Biosorption of Heavy Metals from Aqueous Solutions

by

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Executive Summary

Industrial effluents are a major cause of heavy metal contamination, thus the removal and recovery of heavy metals from effluent streams are essential to the protection of the environment. Conventional technologies are either not able to remove and recover heavy metals to a satisfactory level, or they are too costly to implement. The ability of biomaterials to bind and concentrate heavy metals from dilute aqueous solutions has been well-documented, and offers a potentially cost-effective approach to the removal of heavy metal pollutants from industrial waste waters. However, little has been done with regard to the commercialization of technology based on the use of biosorbents and the objective of this project was therefore to investigate the feasibility of using biomaterials for the removal of heavy metals from aqueous effluents.

The study consisted of several successive steps, first of which was the identification of a suitable biosorbent, followed by characterization of the sorbent and biosorption mechanisms and finally doing some experimental work with packed columns to evaluate the use of adsorbent on an industrial scale.

The metals used in this investigation were mainly zinc, nickel, lead and copper, while the biomaterials were tobacco dust, saw dust, peat moss and seaweed. Limited experiments with other metals, such as cadmium and chromium were also conducted. The biomaterials that were considered are readily available and easy to prepare. An attempt was made to describe the biosorption capacity of the materials in terms of lignin and organic content, but this was not particularly successful.

Screening of biomaterials

Biomaterial screening experiments (including kinetics and adsorption capacity, as well as the regeneration of the sorbents) were conducted in batch adsorption tests with single species solutions. Limited work with multiple species solutions has indicated that interaction between metal species can play

a role in the performance of the biosorbents and that this would have to be taken into account in detailed industrial-scale equipment design.

Characteristics of selected biosorbent

On the whole, the seaweed appeared to be the most effective adsorbent. With the seaweed (marine alga), the adsorption equilibria of Cu, Pb and Cd could be represented by Langmuir isotherms and the capacity of fresh alga for Cu, Pb and Cd was approximately 85-94 mg/g, 227-243 mg/g and 83.5 mg/g respectively. By way of comparison with conventional ion exchange technologies, the performance of the seaweed was slightly better than that of a chelating C467 resin (approximately 80 mg Cu/g) and worse than that of a strong acid IR120 resin (about 101 mg Cu/g) for copper.

The rate of adsorption onto the marine alga was high and appeared to be controlled by both reaction and film diffusion, owing to the non-homogeneity of the algal surface, which contained a variety of functional groups. The alga particle size played an important role in the adsorption behaviour. Coarse alga particles (0.8-1.2 mm) had a higher adsorption capacity and slower adsorption kinetics, and could be regenerated without significant loss of capacity. In contrast, the fine alga particles (0.075 mm) had a lower adsorption capacity and faster adsorption kinetics, and could not be regenerated without significant loss of capacity.

Assessment of packed columns as a potential configuration for industrial implementation

Granulated seaweed was used in a packed column in order to evaluate the design of equipment. A synthetic heavy metal solution comprised of Pb, Ni, Cr,

Cu and Zn with a total concentration of 100 mg/L was passed through the column at a flow rate of approximately 15 BV (bed volume).

For all practical purposes, 100% of the Pb and Cr were removed with approximately 95% of the Cu and 75% of Zn and Ni. Sorption equilibrium was reached within 10 minutes for all heavy metals. The Pb and Cr removal remained constant at sclose to 100%, whereas the other heavy metals peaked close to 90% and then decreased steadily afterwards. The decrease in Ni and Zn concentrations could be attributed to the displacement of these heavy metals with Pb and Cr. This shows that the seaweed is very selective for Pb and Cr and to a lesser extent for Cu.

By using a 2M HCl solution, 95% of the Cr and Pb could be removed within 120 minutes. Initial heavy metal removal was fast, with more than 70% being removed within the first 20 minutes of operation. In summary, although further work still needs to be done to assess the reuse of the seaweed after biosorption and regeneration. the technology appears sufficiently promising to continue with steps towards industrialization.

