

**REMOVAL OF COPPER AND NICKEL FROM SOLUTION BY
THE NON-VIABLE BIOMASS OF THE WATER FERN
AZOLLA FILICULOIDES IN AN UPSCALED FIXED-BED
COLUMN SYSTEM**

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ABSTRACT

The potential of non-viable *Azolla filiculoides* for the removal of Cu and Ni from aqueous solutions and the possibility of scaling up existing lab scale *Azolla* column systems was investigated. The effects of factors such as metal starting concentration, pH and two metals in solution on the removal of Ni and Cu from aqueous solution by dried and crushed *Azolla* biomass were studied in batch systems. Aqueous solutions of Ni with starting concentrations between 1000 and 2000 $\mu\text{mol/l}$ gave the most efficient Ni removal by *Azolla* biomass. For Cu the optimum starting concentration for adsorption was 50 $\mu\text{mol/l}$. The adsorption capacity of both Cu and Ni increased as the starting pH of the sorption media increased. The optimum pH for Ni adsorption was found at pH 7 and for Cu, at pH 5. *Azolla* biomass had a higher maximum binding capacity (q_{max}) for Cu than for Ni at pH 5. The removal of both Cu and Ni showed little or no variation with the presence another metal in solution. Kinetic studies show that both Cu and Ni adsorbed rapidly onto the *Azolla* biomass.

The removal of Cu and Ni from aqueous solutions using non-viable *Azolla* biomass was investigated in a lab scale fixed-bed column and an upscaled 4L column system. The non-viable *Azolla filiculoides* biomass when dried and used in a column for adsorption of Cu and Ni showed good physical stability under many different conditions. Preparation of the biomass before it could be used in the columns was very simple and did not involve any significant pretreatment steps. Prolonged exposure to UV light decreases *Azolla* biomass capacity for Ni and Cu adsorption.

Column adsorption of Cu and Ni from aqueous solutions was successfully upscaled approximately 100 times. Relative to the lab scale column, the 4L column performed better for the uptake of Cu and Ni per gram of biomass. The larger column was also able to operate at relatively higher flow rates. The biomass showed good reusability with little change in the amount of Ni adsorbed in 10 consecutive cycles. Electron micrographs showed little or no change in the physical structure and integrity of the *Azolla* biomass after exposure to mineral acids, Ni solution and high flow rates over 10 consecutive adsorption and desorption cycles. As much as 80% Ni and 70 % Cu was recovered when desorption profiles were generated using 0.1M HCl as a desorption agent.

The 4L column system was also tested using a highly concentrated Ni plating bath solution (Nicrolyte 1). Only 18 % of the Ni could be removed from the expended Nicrolyte 1 plating solution after treating only 25L, indicating that *Azolla* biomass is more suited for removal of metals from more dilute industrial effluents.

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CHAPTER 1

LITERATURE REVIEW

1.1 Water in South Africa and metal pollution

Water is a very important natural resource. It is essential to life, social development and economic progress (Department of Water Affairs, 1986). It is also a finite, though regenerating resource, and needs to be conserved if it is going to last. The responsibility lies with mankind to protect the ability of water resources to sustain long term utilisation (MacKay, 1998). South Africa is faced with the ever growing problem of supplying a rapidly increasing population with water from a very limited water resource (SA Water Bulletin, 1999a). It is estimated that the rising demand for water will exceed the maximum supply by the turn of the century (Department of Water Affairs, 1986).

South Africa's lies in the drought belt of the globe. The average rainfall per annum is about 497 mm, which is well below the world average of about 860 mm. Rainfall is also unevenly distributed. Sixty five percent of the country receives only 500 mm annually and twenty one percent of the country receives less than 200 mm. To put this in perspective, an annual rainfall of 500 mm is usually regarded as the minimum required for successful dry-land farming (Department of Water Affairs, 1986). Another factor is high evaporation rates, which result in South Africa's runoff-to-rainfall ratio being amongst the lowest for any populated region in the world (Kidd, 1997).

Up until the end of the 19th century agriculture accounted for the majority of water use in South Africa. It wasn't until after World War II, as a result of rapid urbanisation and industrialisation, that legislation directed at managing the use of water by industry and curbing pollution caused by industry was introduced. Industry has a dual effect on a country's water supply. Firstly it reduces the quantity of water available to other users, and secondly, industrial effluent that is released back into a water source reduces the quality of the water. The Water Act (Act 54 of 1956) was intended to ensure equitable distribution of water for industrial and other competing users. It was also intended to provide strict control over pollution of water by industry (Kidd, 1997).

Toxic heavy metals have become an important factor in the contamination of our water resources. Pollution of the environment by heavy metals arises as a result of many activities, such as agriculture and sewerage disposal, but mainly as a result of industrial processing (Gadd and White, 1993). Almost all industries discharge at least one trace metal into the environment. In South Africa, where industrial effluents and hazardous waste are becoming a growing problem, the demand for effective biological means to clean up our water is rapidly increasing (SA Water Bulletin, 1999b). Along with the obvious environmental benefit of pollution control is the considerable economic advantages gained from careful waste management. The price of water in urban areas is increasing due to an increasing demand by industry and urban populations. Fines incurred for high pollution levels and the cost of effluent treatment are also contributing factors. There is also the possibility of reclaiming metals from waste effluents or reducing losses during metal processing (Zhao, 1997).

1.2 Electroplating industry

The electroplating industry is an important source of metal pollution when compared to some other manufacturing operations. The discharge of untreated electroplating effluent into natural water sources has contributed to water pollution in many industrial regions (UNEP, 1989). It is estimated that the annual water consumption by the electroplating industry in South Africa is approximately 9 million m³ of which 80 per cent is discharged as effluent. There are a number of sources of waste that result from metal finishing and plating operations, namely, degreasing, acid and alkaline cleanings, spent plating-bath solutions and rinse waters (SA Water Bulletin 1995). Although the spent plating bath solutions contain very high concentrations of potential polluting metals, the rinse waters while relatively lower in metal concentration, account for the largest portion (approximately 90 per cent) of water used for plating (Zhao, 1997). Common plating metals include nickel chromium, copper, zinc, cadmium, lead, iron and tin. For economic and environmental reasons, it is highly recommended that metals from the effluent are recovered and the rinse waters are recycled as far as possible (UNEP, 1989).

Table 1.1 presents the typical effluent characteristics of 16 electroplating factories surveyed in Hong Kong (Chiu *et al*, 1987)

Table 1.1 Characteristics of plating waste effluent discharge from electroplating shops.

Plating operation	Factory		mg/l						
	area (m ²)	pH	Cr	CN	Cu	Ni	Zn	Al	Ag
Cr ⁶⁺ , Ni & Zn	60	4.0	33	6	4	168	250	—	—
Cu, Ni & Cr ⁶⁺	70	5.2	20	4	2	30	—	—	—
Al anodizing	100	2.7	—	—	—	—	—	230	—
Cr ⁶⁺ , Ni & Cr ⁶⁺	120	2.5	31	4	2	13	—	—	—
Ni & Zn	150	8.2	1	—	—	95	10	—	—
Cu, Ni, Cr ⁶⁺ & Ag ⁺	150	1.7	9	1	—	8	—	—	2
Cu, Ni & Cr ⁶⁺	200	4.5	25	6	3	230	—	—	—
Cu, Ni, Cr ⁶⁺ & brass	200	7.0	40	1	11	365	15	—	—
Ni, Cr ⁶⁺ & Au ³⁺	300	5.6	38	—	—	3	—	—	—
Cu, Ni, Cr ⁶⁺ & brass	400	7.7	5	5	30	25	10	—	—
Range	20-1000	1.7 - 8.2	1-40	1-6	1-30	3-365	4-250	10-230	2-3

1.3 Drinking water quality criteria

Drinking water, by definition, should be fit for human consumption. The general criterion for water to be safe for drinking is that the concentration of a contaminant must be lower than the level at which it can be harmful to health. It is therefore necessary to have guidelines that regulate levels of environmental contaminants to ensure against harmful effects to public health and the environment with a large margin of error. Drinking water quality criteria (Table

1.2) represent the maximum level of a contaminant present at the specified concentration such that the water can be consumed with safety (Pieterse, 1989).

Table 1.2: Summary of proposed drinking water quality criteria for application in South Africa (Pieterse, 1989)

Element	Unit	Recommended Limit	Maximum limit
Arsenic	µg/l	100	300
Cadmium	µg/l	10	20
Chromium	µg/l	100	200
Cobalt	µg/l	250	500
Copper	µg/l	500	1000
Cyanide	µg/l	200	300
Gold	µg/l	2	5
Iron	µg/l	100	1000
Lead	µg/l	50	100
Manganese	µg/l	50	1000
Mercury	µg/l	5	10
Nickel	µg/l	250	500
Selenium	µg/l	20	50
Silver	µg/l	20	50
Titanium	µg/l	100	500

1.4 Heavy metal toxicity

Heavy metals can be defined according to their density (Gadd, 1986). They include about forty elements with a density greater than 5, ranging between highly toxic transition metals such as cadmium, lead and mercury; metals toxic at high concentrations such as nickel, copper and cobalt; precious metals and radionuclides (Sanyahumbi, 1999; Wilhelmi, 1997). Some metals, at low concentrations, such as copper, zinc and iron are essential for growth and metabolism in most organisms, although at higher concentrations, these metals and others can have harmful and even fatal effects (Forster and Wase, 1997; Wong and So, 1993; Gadd, 1986; Davies, 1983; Leland *et al*, 1978). The continuous distribution of increasing amounts of toxic metals into the biosphere and their inevitable transfer to the human food chain has become an important environmental issue which may lead to future health risks (Wilhelmi, 1997). Forster and Wase (1997) have identified metals that are of the greatest environmental concern in Table 1.3. Table 1.4 shows the toxicities for some of the metals to various fish species (Forster and Wase, 1997).

Table 1.3: Heavy metals that are of an environmental concern.

Cadmium	Nickel
Chromium	Silver
Cobalt	Tin
Copper	Zinc
Lead	Lanthanides/actinides
Mercury	

Table 1.4: Typical toxicities of heavy metals in fresh water

Metal	Affected species	Effect	Concentration mg/l
Cadmium	Common carp	96 h LC50	22
Chromium	Brook trout	96 h LC50	59
Lead	Brook trout	96 h LC50	3.4
Nickel	Rainbow trout	96 h LC50	45
Zinc	Rainbow trout	48 h LC50	35

'LC50' means that the given concentration will be lethal to 50% of the organisms in the given time.

Toxic heavy metals have been found to have a detrimental effect on many organisms at just about every trophic level. Their toxic effects result from a number of different mechanisms. They have an ability to bind to a variety of ligands causing denaturation of proteins and enzymes, disruption of cell membranes and decomposition of essential metabolites. Heavy metal toxicity is the basis of many fungicidal preparations (Gadd, 1986). Toxicity of heavy metals to phytoplankton is also well known. Copper (Cu), cadmium (Cd), mercury (Hg), zinc (Zn), and lead (Pb) have all been found to inhibit photosynthetic rate in natural phytoplankton populations (Leland *et al*, 1978). In the Red Sea mercury is reported to inhibit phytoplankton photosynthesis by as much as 45%. Cu has been found to repress the growth of phytoplankton cultures at concentrations of 1-5 µg/l. It has also been suggested that any effect on the taxonomic diversity of marine phytoplankton populations could have repercussions at higher

trophic levels. This could affect the feeding and growth of herbivorous zooplankton and filter feeding organisms thereby having important ecological consequences (Davies, 1983). Fresh water shrimps, *Gammarus pulex*, and the marine oyster, *Crassostrea virginica*, have been shown to have reduced viability and poor growth when fed with algae and fungi that has been contaminated with copper and cadmium (Babich and Stotzky, 1978). Cd, Cr, Hg, Ni, and Zn toxicity has been shown in adults of estuarine macroinvertebrate species (Leland *et al*, 1978). The death of cattle in the vicinity of open copper mines and copper foundries has been attributed to chronic Cu toxicity. Cu concentrations in the soil and vegetation in this area exceeded maximum levels. The occurrence of abnormalities in sperm cells of impala (*Aepyceros melampus*) has been shown to be directly correlated to elevated Cu levels in the animals, represented by the concentration of Cu in the liver (Ackerman *et al*, 1999).

The toxicity of heavy metals is influenced by a number of factors. These include metal speciation, which refers to the various chemical forms of a particular metal that exists under certain conditions. One species may be toxic to an organism where as another of the same metal may have no effect. Cd exists as CdCO_3 in fresh water at pH 8 and is unavailable for uptake by organisms. In sea water, at the same pH, Cd is found as CdCl_2 which is totally soluble and available for uptake. Other factors such as an increase or decrease in pH have been show to influence the toxicity of certain metals to microorganisms. Metal complexes tend to dissociate when the pH decreases. Temperature effects the sensitivity of organisms to metals. Water hardness causes reduced toxicity in some metals, but in others such as Hg, toxicity is increased. The presence of soluble or particulate matter, metal chelating agents

(such as EDTA) and inorganic cationic elements all have a reducing effect on bioavailability of the metal and therefore the toxicity of heavy metals (Babich and Stotzky, 1978).

Humans receive their allocation of trace elements from food and water with the link in the food chain being plant life, which also supports animal life. It is well known that microorganisms as well as plants accumulate metals, with these elements becoming concentrated as they progress through the food chain (Gadd, 1990; Volesky, 1990). It is along this route that excess amounts of metal species can lead to toxicity symptoms, to disorders in cellular function, and eventually death. Two metals nickel and copper, that have been chosen as the focus of this study have both been shown to have carcinogenic potential. Copper has been associated with Wilson's disease in which excess copper is deposited in the brain, liver, skin, pancreas and myocardium (Forster and Wase, 1997; Volesky, 1990). Nickel is toxic mainly because it acts as an antagonist to other essential metals, binding to organic acids, nucleotides, amino acids and phospholipid, and interfering with physiological and biochemical processes of cells (Wilhelmi, 1997). Both copper and nickel have been reported to be damaging in fish and plant species (Forster and Wase, 1997; Braune *et al*, 1994).

1.5 Conventional treatment of metal-laden waste

Legislation in most countries requires that heavy metals must be removed from waste water, in accordance with acceptable levels, prior to disposal into a water source, in the ocean or on land (Dean *et al*, 1972). South African legislation dating back to the Water Act 54 of 1956 requires that water used for industrial purposes and effluent must be purified according to standards set under the Act, and such water/effluent must be returned to the same water source

from which it was drawn (Kidd, 1997). For this reason most industries do treat their effluents before they return them to the water source. There are a variety of commonly used treatment methods. Many of them have major shortcomings, often causing further environmental hazards by merely transferring the problem instead of eradicating it.

1.5.1 Chemical precipitation

Chemical precipitation has been generally applied for the treatment of effluents where complex chemical compounds are not involved. It is a relatively low cost and simplistic metal removal procedure. The metals are precipitated out either as hydroxides, carbonates or sulfides depending on the metal and the precipitating agent. Copper, nickel, zinc, cobalt, iron and manganese are precipitated out completely. Metals such as cadmium, lead and mercury have incomplete precipitation and require further treatment with soda ash (for lead) and sodium sulfide (for cadmium and mercury). In the case of chromium a pretreatment step using sulfur dioxide, ferrous sulfate or metallic iron is required before the addition of lime. Chlorination is used to break down complex organic metal compounds before chemical precipitation. There are two major drawbacks to using this form of metal treatment. Firstly, the economic recovery of the metals is ruled out. Secondly the generation of sludge means that an aquatic pollution problem is merely transformed into one associated with solid waste disposal (Forster and Wase, 1997; Dean *et al*, 1972).

1.5.2 Electrodeposition

Electrodeposition also known as electrolysis, involves the electrolytic reduction of the metal and deposition onto the cathode. In this way the metal can be removed from the effluent and

recovered for economic benefit. A drawback of this system is the need for a high concentration of metal in the effluent. It is often necessary to concentrate the metal before this treatment can be employed. In addition, the presence of free acid and other metals can reduce efficiency (Dean *et al*, 1972).

1.5.3 Cementation

This is another method used when the recovery of a metal is a consideration. It is defined as the displacement of a metal from solution or the reduction of any ionic species by contact with a metal in the higher electromotive series. The metal is precipitated as a metallic “sponge”. It has been used for the precipitation of various metals from waste water including silver from photographic processing solutions, copper from printed circuit etching wastes and the reduction of hexavalent chrome from plating effluent. Iron is the most commonly used cementation metal. It is used in the form of shredded, detinned cans. Zinc dust is a good precipitant of gold and silver from cyanide solutions. It also can also be used to recover small amounts of cadmium and mercury. There is, however, incomplete metal removal and a subsequent lime treatment step is still needed (Zhao, 1997; Dean *et al*, 1972).

1.5.4 Solvent extraction

Also known as liquid ion exchange, a particular metal is extracted from a solution using an organic reagent. The reagent reacts preferentially with the desired metal thereby making it soluble in an appropriate organic solvent. Treatment of the solvent with acid releases the metal in a concentrated water-soluble form. This is then subjected to a conventional recovery method. The limiting factor in this form of metal removal is that none of the chelating agents

can be reused after they have been stripped. The cost of the chelating agent is currently higher than the value of the metal, even before the cost of operation and routine chemicals have been considered (Zhao, 1997; Clevenger and Novak, 1983; Dean *et al*, 1972).

1.5.5 Ultrafiltration

Metal laden effluent is forced through a semipermeable membrane at very high pressure. The membrane made from synthetic organic materials, acts like a molecular sieve allowing relatively pure water to pass through while keeping behind a concentrated chemical solution. In the metal finishing industry for example, once the plating salts have been concentrated enough, they can be returned to the bath. The concentration of the plating chemicals is limited by the osmotic pressure of the solution. Another difficulty with ultrafiltration or reverse osmosis lies in maintaining membrane performance. Factors such as operation at the incorrect pH and accumulation of biomatter on the membrane can reduce the life span of the membrane. There is also a high cost involved in setting up, operating and maintaining a reverse osmosis system (Zhao, 1997; Dean *et al*, 1972).

1.5.6 Ion exchange

Ion exchange is a well known procedure. In the case of metal removal, positively charged metal ions are exchanged for protons on the ion exchange resins. In the past results with industrial effluent were not promising due to chemicals in the effluent damaging the resins. The use of ion exchange resins in pre-treated dilute streams may give better results. However, the high price of the materials make ion exchange inappropriate as a method for the treatment of metal-laden effluent (Volesky, 1999; Dean *et al*, 1972).

1.5.7 Activated carbon adsorption

This technique uses activated carbon instead of synthetic resins. It has considerable potential in industrial waste treatment for the removal of trace metals. It has a number of advantages over ion exchange; it generally has higher loading capacities, it is less susceptible to damage from chemicals in the effluent and is lower in cost. Activated carbon adsorption has been used successfully for the removal of gold from cyanide solutions. It has also been used for the removal of hexavalent chromium and the removal of lead from drinking water. It is a widely recognised treatment process, but is also considered to be too expensive for application, especially in developing countries (Dean *et al*, 1972).

1.5.8 Evaporation

Evaporative methods are time consuming and require large open spaces to provide a large enough surface area. For this reason evaporative recovery of metal is not a suitable or widely used method of waste water remediation (Kapoor and Viraraghavan, 1995).

1.6 Biosorption and bioaccumulation

Metals can be removed from solution by an organism/biological system, by both metabolism-dependent and metabolism-independent processes. Biosorption to either living or non-living organisms can occur. It is defined as the process whereby metals in solution are bound and concentrated on ligands or functional groups situated on the outer surface of the cell wall. It is a passive uptake mechanism that requires neither active membrane transport nor metabolic energy. The reaction is rapid and reversible. Bioaccumulation occurs in living cells. It has been defined as a metabolically mediated uptake or concentration of metal ions using living

cells. It is a much slower often irreversible process of intracellular uptake, whereby metals are actively taken up into the cell using the membrane transport system and metabolic energy (Atkinson *et al*, 1998; Sag *et al*, 1998; Tsezos *et al*, 1995; Volesky, 1992). Biosorption shows potential as an efficient and particularly cost-effective wastewater treatment alternative (Volesky, 1999).

