Biosorption of Lead, Copper, Cadmium and Nickel by Anaerobic Biomass

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ABSTRACT

BIOSORPTION OF LEAD, COPPER, CADMIUM AND NICKEL BY ANAEROBIC BIOMASS

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This study will introduce anaerobic granules as a novel type of biosorbent for the removal of lead, copper, cadmium, and nickel from aqueous solutions. The work investigated the equilibrium, batch dynamics and continuous column operation for the biosorption process. Binding capacity experiments using viable biomass revealed a higher value than those for nonviable biomass. Binding capacity experiments using non-viable biomass treated with Ca revealed a high value of metals uptake. The solution initial pH value affected metal sorption. Time dependency experiments for the metal ions uptake showed that adsorption equilibrium was reached almost 30 minutes after metal addition. It was found that the q_{max} for Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+}, were 2.46, 1.74, 1.06 and 0.88 meq/g respectively. The data pertaining to the sorption dependence upon metal ion concentration fitted the Langmiur isotherm model. The kinetics of sorption of Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+} were modelled using a pseudo-second order rate equation.

Column adsorption studies were performed for Pb^{2+}, Cu^{2+}, Cd^{2+}, and Ni^{2+}. The removal of Pb^{2+}, Cu^{2+}, Cd^{2+}, and Ni^{2+}ions from the bed was accompanied by the elution of Ca^{2+} ions from the packed-bed. Ion exchange was identified to be

the dominant mechanism for the biosorption of nickel by the anaerobic biomass. For copper and cadmium 77% and 82% of the total amount adsorbed was attributed to ion exchange respectively. 18% and 15% of the total amount adsorbed of copper and cadmium was attributed to the extent of a complexation process competing with the ion exchange one respectively. For the case of Pb ions it was found out that ion exchange was attributed to be almost 50% of the total uptake mechanism. 30% of the total uptake mechanism was attributed to precipitation mechanism. The remaining 20% was attributed to a complexation process competing with the ion exchange and precipitation.

The affinity order of anaerobic biomass for the four metals under study has been established as: Pb > Cu > Ni > Cd. The selectivity of the biomass for Pb over the other three metals was well exhibited by the results obtained using the flow-through column.

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1 - INTRODUCTION

Heavy metals are toxic pollutants released into the environment as a result of different activities such as industrial, mining and agricultural activities (Esposito et al., 2001). Using the adsorption process for the removal of heavy metals from wastewater has a short history compared to other water purification processes. Adsorption was first observed by Lowitz in 1785 and was soon applied as a process for removal of color from sugar during refining. In the later half of the nineteenth century, American water treatment plants used inactivated charcoal filters for water purification. In 1929 the first granular activated carbon (GAC) units for treatment of water supplies were constructed in Hamm, Germany, and in 1930 at Bay City, Michigan (Montgomery, 1985). Nowadays adsorption on activated carbon is a recognized method for the removal of heavy metals from wastewater while the high cost of activated carbon limits its use in adsorption. A search for a low-cost and easily available adsorbent has led to the investigation of materials of agricultural and biological origin as potential metal sorbents (Hammaini et al., 1999). Biosorption is the ability of certain types of microbial biomass to accumulate heavy metals from aqueous solutions. Microbial biomass could be considered as an ion exchanger of a biological origin (Volesky, 1999). A large number of micro-organisms belonging to various groups,

such as bacteria, fungi, yeasts and algae have been reported to bind a variety of heavy metals to different extents (Volesky, 1990).

Most biosorbents used are suspended in the forms of bioflocs. One of the major operational problems associated with the suspended flocs is separation of biosorbent from the treated effluent. To overcome this drawback, cell immobilization techniques have been developed, but the employment of immobilization procedures is expensive and complex (Liu et al., 2002). Two attempts to market two different types of immobilized microbial biomass, one by BV SORBEX and the other by the US Bureau of Mines did not make a successful commercial application in the market (Tsezos, 2001). This study will introduce anaerobic granules as a novel type of biosorbent for the removal of heavy metals from wastewater. Anaerobic granules possess compact porous structure and excellent settling ability. Such characteristics show that anaerobic granules would be a good candidate as biosorbents for metal removal from wastewater the implication is that the material could be suitable for the continuous flow system. Therefore, the biosorption capacity, kinetics and mechanisms of anaerobic biomass were studied in batch and continuous flow systems.

1.1 Heavy metals pollution

It has been recognized in toxicological studies that heavy metals pose a threat to human health, animals and plants. The toxicity of different types of heavy metal varies. Unlike the majority of organic chemicals that can be either metabolized from tissues by metabolic degradation, heavy metals, once

absorbed, persist in tissues for a long time. In the environment, heavy metals are accumulated in the food chain, which further increases the danger to humans.

Heavy metals are transported into the environment either naturally (e.g. erosion and natural leaching of surface deposits of metal minerals) or from human activities (e.g. mining, smelting, fossil fuel combustion, industrial application of metals). The major source of metal-bearing waste in the mining industry is acid mine drainage (AMD). According to Kalin (1997), 113 million cubic meters of contaminated water are produced annually from mining waste management areas in Canada. Generally, metal-bearing wastewater is discharged from various industrial sources at flow rates between 4 - 1200 m^3/day and the level of contamination varies from parts per million (mg/L) levels to grams per liter levels (Gazea, et al., 1996). The processing cost for remedial action at operating and abandoned mine sites across Canada was estimated to be $ 4 billion CDN over the next twenty years (Filion et al., 1990). The amount of heavy metal pollution is likely to further increase since as high-grade ores are gradually exhausted, low-grade ores are more extensively used in the mining industry. Processing of low-grade ores will produce more liquid waste for the same amount of metal produced. Untreated AMD pollutes receiving streams and aquifers. The impact on the environment can be severe, affecting the aquatic life, the pH of the water, and making a layer of rust-like particles covering river bottoms.

Another main source of heavy metals is the surface finishing industries. These include electroplating, anodizing and chromating operations. The objective

of all of these processes is to enhance the physical appearance, strength and corrosion resistance of surfaces of various objects. The sources of the liquid effluents generated by these processes are mainly from rinse waters and the spent plating baths. According to a survey of Canadian surface finishing industries performed in 1983 (EPS, 1987), 275 companies (128 in Quebec) discharged in total 1,364 m^3 of wastewater. Most of these wastewaters are processed in municipal swage treatment systems while the remainder runs off directly into fresh water bodies. The heavy metals contained within this portion of the wastewater accumulates in the water bodies and posses an environmental hazard. The heavy metal concentration levels in those effluents are shown in Table 1.1. This is approximately 20 times higher than the values allowed according to Federal Guidelines, Table 1.2. Limitations on the acceptable quantities of heavy metals in the discharge of municipal and industrial waste treatment facilities into surface waters have been recognized as a reasonable way to control the pollution.

Table 1.1: Characteristics of wastewater from surface finishing plants (EPS, 1987)

Pollutant	**Concentration in mg/L**
Cu	0 - 36
Cd	0 - 6.2
Ni	0 - 12
Pb	0 - 11.8

Table 1.2: Federal guidelines for metal finishing liquid effluents (EPS, 1987)

Metal	Maximum total concentration (mg/L)
Cu	1.0
Cd	1.5
Ni	2.0
Pb	1.5

The metals chosen for this multi-metal biosorption study were lead, cadmium, copper and nickel. The choice of metals had been made with regard to their industrial use and potential pollution impact. Lead is one of the most threatening heavy metals due to its high toxicity. Lead is used in a wide variety of industrial processes, the consumption of lead is in the order of 3 million tons per year. 40% of which is used in the manufacturing of electrical accumulators and batteries, 20% is used in gasoline, 12% in building construction, 6% in cable coatings, 5% in ammunition and 17% in other usages (Volesky, 1999). Lead has the ability to replace calcium in bones forming a semipermanent reservoir for long-term release well after the initial absorption (Volesky, 1999). The effects of lead on humans include hypertension and brain damage (Wase and Forster, 1997).

Due to cadmium acute toxicity, it has joined lead as being one of the most toxic heavy metals. Cadmium is also used in a wide variety of industrial processes, such as alloy preparation, metal plating and electronic manufacturing. In humans, cadmium can cause serious damage to kidneys and bones (Volesky, 1990).

Copper is one of the most common of the industrial metals. It is mainly used in galvanizing and in manufacturing brass and other alloys. For humans, copper is an essential element, although large doses can have harmful, even fatal, effects. Copper causes 'Wilson's disease' in which excess copper is deposited in the brain, skin, liver and pancreas (Volesky, 1990).

Industrial effluents containing nickel are common, since nickel is used in a large number of industries such as electroplating and batteries manufacturing. There is evidence of nickel carcinogenicity (Volesky, 1990).

1.2 Removal of heavy metals from wastewater

Treatment of industrial effluents containing heavy metals is mainly based on precipitation, ion exchange and activated carbon adsorption. Precipitation is based on the low solubility of heavy metal hydroxides or sulfides. Metal hydroxides and sulfides are formed by adding caustic soda or lime and hydrogen sulfide gas (H_2S) respectively to wastewater that contains heavy metals. The resulting heavy metal hydroxides or sulfides have very low solubility (Ku, 1987). The precipitated solid hydroxides or sulfides may then be separated by various solid-liquid separation techniques such as sedimentation or filtration. Many mining effluents are currently being treated by precipitation. However, there are some limitations to the precipitation method. When the concentration of the contaminant is low, the precipitation reaction is correspondingly very slow. As a result, an increase in the consumption of lime or caustic soda occurs and very large mixing and decanting tanks would be required. Therefore, the removal of

heavy metals by precipitation from diluted effluents is not economical. Furthermore, precipitation processes will generate toxic sludge, which has to be disposed of. The final disposal of sludge is becoming more strictly regulated and corresponding disposal costs are increasing very rapidly. According to Senes (1994) the Canadian mineral sector produces 4 to 12 million m^3 of toxic sludge per year.

Purification of water supplies by activated carbon adsorption has a short history compared to other purification processes. The first granular activated carbon (GAC) units for treatment of water supplies were constructed in Hamm, Germany in 1929, and in 1930 at Bay City, Michigan (Montgomery, 1985). Its use became widespread in the next few decades, for the removal of organic components from aqueous solution. Nowadays, adsorption on activated carbon is a recognized method for the removal of heavy metals from wastewater. According to Ku (1987), activated carbon was used as a polishing step following either metal hydroxide or metal sulfide precipitation in the removal of residual zinc and cadmium from plating wastewaters. Lead and cadmium can be removed from wastewater using granular activated carbon columns, reaching low ppm levels (Periasamy and Namasivayam, 1994; Reed and Berg, 1993). Activated carbon was also used for mercury removal and was reported to be effective (Rigo and Chandler, 1994).

Similar to carbon sorption, the ion exchange process is usually implemented in a continuous-flow column packed with cationic and/or anionic resins. The packed bed reactor is effective for processing high volumes of

effluents with low metal concentrations. The metals would sorb onto the resins in exchange for other metal cations or protons originally present on the ion exchange sites releasing them into the solution. Since heavy metals uptake at low pH is very low, the bound heavy metal cations may be simply eluted from the resin using a low-pH wash (Greene, et al., 1987; Guibal, et al., 1992; Treen-Sears, et al., 1984; Tsezos, 1980). This would make it possible to recover heavy metals in the form of liquid concentrates.

An advantage of using ion exchange is that heavy metals may be sequestered from wastewater selectively when the appropriate resin is chosen. Many ion exchange resins are commercially available for removal of various metals (Anonymous, 1989). Such selectivity is especially useful to recover valuable heavy metals such as gold and silver. However, the higher the selectivity, the more strongly the metals are bound to the resin, and the more difficult it is to desorb them. This would increase the operating cost as more regenerants are used. There are other limitations to the application of ion exchange resins. Chemical degradation can occur with breaking the polymer network or modification of functional groups by a species in the solution. Also organics often cause fouling of the resin particularly gel-type resins (Harland, 1994). The most serious limitation of ion exchange is that ion exchange resins are often expensive in large-scale projects. The prices of resins range from $ 30 - 60 USD per kilogram of resin (on a dry weight basis). Therefore, in order to keep the operating cost of the ion exchange process low, it is applied only to effluents with medium or low levels of heavy metals.

Heavy metals can be also removed by using biological treatment systems. Activated sludge technology is used in most industrial countries to process municipal sewage. However, the resulting bio-sludge is highly toxic and needs to be disposed of. The toxicity of this sludge prevents its use as agricultural fertilizers. Disposal by incineration causes another form of pollution in addition to further increasing sludge disposal cost.

1.3 Biosorption technology

Biosorption is a property of certain types of microbial biomass to concentrate heavy metals from aqueous solution. Only within the past decade has the potential of metal biosorption by biomass materials been well established. It was the work of Lowitz in 1785 that pioneered the field of ion exchange which eventually lead us to the field of biosorption (Montgomery, 1985). Biosorbents could be industrial waste biomass, as is the case in using byproduct biomass from fermentation processes, or naturally occurring substances of biological origin such as algae, which is found in large quantities in the sea (Volesky and Holan, 1995). It is particularly the cell wall structure that is principally responsible for this phenomenon.

In recent years biosorption has been proposed to be a safe and cost effective process for the removal of toxic heavy metals from dilute metal-bearing solutions. Compared with conventional methods for removing toxic metals from industrial effluents, the biosorption process offers several advantages such as the efficiency of biosorption removal of toxic heavy metals is high. Effluents

qualities in the order of only ppb (μg/L) of residual metals can be achieved (Volesky, 1999). Second, biosorption may be implemented over a wide range of operating pH, pressures and temperatures. The main advantage is the lower cost of the biosorbent, since it may be derived from various cheap raw materials. In addition, the possibility of regeneration of loaded biosorbent is important to keep the process costs down and to open the possibility of recovering the metals extracted from the liquid phase, thus minimizing the volume of chemical sludge to be disposed of. These advantages are the primary incentives for searching for and developing full-scale biosorption processes to clean up heavy-metal pollution.

2 – LITERATURE REVIEW

2.1 Overview of biosorption technology

2.1.1 Biomass types

The first major challenge for the biosorption field is to select the most promising types of biomass from an extremely large option of readily available and inexpensive biomaterials. While this task has not been completed, a good number of biomass types have been tested for metal binding capabilities under various conditions. A large number of micro-organisms belonging to various groups, such as bacteria, fungi, yeasts and algae have been reported to bind a variety of heavy metals to different extents. Volesky and Holan (1995) have presented an exhaustive list of microbes and their metal-binding capacities.

2.1.1.1 Activated sludge biosorbents

The ability of activated sludge biomass to accumulate heavy metals from wastewaters has long been recognized (Volesky, 1990). A number of studies have been carried out revealing the capability of activated sludge to accumulate heavy metals. The biosorption of copper and zinc from acid mine drainage (AMD) by nonviable activated sludge in a packed column reactor was reported by

Utgikar et al. (2000). The biomass was not subjected to any processing other than drying and crushing into different particle size fractions. Metal uptake capacity at equilibrium was higher at higher pH values and varied between 2.5 mg/g at pH value 3 to 3.4 mg/g at pH value 3.8 for Zn, and between 1.9 mg/g at pH value 3 to 5.9 mg/g at pH value 3.8 for Cu. Attempts by Hammmaini et al. (1999) were also made to recover cadmium, copper, nickel, lead and zinc in aqueous solution by three different types of biomass: activated sludge, brewer's yeast and barley root. Activated sludge was the most effective to uptake metal ions. Liu et al. (2002) used aerobic granules as a biosorbent for the removal of cadmium from industrial wastewater. The uptake capacity of aerobic granules was in the range of 43 - 566 mg/g depending on the initial Cd^{2+} concentration and biomass concentration. Aerobic granules have excellent settleability and a highly porous structure.

2.1.1.2 Fungi biosorbents

Among micro-organisms, fungal biomass offers the advantage of having a high percentage of cell wall material, which shows excellent metal-binding properties. Kogej and Pavko (2001) studied the biosorption of lead from aqueous solutions in a batch stirred tank reactor and continuous packed bed column reactor. As a biosorbent, self-immobilized biomass in the form of spherical pellets of the fungus *Rhizopus nigricans*, grown in a batch submerged culture was used. Equilibrium lead uptake was 83.5mg/g of dry weight in the batch stirred tank reactor. Sharma et al. (2002) studied the biosorption of zinc by fungal biomass isolated from industrial wastewaters using batch bioreactors. Maximum removal

of zinc was found to be 89% from a synthetic zinc solution and 80% from an actual effluent generated by a color picture tube manufacturing industry. Say et al. (2001) studied the biosorption of Cd, Pb, and Cu ions onto the dry fungal biomass of *Phanerochaete chryosporium*. The maximum uptake capacity of the biomass was 27.8, 85.9 and 26.6 mg/g for Cd, Pb, and Cu respectively. The equilibrium period was reached after 6h for all metals studied. An increase of metal biosorption occurred with increasing pH value from 2.0 to 6.0. The difference between live and non-viable fungal biomass *Mucor rouxii* for the uptake of Pb, Cd, Ni and Zn ions from aqueous solutions was studied by Yan and Viraragham (2003). The biosorption capacity of live biomass was 35.7, 11.1, 8.5, and 7.8 mg/g at pH value 5.0 for Pb, Ni, Cd, and Zn respectively. The biosorption capacity of non-viable biomass was higher being 53.8, 53.9, 20.3 and 20.5 mg/g at pH value 6.0 for Pb, Ni, Cd, and Zn respectively.

2.1.1.3 Algae biosorbents

Another source of potentially metal-sorbing biomass is marine algae which became the candidate of interest due to its bulk availability from water bodies. Several non-living algal biomass types were investigated for biosorption of cadmium and lead in aqueous solution by Prasetyo (1992). The biosorption process was investigated for equilibrium batch as well as dynamic packed bed column modes. Marine algae *Ascophyllum nodosum* was identified for the highest uptake for these metals. *A. nodosum* maximum uptake capacities for lead and cadmium were 250 mg/g of dried biomass and 120 mg/g of dried biomass, respectively. Kaewsarn (2002) used the calcium treated marine algae

Pandina sp. as a biosorbent for the uptake of copper ions from aqueous solutions. The maximum uptake capacity obtained was 0.8 mmol/g at a solution pH value of 5.0. Equilibrium was reached after 30 min. Matheickal and Yu (1999) used Australian marine algae *Durvillaea potatorum* and *Ecklonia radiata* treated with calcium for lead and copper ions removal from aqueous solutions. The maximum adsorption capacity of *Durvillaea potatorum* for lead and copper ions were 1.6 and 1.3 mmol/g, respectively. The corresponding values for *Ecklonia radiate* were 1.3 and 1.1 mmol/g. The maximum adsorption was obtained at a pH value of 4.5. 90% of the adsorption was completed in the first 10 min in batch conditions Kuyucak and Volesky (1989a) reported that brown alga *A. nodosum* exhibited very high cobalt uptake. Kuyucak and Volesky (1989b) also found that marine algae *Sargassum natans* is a potent biosorbent for gold.

2.1.1.4 Other biosorbents

Ozer and Ozer (2003) used yeast biomass *Saccharomyces cerevisiae* for the biosorption of Pb, Ni, and Cr ions. The optimum uptake capacity of Pb, Ni, and Cr was 270.3, 46.3 and 32.6 mg/g respectively. They also observed that the maximum metal uptakes increased with atomic number as follows: Pb>Ni>Cr. Nourbakhsh et al. (2002) studied the biosorption of Cr, Pb, and Cu ions from aqueous solutions on *Bacillus sp.* bacteria. Optimum pH values in single adsorptions of Cr, Pb, and Cu ions were found to be 2.0, 4.5, and 4.0 respectively. The uptake capacity of Cr, Pb, and Cu ions was found to be 123.7, 88, and 108 mg/L respectively. Williams et al. (1998) compared the ability of brown seaweed *Ecklonia maxima*, a seaweed waste, alginate fiber and waste

linseed fiber to remove copper, nickel, and cadmium from single and mixed metal ion solutions. The study has shown that alignate fiber generally exhibited the best overall metal ion uptake and cadmium ions were the most effectively sequestered by the biosorbent. Senthilkumaar et al. (2000) studied the ability of bio-waste obtained from the fruit juice industry (FR) for the uptake of Hg, Pb, Cd, Cu, Zn, and Ni ions. The bio-waste did not have high abilities for the uptake of the metal ions from aqueous solutions. They found out that treating the biosorbent with phosphate would enhance the adsorption capacity of the biosorbent. The order of removal of heavy metals by phosphated FR (P-FR) was Cu>Pb>Ni>Zn>Hg=Cd. Veglio et al. (2003) studied the uptake capacity of olive mill residues (OMR) for the biosorption of copper. The maximum uptake capacity was only 14 mg/g at an optimum pH value of 5.0. Table 2.1 summarizes some of the studied biosorbents in the literature. The values were tabulated from experimental data acquired at sufficiently high initial metal concentrations so that maximal uptake was achieved.

Table 2.1: Uptake of metals by different biosorbents.

Metal	Biomass type	Biomass class	Metal uptake (meq/g)	pH	Reference
Pb	*Phanerochaete chryosporium*	fungus	0.83	6.0	Say et al. (2001)
	Pseudomonas aeruginosa	fungus	0.66	-	Chang et al. (1997)
	Rhizopus arrhizus	fungus	1.22	5.0	Yin et al. (1999)
	Sargassum natans	algae	2.44	3.5	Holan and Volesky (1994)
	Saccharomyces cerevisiae	yeast	2.60	-	Ozer and Ozer (2003)
	Mucor rouxii	fungus	0.52	6.0	Yan and Viraraghavan (2003)
	Durvillaea potatorum	algae	3.20	4.5	Matheickal and Yu (1999)
	Ecklonia radiata	algae	2.60	4.5	Matheickal and Yu (1999)
	Sugar beet pulp	waste	0.712 meq/L	5.5	Reddad et al. (2002)
	Ascophyllum nodosum	algae	2.44	3.5	Holan and Volesky (1994)
Cu	*Phanerochaete chryosporium*	fungus	0.84	6.0	Say et al. (2001)
	Pseudomonas aeruginosa	fungus	0.66	-	Chang et al. (1997)
	R. arrhizus	fungus	1.20	5.0	Yin et al. (1999)
	non-viable activated sludge	waste	0.19	3.8	Utgikar et al. (2000)
	Pandina sp.	algae	1.60	5.0	Kaewsarn (2002)

Table 2.1: Uptake of metals by different biosorbents (con't)

Metal	Biomass type	Biomass class	Metal uptake (meq/g)	pH	Reference
Cu	Olive mill residues (OMR)	Waste	0.44	5.0	Veglio et al. (2003)
	Durvillaea potatorum	algae	2.60	4.5	Matheickal and Yu (1999)
	Ecklonia radiata	algae	2.20	4.5	Matheickal and Yu (1999)
	Sugar beet pulp	waste	0.666 meq/L	5.5	Reddad et al. (2002)
	Anaerobically digested sludge	waste	2.52	-	Artola et al. (1999)
	Aerobic granules	waste	1.88	4.0	Liu et al. (2003)
	Thiobacillus ferrooxidans	bacteria	1.26	-	Ruiz-Manriquez et al. (1998)
Ni	*Saccharomyces cerevisiae*	yeast	1.58	-	Ozer and Ozer (2003)
	Mucor rouxii	fungus	0.69	6.0	Yan and Viraraghavan (2003)
	Sargassum natans	algae	0.82	3.5	Holan and Volesky (1994)
	Ascophyllum nodosum	algae	1.38	3.5	Holan and Volesky (1994)
	Sugar beet pulp	waste	0.404 meq/L	5.5	Reddad et al. (2002)
	Anaerobically digested sludge	waste	1.29	-	Artola et al. (1999)
Cd	*Phanerochaete chryosporium*	fungus	0.49	6.0	Say et al. (2001)
	Mucor rouxii	fungus	0.96	6.0	Yan and Viraraghavan (2003)

Table 2.1: Uptake of metals by different biosorbents (con't)

Metal	Biomass type	Biomass class	Metal uptake (meq/g)	pH	Reference
Cd	*Aspergillus oryzae*	fungus	0.80	5.0	Yin et al. (1999)
	R. arrhizus	fungus	1.12	5.0	Yin et al. (1999)
	Sugar beet pulp	waste	0.434 meq/L	5.5	Reddad et al. (2002)
	Anaerobically digested sludge	waste	2.09	-	Artola et al. (1999)
	Saccharomyces cerevisiae	yeast	0.2-0.8	-	Volesky (1994)
	Saccharomyces uvarium	yeast	0.26	-	Norris and Kelly (1979)
	Ascophyllum nodosum	algae	3.82	5.0	Holan et al. (1993)
	Bacillus sp.	bacteria	0.38	-	Fry et al. (1992)
	Aerobic granules	waste	3.07	4.0	Liu et al. (2003)
	Chlorella vulgaris	algae	1.52	-	Aksu and Kutsal (1990)
	R. oligosporus	fungus	0.70	5.0	Yin et al. (1999)
	R. oryzae	fungus	0.56	5.0	Yin et al. (1999)
	Sargassum natans	algae	2.34	3.5	Holan and Volesky (1994)

2.1.2 Mechanism of biosorption

For better understanding of the relevant mechanisms in metal binding by anaerobic biomass, knowledge about the biomolecular and binding sites in this biomass is necessary. The ability of bacterial cells to bind metals is associated with the components of the cell itself. A general schematic of the bacterial cell is shown in Figure 2.1.

The main components of the cell are water, inorganic salts and mineral elements, proteins, nucleic acids, polysaccharides, and lipids. Water is the most abundant single compound in the cell, it makes almost 70% of the total weight of the cell. Inorganic salts and mineral elements, on the other hand, constitute only a very small fraction of the total dry weight, almost 1% (Lehninger et al., 1993). Nearly all of the solid matter is organic, and is present in four forms:

- Proteins: long strings of amino acids, constitute the largest fraction (beside water) of cells. Amino acids have the amino group ($-NH_2$) in their structure (Reynolds and Richards, 1990).
- Nucleic acids (DNA and RNA): polymers of simple nucleotides.
- Polysaccharides: polymers of simple sugars such as glucose.
- Lipids: grease or oil hydrocarbon derivatives that are only slightly soluble in water. Most lipid molecules contain one or more long-chain fatty acids, of which palmitic acid and oleic acid are parent compounds (Lehninger et al., 1993). These compounds include a long hydrocarbon structure, which usually has a more ionic group (such as –COOH) at one end (Reynolds

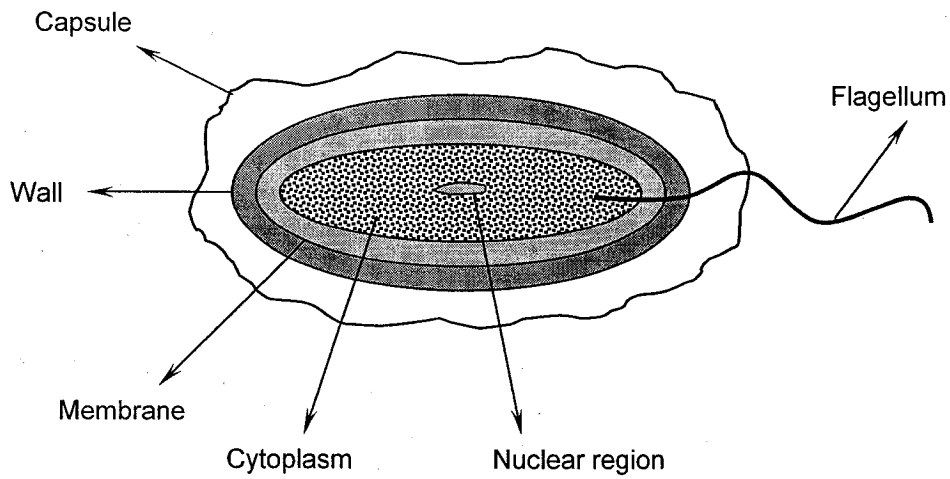

Figure 2.1: A general schematic of bacterial cell

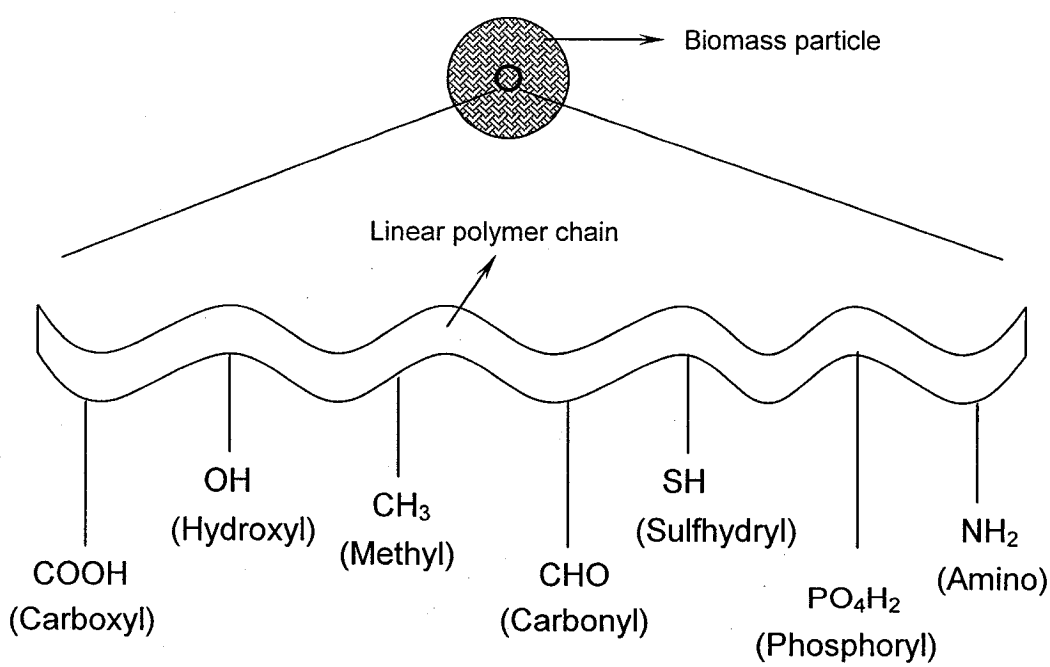

Figure 2.2: Polymer chain, attached to it the different functional groups

and Richards, 1990). Many lipids also contain and alcohol, e.g., glycerol, and some contain phosphate (Lehninger et al., 1993).

These organic compounds contain different surface functional groups such as carboxylic, carbonyl, hydroxyl, amino, phosphoryl, and sulphide groups (Greene et al., 1987). The different functional groups have a high affinity towards heavy metals that they can complex the metal ions (Delgado et al., 1998). According to Buffle (1988) the main functional groups on polysaccharides in bacteria, other than OH groups, are the carboxyl and amino groups. The chelating ability of polysaccharides was related to their content of carboxyl and hydroxyl groups (Kaplan et al., 1987). The carboxylate, sulfate and phosphate groups were proposed by Tobin et al. (1984) and Figueira et al. (1997) to be actively involved in metal ions uptake along with hydroxyl groups. Figure 2.2 illustrates a simplified polymer chain, attached to it the different functional groups involved in sequestering heavy metals from solution.

Several different mechanisms have been proposed to explain the uptake of metals by non-living microbial biomass, including microprecipitation, ion exchange, and complexation. Because of the complexity of most cell walls it is very likely that all of these processes of binding will take place in the system at the same time (Volesky, 1990). Initially metal ions diffuse from the bulk solution to the surface of the microbial cell where they bind to the active sites formed by the presence of various chemical functional groups (Figure 2.3).

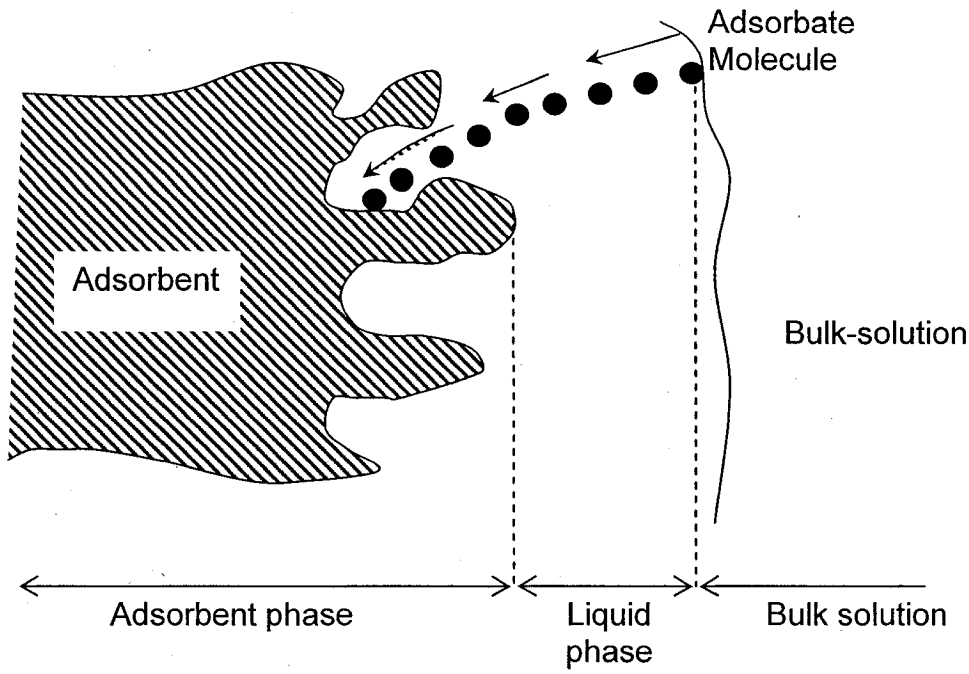

Figure 2.3: Adsorption of molecules into the adsorbent surface (after Hand et al., 1981)

In the context of this work, the term ion-exchange refers to binding of a solute (usually: metal cation) to a site, which is previously occupied by another cation. The second ion is released into the solution upon the binding of the first ion.

Micro – precipitation is the deposition of electrically neutral material at the surface of the biomass and does not necessarily involve a bond between the biomass and the deposited layer. Micro-precipitation may, however, be facilitated by initial binding of metal ions to reactive sites of the biomass, which serve as nucleation sites for further precipitation (Mayers and Beveridge, 1989). This

process is not limited to a mono-layer (or saturation of sites): cells can accumulate several times their dry weight in metal.

Complexation plays an important role in metal-ligand interaction (Westall, 1987, pp.4-7). A complex (also referred to as a coordination compound) is a poly-atomic molecule that consists of one or more (then: poly-nuclear complex) central atoms (usually metal cations) surrounded by ligands that are attached to it. Complexes can be neutral or positively or negatively charged. The number of coordinating atoms (in the ligand) which are directly attached to the central atom is called coordination number and can be larger than the valence of the central atom (most common coordination numbers: 4 and 6). If one ligand is attached to the central atom through two or more coordination atoms, then the complex is called a chelate.

2.1.3 Binding forces

In general, the forces between atoms or molecules can be classified into chemical and physical ones. Chemical adsorption, or chemisorption, is based on electrostatic forces. The difference between physical adsorption and chemisorption is not distinct; the former is less specific for which compounds sorbed to which surface sites, has weaker forces and energies of bonding, operates over longer distances, and is more reversible. In chemical adsorption, the attraction between adsorbent and adsorbate approaches that of covalent or electrostatic chemical bond between atoms, with shorter bond lengths and higher bond energies.

Physical forces can be subdivided into electrostatic and London – van der Waals forces (Myers, 1991, pp.41). In the resulting bonds, the electrons stay in their original systems. Electrostatic forces between ions or between ions and dipoles extend over a long range and are the strongest among the physical bonds (Myers, 1991, pp.41). The magnitude of the force is proportional to the charge of each ion and inversely proportional to the square of the distance between the ions. Other physical interactions among molecules, based on electrostatic force, include dipole-dipole interactions, dispersion interactions, and hydrogen bonding. When two polar compounds are near each other, they tend to orient their charges to lower their combined free energy; negative charges of one tend to approach positive charges of the other. When electrostatic forces among the charges of the two molecules are summed, the net dipole-dipole interaction is an attraction between the two. Hydrogen bonding is a special case of dipole-dipole interaction in which the hydrogen atom in a molecule has a partial positive charge and attracts an atom on another molecule that has a partial negative charge. When two neutral molecules that lack permanent dipoles approach each other, a weak polarization is induced in each because of quantum mechanical interactions between their distributions of charge. The net effect is a weak attraction between the molecules, known as the dispersion interaction or the London-van der Waals force (Russell, 1980).

2.1.4 Mechanisms in the literature

Muzzarelli et al. (1980) considered that chelation was the main mechanism for cupric ions binding to chitisan membranes. Darnall et al. (1986),

Volesky (1990) and Sharma and Forster (1994) attributed the biosorption of heavy metals to chemical sorption. Treen-Sears et al. (1984) presented evidence for the ion exchange of the uranyl ion by *Rhizopus* fungal biomass. They reported that in column applications two moles of protons were released for one mole of uranyl sequestered. Crist et al. (1993) and Schiewer and Volesky (1995) confirmed that ion exchange played an important role in biosorption of heavy metals onto brown algal biomass. Reddad et al. (2002) attributed the biosorption of heavy metals onto sugar beet pulp to ion exchange and chelation mechanisms. According to Chen et al. (1990) the Cu binding to peat at low metal concentrations was predominantly ion exchange (with H, Ca, Mg) while at higher concentrations binding of copper nitrate complexes occurred. Utgikar et al. (2000) indicated that at a pH value of 3.8, ion-exchange was the predominant mechanism of Cu and Zn ions uptake by non-viable activated sewage sludge. Jianlong (2002) used yeast biomass *Saccharomyces cerevisiae* originated from beer fermentation industry to remove copper ions from aqueous solutions. He studied the role played by various functional groups in the cell wall of the biomass in biosorption of copper. It was found out that the carboxylic and amine groups were the primary groups involved in the uptake of copper ions.

2.1.5 Fixed bed column

Large-scale application of biosorption in industry is ultimately the main goal for biosorption research. Some metal sequestering biosorbents have been commercialized. For instance, the algal biosorbent AlgaSORB which employed a fresh water algae *Chlorella vulgaris* and AMT-Bioclaim which employed *Bacillus*

biomass were developed for wastewater treatment and metal recovery (Gupta et al., 2000).

The process of metal recovery by biosorbent materials is basically a solid-liquid contact process consisting of the metal uptake cycle and the metal desorption cycle. Appropriate contact between the solution and the solid phase can be accomplished by the fixed packed-bed contactor. The contactor is represented by a column arrangement where the biosorbent is packed into a solid bed, which does not normally move. The liquid usually trickles through the bed in a down flow or up flow arrangement. Continuous flow systems have some process application advantages over batch type operations. In packed bed systems, fluid flows continuously through a column of adsorbent allowing its more efficient application. In order to make the biosorption process truly continuous, pairs of columns are employed in parallel so that during the regeneration and rinsing of one of the columns the other is being loaded with heavy metals (Volesky, 1990). Figure 2.4 demonstrates biosorption and desorption cycles that take place alternately.

Removal of heavy metals by biosorption is suited as a 'polishing' step because it is possible to reach drinking water quality of the treated water (initial concentrations for example 1 – 100 mg/L, final concentration < 0.01 – 0.1 mg/L), especially in packed-bed applications. In order to prevent unnecessarily rapid exhaustion of the sorption capacity when the metal concentrations in the wastewater to be treated are high (>100 mg/L), it may be desirable to use a different technique such as precipitation (UNEP, 1989).

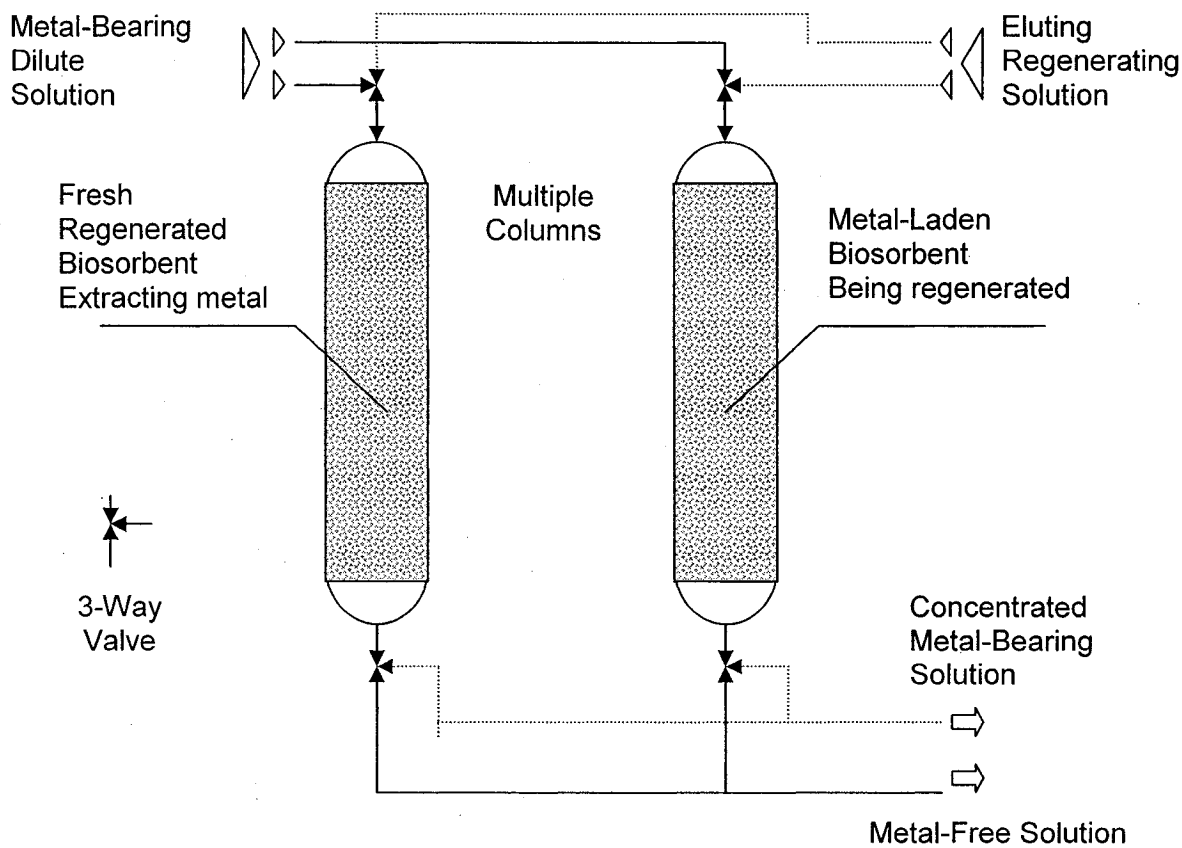

Figure 2.4: Flow sheet of possible biosorption application (Volesky, 1990)

The design of a fixed bed reactor involves estimation of the shape of the breakthrough curve and the appearance of the breakpoint. When contaminated water is passed through a long enough bed of adsorbent (sufficient contact time), there would be no contaminant in the effluent until all of the contaminant break through at once and all of the adsorbent is exhausted.

