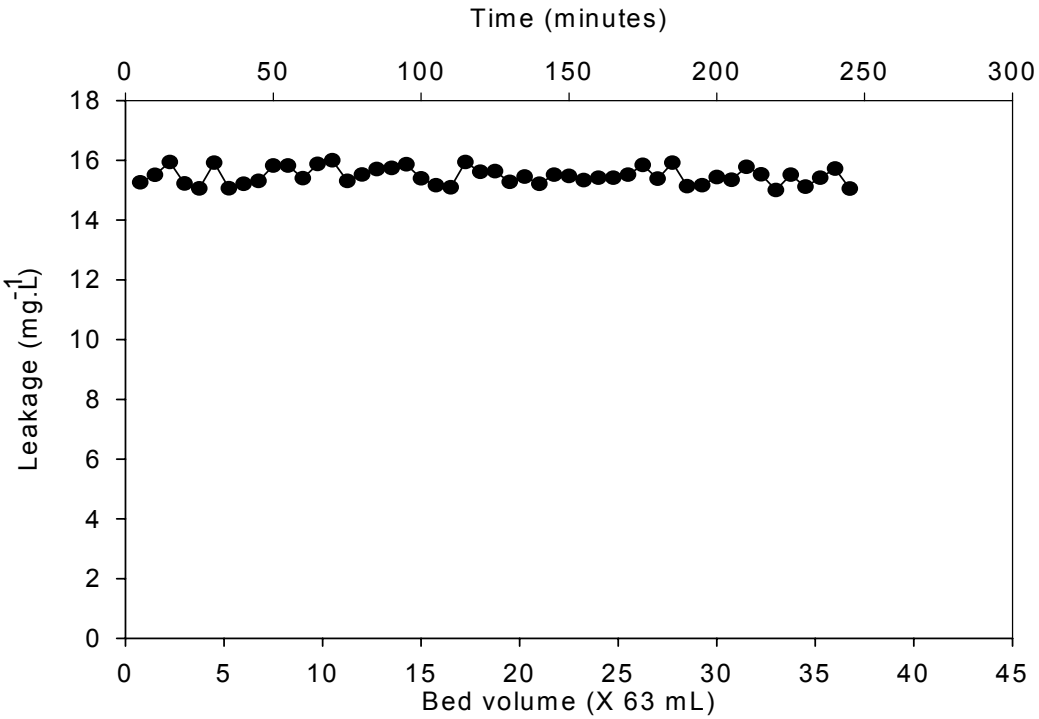


Figure 4.19 Column breakthrough curve for *Citrobacter* N14cycle 1 used to treat Ag^{2+} from outlet of drag-out bath at Natal Electroplater