The physico-chemical process of biosorption tends to be an exothermic reaction that may involve one or a combination of metal-binding mechanisms including adsorption, ion exchange, complexation, chelation, coordination, and microprecipitation. Metallic cations are attracted to negatively charged groups on the cell surface. Cell walls are composed of polysaccharides, proteins and lipids that provide a variety and abundance of functional groups for biosorption reactions. These anionic ligands include amino, phosphoryl, carboxyl, sulfhydryl and hydroxyl groups (Juang *et al*, 1999; Volesky, 1990; Kuyucak and Volesky, 1988; Volesky, 1987). Biosorption is dependent on temperature, pH and metal concentration.

1.7 The Langmuir model for sorption isotherms

The sorption process involves a solid phase (biosorbent) and a liquid phase (solvent, water) containing the dissolved metal species (sorbate). The sorbate is attracted to the functional groups on the sorbent and bound via different mechanisms. This process will continue until such time as equilibrium is reached between the bound and unbound sorbate. The ability of a biomass as a biosorbent can be measured in terms of the amount of metal that remains bound to the biomass at equilibrium. In order to quantify this it is common practice to calculate the metal uptake (q) as the amount of metal bound by the weight of biosorbent (Volesky, 1999).

$$q = V(C_i - C_f) / S$$

Where: V is the volume of the metal bearing solution in contact (batch) with the sorbent (L)

C_i and C_f are the initial and the equilibrium metal concentrations that are calculated analytically ($\mu\text{mol/l}$)

S is the dry weight of the biosorbent added to the metal solution (g)

The Langmuir sorption model, used to describe the q vs C_f relationship, has a hyperbolic equation:

$$q = (q_{\max} \times C_f) / (K_d + C_f)$$

Where: $q_{\max}$ is the maximum sorbate uptake under the given conditions ($\mu\text{mol/g}$)

K_d is the dissociation constant

The Langmuir sorption model makes the following assumptions:

1. Metal ions are chemically adsorbed at a fixed number of well defined sites.
2. Each site can hold only one sorbate ion.
3. Each site is energetically equivalent.
4. Ions adsorbed on neighboring site do not interact with each other.

There are a few shortcoming with these assumptions and therefore with the model. It is clear from section 1.6 above that metal biosorption involves a number of different mechanisms, and not just one, for example adsorption. Other mechanisms involved may not adhere to these assumptions. The model needs to be considered in context, which is that it is a mathematical

model that is able to follow the experimental data. In other words the model has been adopted for the estimation of the maximum metal uptake that can be achieved by a biosorbent under specified conditions (Volesky, 1999; Zhao and Duncan, 1997a).

1.8 Microbial biosorbents

Over the years there have been discoveries of many materials that have a high affinity for a variety of metals and therefore have promising potential as biosorbents. Many of these are likely to be very competitive and cost-effective bioremediation systems. The criteria for evaluating a commercially viable biosorbent are as follows (Atkinson *et al*, 1998; Zhao, 1997; Van Hille, 1995):

1. The uptake and release of the metal should be efficient and rapid.
2. The active biosorbent agent should be produced at low cost and should be reusable up to 100 cycles.
3. The particle size, shape and mechanical properties of the biosorbent material should be suitable for use in continuous flow systems in freely mixed, packed bed, or fluidised bed configurations.
4. Removal of biosorbent from solutions should be cheap, efficient and rapid.
5. The sorbent should be metal selective in order to select single metals from a solution containing various metallic species.
6. Desorption (separation of the metal from the sorbent) should be metal selective, economically feasible and the loss of sorbent should be minimum.

Microbial biosorbents may be divided into 3 categories: Algal, fungal and bacterial.

Research has shown a variety of algae to be good biosorbents. Depending on the organism, algal biomass can be used as viable or non-viable, free or immobilized (Garnham, 1997). Microalgae such as *Chlorella vulgaris* can be immobilized onto Ca-alginate, a biopolymer. Other biopolymers that can be used for immobilizing algae include glutaraldehyde, agarose and cellulose acetate. Immobilizing biomass in a biopolymeric matrix can improve biomass performance in a continuous-flow system, it can result in increased biosorptive capacity, increased biomass concentration and facilitate separation of the metal solution from the biomass (Aksu *et al*, 1998). *Chlorella vulgaris* performed very well as a biosorbent for gold as well as for copper and hexavalent chromium (Aksu *et al*, 1998; Garnham, 1997; Nourbakhsh *et al*, 1994). In a recent study, free *Phormidium laminosum* removed up to 99, 95, 85, and 93 percent of Cu, Fe, Ni and Zn respectively from a synthetic solution in a batch system (Blanco *et al*, 1998). Using Langmuir isotherms *Phormidium* sp. was also reported to have a maximum specific adsorption of 9600 mg/kg, 13600 mg/kg, 5700 mg/kg, 10100 mg/kg for Cd, Pb, Ni and Cu respectively at pH 5 (Wang *et al*, 1998). Macroalgae such as the seaweeds *Gracilaria conferta*, *Eisenia bicyclis*, and *Sargassum* sp. do not need to be immobilized. Binding of metals including Ni, Cu, Pb, Zn, Cd, Cr and Fe were found to be strongly pH dependent (Ramelow *et al*, 1992). It is thought that brown algae are better suited for metal binding, due to their high polysaccharide content (Volesky and Holan, 1995, Crist *et al*, 1981).

Both living and dead fungal cells of *Penicillium*, *Aspergillus*, *Rhizopus*, *Saccharomyces*, *Mucor* and *Trichoderma* can remove heavy metals from solution (Kapoor and Viraraghavan, 1998; Tobin *et al*, 1994). Fungal cells have been used in powdered or immobilised form in

batch systems. Powdered *Aspergillus niger* immobilised in a polysulfone-DMF-matrix and beaded, was used in a column system to remove nickel, copper, cadmium and lead (Kapoor and Viraraghavan, 1998). The yeast *Saccharomyces cerevisiae* has been used for the removal of heavy metals from solution. Copper, cadmium and cobalt were removed from solution by isolated yeast cell walls. Binding isotherms for Cu, Cd, and Co showed $q_{\max}$ values of 0.4, 0.17 and 0.11 $\mu\text{mol/mg}$ at pH 4.75, respectively. In the same study, blocking of amino, carboxyl and hydroxyl groups on the cell wall caused a decrease in copper accumulation onto the cell walls. It was concluded that these groups play a role in the binding of copper to the yeast cell wall (Brady and Duncan, 1994). Immobilised, non-viable, whole yeast cells have been shown to remove copper from aqueous solutions. The removal of copper using immobilised yeast cells showed better adsorption at higher pH values (Stoll and Duncan, 1997).

Bacteria have also been used as a biosorbent of heavy metals. The isolated cell walls of *Enterococcus hirae* removed 2.03 μmol of Ni per mg of cell wall at pH 6.5 (Bossrez *et al*, 1997). Ni, Cu, Mn and Pb have been removed from aqueous solution using free cell of *Arthrobacter* sp.. The highest values of specific uptake generated from Langmuir isotherms for copper and nickel were 148 mg/g and 13 mg/g, respectively (Veglio *et al*, 1997). Fungal microorganisms have been the subject of increased interest as potential biosorbents of heavy metals from aqueous solutions.

Another material that has shown potential as a biosorbent of heavy metals, is chitosan. Chitosan is a biopolymer, also called poly-D-glucosamine, occurring in Mucorales cell walls

and is processed from chitan. Chitosan has been used to remove uranium, Cu and Hg (Juang *et al*, 1999; Jansson-Charrier *et al*, 1995) .

1.10 The use of plant materials as biosorbents

Plant material is broadly available and relatively inexpensive. In many cases plant material is discarded as an agricultural waste product. There is also the problem of alien plants invading and destroying natural ecosystems. In some cases indigenous plants have become a problem where their natural control mechanisms have disappeared due to human intervention. Therefore, in some cases, harvesting a plant for bioremediative purposes would also solve the immediate environmental problem that the plant may be causing. Another advantage that plant material has as a biosorbent is that plant tissue is generally water insoluble and has good mechanical strength and rigidity to avoid the sometimes complicated task of immobilization for a continuous-flow process. Various types of biomass derived from plants are reported to have shown potential for the removal of a range of metals from solution (Table 1.5).

Sago processing waste, from the production of sago flour in Malaysia has, which is both a waste and a pollutant, was used to adsorb lead and copper ions from solution. The sago waste had a greater adsorption capacity for lead (46.6 mg/g) than it did for copper (12.4 mg/g) (Quek *et al*, 1998). Leaves from the typha plant, commonly known as Cattails, have been shown to remove Hg and Cd from aqueous solutions. The adsorption capacity of cattails was relatively low (4.8 mg/g for Hg and 4.9 mg/g) when compared with other biosorbent materials (Krishnan *et al*, 1987). Modified agricultural byproducts such as bagasse, flour waste, paddy

husk, paddy straw, onion skin and garlic skin have been investigated for their adsorption capacities of metals such as Cu, Pb, Zn and Cd (Kumar and Dara, 1980).

Table 1.5: Summary of data for the sorption of Ni, Cu, Cr, Cd and Zn, metals commonly found in electroplating effluent, by biomass derived from plant material.

Sorbent	Metal	pH	q _{max} (mg/g)
Activated carbon	Cd	6.3-7.2	11.1
	Cr	2.7	145.0
	Cr	-	57.7
	Ni	6.5-7.3	8.1
	Cu	5.4-5.9	13.8
	Zn	6.3-6.8	6.2
Straw	Cd	6.3	7.7
	Ni	6.5	6.4
	Cu	5.0-5.4	6.4
	Zn	6.3	5.3
Imunodiacetic acidcellulose	Ni	7	20.8
	Cr	5	48.9
	Cu	4	20.5
Water hyacinth roots	Cu	5.5	20.9
	Ni	5.0-6.6	11.6
Rice hulls	Cd	-	21.4
	Cr	-	164.3

(Zhao, 1997)

1.11 *Azolla filiculoides* as a biosobent

Azolla filiculoides is a small aquatic annual or perennial heterosporous fern that grows on dams and rivers, forming dense mats that cover large areas. It has very fine fronds, called macrophytes. Each frond consists of two lobes, an aerial dorsal lobe (chlorophyllous) and a partially submerged ventral lobe, for buoyancy. It has a symbiotic relationship with an endophytic blue-green alga (*Anabaena azollae*) that lives in the dorsal leaf cavity of the water fern. The endosymbiont fixes nitrogen for both organisms while the fern provides a protective environment and a fixed carbon source (Kiguli *et al*, 1999).

It is widely distributed in South Africa especially on still and slow moving water bodies. It originates from parts of South and North America, and thrives in tropical, subtropical and warm and temperate regions. It was introduced to South Africa as an ornamental during the 19th century (Kiguli *et al*, 1999). *Azolla* does not require any special growth conditions and can grow in water of extremely poor quality (Priel, 1995). It has an enormous reproductive capability, via production of spores as well as vegetative growth (EPM, 1996). The fern forms dense mats (5-20 cm) on the surface of the water, on which terrestrial plants are able to grow to form a type of sud vegetation (Ashton and Walmsey, 1976). This results in reduction of drinking water quality from bad odors, colour and turbidity. It also causes major problems to the ecology of the ecosystems underneath the mat (de Wet *et al*, 1990). These characteristics have led to *Azolla filiculoides* being included on the list of declared weeds (Kiguli *et al*, 1999).

Azolla filiculoides has been shown to accumulate the heavy metals Fe, Cu, Ni, Pb, Zn, Mn and Cr. The ability of *Azolla* to adsorb heavy metals has been investigated under laboratory and natural environmental conditions (de Wet *et al*, 1990). Another study showed that viable *Azolla* has a high binding capacity for cadmium, copper and uranium, accumulating as much as 6.021, 5.365 and 5.082 mg/l of the metals, respectively, when grown in the presence of 10 mg/l metal (Sela *et al*, 1988). Non-viable *Azolla* biomass has been used for the biosorption of Cr and Ni. *Azolla* biomass showed a $q_{\max}$ of 72 mg/g at pH 2 and 43.4 mg/g at pH 6.5 for Cr and Ni respectively (Zhao and Duncan, 1997a and b).

1.12 Research aims

The primary aim of this research project was to investigate the potential for an upscaled packed bed column system for the removal of Ni and Cu from aqueous solutions by non-viable *Azolla filiculoides* biomass. Preliminary studies investigated the effects that parameters such as pH and metal starting concentrations had on the removal of Ni and Cu from aqueous solutions by *Azolla* in batch systems. Sorption isotherms were generated for Ni and Cu to determine the maximum binding capacities of *Azolla* biomass at different pHs. Competition studies were performed with Cu and Ni in binary metal solution to investigate how the two metals influence the adsorption of each other onto the biomass.

A 4L packed bed column system was designed in order to investigate the upscale potential of the *Azolla* adsorption system. Preliminary studies were done with lab scale columns in order to compare the performance of the 4L column. The upscaled column was tested with different *Azolla* biomass and different flow rates. Desorption profiles were generated for Cu and Ni to

investigate the recovery of the biomass-bound metals by elution with dilute mineral acids. Repeated adsorption-desorption studies were carried out to determine the potential re-usability of the biomass over 10 Ni removal/recovery cycles in the 4L fixed bed column system. The removal of Ni from a concentrated nickel plating effluent was also tested on the 4L column. Scanning electron micrographs were taken of *Azolla* biomass after subsequent removal/recovery cycles in order to observe a change in the physical integrity of the biomass.

CHAPTER TWO

THE REMOVAL OF COPPER AND NICKEL FROM AQUEOUS SOLUTION IN BATCH SYSTEM USING NON-VIABLE

***Azolla Filiculoides* BIOMASS**

2.1 INTRODUCTION

Previous investigations have shown that plant material is able to bind heavy metals and remove them from solution (Ho Lee *et al*, 1998; Quek *et al*, 1998; Bryant *et al*, 1992; Gosset *et al*, 1986). The effects of parameters such as pH and concentration of metals in solution need to be studied in order to establish the capacity of any given biomass for the biosorption of a metal (Veglio *et al*, 1997; Pascucci and Sneddon, 1993).

Azolla filiculoides was chosen as a potential candidate due to its metal binding capabilities, its weed status in South Africa and its availability on nearby dams and rivers. It also meets the economic and mechanical requirements of a sorbent. The use of viable *Azolla* for the remediation of metal-laden waste water is limited due to the slow process of removing metals in ponding systems. Attempts have been made using non-viable *Azolla* biomass in filter containers. This method was considered to be a more efficient process for removing metals from solutions (Priel, 1995).

Copper and nickel are two major contaminants commonly found in electroplating effluents (Binnie and Partners, 1987). The electroplating industry adds 0.39 million tons per year of metals including Cu and Ni to the environment. Metals discharged into the environment can have a large environmental, public health and economic impact (Brower *et al*, 1997). Cu and Ni have toxic effects on living organisms at every trophic level, including humans is well documented (Ho Lee *et al*, 1998; Wong and Fung, 1997; Nourbakhsh *et al*, 1993 Bryant *et al*, 1992). Many of the conventional methods for the removal of these metals from the effluents are relatively expensive or impractical due to the difficulty in treatment of the resulting solid wastes (Wong and Fung, 1997). Biological methods for the treatment of metal-laden waste water are therefore receiving increased interest.

Although a lot of work has been done on the study of adsorption of metals from single metal solutions it is important to realise that single toxic metallic species rarely exist in natural and waste waters. The presence of more than one metal in solution leads to interactive effects with the possibility of competition between the metals for the binding sites on the biomass. Factors affecting this competition include, the combination of the metal in solution and the concentration that they are present in (Sag and Kutsal, 1998 and 1997; Pascucci, 1993). Copper and nickel are two metals that are frequently found together in electroplating effluents. It is important to determine how the adsorption of Cu or Ni is effected by the presence of the other metal in a binary metal solution.

This chapter examined the sorption of Ni and Cu under different conditions of pH, starting concentrations and the adsorption of Ni and copper in binary metal solutions. Langmuir

sorption isotherms were generated to establish the maximum binding capacity of *Azolla* biomass for Ni and Cu at different pH values and for the binary metal solutions. Adsorption kinetics of Ni and Cu were also studied.

2.2 MATERIALS AND METHODS

2.2.1 Collection and preparation of biomass

Azolla filiculoides biomass was collected on local farm damns in the Albany district around Grahamstown and then grown up in a small pond that only received sun in the morning. No nutrients were added for *Azolla* growth. As it was needed *Azolla* was harvested and then dried in an oven at a constant temperature of 50°C for approximately 24hrs. Once the biomass was dried, it was ground through a sieve (1mm²) to achieve a constant size.

2.2.2 Metal Solutions

All solutions were made up using double distilled water (ddH₂O) to avoid contamination from other metals. All chemicals used were of analytical grade and were obtained from Saarchem (Pty) Ltd, Krugersdorp, SA. All glassware was washed prior to use with 25 % nitric acid and rinsed with ddH₂O to remove any metal that may be bound to the glass. Cu solutions were made by weighing out the appropriate amount of anhydrous cupric sulphate (CuSO₄, Mr: 159.6) and dissolving it in a specific volume of water to obtain the desired concentration. Ni solutions were made up by weighing out the appropriate amount of nickel chloride (NiCl₂·6H₂O, Mr 237.71) and dissolving it in double distilled water.

2.2.3 Preparation of binary metal solutions

In order to determine the adsorption characteristics of Cu in the presence of Ni, the initial concentrations of Cu were varied between 50 and 500 $\mu\text{mol/l}$ while the Ni concentration was held constant at 50, 100, 200, 300, 400 and 500 $\mu\text{mol/l}$. In order to determine the adsorption characteristics of Ni in the presence of Cu, the initial concentrations of Ni were varied between 50 and 500 $\mu\text{mol/l}$ while the Cu concentration was held constant at 50, 100, 200, 300, 400 and 500 $\mu\text{mol/l}$.

2.2.4 pH profiles

The pH of the metal solutions were adjusted to the desired pH using 0.1M or 1M hydrochloric acid (HCl) and 0.1M or 1M sodium hydroxide (NaOH) where necessary.

2.2.5 Batch experiments with copper

All experiments were conducted in duplicate. 0.4g of dried, crushed *Azolla* biomass was added to 100ml of CuSO_4 solution of a specific concentration, 50 – 20000 $\mu\text{mol/l}$, in a 300ml Erlenmeyer flask. Starting pH's were set to the desired pH and were maintained throughout the experiment by adjusting the pH every half-hour. The flasks were incubated at 20°C on a rotary shaker at 200rpm for 6 hours. 5ml samples were taken from each flask at the end of the incubation and filtered through a Millipore filter system using a 25mm diameter, 0.45 μm pore size cellulose acetate filter. Control experiments excluding the biomass were performed to determine the effect of precipitation of Cu on the uptake of Cu by the cellulose acetate filters, this was found to be negligible.

2.2.6 Batch experiments with nickel

Batch experiments with Ni were carried out in the same way as with Cu except that NiCl_2 was used to make up the metal solutions to the desired concentrations, 50 – 15000 $\mu\text{mol/l}$.

2.2.7 Biosorption studies with binary metal solutions

Batch experiments with two metals in solution were carried out in the same way as with the single metals, except that the *Azolla* was incubated with the binary metal solutions.

2.2.8 Kinetics experiments with Copper and Nickel

Experiments were performed in duplicate. 0.8 g of dried *Azolla* biomass was added to 200ml of 1000 $\mu\text{mol/l}$ Cu and Ni solutions, in 500ml Erlenmeyer flasks. The flasks were incubated on a rotary shaker at 200 rpm for 6hrs, at 20°C. The pH was set at the start of the experiment at pH 5, and was not adjusted during the incubation. A 2 ml sample was taken a minute after the biomass came into contact with the metal solution. Subsequent samples of 2 ml were taken at 3 min, 6min, 10min and then every 10 min for the first hour, every 30 min for the next 3 hours and thereafter once an hour. All the samples were filtered in the same manner as in the batch experiments.