Zulfadhly et al. (2001) studied the ability of the macro fungus *Pycnoporus sanguineus* to adsorb lead, copper and cadmium in a fixed-bed column. They studied the effect of column bed height, flow rate and initial metal concentration on the breakthrough profile of the bed. Biomass of any kind cannot be used directly in the standard sorption process. Usually if it is very soft, and without reinforcement and granulation, it cannot be used in column operations. The fungus *M. rouxii* biomass immobilized in polysulfone in the form of spherical beads was used by Yan and Viraraghavan (2001) to adsorb lead, cadmium, nickel and zinc. The biomass had very low uptake capacities especially for nickel and cadmium since breakthrough occurred within the first few bed volumes. Two different sorbent immobilization techniques, cross-linking and entrapment were developed for the packed bed studies. It was observed that *A. nodosum* cross-linking with formaldehyde gave the best results for practical use. Kogej and Pavko (2001) studied the biosorption of lead from aqueous solutions in a batch stirred tank reactor and continuous packed bed column reactor. As a biosorbent, self-immobilized biomass in the form of spherical pellets of the fungus *Rhizopus nigricans*, grown in a batch submerged culture was used. Equilibrium lead uptake was 83.5mg/g of dry weight in the batch stirred tank reactor. However, for the fixed bed column reactor the experiments showed varying and a lower lead uptake 56 mg/g of dry weight ± 22%. The corresponding lower metal uptake efficiency was found to be due to the packed bed non-uniformity. Therefore, the formation of channels and zones of unexploited biomass within the bed were expected.

The main requirement of an industrial sorption system is that the sorbent can be utilized as a fixed or expanded bed and it should not cause much pressure drop across the bed. Some types of biomass have to be immobilized in a synthetic polymer matrix (Jeffers and Corwin, 1993) in order to yield particles with the required mechanical properties (Mahan and Holcombe, 1992). The biosorbent material that will be used in this study is anaerobic biomass. Unlike most of other biomass, immobilization or stiffening is not necessary prior to using the biomaterial. The particulate biomass was found to possess high mechanical strength. Even under aggressive chemical environments (acidic or base conditions) the biomass demonstrated high stability with no visible structural damage. This implies that the biomass could be suitable for the continuous flow system. The limited availability of granulated biosorbents makes this biomass advantageous over other biosorbents.

2.1.6 Desorption and reuse of biosorbents

Biosorption can be used to treat industrial effluents containing heavy metals or to recover precious metals from processing solutions (Davis et al., 2003). The efficiency of biosorption technology depends not only on the efficiency of removal of heavy metals from aqueous solutions but also on the efficiency of regeneration of biosorbent after metals adsorption. The efficiency of desorbing agent or the eluant is often expressed by the S/L ratio, i.e. solid to liquid ratio. The solid represents the solid sorbent (mg dry weight) and the liquid represents the amount of eluant applied (in mL). High values of S/L are desirable for complete elution and to make the process more economical (Gupta et al.,

2000). The elution process does not significantly reduce the binding capacity of the biomass and several cycles may be employed. Hydrochloric acid has been recognized as an effective eluant (Brooks, 1991; Holan et al., 1993). Desorption of heavy metals from the biomass is the result of a competition between protons and heavy metal ions under acidic conditions. Active site protonation is favored at low solution pH levels and the competition between protons and metals has been documented by many researchers (Brooks, 1991; Crist et al., 1990; Fourest and Roux, 1992). Generally, increasing the acidity leads to more effective removal of metals from the biomass (Gupta et al., 2000). In addition to the mineral acids, many other elution agents, such as $NaHCO_3$, Na_2CO_3, $(NH_4)_2SO_4$, were also used for desorption of bound heavy metals from various types of biomass. Carbonate and bicarbonate have proven to be effective for the desorption of uranium from fungal biomass (Tsezos, 1984). Desorption process should ensure three major issues: high elution efficiency, low biomass damage and low cost.

After the desorption process, the biosorbent is regenerated and a highly concentrated metal solution is obtained. Co-precipitation, flocculation or electro-winning could be used to treat the generated concentrate. Co-precipitation would generate a toxic sludge whereas the solid metal, a more desirable end product, would be recovered from the concentrate by using the electro-winning process (Davis et al., 2003).

2.1.7 Presence of other ions

Other ions in the solution may compete with the metal ion of interest for sorption sites. The binding of this metal ion is then decreased. The amount of inhibition depends on the binding strength of the respective ions to the biomass.

The biosorption of copper and zinc from acid mine drainage (AMD) by nonviable activated sludge in a packed column reactor was reported by Utgikar et al. (2000). Equilibrium uptakes from binary mixtures were 30% lower than single component solution uptakes for both metals, indicating some competition between the two metals. Artola et al. (1999) reported the competition between four metal ions, cadmium, copper, nickel and zinc for the binding sites of anaerobically digested sludge. Hammaini et al. (2002) reported the effect of the presence of lead on the biosorption of cadmium, copper and zinc by activated sludge. The study indicated a competitive uptake of the different metals with Pb being preferably adsorbed. It was found out that when the concentration of Pb and Cu, Pb and Cd, and Pb and Zn were the same in each system, about 97%, 88%, and 99% of the total metal uptake was due to Pb uptake in each system. Kaewsarn (2002) found out that the presence of Na ions did not interfere with binding capacity of copper ions onto the calcium treated marine algae *Pandina sp.*. While the presence of K, Mg, and Ca ions (one at a time) at a concentration of 10mM reduced the removal efficiency of copper by 4%, 11%, and 13% respectively. Say et al. (2001) studied the biosorption of Cd, Pb, and Cu ions onto the dry fungal biomass of *Phanerochaete chryosporium*. The maximum uptake capacity of the biomass was 27.8, 85.9 and 26.6 mg/g for Cd, Pb, and Cu

respectively. The competitive biosorption capacity of the fungal biomass was lower than the single metal ion biosorption. The biosorption capacity of the biomass with the presence of other metals was found to be, 7.8, 16.9, and 7.6 mg/g for Cd, Pb, and Cu respectively. Matheickal and Yu (1999) used Australian marine algae *Durvillaea potatorum* and *Ecklonia radiata* treated with calcium for lead and copper ions removal from aqueous solutions. The results of their study showed that the biosorbents have much higher relative affinities for the heavy metal ions than for the light metal ions. The presence of calcium ions, at a 10mM concentration, reduced the removal efficiency by around 10-18%, while Mg ions reduced the efficiency by around 5-10%. The presence of Na and K ions had minimal effect on the uptake capacity. A study carried out by Kuyucak and Volesky (1989a) demonstrated that the presence of multi-metal species in solution had no significant effect on the biosorption of gold by *Sargassum natans*. However, further investigation reported in their study using a reinforced *Ascophyllum nodosum* biomass indicated a significant negative effect of zinc and particularly copper on the uptake of cadmium ions. From the different literature sources it can be generally concluded that the light metals bind less strongly than the heavy metal ions. Therefore, the former do not strongly interfere with the binding of the later.

2.1.8 Effect of pH

One of the most important variables affecting the biosorption process is the pH value of the solution. Since the early studies of the biosorption phenomenon, it has been known that the uptakes of heavy metal cations by most

biomass types decrease as the pH value of the metal solutions decreases from pH value 6 to 2.0 (Figure 2.5) (Tsezos, 1980). At lower pH values the active sites of the biomass are protonated and therefore competition between protons and metal ions for the sorption site occurs (Green et al., 1987; Tobin et al., 1984). At low enough pH values, almost all sites become protonated and complete desorption of the bound metal ion is possible (Aldor et al., 1995). Decreasing the pH value to extreme acidic conditions may damage the structure of the biosorbent material. Microscopic observations have shown distorted cells; significant weight loss and decrease in the sorption capacity have been observed (Kuyucak and Volesky, 1989b). With further increase of pH value (>5.5) most heavy metals would precipitate. At those high pH values precipitation may contribute to the overall removal of metals from solution.

Furthermore, the pH of a solution can be changed as a result of biosorption. Experiments performed in closed batch systems without pH adjustment revealed that the sorption of heavy metals onto acid washed biomass led to a decrease of the pH value in the solution (Crist et al., 1981). Holan et al. (1993) observed a decrease of pH value from 5 to 3.5 in sorption experiments with crosslinked *A. nodosum.* An increase of pH during sorption was reported by Kuyucak and Volesky (1989a), the release of carbonate ions from *Ascophyllum* biomass could be responsible for the observed increase in solution pH.

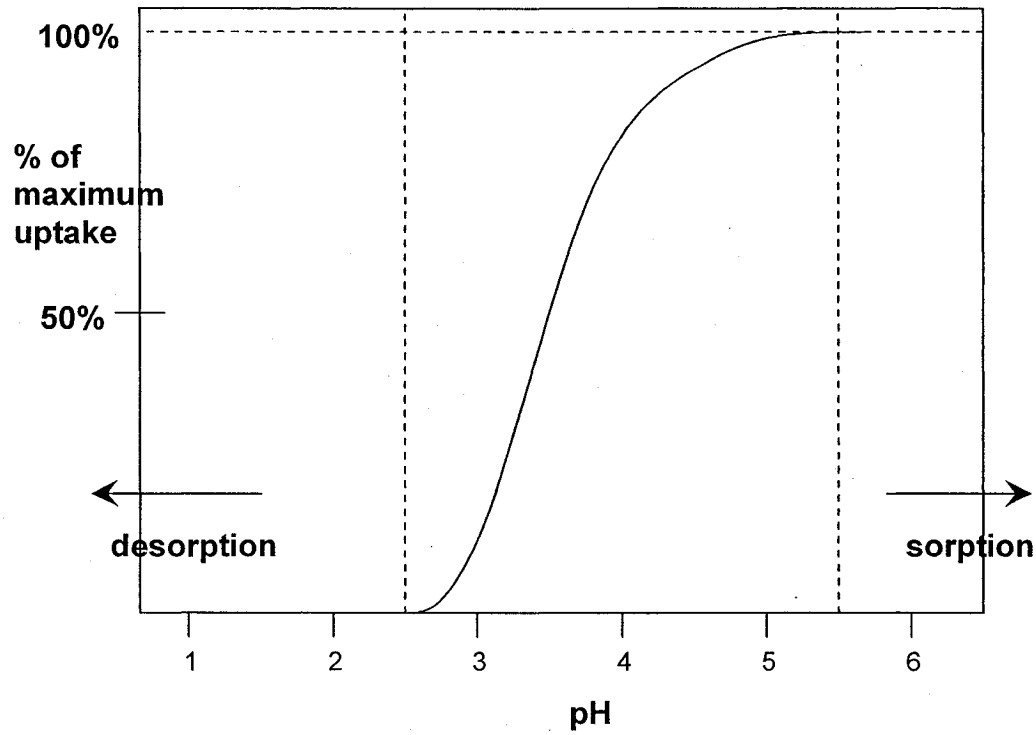

Figure 2.5:Typical dependence of metal uptake on pH value (Kratochvil and Volesky, 1998)

2.1.9 Equilibrium time

Most of the sorption process is completed in less than one hour. Ninety percent of the equilibrium binding of Cd by *Ascophyllum nodosum* was reached in 30 min., the equilibrium was attained after ~300 min (Volesky and Prasetyo, 1994). Investigations by Yang and Volesky, 1996, showed that 50% of the Cd adsorption by *Sargassum* was achieved in less than three minutes and that the equilibrium was reached in 30 min. Equilibrium was reached after 30 min for the uptake of copper onto the calcium treated marine algae *Pandina sp.* (Kaewsarn, 2002). 90% of the adsorption of lead and copper onto Australian marine algae

Durvillaea potatorum and *Ecklonia radiate* was completed in the first 10 min (Matheickal and Yu, 1999).

2.1.10 Temperature effect

Overall, the effect of temperature is small compared to other influencing factors (Tsezos, 1990). Greene and Darnall (1988) found that the distribution ratio (metal bound/ metal in solution) for biosorption of Cd, Pb, Zn, Ni, and Cu to *Spirulina* algae increased by only ~ 20% when the temperature was raised from 4°C to 55°C. Hammaini et al. (1999) found out that temperature-related effects on biosorption of Cd, Cu, Ni, Pb, and Zn ions to activated sludge biomass were not significant. Over the range of temperature investigated (20°C – 60°C) no difference on biosorption capacity for the five metal ions was observed. The biosorption on viable-biomass is strongly affected by temperature. However, biosorption by non-viable biomass is metabolism-independent, and therefore, temperature is not expected to have a significant effect on the metal ions uptake (Hammaini et al., 1999).

2.2 Summary

Heavy metal pollution represents a serious environmental problem. The conventional treatment of wastewater containing heavy metals by precipitation and by ion exchange has its limitations due to the low efficiency of metals removal at low metal concentrations, and due to a relatively high operating cost, respectively. These shortcomings have created a strong incentive for developing an innovative process of biosorption of heavy metals. Biosorption refers to the

binding and concentration of heavy metals from dilute solutions to certain types of inactive, dead biomass. In contrast to the conventional treatments, biosorption offers low cost and high metal removal efficiency from diluted solutions.

Wastewater treatment using biosorbent on an industrial scale is likely to be a continuous process employing flow-through biosorption columns. Therefore studying the removal of toxic heavy metals in flow-through biosorption columns, and exploring the possibility of using the biosorbent in such columns is necessary. Furthermore, the effect of wastewater composition on the limitations, efficiency, and design of a biosorption process should be addressed.

Three attempts to commercialize immobilized biomass biosorption in the fields of wastewater treatment and metal value recovery did not manage to succeed. Two attempts to market two different types of immobilized microbial biomass, one by BV SORBEX and the other by the US Bureau of Mines are not known to have made a successful commercial application in the market (Tsezos, 2001). Most types of biomass have to be immobilized in a synthetic polymer matrix in order to yield particles with the required mechanical properties. The biosorbent material that will be used in this study is anaerobic biomass. It possesses compact porous structure and excellent settling ability. Unlike most of other biomass, immobilization or stiffening is not necessary prior to using the biomaterial. The particulate biomass was found to possess high mechanical strength. Even under aggressive chemical environments (acidic or base conditions) the biomass demonstrated high stability with no visible structural

damage. The limited availability of granulated biosorbents makes this biomass advantageous over other biosorbents.

3 – OBJECTIVES

Most biosorbents studied in the literature are suspended in the form of bioflocs. One of the major operational problems associated with the suspended flocs is separation of biosorbent from the treated effluent. To overcome this drawback, cell immobilization techniques have been developed, but the employment of immobilization procedures is expensive and complex. This study will look into the feasibility of anaerobic granules as a novel type of biosorbent for the removal of cadmium, copper, nickel, and lead from aqueous solution. Anaerobic granules are microbial aggregates with a strong, compact and porous structure and excellent settling ability. Anaerobic sludge obtained from a wastewater treatment plant in the province of Quebec for cheese production (Agropur, Notre Dame de Bon Conseil, Quebec) was used.

The main objective of this work was to investigate the equilibrium, batch dynamics and continuous column operation for the biosorption process. The biosorption of lead, copper, cadmium and nickel was studied in a continuous flow system in order to establish the link between the biosorption equilibrium isotherms and the performance of biosorption columns. The affinity order of anaerobic biomass for the four metals under study was investigated. Issues

regarding the limitations of biosorption process imposed by the wastewater composition in flow-through columns was also investigated.

The scope of work for this study was as follows:

Equilibrium studies

- To compare between the biosorption capacity of nonviable and viable anaerobic sludge for recovery of metal ions in order to determine the state of biomass to be used in the packed bed column reactor.
- To determine the effect of the ionic form of the biosorbent on metal removal to be studied.
- To determine the optimum pH value corresponding to a higher uptake value of each metal ion tested.
- To determine the time for adsorption equilibrium in order to determine the detention time to be used in the fixed bed column reactor.
- To obtain metal sorption isotherms. These isotherms determined the maximum biosorption capacity of the anaerobic biomass for the recovery of each metal ion tested.

Biosorption column studies

- To evaluate the binding of metals under flow conditions. Breakthrough curves for the four metals studied were established.
- To investigate the effect of multi-metal systems on the uptake capacity of each metal ion studied.

- To determine the metal-binding mechanisms that function in sequestering the metallic species onto the biosorbent.
- To use a scale-up approach to design the biosorption column.

Figure 3.1 shows the sequence of tests performed in this study.

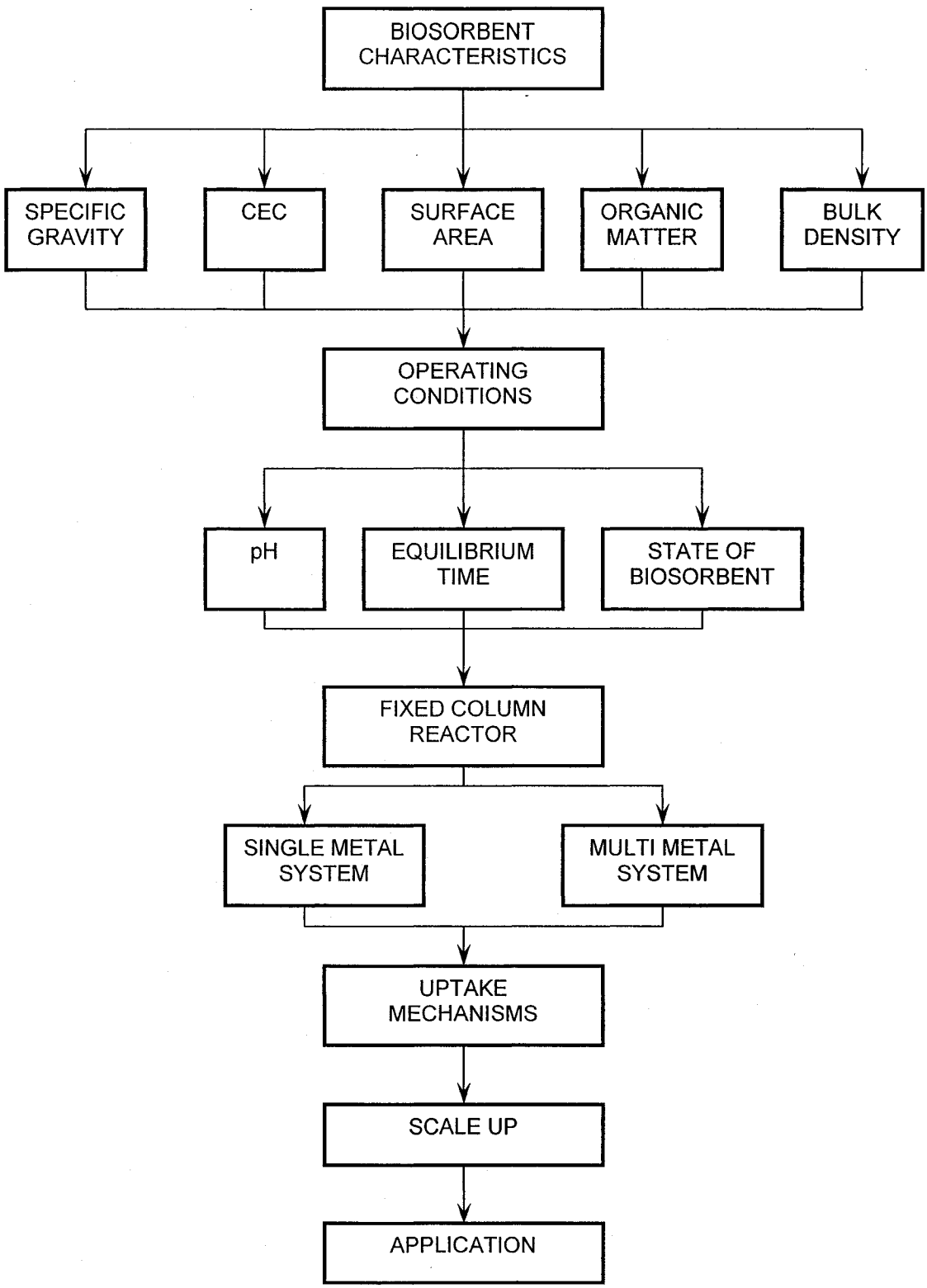

Figure 3.1: Adsorption process development steps

4 - MATERIALS AND METHODS

4.1 Biomass

Anaerobic sludge was obtained from an anaerobic wastewater treatment plant in the province of Quebec for cheese production (Agropur, Notre Dame de Bon Conseil, Quebec) (Figure 4.1).

The term 'untreated biomass' shown in Figure 4.2 refers to the sludge which was first centrifuged for 20 minutes at 3000 rpm; the pellets obtained were dried at 50°C for 6 days. Subsequently, the dried biomass was ground and sieved into different size fractions. The fraction collected between mesh sizes 16 and 20, corresponding to a particle size ranging from 0.84 mm to 1.18 mm, was used in the experiments.

H-biomass was prepared by first combining dried and sieved biomass with 0.02 M HCl in a 2 L beaker for 3 hours at a biomass concentration of 20 g/L and pH value close to 2.5. Next, the acid solution was drained and the biomass was washed with distilled water 4 or 5 times. Finally, the wet protonated biomass was dried in the oven at 50°C.

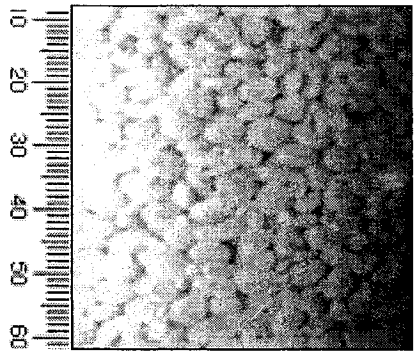

Figure 4.1: Anaerobic sludge obtained from an anaerobic wastewater treatment plant for cheese production

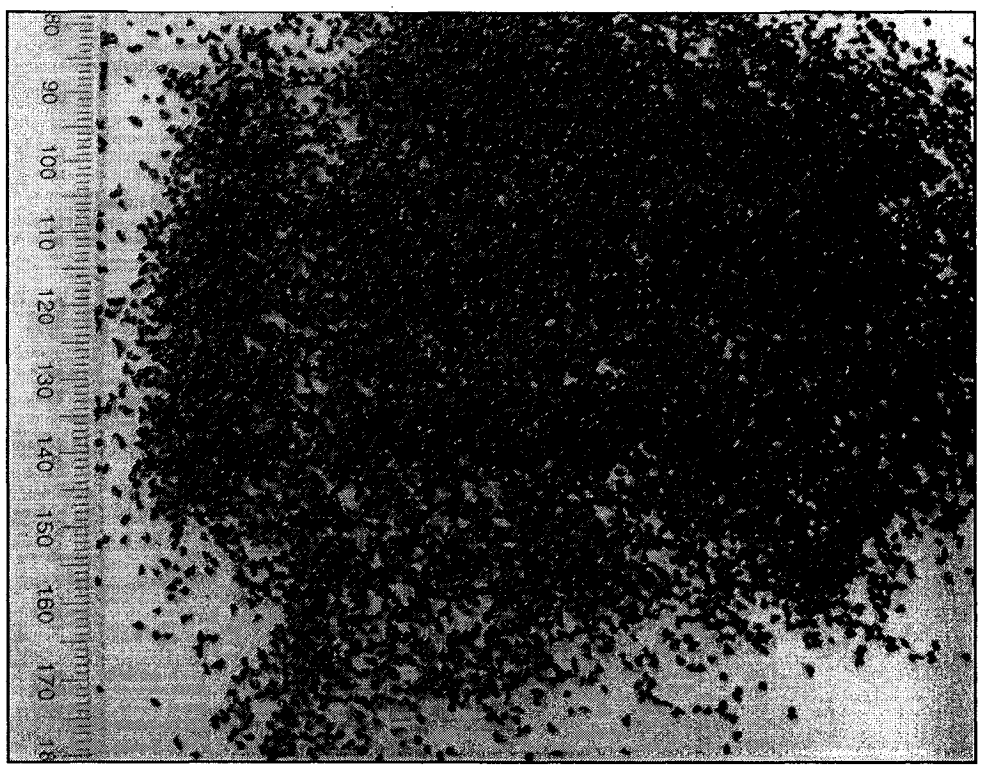

Figure 4.2: Dried and sieved biomass (fraction collected between mesh sizes 16 and 20, corresponding to particle sizes ranging from 0.84 mm to 1.18 mm)

K-biomass and Ca-biomass were prepared by the same method as H-biomass but combining the dried and sieved biomass with 0.02 M KOH and 0.01 M $Ca(OH)_2$, respectively at a pH value close to 11.5. The base solution was drained and the biomass was washed with distilled water 4 or 5 times. Finally, the wet biomass was dried in the oven at 50°C.

4.2 Biomass characteristics

4.2.1 Chemical oxygen demand (COD)

The Chemical Oxygen Demand (COD) test measures the chemical oxidant required to break down organics. COD is an indicator of the concentration of organics in the solution. The Closed Reflux, Colorimetric Method from American Public Health Association (1995) was used.

The biomass was diluted 50, 100 and 200 times, 2.5 mL of the diluted samples were added to the standard tube which contains 7.5 mL of the digestion solution (commercially available pre-measured solution containing a 7.5 mL mixture of sulfuric acid, potassium dichromate, silver sulfate, mercuric sulfate, and sulfuric acid in twist cap digestion vials, and potassium hydrogen phthalate (KHP) standard (425 mg KHP /L distilled water; having a theoretical COD of 500 mg O_2/L). The final dilution of the biomass was 200, 400 and 800 times. The samples were digested for two hours at 150°C. The tubes are then cooled and the test color was measured using a Perkin-Elmer Lambda 2S Spectrophotometer with cell adapters for COD vials. The standard vials cover a COD range of 0 - 125 mg O_2/L (in the final 10 mL digestion mixture). Since this

2.5 represents a 4X dilution (2.5 mL to 10.0 mL) for digestion, this corresponds to a maximum working range of 0 - 500 mg O_2/L. the 800X dilution had a COD value within the working range:

COD of the biomass = 59, 600 mg O_2/L

4.2.2 Settled sludge volume and solids

According to the American Public Health Association (1995), an Imhoff cone was filled to the 1-L mark with a well-mixed sample of the sludge. The sample was left to settle for one hour. The settleable solids were found to be 940 mL/L.

The total solids of the sludge were determined. A well-mixed sample of 10 mL of the sludge was evaporated in a weighed dish and dried to constant weight in an oven at 105°C. The increase in weight over that of the empty dish represents the total solids (TS), the test was done in triplicate, and gave TS = 42,600 mg/L. To determine the total volatile solids (TVS) the residue from the previous procedure was ignited to a constant weight at 550°C. The weight lost on ignition is the volatile solids, the test was done in triplicate, and determined to be TVS = 31,300 mg/L. TVS offers a rough approximation of the amount of organic matter present in the solid fraction of the sludge. The other part of the sludge is the inorganic part which is represented by the total fixed solids (TFS), and determined as TFS = 42,600 mg/L - 31,300 mg/L = 11,300 mg/L. From these results it can be seen that almost 75% of the solid fraction of the sludge is

organic matter. The other 25% is inorganic matter which consists of inorganic salts and mineral elements.

To determine the total suspended solids (TSS), a 3 ml sample of the unsettled part in the Imhoff cone was filtered through a weighed standard glass filter and the residue retained on the filter was dried to a constant weight at 105°C. The increase in weight of the filter represents the total suspended solids, the test was done in triplicate and TSS = 1400 mg/L. To determine the total volatile suspended solids (TVSS) the residue from the previous procedure was ignited to a constant weight at 550°C. The weight lost on ignition is the volatile solids, the test was done in triplicate, TVSS was found to be almost the same as the TSS, which indicated that all of the TSS was volatile (organic matter). The characterization of the solids of the biomass is summarized in Table 4.1.

Table 4.1: Characterization of anaerobic biomass solids

Sludge Solid Characteristics	**Concentration (mg/L)**
Total Solids (TS)	42,600
Total Volatile Solids (TVS)	31,300
Total Fixed Solids (TFS)	11,300
Total Suspended Solids (TSS)	1,400
Total Volatile Suspended Solids (TVSS)	1,400

4.2.3 Biomass bulk density

Bulk density is a measure of the weight of the biomass per unit volume (g/mL), usually given on a dry basis. Variation in bulk density is attributable to the relative proportion and specific gravity of solid organic and inorganic particles and to the porosity of the biomass. Since the biomass will not be dry during its use we were interested in measuring the wet bulk density of the biomass.

The wet bulk density of the biomass was measured by saturating a 1.5 g (dry weight) of the biomass (2.0 mL) with distilled water and measuring the increase of volume with time. The volume was measured until constant volume was achieved almost after 24 hours (5.87 mL). Three samples were taken and the average of the three samples was used to calculate the bulk density:

Wet bulk density = 0.26 g (dry weight)/mL(wet volume)

4.2.4 Specific gravity

The specific gravity of the biomass is the ratio of the masses of equal volumes of biomass and distilled water. The specific gravity of the biomass was determined according to the American Public Health Association (1995) by comparing the mass of known volume of biomass sample at a specific temperature to the mass of the same volume of distilled water at 4°C. Three samples were taken and the average of the three samples was used to calculate the specific gravity of the biomass:

Specific Gravity (SG) = 1.75

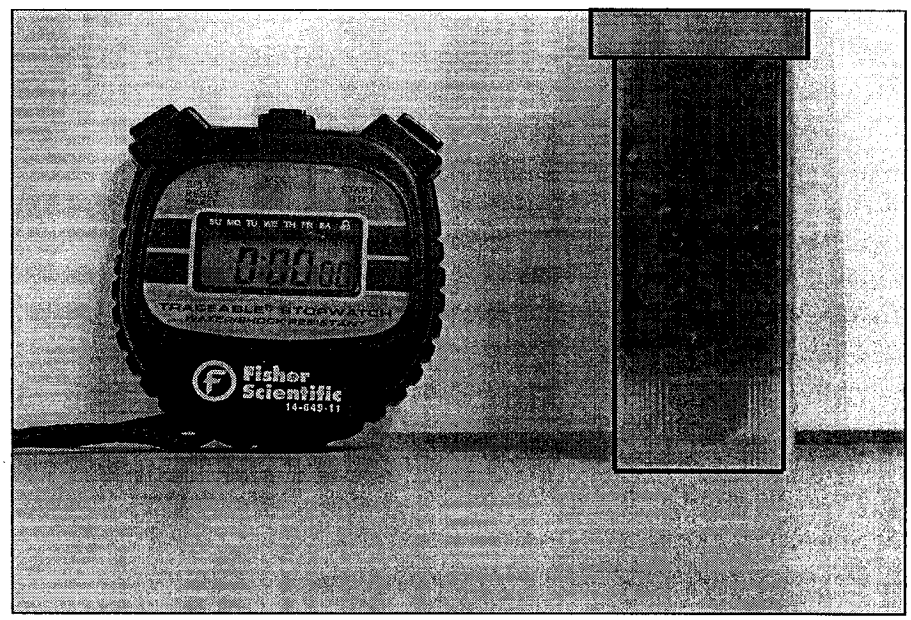

Figure 4.3: Biomass suspended in distilled water at time zero

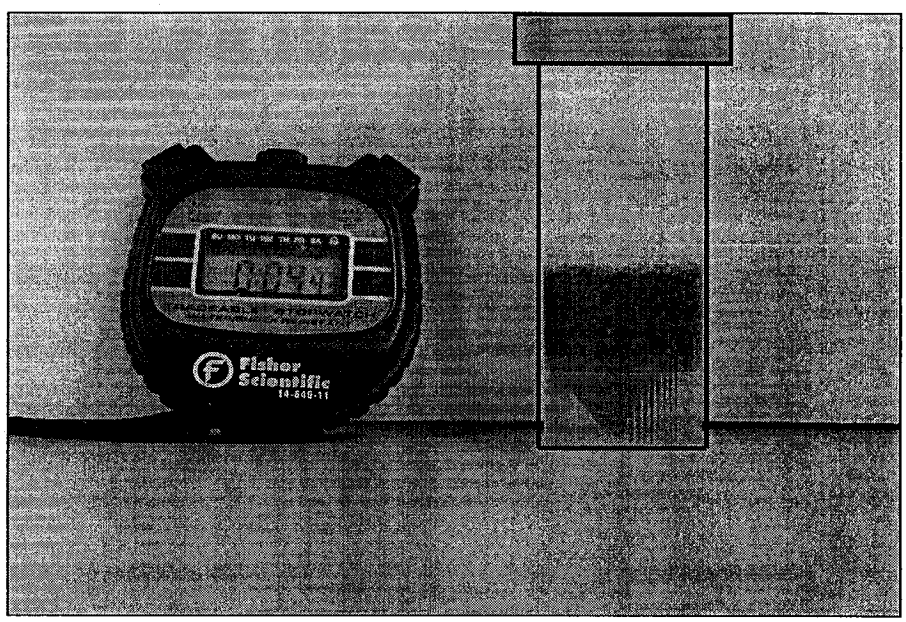

Figure 4.4: Biomass settled after almost 5 seconds

Figures 4.3 and 4.4 show that the biomass has high settleability where after almost 5 seconds of initial agitation all of the biomass settles. The very fast settling observed with the anaerobic biomass represent an advantageous aspect when water treatment systems are designed. The implication is that the material could be suitable for the continuous flow system.

4.2.5 Cation exchange capacity (CEC)

The ability of the biomass to bind cations by ion exchange mechanism is proportional to the number of negative charges per unit weight of the biomass. The ion exchange capacity of the biomass to hold cations, i.e. the number of negatively charged sites per unit of biomass mass, could be quantitatively expressed in a measured property known as cation exchange capacity (CEC).

There are a number of ways to measure CEC. Most are based on the principle of cation displacement. This refers to a process in which the cations occupying the exchange complex are displaced by 'flooding' the biomass with excess cations from reagents such as ammonium acetate (NH_4^+ is the displacing cation). 20 mL of 1M potassium acetate was added to 1g of the biomass in a centrifuge tube. The samples were shaken on a shaker for 30 minutes at a speed of 75 rpm. The samples were centrifuged for 10 minutes. The clear supernatant was discarded. This process was done twice to ensure effective washing of the biomass. 20 mL of distilled water was added to the biomass and the samples were shaken for 30 minutes at a speed of 75 rpm with an adjustable reciprocating orbital shaker (AROS-160-Thermolyne). The samples were centrifuged for 10 minutes. The clear supernatant was discarded. This process

was done twice. 25 mL of 1 M ammonium acetate was added to the biomass. The pH of the solution was 5.5. The samples were shaken for 30 minutes at a speed of 75 rpm. The samples were centrifuged for 10 minutes. The clear supernatant was collected in a clean 50 mL centrifuge tube. This process was repeated twice pouring the clear supernatant into the same centrifuge tube. The concentration of K was measured by atomic adsorption. Using the value for the concentration of K, the CEC value was determined. The test was done in triplicate and the average CEC value was:

CEC = 94 meq/100g

4.2.6 Surface area

The surface area of the dried and sieved biomass was determined using the ethylene glycol monoethyl ether (EGME) method (Heilman et al., 1965). 3.0 g of the biomass were weighed into an aluminum weighing dish. The samples were then placed in a vacuum desiccator over P_2O_5. The desiccator was then evacuated for one hour using a vacuum pump. The samples were dried until a constant weight was achieved.

The P_2O_5 dried biomass was then wet, with approximately 3 mL of EGME to form a biomass slurry. The sample – EGME slurry was placed in a Petri dish and then into the vacuum desiccator containing $CaCl_2$ – EGME solvate on the bottom of the desiccator. The $CaCl_2$ – EGME solvate was prepared by weighing 120 g of $CaCl_2$ into a 1 L beaker and dry in an oven at 120 °C for one hour to remove all traces of water. 30 g of EGME were weighed into a 400 mL beaker

and then added to 100 g of the hot $CaCl_2$. They were then mixed thoroughly with a spatula. After the $CaCl_2$-EGME solvate has cooled it was uniformly spread over the bottom of the dessicator. The desiccator was then evacuated for one hour using a vacuum pump. The desiccator was left closed for one hour, then the samples were weighted. This process was repeated until constant weight was reached.

It has been calculated that $2.86X10^{-4}$ g EGME = monolayer of 1 m^2 surface area (Heilman et al., 1965). Therefore, the biomass surface area can be calculated by knowing the weight of EGME retained on the surface of the biomass. The surface area of the biomass was found to be:

Surface area = 6.0 m^2/g

Table 4.2 summarizes the physical properties obtained for the anaerobic biomass.

Table 4.2: Physical properties of the anaerobic biomass

Parameter	**Value**
Cation exchange capacity (CEC) (meq/g)	0.94
Organic matter (%)	75
Surface area (m^2/g)	6.0
Specific gravity (SG)	1.75
Wet bulk density (g/mL)	0.26

4.3 Batch experiments

Solutions of Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} were prepared in distilled water using the metal salts $CuCl_2$, $NiCl_2$, $CdCl_2$ and $PbCl_2$ respectively. The solutions were stored at room temperature. The solubility of the metal salts is summarized in Table 4.3. 0.5 g of dried and sieved biomass was suspended in 50 mL of metal solutions in 50 mL centrifuge tubes. The tubes were placed on a shaker at 150 rpm, and left to equilibrate for 24 hours (Figure 4.5). Uptake of metals by the biomass was determined from the difference of metal concentration in the initial and final supernatant solutions. The pH of the solutions before and during the sorption experiments was monitored using an AR-25 Fisher Scientific pH meter. The initial pH value was adjusted to 5.0 using a 1M hydrochloric acid solution. Since there was potential for metal adsorption onto the surface of the glassware or plastic ware, metal-free and biosorbent-free blanks were used as controls.

4.4 Metal concentration analysis

Dissolved metal concentrations in solution were determined by a flame atomic absorption (AA) spectrophotometer (Model Analyst 100 Perkin Elmer). The characteristic wavelengths used in AA analysis for various metals are listed in Table 4.3. Standard solutions for metals tested were also prepared.

Samples were filtered using Whatman 42 filter paper before they were measured by the AA machine. To reduce interference by organic matter and to convert metal associated with particulates to the free metal form that can be

determined by AA a cold digestion was used by adding hydrochloric acid to the samples to make a 4N solution of HCl.

Table 4.3: Solubility of the different metal salts used in the study (Handbook of chemistry and physics, 1979)

Metal Salt	Solubility, in grams per 100 mL
PbCl2	0.99
CuCl2	70.6
CdCl2	140
NiCl2	254

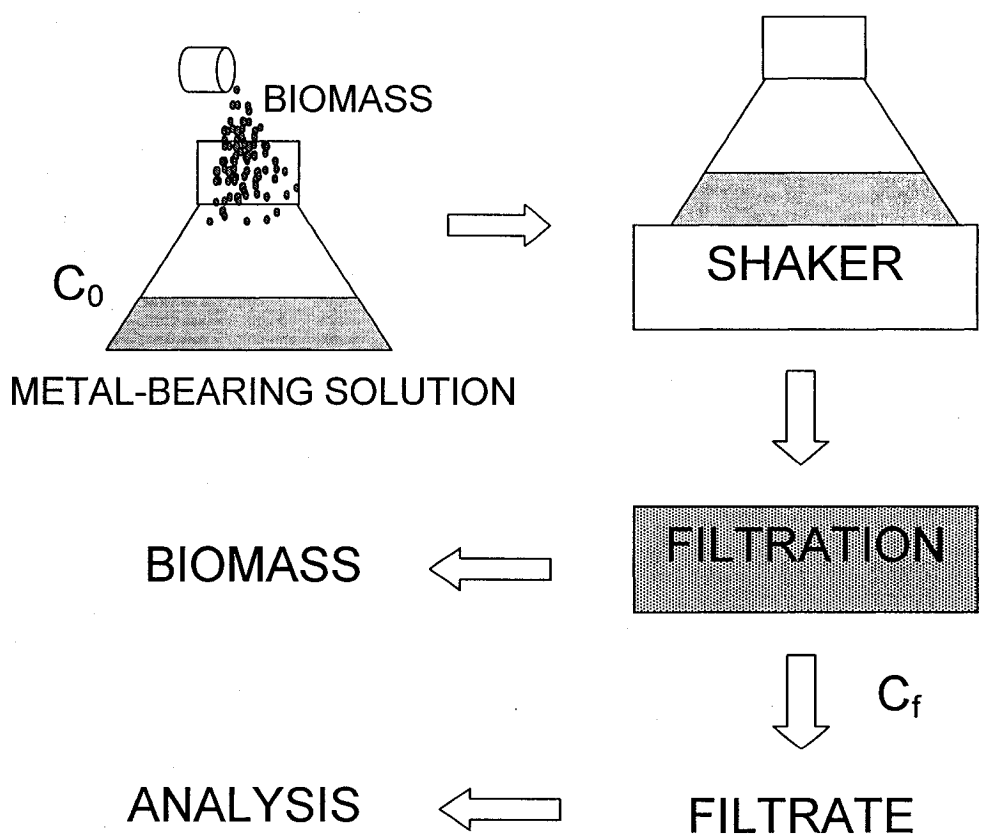

Figure 4.5: Scheme of batch experiments

Table 4.4: The characteristic wavelengths used by AA for studied metals

Metal	Wavelength (nm)
Pb	283.3
Cd	228.8
Cu	324.8
Ni	346.2

The metal uptake, q, was evaluated from the following equation:

$$q = (C_0 - C_f)V/M$$

where C_0 is the initial concentration of metal ions in the solution (mg/L), C_f is the equilibrium concentration of metal ions in the solution (mg/L), V is the volume of solution in the flask (mL) and M is the mass of biomass (g).