4.3.2.4 Results for Cu^{2+} bath

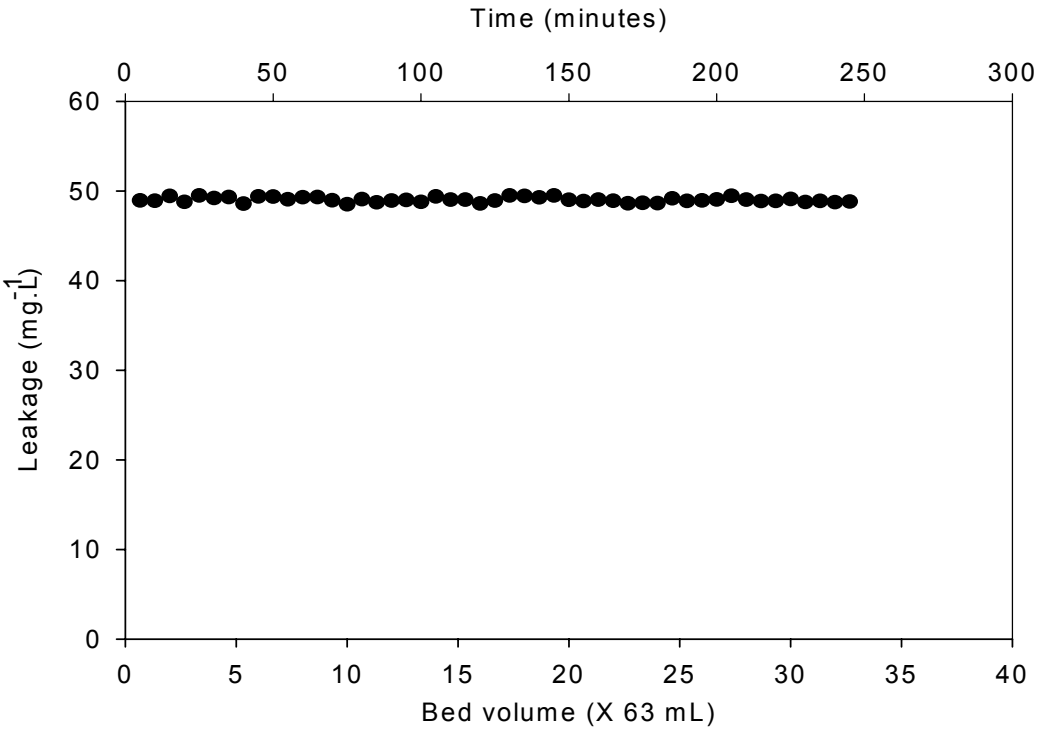


Figure 4.20 Column breakthrough curve for *Citrobacter* N14 cycle 1 used to treat Cu^{2+} from outlet of drag-out bath at Natal Electroplater

Figures 4.19 and 4.20 represent the breakthrough curves for *Citrobacter* N14 used to treat Ag^{2+} and Cu^{2+} respectively. The figures show that the imported biomass was unable to remove these metals from solution.

4.4. DISCUSSION

Discharges from the electroplating industry usually contain cyanide complexes and hexavalent chromium as well as salts of several heavy metals such as copper, zinc and nickel. The toxic nature of these compounds can cause severe problems to biological sewage treatment works and for this reason most receiving authorities stipulate maximum concentrations which should not be exceeded. In order to comply with these limits, some form of treatment is necessary (Binnie and Partners, 1987).

The nickel influent concentration varied between 55 and 75 $mg.l^{-1}$ at pH 5. Both *Bacillus subtilis* and *Citrobacter* N14 columns showed five almost reproducible cycles before a decrease in uptake capacity was observed. Ni^{2+} removal by *Bacillus subtilis* fluctuated between 71.948 and 87.892 mg in the first four cycles (Figures 4.2,4.3 4.4 and 4.5). Breakthrough occurred at 16 BV for cycle 1 (Figure 4.2), and increased to 20 BV for cycle 3 (Figure 4.4). Thereafter the bed volumes for cycle 4 and 5 decreased to 13 and 14 BV respectively. Cycle 4 removed the maximum amount of Ni^{2+} (87.892 mg) (Figure 4.5), and the desorption of Ni^{2+} from this cycle was the lowest (52.03 %) (Table 4.1). The fifth cycle removed the least Ni^{2+} (58.500 mg) (Figure 4.6). More than 90% of Ni^{2+} was recovered for each of the first three cycles followed by rapid dissolution. Breakthrough occurred between 13 and 20 BV and was independent of the mass biosorbed. The mass of Ni^{2+} adsorbed was much higher at a higher bed volume of 40.

Bacillus subtilis column was unsuccessful in Cr removal (Figures 4.7 and 4.8), Ag^{2+} (Figure 4.9) and Cu^{2+} (Figure 4.10).

Ag^{2+} and Cu^{2+} occurred as their cyanide complexes. These complexes are very stable and do not interact with the resin either chemically or physically. For biosorption to be successful, the cyanide complex has to be displaced by another large anion however the laboratory is not equipped to carry out cyanide experiments.

The Ni^{2+} effluent pH was 5 and the concentration varied between 55 and 75 $mg.L^{-1}$. The largest

amount of Ni^{2+} adsorbed by *Citrobacter* at breakthrough was 95.56 mg during cycle three (Figure 4.13). Best biosorption was obtained in the second (83.67 mg) and third (95.56 mg) cycles . The fifth cycle produced the lowest precipitation with *Citrobacter* removing a mass of 41.209 mg Ni^{2+} (Figure 4.15). Breakthrough decreased through the cycles except cycle 2, here a slight increase was observed. Metal recovery was reasonably reproducible and fluctuated between 77.748 and 96.62%. Breakthrough of the column occurred between 12 and 29 BV. 96.63% of Ni^{2+} was recovered by desorption during the fifth cycle (Table 4.2).