2.2.9 Metal analysis

The filtered samples were analysed for Cu and Ni concentration using a GBC 909 atomic absorption spectrophotometer (AAS). Standard metal solutions were purchased from Saarchem (Pty) Ltd, Krugersdorp, SA, and the appropriate concentration made by dilution with de-ionised water. The operation parameters for are set out in table 2.1 below

Table 2.1: Atomic adsorption spectrophotometer operating conditions (Rothery, 1980).

Element	Flame	Wavelength (nm)	Working range ($\mu\text{mol/l}$)
Copper	A-A*	324.7	1 - 80
		327.4	40 - 160
		217.9	100 - 500
		222.6	700 - 3000
		249.2	3000 - 11000
Nickel	A-A	232.0	1 - 130
		341.5	100 - 500
		351.5	500 - 1400

* A-A = Air - Acetylene

2.3 RESULTS AND DISCUSSION

2.3.1 Effect of starting concentration

Figure 2.1 shows the percentage Ni removal by the *Azolla* biomass after incubation for 6 hours for a range of starting concentrations of Ni. The *Azolla* concentration of 4g/l and the pH of 5 were chosen based on similar experiments performed previously (Zhao, 1997). This experiment was done in order to establish the optimum Ni starting concentration for Ni adsorption by *Azolla* biomass. The percentage uptake increased as the Ni starting concentration increased from 50 $\mu\text{mol/l}$ to 500 $\mu\text{mol/l}$ with maximum adsorption of 59% achieved at 1500 $\mu\text{mol/l}$ and then decreasing as the starting concentration increased. Ion exchange is thought to be the main mechanism involved in the biosorption process and involves the competition between metal ions and protons for binding sites on the biomass (Volesky, 1999; Goncalves and Boaventura, 1998; Nourbakhsh *et al*, 1993). The poor adsorption at the lower concentrations could be due to not enough competition with protons for binding sites by relatively few Ni ions. The lower percentage adsorption at the higher concentrations would simply be due to Ni being present in excess, not due to poor adsorption.

Figure 2.2 shows the effect of Cu starting concentration on the biosorption of Cu by *Azolla* biomass. The adsorption of Cu showed a different effect to that of Ni. The maximum percentage adsorption for Cu was 70% and this was achieved at a starting concentration of 50 $\mu\text{mol/l}$. This might be an indication that *Azolla* biomass has a higher affinity for Cu than for Ni.

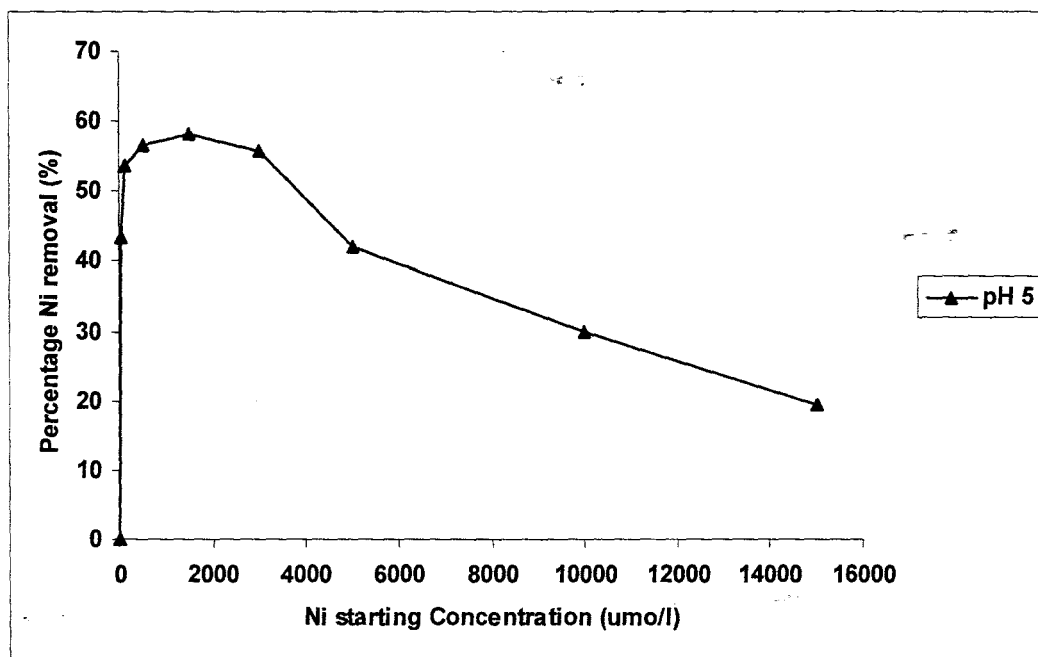


Figure 2.1: Percentage Ni removal in a batch system with varying Ni starting concentration. Biomass concentration 4g/l, pH5, 20°C, 200rpm.

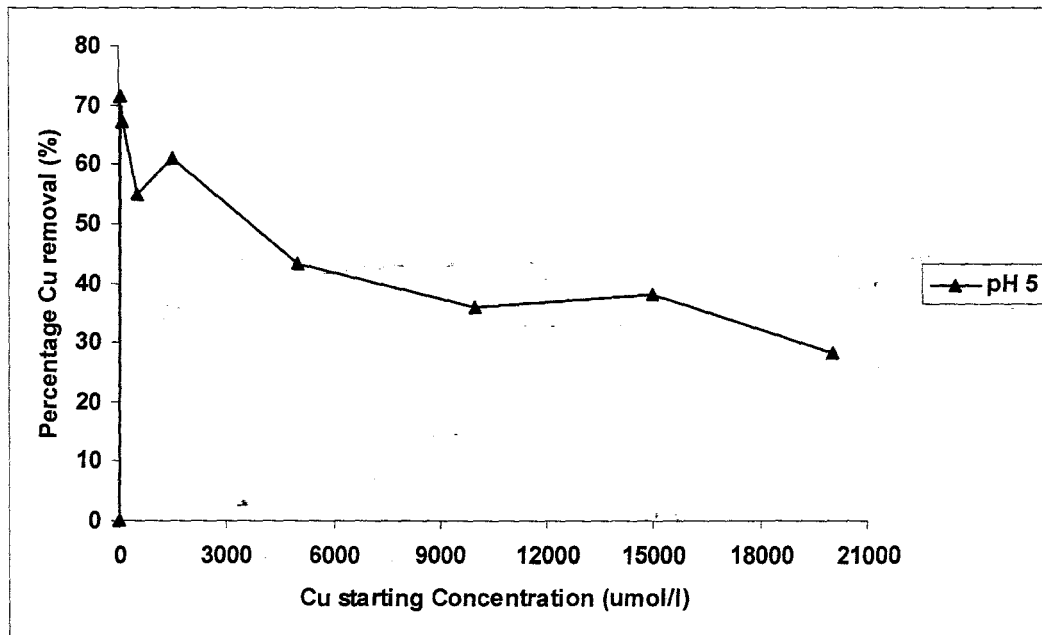


Figure 2.2: Percentage Cu removal in a batch system with varying Ni starting concentration. Biomass concentration 4g/l, pH5, 20°C, 200rpm.

The pH of the solutions were checked and adjusted back to pH 5 every half hour. Figure 2.3 and 2.4 show the pH profiles for the adsorption of Ni and Cu over time for the different starting concentrations, respectively. For Ni there is an initial increase in pH with the largest increase occurring with the 50 $\mu\text{mol/l}$ Ni starting concentration. The changes in the pH decreased until after about two and a half hours, where there was little or no change in the pH. For the removal of Cu at pH 5 there was a decrease in pH, as much as 1.8, for the higher concentrations. For the lower starting concentrations the pH increased by as much as 2. There was a more gradual decrease in the change of pH for Cu removal with equilibrium being reached only after four hours of incubation.

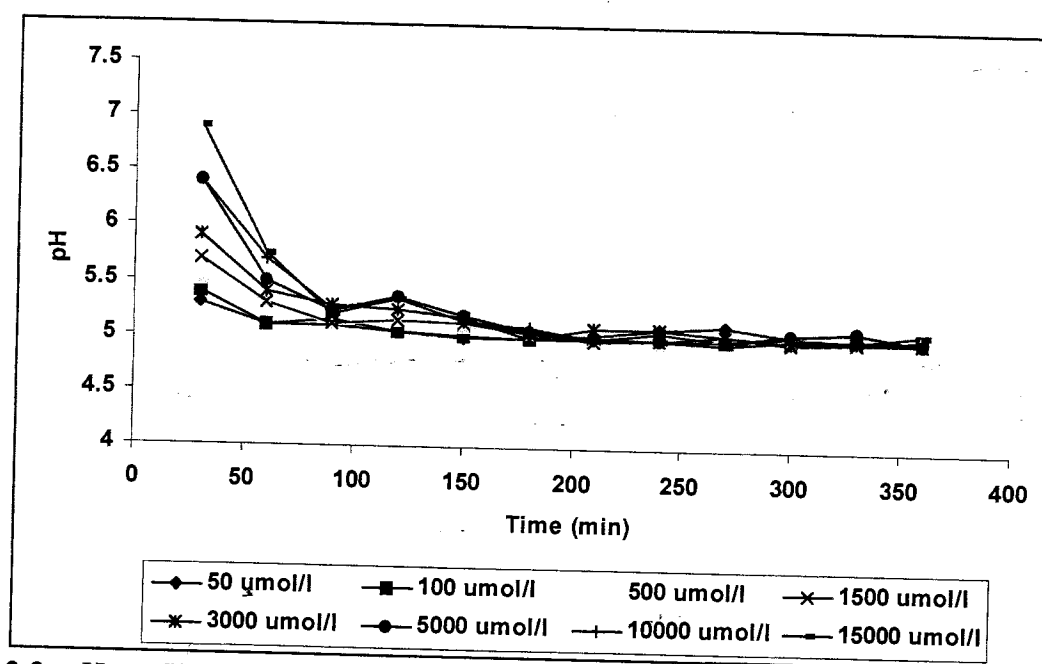


Figure 2.3: pH profile for the removal of Ni by *Azolla* biomass in a batch system at different Ni starting concentrations, 4g/l *Azolla* pH 5, adjusted, 20°C, 200rpm

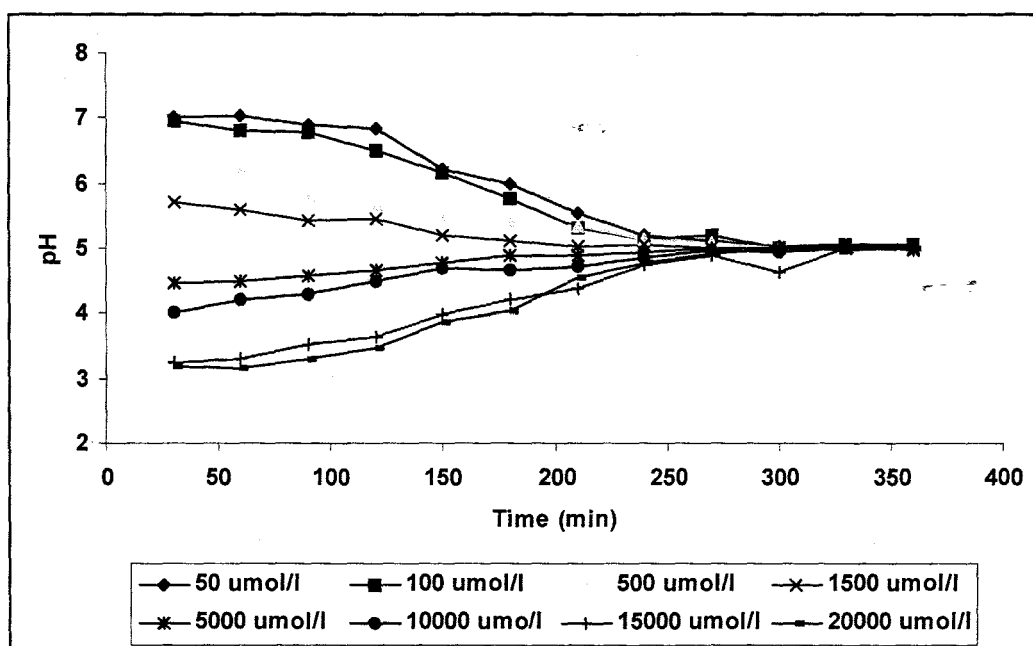


Figure 2.4: pH profile for the removal of Cu by *Azolla* biomass in a batch system at different Cu starting concentrations, 4g/l *Azolla* pH 5, (adjusted), 20°C, 200rpm

Cu and Ni showed very different pH profile indicating that they possibly involve different binding mechanisms onto binding sites, or bind to different functional groups on the *Azolla* biomass. The binding of Cu and Ni to the biomass is evidently very dependent on pH. This is supported by other reports in the literature (Quek *et al*, 1998; Brower *et al*, 1997; Wong and Fung, 1997). The pH variations encountered when different metals bind to a biomass are said to be possibly as a result of the acidic properties of carboxylic and phenolic functional groups present in humic substances. They are also thought to be due to ion exchange reactions, i.e. proton release when metal cations bind to the biomass (Gosset *et al*, 1986).

2.3.2 Effect of pH

Batch experiments were carried out on both Cu and Ni to study the effect that pH has on the removal of the metal from solution by *Azolla* biomass. These results were characterized using Langmuir sorption isotherms, described in the literature review, in order to generate a $q_{\max}$ value for comparison of binding capacity at different pHs. Figure 2.5 and 2.6 show the binding isotherms for Ni at pH 3, 4, 5 and 7, and for Cu at pH 3, 4 and 5, respectively. The $q_{\max}$ values generated from the sorption isotherms for Ni and Cu are summarized in table 2.2 and 2.3, respectively.

The data for the experiments with Ni for pH 3, 4, and 5 followed the Langmuir model very well with correlation coefficients (R^2 value) higher than 0.979 (Table 2.2). The correlation for the sorption isotherm at pH 7 was not as good, with an R^2 value of only 0.947 (Table 2.2). The best removal of Ni from aqueous solution by *Azolla* biomass was achieved at pH 7, with a $q_{\max}$ of 2105,88 $\mu\text{mol/g}$ (123.59 mg/g). However, it must be noted that this result might not be entirely accurate since the correlation coefficient was slightly lower than that of the other experiments. In other words, the data from the pH 7 experiment did not fit the model as well as the other pHs. This could be due to the possibility of small amounts of precipitation of Ni out of solution at the higher pH, although control experiments did not show any evidence of this. Metal precipitation is likely to occur at pH 7, but only at higher concentrations (17000 $\mu\text{mol/l}$) (Holan and Volesky, 1994). Over all, the sorption of Ni on *Azolla* biomass was more efficient with increasing incubation pHs. The maximum Ni uptake at pH5 was found to be 869.2084 $\mu\text{mol/g}$ (51.013 mg/g), whilst lower maximum uptake values were found for pH 4 (729.3057 $\mu\text{mol/g}$ or

42.8 mg/g)) and pH 3 (667.38 $\mu\text{mol/g}$ or 39.17 mg/g) Similar results have been reported in literature (Zhao and Duncan, 1998).

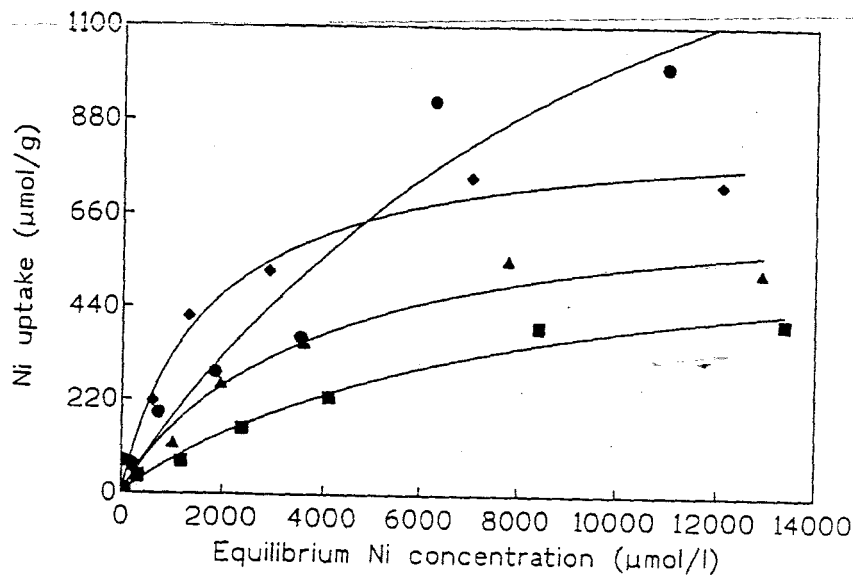


Figure 2.5: Binding isotherms for the removal of Ni from solution by *Azolla* biomass in a batch system at different incubation pH: ■ pH3, ▲ pH4, ◆ pH5 and ● pH7. *Azolla* concentration 4g/l, temp 20°C, 200rpm.

Table 2.2: Langmuir constants of Ni-*Azolla* binding at 20°C

Incubation pH	q_{max} ($\mu\text{mol/g}$)	Correlation (R^2)
3	667.38	0.986
4	729.3507	0.979
5	869.2084	0.991
7	2105.88	0.947

The R^2 values for Cu removal by *Azolla* biomass at different pHs were not as good as those found for Ni, with 0.975 being the highest that was achieved (Table 2.3). In general, the adsorption of Cu to the biomass was also found to increase as the incubation pH was increased. This was also found in other Cu adsorption studies using different biomass (Ho Lee *et al*, 1998; Quek *et al*, 1998). *Azolla* biomass showed a good affinity for Cu with the best removal of 2406.15 $\mu\text{mol/g}$ (152.87 mg/g) achieved at pH 5, lower q_{max} values were obtained for pH 4 (1197.315 $\mu\text{mol/g}$ or 76.07 mg/g) and for pH 3 (941.7382 $\mu\text{mol/g}$ or 59.83 mg/g) (Table 2.3).

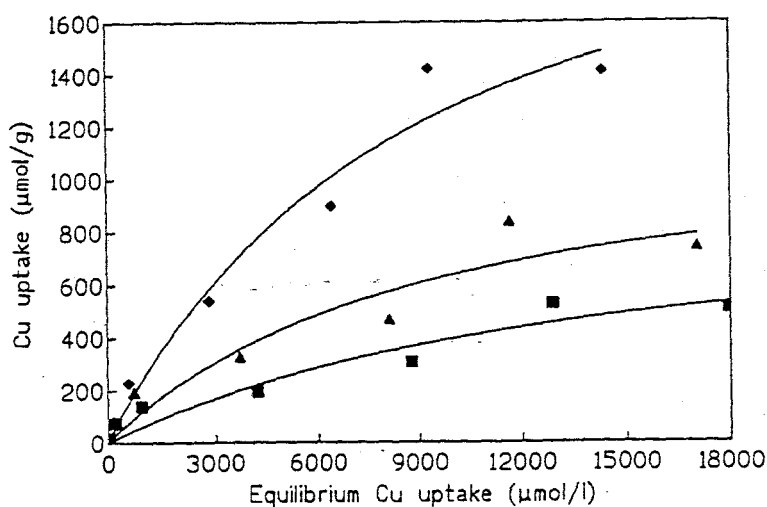


Figure 2.6: Binding isotherms for the removal of Cu from solution by *Azolla* biomass in a batch system at different incubation pH: ■ pH3, ▲ pH4 and ♦ pH5. *Azolla* concentration 4g/l, temp 20°C, 200rpm.

Table 2.3: Langmuir constants of Cu-*Azolla* binding at 20°C

Incubation pH	q_{max} (μmol/g)	Correlation (R²)
3	941.7382	0.933
4	1197.315	0.931
5	2406.15	0.975

2.3.3 Effect of two metals in solution

Sorption isotherms using the Langmuir model were generated from the data to characterize this effect. Figure 2.7 shows the sorption isotherms for the removal of Ni by *Azolla* biomass and the effect of Cu present at different concentrations. The data for the binary metal studies showing the effect of Cu on adsorption of Ni showed good correlation to the Langmuir model with R² values higher than 0.968 (Table 2.4). The q_{max} values for the removal of Ni from solution by *Azolla*, under these experimental conditions, varied greatly, but showed no trend in terms of the different copper concentrations (Table 2.4 and Figure 2.7).