All the equilibrium biosorption experiments were performed at room temperature (23 - 25°C, maintained by a central air conditioning system).

4.5 Scanning electron microscopy

The scanning electron microscope (SEM) (JEOL JSM-840A) was used to take microscopic pictures of the biomass. The SEM has unique capabilities for analyzing surfaces. It is analogous to the reflected light microscope. Whereas the reflected light microscope forms an image from light reflected from a sample surface, the SEM uses electrons for image formation. Electrons have a much shorter wavelength than light photons, and shorter wavelengths are capable of generating higher-resolution information. Enhanced resolution in turn permits

higher magnification without loss of details. The SEM micrographs can maintain the three-dimensional appearance of textured surfaces (Gabriel, 1985). The combination of high resolution up to 4 nm, extensive magnification range (10 to 300,000X), and high depth of field (accelerating voltage 0.2 to 40 Kv) makes the SEM uniquely suited for the study of the surface of the anaerobic biomass.

The SEM is frequently equipped with a spectrometer capable of detecting X-rays emitted by the specimen during electron-beam excitation. These X-rays carry a characteristic energy and wavelength, which when measured will reveal the elemental composition of the specimen. X-ray analysis can be performed on a wide variety of sample types containing elements that covers a large portion of the periodic table. The minimum detectability limit of X-ray analysis is roughly 10^{-16}g (Gabriel, 1985). X-ray analyses were performed on the Ca-biomass and on biomass samples saturated with the four different heavy metals studied.

4.5.1 Sample preparation for SEM

Sample stability is extremely important during the SEM analysis; any movements other than the normal motions used to manipulate the sample are undesirable because a given field of view will shift, interfering with photography. Aluminium stubs (diameter 1.2cm) were used to mount the samples on. After cleaning the stubs with methanol conductive carbon tape was cut and placed on the surface of the stubs. The biomass, which was saturated with the different heavy metals studied, was sprinkled onto the carbon tape.

4.5.2 Conductive coating of samples

Examination of uncoated, nonconductive specimens in the SEM is difficult because the specimens behave like insulators by adsorbing electrons, and accumulate a net negative charge. Consequently, the specimen deflects the electron beam and image quality is degraded. Such specimens may be examined at low accelerating voltages, but magnification and resolution are limited. The best method for improving image quality over the entire magnification range of the SEM is to deposit a conductive thin film over the specimen surface (Gabriel, 1985).

The metallic thin film was prepared using the sputtering system Hummer 6 using gold and palladium to make the thin film. Gold and palladium were used because they can form very thin films without suppressing fine surface features.

4.6 Continuous flow column tests

The experimental set-up of the fixed-bed column is schematically shown in Figure 4.6. 11 g of dry biomass were packed into a 15 cm long column of 2.0 cm inner diameter. A bed of glass spheres was placed at the bottom of the column prior the active biomass bed to ensure homogenous distribution of the feeding solution.

The biomass was first washed by slowly flooding the column with distilled water from the bottom. After washing the biomass with distilled water and prior to the sorption of heavy metals the biomass was washed with a 0.1 M $CaCl_2$

solution in order to prepare the Ca-biomass. As can be seen from Figure 4.6 metal solutions were pumped from a 50 L storage tank into the column. The pH value in the storage tank was adjusted to 4.0 using a 1M hydrochloric acid solution. The metal solutions were fed into the column from the bottom, at a flow rate equal to 1.5mL/min allowing 30 minutes of detention time in the column. Samples of column effluent were collected from the top by means of a fraction collector (FC203 Gilson) at preset time intervals and were analyzed for heavy metals content using AA. When the biomass in the column was saturated with metal, that is, the final column effluent concentration approached the influent concentration, the column feed was switched to distilled water for several hours and then followed by a 0.5 M $CaCl_2$ solution in order to elute the heavy metals. The collection and analysis of the elution outlet samples was the same as for the sorption protocol. The pH value in the effluent was also measured using an AR-25 Fisher Scientific pH meter.

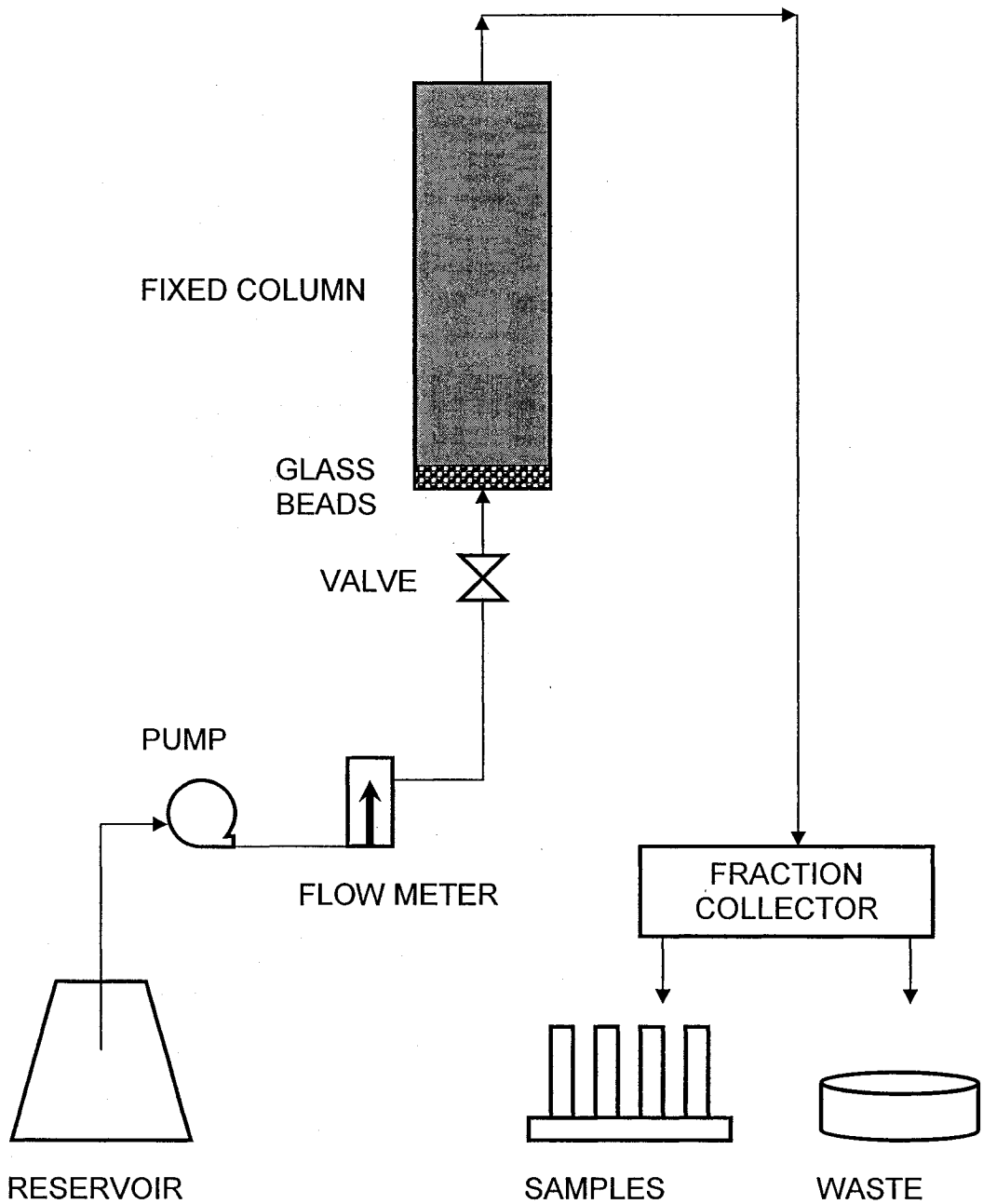

Figure 4.6: The experimental arrangement of the continuous flow biosorption column

5 - RESULTS AND DISCUSSION

5.1 Batch tests

5.1.1 Nonviable and viable biomass

As mentioned before biosorption is a process whereby certain types of inactive, dead biomass may bind and concentrate heavy metals from aqueous solutions. In contrast to the passive nature of biosorption, bioaccumulation occurs in living cells and is metabolically driven. A comparison between the biosorption capacity of nonviable and viable anaerobic biomass for recovery of metal ions was conducted in order to determine the state of biomass to be used in the packed bed column reactor. The biosorption process was investigated in equilibrium batch tests. The solution was left to equilibrate for 24 hours.

Figure 5.1 shows that for the four metal ions studied the uptake capacity of the viable biomass was higher than that of the nonviable biomass. Different possible explanations why sorption was higher for the viable biomass could be introduced. First two different processes are involved in metal ion uptake by viable and non-viable biomass. The first uptake process is independent of cell metabolic activity, and is referred to as biosorption or passive uptake. It involves the binding of metal ions to the cell surface. The second uptake process involves

the uptake of metal ions into the cell across the cell membrane, this process is referred to as intracellular uptake, active uptake or bio-accumulation (Volesky, 1990). The metal uptake by active mode has been observed for metals such as Cu, Cd, Ni, Zn, Mn, Sr, Co, Mg, and Ca (Wase and Forster, 1997). The first process occurs by both viable and non-viable biomass, the second process, which is metabolism-dependent, happens only in viable biomass. For viable biomass the metal uptake is also facilitated by the production of metal-binding proteins (Wase and Forester, 1997). Therefore, the higher uptake capacity of the viable biomass observed in Figure 5.1 could be due to the active uptake of the biomass. Active uptake by the cell membrane can be highly selective and often irreversible unless the living system is destroyed. The second possible explanation why sorption was higher for the viable biomass is the difference of surface area of viable and non-viable biomass. The biomass was killed by basically drying the biomass in the oven. According to Lehninger et al. (1993) water is the most abundant single compound in the cell and it makes almost 70% of the total weight of the cell. Thus by drying the biomass into the form of pellets the surface area of the cell would decrease, i.e. less area is exposed to the metal ions, therefore, less metal uptake. Finally according to Yan and Viraraghavan (2000) heat treatment of the biomass could cause a loss of amino-functional groups on the cell surface. Amino-functional groups are among the functional groups that would contribute to the binding of heavy metals as was discussed in section 2.1.2.

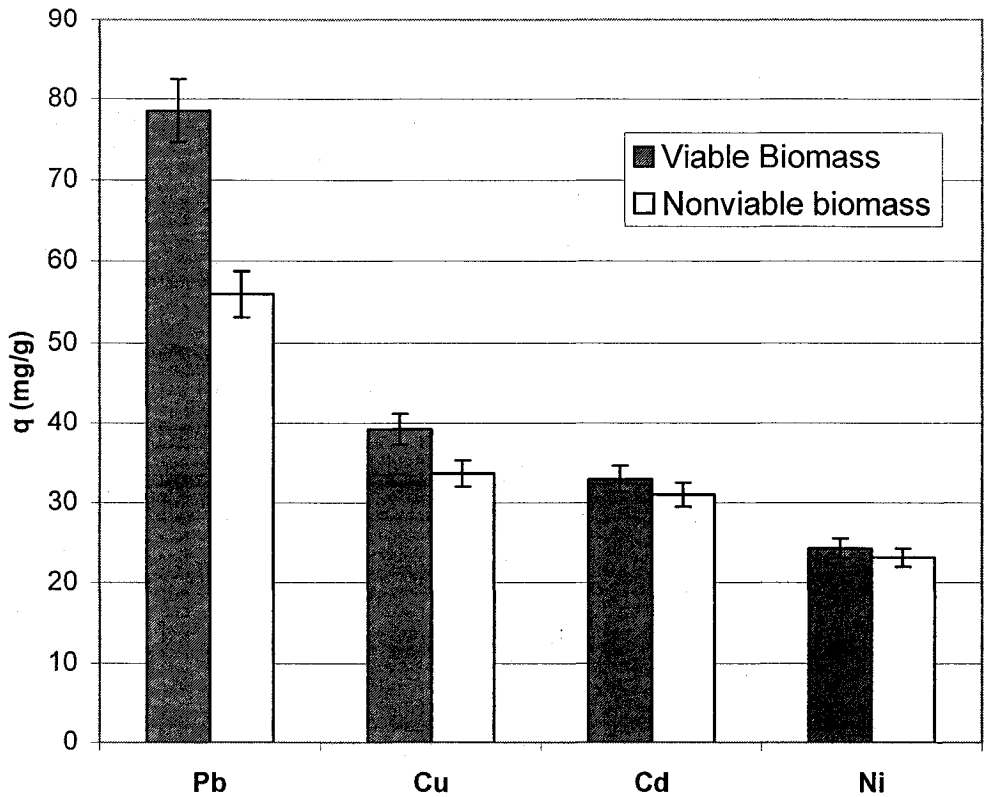

Figure 5.1: Comparison of q value for viable and nonviable biomass for different metals

From the results in Figure 5.1, it was found that the efficiency of non-viable cells in biosorbing metal ions may be less than that of the living cells. However, the use of non-viable biomass offers the following advantages over viable cells:

- The metal removal system is not subject to toxicity limitations.
- No requirements for growth media and nutrients.
- Biosorbed metal ions can be easily desorbed and biomass can be reused.
- Much simpler process control.

- The biomass can be stored for a period of time.
- Biosorption tends to be very fast.

Therefore, to overcome the disadvantages of using viable biomass, non-viable biomass was used in this study.

5.1.2 Effect of the ionic form of biosorbent on metal removal

Recent studies of biosorption phenomenon revealed that biosorbents, very much like synthetic ion exchange resins, can be prepared in different ionic forms, i.e., Na^+, Ca^{++}, H^+, etc., by washing the biomass with mineral acids, salts and/or basis (Figueira et al., 2000, Crist, et al., 1991, Fourest and Roux, 1994, Jeffers, et al., 1991). According to Yan and Viraraghavan (2000) pre-treatment of the biomass would expose more available binding sites for metal biosorption and would remove surface impurities from the biomass.

Untreated biomass generally contains light metal ions such as K^+, Na^+, Ca^{2+}, and Mg^{2+}. Treated biomass generally implies one of two chemical alterations. The first is protonation of the biomass with a strong acid such as HCl whereby the proton displaces the light metal ions from the binding sites. In the second, the biomass is reacted with an aqueous solution of a given ion at high concentration so that the majority of sites are occupied by, for example, calcium or potassium:

(Ca^{2+} + light metal ions. Biomass $\leftrightarrow$ Ca. Biomass + light metal ions)

Consequently, questions arise as to what ionic form should be used for removing heavy metals, and what chemicals should be selected for desorbing the metals, in order to assure the optimal performance of the biosorption column.

The performance of H, K and Ca-biomass was studied and compared to the untreated biomass. The biosorption process was investigated in equilibrium batch tests. Figure 5.2 shows that Ca-biomass has a q value higher than that for the untreated biomass in the case of the four metals tested. For the K-biomass the q value was close to that of the untreated biomass in the case of copper and it was less in the case of nickel. The q value of the K-biomass for lead and cadmium was higher than that for the untreated biomass. The performance of the H-biomass was not good since the q value was less than that of the untreated biomass for all metals tested.

The pH value of the solution containing the heavy metals before adding it to the treated biomass was adjusted to 5.0 for all four cases. Eventually the biomass will be used in a fixed column reactor and since the pH control inside the column is difficult the pH value was not adjusted during the batch test. For the case of K and Ca treated biomass the pH value of the supernatant solution after the test remained almost constant. However, for the H-biomass the pH value of the supernatant solution decreased to almost 3.0. The lower pH value in turn would reduce the biosorption capacity significantly, as would be discussed in the following section. The type of counter-ion, i.e. the ionic species released from the biomass in exchange for the heavy metal, is determined by the type of chemical used for washing the biomass prior to the sorption of the heavy metal.

One disadvantage of using the H-biomass is that biosorption by protonated biomass would be accompanied by release of protons. This is reflected in the lowering of the solution pH. From these results, the Ca-biomass was used to carry out the study in order to try to stabilize the pH fluctuation in the fixed column as would be discussed in the following sections.

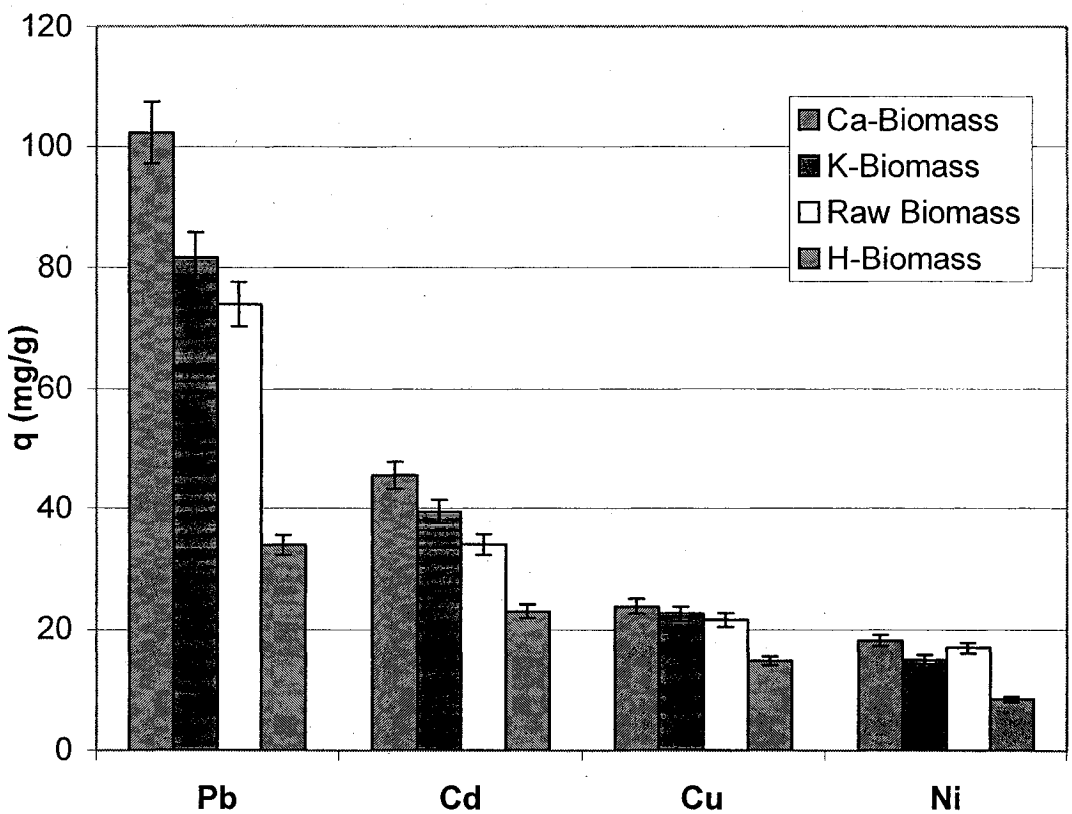

Figure 5.2: Effect of different ionic forms of biomass on metals uptake

5.1.3 Effect of pH

Since pH is one of the main variables affecting the biosorption process (Lezcano et al., 2001), the optimum pH value for the uptake of metals was determined.

The Ca-biomass was used in these tests since it was found to have the highest q value. Six different pH tests, chosen within the solubility range of the metals used, were carried out.

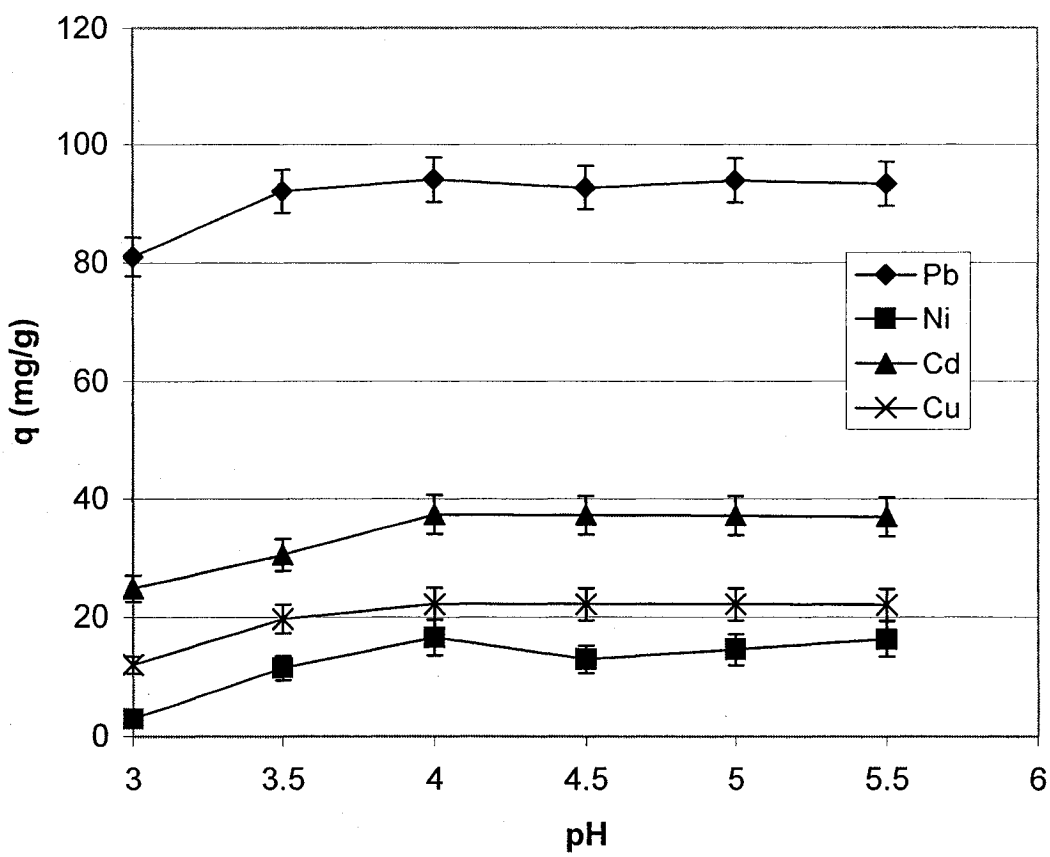

Figure 5.3: Effect of pH on the biosorption of the four metals

Figure 5.3 shows that over the pH range 4 to 5.5, pH-related effects were not significant. Meanwhile, at the pH values of 3.5 and 3 the q values started to decrease. At low pH, protons would compete for active binding sites with metal ions. The protonation of active sites thus tends to decrease the metal sorption. At a low enough pH, almost around 2.0, all the binding sites may be protonated, thereby desorbing all originally bound metals from the biomass (Gupta et al., 2000; Aldor et al., 1995).

An additional possible explanation why sorption increases with increasing pH is that the solubility of many metals in solution decreases with increasing pH. A further possible explanation of increasing sorption with increasing pH is that hydrolyzed species have a lesser degree of hydration, i.e. less energy is necessary for removal or reorientation of the hydrated water molecules upon binding (Schiewer and Volesky, 1995). At a further increase of pH the solubility of metals decreases enough for precipitation to occur. This should be avoided during sorption experiments where distinguishing between sorption and precipitation metal removal becomes hard (Schiewer and Volesky, 1995).

5.1.4 Time profile of metals sorption

As the adsorption process proceeds, the sorbed solute tends to desorb back into the solution. Eventually the rates of adsorption and desorption will attain an equilibrium state. When the system reaches the sorption equilibrium, no further net adsorption occurs. The time at which the adsorption equilibrium will occur was determined in order to determine the retention time that would be used

in the fixed bed reactor. The adsorption rate tests were performed on an equilibrium batch basis. 0.5 g of the Ca-biomass was contacted with 50 mL of metal bearing solution of 100 mg/L concentration. The biomass was kept in contact with the metal-bearing solution for different time periods (15, 30, 45 and 60 minutes). Time zero samples were also taken. In these samples the biomass was directly separated from the metal-bearing solution within less than one minute contact time.

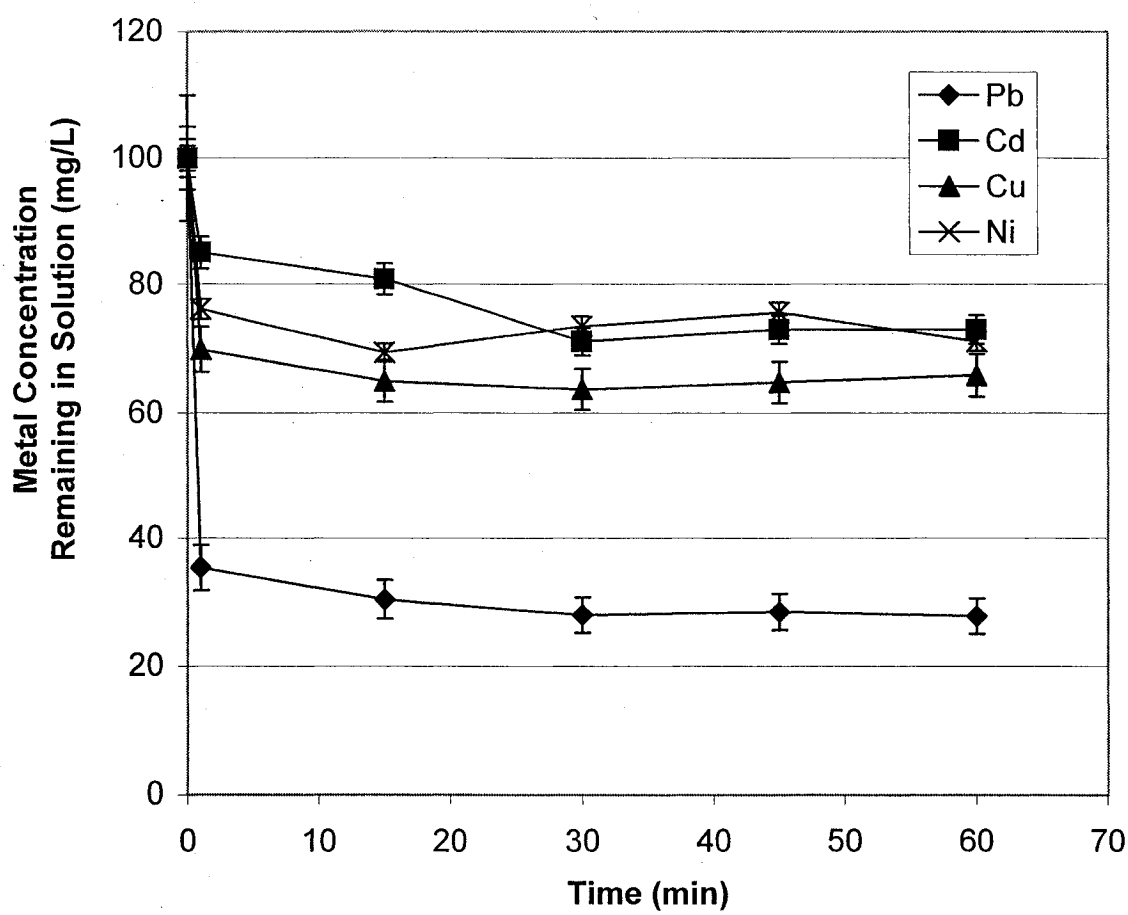

Figure 5.4: Time profile of metal ions sorption

Figure 5.4 shows that the rate of metal uptake was very fast. Within the first 5 minutes of contact, almost 75% of the total metal uptake was completed. Adsorption equilibrium was reached almost 30 minutes after biomass addition. These results show that the actual chemical reaction of metal ion binding to the biomass is a fast phenomenon. The very fast sorption observed with the anaerobic biomass represent an advantageous aspect when water treatment systems are designed. The implication is that the material would be suitable for the continuous flow system. From these results, the 30 minutes time was chosen as the contact time in the packed bed column reactor.

5.1.5 Biosorption isotherms

To describe the distribution of the solute in the solid phase and the liquid phase at equilibrium condition, it is necessary to express the amount of solute adsorbed per unit weight of sorbent, q, as a function of the residual equilibrium concentration, C_f, of solute remaining in solution. The expression of this relationship is termed an adsorption isotherm.

Sorption isotherms were experimentally determined for the Ca-biomass. These isotherms were derived at a pH value of 5.5 and at room temperature. A 500 mL metal solution of 10 meq/L of Cu^{2+}, Ni^{2+}, Cd^{2+} and Pb^{2+} was prepared from $CuCl_2$, $NiCl_2$, $CdCl_2$ and $PbCl_2$ respectively. Samples of solutions were diluted to prepare working metal concentrations. The experiment was performed at sufficiently high initial metal concentrations so that maximal uptake would be achieved.

From Figures 5.5 to 5.8, it can be seen that for the biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} initially the uptake capacity increases in a linear way with rising equilibrium concentration. Uptake capacity is eventually limited by the fixed number of uptake active sites on the biomass and a resulting plateau can be observed. This plateau would represent the maximum uptake capacity of the biomass for each metal ion. From Figures 5.5 to 5.8 it was found that the q_{max} for Pb^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+}, are 255, 60, 55 and 26 mg/g respectively (1.23, 0.53, 0.87, and 0.44 mmoles/g respectively). The q_{max} values of the anaerobic biomass were compared with the metal adsorption capacities reported for other adsorbents, namely, activated carbon, waste sorbents, fungus, brown alga and some commercial cation exchange resins (Table 5.1). The metal removal capacity of anaerobic biomass was higher than that of activated carbon in the granular or powder form. Relating to solid waste and fungus, anaerobic biomass appeared to be more efficient in metal uptake than sugar beet pulp, activated sludge, *Penicillium chrysogenum*, and *Rhizopus arrhizus* fungus. More generally, lead removal capacity of anaerobic biomass was higher than most of the biosorbents. The considerably lower cost of the anaerobic biomass, its physical characteristics and the high uptake capacity of the heavy metals makes it a very attractive biosorbent.

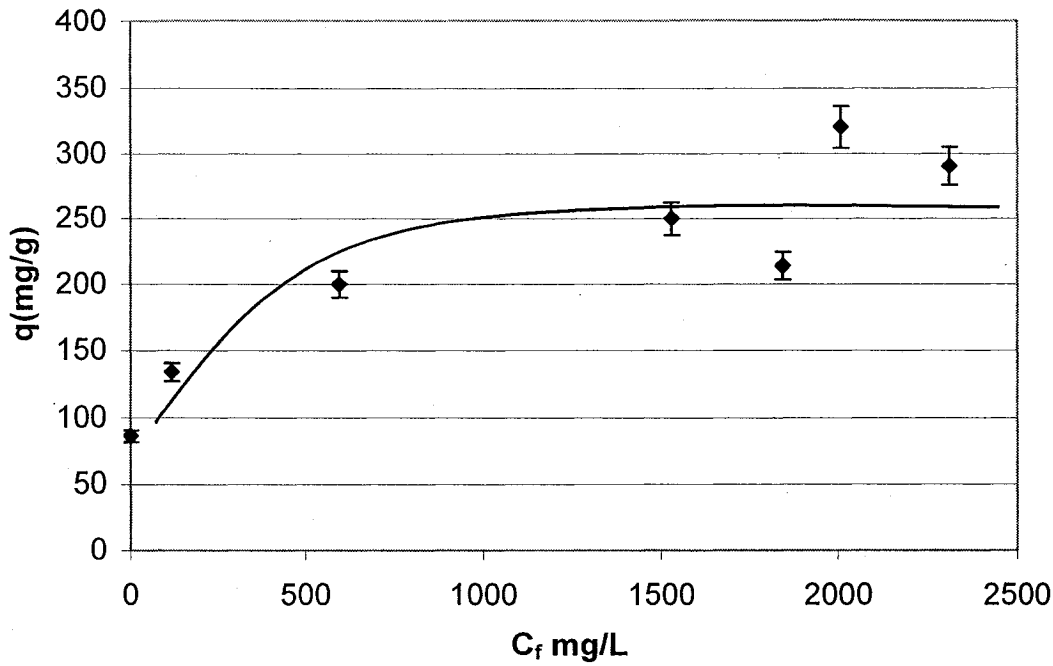

Figure 5.5: Biosorption isotherm for Pb

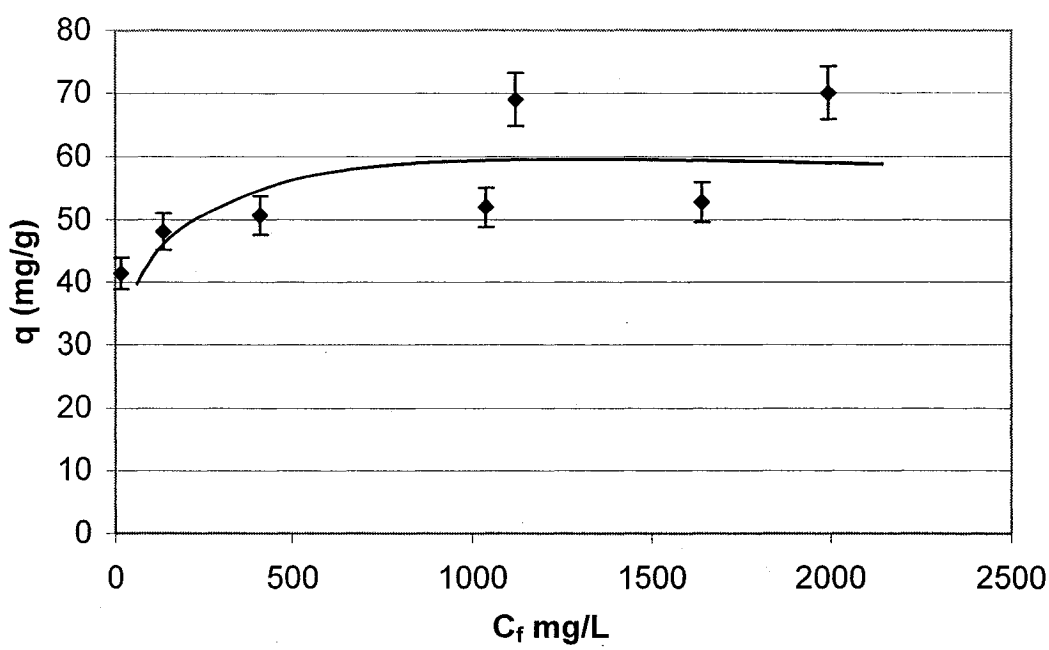

Figure 5.6: Biosorption isotherm for Cd

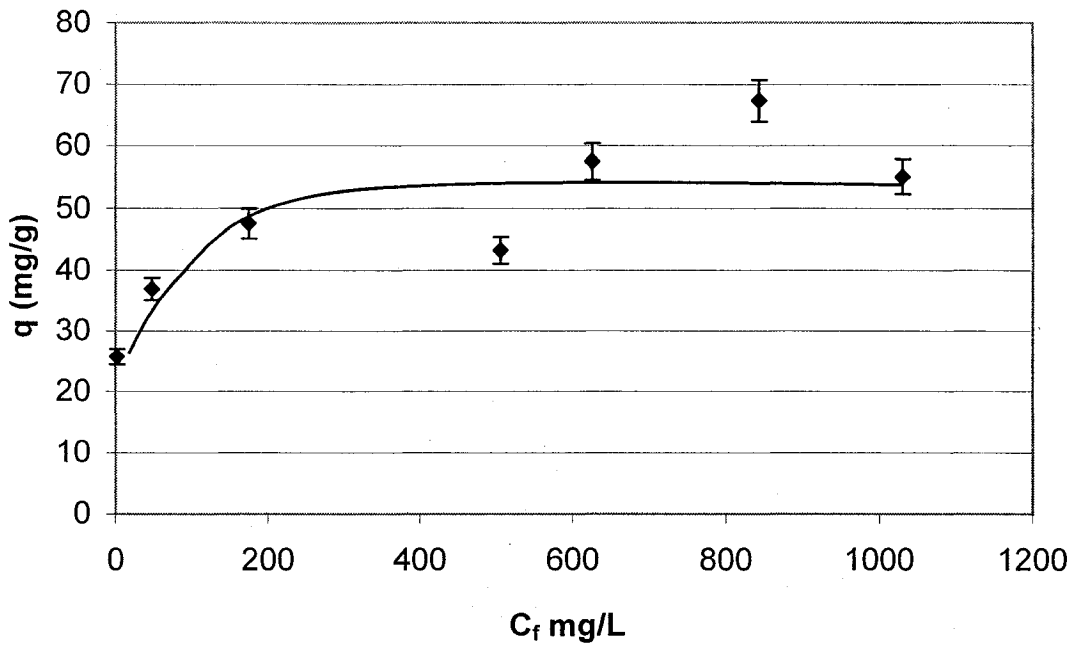

Figure 5.7: Biosorption isotherm for Cu

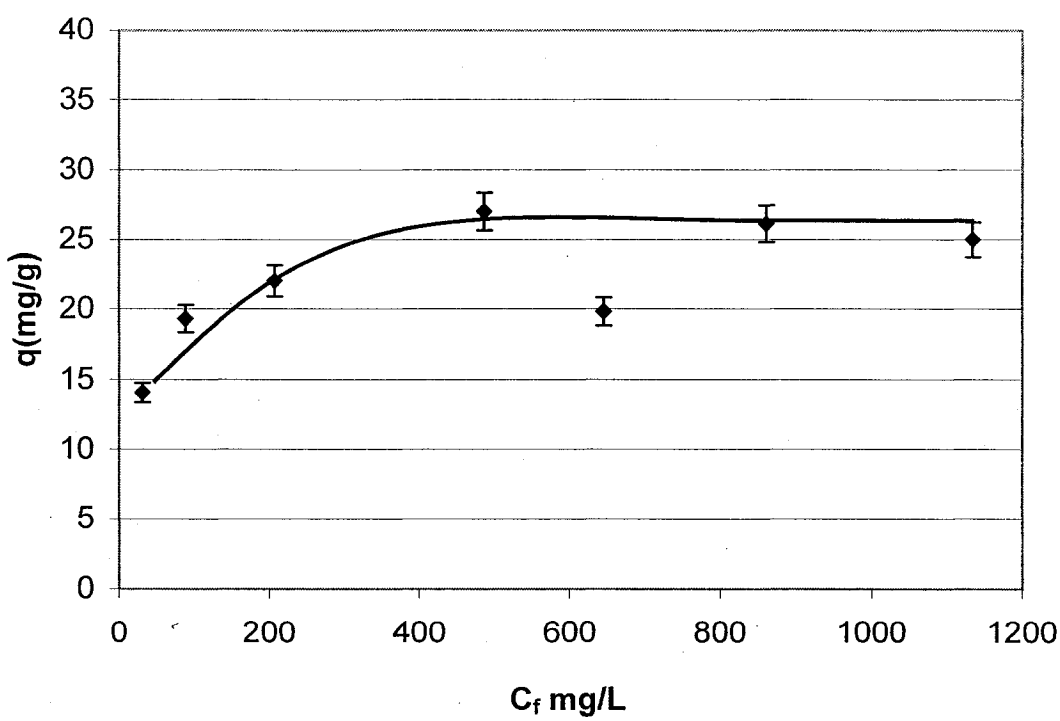

Figure 5.8: Biosorption isotherm for Ni

Table 5.1: Comparison of metal adsorption capacities (mmoles/g) of activated carbon, commercial resins, brown alga, fungus, and selected solid waste.

Adsorbent		**Pb**	**Cu**	**Cd**	**Ni**	**pH**	**Reference**
Activated carbon (AC)	Granular AC	0.08	0.08	0.03		5.0	An et al. (2001)
	Powder AC	0.13	0.07	0.03		5.0	An et al. (2001)
Commercial resins	Duolite GT-73	0.59	0.97	0.94	0.97	4.8	Vaughan et al. (2001)
	Amberlite IRC-718	1.40	2.00	2.30	2.20	4.8	Vaughan et al. (2001)
	Lewatit TP207	0.96	1.34		0.44		Brown et al. (2000)
Brown alga	*Ascophyllum nodosum*	1.31		1.18	0.69	3.5	Thomas et al. (2003)
	Sargassum natans	1.22		1.17	0.41	3.5	Thomas et al. (2003)
Fungus	*Penicillium chrysogenum*	0.59	0.14	0.50			Volesky and Holan (1995)
	Rhizopus arrhizus	0.44	0.16	0.24	0.31		Volesky and Holan (1995)
Waste sorbents	Sugar beet pulp	0.36	0.33	0.22	0.20	5.5	Reddad et al. (2002)
	Activated sludge	0.43	0.77	0.61	0.31	5.5	Hammaini et al. (1999)

5.1.6 Adsorption isotherm models

Several mathematical models have been developed to quantitatively express the relationship between the extent of sorption and the residual solute concentration. The most widely used models are the Freundlich adsorption isotherm model and the Langmuir adsorption isotherm model.