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THE REMOVAL OF HEAVY METALS FROM WATER BY THE USE OF BIOMATERIALS

The Steering Committee responsible for this project, consisted of the following persons:

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1. Introduction

Heavy metals, due to its non-biodegradability and persistence, accumulate in the environment, and can be detrimental to a variety of living species. Industrial effluents are a major cause of heavy-metal contamination, thus the removal and recovery of heavy metals from effluent streams is essential to the protection of the environment. Conventional technologies are either not able to remove and recover heavy metals to a satisfactory level, or they are too costly to implement. Biosorption, which is the ability of certain microbial biomaterials to bind and concentrate heavy metals from even the most dilute aqueous solutions, offers a technically feasible and economically attractive alternative.

Various biomaterials produced or harvested from natural sources or agricultural products, mostly in metabolic inactive states, have been used in the treatment of heavy metal effluents by biosorption. They include microorganism biomaterials (such as bacteria, fungi, algae and yeast) and lignin cellulose biomaterials (such as peat moss, rice straw coconut husks, waste coffee powder, dried plant leaves, etc.). However, due to the complex, heterogeneous composition and structural property of these biomaterials as well as the wide chemical spectrum of heavy metals, the knowledge with regard to the industrialization of biosorption process is still limited.

In this project the aim is to use a suitable biomaterial to effect the removal of heavy metals from solution. The metals used in this investigation are zinc, nickel, lead and copper, while the biomaterials were tobacco dust, sawdust, peat moss and seaweed. All of these biomaterials are readily available and easy to prepare. In order to standardize the biomaterials, lignin content and organic percentage of the biomaterials were used as common elements, and were used for comparative purposes.

2. Literature Review of accumulated experience and the outlook for technology development

2.1 Introduction

Heavy metals, which are released from industry, mining and agriculture activity, tend to accumulate in the environment due to its non-biodegradability and persistence. The increasing accumulation of the heavy metals in the environment can be detrimental to a variety of living species, including man. Thus, removal of heavy metals from industrial, mining and agriculture effluents has been an important priority in the tightening and enforcement of environmental regulations. The conventional treatments used to remove heavy metals from waste effluents are precipitation, coagulation, reduction and membrane processes, ion exchange and adsorption. However, the application of such processes is often found limited because of technical and/or economic constraints. For example, precipitation processes can not guarantee the metal concentration limits required by regulatory standards and produce wastes that are difficult to treat; on the other hand, ion exchange and adsorption processes are comparatively effective but require expensive adsorbent materials and difficult plant management. Thus, the search for a new cost - effective alternative to the conventional heavy metal wastewater treatment process is in demand.

It was observed by several workers, mostly microbiologists, that microbial cells had the ability to concentrate, in their cellular mass, metals that existed in dilute concentrations in their aqueous environment. As a result the idea of the use of biomaterial for the uptake of heavy metals has been extensively studied for the last two decades.

Generally, biosorption is a property of certain types of inactive, dead, microbial biomaterials to bind and concentrate heavy metals from even very dilute aqueous solutions. Biomass exhibits this property, acting just as a chemical

substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria, which was found responsible for this phenomenon. Opposite to biosorption is metabolically driven active bioaccumulation of heavy metals by living cells.

Overall, compared with the conventional heavy metal removal methods, the potential advantages of biosorption process includes (Zümriye, 1997):

- Use of naturally abundant renewable biomaterials that can be cheaply produced;
- Ability to treat large volumes of wastewater due to rapid kinetics;
- High selectivity in terms of removal and recovery of specific heavy metals;
- Ability to handle multiple heavy metals and mixed wastes;
- High affinity, reducing residual metals to below 1 ppb in many cases;
- Less need for additional expensive reagents which typically cause disposal and space problems;
- Operation over a wide range of physiochemical conditions including temperature, pH, and presence of other ions (including Ca^{2+} and Mg^{2+});
- Relatively low capital investment and low operational cost;
- Greatly improved recovery of bound heavy metals from the biomass;
- Greatly reduced volume of hazardous waste produced.