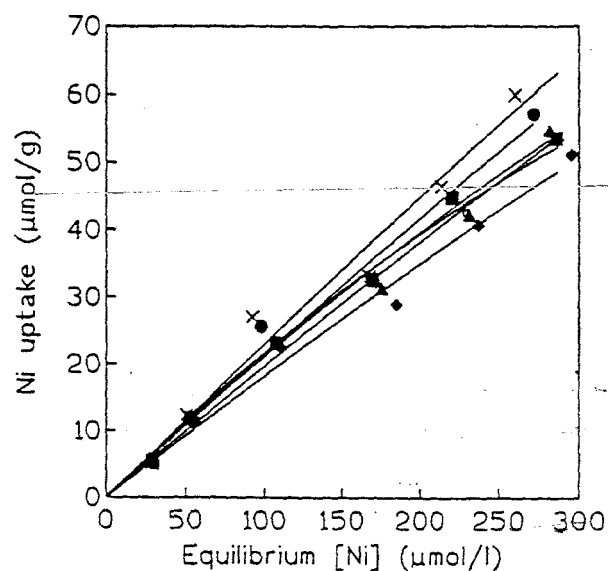


Figure 2.7: Binding isotherms for the removal of Ni from solution by *Azolla* biomass in a batch system with varying concentrations of Cu in a binary metal solution: ■ 50, ▲ 100, ◆ 200, ● 300, + 400, x 500. *Azolla* concentration 4g/l, temp 20°C, 200rpm.

Table 2.4: Langmuir constants of Ni-*Azolla* binding at 20°C in a binary solution with copper.

Copper concentration	$q_{\max}$ (μmol/g)	Correlation (R^2)
50	331.5295	0.994
100	688.3648	0.987
200	463.252	0.985
300	829.6466	0.981
400	809.7553	0.968
500	212.548	0.983

Figure 2.8 shows the percentage nickel removal at different Ni starting concentrations with Cu present at various concentrations. This figure represents the same data that has not been fitted to a mathematical model. From this graph it seems as if there is little or no effect on the removal of Ni from solution by the presence of Cu at varying concentrations. The maximum percentage Ni removal in a binary metal solution was 54% which was slightly higher than the percentage removal for Ni in solution on its own (Figure 2.1) in the same concentration range, at the same pH.

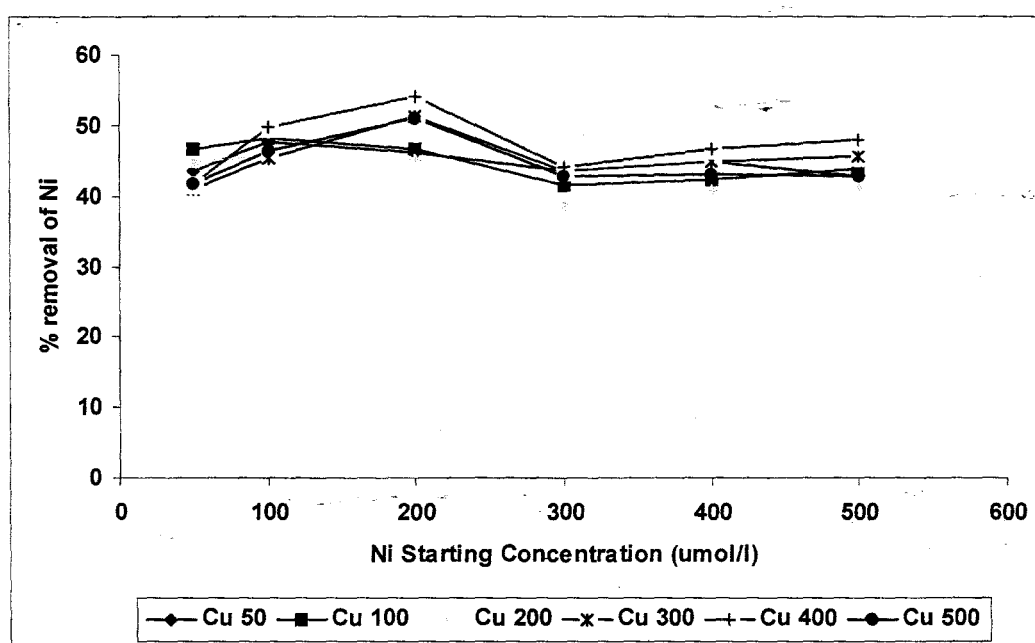


Figure 2.8: Percentage removal of Ni from binary metal solutions in the presence of Cu at varying concentrations, *Azolla* concentration: 4g/l, pH 5, 20°C, 200rpm.

Figure 2.9 shows the sorption isotherms for the removal of Cu by *Azolla* biomass and the effect of Ni present at different concentrations. Some of the data fitted the Langmuir model well with R^2 values of 0.974 and 0.982, with other data there was a lower correlation with correlation coefficients less than 0.96 (Table 2.6). The isotherms seemed to indicate that there was little or no difference in the adsorption of Cu when the concentration of Ni in the binary solution was increased from 50 to 500 $\mu\text{mol/l}$ (Figure 2.9 and Table 2.5).

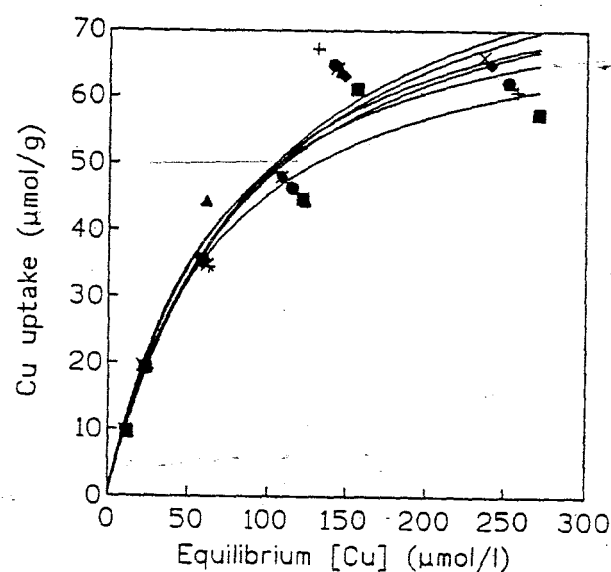


Figure 2.9: Binding isotherms for the removal of Cu from solution by *Azolla* biomass in a batch system with varying concentrations of Ni in a binary metal solution: ■ 50, ▲ 100, ◆ 200, ● 300, + 400, x 500. *Azolla* concentration 4g/l, temp 20°C, 200rpm.

Table 2.5: Langmuir constants of Cu-*Azolla* binding at 20°C in a binary solution with nickel.

Nickel concentration	$q_{\max}$ ($\mu\text{mol/g}$)	Correlation (R^2)
50	76.0896	0.953
100	80.3415	0.925
200	94.0712	0.982
300	87.00447	0.948
400	96.38053	0.974
500	86.15255	0.917

The same conclusion could be obtained from plotting the data as percentage removal of copper from binary metal solutions in the presence of nickel at varying concentrations (Figure 2.10). The percentage removal of copper was as high as 80% at the lower Cu starting concentrations and then it decreased to as low as 50% as the starting concentration of Cu increased. This is almost identical to the percentage removal of copper in a single metal solution (Figure 2.2).

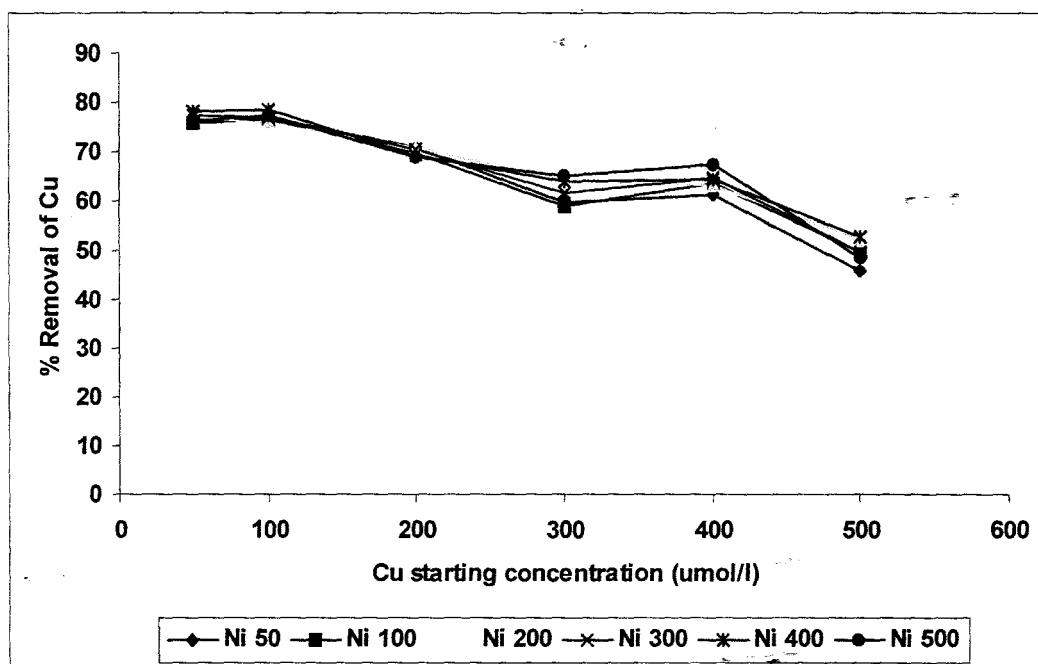


Figure 2.10: Percentage removal of Cu from binary metal solutions in the presence of Ni varying concentrations, *Azolla* concentration: 4g/l, pH 5, 20°C, 200rpm.

2.3.4 Kinetics experiments with copper and nickel

The rate at which Ni and Cu bind to the *Azolla* biomass is an important parameter when considering the use of this system as a practical bioremediation system. It will give an indication of the time that the metal laden effluent needs to be in contact with the biomass in order to get maximum adsorption. Kinetics experiments were performed with Ni at a starting concentration of 1000 $\mu\text{mol/l}$, at pH 5, 20°C. Figure 2.11 shows the removal of Ni by *Azolla* biomass over a period of 5 hours. There is an initial rapid adsorption of Ni for the first 10 minutes after which there is little or no adsorption of Ni from solution.

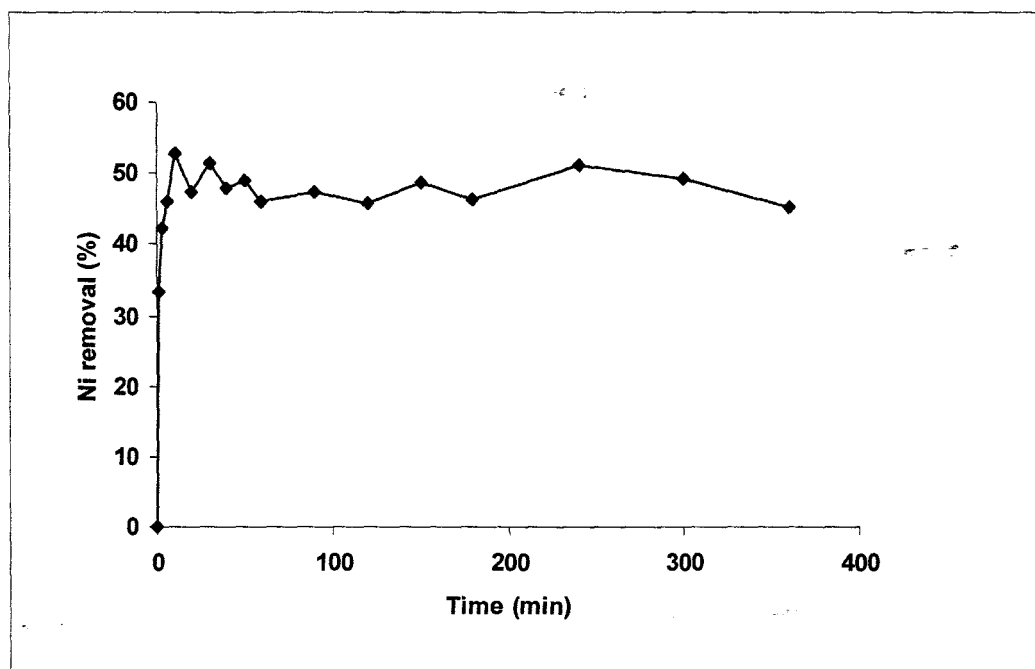


Figure 2.11: The effect of contact time on the adsorption of Ni by *Azolla*, concentration 4g/l, pH 5, 20°C, 200rpm, sampled over 5 hours.

The adsorption of nickel from solution under the same conditions, sampled over a period of 60 minutes is shown in figure 2.12. 100 % of the total metal removed was removed in the first 10 minutes of the reaction, with a maximum metal removal of 52 %. Equilibrium was then reached after 10 minutes with little or no metal being removed

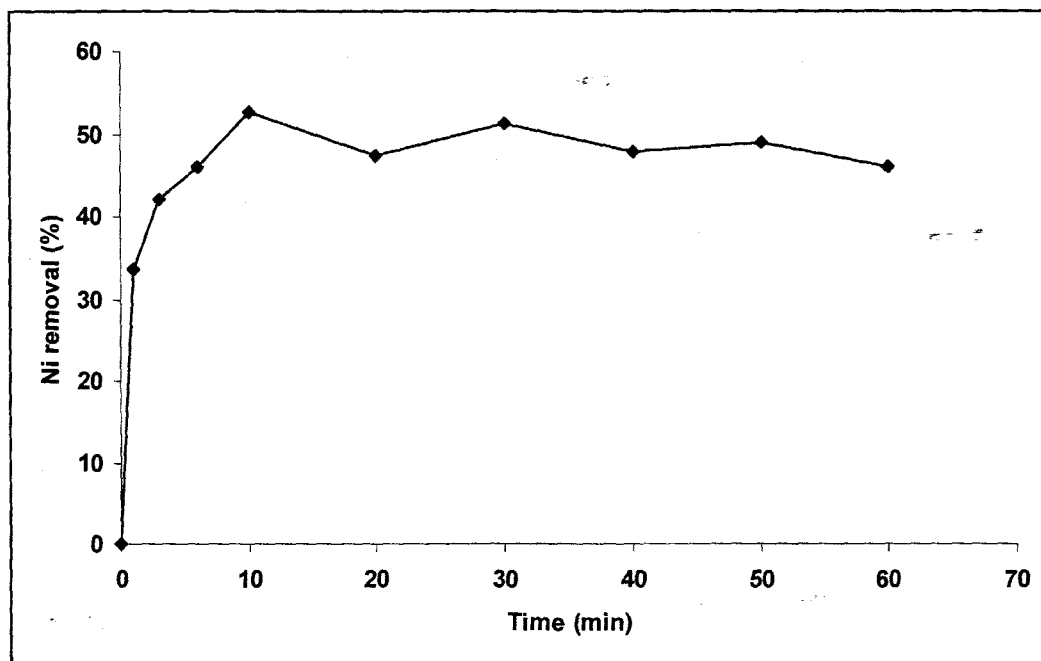


Figure 2.12: The effect of contact time on the adsorption of Ni by *Azolla*, concentration 4g/l, pH 5, 20°C, 200rpm, sampled over 60 minutes.

Figure 2.13 shows the removal of Cu from cupric sulfate solution with a starting concentration of 1000 $\mu\text{mol/l}$, at pH 5, by *Azolla* over a period of 5 hours at a 20°C. There is an initial rapid uptake of Cu in the first 30 minutes of the reaction with the Cu removal leveling off to a maximum of 62%.

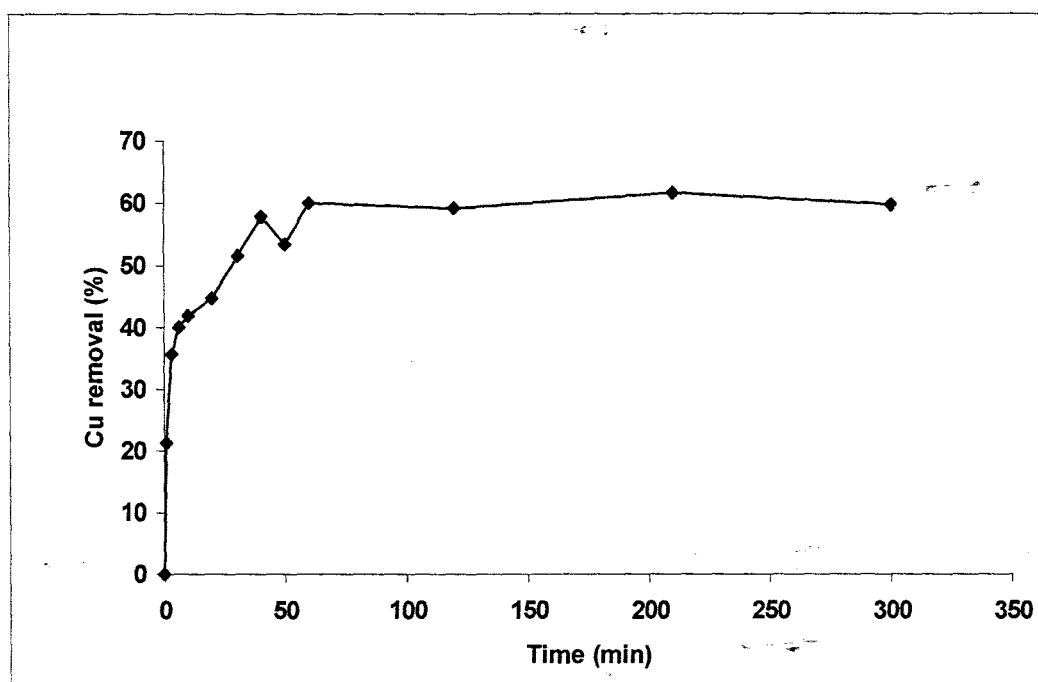


Figure 2.13: The effect of contact time on the adsorption of copper by *Azolla*, concentration 4g/l, pH 5, 20°C, 200rpm, sampled over 5 hours.

Figure 2.14 shows the removal of Cu during the first hour of the experiment under the same conditions as above. This figure highlights the initial rapid rate of Cu removal. Seventy percent of the total metal removed was removed in the first 20 minutes, followed by a more gradual sorption. Ni was adsorbed onto the *Azolla* biomass faster than Cu. The initial rapid removal of Cu and Ni has been reported in other studies (Goncalves and Boaventura, 1998; Chang *et al*, 1997; Galun *et al*, 1987). The importance of rapid adsorption is relevant in a continuous flow system because it enables an optimum metal uptake at a high flow rate.