5.1.6.1 Freundlich adsorption isotherm model

The Freundlich isotherm, which is an empirical formulation, is expressed as:

$$q = K\, C_f^{1/n} \qquad (5.1)$$

where,

q : amount of solute adsorbed per unit weight of sorbent, (mg/g)

C_f : solute equilibrium concentration, (mg/L)

K and n: experimental constants, K is an indication of the adsorption capacity of the adsorbent; n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity.

To facilitate the fitting of the model to the experimental data and its parameter evaluation, equation 5.1 can be transformed into an expression of linear form:

$$\mathrm{Ln}\, q = \mathrm{Ln}\, K + (1/n)\mathrm{Ln}\, C_f \qquad (5.2)$$

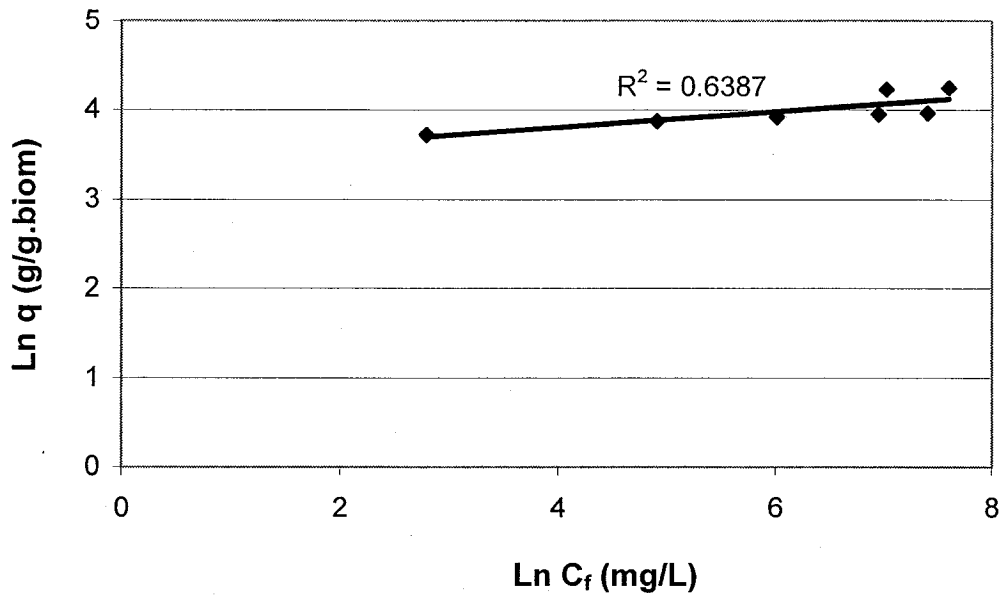

Figure 5.9: Freundlich adsorption isotherm model for Cd

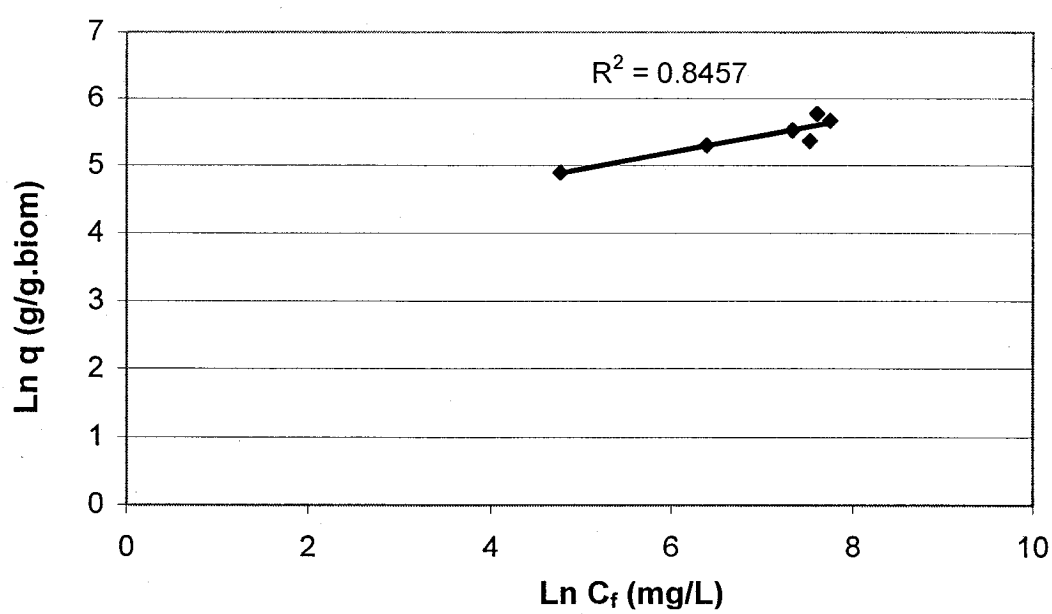

Figure 5.10: Freundlich adsorption isotherm model for Pb

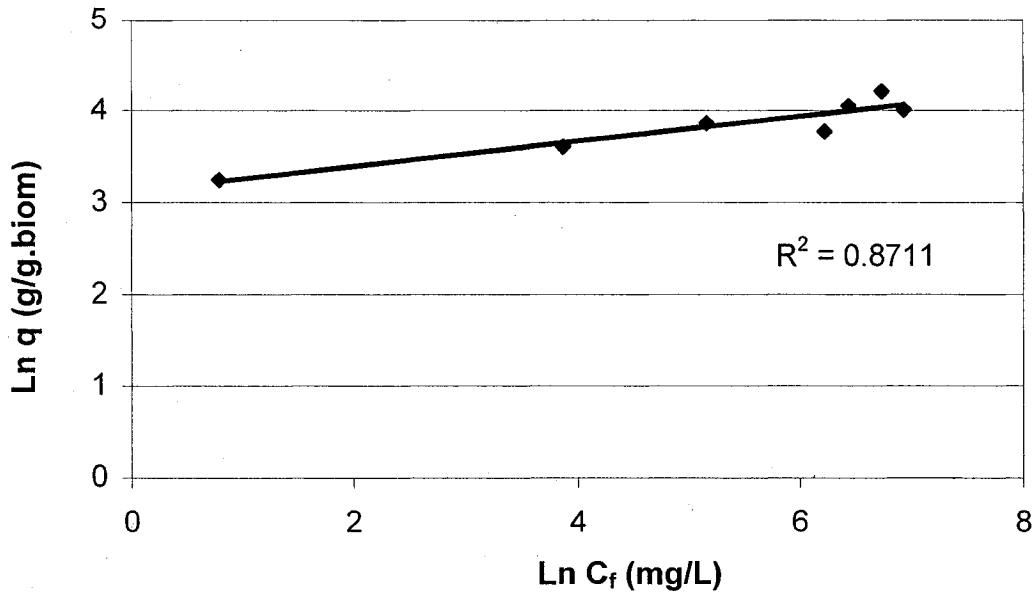

Figure 5.11: Freundlich adsorption isotherm model for Cu

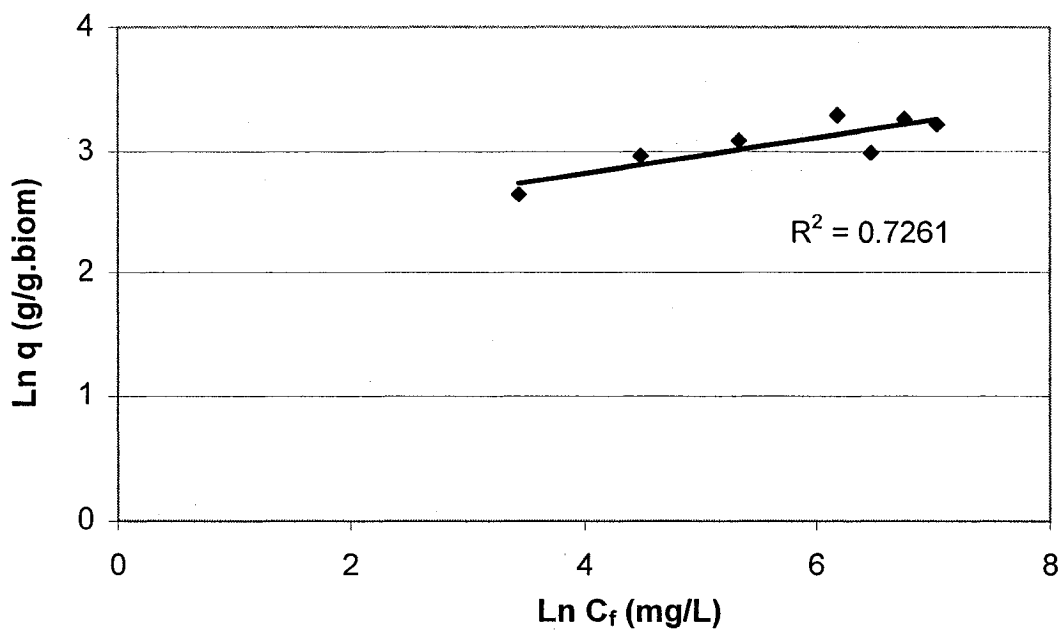

Figure 5.12: Freundlich adsorption isotherm model for Ni

As shown in Figures 5.9 to 5.12 the Freundlich isotherm model did not fit our data very well. The Freundlich model does not indicate a finite uptake capacity of the sorbent, the surface concentration of adsorbate does not approach a saturating value as C increases, and therefore it will frequently represent the adsorption equilibrium over a limited range in solute concentration. As shown in Figures 5.5 to 5.8 the biosorption of Pb^{2+}, Cd^{2+}, Cu^{2+} and Ni^{2+} is eventually limited by the fixed number of uptake active sites on the biomass and a finite uptake capacity is observed which represents the maximum uptake capacity of the biomass for each metal ion.

5.1.6.2 Langmuir adsorption isotherm model

This model provides a simple mechanistic picture of the adsorption process and gives rise to a relatively simple mathematical expression. The Langmuir model assumes that the rate of adsorption is proportional to the concentration of the solute in the fluid phase and to the adsorbent surface. Adsorption may occur only when a molecule of the solute in the fluid phase strikes the surface of the biosorbent. The Langmuir model can be derived as follows: If the fraction of the surface covered by an adsorbed solute i is denoted by θ_i, the fraction that is bare will be $1 - \theta_i$. The concentration of i in the liquid phase is C and the rate of adsorption (r_{ads}) is given by:

$$r_{ads} = K_a C (1 - \theta_i) \qquad (5.3)$$

The rate of desorption depends only on the fraction of the surface coverage (the number of solute molecules that are adsorbed) and can be expressed as

$$r_{des} = K_d \, \theta_i \qquad (5.4)$$

At equilibrium, the rate of adsorption and desorption are equal,

$$K_a \, C \, (1 - \theta_i) = K_d \, \theta_i \qquad (5.5)$$

The fraction of the sorbent surface occupied by species i is then

$$\theta_i = K_a \, C \, / \, (K_d + K_a C) \qquad (5.6)$$

or

$$\theta_i = (K_a/K_d) \, C \, / \, (1 + (K_a/K_d)C) \qquad (5.7)$$

or

$$\theta_i = b \, C \, / \, (1 + bC) \qquad (5.8)$$

where b, is the adsorption equilibrium constant. The fraction of the surface occupied, θ_i, is equal to the ratio of the amount of solute adsorbed to the maximum which would be adsorbed:

$$\theta_i = q \, / q_{max} \qquad (5.9)$$

The last two equations can be combined to give a relationship between the concentration of the solute and the amount of it that is adsorbed:

$$q = q_{max} \, b \, C_f \, / \, (1 + bC_f) \qquad (5.10)$$

where:

q : amount of solute adsorbed per unit weight of sorbent, (mg/g)

C_f : solute equilibrium concentration, (mg/L)

q_{max} : maximum uptake capacity of the sorbent, (mg/g)

b: adsorption equilibrium constant ($k_{adsorption}$/ $k_{desorption}$)

The term q_{max} is supposed to represent a fixed number of surface sites in the sorbent, and it should, therefore, be constant and it is determined solely by the nature of the sorbent. Equation (5.10) shows that at low values of C_f, the term bC_f in the denominator will be relatively small compared to unity, and the solute adsorbed will be linearly dependent on C_f. Under those conditions, equation (5.10) can be reduced to a linear form:

$$q = q_{max} b C_f \qquad (5.11)$$

At high equilibrium concentrations, the term bC_f in the denominator of equation (5.10) will be relatively large compared to unity, and the active sites of the sorbent are nearly saturated. In this case, q will be approaching q_{max}.

To facilitate the fitting of the model to the experimental data and its parameter evaluation, equation 5.10 can be transformed into an expression of linear form:

$$C_f / q = 1/ bq_{max} + C_f / q_{max} \qquad (5.12)$$

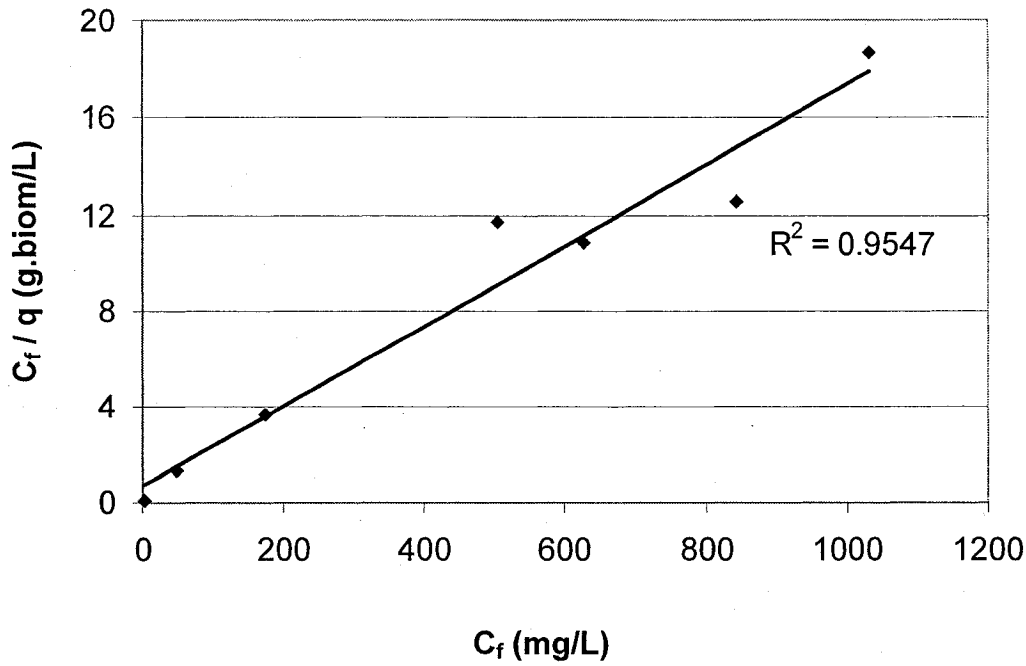

Figure 5.13: Langmuir adsorption isotherm for Cu

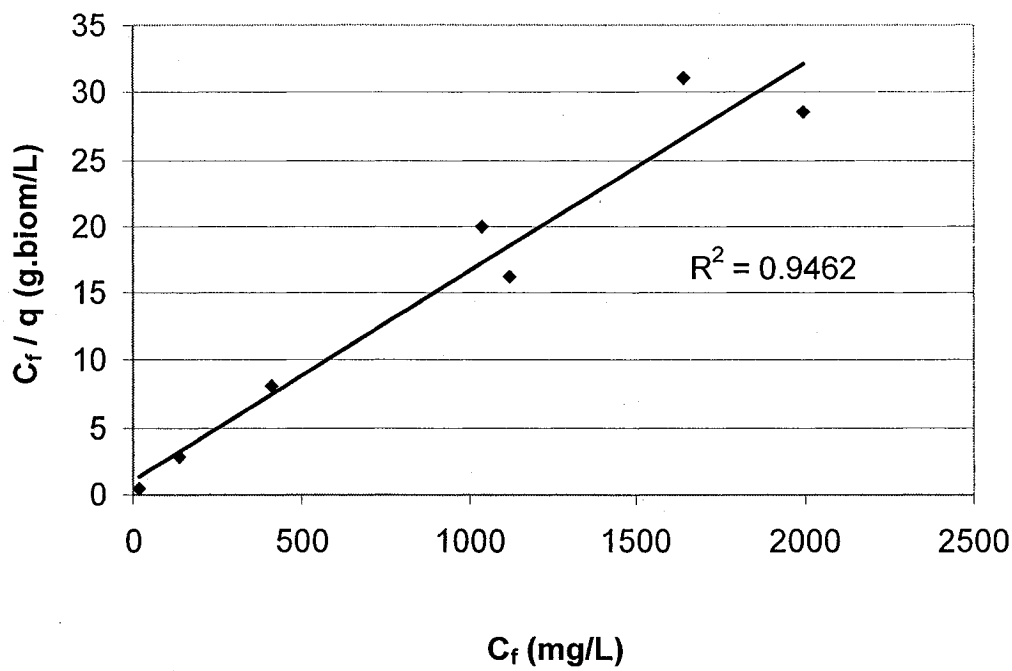

Figure 5.14: Langmuir adsorption isotherm for Cd

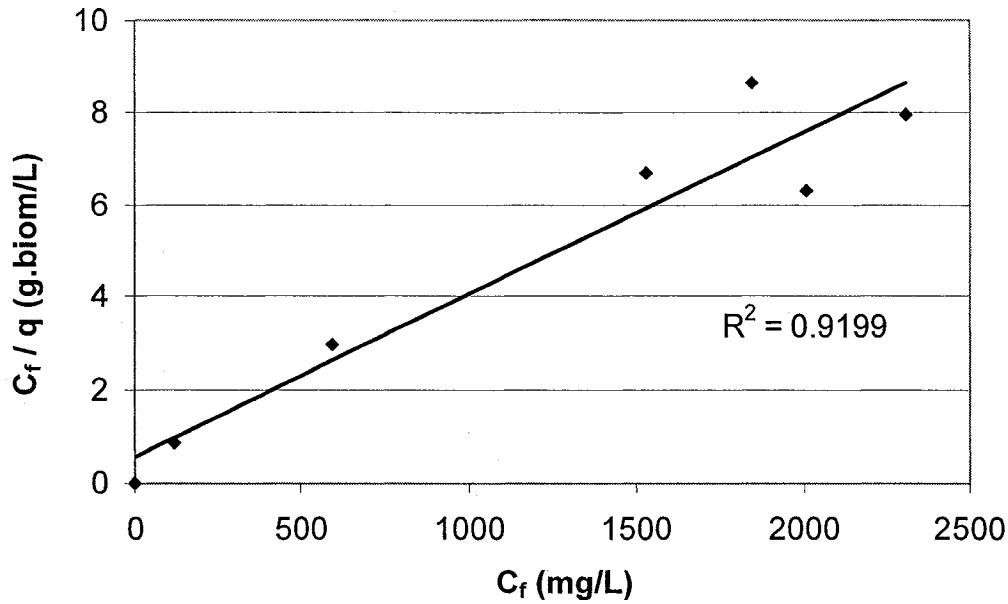

Figure 5.15: Langmuir adsorption isotherm for Pb

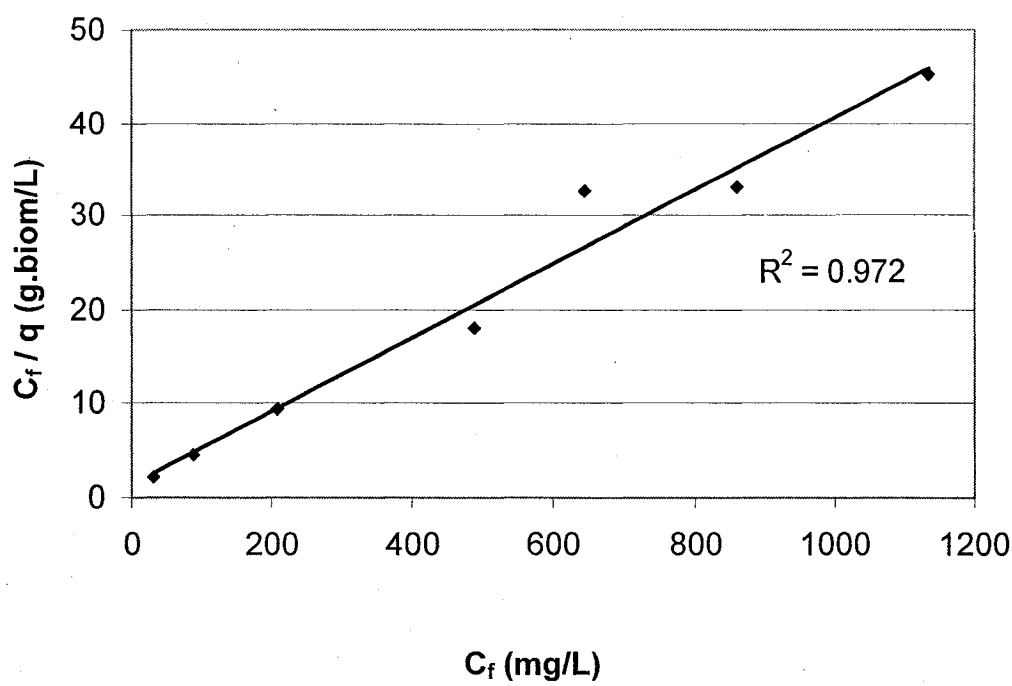

Figure 5.16: Langmuir adsorption isotherm for Ni

As shown in Figures 5.13 to 5.16, it was found that the data pertaining to the sorption dependence upon metal ion concentration fitted the Langmuir isotherm. Experimental C_f and q data were used to evaluate the Langmuir constants q_{max} and b for all metals tested. Results are summarized in Table 5.2.

Table 5.2: Langmuir biosorption isotherm model parameters and experimental q_{max}

Metal	**q_{max} (mg/g) Langmuir model**	**q_{max} (mg/g) Experimental**	**b (L/g)**	**R^2**
Pb	286	255	0.0063	0.92
Cd	64	60	0.0134	0.95
Cu	60	55	0.024	0.95
Ni	25	26	0.03	0.97

The Langmuir model has been used successfully to describe equilibrium biosorption (Utgikar et al., 2000; Say et al., 2001; Yin et al., 1999; Yan and Viraraghavan, 2003; Kaewsarn, 2002; Ozer and Ozer, 2003; Veglio et al., 2003; Matheickal and Yu, 1999; An et al., 2001). The Langmuir model was originally derived for the adsorption of gas on to activated carbon with the assumption of (1) there is a limited area available for adsorption, (2) the adsorbed solute material on the surface is only one molecule in thickness, and (3) the adsorption is reversible and an equilibrium condition is achieved (Reynolds and Richards, 1992). Its two parameters reflect the maximum gas uptake and the affinity of the gas component on the sorbent. As it was shown in section 5.1.5 for the biosorption of heavy metals, initially the uptake increases in a linear form with

rising equilibrium concentration. Uptake is eventually limited by the fixed number of active sites and a resulting plateau can be observed. This phenomenon is well depicted by Langmuir isotherms. The maximum metal uptake may well be described by Langmuir parameter, q_{max}, as shown in Table 5.2. In this study the Langmuir model was applied because it provides information on uptake capabilities and it is capable of reflecting the equilibrium sorption process behaviour. The values obtained from the Langmuir model (q_{max} and b) can provide information in the screening of the sorbent. The more favourable sorbent is indicated by the higher value of the slope of an adsorption isotherm. It means that potentially "good" sorbent can be comparatively evaluated from values of q_{max} and b. However, the Langmuir model sheds no light on the mechanistic aspects of sorption. The nature of the binding processes in biosorption is largely unknown. Several different mechanisms have been proposed to explain the uptake of metals by non-living microbial biomass, including microprecipitation, ion-exchange, and complexation/chelation. Further investigations was carried out in the following sections to evaluate what are the mechanisms involved in the uptake process of each metal ion studied by the anaerobic biomass.

5.1.7 Sorption kinetics

The form of the rate equation of the sorption process can be defined either based on the unit volume of the solution or unit mass of the sorbent:

$$r = (1/V)dM_i/dt = dC_i/dt \qquad (5.13)$$

or

$$r = (1/m)\, dM_i/dt = dq/dt \qquad (5.14)$$

Where:

r :rate of sorption (meq/g.min)

M_i : amount of solute i adsorbed (mg)

C_i: concentration of solute I (mg/L)

V: unit volume of the solution (mL)

t: time (min)

m: unit mass of the sorbent (g)

q: metal uptake by the sorbent (mg/g)

The kinetics of sorption of Pb^{2+}, Cu^{2+}, Ni^{2+} and Cd^{2+} were modelled using a pseudo-second order rate equation developed by Ho and MacKay (2000). Initially, it must be assumed that the sorption follows the Langmuir model, which agrees with chemisorption as being the rate controlling mechanism and this has been confirmed previously. The rate of the pseudo-second order reaction may be dependent on the amount of metal ion on the surface of the biosorbent and the amount of metal ion sorbed at equilibrium. The kinetic rate equation is:

$$dq_t/dt = k(q_e - q_t)^2 \qquad (5.15)$$

where:

k: rate constant of sorption (g/mg.min)

q_e: amount of metal ion sorbed at equilibrium (mg/g)

q_t: amount of metal ion sorbed at time t (mg/g)

Equation 5.15 can be rearranged to obtain:

$$t/q_t = (1/kq_e^2) + (1/q_e)t \qquad (5.16)$$

if the initial sorption rate is:

$$v_o = kq_e^2 \qquad (5.17)$$

then equation (5.16) becomes:

$$t/q_t = (1/v_o) + (1/q_e)t \qquad (5.18)$$

The constants can be determined experimentally by plotting t/q_t versus t. The data obtained from section 5.1.4 were used to apply the proposed model. The adsorption rate tests were performed on an equilibrium batch basis. 0.5 g of the Ca-biomass was contacted with 50 mL of metal bearing solutions of 100 mg/L concentration. The biomass was kept in contact with the metal-bearing solution for different time periods (15, 30, 45 and 60 minutes). Figure 5.17 shows lead, copper, nickel and cadmium data plotted (t/q_t versus t) so that the v_o values in equation (5.18) can be determined. These values are given in Table 5.3. As shown in Figure 5.17 and Table 5.3, the regression coefficients for the linear plots are very good. Also it can be seen that the initial rate of sorption (v_o) values ranged from 0.026 meq/g.min for Cd^{2+} to 0.5 meq/g.min for Ni^{2+} ions. From a comparison of the initial sorption rates between the metal ions studied, the following tendency was observed:

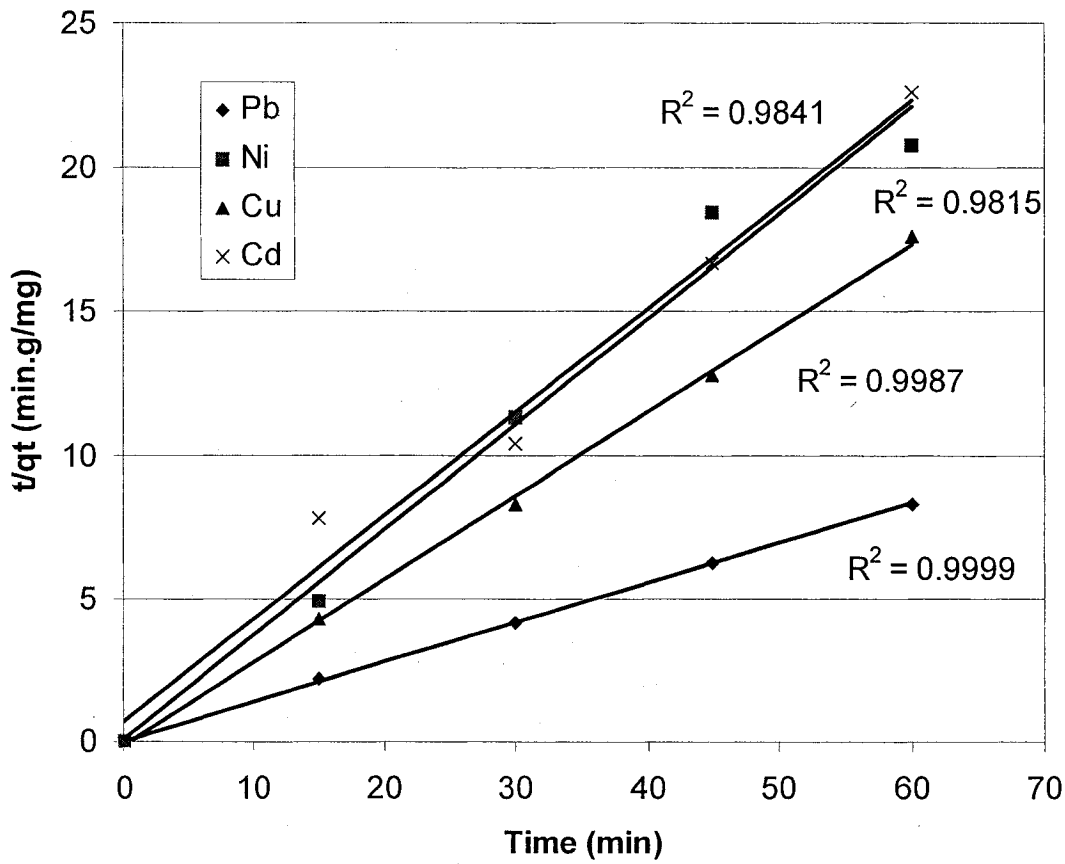

Figure 5.17: Second order model for the sorption kinetics of Pb, Cu, Cd, and Ni

Table 5.3: Lead, copper, cadmium and nickel initial sorption rate (v_o) derived from the second order kinetic model

Metal	**v_o (meq/g.min)**	**R^2**
Pb	0.28	0.9999
Cu	0.20	0.9987
Cd	0.026	0.9815
Ni	0.50	0.9841

$$Ni > Pb > Cu > Cd$$

By comparing the amount of metal sorbed at q_{max} at equilibrium obtained from the isotherm tests a different order was obtained:

$$Pb > Cu > Cd > Ni$$

With Ni^{++} ions being the least to adsorb. Hence, despite Ni^{++} ions being more weakly adsorbed than other metals, their removal showed the fastest kinetics.

5.2 Column Tests

5.2.1 Biosorption in a continuous system

Most separation and purification processes that use sorption technology in industry employ continuous flow columns. During this process, the influent is continuously percolated through a sorbent filled column with the undesirable species being retained. The column sorbent gradually becomes saturated whereby this process begins at the feed zone and gradually progresses to the exit. When the sorbate concentration in the effluent stream reaches a pre-defined level, column operation is terminated. At this point the regeneration process may begin before activation of the next cycle operation.

The breakthrough curve, which represents the history of effluent concentration as a function of time, is characteristic for any given continuous flow column system. The breakthrough time represents the duration of ongoing sorption until a pre-defined exit threshold concentration is reached. Three typical types of breakthrough curves may be observed. They either display:

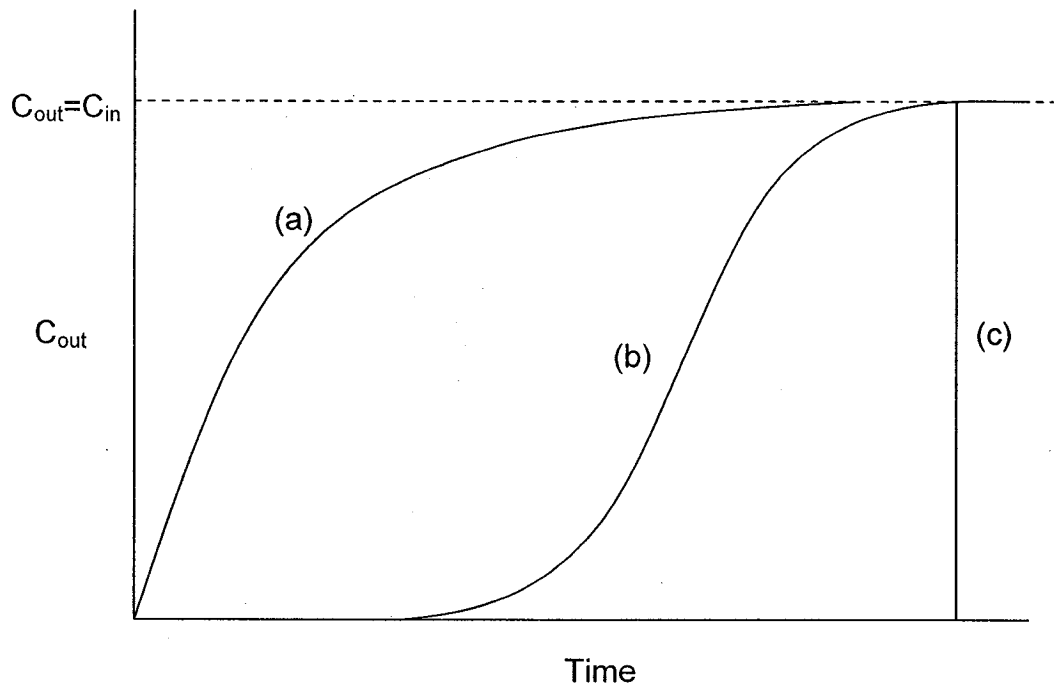

Figure 5.18: Schematic representation of breakthrough curves.
(a) Poor sorption (b) normal sorption (c) and strong sorption

(a) poor sorption, (b) normal sorption or (c) strong sorption, whereby no mass transfer zone occurs in the latter. These breakthrough curves are illustrated schematically in Figure 5.18.

5.2.1.1 Heavy metals biosorption breakthrough curves

For the operation of a Cu, Ni, Cd, and Pb biosorption column, an influent, pH 4.0 and an initial concentration of 40 ppm of heavy metals, with an upward flow rate of 1.5mL/min was used. The volume of the bed was 45 mL with an average residence time of approximately 30 minutes. The column was loaded with 11g (dry weight) of anaerobic biomass. Figures 5.21 to 5.24 illustrate the

resulting breakthrough curves for Cu, Ni, Cd, and Pb respectively. The heavy metals concentration values at the column exit are plotted as a function of a dimensionless volume V/V_o (with reference to the bed volume). Comparing Figures 5.21 to 5.24 with Figure 5.18, these heavy metal breakthrough curves represent a "normal sorption" curve with a commonly observed S shape (Matheickal and Yu, 1999; Kogej and Pavko, 2001; Mahan and Holcombe, 1992; Yan and Viraraghavan, 2001). It can be seen from Figures 5.21 through 5.24 that there was a period of time where the heavy metal concentration in the effluent remained zero until a certain period of time then the concentration of metal started to increase gradually. This is due to the formation of the mass transfer zone in the column. Once the solution containing the heavy metal becomes exposed to the fresh layer of the biomass the metal ions are adsorbed onto the biomass until the amount adsorbed is in equilibrium with the influent concentration. At this time the biomass is loaded to capacity and that portion of the biomass becomes exhausted. Above this line which is progressing in the direction of the flow, adsorption is happening where the metal ion is being actively transferred from the liquid onto the biomass. The mass transfer zone will move up through the column until it reaches the effluent port, whereupon the heavy metal concentration in the effluent begins to rise. In this process arrangement, metal-bearing solution percolates through the bed of active biomass which would act like a series of batch contactors. Consequently, the biomass would be loaded up to its maximum capacity yielding the q_{max} value.

Figure 5.19 shows the progress of the mass transfer zone as it gradually moves through the column bed.

For the case of lead, the breakthrough took place after 978 bed volumes at that point the exit lead concentration was 1.5 ppm, which is the maximum concentration allowed to be discharged into the surface water according to the federal guidelines (Table 2). Approximately 44 L of 40 ppm lead influent were processed before breakthrough. To reach the breakthrough point it took almost 21 days assuming a 30 minute detention time in the column. The total lead removed by 11.0g (dry) of biomass was 1760 mg (17.0 meq), yielding an average lead biosorption capacity of 160 mg/g (1.55 meq/g). The ultimate capacity of the biomass was calculated using the following equation:

$$\text{Biomass capacity} = C_oV_f - C_o\int(C_f/C_o)dv \qquad (5.19)$$

$C_o\int(C_f/C_o)dv = C_o$ (Area under the breakthrough curve)

The area under the breakthrough curve was calculated by the trapezoidal rule, where the area underneath the breakthrough curve was divided into a certain number of trapezoids. The area of each trapezoid was calculated and all areas were added up to give the total area underneath the curve.

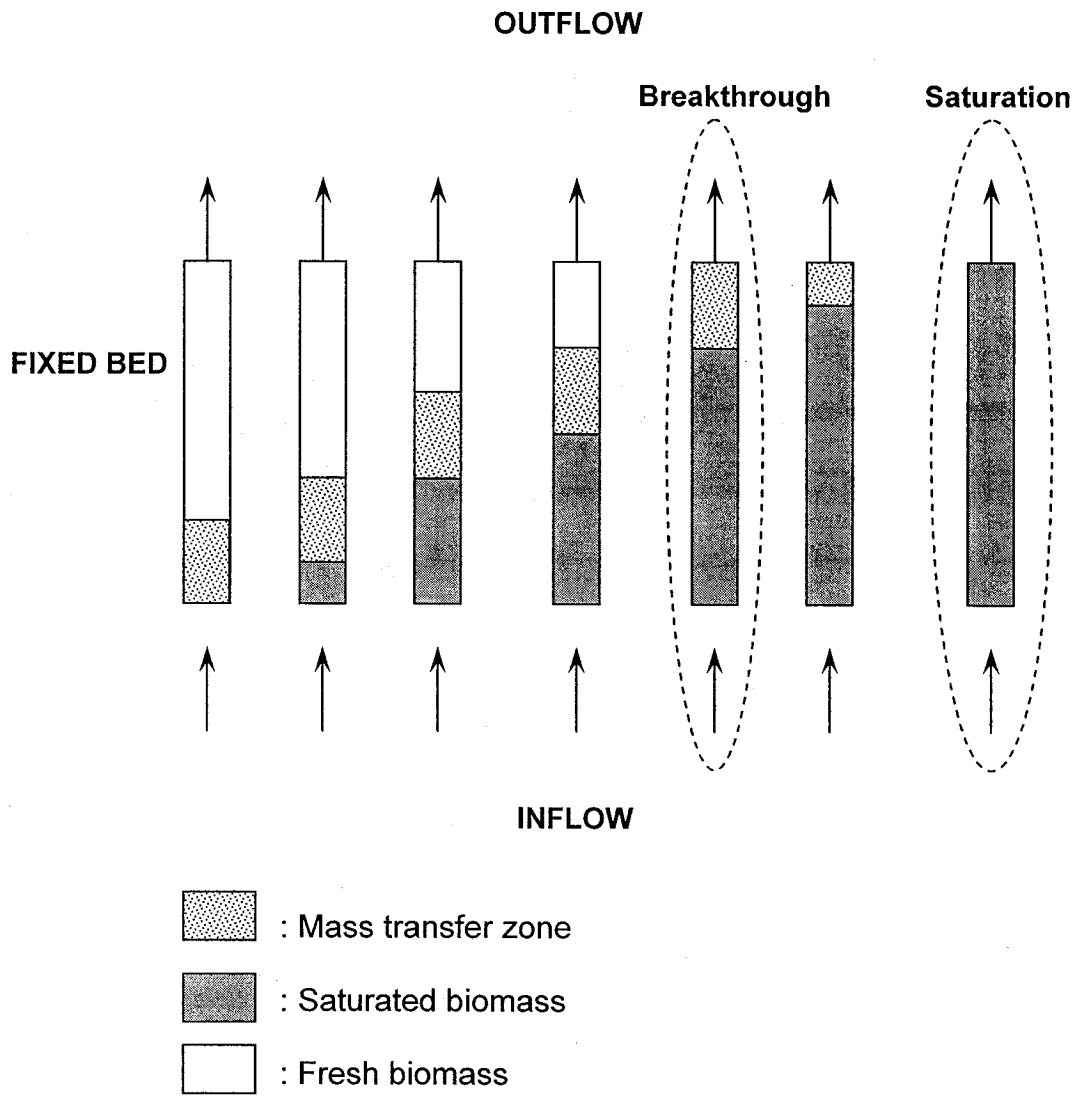

Figure 5.19: The movement of the mass transfer zone through a fixed bed column

Normally when the allowable discharge concentration is reached in the effluent of the bed, the bed should be stopped and the regeneration process could then be started. In our case the column was kept running until the biomass

was totally saturated (concentration in the effluent = concentration in the influent). This was done in order to study the shape of the breakthrough curves and to calculate the maximum uptake capacity of the bed. Also it is not possible to design a column accurately without the whole breakthrough curve. Total saturation of the column with lead took place after 1482 bed volumes. At that point the exit lead concentration was 40 ppm which is equal to the influent concentration. Approximately 67 L of 40 ppm lead influent was processed for total saturation of the biomass. Using equation 5.19, it was found that the total lead removed by the biomass to reach saturation point was 2109 mg (20.4 meq), yielding an average lead biosorption capacity of 191.8 mg/g (1.85 meq/g). At the point of complete exhaustion, the entire biosorption column is in equilibrium with the influent and effluent flows. Therefore, the uptake capacity of the biomass in the column test at the saturation point should be comparable with the maximum uptake capacity of the biomass obtained from the batch tests (q_{max}). Comparing these two values it could be seen that for the case of lead the uptake capacity by the biomass in the column test was almost 26% less than that obtained from the batch tests. From equation 5.19, the area under the breakthrough curve represents the unused part of the column which was calculated to be almost 17% of the total column. This unused part of the column was responsible for the decrease in the uptake capacity of the biomass in the column test. The corresponding unused part of the biosorption column is a consequence of the formation of channels and zones of unexposed biomass within the bed column. The saturation capacity, operating capacity, and breakthrough and saturation

volume for the three other metals studied are summarized in Tables 5.5, 5.6, and 5.7.