2.2 Biosorbents

A wide spectrum of biological materials has shown potential for heavy metal removal. A large number of biomass types have been tested for their metal-binding capacity under various conditions. These include agricultural wastes (such as rice straw coconut husks, waste coffee powder, dried plant leaves), sewage sludges, and microbial cells in the whole or part (such as bacterial, fungal, algal and yeast) and peat moss (Orhan and Buyukgungor, 1993; Zouboulis *et al.*, 1999; Davis *et al.*, 2000; Malterer *et al.*, 1996).

Table 1 gives various examples of natural biosorbents (in raw form or after limited chemical treatment) and summarizes the biosorption efficiency of these materials [Junter *et al.*, 2001]. Considering the diversity of the tested materials and the variations in experimental conditions, the quoted efficiencies are relatively homogeneous. As a rule, however, materials of marine origins (seaweeds, chitin/chitosan) offer a high metal-binding potential whereas modified materials (sawdust, apple residues, chitin) display higher biosorption capabilities than raw counterparts.

Table 2.1 - Examples of natural (crude or chemically modified) materials of animal or vegetal origins tested for heavy metal removal

Materials	Metal tested	Metal binding efficiency
Plant roots - from tomato and tobacco	Sr	Maximum adsorption capacity (mg Sr g^{-1} dry biomass): 25.8 (tomato), 18.3 (tobacco). Overall adsorption loading in continuous operation (biomass-loaded carrageenan beads in packed bed column; [Me]i, 10 mg Sr dm^{-3}, flow rate, 0.5 cm^3 min^{-1}; 25°C; pH 5.35-5.95): 3.35 mg Sr g^{-1} biomass
- from weeds (Amaranthus spinosus and Solanum nigrum	Cu(II)	Maximum adsorption capacity (mg Cu g^{-1} dry biomass): 13.1 (A. spinosus), 9.7 (S. nigrum). In continuous experiments (biomass-loaded alginate beads in packed-bed column; [Me]i, 100 mg Cu dm-3, flow rate, 0.5 cm3 min^{-1}): 15.6 mg Cu g^{-1} (A. spinosus), 11.6 mg Cu g^{-1} (S. nigrum)
Plant and tree leaves - waste tea leaves	Hg (II)Pb, Cd, Zn	Adsorption capacity (mg Hg g^{-1} dry leaves): 175 (redwood), 250 (senna). Acid-washed (10^{-2} M $HN0_3$) and oven-dried (110°C). Maximum adsorption capacity (mg g^{-1}): 79 (Pb), 32 (Cd), 12 (Zn)
Tree bark - from black oak - from Pinus pinaster	Pb, Hg (II), Pd, Ag, Zn, Cd Pb, Cu (II), Zn	Metal uptake (mg g^{-1} dry bark): 153 (Pb), 124 (Hg), 96 (Pd), 79 (Ag), 41 (Zn), 26 (Cd). Bark pretreated with acidified formaldehyde solution. Adsorption capacity (mg g^{-1} dry wt.) at 22°C and an initial pH of 6.3: Pb, 4.2 100 mg dm-3); Cu, 2.3 ([Me]i 50 mg dm^{-3}); Zn, 1.9 ([Me]i 50 mg dm^{-3})
Sawdust - untreated, oven-dried (110°C) and sieved - from Red Fir (Abies magnifica), untreated - from spruce (Picea engelmanii), crosslinked and chemically modified (phosphorylated)	Cr(VI)Cr (VI),Cu (II)Pb,Cd, Ni	Maximum adsorptive capacity: 39.7 mg g^{-1} dried sawdust ([Me]i 1000 mg dm^{-3}; pH 2.0; 25°C). Maximum adsorption capacity (mg g-1 air dried sawdust): 10.1 (Cr), 7.1 (Cu). Adsorption rate: 1.5 mg Cu g^{-1} h^{-1}([Me]i , 200 mg dm^{-3}; 60 g sawdust dm^{-3}; pH 5; 45°C) - somewhat lower rate for Cr. Highest metal uptake (mg g^{-1} biosorbent): 224 (Pb), 56 (Cd), 26 (Ni). Raw sawdust: 15 mg Pb g-1
Coconut husk fibres	Cr(VI)	Oven-dried at 100°C, ground and treated successively with NaOH and HNO_3. Maximum adsorption capacity at pH 2.0: 29 mg g^{-1}