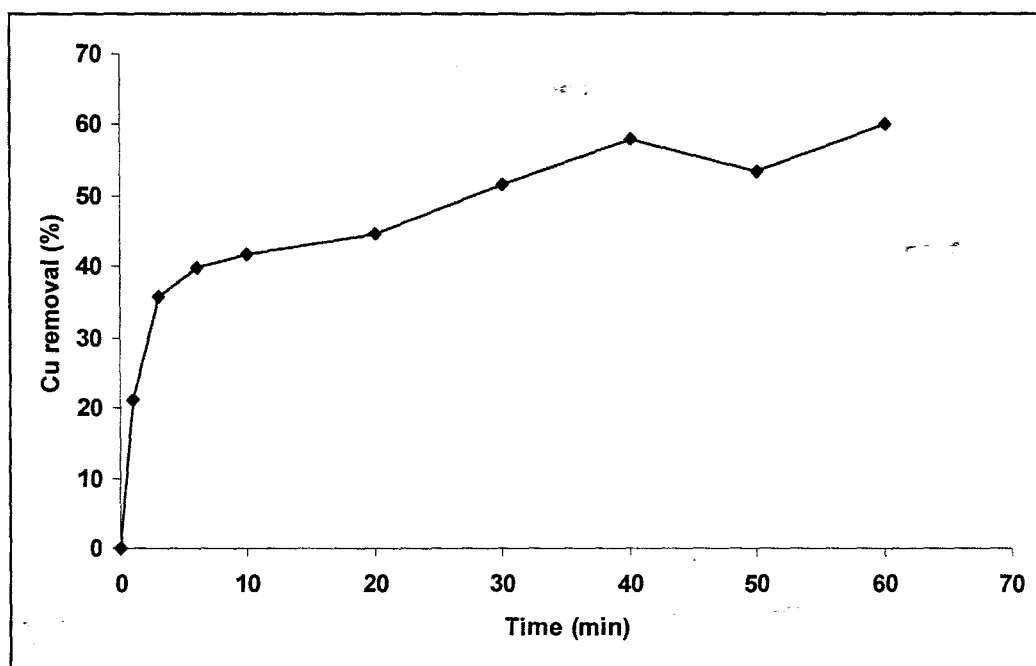


Figure 2.14: The effect of contact time on the adsorption of copper by *Azolla*, concentration 4g/l, pH 5, 20°C, 200rpm, sampled over 60 minutes.

2.4 CONCLUSION

Non-viable *Azolla filiculoides* biomass showed potential as a biosorbent of Cu and Ni from solution. Aqueous solutions of Ni with starting concentrations between 1000 and 2000 $\mu\text{mol/l}$ gave the most efficient Ni removal by *Azolla* biomass. For Cu the optimum starting concentration for adsorption was 50 $\mu\text{mol/l}$. The adsorption capacity of both Cu and Ni increased as the starting pH of the sorption media increased. The optimum pH for Ni adsorption was found at pH 7 and for Cu, at pH 5. *Azolla* biomass had a higher maximum binding capacity (q_{max}) for Cu than for Ni at pH 5. This could be an indication of a difference in the effect of pH on the binding capacity of *Azolla* biomass for different metals. Therefore, it would

be expected that *Azolla* biomass has a higher affinity for Cu at pH 5 and a higher affinity for Ni at pH 7.

The removal of both Cu and Ni showed little or no variation with the presence the other metal in solution, under the conditions of this experiment. It has been suggested that different metals bind to different sites on a biomass, because of the different affinities of the ligands for metals (Sag and Kutsal, 1996a). This could be a possible explanation for the apparent independence of copper and nickel binding to *Azolla*. This hypothesis is supported by the different pH profiles of copper and nickel binding.

Kinetic studies showed that both the binding of Cu and Ni to *Azolla* biomass is characterised by a rapid initial adsorption step. Ni is adsorbed more rapidly than Cu with 100% of the Ni that was adsorbed, being bound in the first 10 min of contact with the *Azolla* biomass. This characteristic is an advantage when applying the biomass in a fixed-bed column adsorption system.

CHAPTER THREE

THE REMOVAL OF COPPER AND NICKEL FROM AQUEOUS SOLUTION USING NON-VIABLE *Azolla filiculoides* IN AN UPSCALED FIXED-BED ADSORPTION COLUMN

3.1 INTRODUCTION

Batch systems are usually limited to the treatment of small quantities of waste water. For this reason it is necessary to investigate a continuous flow fixed-bed system for the removal of Cu and Ni from aqueous solutions (Zhao and Duncan, 1997b). Biosorption depends on binding equilibrium. Therefore, batch systems are unable to completely remove heavy metals from solution. Fixed-bed systems/reactors of biomass possess numerous theoretical plates of equilibrium. This allows as much as 99% metal removal, as each subsequent theoretical equilibrium plate removes more metal (Brady *et al*, 1994). There has been considerable interest in the potential process applications of bioremediation, in order to develop a treatment system for the large-scale treatment and the recovery of metals from waste or process streams (Tsezos *et al*, 1993).

There are a number of proposed reactor types. These include batch or continuous stirred tank reactors, fluidized bed, moving bed and packed-bed columns. Norber and Rydin (1983) describe a batchwise flocculation process, where the biomass can be regenerated and therefore be recycled. A reactor design described by Brady *et al* (1994) makes use of a hollow fiber

cross-flow membrane filtration system for immobilizing yeast biomass. This system was chosen as an alternative to gel immobilization, as cost and diffusion rates become limiting factors, when working on a larger scale. The advantage of a cross-flow microfiltration system (CFMF) is that it operates at low pressures and at high flux rates. The maximum volume treated that was reported was 20L with as much as 1000 μmol of metal (Cu) being accumulated onto 14g of yeast biomass. Another proposal for the treatment of metal laden effluent on a larger scale is a continuous stirred tank reactor (CSTR) train followed by separators, but this has been shown to have limited practical application (Tsezos *et al*, 1988).

Of all the reactor types that have been considered, packed-bed (also fixed-bed) columns are the most widely used and considered to have the best potential for metal removal on a larger scale. Packed-bed adsorption has a number of advantages that are related to its process engineering. It is a simple and high yield operation that can be easily scaled up from a laboratory scale procedure. The stages in the separation protocol can be automated and high degrees of purification can be achieved (Aksu and Kutsal, 1997). In general, columns are always vertical, cylindrical shells, closed on top and the bottom, but including a 'man hole' for access to the biomass. Columns should include perforated adsorbent bed supports to house the biomass and prevent cavitation of the adsorbent bed. Another consideration that needs to be addressed when designing a fixed bed adsorption column is that the adsorbent needs to be regenerated and reused repeatedly (Johnston, 1972). The economical and ecological success of a bioremediation process for metal laden effluent will depend, not only on optimum biosorption, but also the ease of metal recovery and the ability to regenerate the biomass (Bux *et al*, 1995). An added advantage of *Azolla filiculoides* as a biomass is that no immobilization

of the biomass is required. This will save on considerable cost of an appropriate immobilization material.

In this chapter the potential for an upscaled application of biosorption of Cu and Ni to *Azolla* biomass is evaluated with the upscaling of a normal lab scale column approximately one hundred times. Preliminary studies are done with lab-scale columns in order to compare the performance of the upscaled fixed-bed column system. For the purpose of recovering the metals from the column biomass and reusing the biomass a desorption profile was performed by washing the column biomass with dilute mineral acid after adsorption of Cu and Ni. Reusability of the column was tested with 10 sorption/desorption cycles with Ni. The capacity of the column to treat actual effluent was tested by treating a concentrated Ni laden effluent obtained from a Ni plating factory.

3.2 MATERIALS AND METHODS

3.2.1 Upscaled 4L fixed-bed adsorption column system

A normal lab scale column (bed volume 49 ml) with which fundamental column experiments were performed was upscaled approximately 88 times to a bed volume of 4.3295L (Figure 3.1). The column is made out of PVC drain piping with a 10.5cm diameter. The advantage of PVC plastic as a material for the column is that metal will not bind to the column as in the case of a glass column for instance. Another advantage is the relatively low cost of the materials. The column cost approximately R75 to manufacture. On either end are fine nylon sieves, reinforced with plastic grids, followed by screw-on stop-ends to seal the column. The

biomass is housed between sieves. There is a 1.5cm gap between the sieve and the inflow pipe at the bottom stop-end, which forms a pseudo showerhead to eliminate channeling. When the biomass is loaded into the column it forces the sieve out into a convex shape. This will also aids in the prevention of channeling, as the effluent flowing in will be forced to the sides of the column. Sieves and stop-ends on both ends can be easily removed to make cleaning the column convenient. The biomass is loaded from the top of the column with the bottom stop-end in place. The column can hold approximately 500g of firmly packed *Azolla* biomass.

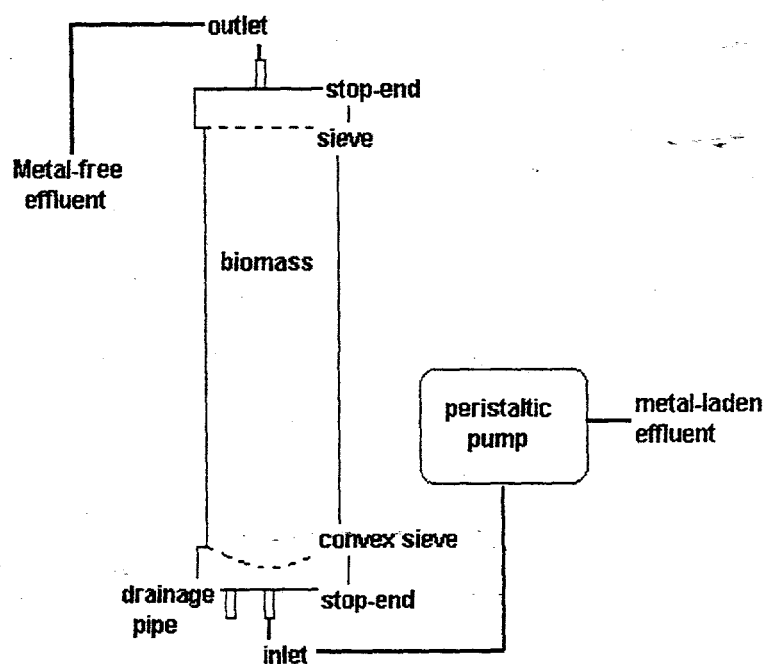
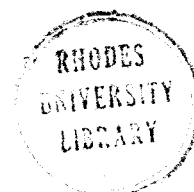


Figure 3.1: Schematic diagram of 4L fixed-bed adsorption column system using *Azolla* biomass.



3.2.2 Biomass

Azolla biomass for the normal lab scale experiments was obtained from the laboratory *Azolla* pond. With the large scale column, however, at the out-set of doing these experiments there was a shortage of available fresh *Azolla*. It was no longer growing on the dams where it was normally found. The laboratory *Azolla* pond could not produce enough *Azolla* to sustain the amount of needed for these large scale experiments experiments. Two alternative sources of old *Azolla* were located. The first was dried *Azolla* that had been harvested approximately a year earlier, dried under indirect sunlight in the lab, and stored in relatively air tight black plastic bags (Biomass 1). The second was obtained from the Botany Department at Rhodes University. This *Azolla* had been harvested about 8 months ago, also dried in the sun, but then stored under much harsher conditions, in sunlight on the floor of a large green house (Biomass 2). Both biomasses were used without further treatment or grinding.

3.2.3 Column adsorption of nickel

A normal lab scale column (bead volume 49ml) was used for preliminary column experiments with Ni. *Azolla* biomass was cultivated in the laboratory pond and prepared as in section 2.2.1, except that it was not ground through a sieve. 5g of *Azolla* biomass was packed into a glass column of 25mm internal diameter to achieve a bead volume of 49ml. A feed solution of 1000 μ mol/l $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ was pumped upwards through the column using a peristaltic pump (Watson Marlow, 504S). The flow rate was maintained at 200ml/hour. The pH of the solution was adjusted to pH 5. The working temperature was kept at approximately 20°C. The elute was collected in 20ml fractions. Fractions were analyzed for Ni concentration.

3.2.4 Column adsorption of copper

Experiments with Cu were done in the same way and under the same conditions as with Ni, except that a feed solution of 1000 μ mol/l CuSO₄ was pumped upwards through the column.

3.2.5 Adsorption of nickel using upscaled 4L fixed-bed adsorption column system.

500 g of Azolla biomass was packed into the 4L column. The biomass was washed with 10 l of tap water. Feed solutions of 1000 μ mol/l NiCl₂.6H₂O were pumped upwards through the column at 300, 400, 450 and 500 ml/min, using a peristaltic pump (Watson Marlow). The pH of the solutions were not adjusted and remained at a pH of approximately 6.3, the pH at which the 1000 μ mol/l NiCl₂.6H₂O solution was when made up. The temperature was maintained at approximately 20°C. Due to the large volume of the solutions that were passed through the column, entire fractions could not be collected. Instead, a sample was collected every 10, 15 or twenty minutes, depending on the flow rate. Samples were analyzed for nickel concentration. Both forms of biomass were used separately to test their different adsorption capacities.

3.2.6 Desorption of nickel from column biomass

A feed solution of 2000 μ mol/l NiCl₂.6H₂O was passed upwards through the 4L column, in order to saturate the column with Ni, at a flow rate of about 400ml/min, at 20 °C. Biomass 2 was used in the column. The column was then washed by passing 10L of 0.1 M HCl upwards through the column at a flow rate of approximately 100ml/min. Whole fractions could not be collected because of the large volumes, so a sample was taken every 10min and analyzed for concentration of Ni.

3.2.7 Desorption of copper from column biomass

Experiments with Cu were done in the same way and under the same conditions as with Ni, with a feed solution of 2000 μ mol/l CuSO₄ was pumped upwards through the 4L column, in order to saturate the column with Cu.

3.2.8 Regeneration of column biomass

After desorption of the metal, the *Azolla* biomass was regenerated by washing the column with hydrochloric acid. 10L of 0.1M HCl was circulated upwards through the column at approximately 450ml/min for about an hour. After acid washing 2 bead volumes (approximately 8L) of 0.1M NaOH passed through the column to reverse the acidity of the biomass. The column was then washed with 10L of tap water. The reconditioning of the regenerated biomass was considered necessary because pH values of the acid-washed biomass were extremely low (1-2) with the result that metal uptake was markedly decreased.

3.2.9 Removal of nickel from expended Nicrolyte 1 plating solution

Expended Nicrolyte 1 plating solution was obtained from LUK Africa. Nicrolyte 1 is an electrolysis nickel plating process for the deposition of a nickel-phosphorous alloy on to metal surfaces. The starting Ni concentration of the effluent was measured on the AAS and was found to be 59636.5 μ mol/l and the pH was measured as pH 5. Effluent (25L) was passed

through the 4L column at a flow rate of 300ml/min. The column was packed with 500g of *Azolla* biomass 2. The elute was collected every 5 min and measured for Ni concentration.

3.3 RESULTS AND DISCUSSION

3.3.1 Removal of Ni and Cu by *Azolla* biomass in a lab scale fixed-bed column

The removal of Ni and Cu by *Azolla* biomass using a lab scale column was initially studied in order to establish a comparison with metal removal in an upscaled system. Breakthrough curves are usually used when assessing the performance of fixed bed column adsorption (Wong and Niedzwiecki, 1982). The breakthrough point for the column operation was defined as the volume when the exit concentrations of Ni and Cu reached 40 % of the influent concentration. At this point it is said that the column has reached 40 % saturation. 100 % saturation of the biomass would be when the exit concentrations were the same as that of the influent concentration. Figure 3.2 shows the breakthrough curve for the removal of Ni from solution by 5g of *Azolla* in a lab scale packed bed column. Initially there was very good removal of Ni. As more influent passed through the column the removal of Ni dropped steadily as the column became more and more saturated. The breakthrough point of 40 % saturation of biomass was reached after 680 ml of 1000 $\mu\text{mol/l}$ Ni solution had been passed through the column. The pH profile shows an initial increase in pH from a starting pH of 5 to pH 6.8. There was a gradual decrease in the pH as the biomass became more saturated. Similar results have been reported in the literature with Ni as well as other metals (Zhao and Duncan, 1997b and 1998).

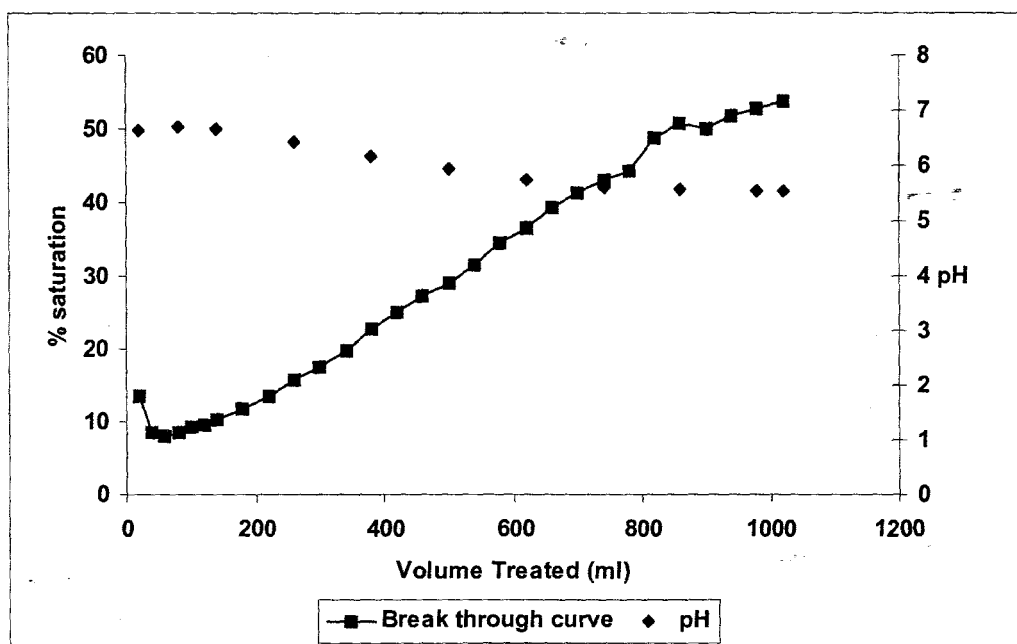


Figure 3.2: Breakthrough curve for a 5g *Azolla* biomass column removal of Ni at a flow rate of 200 ml/hour. Influent pH 5 and Ni, 1000 $\mu\text{mol/l}$.

The cumulative removal of Ni is shown in figure 3.3. A maximum of 92 % removal was achieved at the initial stages of the experiment. Thereafter the percentage of metal removal decreased rapidly. After 680 ml (the volume treated at break through point) of Ni solution had passed through the column, 49 % of the total metal had been adsorbed onto the biomass. At breakthrough point the Ni uptake of *Azolla* biomass was 68 $\mu\text{mol/g}$ (3.91 mg/g).

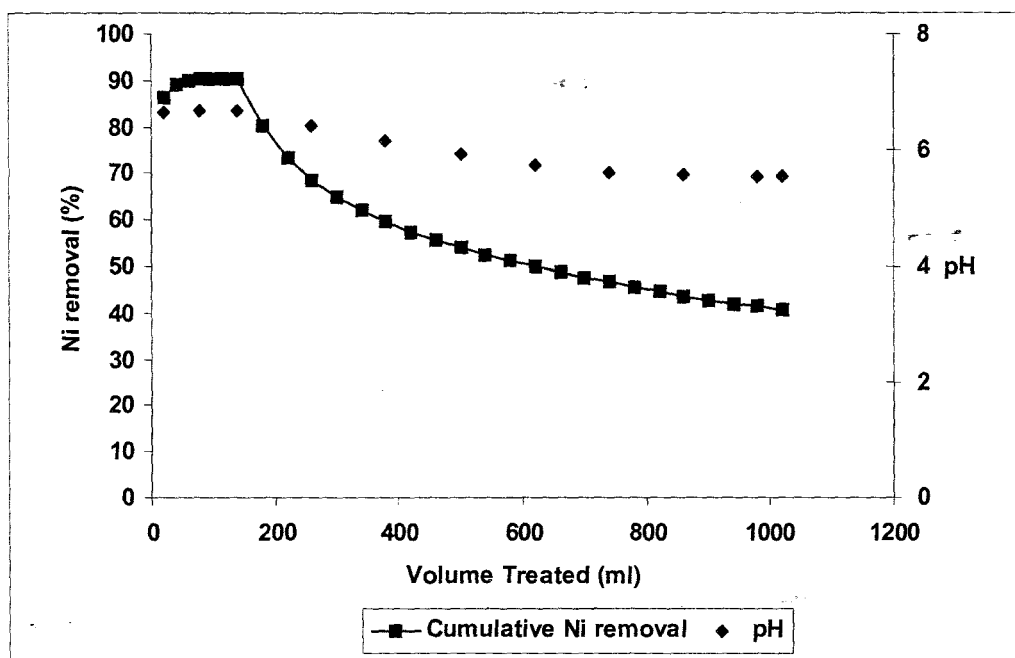


Figure 3.3: The cumulative removal of Ni by 5g of *Azolla* biomass in a fixed-bed column system, at a flow rate of 200ml/hour. Influent pH 5 and Ni, 1000 $\mu\text{mol/l}$.