The overall performance of flow-through columns is very much related to the length and shape of the ion exchange zone which develops in the column during sorption and regeneration as shown in Figure 5.20. As the loading or regeneration of the biosorbent progresses, the zone moves along the column in the direction of the liquid flow. The length of the sorption zone, Z_S, was defined by Ruthven (1984) and it is related to the column height, Z, and the breakthrough time, T_B, and the total saturation time, T_S.

$$Z_S = Z\,[1 - (T_B/T_S)] \qquad (5.20)$$

The time of ion-exchange zone was represented as $T_Z = T_S - T_B$

Clearly, the shorter the zone, the longer the service time of the column during the loading stage. Correspondingly, also the more complete the desorption of metals from biosorbents during the regeneration stage. The length of the mass transfer zone for all four metals and other design parameters are summarized in Tables 5.4 to 5.7.

For the case of metal ions sorbing onto Ca-saturated biomass, two different scenarios of column performance depending on the respective affinities of the metal ions and Ca towards the sorbent material could occur (Figure 5.20). If the metal ion is more strongly bound to the biomass than the Ca cation, i.e. the affinity of metal species>Ca^{2+}, then the zone which develops in a column is short and retains its shape as it moves down the column. However, if the affinity of

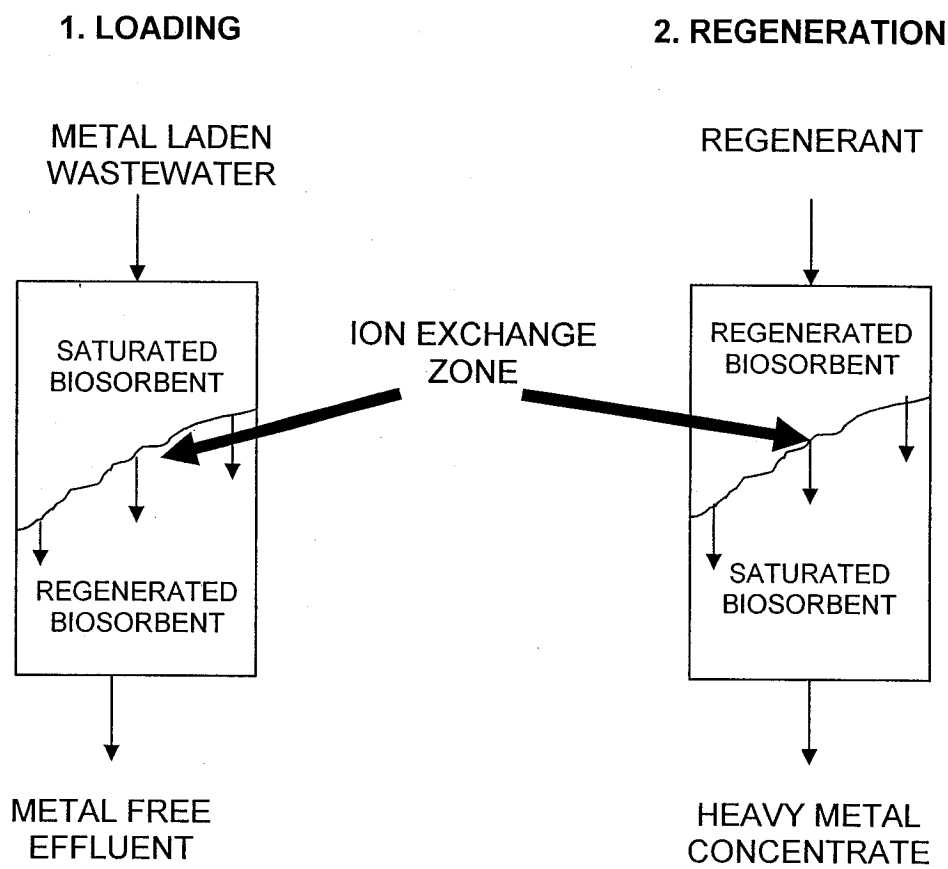

Figure 5.20: Development of ion exchange zones in flow-through columns

Ca^{2+} cations is greater than that of the metal species, the zone spans across a large section of the column, and is prone to further broadening as the operation progresses. This would result in a poor sorption breakthrough curve as shown in Figure 5.18. However, breakthrough curves of the studied metals represented a "normal sorption" curve with a commonly observed S shape, Figures 5.21 through 5.24. It could be concluded from the shape of the breakthrough curves that the affinity of the metal ions studied towards the anaerobic biomass is higher

than the affinity of Ca cations. The length of the ion-exchange zone for lead, cadmium, copper, and nickel was found to be 2.7, 2.9, 3.7, and 3.6 cm respectively. From these results it could be also concluded that lead and cadmium are more likely to desorb Ca cations more than copper and nickel due to the shorter ion-exchange zone of Pb and Cd.

A high degree of biomass utilization or regeneration is achieved only if the metal ion sorbing onto the biomass has a higher affinity than the species originally present in the sorbent. Therefore, the selection of the ionic form of the biosorbent for the loading stage, should assure that it would follow the pattern of strongly binding A replacing weakly bound B. It could be seen from the design parameters that the Ca-biomass followed this pattern. This could be another reason why the biomass was treated with Ca ions in the first place. In future sections the data obtained from packed bed experiments will be used for the scale-up of the sorption process.

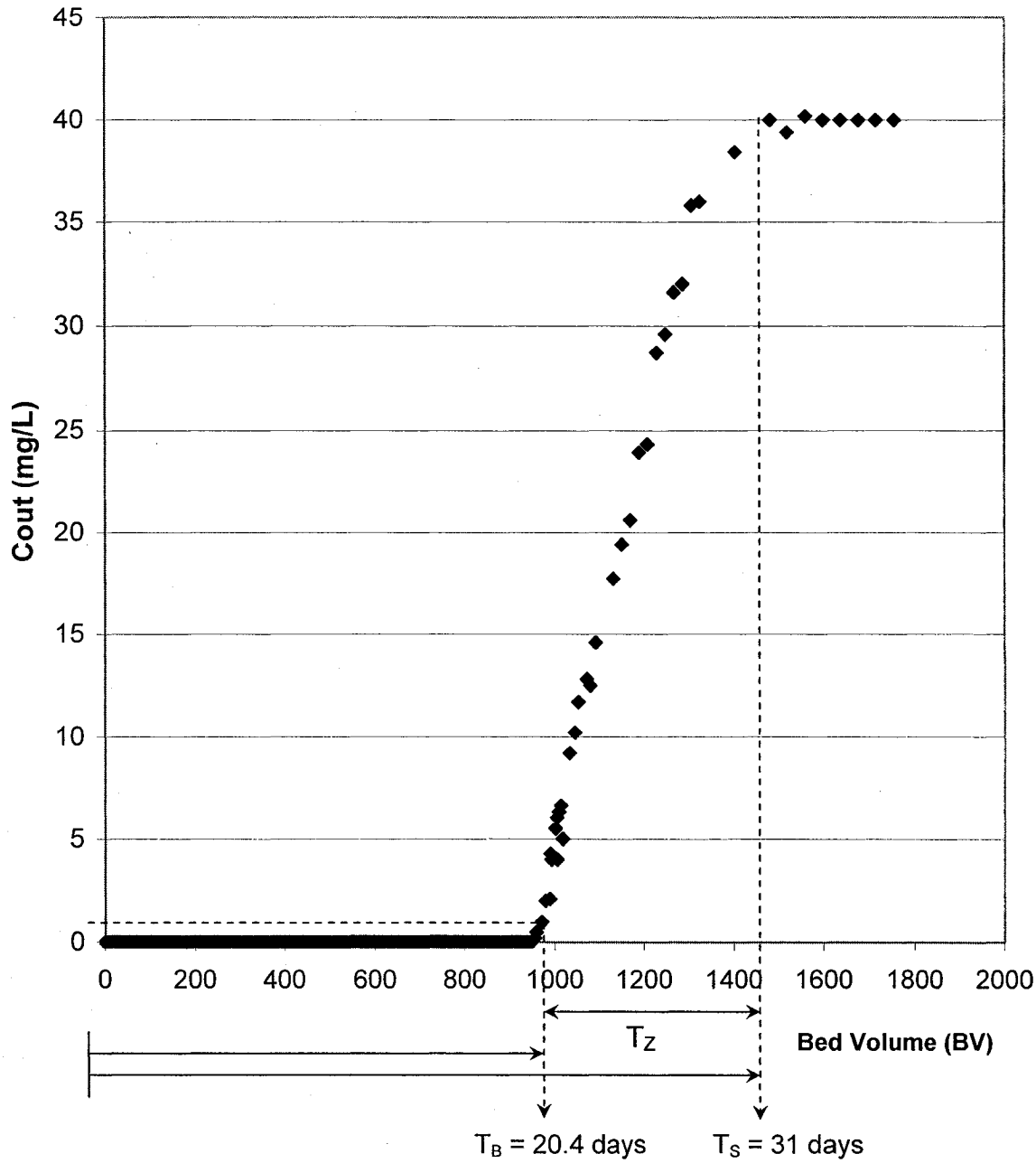

Figure 5.21: Lead biosorption breakthrough curve showing the breakthrough time (T_B), the total saturation time (T_s) and the time of mass-transfer zone (T_Z)

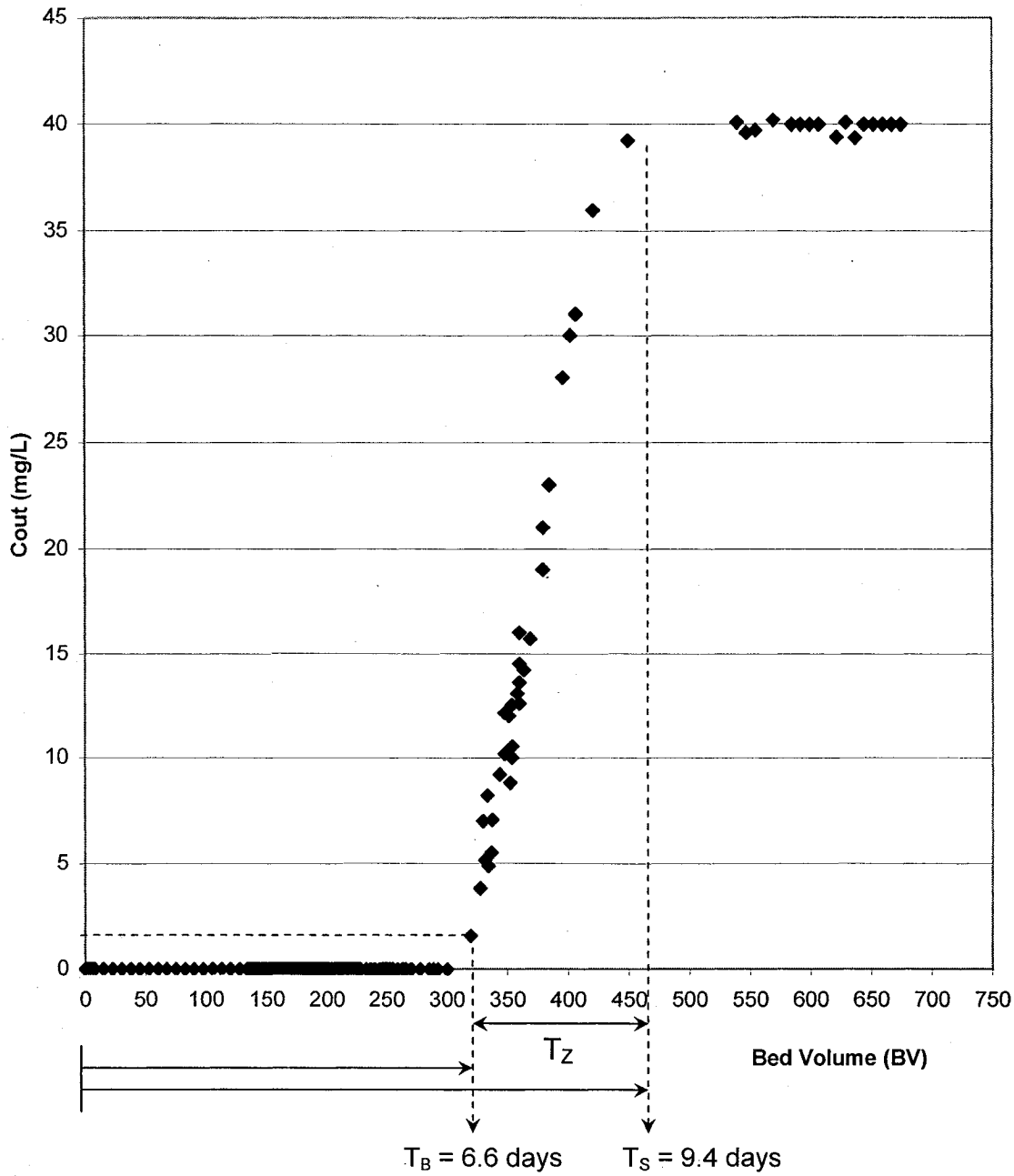

Figure 5.22: Cadmium biosorption breakthrough curve showing the breakthrough time (T_B), the total saturation time (T_s) and the time of mass-transfer zone (T_Z)

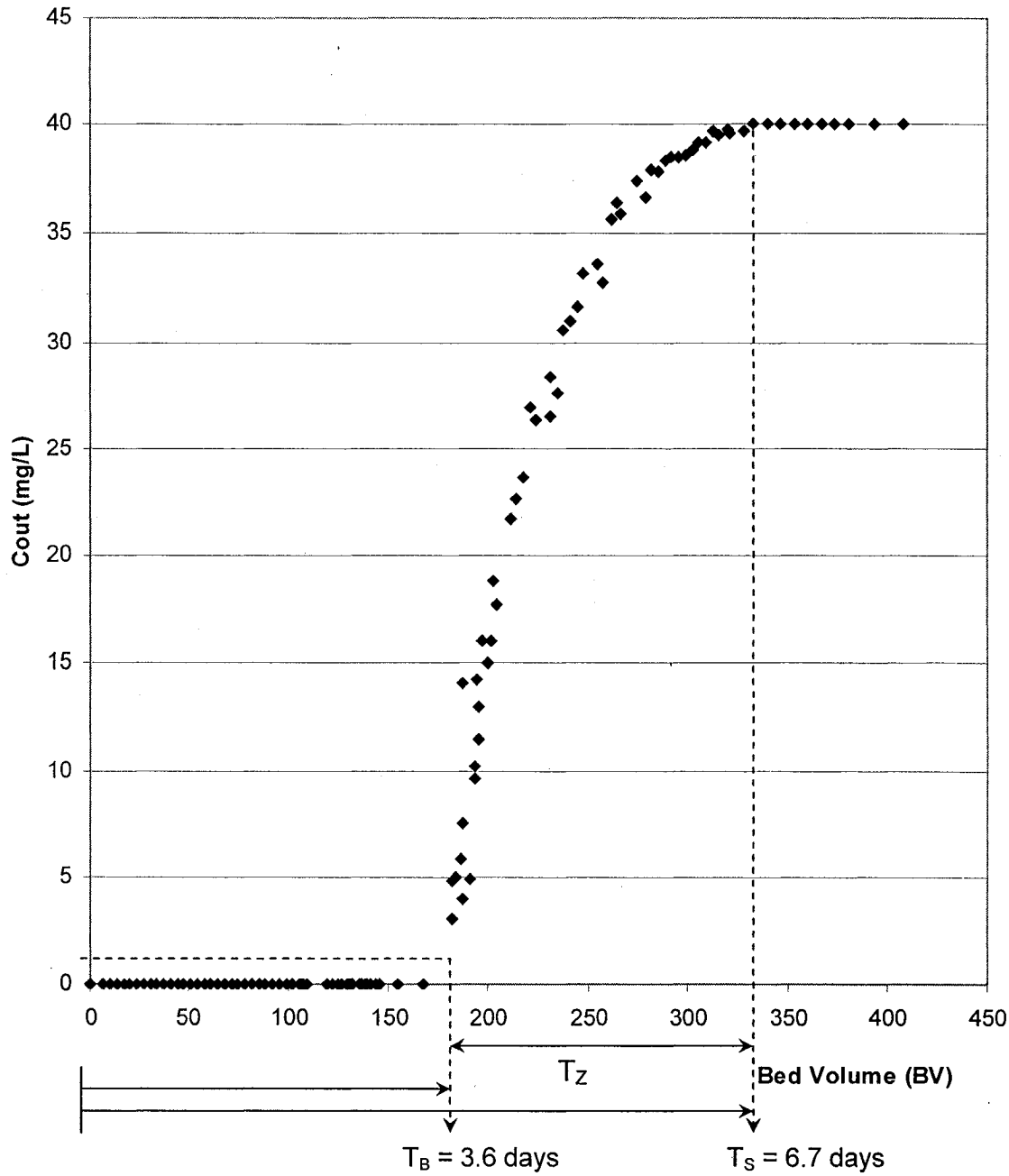

Figure 5.23: Copper biosorption breakthrough curve showing the breakthrough time (T_B), the total saturation time (T_s) and the time of mass-transfer zone (T_Z)

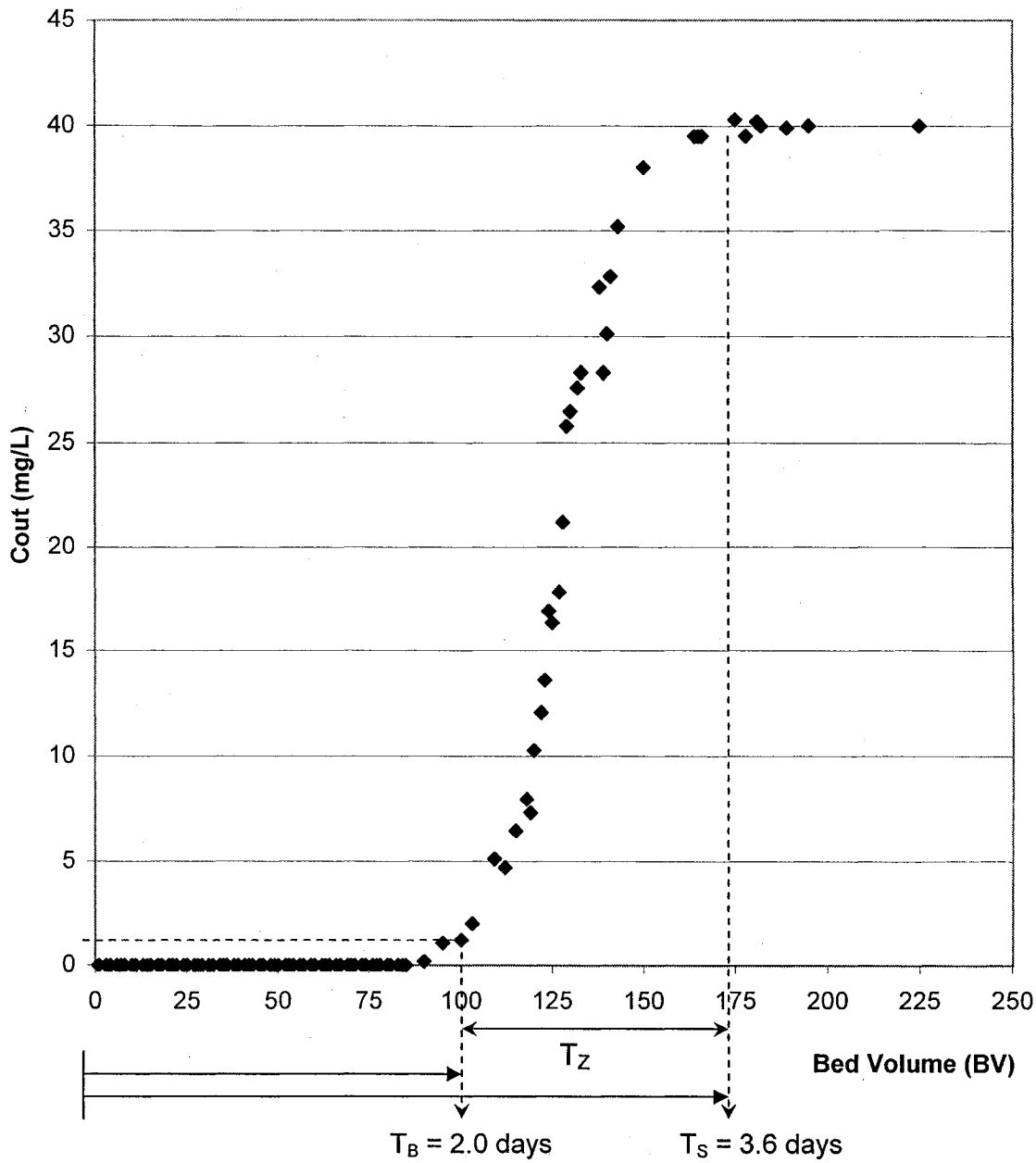

Figure 5.24: Nickel biosorption breakthrough curve showing the breakthrough time (T_B), the total saturation time (T_s) and the time of mass-transfer zone (T_Z)

Table 5.4: Design parameters for lead obtained from column breakthrough curve

Parameter	Value
Breakthrough Volume (V_B) (L)	44
Saturation Volume (V_S) (L)	67
Ion-exchange Zone Volume (V_Z) (L)	23
Ion-exchange Zone length (cm)	2.7
Operating Capacity (mg/g)	160
Ultimate Capacity (mg/g)	192
Fraction of the capacity used (%)	83

Table 5.5: Design parameters for cadmium obtained from column breakthrough curve

Parameter	Value
Breakthrough Volume (V_B) (L)	6.6
Throughput Volume (V_T) (L)	9.4
Ion-exchange Zone Volume (V_Z) (L)	2.8
Ion-exchange Zone length (cm)	2.9
Operating Capacity (mg/g)	50
Ultimate Capacity (mg/g)	61
Fraction of the capacity used (%)	82

Table 5.6: Design parameters for copper obtained from column breakthrough curve

Parameter	Value
Breakthrough Volume (V_B) (L)	3.6
Throughput Volume (V_T) (L)	6.7
Ion-exchange Zone Volume (V_Z) (L)	3.1
Ion-exchange Zone length (cm)	3.7
Operating Capacity (mg/g)	28
Ultimate Capacity (mg/g)	40
Fraction of the capacity used (%)	70

Table 5.7: Design parameters for nickel obtained from column breakthrough curve

Parameter	Value
Breakthrough Volume (V_B) (L)	2.0
Throughput Volume (V_T) (L)	3.6
Ion-exchange Zone Volume (V_Z) (L)	1.6
Ion-exchange Zone length (cm)	3.6
Operating Capacity (mg/g)	15
Ultimate Capacity (mg/g)	21
Fraction of the capacity used (%)	71

5.2.1.2 pH in column bed

As mentioned in section 5.1.2, like synthetic ion-exchange resins, biosorbents can be prepared in different ionic forms such as the protonated (H-form) or saturated with Ca, Mg, Na, etc., by washing the biomass with mineral salt solutions. The type of counter-ion, i.e. the ionic species released from the biomass in exchange for the heavy metal, is determined by the type of chemical used for washing the biomass prior to the sorption of the heavy metal. One disadvantage of using the H-biomass is that biosorption by protonated biomass would be accompanied by release of protons. During continuous flow column operation, this is reflected in the lowering of the solution pH throughout the column. This lower solution pH value in turn would reduce the biosorption capacity significantly as was discussed previously in section 5.1.3.

Since pH control inside the column is difficult, calcium biomass was used to stabilize the pH fluctuation. As shown in Figures 5.25 to 5.28, while the feed pH value was 4.0 for the four metals studied, the exit solution pH increased to a value around pH 5.0 to 5.5 for all of the experiments before the breakthrough point. At this pH value all metal ions studied in the column would be in the soluble state. Table 5.8 shows the precipitation pH values (pH_{prec}) of all metal salts used.

Table 5.8: pH precipitation for different metal salts

Metal Salt	Precipitation pH value (pH_{prec})
$PbCl_2$	6.0
$CuCl_2$	7.0
$CdCl_2$	9.0
$NiCl_2$	8.5

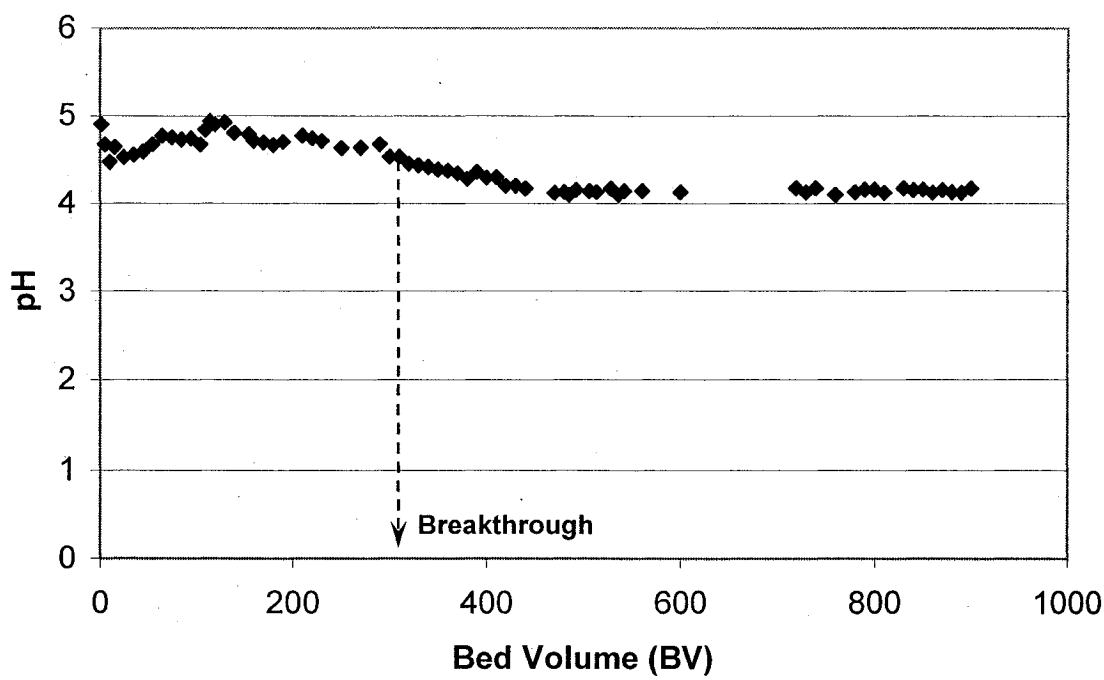

Figure 5.25: pH profile for Cd biosorption in the fixed column

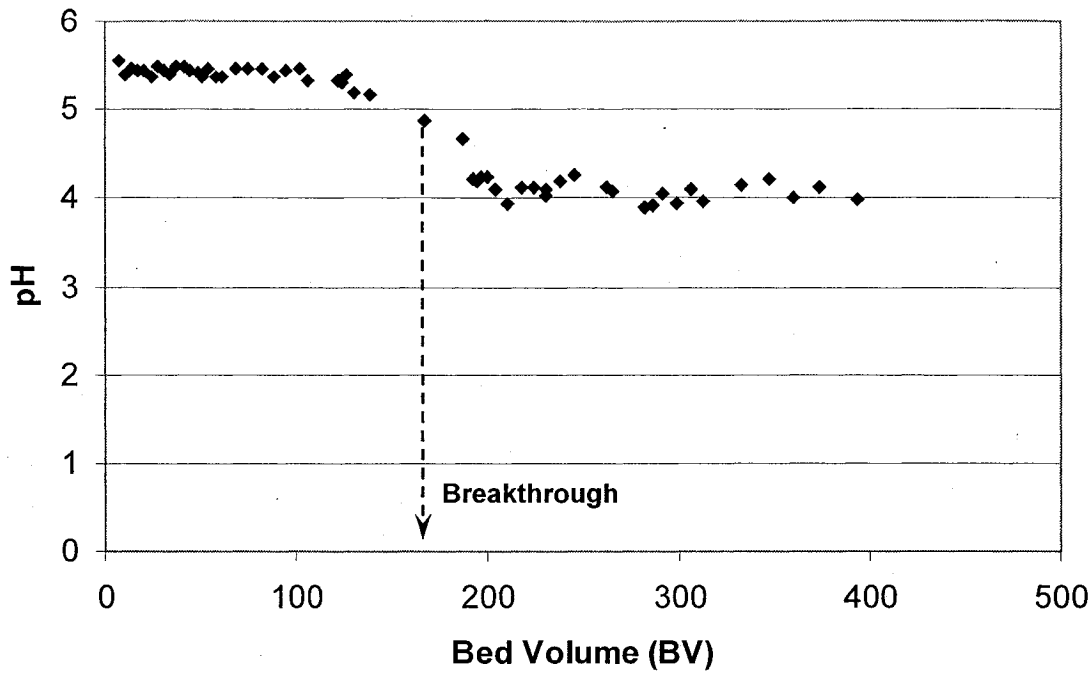

Figure 5.26: pH profile for Cu biosorption in the fixed column

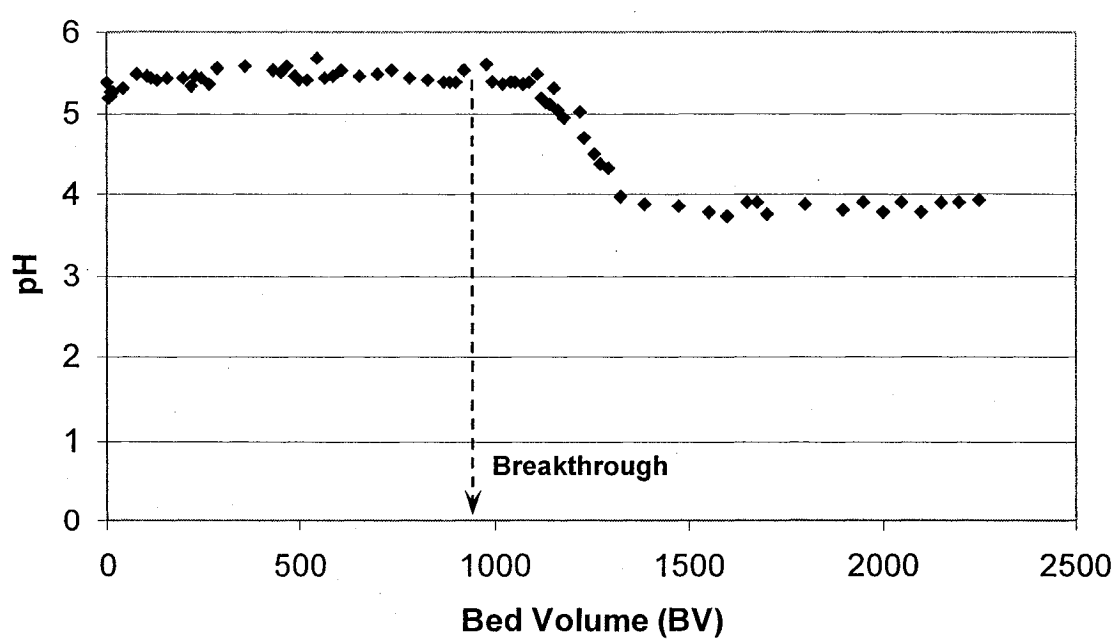

Figure 5.27: pH profile for Pb biosorption in the fixed column

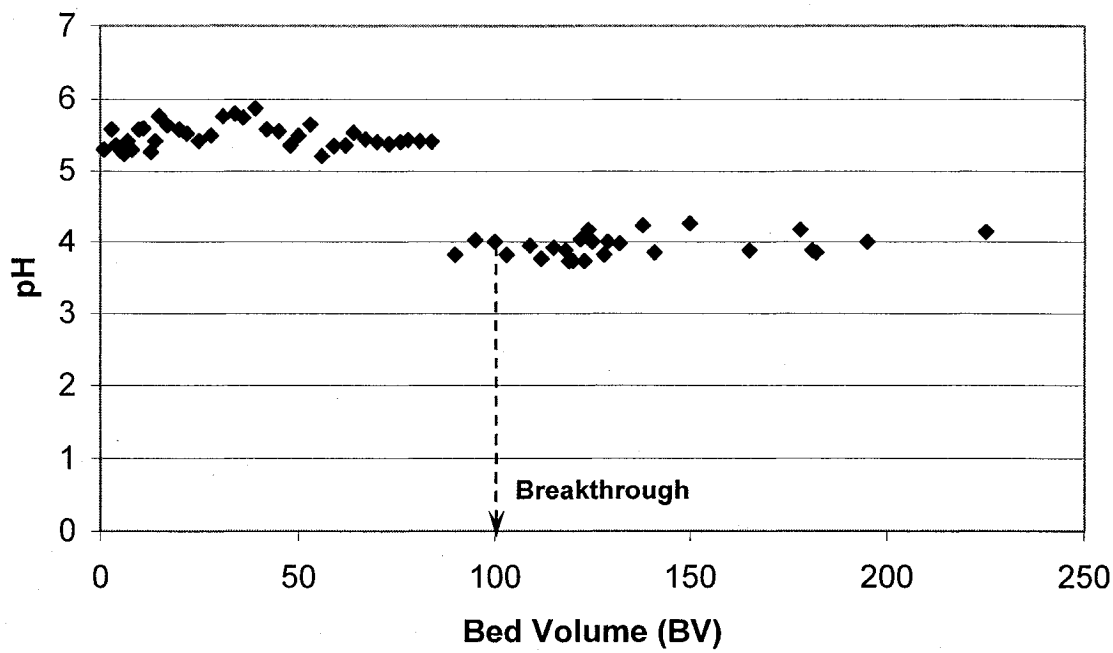

Figure 5.28: pH profile for Ni biosorption in the fixed column

At the breakthrough point the pH value decreased to the value of the feed solution. The increase of the pH value could be due to the adsorption of the H^+ ions from the solution and as the column became saturated less adsorption of H^+ occurred which returned the pH value to its feed value as the breakthrough occurred.

5.2.1.3 Desorption

Once the biomass was saturated with metal ions in the column it was important to regenerate the biomass for the recovery of metal ions as well as the use of the biomass for biosorption. The column desorption studies were carried out by passing 0.5 M $CaCl_2$ solution with an initial pH value of 4.0 and an upward

flow rate of 1.5 mL/min. The average residence time in the column was approximately 30 minutes. Figures 5.29 to 5.32 show the concentration history of Cd, Cu, Pb and Ni in the column effluent during desorption process respectively. It can be seen that the elution of Pb, Cu, Cd, and Ni was completed within 35, 31, 38, and 25 bed volumes respectively. The concentration of Pb, Cu, Cd, and Ni reached a fairly high value of 6.7, 1.1, 1.6, and 1.9 g/L respectively. Using the trapezoidal rule to calculate the amount of eluted metals during the desorption process it was found that 16.38, 10.22, 10.90 and 7.71 meq was eluted of Pb, Cd, Cu and Ni respectively. A comparison between the total Pb, Cu, Cd and Ni retained and the total amount of metal collected by the $CaCl_2$ eluant shows that almost 80%, 82%, 85%, and 98% of the metal was recovered, respectively.

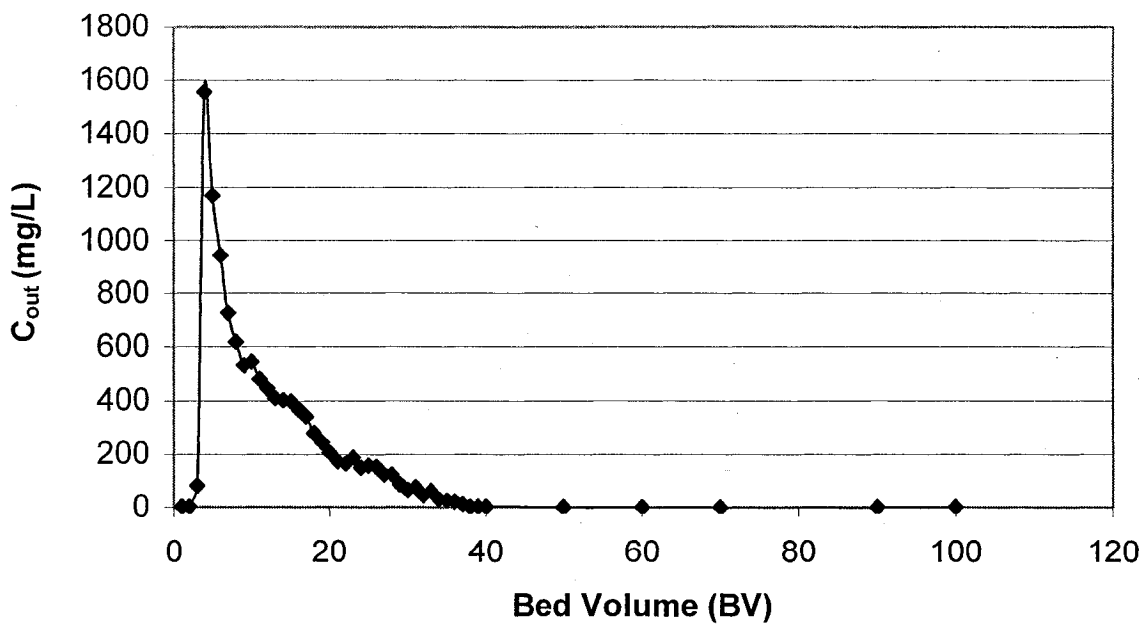

Figure 5.29: Desorbing Cd from a column with 0.5M $CaCl_2$

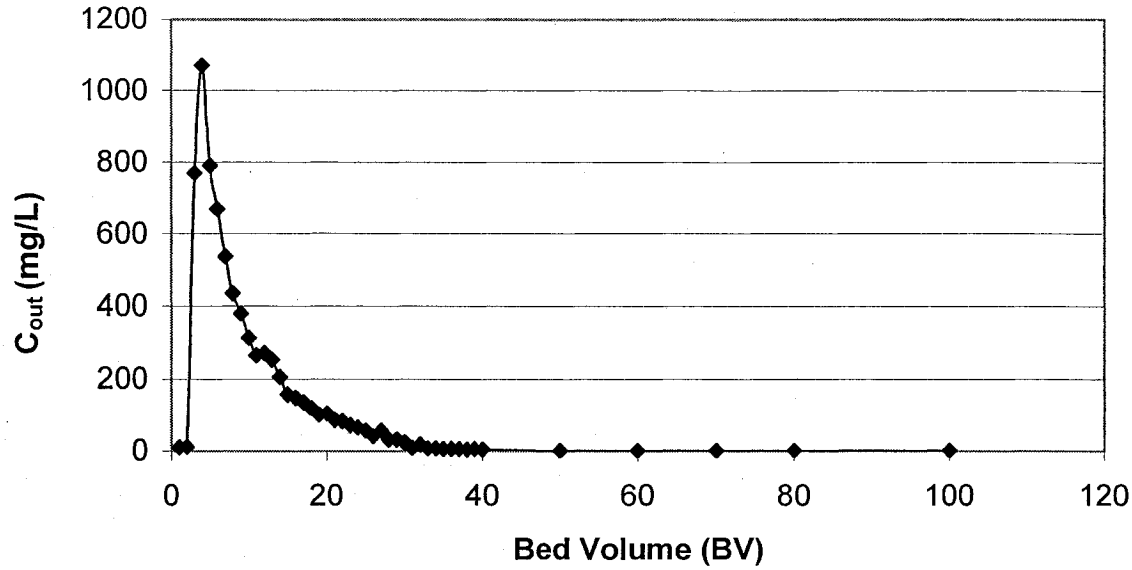

Figure 5.30: Desorbing Cu from a column with 0.5M $CaCl_2$

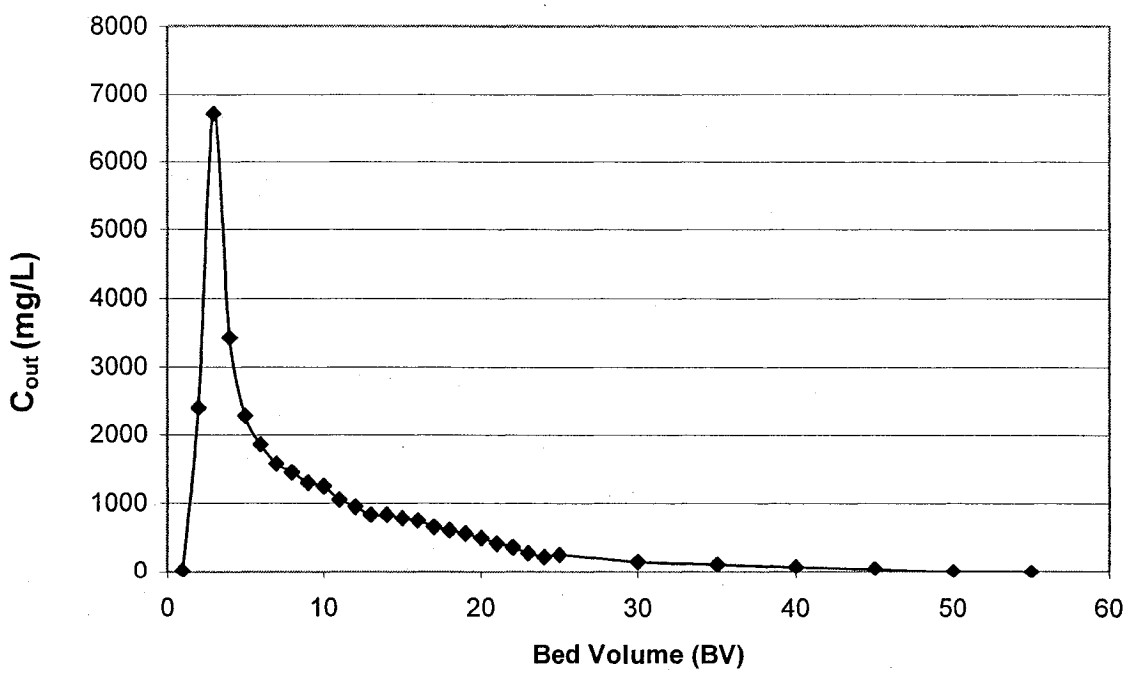

Figure 5.31: Desorbing Pb from a column with 0.5M $CaCl_2$

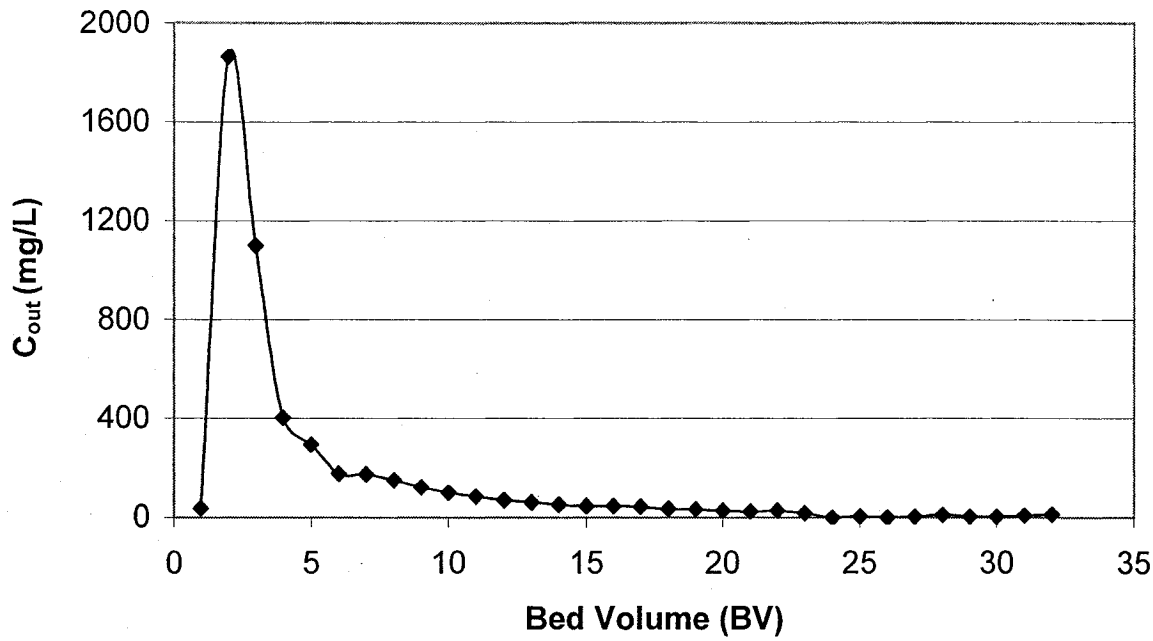

Figure 5.32: Desorbing Ni from a column with 0.5M $CaCl_2$

The trailing edge of an elution peak is a mirror image of the breakthrough curve of the regenerant from a column saturated with heavy metals. It follows that the trailing edges of elution peaks can be either sharp or broad, depending on the type of equilibrium between the regenerant and the heavy metal to be desorbed from the biomass. Therefore, the tailing observed in Figures 5.29 to 5.32, can be explained by the fact that the uptake of the heavy metals is more favorable than the uptake of Ca ions giving rise to the broad Ca breakthrough. During the process of desorption the biomass is successfully converted back to the calcium-saturated form. As a result the process of desorption would include

two process at the same time which are desorption and treatment of the biomass for the second round of usage.