Maize cob	Cr(VI)	Untreated material [oven-dried (110°C) and sieved]. Maximum adsorptive capacity: 13.8 mg g^{-1} dried cob ([Me]i, 300 mg dm^{-3}; pH 1.5; 25°C)
Rice bran	Cu (II), Cr (III), Zn, Co, Ni	Defatted, extrusion-stabilized bran. Maximum adsorption capacity (mg g^{-1}): 38.4 (Cu), 32.9 (Cr), 24.5 (Zn), 9.4 (Co), 6.8 (Ni)
Exhausted coffee grounds	Hg (II)	Pretreatment with 0.5 M NaOH and 0.5 M HCl, successively, then oven-drying at 105°C. Maximum sorption capacity (pH 3-11): ca. 80 mg g^{-1}
Palm pressed fibres	Cr(VI)	Oven-dried at 100°C, ground and treated successively with NaOH and HNO_3. Maximum adsorption capacity at pH 2.0: 14 mg g^{-1}
Sugar cane bagasse	Cr(VI)	Untreated material {oven-dried (110°C) and sieved}. Maximum adsorptive capacity:13.4 mg g^{-1} dried bagasse ([Me]i, 500 mg dm^{-3}; pH 2.0; 25°C)
Sugar-beet pulp	Cu (II). Pb, Cd, Zn, Ni, CaCr (VI)	Sugar-free pulp dried by solvent exchange and air-drying. Maximum binding capacity a of the acidic form (mg g^{-1} pulp) in 0.1 M $NaNO_3$: 19.7 (Cu), 60.1 (Pb), 26.4 (Cd), 16.0 (Zn), 10.6 (Ni) ([Me]i = 10 mM, 14.55 g pulp dm^{-3}, initial pH 7.2, 25°C Untreated material oven-dried (110°C) and sieved]. Maximum adsorptive capacity: 17.2 mg g^{-1} dried pulp ([Me]i, 500 mg dm^{-3}; pH 2.0; 25°C)
Apple residues - raw - phosphated	Cu (II), Zn, Ni	Saturation capacity (mg g^{-1} dry residues): 12.7 (Cu), 9.8 (Zn), 9.1 (Ni), 51.2 (Cu), 46.7 (Zn), 39.3 (Ni)
Marine macroalgae (brown algae: A. nodosum, Sargassum natans, Fucus vesiculosus, ...)	CoCdPb, Ni	Biosorption capacity of Ascophyllum nodosum > 160 mg g^{-1} (25°C, pH 4)Maximum adsorption capacity a of A. nodosum (raw biomass): 215 mg Cd g-1 dry weight (26°C, pH 4.9). Crosslinked biomass: 117-149 mg g^{-1}, depending on the crosslinking agentMaximum adsorption capacity a (mg g^{-1}) of native A. nodosum biomass (pH 3.5, 25°C):272 (crosslinked: 177-359) mg Pb g^{-1}; 41 (crosslinked 24-30) mg Ni g-1. Corresponding data for F.vesiculosus: 229 (crosslinked 301-363) mg Pb g^{-1}; 23 (crosslinked 31) mg Ni g^{-1}
Chitin - raw - deacetylated (chitosan)	Cu (II), Cd, Cr (III), Zn, PbPd, Au(*) Hg(II) Pt(**), PbCu (II), Cr (III), Ni, Zn, Fe (III)	Removal of metals (mg g^{-1}) by commercial chitin from a mixture of metals ions containing 1 mg cm-3 of each metal (pH 6, 25°C): 25.0 (Cu), 15.8 (Zn), 40.1 (Cd), 47.8 (Cr), 38.0 (Pb)Metal removal efficiency of chitosan (mg g^{-1}) in the same test conditions as above: 70.5 (Cu), 20.0 (Zn), 54.3 (Cd), 53.4 (Cr), 40.3 (Pb) Metal uptake (mg g-1 chitosan) from 200-400 mM metal solution: 668 (Pd), 1150 (Au), 882 (Pt), 1123 (Hg), 823 (Pb)Chitosan from prawn waste. Quantity of metal adsorbed (mg g-1): Fe, Cu, Cr 1500 Zn > 1800, Ni 300-350 ([Me]i = 25-100 mM)
Fish scales	Cu (II), Cd, Cr (III),Zn, Pb	Adsorption capacity of porgy scales (mg g-1): 57.2 (Cu), 17.6 (Zn), 44.5 (Cd), 51.7 (Cr), 86.8 (Pb)