Figure 3.4 shows the breakthrough curve for the removal of Cu from solution by 5g of *Azolla* in a lab scale packed bed column. Initially there was very good removal of Cu. The removal of Cu from solution steadily decreased as more influent passed through the column and the column became more saturated. The breakthrough point of 40 % saturation of biomass was reached after 720 ml of 1000 $\mu\text{mol/l}$ Cu solution was passed through the column. As with Ni, pH profile showed an initial increase in pH from a starting pH of 5 to pH 6.7. There was a gradual decrease in the pH as the biomass became more saturated. There was a greater decrease in pH with Cu than with Ni.

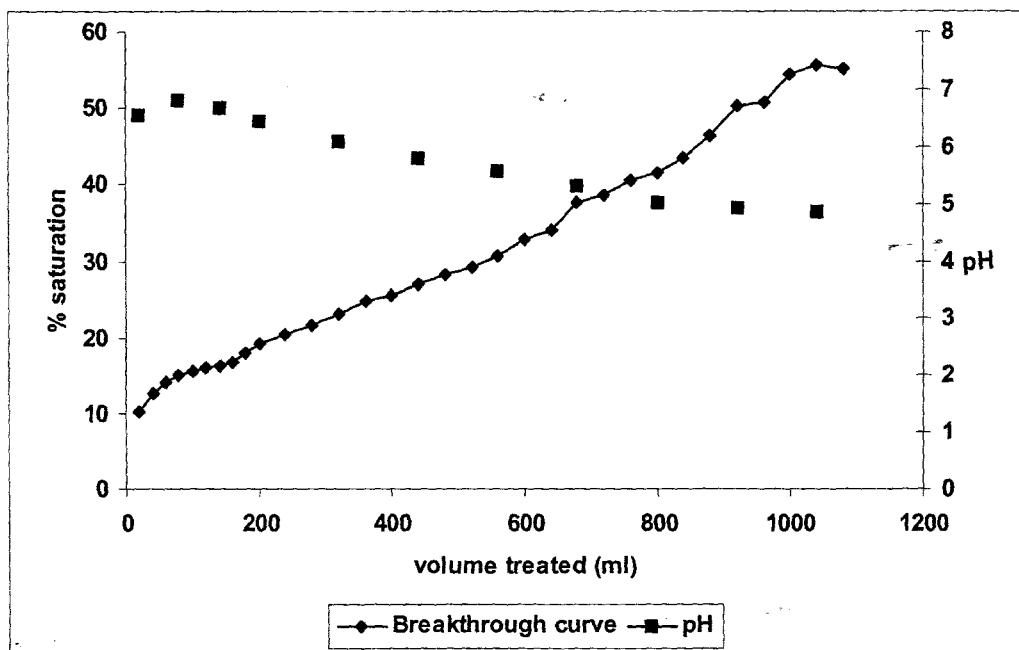


Figure 3.4: Breakthrough curve for a 5g *Azolla* biomass column removal of Cu at a flow rate of 200 ml/hour. Influent pH 5 and Ni, 1000 $\mu\text{mol/l}$.

Figure 3.5 shows the cumulative removal of Cu. The maximum removal of Cu was not as good as for Ni, with a maximum of 90 % Cu removal. After 720ml (the volume treated at break through point) of Cu solution had passed through the column, 50 % of the total metal treated had been removed. At breakthrough point the Cu uptake of *Azolla* biomass was 72 $\mu\text{mol/g}$ (5.57 mg/g).

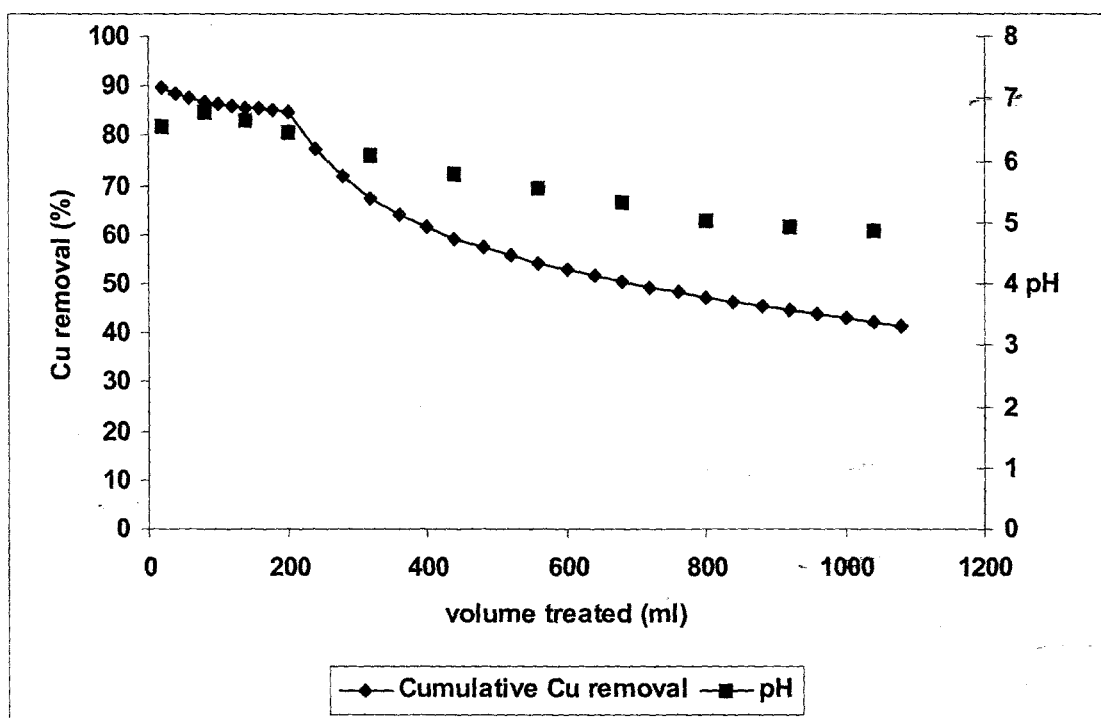


Figure 3.5: Cumulative removal of Cu from solution in a 5g *Azolla* biomass column at a flow rate of 200 ml/hour. Influent pH 5 and Cu, 1000 $\mu\text{mol/l}$.

3.3.2 Comparison of biomass 1 and biomass 2 in an upscaled 4l column

Two different forms of *Azolla* biomass, biomass 1 and biomass 2 (see Materials and Methods section 3.2.2) were tested for their capacity to adsorb Ni from solution in an upscaled 4L packed bed column system. Figure 3.6 shows the breakthrough curves for the removal of Ni from aqueous solution by *Azolla* biomass in a 4L packed bed column, at a flow rate of 300 ml/min. Initially both forms of biomass showed good removal of Ni. Biomass 2 started to saturate after about 20L of Ni solution had been passed through it. Biomass 1 did not become saturated after treating almost 60L of Ni solution. The maximum percentage saturation of

biomass 2 was about 11 % after treating 85L, and that of biomass 1 was 12 % after treating 115L. A breakthrough point could not be reached after treating 80L of Ni solution by both biomass 1 and 2. This is a proportionally better performance than the lab scale column. The pH profile of the bigger column followed the same trend as that of the smaller lab scale column.

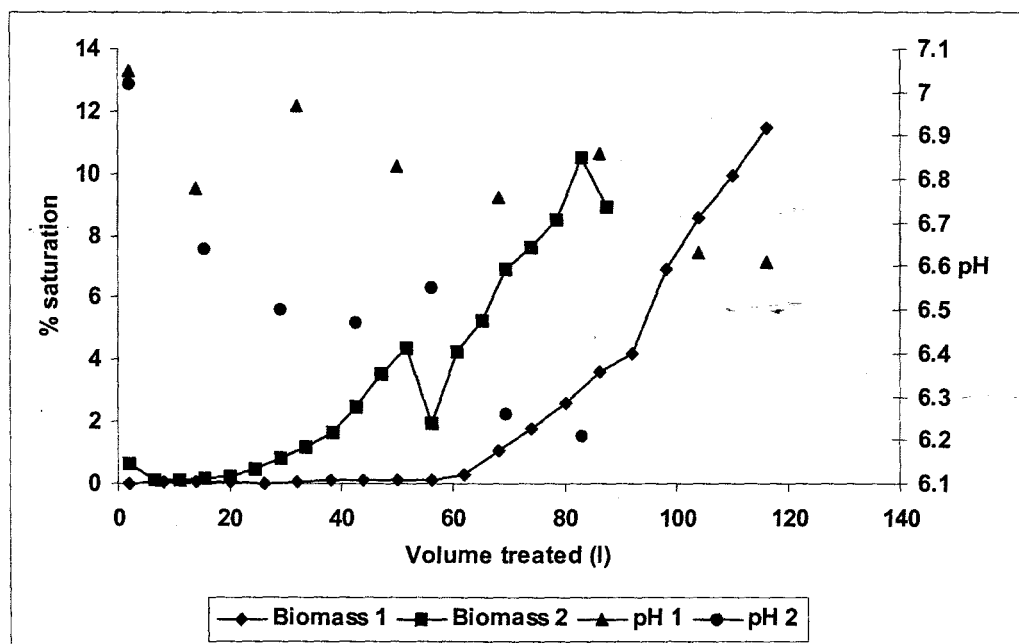


Figure 3.6: Breakthrough curves for the removal of Ni from solution by *Azolla* biomass 1 and 2 in an upscaled 4L fixed-bed column system. Flow rate 300ml/min, 400g of biomass 1, 500g of biomass 2, influent pH 6.3, Ni 1000 $\mu\text{mol/l}$.

The cumulative removal of Ni from aqueous solution by biomass 1 and biomass 2 is shown in figure 3.7. Initially 100 % removal of Ni was achieved by biomass 1. The maximum percentage Ni removal by biomass 2 was marginally less than that of biomass 1, at 99.8 %. Biomass 1 was able to remove more than 97 % of the total Ni that was passed into the system. Even though a breakthrough point was not reached after treating 115L of Ni solution, biomass

2 achieved a Ni uptake of 278.875 $\mu\text{mol/g}$ (16.037 mg/g). Biomass 2 was able to remove more than 96% of the total Ni that was fed into the system. After treating 82L of Ni solution, biomass 2 had a Ni uptake of 164 $\mu\text{mol/l}$ (9.43mg/g). Due to the relatively good performance of both types of biomass in removing Ni, it was not considered necessary to also test their performance with Cu.

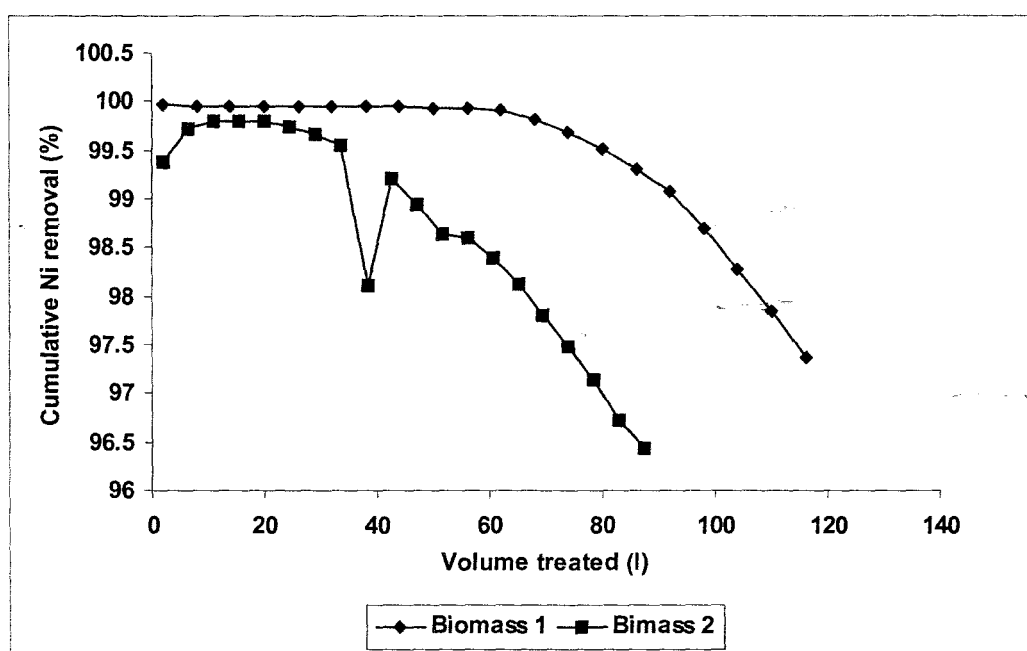


Figure 3.7: The cumulative Ni removal by *Azolla* Biomass 1 and 2 in an upscaled 4L fixed-bed column system. Flow rate 300ml/min, 400g of biomass 1, 500g of biomass 2, influent pH 6.3, Ni 1000 $\mu\text{mol/l}$.

3.3.3 Effect of flow rate on Ni removal in a 4L fixed-bed column.

The most critical parameter for the biosorption of metal is the pH of the adsorption medium (Aksu and Kutsal, 1998). In the batch experiments with Ni and *Azolla* the adsorption capacity of *Azolla* biomass increased as the initial pH of the adsorption medium increased to the value of 7. The pH of the adsorption medium for the removal of Ni from solution by the 4L packed

bed column system was measured at pH 6.3. No adjustments were made to the pH of the adsorption medium due to difficulties imposed by the large volumes. This was deemed not to be necessary as the pH of the medium was close enough to the optimum pH, as determined in the batch experiments. The effect of flow rate was investigated to establish the maximum throughput for optimum adsorption of Ni.

Figure 3.8 shows the breakthrough curves for the removal of Ni by *Azolla* in an upscaled 4L packed bed column system at 4 different flow rates: 300ml/min, 400ml/min, 450ml/min and 500ml/min. The breakthrough point of 40 % saturation was not reached after treating 87L of Ni solution for all of the flow rates. The minimum retention time (the time that the metal solution remains in contact with the biomass before it passes through the column) needed and therefore the optimum flow rate is determined by the rate that the metal is adsorbed by the biomass. In Chapter 2 it was found that Ni has a high rate of adsorption onto the *Azolla* biomass. 100 % of the Ni that was adsorbed in the kinetic study, was adsorbed in less than 10 minutes of contact between the metal and the biomass. The minimum retention time that could be achieved by 500 ml/min is about 9 minutes. Therefore, it is possible, that the maximum flow rate that was achieved would not significantly effect the total metal adsorbed.

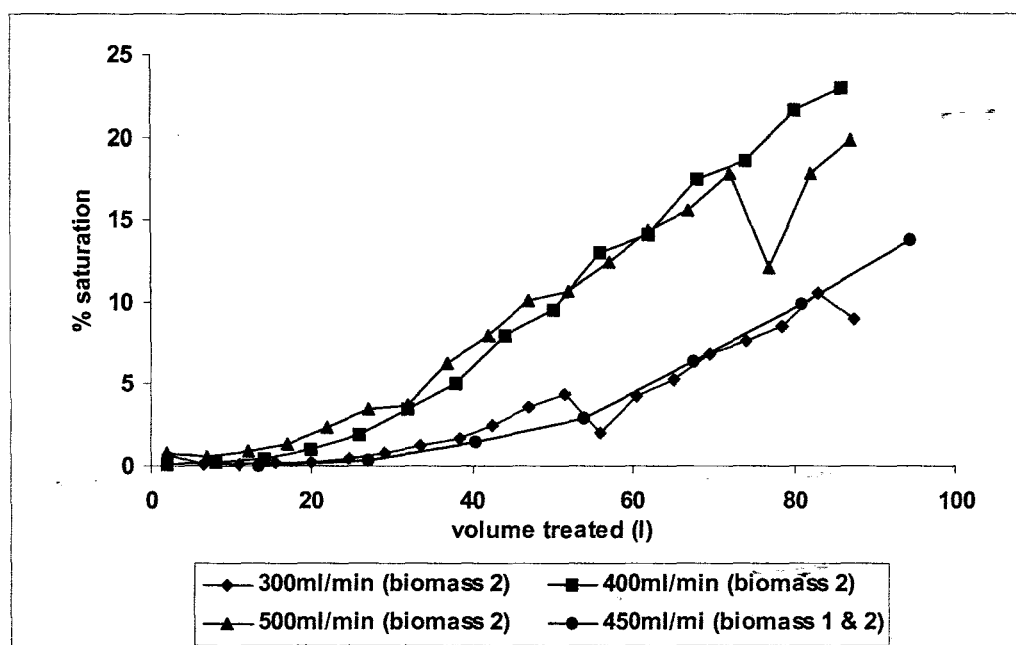


Figure 3.8: Breakthrough curves for the removal of Ni from solution by *Azolla* biomass in an upscaled 4L fixed-bed column system at 4 different flow rates: 300ml/min, 400ml/min, 450ml.min and 500ml/min. 500g of biomass, influent pH 6.3, Ni 1000 $\mu\text{mol/l}$.

Figure 3.9 shows the cumulative removal of Ni from aqueous solution by *Azolla* biomass in a 4l packed bed column system at different flow rates. 100 % removal of Ni was achieved at flow rates 300ml/min, 400ml/min and 450ml/min. At 500ml/min the maximum percentage Ni removal was 99%. The percentage Ni removal of the total amount of Ni fed into the system after 87L of Ni solution was treated was 96%, 90.4%, 95% and 90% at 300ml/min, 400ml/min, 450ml/min and 500ml/min, respectively. Due to the limitations in the amount of biomass available, the experiment performed at 450ml/min had a mixture of biomass 1 and 2. Even though the breakthrough point was not reached with the volumes that were treated, a Ni

uptake of $167.04\mu\text{mol/l}$, $157.296\mu\text{mol/l}$, $165.3\mu\text{mol/l}$ and $156\mu\text{mol/l}$ was achieved at these respective flow rates.

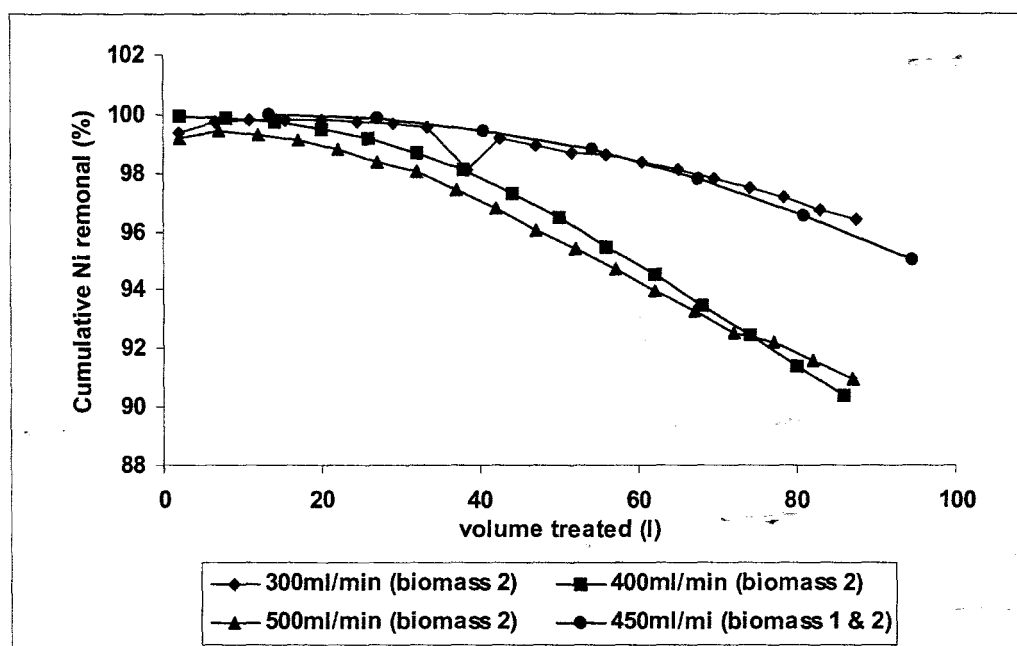


Figure 3.9: The cumulative Ni removal by *Azolla* Biomass in an upscaled 4L fixed-bed column system at a flow rate of 300ml/min, 400ml/min, 450ml/min and 500ml/min. 500g of biomass, influent pH 6.3, Ni $1000\mu\text{mol/l}$.