5.2.2 Biosorption mechanisms by anaerobic biomass

As mentioned before in section 2.1.2 the ability of bacterial cells to bind metals is associated with the components of the cell itself. The main components of the cell are water, inorganic salts and mineral elements, proteins, nucleic acids, polysaccharides, and lipids. Water is the most abundant single compound in the cell it makes almost 70% of the total weight of the cell. 75% of the solid fraction of the biomass was found to be organic matter. Inorganic salts and mineral elements, on the other hand, constitute 25% of the total dry weight (Section 4.2.2). The organic compounds contain different surface functional groups such as carboxylate, carbonyl, hydroxyl, amino, phosphoryl, and sulphide groups (Greene et al., 1987). The different functional groups have a high affinity towards heavy metals that they can complex the metal ions (Delgado et al., 1998).

Several different mechanisms have been proposed to explain the uptake of metals by non-living microbial biomass, including microprecipitation, ion exchange, and complexation (section 2.1.4). Because of the complexity of most cell walls it is very likely that all of these processes of binding will take place in the system at the same time. Ion exchange was believed to be the principal mechanism for metal uptake. In order to investigate the role of ion exchange in biosorption by anaerobic biomass, sorption experiments were carried out using a

flow-through column packed with Ca-biomass. The fact that the biomass was treated with calcium implies that the majority of sites on the cell wall are occupied by calcium. In the context of this work, the term ion exchange refers to binding of a solute (usually: metal cation) to a site which was initially occupied by another cation and this second ion is released upon the binding of the first ion. In our case the released cation in exchange for the heavy metal, would be calcium.

Figures 5.33, 5.34 and 5.35 show the concentration of nickel, copper, cadmium and calcium in the column effluent during the saturation of the packed-bed of Ca-biomass with Ni, Cu, and Cd ions respectively. As can be seen from these figures, the removal of Ni, Cu, and Cd ions from the bed was accompanied by the elution of Ca^{2+} ions from the packed-bed. The fact that the breakthrough curves of Ni, Cu and Cd ions and the elution curve of Ca^{2+}, formed a mirror image of each other, indicated that 1) Ni, Cu and Cd ions were exchanged for Ca^{2+} in the biomass, and 2) the biosorption process of nickel, copper and cadmium can be mainly accounted for by ion exchange with calcium. The amounts of Ni, Cu and Cd ions adsorbed by the biomass were calculated using equation (5.5) (section 5.2.1.1). The amount of Ca ions eluted was calculated by calculating the area under the curve of eluted Ca ions in Figures 5.33 to 5.35 by using the trapezoidal rule. Where the area underneath the curve of eluted Ca ions was divided into a certain number of trapezoids. The area of each trapezoid was calculated and all areas were added up to give the total area underneath the curve. The amount of adsorbed Ni ions was found to be 7.87 meq which is almost equal to the amount of eluted Ca ions (7.75 meq).

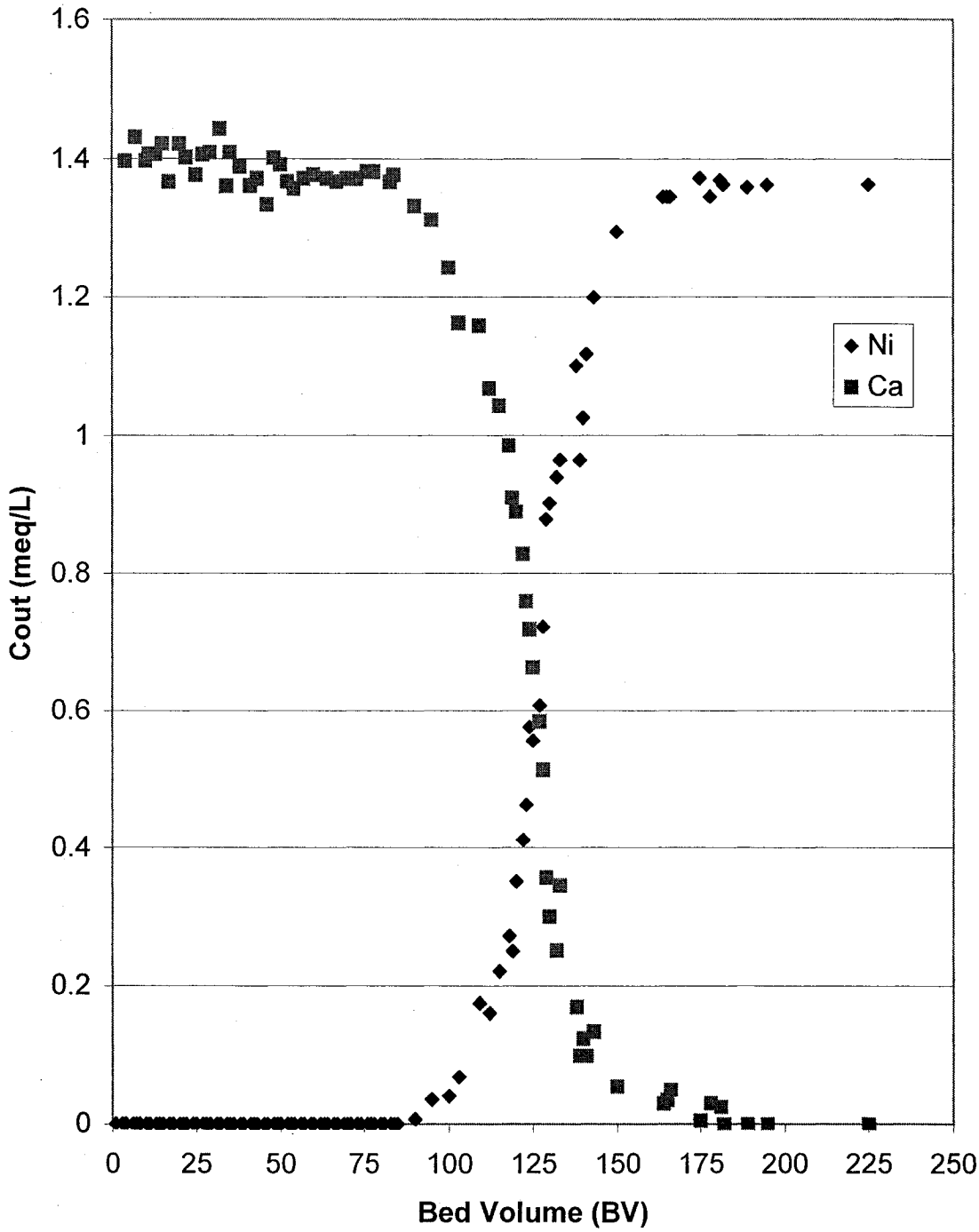

Figure 5.33: Adsorption of Ni^{2+} ions and desorption of Ca^{2+} ions in a packed-bed column of Ca-biomass
(Bed volume = 45 mL, Flowrate = 1.5 mL/min, C_o(Ni) = 1.4 meq/L)

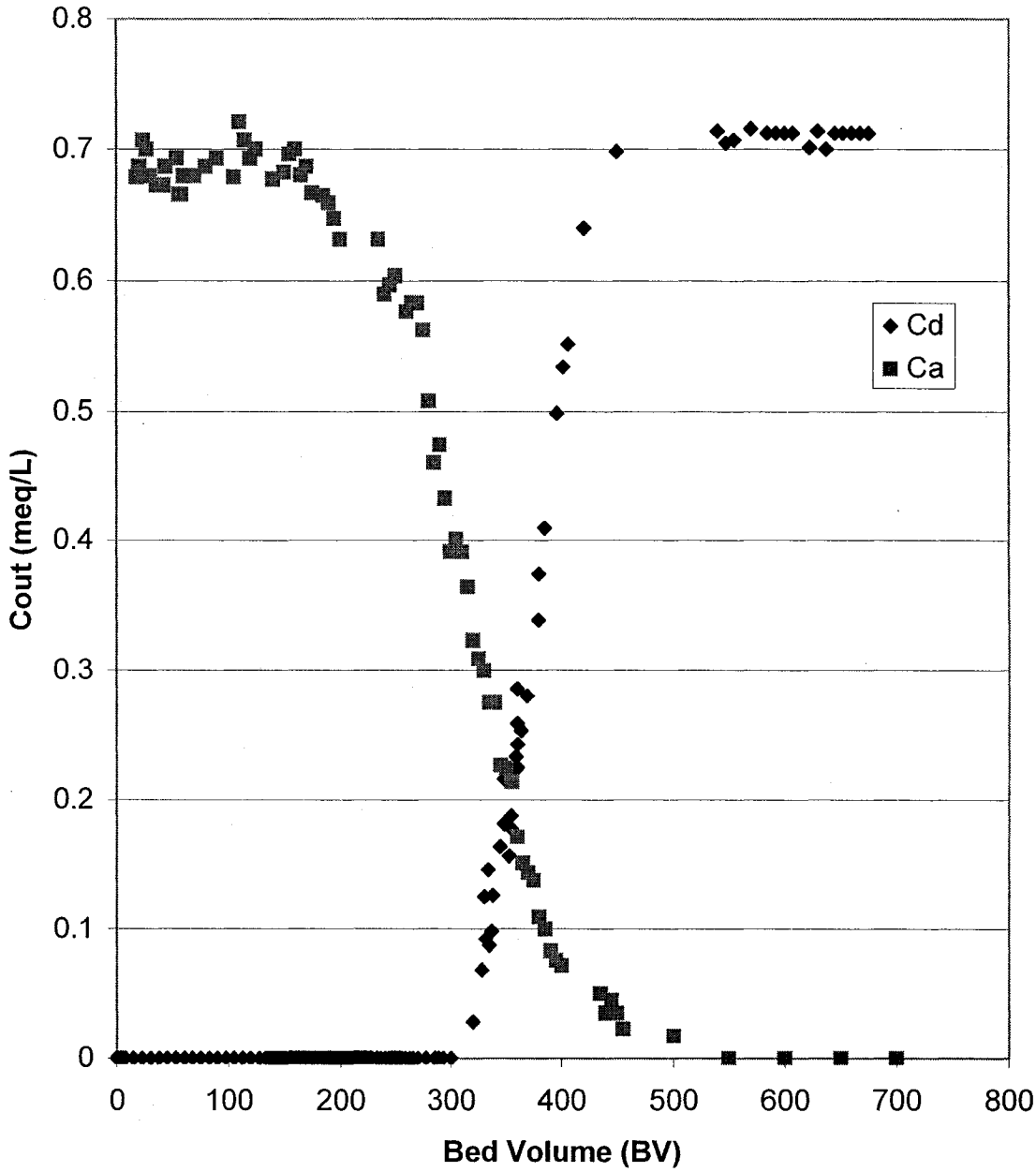

Figure 5.34: Adsorption of Cd^{2+} ions and desorption of Ca^{2+} ions in a packed-bed column of Ca-biomass
(Bed volume = 45 mL, Flowrate = 1.5 mL/min, C_o(Cd) = 0.71 meq/L)

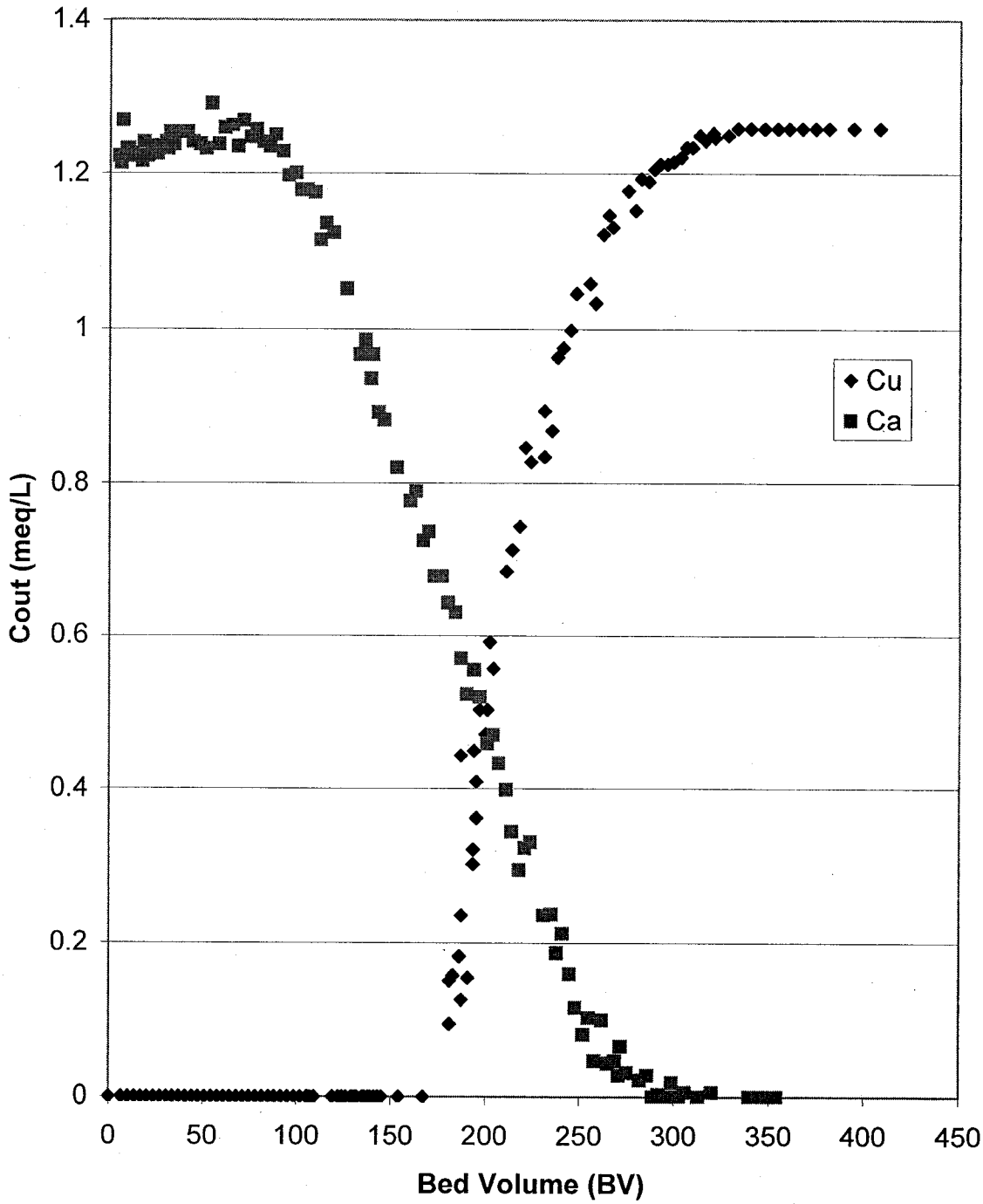

Figure 5.35: Adsorption of Cu^{2+} ions and desorption of Ca^{2+} ions in a packed-bed column of Ca-biomass
(Bed volume = 45 mL, Flowrate = 1.5 mL/min, $C_o(Cu)$ = 1.25 meq/L)

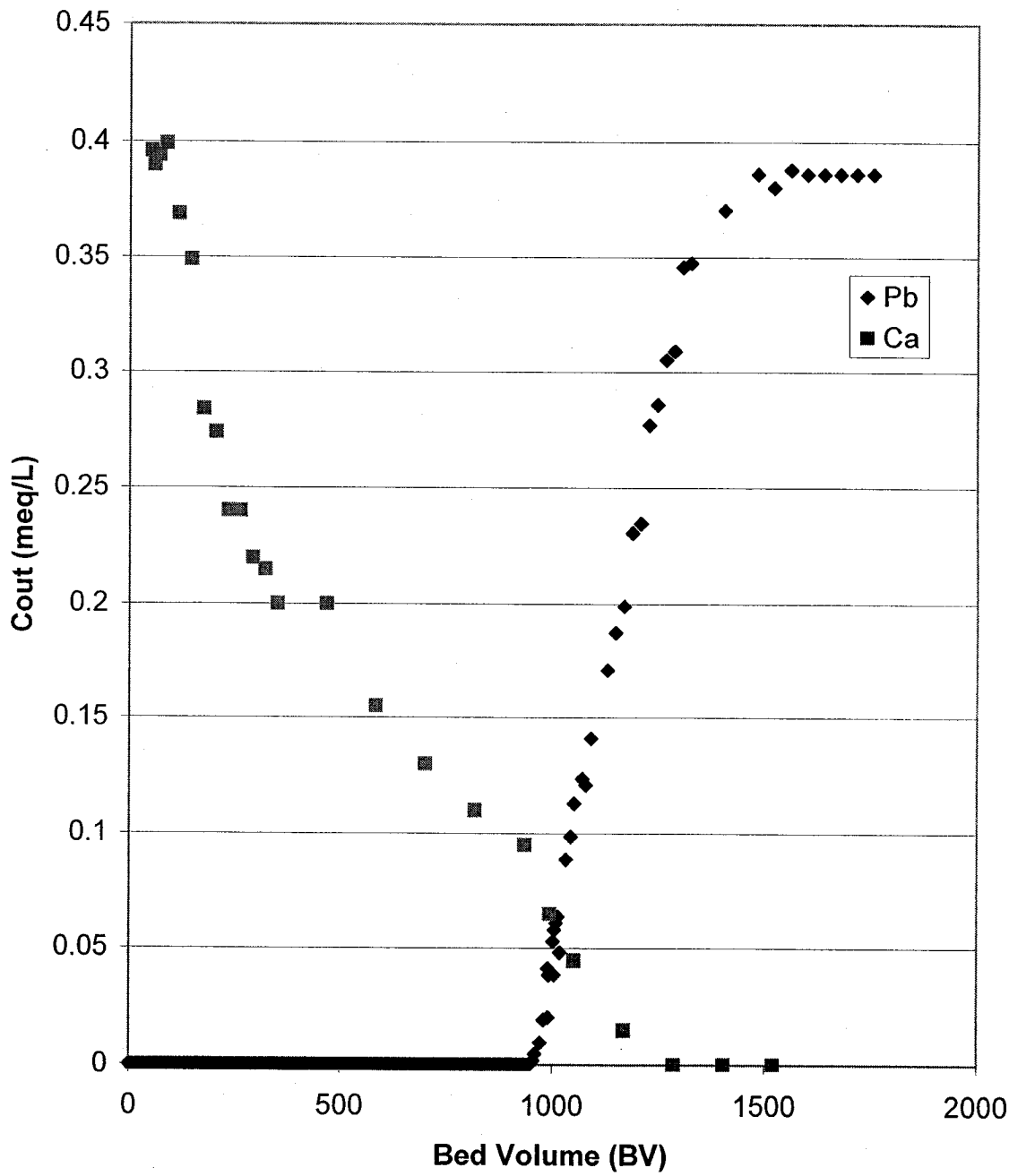

Figure 5.36: Adsorption of Pb^{2+} ions and desorption of Ca^{2+} ions in a packed-bed column of Ca-biomass
(Bed volume = 45 mL, Flowrate = 1.5 mL/min, C_o(Pb) = 0.38 meq/L)

This shows that the biosorption process of nickel can be accounted for by ion exchange with calcium. Comparing the amount of adsorbed Cu and Cd ions (13.3 and 12.0 meq respectively) with the amount of eluted Ca ions (10.2 and 9.84 meq respectively) shows that ion exchange was accounted for 77% and 82% of the total biosorption process respectively.

Figure 5.36 shows the elution of calcium in the column effluent during the saturation of the packed-bed of Ca-biomass with Pb ions. As can be seen from the figure, the breakthrough curve of Pb ions and the elution curve of Ca ions, did not form a mirror image of each other. Comparing the amount of Pb adsorbed (20.36 meq) with the amount of Ca eluted (10.42 meq) it could be found that ion exchange was accounted for only 51% of the total biosorption process.

For the case of Pb, Cu and Cd ions, the difference between the adsorption amount and the Ca^{2+} desorbed amount can be attributed to the extent of complexation/chelation and precipitation mechanisms completing the ion exchange one. The extent of these mechanisms depends on the nature of the metal. As mentioned before because the amount of Ni^{2+} adsorbed and amount of Ca^{2+} desorbed were almost similar. Ni ions seemed to be exclusively adsorbed by an ion exchange mechanism.

In order to further investigate the existence of the complexation/chelation and precipitation processes results from the desorption tests were used. Metal ions that are ionically bound and precipitated on the surface of the biomass would be able to desorb at the proper pH value. The initial pH value of the regenerant solution was 4.0. During the desorption process the pH value of the

effluent dropped to a value around 3.0. At this pH value all metals in the column would be in the soluble state (Table 5.8). In section 5.2.1.2 it was mentioned that during the biosorption process while the feed pH was 4.0 for the four metals studied, the exit pH increased to a value of (pH 5.0 ~ 5.5). That behaviour was explained due to the adsorption of H ions onto the biomass. The fact that the pH value of the influent decreased during the desorption process could be explained by the release of the adsorbed H ions on the biomass into the solution.

From Figures 5.29 to 5.31 it was found out that 85%, 82% and 80% of Cd, Cu and Pb ions were desorbed respectively. For each metal, the difference between the amount adsorbed and the amount desorbed can be attributed to the extent of complexation/chelation process competing with the ion exchange one:

(Amount of complexed metal ion = Total amount of adsorbed ion –
Amount of desorbed ion)

It was found that for Pb, Cu and Cd ions almost 20%, 18% and 15% of the total amount adsorbed may be attributed to the complexation/chelation process.

For each metal ion the difference between the amount of desorbed ion and the amount of eluted Ca^{2+} ions at the proper pH value can be attributed to the precipitation process competing with the ion-exchange and complexation/chelation:

(Amount of precipitated metal ion = Total amount of desorbed ion –

Amount of eluted Ca^{2+} ions)

For Cu and Cd ions the amount of desorbed ions was almost identical to the amount of eluted Ca^{2+} ions. This could indicate that no precipitation occurred for these two metal ions. For the case of lead the difference between the amount desorbed and the amount of eluted Ca^{2+} ions, 29%, can be attributed to the extent of precipitation mechanisms competing with the ion exchange and complexation/chelation one. Figures 5.25 to 5.28 in section 5.2.1.2 showed that during the biosorption process the pH value increased to a value of 5.0 to 5.5. For the case of Cu, Cd, and Ni ions at this pH value they are totally soluble in the solution since the precipitation pH value for these metals was found to be around 7 to 9 (Table 5.8). For the case of Pb ions it was found out that precipitation would occur at a pH value around 6.0 (Table 5.8) which is close to the pH value inside the adsorption column. According to SenGupta (2002) as the solution pH approaches pH_{prec}, surface precipitation becomes an important uptake mechanism. Reed and Matsumoto (1993) reported that surface precipitation could occur at pH values ½ to 1 pH unit lower than the pH at which metal precipitation occurs. Reasons for the behavior included the following: (1) the surface of the biomass would behave as a "nucleus" for metal precipitate formation; and (2) a locally high concentration of metal may exist on the biomass surface, increasing the opportunity for precipitation to occur. Apparently, in the case of Ni, Cu, and Cd most metal binding occurs after initial ion exchange with

calcium cations which are chemically bonded on the chemically active sites of the biomass. Binding of lead to the cell walls might 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 those same sites create a condition on the surface of the biomass whereby additional lead removal can occur via surface precipitation (i.e., the first layer of lead is by ion exchange and additional layers are due to the formation of metal precipitate). The deposition of lead on the surface of the biomass would provide a nucleus which would nucleate the deposition of more metal as a chemical precipitate. Table 5.9 summarizes the amounts of adsorbed and desorbed metal ions and the amount of eluted Ca^{2+} ions during the adsorption of each metal ion. The presumed mechanisms for the uptake of the metal ions are reported in Table 5.10.

Figures 5.37 to 5.40 provide a schematic presentation of the uptake mechanisms by the anaerobic biomass for lead, cadmium, copper and nickel respectively. To further investigate the possible precipitation of lead ions on the surface of the biomass scanning electron microscopy and X-ray analysis were performed on different samples of the biomass.

Table 5.9: Amounts of adsorbed and desorbed metal ions and amount of eluted Ca ions during the adsorption of each metal ion.

Metal	Amount adsorbed (meq)	Amount desorbed (meq)	Amount of Ca ions eluted (meq)
Pb	20.36	16.38	10.42
Cu	13.30	10.90	10.20
Cd	12.00	10.22	9.84
Ni	7.87	7.71	7.75

Table 5.10: Adsorption mechanisms for different metal ions

Metal	Adsorption mechanisms		
	Ion exchange	Complexation	Precipitation
Pb	51%	20%	29%
Cu	77%	18%	0%
Cd	82%	15%	0%
Ni	98%	0%	0%

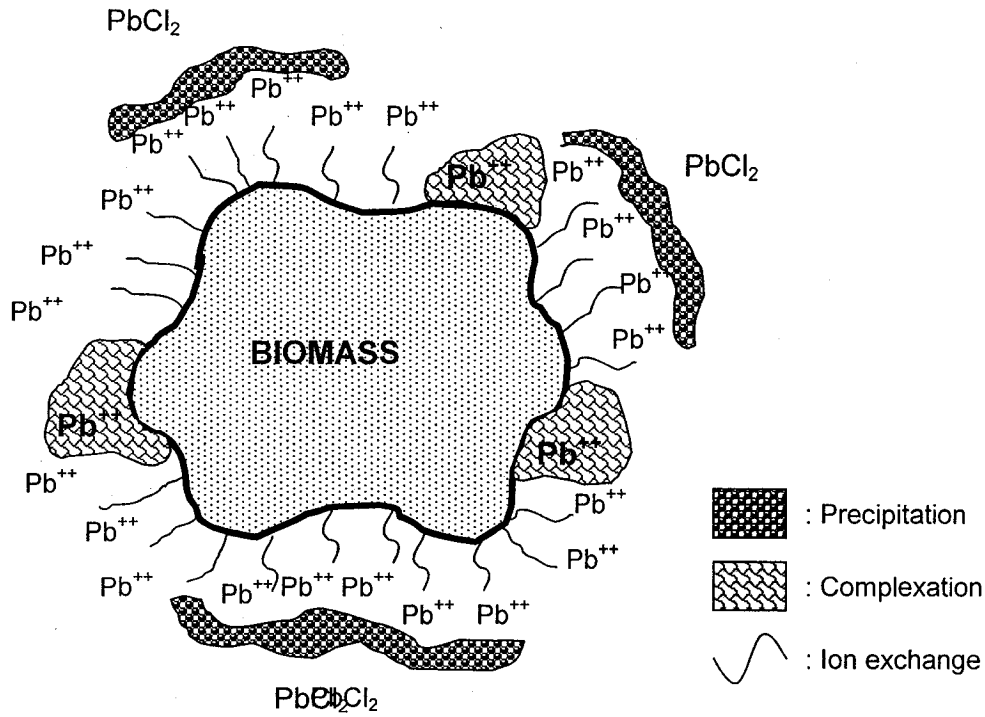

Figure 5.37: Lead uptake mechanisms by anaerobic biomass

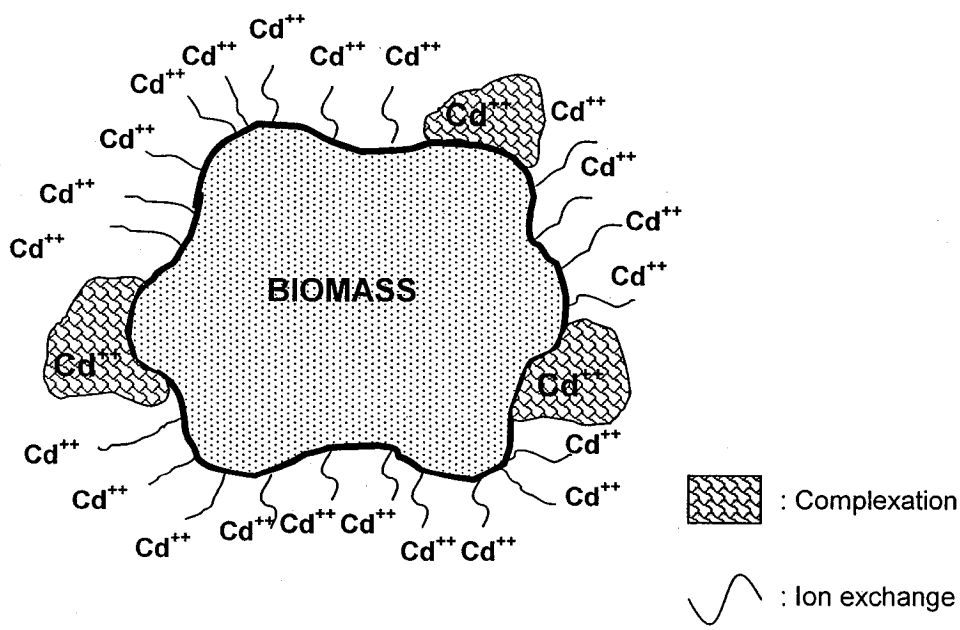

Figure 5.38: Cadmium uptake mechanisms by anaerobic biomass

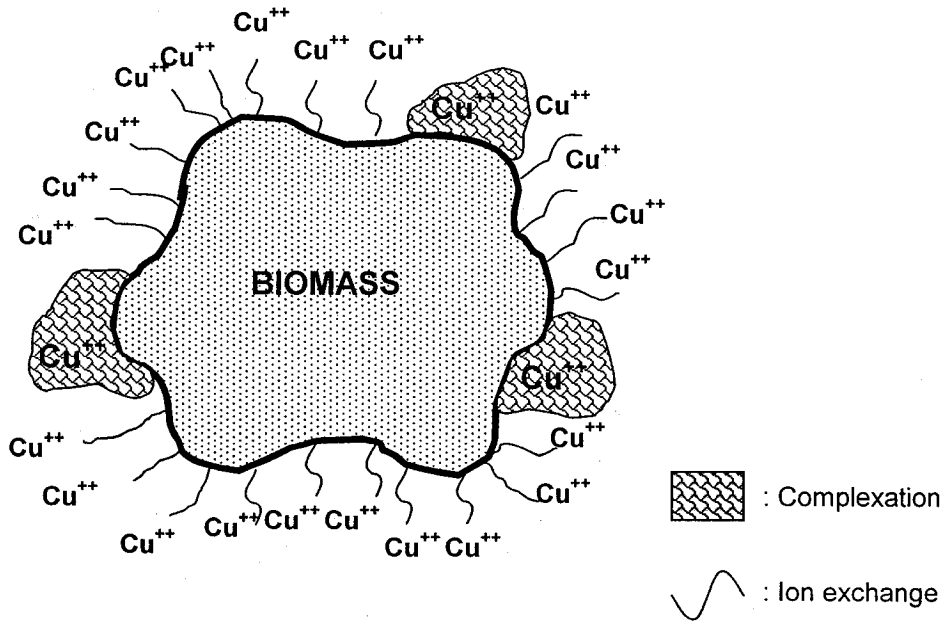

Figure 5.39: Copper uptake mechanisms by anaerobic biomass

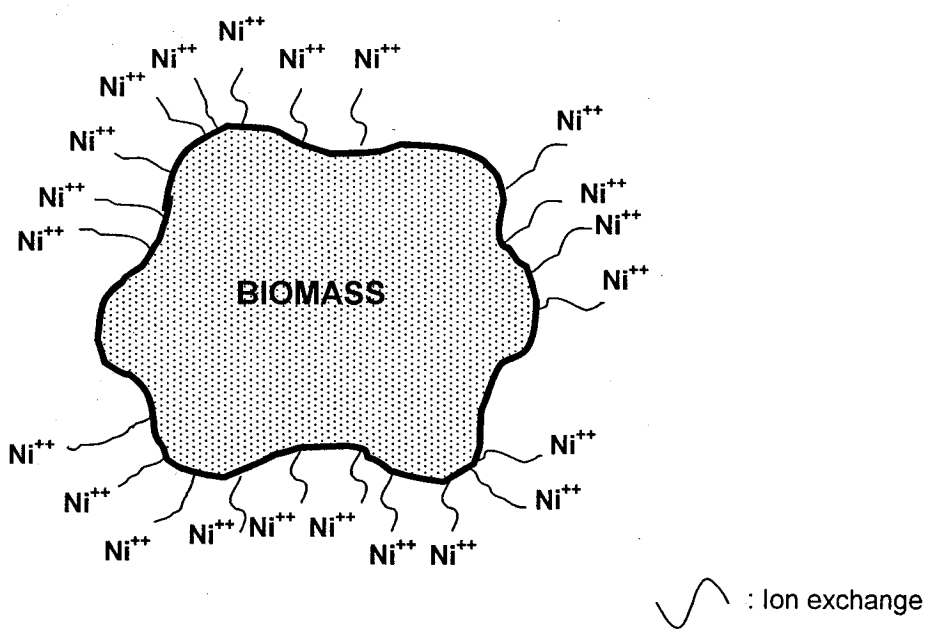

Figure 5.40: Nickel uptake mechanism by anaerobic biomass

5.3 Scanning electron microscopy

The scanning electron microscopy (SEM) is frequently equipped with a spectrometer capable of detecting X-rays emitted by the specimen during electron-beam excitation. These X-rays carry a characteristic energy and wavelength, which when measured will reveal the elemental composition of the specimen. X-ray analysis can be performed on a wide variety of sample types containing elements that covers a large portion of the periodic table. In order to further investigate what is happening on the surface of the biomass SEM and X-ray analysis were performed on the untreated biomass, Ca-biomass, and biomass saturated with Pb, Cu, Ni, and Cd respectively. Figure 5.41 presents the characteristic spectra of the untreated biomass which shows the presence of different metals on the surface of the biomass such as (K, Na, Al, and S) where each element is identified by the presence of peaks at the characteristic spectra. Figure 5.42 shows that after washing the biomass with a high concentration of $CaCl_2$, Ca ions would wash away the different metal ions found on the surface of the biomass so that the majority of sites are occupied by calcium ions. SEM pictures of the untreated biomass and Ca-biomass, Figures 5.47 and 5.48 in sections A and B respectively show that the untreated biomass contained agglomerates on the surface of the biomass before washing. Section B of these figures shows that washing the biomass with $CaCl_2$ solution would remove the fine agglomerates and other surface impurities from the surface of the biomass. Figures 5.43 to 5.46 present the X-ray analysis of the biomass after saturation with Pb, Cu, Ni, and Cd respectively. These figures show the presence of these

metals on the surface of the biomass. As mentioned in the previous section binding of lead to the cell walls might 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 those same sites create a condition on the surface of the biomass whereby additional lead removal can occur via surface precipitation. Figure 5.43 shows that after the biomass was saturated with lead ions the presence of Cl ions was also observed, this could indicate the formation of $PbCl_2$ as a precipitate on the surface of the biomass. Although metal solutions were all prepared from metal salts only in the case of lead Cl ions could be found on the surface of the biomass. It could also be seen from Figures 5.47 and 5.48 that in the case of the biomass saturated with lead, the surface of the biomass is more uniform than in the case of the biomass saturated with other metals. This could also indicate the formation of a layer of $PbCl_2$ on the surface of the biomass which makes the surface of the biomass more uniform.