The chromate influent concentration varied from 250 to 500 $mg.l^{-1}$ at a pH of approximately 6.5. 212.14 mg was adsorbed during the first cycle (Figure 4.16). Biosorption of Cr^{6+} decreased to 49.151 g during the 3rd cycle (Figure 4.18). Desorption of metals by acid deteriorated rapidly through the cycles. During cycle 1, 97.537% was desorbed and at the end of cycle3, 26.60% was recovered from the biomass. The column became saturated with chromate hence biosorption for cycles 2 and 3 deteriorated. An increase in acid concentration had no effect on desorption.

Citrobacter N14 was unable to remove Ag^{2+} and Cu^{2+} from the electroplating effluent baths (Figures 4.19 and 4.20).

4.5 CONCLUSIONS

Both technologies were able to remove high amounts of nickel from the drag out baths. *Bacillus subtilis* removed 89.89 mg of Ni^{2+} during the fourth cycle at a breakthrough of 20 BV, and at 40 BV removed 113.15 mg of Ni^{2+}. 98.865% of Ni^{2+} was desorbed from the *B. subtilis* column during the second cycle. The *Bacillus subtilis* column was unsuccessful in removing Cr, Ag^{2+} and Cu^{2+}

Citrobacter N14 removed 95.56 mg of Ni^{2+} during the third cycle at a breakthrough of 20 BV. At 40 BV, 102.877 mg of Ni^{2+} was removed. 94.59 of Ni^{2+} was desorbed from the *Citrobacter* N14 column. 212.14 mg of Cr^{6+} was adsorbed during the first cycle by *Citrobacter* N14 at a bed

volume of 20. At 40 BV, 360.23 mg of Cr^{6+} was removed. *Citrobacter* N14 was unable to remove Ag^{2+} and Cu^{2+}.

Citrobacter N14 proved to be a better remediating agent than *Bacillus subtilis* in treating electroplating effluent containing Ni^{2+} and Cr^{6+} ions.

CHAPTER FIVE

COST ANALYSIS OF IMPORTED BIOMASS AND TRANSFER TO INDUSTRY TO REMOVE HEAVY METALS FROM METAL FINISHING OPERATIONS

5.1 INTRODUCTION

The metals finishing industry is concerned with pollution and wastes generated by all processes but especially those generated by the use of four specific materials in finishing processes: (1) the use of cadmium as a plating material, (2) the use of chromium as a plating material, (3) the use of cyanide-based electroplating solutions, and (4) the use of copper/formaldehyde- based electro less copper solutions (Williams and Randall, 1994). Each process stage within the electroplating sequence is followed by a rinse stage, removing traces of process solution which adhere to the article being plated. These rinse flows contain quantities of the various process solutions which are subsequently carried to drain (Binnie and Partners, 1987).

Assessment of the feasibility of a particular biosorbent to be applied for soluble metal immobilisation and possible recovery required several factors to be taken into consideration. These include initial capital expenditure, cost of acquisition of biomass, growth requirements of biomass, immobilisation of biomass and metal loading capacity of biomass which relates directly to the volume of biosorbent required to remove a specific quantity of metal. For metal recovery to become successful at an industrial scale, it is imperative that the biosorbent material exhibits some degree of metal selectivity and that the metals are of economic importance (Bux *et al*, 1997).

5.2 COST ANALYSIS

5.2.1 Growth of microorganisms

Three biomasses, *Bacillus subtilis* (Thapar Centre for Research and Development, Patiala, India), *Citrobacter* N14 (ISIS Innovations Ltd, Oxford) and *Chlamydomonas reinhardtii* (Ohio State University, Ohio) were imported and used as remediation agents for the treatment of

effluents produced by Natal Electroplaters, Durban, South Africa. Four kilograms of *Citrobacter* cost R1 122.29 (£60) to import from Oxford and 0.217 kilograms of *C. reinhardtii* cost R788.20 (including delivery) to import from Ohio. *Bacillus subtilis* was obtained without cost.