3.3.4 Desorption profile for the recovery of Ni and Cu in a 4L fixed-bed column.

Studies have been carried out with a number of desorbing agents. HCl has been reported to be a very efficient desorption agent of metal ions. High concentrations of protons made available by the HCl are thought to dislodge the metal ions from binding sites (Bux *et al*, 1995). Figure 3.10 shows the desorption profiles for the removal of Ni and Cu using 10L of 0.1M HCl. Cu and Ni showed similar desorption profiles. There was an initial increase in the amount of Ni

and Cu removed per fraction of desorption liquor. Thereafter the amount of Ni and Cu being removed decreased steadily as the amount of the metal bound to the biomass decreased.

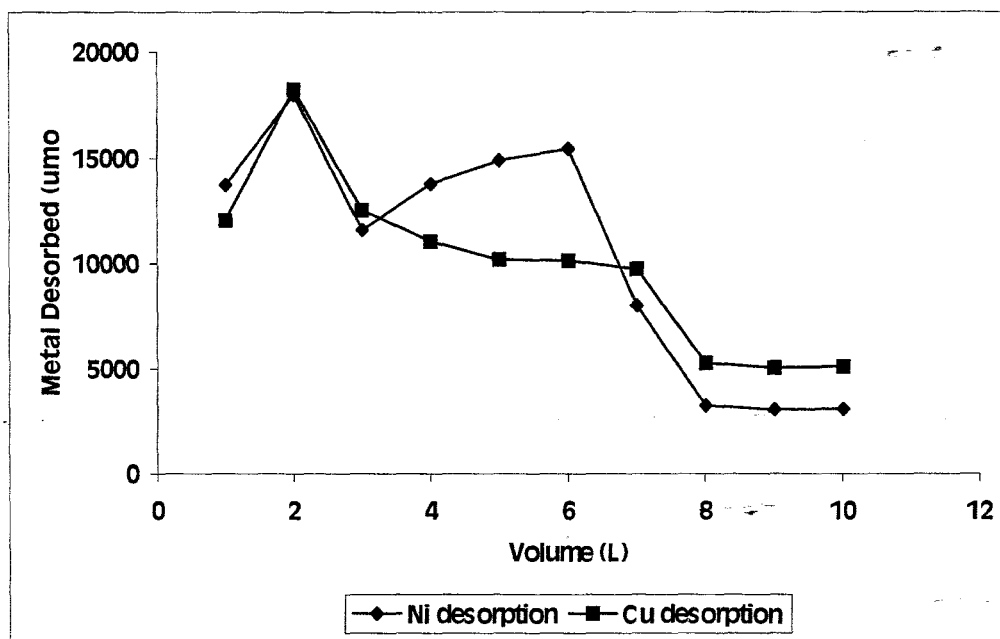


Figure 3.10 Desorption profile for the recovery of Ni and Cu from *Azolla* biomass in a 4L fixed-bed column, using 10L of 0.1M HCl. Flow rate 100ml/min. Temp 20°C.

Figure 3.11 shows the amount of Ni and Cu adsorbed in the saturation step, the amount of Ni and Cu removed and the percentage recovery of Ni and Cu. Recovery of up to 80% of Ni was achieved using 0.1M HCl in a volume of 10L. Only as much as 70% recovery of Cu was achieved using 0.1M HCl in a volume of 10L 0.1M.

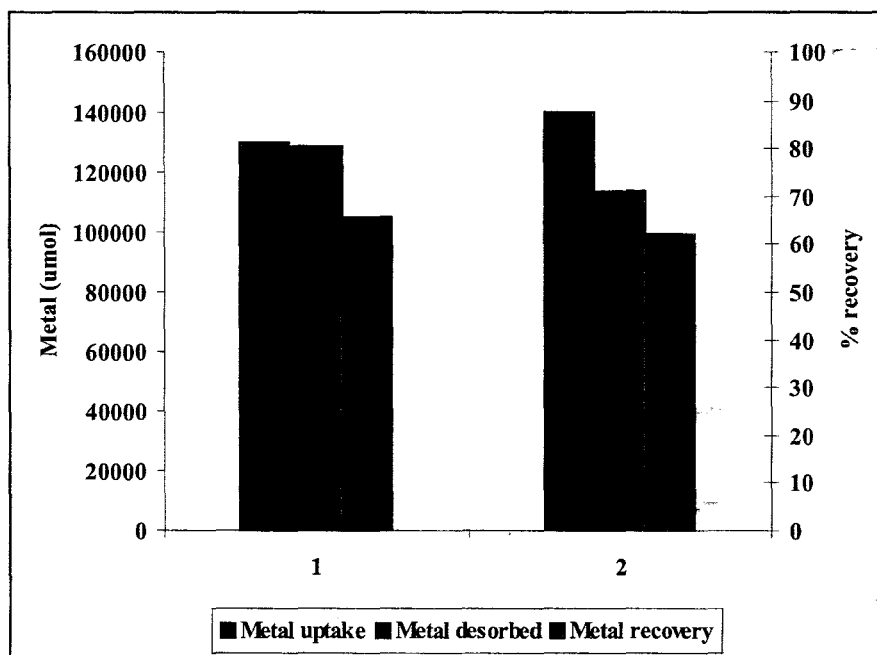


Figure 3.11: Saturation, desorption and recovery of Ni (1) and Cu (2) from *Azolla* biomass in a 4L fixed-bed column, using 10L of 0.1M HCl. Flow rate 100ml/min. Temp 20°C.

3.3.5 Reusability of *Azolla* biomass in a 4L fixed-bed column

Reusability of the *Azolla* Biomass was tested by running 10 subsequent adsorption/desorption cycles using Ni with the same biomass in the 4L column. Cu was not tested since Ni and Cu showed similar sorption and desorption patterns. Desorption was performed differently to the desorption profile generated in section 3.3.4. 10L of 0.1M HCl was circulated through the column at 400ml/min for about an hour in order to try and utilize the desorption solution fully. The column was reconditioned using 2 bed volumes (approximately 8L) of 0.1M NaOH.

Figure 3.12 shows the breakthrough curves for the removal of Ni by *Azolla* in an upscaled 4L fixed-bed column system over 10 consecutive adsorption/desorption cycles. The biomass showed excellent reusability over 10 cycles with a total of 1620L of 1000 μ mol/l Ni solution treated.

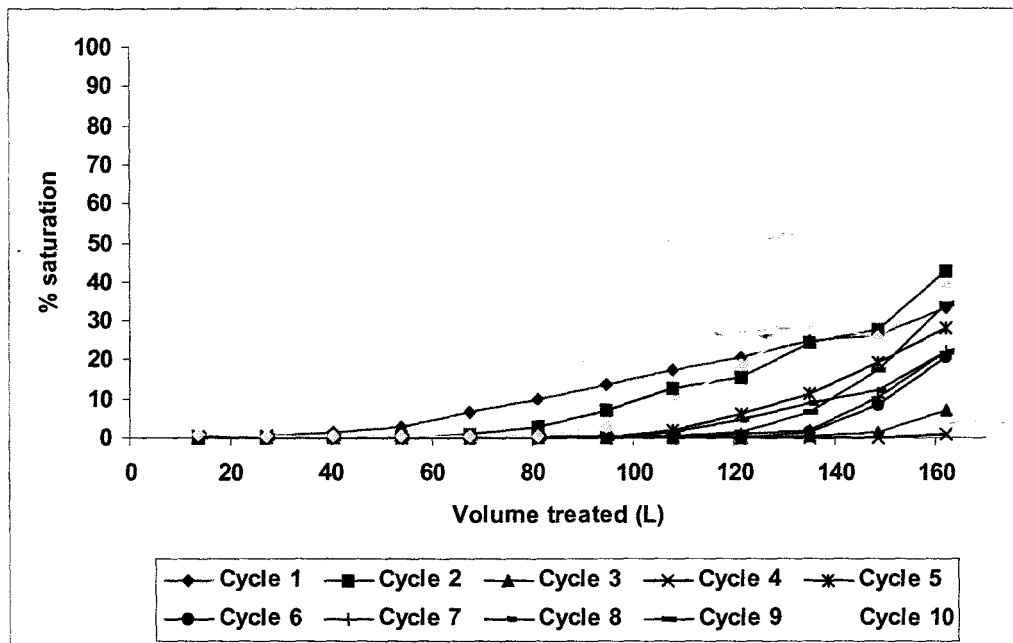


Figure 3.12: Breakthrough curves for the removal of Ni from solution by *Azolla* biomass in an upscaled 4L fixed-bed column system for 10 consecutive adsorption/desorption cycles. Flow rate 450ml/min. 500g of biomass, influent pH 6.3, Ni 1000 μ mol/l.

The percentage removal of Ni increased after the second cycle with the best removal taking place in cycle 4 (figure 3.13). In cycle 4, 99.7% of the Ni was removed from the influent solution that was passed through the column. The improved performance in the third cycle suggested that the reconditioning step with 0.1M NaOH enhanced the performance of the biomass. It is possible that the increase in the pH opened up further binding sites for Ni. The

pH of the column after reconditioning was recorded at between 7.3 and 8 for each of the cycles (Table 3.1). The column did start to show some decline in performance, based on percentage metal removal after treating 160L, towards the end of the 10 cycles.

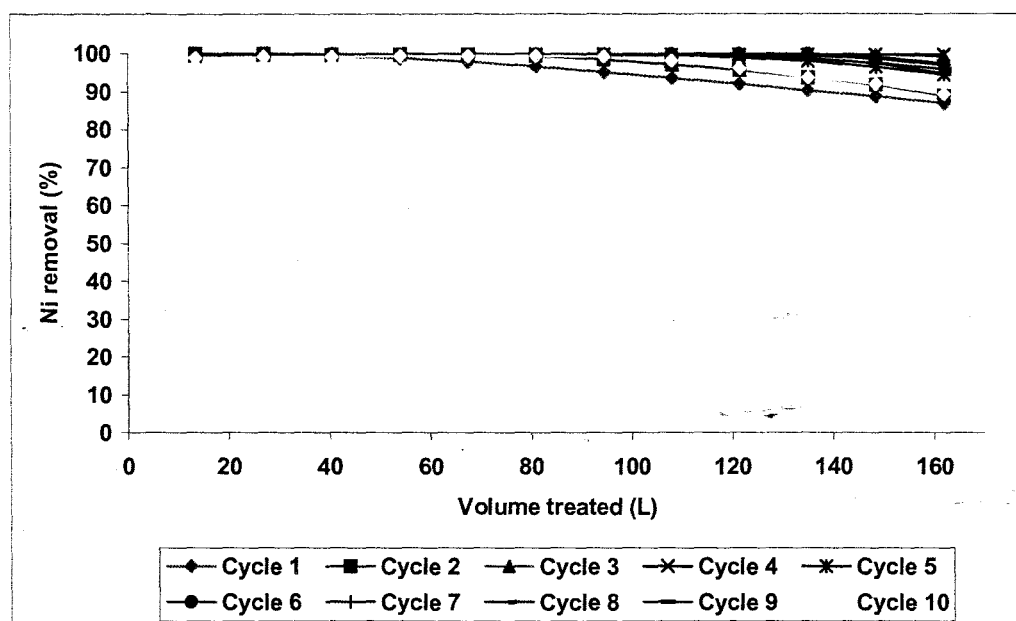


Figure 3.13: The cumulative Ni removal by *Azolla* Biomass in an upscaled 4L fixed-bed column system for 10 consecutive adsorption/desorption cycles. Flow rate 450ml/min. 500g of biomass, influent pH 6.3, Ni 1000 $\mu\text{mol/l}$.

Figure 3.14 shows the total Ni adsorbed, desorbed and the percentage recovery of Ni for the 10 consecutive adsorption/desorption cycles. A total of 1528.126mmol of Ni was absorbed onto the *Azolla* biomass during the 10 cycles. As much as 79% of the Ni was recovered. A summary of the data from this adsorption study is given in table 3.1

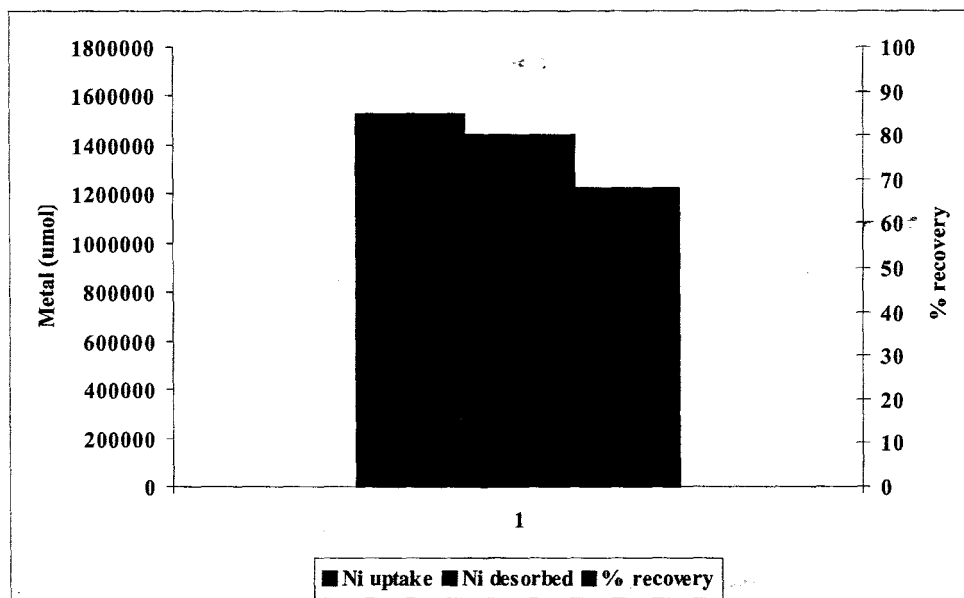


Figure 3.14: Saturation, desorption and recovery of Ni from *Azolla* biomass in a 4L fixed-bed column after 10 consecutive adsorption/desorption cycles.

Table 3.1: Summary of the Ni uptake, the Ni desorbed and the % recovery at the end of each cycle

Cycle #	Column pH	Ni uptake (μmol)	Ni Desorbed (μmol)	Recovery (%)
1	5.9	140.7575	110.1947	78.27
2	7.3	143.9649	110.08708	70.07
3	7.3	160.6688	112.7551	70.18
4	8.1	161.5244	138.7399	86
5	7.65	152.8503	125.7896	82.3
6	7.52	157.8048	139.1209	88.16
7	7.4	157.0262	125.5887	79.98
8	7.12	155.6261	130.8274	84.26
9	7.32	153.514	127.1934	82.85
10	7.3	144.7525	110.1136	76.07

3.3.6 Removal of nickel from expended Nicrolyte 1 plating solution

The breakthrough point of 40% was reached within the first five liters of Nicrolyte 1 plating solution passing through the column (Figure 3.15). The column biomass was saturated after 20L of effluent was treated.

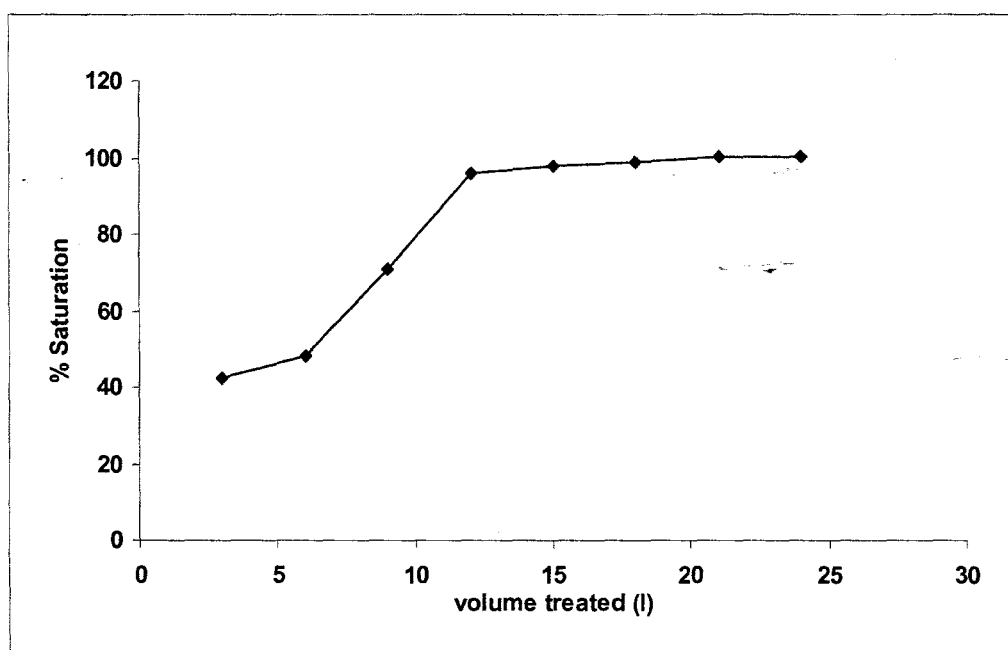


Figure 3.15: Breakthrough curve for the removal of Ni from expended Nicrolyte 1 plating solution by *Azolla* biomass in an upscaled 4L fixed-bed column system. Flow rate 300ml/min. 500g of biomass, influent pH 5, Ni 1000 $\mu\text{mol/l}$.

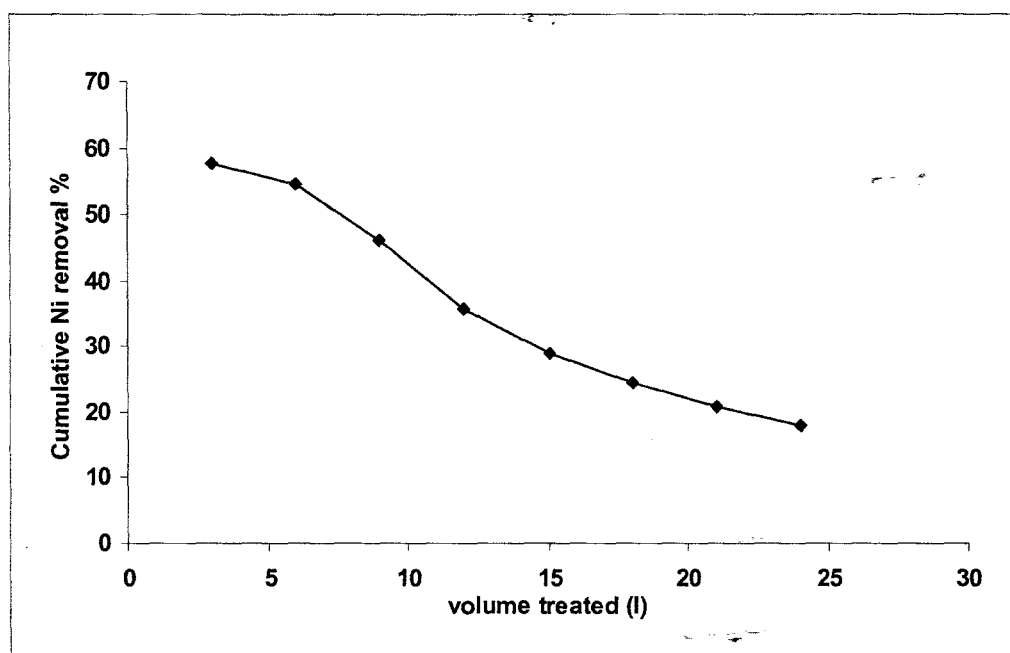


Figure 3.16: The cumulative Ni removal by *Azolla* Biomass in an upscaled 4L fixed-bed column system from expended Nicrolyte 1 solution. Flow rate 300ml/min. 500g of biomass, influent pH 5, Ni 59 637 $\mu\text{mol/l}$.