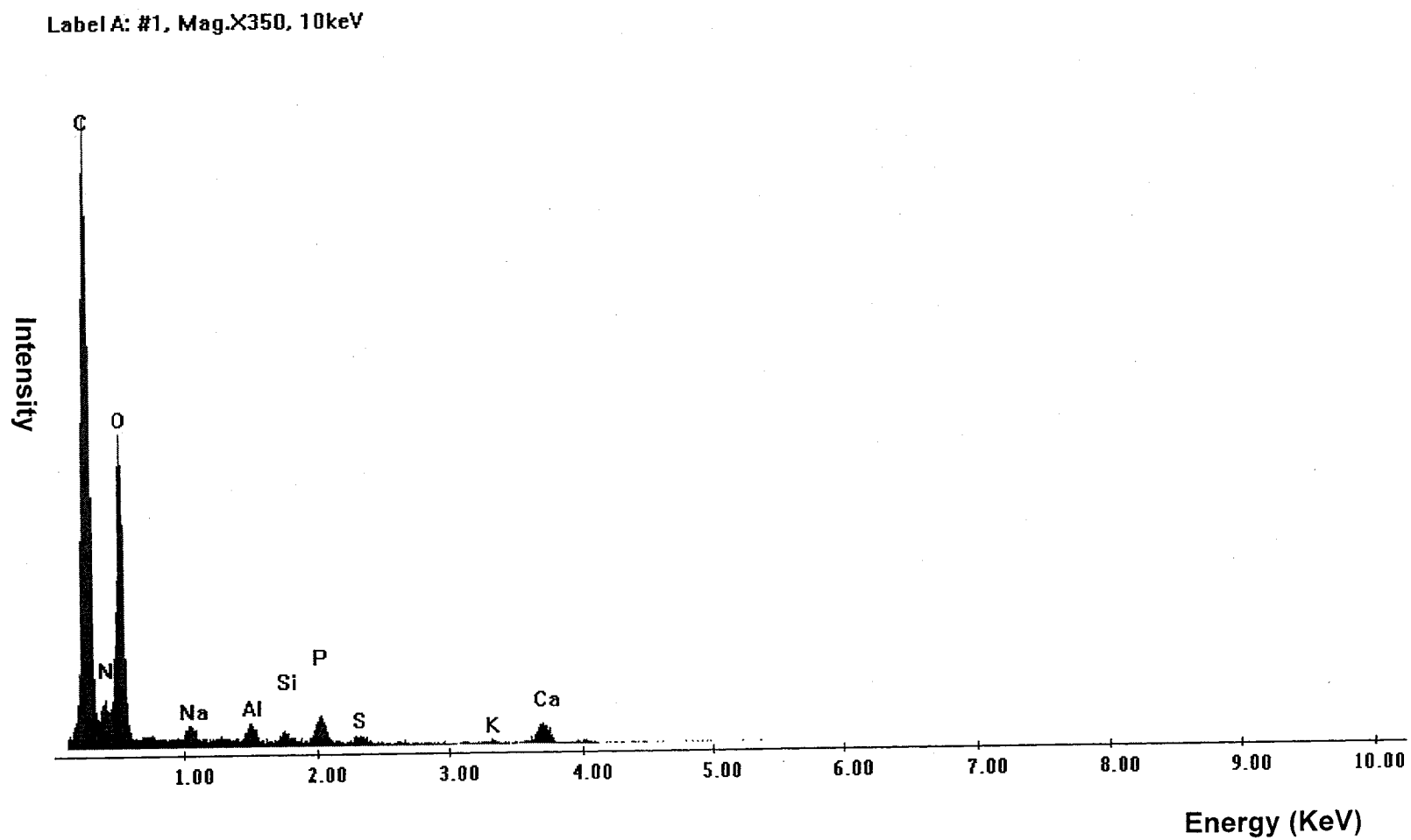

Figure 5.41: Characteristic X-ray spectrum of untreated biomass

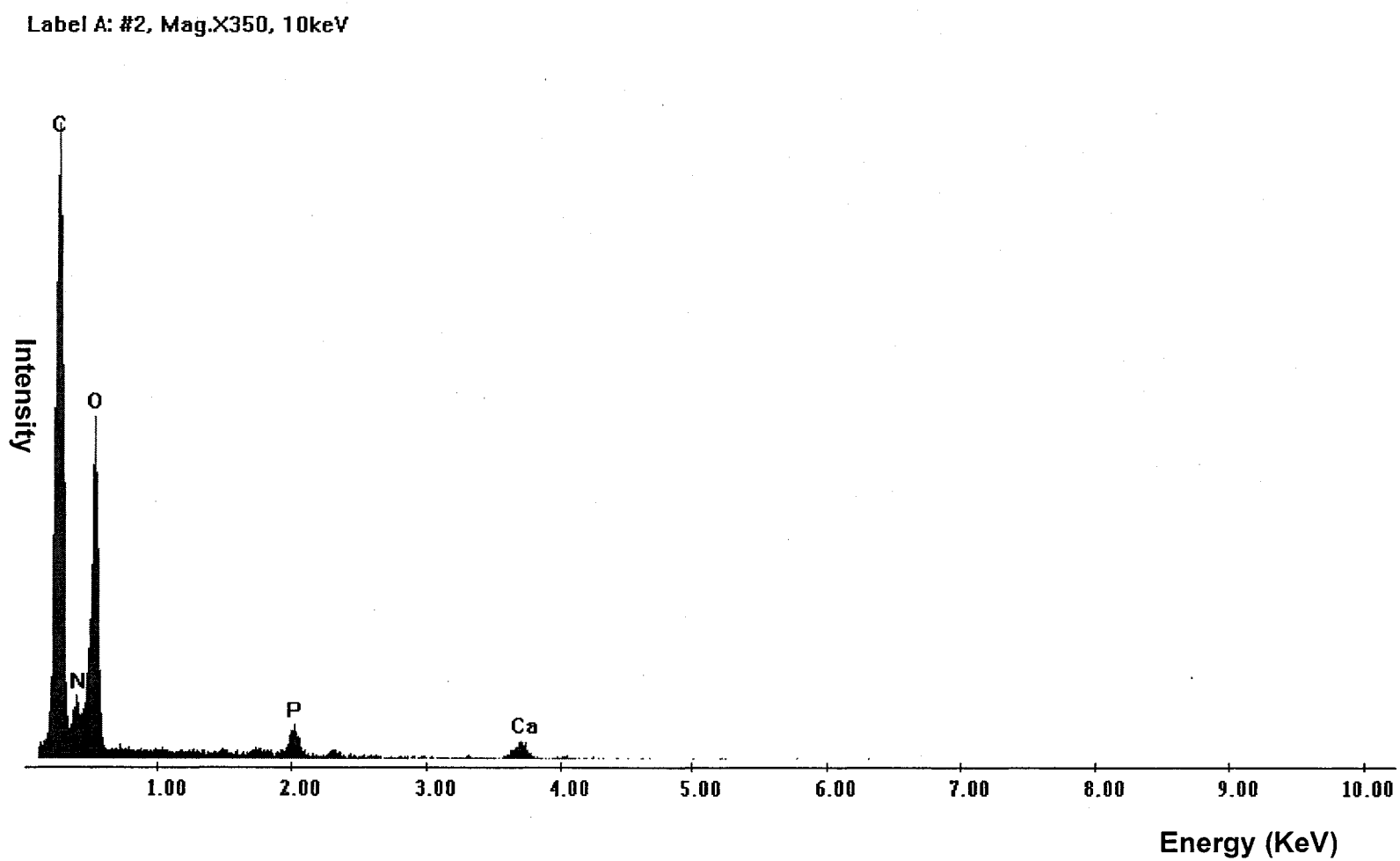

Figure 5.42: Characteristic X-ray spectrum of Ca-biomass

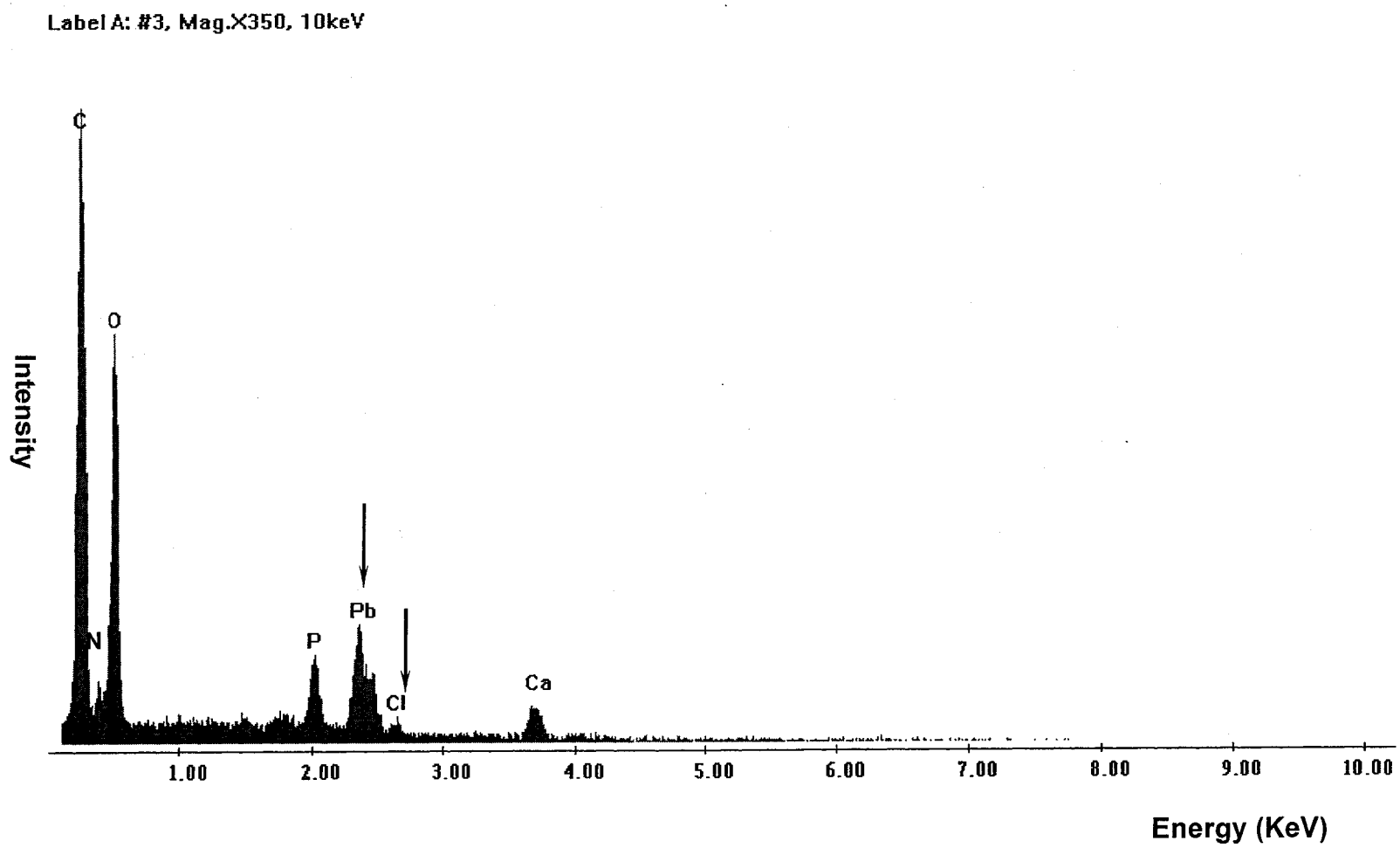

Figure 5.43: Characteristic X-ray spectrum of Ca-biomass saturated with Pb

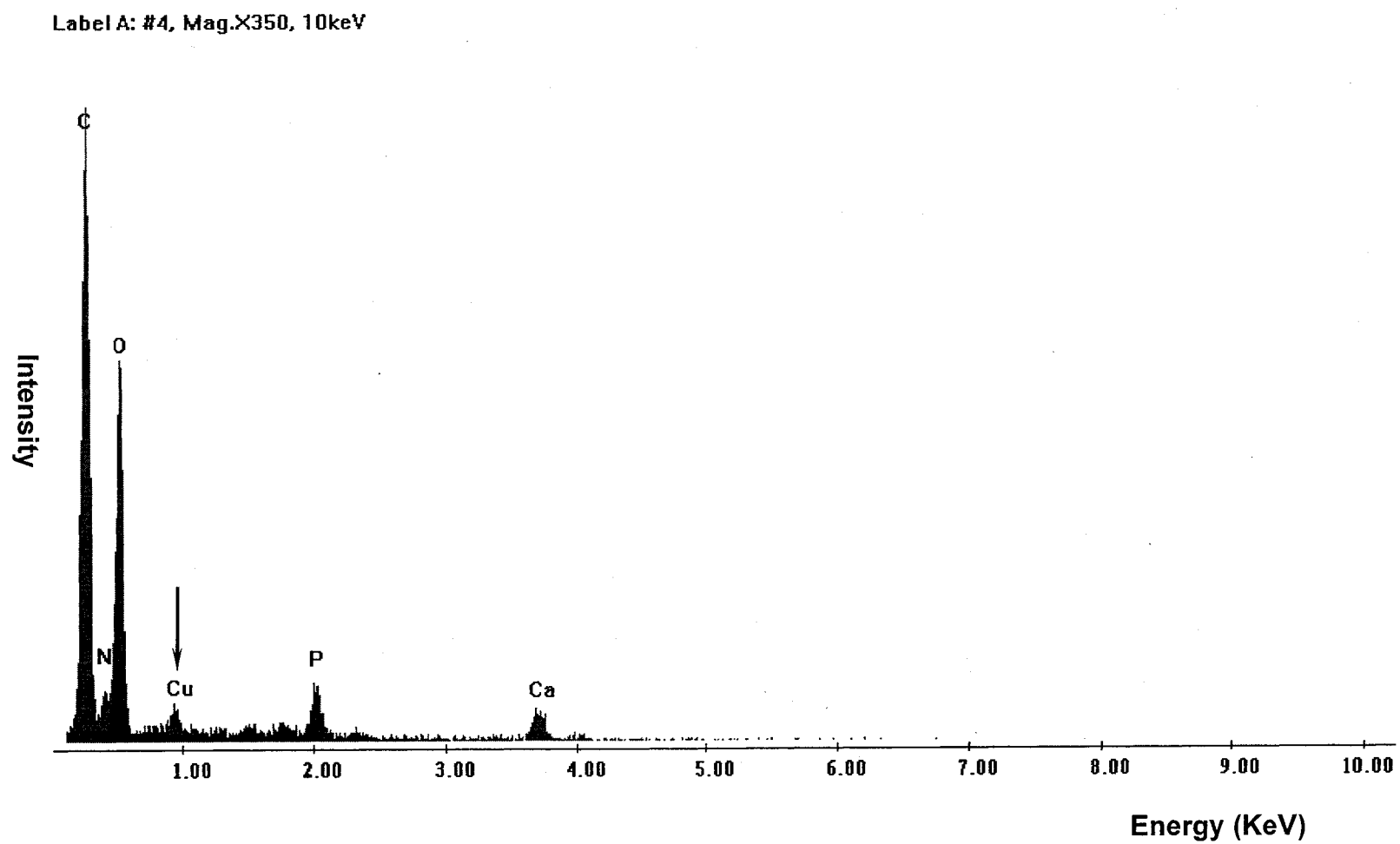

Figure 5.44: Characteristic X-ray spectrum of Ca-biomass saturated with Cu

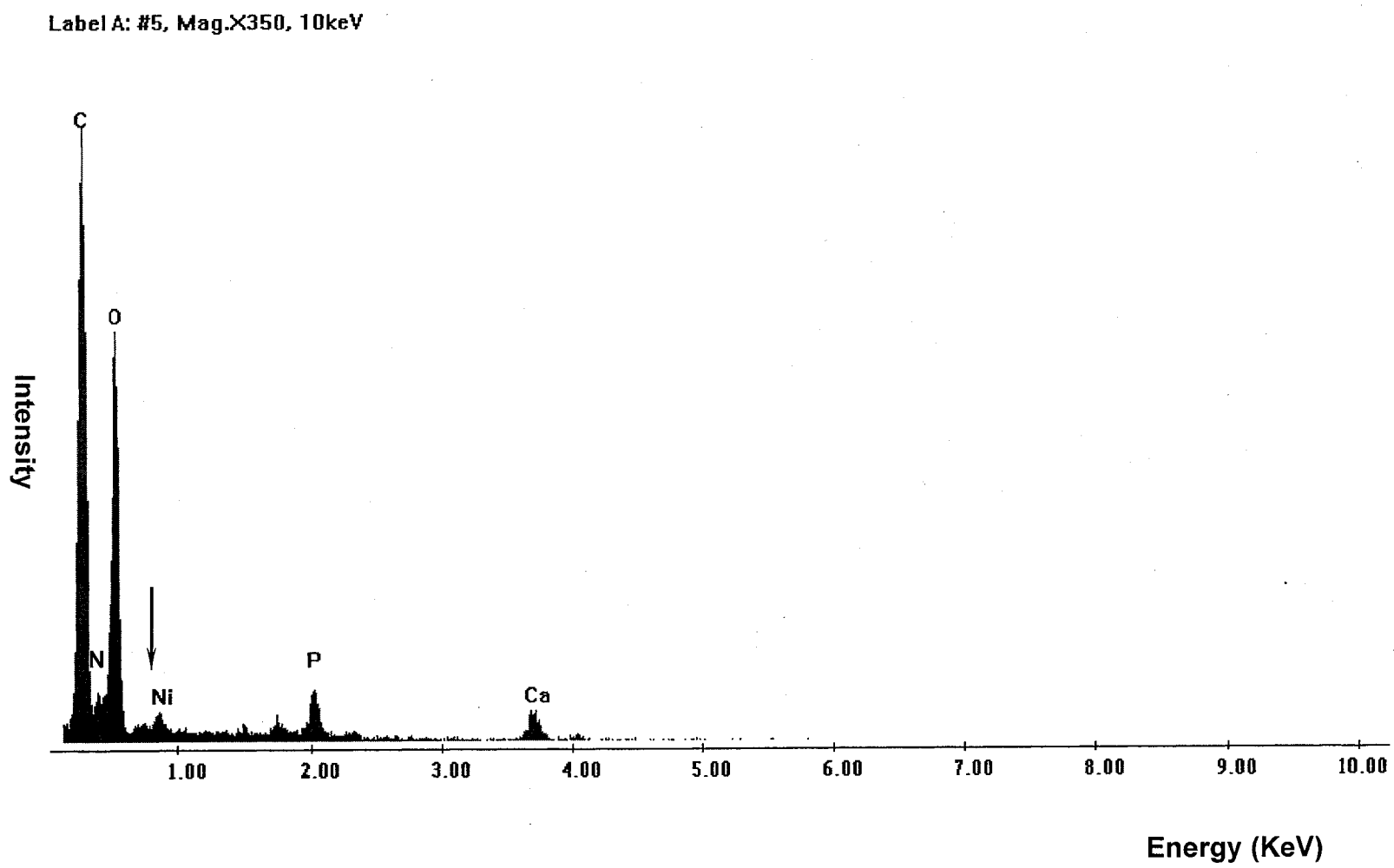

Figure 5.45: Characteristic X-ray spectrum of Ca-biomass saturated with Ni

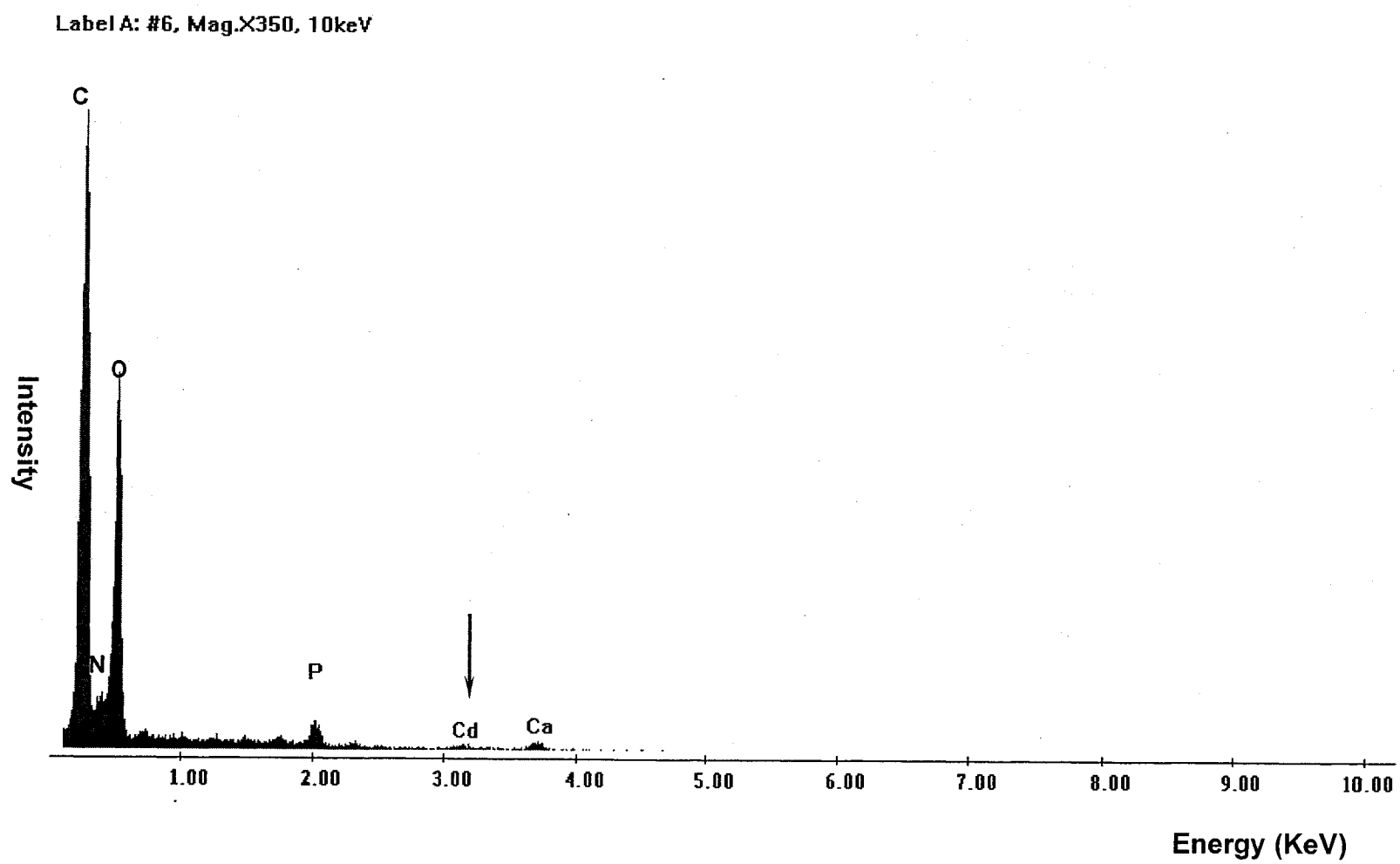

Figure 5.46: Characteristic X-ray spectrum of Ca-biomass saturated with Cd

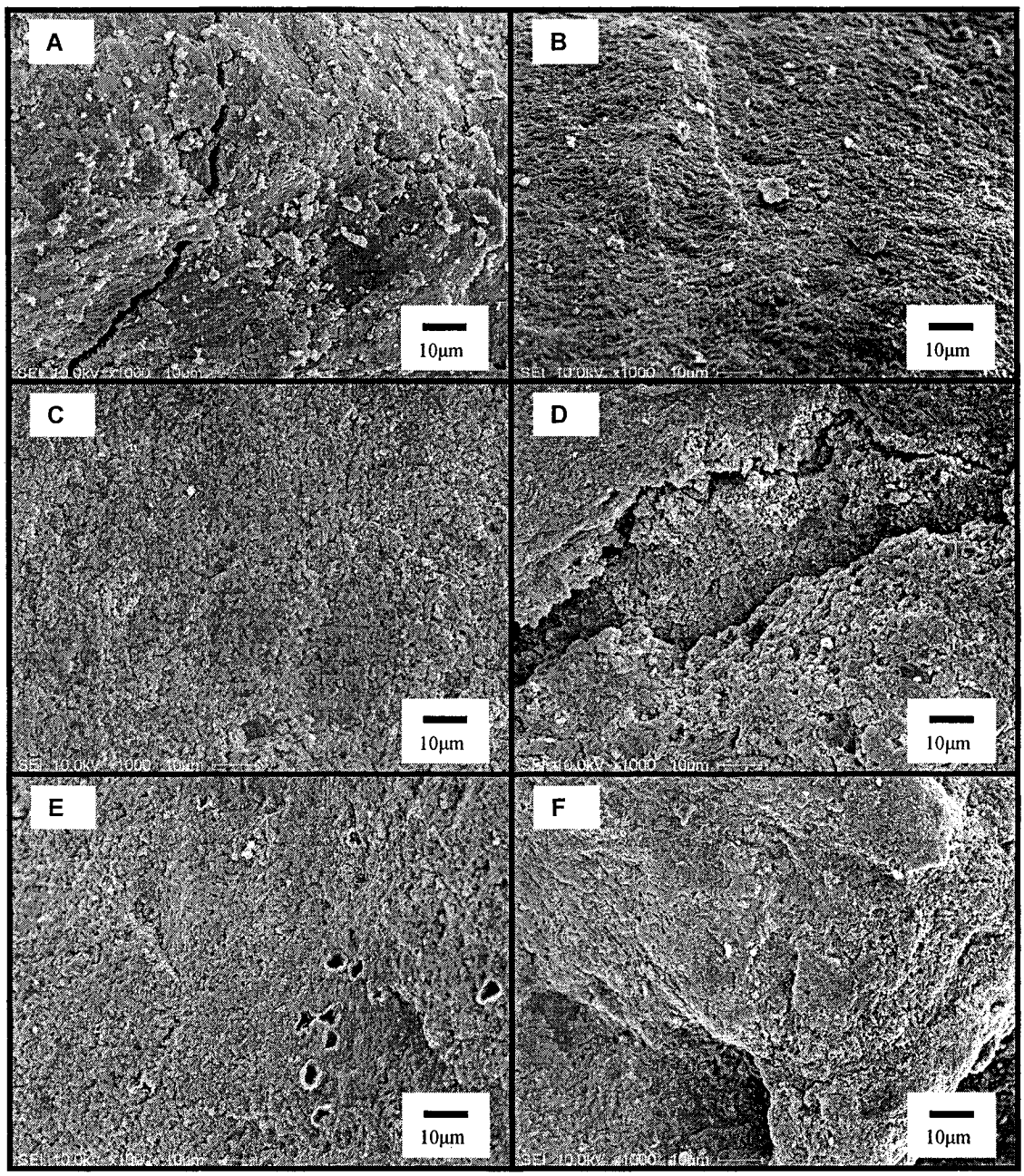

Figure 5.47: Scanning electron micrographs of anaerobic biomass at 1000X magnification. **A)** untreated biomass **B)** Ca-biomass **C)** Ca-biomass saturated with Pb **D)** Ca-biomass saturated with Cu **E)** Ca-biomass saturated with Ni **F)** Ca-biomass saturated with Cd

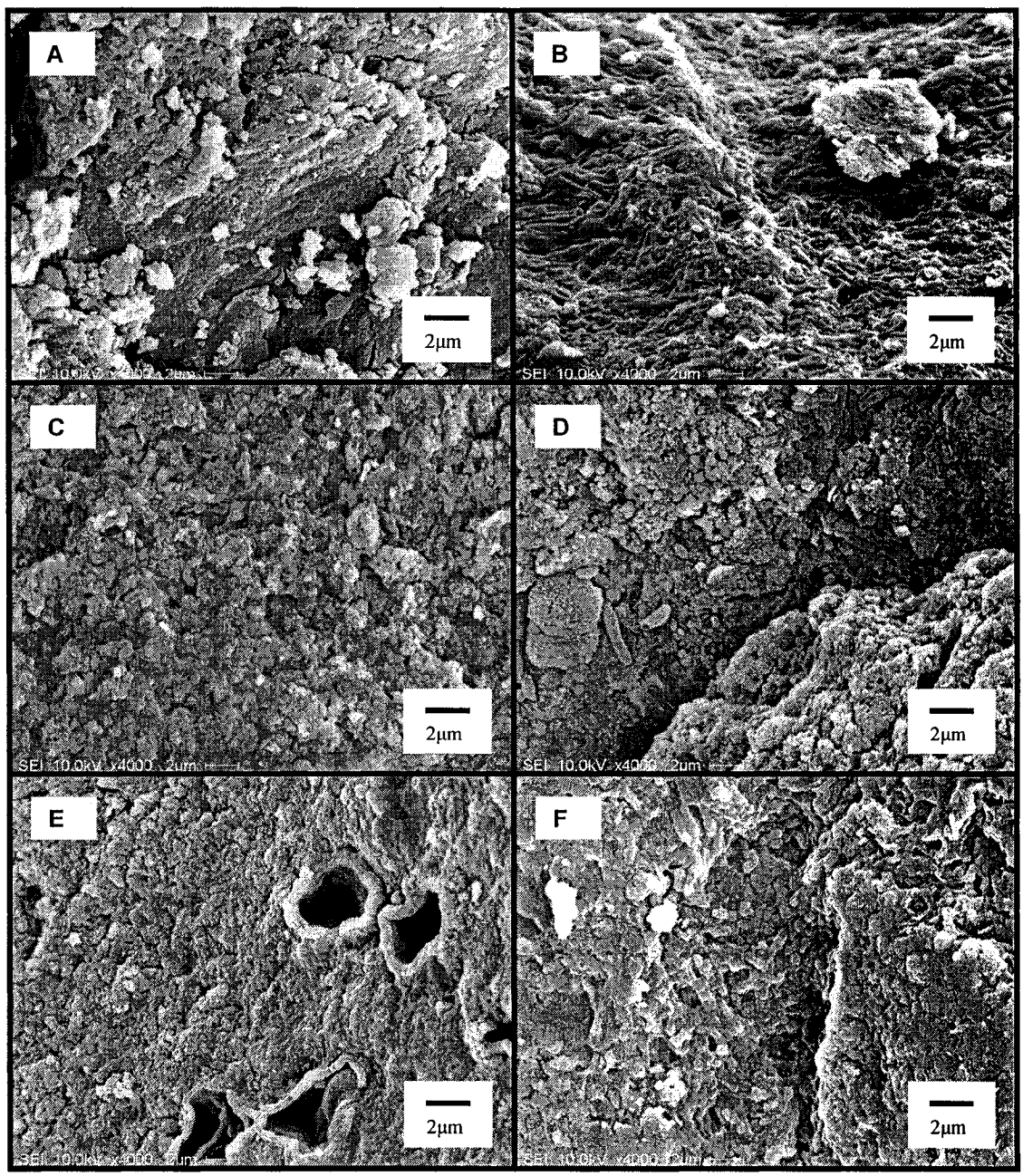

Figure 5.48: Scanning electron micrographs of anaerobic biomass at 4000X magnification. **A)** untreated biomass **B)** Ca-biomass **C)** Ca-biomass saturated with Pb **D)** Ca-biomass saturated with Cu **E)** Ca-biomass saturated with Ni **F)** Ca-biomass saturated with Cd

5.4 Metal competition

Multi-metal systems are usually present in effluents from different industries. The presence of other metal ions can derive in the modification of the biosorption equilibrium uptake capacity determined from a single-metal system. The possible competition between heavy metals for the binding sites on the microorganisms cell wall requires a study of the effects derived from the presence of other metal ions on the adsorption of the desired one.

Two-metal sorption systems were examined containing always two out of the four metals of interest: Pb, Cd, Cu, and Ni. All samples were prepared with the same molar concentration of all metals present. A high initial concentration of 12.5 meq/L was chosen in order to reach the maximum adsorption capacity obtained in individual tests.

Pb-system

Figure 5.49 shows the uptake capacity of lead by the biomass in a mixed system, results without any added competing metal are also presented. Figure 5.49 demonstrates that the single-metal sorption uptake capacity of the biomass for Pb was slightly inhibited by the presence of the other heavy metals in the system. The presence of Cu and Cd cations in separate systems reduced the biomass uptake capacity of Pb by only 6%. The presence of nickel reduced it by 11%.

Cu-system

Figure 5.50 shows the uptake capacity of copper by the biomass in a mixed system, results without any added competing metal are also presented.

Figure 5.50 demonstrates that the single-metal sorption uptake capacity of the biomass for Cu showed very little sensitivity to the presence of Ni cations, where only 9% reduction on the uptake capacity was observed. This could indicate the higher selectivity of the biomass for Cu over Ni. The presence of Cd cations inhibited the uptake of the Cu cations, the biomass uptake capacity for Cu was reduced almost 25% than the single Cu uptake capacity. The presence of Pb cations inhibited the uptake of the Cu cations, the biomass uptake capacity for Cu was reduced almost 71% than the single Cu uptake capacity which indicated the high favorability of Pb cations to be adsorbed onto the anaerobic biomass compared to Cu cations.

Ni-system

Figure 5.51 shows the uptake capacity of nickel by the biomass in a mixed system, results without any added competing metal are also presented. Figure 5.51 demonstrates that the single-metal sorption uptake capacity of the biomass for Ni showed very little sensitivity to the presence of Cd cations, compared to the presence of other metal ions, where a 14% reduction on the uptake capacity was observed. This could indicate the higher selectivity of the biomass for Ni over Cd. The presence of Cu cations inhibited the uptake capacity of Ni by 30% which is higher than the case in the Cu-system where Cu showed very little sensitivity to the presence of Ni cations, this could indicate the higher selectivity of the biomass for Cu than Ni. The same trend was found in the case of the presence of Pb cations were Pb inhibited the uptake capacity of Ni by 37%, while

Pb showed very little sensitivity to the presence of Ni cations, this would also indicate the higher selectivity of the biomass for Pb over Ni.

Cd-system

Figure 5.52 shows the uptake capacity of cadmium by the biomass in a mixed system, results without any added competing metal are also presented. Figure 5.52 indicates that Cd was the least favourable to be adsorbed by the biomass among the three other metals studied. The presence of Ni cations inhibited the uptake capacity of Cd by 36%, which is higher than the case in the Ni-system where Ni uptake capacity was reduced by only 14% in the presence of Cd cations. The same trend was found in the case of the presence of Pb cations, which inhibited the uptake capacity of Cd by 66%, which is higher than the case in the Pb-system where Pb showed very little sensitivity to the presence of Cd cations. The presence of Cu cations inhibited the uptake capacity of Cd by 60% which is higher than the case in the Cu-system where Cd inhibited the uptake capacity of Cu by 25%.

The results from competition experiments of equimolar binary solutions of Pb, Cd, Cu, and Ni, are summarized in Table 5.11. Table 5.11 summarises the percentage of removal and adsorption capacity values obtained in each test. The total metal adsorbed (last column of Table 5.11) increases in all cases compared to the values obtained for each metal in single metal tests. In spite of that, the total capacity of adsorption is always lower than the sum of the individual adsorption capacities of each metal taking part in the test. The decrease of

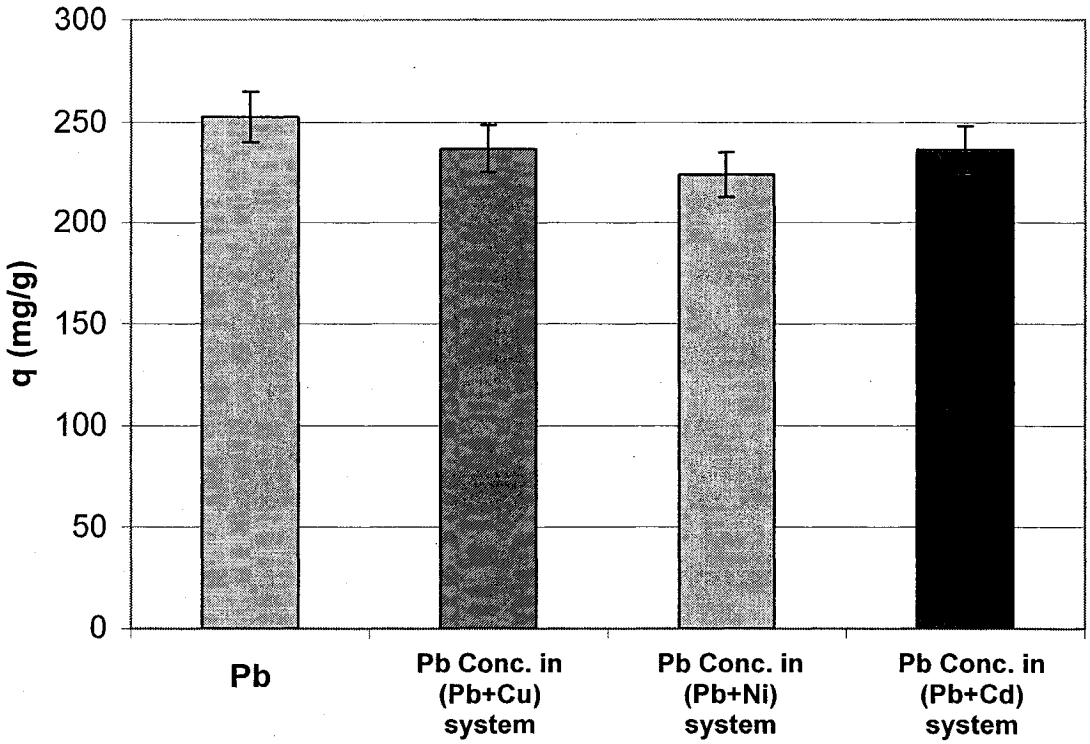

Figure 5.49: Pb competition with Cu, Ni, and Cd

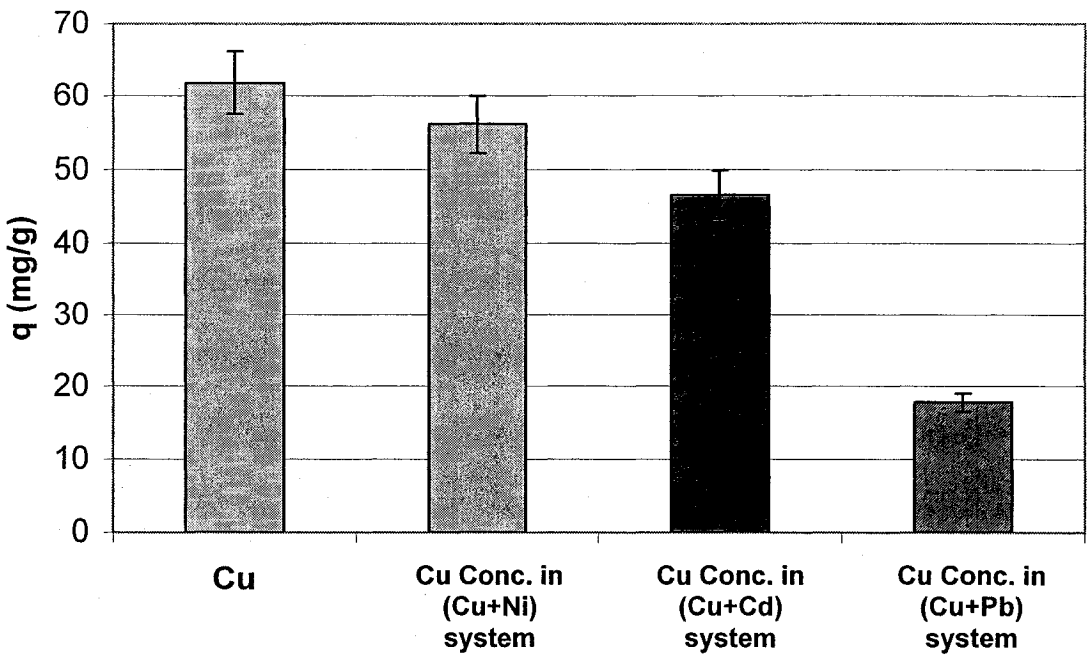

Figure 5.50: Cu competition with Pb, Ni, and Cd

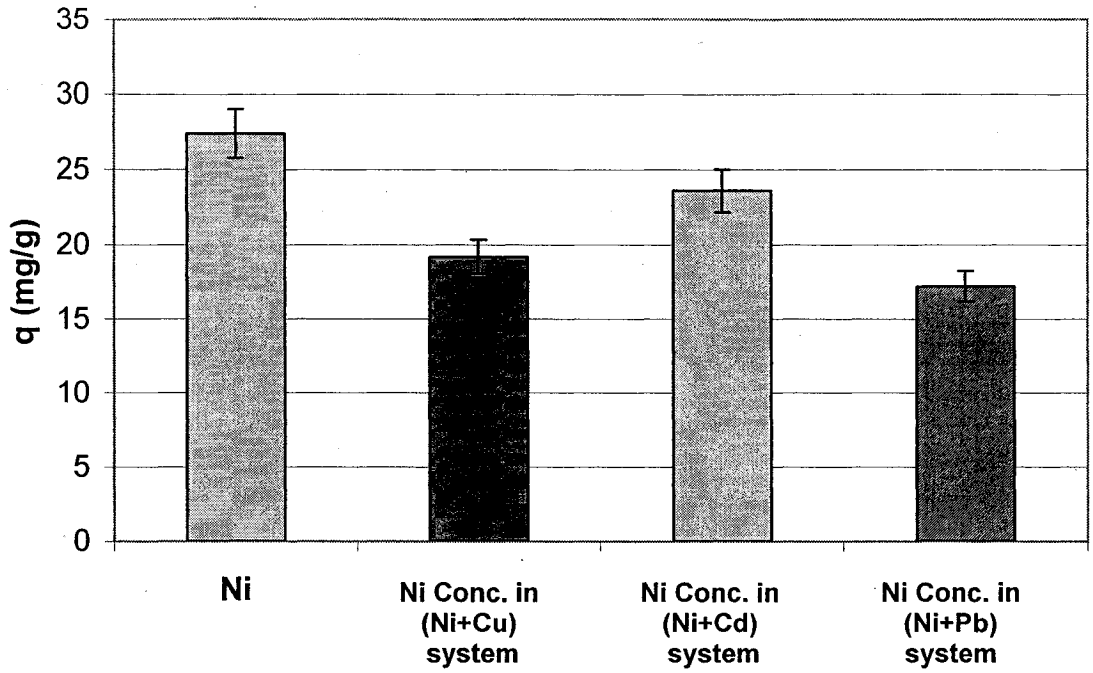

Figure 5.51: Ni competition with Pb, Cu, and Cd

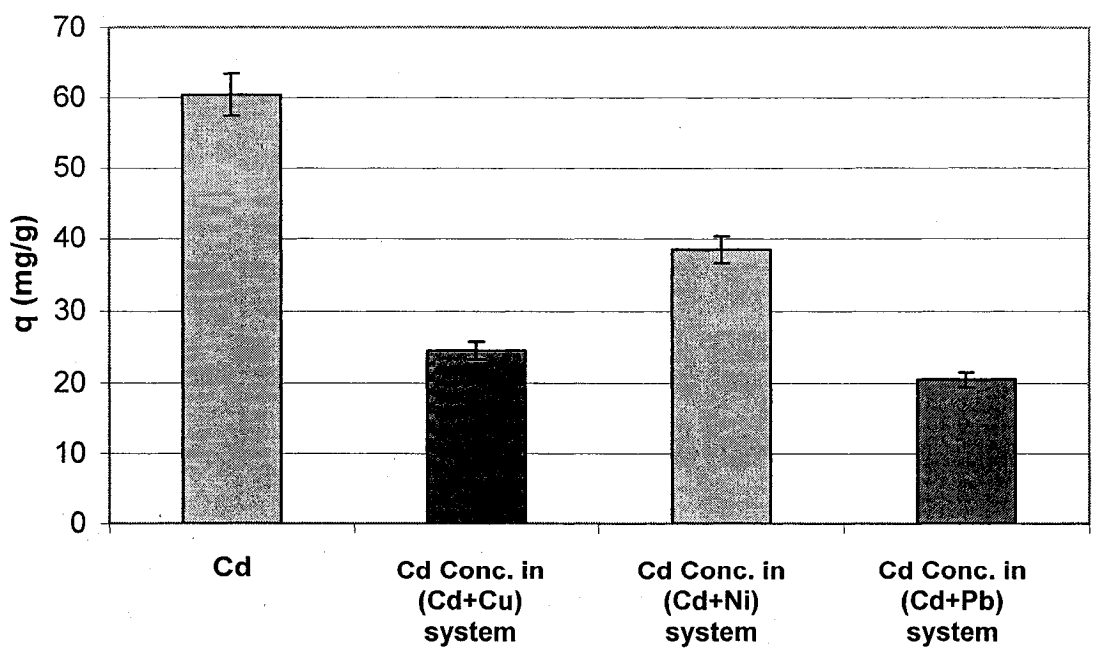

Figure 5.52: Cd competition with Pb, Cu, and Ni

Table 5.11: Single metal uptake capacity and competition results in binary systems at initial metal concentration 12.5 meq/L.