Bacillus subtilis and *Citrobacter* N14 were grown in nutrient broth. A total of seven L of nutrient broth was required for starter cultures and mass cultivation of each of the bacteria. For this volume of broth, 112 g of broth was needed. Sodium beta glycerophosphate was added to *Citrobacter* N14 to increase its phosphatase activity. The growth of each of the bacteria in three L of nutrient broth yields approximately 10 g of cells. Only five g of dry biomass is used for immobilisation and packing into columns. Therefore from the amount of cells produced by growing in three L of nutrient broth, 2 columns for each biomass could be prepared. The total cost of growing *Bacillus subtilis* and *Citrobacter* N14 was R61.60. Alternatives eg. Corn Steep Liquor are being considered as a growth medium for the bacteria since it is much cheaper to use.

Only 0.217 kg of *C. reinhardtii* was present on the plate culture that was imported from Ohio. Three L of TAP medium only yields 2 g of the alga, after innoculation with the starter culture and an incubation period of longer than 24 to 48 hrs. The cost of preparing 3 L of TAP medium is R32.58. Approximately 5 g of biomass is required for immobilisation, so the total cost of growing *C. reinhardtii* is R 81.45. Since the alga requires light to grow, the growth period of cultivation is too long and not feasible. For this reason, the alga was not used in the pilot scale study. Another important reason for not using this biomass to remediate effluent was its inability to remove large amounts of heavy metals from solution during the batch experiments.

5.2.2 Column and Associated Equipment

The columns were constructed from Grade 316 stainless steel (250 mm x 270 mm) and contained 5 g of immobilised biomass, yielding an approximate packing density of 40 $g.L^{-1}$. The cost of two columns and accessories was R600.00.

5.2.3 Immobilisation of Microorganisms

Polysulfone was used as the immobilising agent for the two technologies. 25 g of polysulfone was dissolved in 100 ml of dimethlyformamide. Cost for immobilisation of each of the two technologies was R409.40.

5.2.4 Desorption of Metal from Column

Desorption of metals from the column was carried out using 500 mL of 0.2 M HCl at a cost of R15.20, and then the column was reconditioned with 500 ml 0.2 M NaOH, at a cost of R17.50. Since the columns had to be desorbed and reconditioned between cycles, the total cost was R98.10.

5.2.5 Summary of Cost

Table 1 Summary of cost of two technologies

	Bacillus subtilis	***Citrobacter N14***
Fixed costs		
Aquisition of biomasses	-	R 1 122.29
Bioreactor and peripherals	R300	R300.00
TOTAL	R300.00	R 1 422.29
Consumables		
Growth of organisms	R30.80	R30.80
Immobilisation	R409.40	R409.40
Desorption of metals		
Acid (HCl)	R15.20	R15.20
Base (NaOH)	R17.50	R17.50
Water	-	-
Total for 3 desorption cycles	R98.10	R98.10
TOTAL	R538.30	R538.30

5.2.6 Treatment of Electroplating Effluents

5.2.6.1 Current conventional treatment

Discharges from the electroplating industry usually contain several heavy metals, such as copper, zinc and nickel. As a result of the toxicity of these compounds from industrial effluents, removal has become an important priority that is reflected in a tightening and enforcement of environmental changes. Large volumes of industrial heavy metal bearing wastewaters require efficient and very cost effective treatment (Davis *et al*, 2000).

Where cyanide based solutions are in use, the cyanide content should be oxidised to less toxic cyanate. Metals complexed with cyanides are not easily removed, so cyanides must be destroyed first before treatment. The most common method is alkaline chlorination, usually by the addition of hypochlorite. Chromate bearing wastes must first undergo reduction from the hexavalent to the trivalent state to facilitate subsequent precipitation and removal. The simplest way to achieve this is by placing a tank filled with sodium metabisulphite immediately after the chrome drag out bath. Local authorities in South Africa impose limits on the concentration of heavy metals allowed in final effluent streams. In most cases at least two limits are set, one for the concentration of individual metals and a separate limit for all heavy metals present. Of the many methods available for the removal of heavy metals, precipitation is the most common and will only be effective if the cyanide and chromium streams have first been treated separately (Binnie and Partners, 1987)..