Figure 3.16 shows the cumulative percentage removal of Ni from expended Nicrolyte 1 solution. The highest percentage Ni removal was 57%. Of the total amount of Ni that was passed into the column only 18% was removed after treating 25L of effluent. At breakthrough point Ni uptake was 206 $\mu\text{mol/g}$. Although the percentage Ni removal was low, due to the very high Ni concentration of Nicrolyte 1, the metal uptake of the biomass was slightly improved compared with the Ni uptake from synthetic Ni solutions at much lower inflow concentrations. The elute from the column would have to be treated in subsequent columns connected in series to achieve satisfactory metal removal.

3.4 CONCLUSION

Column adsorption of Cu and Ni from aqueous solutions was successfully upscaled approximately 100 times from a lab scale fixed-bed column containing 5g of *Azolla* biomass to a 4L fixed-bed column containing 500g of *Azolla* biomass. Relative to the lab scale column, the 4L column performed better for the uptake of Cu and Ni per gram of biomass. The larger column was also able to operate at relatively higher flow rates.

The non-viable *Azolla filiculoides* biomass when dried and used in a column for adsorption of Cu and Ni showed good physical stability under many different conditions. The biomass needs no special pretreatment or immobilisation steps before it can be utilised in fixed-bed columns for the removal of Ni and Cu from solution. The biomass can simply be cultivated in ponds or removed from dams and rivers where it is causing a problem, dried at relatively low temperatures (30-50°C) and then packed into the column. The dried biomass can also be stored for long periods of time. Care should generally be taken to store *Azolla* biomass in darkness as prolonged exposure to UV light decreases its capacity for Ni and Cu adsorption. Different storage conditions of biomass used in the large scale systems in this study did not have markedly different adsorption characteristics.

The biomass showed good reusability with little change in the amount of Ni adsorbed in 10 consecutive cycles. There was a slight decrease in the column performance over the last 2 cycles but that is more likely due to the biomass becoming more saturated than to incomplete desorption. As much as 80% Ni was recovered and 70 % Cu was recovered when desorption

profiles were generated using 0.1M HCl as a desorption agent. Regeneration of *Azolla* biomass with 0.1M NaOH in between consecutive Ni adsorption/desorption steps enhanced the performance of the biomass and resulted in good recovery of Ni.

Only 18 % of the Ni could be removed from the expended Nicrolyte 1 plating solution, containing a very high Ni concentration, after treating only 25L. However, the *Azolla* biomass still showed good uptake at the breakthrough point. The 4L fixed-bed column adsorption system using *Azolla* biomass would, therefore, be more suited to more dilute metal laden effluents.

CHAPTER FOUR

SCANNING ELECTRON MICROSCOPY OF NON-VIABLE

***Azolla filiculoides* BIOMASS**

4.1 INTRODUCTION

The ability of *Azolla* biomass 1 and biomass 2 to remove Ni from solution was investigated in chapter 3. Biomass 1 was found to have a much greater capacity for the adsorption of Ni under the conditions of the experiment. The physiological and physical state of a biomass is known to affect the biosorption ability of that biomass (Sanyahumbi, 1999). Due to the difference in the treatment of the two forms biomass before they were used in the 4L column for the removal of Ni and Cu, it was thought that their physical state might reveal the difference in their biosorptive abilities.

The reusability of a biomass is an important consideration in the evaluation of the potential of a biomass as an economically and ecologically viable biosorbent. A non-viable *Azolla* biomass was chosen because it offers a good potential for biomass recycling. A non-viable biomass is generally able to withstand potentially destructive regeneration processes such as desorption of metal ions by mineral acids followed by a neutralisation step with a basic solution. The biomass needs to keep its metal binding capacity as well as maintain its structural integrity over many cycles of adsorption and regeneration.

Scanning electron microscopy (SEM) has been used to study the physical state of a biomass before and after exposure to metals in solution (Lawrence *et al*, 1998). An attempt was made to use SEM to observe the difference between *Azolla* biomass 1 and 2 and to study the effect of continuous recycling of *Azolla* biomass for the removal of Ni from solution on the physical integrity of the biomass.

4.2 MATERIALS AND METHODS

4.2.1 Biomass samples for SEM

Azolla biomass 1 and 2 were obtained and prepared as described in chapter 3. Samples were taken before biosorption took place and placed immediately into 2.5% glutaraldehyde in phosphate buffer fixative solution.

Samples of *Azolla* biomass were taken in between each adsorption/desorption cycle, immediately after regeneration with NaOH. Samples were placed into 2.5% glutaraldehyde in phosphate buffer fixative solution.

4.2.2 Preparation of sample for SEM

Samples were removed from the fixative and washed well with cold phosphate buffer. The samples were then dehydrated by washing them with a series of ethanol concentrations ranging from 30 to 100%. The ethanol was removed and the samples were washed with 75 : 25 then 25 : 75 ethanol : amyl acetate mixture. Samples were exposed to liquid CO₂ at 80 atmospheric pressure to achieve critical point drying. *Azolla* samples were then mounted onto

copper stubs, gold coated in a sputter chamber and subsequently observed with a JEOL JSM-840 scanning electron microscope.

4.3 RESULTS AND DISCUSSION

4.3.1 Comparison of *Azolla* biomass 1 and biomass 2

Figure 4.1 shows the electron micrograph for *Azolla* biomass 1 at 400x and 2000x magnification (**A** and **B** respectively) and for biomass 2 at 400x and 2000x magnification (**C** and **D** respectively). An attempt was made to try and observe the differences in the physical structure of the two biomass in order to explain the difference in their biosorption performance. It must be noted that electron micrographs were difficult to interpret and observations made were subjective. Comparison of both the 400x and the 2000x magnification micrographs of biomass 1 and biomass 2 indicate that biomass 2 has a more compacted structure. This could be the result of shrinking and perishing under exposure to sunlight.

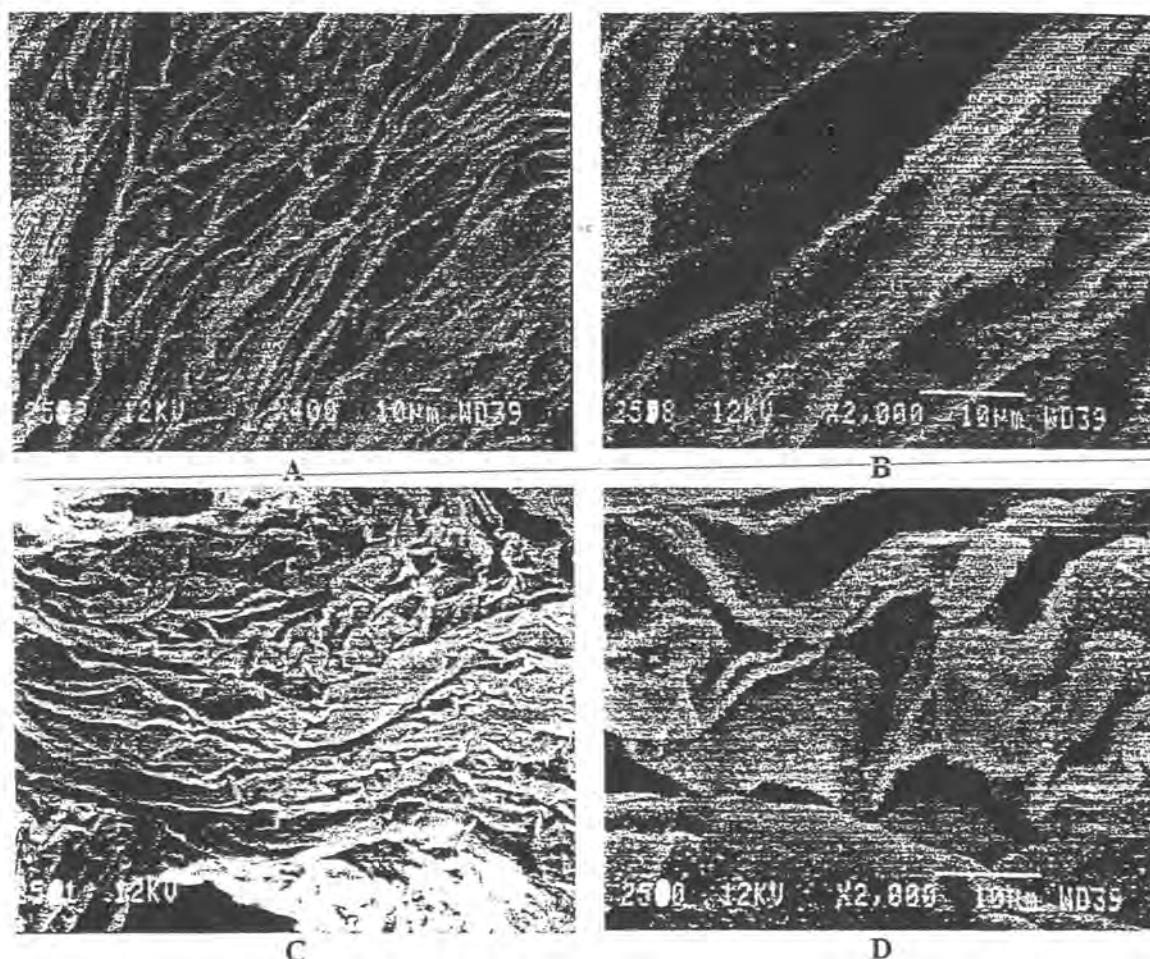


Figure 4.1: Scanning electron micrograph showing the physical state of whole *Azolla* biomass **A:** biomass 1 at 400x magnification **B:** biomass 1 at 2000x magnification **C:** biomass 2 at 400x magnification **D:** biomass 2 at 2000x magnification.

4.3.2 Comparison of *Azolla* biomass during regeneration cycles

Figure 4.2 shows SEM micrographs of *Azolla* biomass at the start of each adsorption cycles at 400x magnification. Attempts to observe the same biomass structures proved very difficult. It did seem clear from the micrographs that there was little change in the physical integrity of the biomass in subsequent cycles. This observation seems realistic in relation to the fact that there was very little change in Ni binding capacity of the biomass over 10 adsorption/desorption cycles.

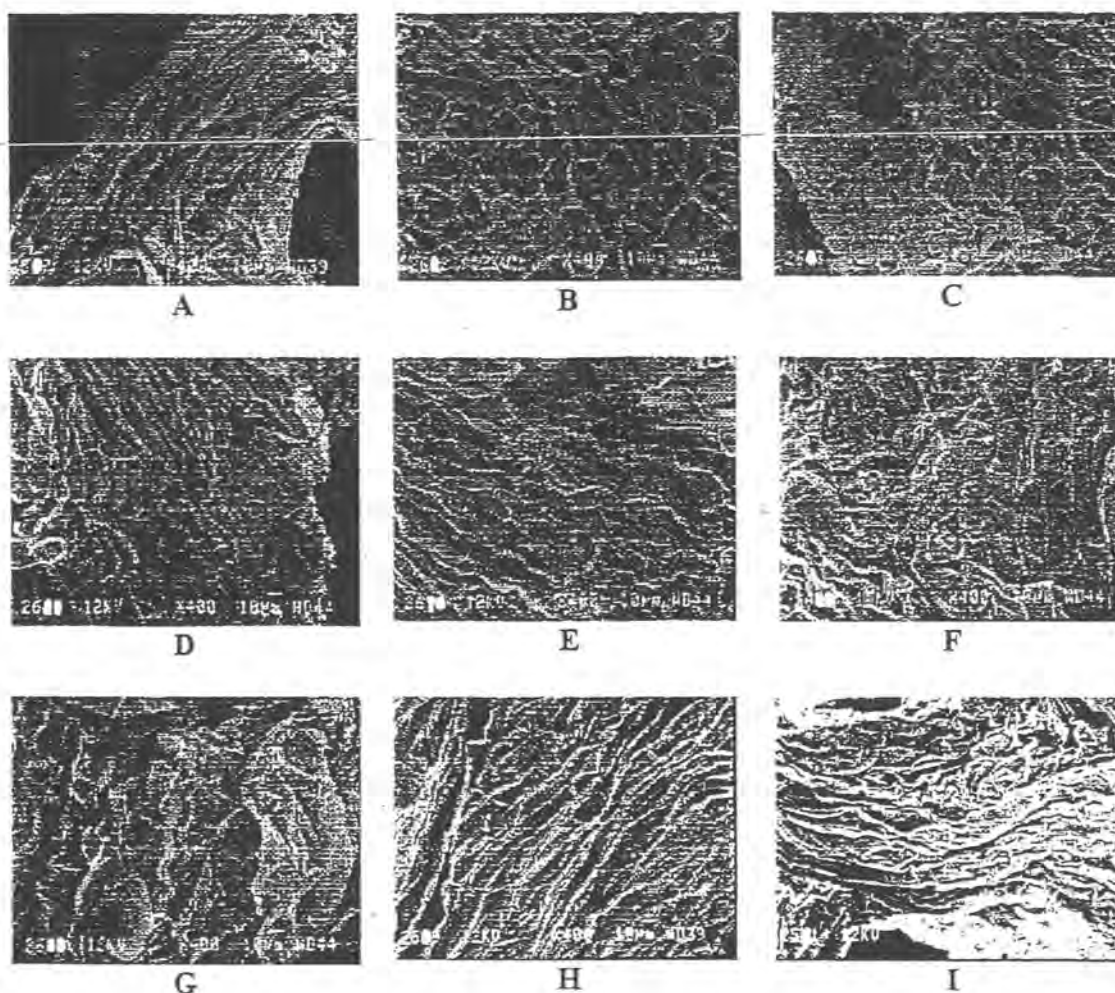


Figure 4.2: Scanning electron micrographs showing the physical state of whole *Azolla* biomass at the start of each adsorption cycle **A:** cycle 1 **B:** cycle 2 **C:** cycle 3 **D:** cycle 4 **E:** cycle 5 **F:** cycle 6 **G:** cycle 7 **H:** Cycle 8 **I:** cycle 9 **J:** cycle 10

4.4 CONCLUSION

Electron micrographs of biomass 1 and 2 revealed a more compacted structure for biomass 2 compared with biomass 1. The compacted structure of biomass 2 could have inhibited the access of the metal solution to parts of the biomass thereby masking the availability of some of the binding sites. This could have resulted in the biomass 2 not performing as well as biomass 1.

The physical structure and integrity of the *Azolla* biomass was not effected by the exposure to mineral acids, Ni solution and high flow rates over 10 consecutive adsorption and desorption cycles. The cellulose structure of *Azolla* biomass gives it a robust structure that is not easily disrupted (Sanyahumbi, 1999). The strong physical integrity of *Azolla* biomass allows it to meet the structural requirements of a good biosorbent material.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSION

Focus on toxic heavy metals has increased over the last two decades, due to the harmful effects that these metals have had on the environment. Industrial development is an important part of the social and economic development of any country. Almost all industries discharge at least one trace metal into the environment. In South Africa, industrial effluents and hazardous waste is a growing problem. In order to protect the environment and natural watercourses from pollution by toxic heavy metals restrictions on the discharge of effluents containing heavy metals have intensified over the past few years (Wilson and Edyvean, 1994). This project evaluated the alternative of biosorption using non-viable *Azolla filiculoides* for the removal of Cu and Ni from aqueous solutions and the possibility of scaling up existing lab scale *Azolla* column systems.

The electroplating industry is an important source of metal pollution when compared to some other manufacturing operations. Copper and nickel are two metals that are commonly found together in electroplating effluents. The toxicity of heavy metals, including copper and nickel are well known. They have been found to have harmful effects on various organisms on virtually every trophic level. They have a tendency to accumulate in the human food chain leading to future health risks.

Conventional methods used for the removal of metals from electroplating effluents include chemical precipitation, electrodeposition, cementation, solvent extraction, ultrafiltration, ion exchange, activated carbon adsorption and evaporation. These methods have been used in industry with limited success, many of them having major shortcomings, often causing further environmental hazards by merely transferring the problem instead of eradicating it. For this reason interest in biosorption as an alternative for the removal of metals from waste water has increased. Biosorbents are as efficient, if not more so, as conventional metal removal methods, and they are cleaner and more 'environmentally friendly' technologies, whose commercial application is likely to be cheaper than conventional methods.

It is generally accepted that dead biomass has a number of advantages over viable biomass for the biosorption of metals from aqueous solutions. *Azolla filiculoides* status as a weed in South Africa, as well the fact that it is freely available and fulfills the criteria of a good biosorbent material makes it an excellent candidate for the industrial application of a metal biosorbent.

The effects of factors such as metal starting concentration, pH and two metals in solution on the removal of Ni and Cu from aqueous solution by dried and crushed *Azolla* biomass were investigated in batch systems. The effects of these parameters need to be studied in order to establish the capacity of any given biomass for the biosorption of a metal. Aqueous solutions of Ni with starting concentrations between 1000 and 2000 $\mu\text{mol/l}$ gave the most efficient Ni removal by *Azolla* biomass. For Cu the optimum starting concentration for adsorption was 50 $\mu\text{mol/l}$. The adsorption capacity of both Cu and Ni increased as the starting pH of the sorption media increased. The optimum pH for Ni adsorption was found at pH 7 and for Cu,

at pH 5. *Azolla* biomass had a higher maximum binding capacity ($q_{\max}$) for Cu than for Ni at pH 5. The fact that adsorption of Cu had a lower optimum starting concentration and the higher maximum binding capacity, might indicate that *Azolla* biomass has a higher affinity for binding Cu than it does for Ni. The removal of both Cu and Ni showed little or no variation with the presence of the other metal in solution, under the conditions of this experiment. It is possible that Cu and Ni bind to different sites on a biomass, because of the different affinities of the ligands for metals. Kinetic studies show that both the binding of Cu and Ni to *Azolla* biomass is characterised by a rapid initial adsorption step. This characteristic is beneficial when applying the biomass in a fixed-bed column adsorption system. Ni is adsorbed more rapidly than Cu.

The removal of Cu and Ni from aqueous solutions using non-viable *Azolla* biomass was investigated in a lab scale fixed-bed column system. These results were compared to those obtained in an upscaled 4L fixed bed column system, in order to evaluate the systems upscale potential. The non-viable *Azolla filiculoides* biomass when dried and used in a column for adsorption of Cu and Ni showed good physical stability under many different conditions. Preparation of the biomass before it could be used in the columns was very simple and involved no complicated pretreatment steps. Care should be taken to store *Azolla* biomass in darkness as prolonged exposure to UV light decreases its capacity for Ni and Cu adsorption.

Column adsorption of Cu and Ni from aqueous solutions was successfully upscaled approximately 100 times from a lab scale fixed-bed column containing 5g of *Azolla* biomass to a 4L fixed-bed column containing 500g of *Azolla* biomass. Relative to the lab scale

column, the 4L column performed better for the uptake of Cu and Ni per gram of biomass. The larger column was also able to operate at relatively higher flow rates. The biomass showed good reusability with little change in the amount of Ni adsorbed in 10 consecutive cycles. Electron micrographs showed little or no change in the physical structure and integrity of the *Azolla* biomass after exposure to mineral acids, Ni solution and high flow rates over 10 consecutive adsorption and desorption cycles. As much as 80% Ni was recovered and 70 % Cu was recovered when desorption profiles were generated using 0.1M HCl as a desorption agent.

The 4L column system was also tested using a highly concentrated Ni plating bath solution (Nicrolyte 1). Only 18 % of the Ni could be removed from the expended Nicrolyte 1 plating solution after treating only 25L. However, the *Azolla* biomass still showed good uptake at breakthrough point. The 4L fixed-bed column adsorption system using *Azolla* biomass would be more suited to more dilute metal laden effluents.

It may be concluded that non-viable *Azolla filiculoides* biomass is an efficient biosorbent of Ni and Cu from aqueous metal solutions. It also shows good potential for an upscaled application in the treatment of relatively dilute metal solutions.

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