Metal			Metal			Total metal adsorbed (mg/g)
	mg/g	% removal		mg/g	%removal	
Cu	61.83	77.8	Cu	56.1	70.6	75.3
			Ni	19.2	44.0	
			Cu	46.6	58.7	71.1
			Cd	24.5	17.4	
			Cu	17.8	22.4	254.8
			Pb	237.0	93.8	
Ni	27.4	62.7	Ni	19.2	44.0	75.3
			Cu	56.1	70.6	
			Ni	23.6	54.1	62.1
			Cd	38.5	27.4	
			Ni	17.2	39.3	239.7
			Pb	222.5	85.6	
Pb	252.6	97.5	Pb	237.0	93.8	245.8
			Cu	17.8	22.4	
			Pb	223.9	86.4	241.1
			Ni	17.2	39.3	
			Pb	236.4	91.3	256.9
			Cd	20.5	14.6	
Cd	60.5	43.1	Cd	24.5	17.4	71.1
			Cu	46.6	58.7	
			Cd	38.5	27.4	62.1
			Ni	23.6	54.1	
			Cd	20.5	14.6	256.9
			Pb	236.4	91.3	

adsorption capacity compared to the single metal systems observed for all metals with exception of lead, reflects the existence of a competition between the four metals studied for the binding sites present in the cell wall.

The affinity order of anaerobic biomass for the four metals under study has been established as:

$$Pb > Cu > Ni > Cd$$

The affinity order obtained from single metal tests was almost in agreement with the affinity order in binary metal system only for the case of Cd and Ni where the uptake capacity of cadmium was higher than that of nickel. The affinity order in a single metal system was established to be:

$$Pb > Cu > Cd > Ni$$

Thus the higher uptake capacity of the biomass for a certain metal does not mean that that metal is more preferable by the biomass than other metals.

The results of this study suggest that the available binding sites on the biomass may have different affinity towards specific metal ions. The Pearson classification categorized metals into three broad categories: those that are polarizable or "soft", those that are non-polarizable or "hard" and those that are bordline (Williams et al., 1998). Lead, copper and nickel ions are classified in the bordline category according to this classification, while Cd ions fall into the soft category (SenGupta, 2002). According to Buffle (1998) soft cations form more stable complexes with soft donors while hard cations prefer hard donors.

Examples of hard ligands are carbonate, phosphate, sulfate, carboxylate and hydroxyl groups (Bell, 1977; Nieboer and Richardson, 1980) soft ligands include sulfhydryl and amino groups. The fact that cadmium ions were the least favorable by the biomass could indicate that the main functional groups involved in heavy metals sequestering are mainly hard ligands (i.e. carbonate, phosphate, sulfate, carboxylate and hydroxyl groups).

The binding strength of a metal ion to the biomass is dependent upon other different factors such as, the hydration and hydrolysis effects, ionic binding and covalent binding of a metal ion. Each will now be examined:

Hydration effects

According to Buffle (1988) electrostatic binding of metal ions is associated with a change in the orientation of the hydrated water molecules. If the hydrated water molecules are not held strongly by the metal ion the change in the hydration state can occur more easily. The effective hydrated radius is larger than the crystal ion radius (Table 5.12). In general the larger the effective hydrated radius the larger the hydration energy of an ion (i.e. the smaller the crystal radius) (Russell, 1980).

According to Jain and Wagner (1980), when the binding is weak hydration effects can be dominating. In this case, larger ions (comparing crystal radii of ions of the same charge) that are less strongly hydrated are preferably accumulated at the interface.

Table 5.12 shows that lead which has the highest affinity order for being adsorbed by the biomass has the lowest hydration radius while cadmium, i.e. the

least favorable by the biomass, has the highest hydration radius. This coincides with the fact that less strongly hydrated ions are preferably accumulated at the interface. It can be also seen from this table that lead is the largest ion among the studied metal ions (comparing crystal radii of ions). According to Haug and Smidsrod (1970) larger ions may fit into a binding site and bind to several groups simultaneously.

Ionic binding

Negatively charged groups can attract metal cations. The higher the charge density of both biosorbent and metal ion the stronger the interaction. Electrostatic effects may become the dominant factor if a strong electric field is present such that higher charge density ions are bound more strongly (Jain and Wagner, 1980). Since the cation retains its hydrated water molecules in aqueous solution, the hydrated cation radius is more characteristic for electrostatic attraction. According to Marcus and Kertes (1969), the selectivity increases with increasing charge and decreasing hydrated radius. Phillips and Williams (1965) used the charge density z^2/r_{hyd} (with r_{hyd} being the cation hydrated radius) as a measure for the strength of ionic binding. Table 5.12 shows that the ionic binding strength of the studied metal ions is almost the same. According to Pauling (1967), the ratio between ionic binding to the total binding strength is $1 - \exp(-\Delta x^2/2)$ where Δx is the difference between the electronegativity (x) of the metal ion and the one of oxygen. Since the parameter z^2/r_{hyd} expresses the ionic binding strength and the parameter $1 - \exp(-\Delta x^2/2)$ is supposed to characterize the relative contribution of ionic binding to the total binding strength, one can

obtain an indicator for the total binding strength by dividing z^2/r_{hyd} over $1 - \exp(-\Delta x^2/2)$. This parameter will be called ξ and it is listed in Table 5.12.

Table 5.12 shows that the more adsorbed lead and copper ions have a higher total binding strength than the lower affinity metal ions nickel and cadmium.

Covalent binding

Sharing of the electrons is involved in covalent binding. According to Dean (1985) the more similar the electronegativities of the metal ion and the coordinating atom of the ligand, the higher the covalent character of the bond. The selectivity (or binding strength) increases with increasing polarizability of the ion (Marcus and Kertes, 1969). Nieboer and McBryde (1973) introduced the parameter $x^2(r_{cryst} + 0.85A^o)$ as a measure for the strength of covalent binding, where $85A^o$ stands for the contribution of N or O donors to the bond distance. Table 5.12 shows that more adsorbed lead and copper ions have higher strength of covalent binding than the lower affinity metal ions nickel and cadmium.

Hydrolysis of metals

According to Alloway (1990) the selectivity of metals to be adsorbed is increased with those metals that are most able to form hydroxy complexes. Therefore, the pK (equilibrium constant) values of the reaction $M^{2+} + H_2O = MOH^+ + H^+$ determine the adsorption behavior of the different metals. Selectivity increases with decreasing pK values. According to Reddad et al. (2002) lower pK values would lower the degree of solvation of metal ions, thus enabling them to better approach the solid surface and exhibiting significant adsorption as for lead

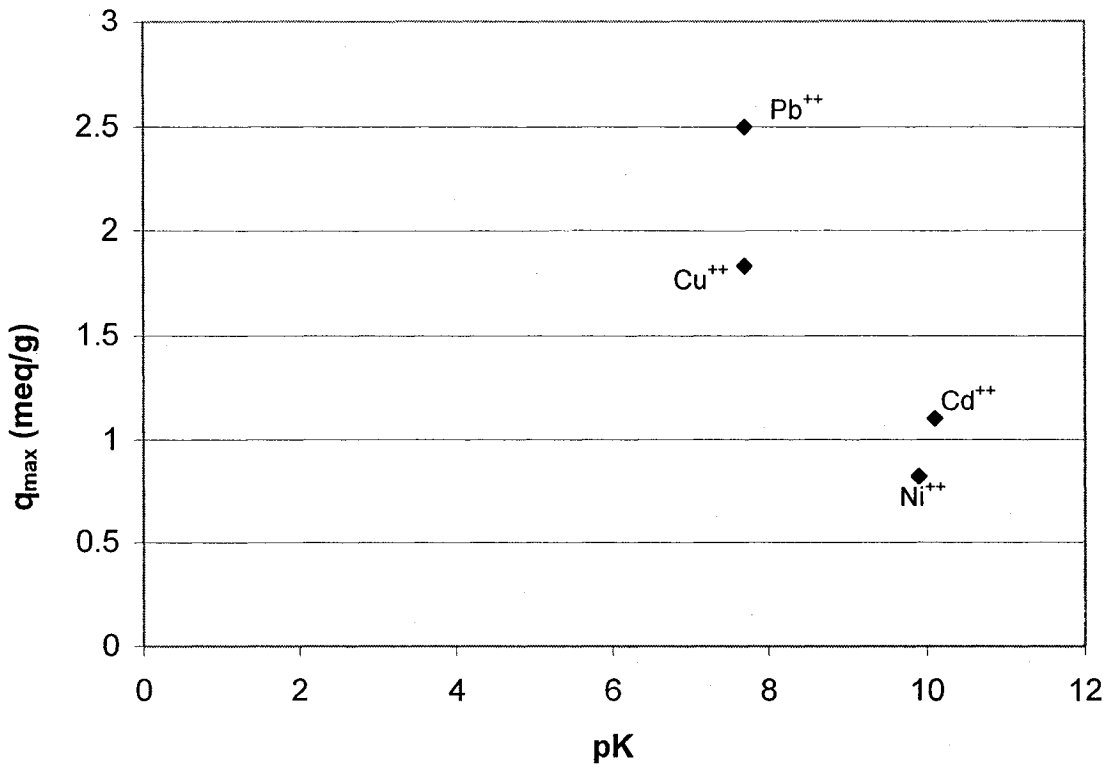

Figure 5.53: Relationship between q_{max} versus the first hydrolysis constant pK of metal ions

Table 5.12: Parameters characterizing the binding strength of metals

Metal	z^a	$r_{cryst}{}^b$ (A°)	$r_{hyd}{}^c$ (A°)	x^d	$z^2/r_{cryst}{}^e$ (1/A°)	$z^2/r_{hyd}{}^f$ (1/A°)	B^g (A°)	pK^h	ξ^i (1/A°)
Pb	2	1.19	4.01	1.8	3.36	1.00	6.61	7.7	1.94
Cu	2	0.73	4.19	2.0	5.48	0.95	6.32	7.7	2.22
Ni	2	0.69	4.04	1.8	5.80	0.99	4.99	9.9	1.92
Cd	2	0.95	4.26	1.7	4.21	0.94	5.20	10.1	1.69

[a] Charge (Russell, 1980)
[b] Crystal radius (Evans, 1993)
[c] Hydrated ion radius (Marcus and Kertes, 1969)
[d] Electronegativity (Russell, 1980)
[e,f] (Phillips and Williams, 1965)
[g] Parameter for covalent binding $x^2(r_{cryst} + 0.85)$ (Nieboer and McBryde, 1973)
[h] Equilibrium constant (Alloway, 1990)
[i] Parameter for total binding strength = $(z^2/r_{hyd}) / (1 - \exp(-\Delta x^2/2))$

and copper (Table 5.12). According to Reddad et al. (2000) relating to the hydrolysis constant, a linear relationship could be drawn with the maximum adsorption capacities q_{max} as shown in Figure 5.53.

Overall the tendency of stronger binding for lead and copper is probably due to the high covalent binding (characterized by $x^2(r_{cryst} + 0.85A^o)$), lower pK values, smaller hydrated radius (except for the relation between Ni and Cu), and stronger over all binding (characterized by ξ).

In general, it could be concluded that the binding strength increases with:

- Increasing ionic radius and decreasing charge, if binding is weak and largely due to hydration effect.
- Decreasing hydrated radius and increasing charge, if binding intermediately strong and due to electrostatic effects.
- Decreasing electronegativity difference, if binding is strong and covalent.

5.5 Effect of wastewater composition

As can be seen from the previous section the feasibility and efficiency of a biosorption process depends not only on the properties of the biosorbents, but also on the composition of the wastewater. To confirm the competition between metals for the binding sites of the anaerobic biomass established in binary metal systems, adsorption test in a flow-through column was undertaken. The column was fed with an equimolar mixture of Pb, Cd, Cu, and Ni.

The selectivity of the biomass for Pb over the other three metals is well exhibited by the results obtained using the flow-through column. Figure 5.54

displays the concentrations of Pb, Cd, Cu, and Ni in the column effluent as a function of bed volumes for the sorption experiment during which the column packed with the biomass in Ca-form was fed with an equimolar mixture of the metals (2 meq/L). As can be seen in Figure 5.54 Ni and Cd, due to their low affinity, broke through the column faster than Pb and Cu at the 4 and 15 bed volume mark respectively. Also Cu broke through the column faster than Pb at the 30 bed volume mark. Lead was the last metal to break through the column at the 150 bed volume mark. At approximately the 20, 25, 55, 200 bed volume mark, the concentration of Ni, Cd, Cu, and Pb in the column effluent plotted in Figure 5.54 reached the level of Ni, Cd, Cu, and Pb in the feed, i.e. $(C/C_o) = 1$, respectively. At this point the column feed is completely in equilibrium with all metal ions sorbed on the biomass. Subsequently, thereafter, Ni, Cd, and Cu were no longer taken up by the biosorbent and hence trickled through the packed-column as an inert. The fact that the C/C_o for Ni, Cd, and Cu continued rising above 1 in Figure 5.54 even after 20, 25, and 55 bed volumes respectively may be explained by the ion exchange between Ni, and Cd and Cu and Pb, whereby Pb and Cu from the solution were displacing Cd and Ni bound to the biosorbent. Pb also displaced Cu bound to the biosorbent. Since no more Ni, Cd and Cu were being sorbed from the liquid beyond 20, 25, and 55 bed volumes respectively, the released Ni, Cd, and Cu increased the overall concentration of Ni, Cd, and Cu in the liquid above the level present in the column influent. This phenomenon is commonly referred to as "overshooting" (Kratochvil and Volesky, 1998; Figueira et al., 2000). The overshooting of Ni and Cd is caused due to ion

exchange effect, whereby the high affinity Cu and Pb desorb the low affinity Ni and Cd which had previously sorbed onto the biomass in the bed. The high affinity Pb also desorbed the lower affinity Cu which had previously sorbed onto the biomass this caused the overshoot of Cu. The fact that no overshoot of Pb occurred shows the high affinity of Pb to the biomass compared with the other three metals. The three other metals could not desorb Pb bound to the biosorbent. Therefore, the overshoot provides yet another piece of evidence that the principal mechanism of biosorption in anaerobic biomass is ion exchange.

The overshoot peak depends on the relative affinity of the individual metals for the biosorbent. Comparing the peaks obtained from Figure 5.54, the affinity order of anaerobic biomass for the four metals under study would be established as:

$$Pb > Cu > Ni > Cd$$

The affinity order obtained from binary metal tests is in agreement with the affinity order in the mixed system.

As shown in Figure 5.55 the removal of the metal ions from the bed was again accompanied by the elution of Ca ions from the packed-bed. The concentration of Ca ions reached a fairly high value of 20.2 meq/L. The total amount of eluted Ca was almost 10.9 meq. The high peak value of desorbed Ca ions compared to the other metals indicates the lower affinity of Ca ions.

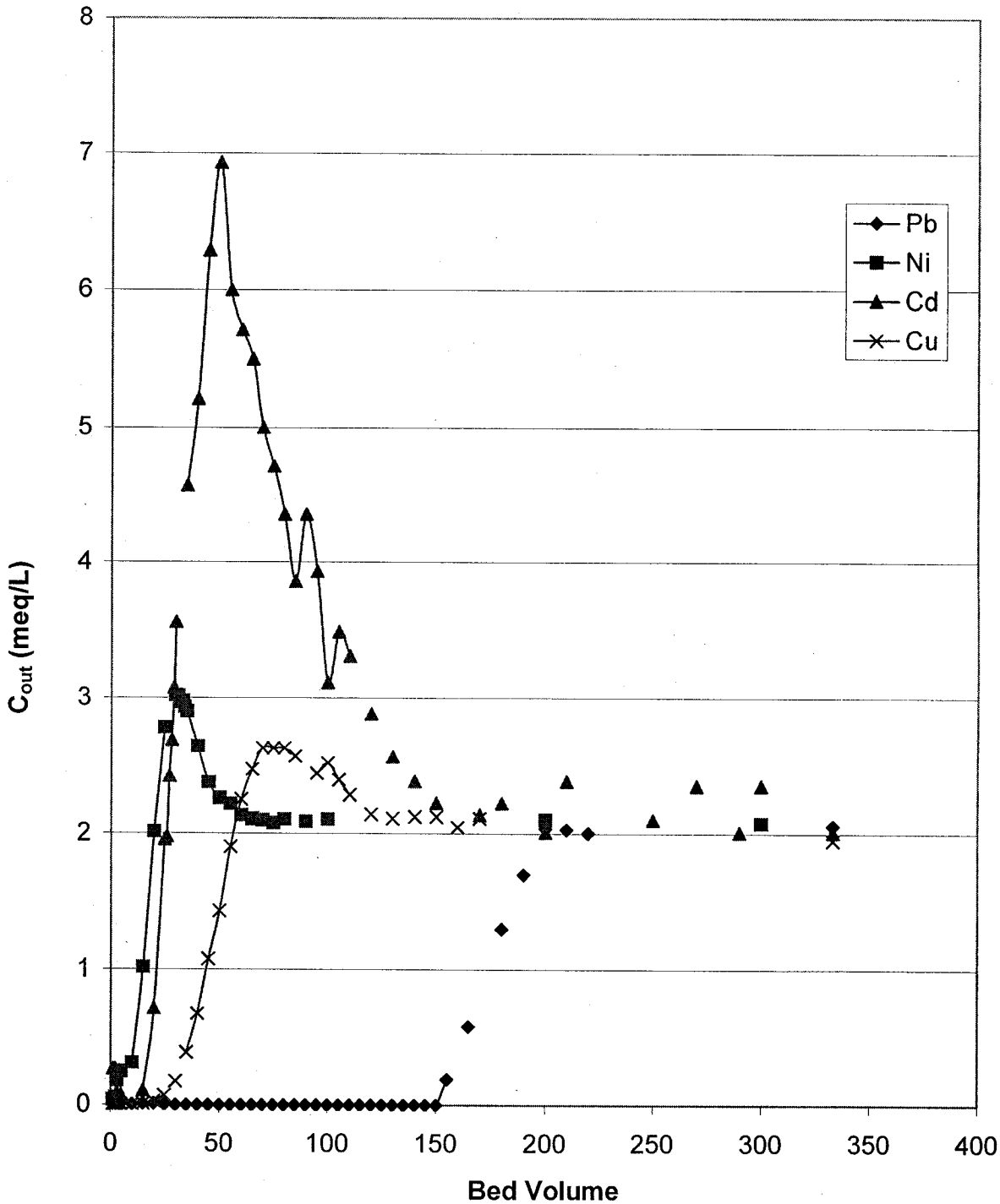

Figure 5.54: Breakthrough curves of Ni, Cd, Cu, and Pb from a flow-through column treating and equimolar mixture of Ni, Cd, Cu, and Pb (Bed volume = 45 mL, Flow rate = 1.3 mL/min, C_0= 2 meq/L)

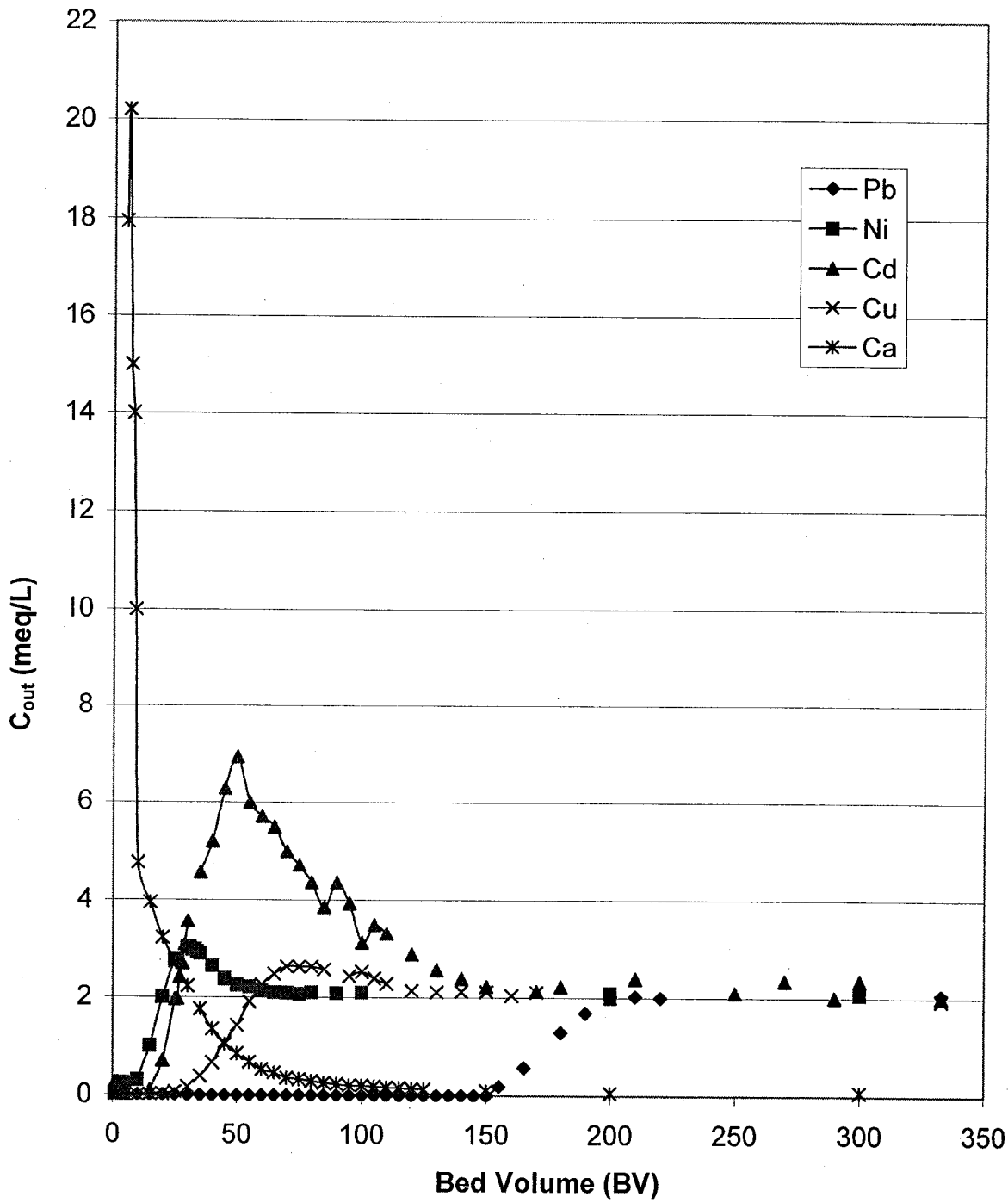

Figure 5.55: Elution of Ca cations during the biosorption of Ni, Cd, Cu, and Pb from a flow-through column treating and equimolar mixture of Ni, Cd, Cu, and Pb (Bed volume = 45 mL, Flow rate = 1.3 mL/min, C_0= 2 meq/L)

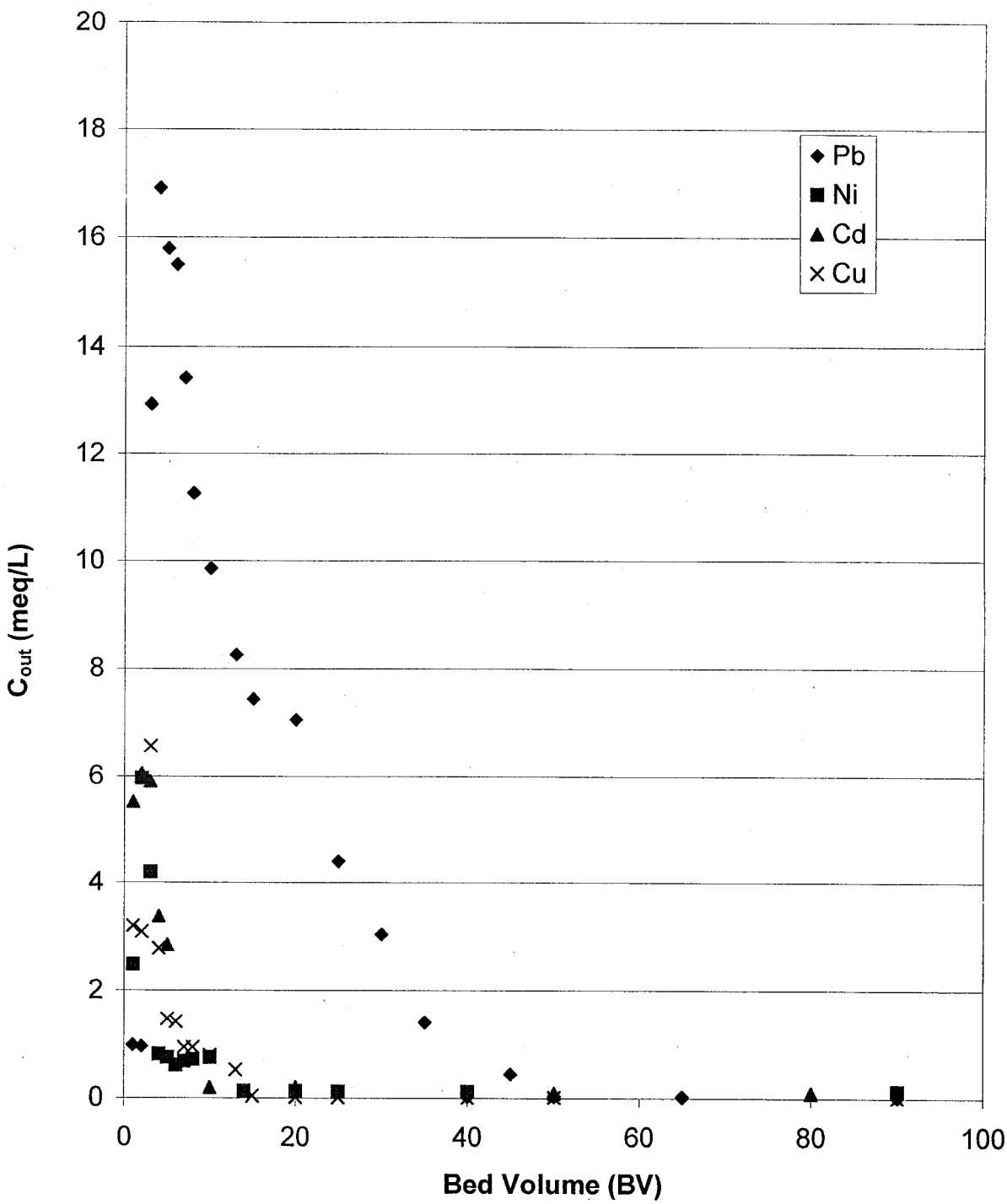

Figure 5.56: Desorption of Pb, Cd, Cu, and Ni from a packed-bed column using 0.5M $CaCl_2$ (Bed Volume = 45 mL, Flow rate = 1.3 mL/min)

Figure 5.56 shows the elution peaks of Pb, Cd, Cu, and Ni obtained during desorption carried out with 0.5M $CaCl_2$. The peaks of Cu, Cd, and Ni are much smaller than the peak of Pb, which is in agreement with the higher selectivity of the biomass for Pb. The smaller peaks of Cu, Cd, and Ni are due to the fact that desorption of these metals occurred during the adsorption of Pb into the biomass.

The condition under which overshoots occur can be formulated as follows: Low-affinity species present in the feed solution would overshoot in the column effluent only if a species with higher affinity is present in the feed solution. Consequently, a metal species may or may not overshoot in the column effluent depending on the metal species in the influent solution. Effluents containing one toxic species at a relatively high level in combination with one or more heavy metals at low levels that may not be considered toxic represent a special class of heavy-metal pollution. A typical example is an effluent produced at a copper mine containing a high level of Cu and traces of Cd and Ni (Kratochvil and Volesky, 1998). Usually, only the toxic metal with the high concentration is targeted for removal and thus it is expected that the affinity and the concentration of this metal will determine the efficiency of the biosorption process. Therefore, it is recommended to use the anaerobic biomass for the treatment of such industrial effluents in order to overcome the overshoot phenomenon. Since lead showed little effect for the presence of other metal species it would be our target metal for treatment. In the following section scale up of the biosorption process will be performed considering lead as our targeted pollutant.

5.6 Scale up of the process

It is not possible to design a column accurately without a test column breakthrough curve for the liquid of interest and the adsorbent solid to be used, this is what was done in the previous sections. In the subsequent sections, a scale-up and kinetic approach to design adsorption column is presented. The main advantage of using the scale-up method is its simplicity. The advantage of using the kinetic approach is that the breakthrough volume, V_b, may be selected in the design column to a desired value. In both of the approaches, the data obtained from the breakthrough curves will be used. As shown in the previous section, lead did not overshoot in the presence of other metal species because of its high affinity to be adsorbed on to the biomass. Since lead uptake capacity was slightly affected by the presence of other metal species, it would be our target metal for the scale up process.

5.6.1 Scale-up approach

This method was developed by Fornwalt and Hutchins (1966) for the design of carbon adsorption columns. The principal experimental information required is the breakthrough curve obtained from a test column, that has been operated at the desired flow rate in terms of bed volumes per unit time, Q_b, as the design column. The flow rate will be the same as the design column and will have the same contact time as the test column. Since the contact times are the same, it is assumed that the volume of liquid treated per unit mass of adsorbent,

$\bar{v}_b$, for a given breakthrough in the test column is the same as for the design column.

According to Reynolds and Richards (1992) the bed volume of the design column is given by

$$\text{Bed volume (BV)} = Q/Q_b \qquad (5.21)$$

where Q is the design liquid flow rate. The mass or weight of the adsorbent, M, for the design column is determined from

$$M = (BV)(\rho_s) \qquad (5.22)$$

where ρ_s is the adsorbent bulk density which was calculated in Chapter two. From the breakthrough curve for the laboratory column, the breakthrough volume, V_b, is determined for the allowable effluent solute concentration, C_a (Table 2). the volume of liquid treated per unit mass of adsorbent, $\bar{v}_b$, is then determined by

$$\bar{v}_b = V_b / M \qquad (5.23)$$

Where M is the mass of the adsorbent in the test column (11.0g). The mass of adsorbent exhausted per hour, M_t, for the design column is computed from

$$M_t = Q / \bar{v}_b \qquad (5.24)$$

Where Q is the design liquid flow rate, the breakthrough time T, is

$$T = M / M_t \qquad (5.25)$$

Where M is the mass of adsorbent in the design column. The calculated breakthrough volume, V_b, for the allowable breakthrough concentration, C_a, for the design column is

$$V_b = QT \qquad (5.26)$$

If the calculated breakthrough time, T, from equation 5.5 or calculated breakthrough volume V_b, from equation 5.6 is not acceptable, another liquid flow rate, Q_b, to give the required time or volume should be determined from the available breakthrough data.

This method can be applied to our case study for a wastewater treatment plant having a flow of 150 m^3/d with an initial lead concentration of 40 mg/L. Our column test was operated at a 2.0 BV/h to ensure the 30 min of contact time. Table 5.13 presents the design parameters for a biosorption column, obtained from the scale-up approach, operated to treat industrial effluent containing lead at an initial concentration of 40 mg/L. From Table 5.13 it can be found that almost a 3.0 m^3 biosorption column packed with anaerobic biomass would treat a volume of 3022 m^3 of an industrial effluent containing lead at a 40 mg/L concentration.

Table 5.13: Design parameters of the biosorption column obtained from the scale-up approach for lead

Design Parameter	Value
Usage rate (Kg/h)	1.6
Breakthrough time (day)	20.1
Breakthrough volume (m^3)	3021.8
Mass of biomass (Kg)	763.9
Bed volume (m^3)	3.1

5.6.2 Kinetic approach

This method uses a kinetic equation based on the derivation by Thomas (1948). The kinetic equation may also be derived from an extension of the Bohart and Adams (1920) equation. Breakthrough curves obtained from experimental tests is required for this approach.

The equation of Thomas can be introduced in the following design equation (Reynolds and Richards, 1992):

$$\ln [(C_o/C) - 1] = [(k_1 q_{max} M)/Q] - [(k_1 C_o V)/Q] \qquad (5.27)$$

where

C: effluent solute concentration (mg/L)

C_o: influent solute concentration (mg/L)

k_1: rate constant (L/sec.kg)

q_{max}: maximum uptake of sorbed solute (kg/kg)

M: mass of adsorbent (kg)

V: throughput volume (L)

Q: flow rate (m^3/sec)

From equation 5.27, it can be seen that drawing the relation between ln $[(C_o/C) - 1]$ and V will give a straight-line with a slope of $(k_1C_o)/Q$ and an intersection with the y-axis at a point equal to $(k_1q_oM)/Q$ (Figure 5.57).

The experimental column used to obtain the breakthrough curves for the kinetic design approach should be operated at the same flow rate in terms of bed volumes per hour as the design column. Applying this method on our case study assuming that a wastewater treatment plant having a flow of 150 m^3/d. Our column test was operated at a 2.0 BV/h to ensure the 30 min of contact time. Table 5.14 presents the design parameters for a biosorption column, obtained from the kinetic approach, operated to treat industrial effluent containing lead at an initial concentration of 40 mg/L. It could be seen from Table 5.14 that to treat a volume of 1000 m^3 of an industrial effluent containing lead at a 40 mg/L concentration the required volume of the biosorption column, packed with anaerobic biomass, would be 1.7 m^3.

Table 5.14: Design parameters of the biosorption column obtained from the kinetic approach for lead

Design Parameter	Value
Maximum uptake capacity, q_{max} (kg/kg)	0.19
Rate constant (L/sec.kg)	0.16
Breakthrough volume (m^3)	1000
Mass of biomass (kg)	420
Bed volume (m^3)	1.70

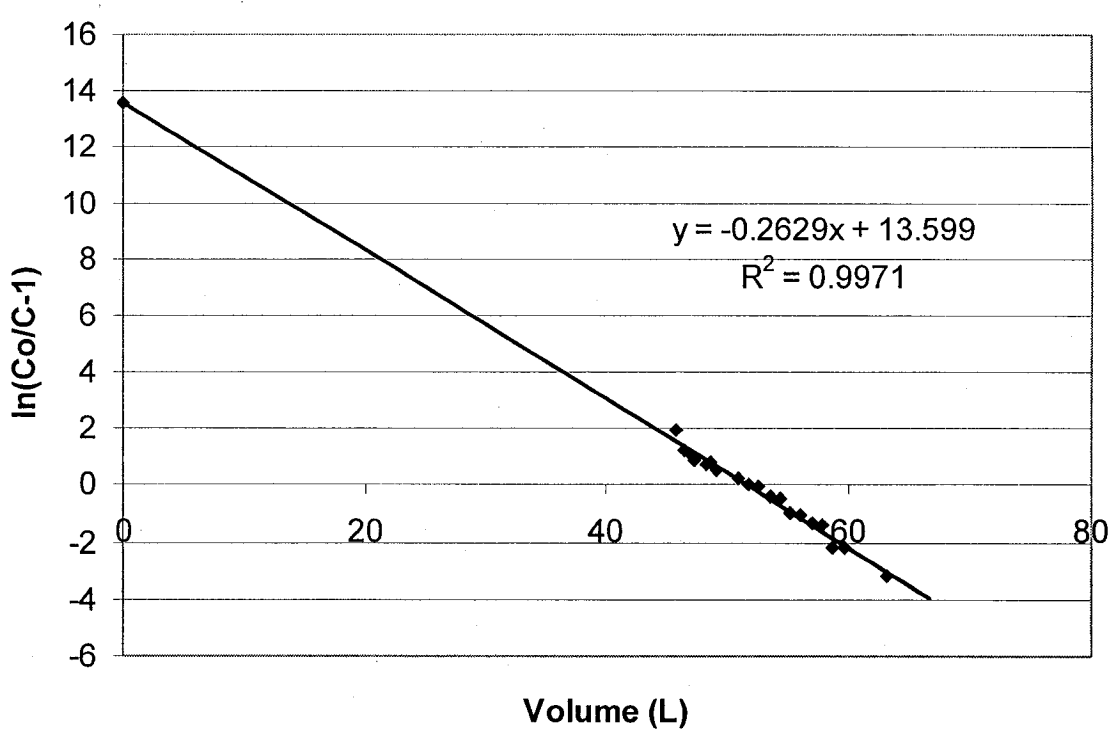

Figure 5.57: Kinetic approach scale up for Pb

6 – CONCLUSIONS, ORIGINAL CONTRIBUTIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

6.1 Conclusion

It was found that the efficiency of non-viable cells in biosorbing metal ions was less than that of the living cells. But in order to overcome the disadvantages of using viable biomass, non-viable biomass was to be used.

Ca treated biomass had the highest uptake value than that for the untreated biomass and the other ionic forms of the biomass studied (i.e. H and K) in the case of the four metals tested.

The solution pH affected metal sorption capacity, which increased with rising pH. It was found out that over the pH range 4 to 5.5, pH-related effects were not significant. Meanwhile, at the pH value 3.5 and 3 the q value started to decrease.

The rate of metal uptake by anaerobic biomass was found out to be very fast. Within the first 5 minutes of contact, almost 75% of the total metal uptake was completed. Adsorption equilibrium was reached almost 30 minutes after biomass addition. These results show that the actual chemical reaction of metal ion binding to the biomass is a fast phenomenon. The very fast sorption

observed with the anaerobic biomass represent an advantageous aspect when water treatment systems are designed the implication is that the material would be suitable for the continuous flow system.

The maximum uptake capacity of the biomass, q_{max}, for Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+}, was found to be 255, 55, 60 and 26 mg/g respectively (2.46, 1.74, 1.06 and 0.88 meq/g respectively). Lead removal capacity of anaerobic biomass was found out to be higher than most of the biosorbents available in the literature. The data pertaining to the sorption dependence upon metal ion concentration fitted the Langmuir isotherm. The kinetics of sorption of Pb^{2+}, Cu^{2+}, Cd^{2+} and Ni^{2+} were modelled using a pseudo-second order rate equation.

Biosorption of heavy metals in a continuous flow column packed with Ca treated anaerobic biomass was shown to be highly effective. The average biosorption column capacity for lead, copper, cadmium and nickel before breakthrough occurred was 1.55, 0.88, 0.89 and 0.51 meq/g respectively. Biosorption of heavy metals was accompanied by Ca ions release from the Ca treated biomass. While the feed pH was 4.0 for the four metals studied, the exit solution pH increased to a value around (pH 5.0 ~ 5.5) for most of the experiments before the breakthrough point. The heavy metals saturated column was regenerated by rinsing with 0.5 M $CaCl_2$ solution. The total amount of metal collected by the $CaCl_2$ eluant showed that almost 78%, 79%, 83%, and 100% of Pb, Cu, Cd and Ni was recovered respectively.

Ion exchange was identified to be the dominant mechanism for the biosorption of nickel by the anaerobic biomass. For copper and cadmium 80% of

the total amount adsorbed was attributed to ion exchange. The remaining 20% of the total amount adsorbed was attributed to the extent of a complexation/ chelation process completing the ion exchange one. For the case of Pb ions it was found out that ion exchange was attributed to be almost 50% of the total uptake mechanism. 30% of the total uptake mechanism was attributed to the extent of a precipitation mechanism. The remaining 20% was attributed to the extent of a complexation/chelation process completing the ion exchange and precipitation one.

The affinity order of anaerobic biomass for the four metals under study has been established as:

$$Pb > Cu > Ni > Cd$$

Issues regarding the limitations of biosorption process imposed by the wastewater composition in flow-through columns were addressed by studying the breakthrough curves obtained from a fixed bed column fed with an equimolar mixture of Pb, Cd, Cu, and Ni. The selectivity of the biomass for Pb over the other three metals was well exhibited by the results obtained using the flow-through column. The fact that lead was the last metal to break through the column and that no overshoot of Pb occurred showed the high affinity of Pb to the biomass compared with the other three metals.

Finally, it could be concluded that anaerobic biomass is a promising biosorbent that could make a successful commercial application in the market due to its particulate shape, porous structure, excellent settling ability, high

mechanical strength, very fast sorption rate, and high selectivity and uptake capacity for lead. Using anaerobic biomass it was possible to reach very low metal concentration of the treated wastewater (initial concentrations of 40 mg/L, final concentration 0.00 – 0.01 mg/L).

6.2 Original contributions

- Anaerobic granules were introduced as a novel type of biosorbent for the removal of lead, copper, cadmium, and nickel from aqueous solutions. Unlike most of the other biomass, immobilization or stiffening is not necessary prior to using the biomaterial.
- The influence of the state of the biomaterial (viable or non viable), solution pH, and ionic form of the biomaterial on the biosorption of the four studied metals was studied.
- It has been demonstrated that the anaerobic biomass can sorb lead, copper, cadmium and nickel effectively. The maximum uptake capacity of the biomass was found to be 2.46, 1.74, 1.06 and 0.88 meq/g for lead, copper, cadmium and nickel respectively.
- The biosorption of lead, copper, cadmium and nickel was studied in a continuous flow system to establish the link between the biosorption equilibrium isotherms and the performance of biosorption columns.
- The uptake mechanisms by the anaerobic biomass for each metal studied were identified.

- The affinity order of anaerobic biomass for the four metals under study has been established.
- Issues regarding the limitations of biosorption process imposed by the wastewater composition in flow-through columns were addressed.

6.3 Recommendations for future work

- Investigate the uptake capacity and stability of the biomass as a function of the number of adsorption-desorption cycles.
- To evaluate the biosorption process for different types of wastewater of varying metal content.
- Evaluate the biosorption process using the viable biomass taking into account the LC50 's of the biomass for the different metals
- Investigate the potential of using the biosorption process as a remediation process for contaminated underground water.
- Establish a mathematical model that would link between the Langmuir model for batch tests and breakthrough curves in the fixed bed column.

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