5.2.6.2 Treatment of effluent using microorganisms

The metal binding capabilities of various algae, fungi, yeast and bacteria accumulate hazardous metals from waste water through functional groups such as ketones, aldehydes and carboxyls on their cell walls (Nourbakhsh *et al*, 2002). Algae and fungi have shown tremendous potential in various biosorption applications. *D. potatorum* has been used to remove lead and copper from aqueous solutions (Matheickal and Yu, 1999b), *S. natans* is involved in gold binding (Kuyucak and Volesky, 1990) and *C. vulgaris* has been shown to bind uranium (Wilde and Bennemann, 1993). A commercial algal biosorption system (called AlgaSORB) for the removal of uranium was available on the market (Hu and Reeves, 1997). Immobilised *Pseudomonas aeruginosa* has

been used to remove lead (Chang *et al*, 1998) and copper and cadmium (Chang *et al*, 1997) from contaminated water. Non living *Steptomyces rimosus* was successful in removing zinc in batch biosorption studies (Mameri *et al*, 1999).

5.2.6.3 Treatment methods used by Natal Electroplaters

The direct chemical costs, on a monthly basis, to control the heavy metals in Natal Electroplating's effluent is R390.00 for caustic soda and R105.00 for sodium hypochlorite. This is a total of R495 a month. Indirect costs include extra samples taken by the Metro Waste Department as a result of samples falling outside of the metro parameters. This is charged at R238.00 a sample. More than 10 samples are taken for treatment and this can amount up to more than R2 380.00. The average volume of the drag-out bath is 1000 L, but the volume treated varies on a day to day basis. No record is taken of this volume (Foaden, M. 2002).

5.2.7 Comparison and Efficiency

The direct costs for effluent treatment at Natal Electroplaters is R495.00 to treat volume of up to 1000 l a day. This excludes the extra cost of taking samples to the Metro Department. If an average of ten samples are treated every month, then the total cost for effluent treatment is R2875. The *B. subtilis and Citrobacter* N14 columns were able to treat 2 L of effluent in 4 hrs during1 cycle. The column lasts for 4 cycles before the packing has to be changed. This means that at least 8 L of effluent can be treated. Repacking of 1 column costs R472.90. If the imported technologies are to be used to treat the same volume of effluent (1000 L), the cost amounts to R59 112.50. Also *B. subtilis* was unable to remove Cr, Cu and Ag, and Citrobacter N14 was unable to remove copper and silver.

With regards to cost of effluent treatment, it seems that the two technologies are far more costly to use than the conventional methods currently being used. The *B. subtilis* and *Citrobacter* N14 columns can be used for four cycles before a decrease in the metal recovered is observed. The two technologies have not proved themselves cost-effective and it is not recommended that they be imported and used at this stage for metal plating effluent remediation.

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7. APPENDICES

Appendix One

Nutrient broth

35 grams of nutrient broth base was dissolved in 1000 milliliters of distilled water in a 1000 millilitre shott bottle. The contents were mixed with a stirrer. The broth was sterilised by autoclaving at 121^0C for 15 minutes. After the broth was cooled, it was inoculated with the starter culture.

Appendix Two

Tris Acetate Phosphate (TAP) medium for *Clamydomonas reinhardtii*

Stock Solution	For 1 L
1M Tris base	20 ml
Phosphate Buffer II	1 ml
Hutner's Trace Metals	1 ml
Solution A	10 ml
Glacial acetic acid	1 ml

The above components make up the TAP medium. To prepare each of the components individually:

Phosphate Buffer II:

Dissolve 10.8 grams of K_2HPO_4 and 5.6 grams of KH_2PO_4 in 100 ml of water.

Solution A:

Dissolve 20 grams of NH_4Cl5 grams of $MgSO_4 \cdot 7H_2O$ and 2.5 grams of $CaCl_2 \cdot 2H_2O$ in 500 ml of water.

Hutner's Trace Metals

Dissolved 50g of acid free EDTA in 250 ml of ddH_2O. Heated to dissolve.

Then dissolved the following, one by one, in order,

11.4 grams BO_3H_3,

22 grams $ZnSO_4 \cdot 7H_2O$,

5.06 grams $MnCl_2 \cdot 4H_2O$

4.99 grams $FeSO_4 \cdot 7H_2O$

1.61 grams $CoCl_2 \cdot 6H_2O$

1.57 grams $CuSO_4 \cdot 5H_2O$

1.1 grams $Mo_7O_{24}(NH_4)_6 \cdot 4H_2O$