Wool fibres	Hg (II), Pb, Cd, Zn, Cu (II), Ni	Wool fibres cleaned from grease with petroleum ether. Average uptake rate in mg metal g^{-1} dry wt. fibres h^{-1} (50°C, pH 5, contact for 60 min): 26.5 (Hg), 16.0 (Pb), 5.5 (Cu) ([Me]i =200 mg dm^{-3}); 7.5 (Cd), 7.2 (Zn) ([Me]i =100 mg dm^{-3}); 4.0 (Ni) ([Me]i =50 mg dm^{-3})
Egg shell membrane	Au (*), Pt (**), U (***), Pd	Adsorption capacity (25°C, contact for 3 h in 3 mM metal solution): 550 mg Au g^{-1} dessicated hen egg shell membrane (pH 4); 270 mg Pt g^{-1} (pH 2-4); 240 mg Pd g^{-1}; 280 mg U g^{-1} (pH 6)
Bone gelatin	Cu (II)	Gel beads of gelatin (25% wt.) + propylene glycol alginate (2% wt.) crosslinked with NaOH. Adsorption capacity at pH 5.5: 30 mg Cu g^{-1} dry gel
(*) As $AuCl_4^-$	(**) As $PtCl_4^{2-}$	(***)As UO_2^{2+}

Many living microorganisms are also known to be capable of accumulating metal species within their structures. For instance, numerous genera of cyanobacteria have been isolated from metal contaminated environments such as zinc-enriched water, mine tailings containing high concentrations of zinc (22.8 mg/l), nickel (0.43mg/l) and lead (0.28 mg/l) and copper rich soils. Subsequently, many of these isolates have proven to be naturally tolerant to these metals and laboratory studies have demonstrated that metal-tolerance can be selected for [Fiore *et al.*, 1997].

Researchers involved in the US Department of Energy's (DoE) natural and accelerated bioremediation research programme (NABIR) are investigating ways of using micro-organisms to clean up over 120 DoE sites that have been contaminated by metals such as zinc, lead and chromium, and radionuclides such as strontium, techtinium and uranium. Part of the programme is being undertaken at Pennsylvania State University where they are investigating the way that natural organic materials can help bacteria reduce iron in the soil. In an iron-reducing environment heavy metals and radionuclides stick to the iron or can be converted into less hazardous forms [Fisher, 2001].

Fisher (2001) is using a laboratory-model system consisting of iron reducing bacteria, iron oxide and natural organic material in an anaerobic (oxygen- free)

chamber to study the interactions between the bacteria, which 'breathe' iron and convert the ferric form to the ferrous form and organic matter. She found that the natural organic materials accelerate the reduction process via several different methods. They hope to isolate each method and understand the mechanisms behind them, with the aim of developing an additive that could be introduced to contaminated soils and ground water to stimulate iron reduction.

However, non-living biomass generally appears to present specific advantages compared to the use of living microorganism. For instance, the former may be obtained by much lower cost, it is not subject to metal toxicity, the nutrient supply is not necessary and it can be used for many process cycles [Zümriye, 1997]. It is therefore noticed that much more effort was taken for exploration of the biosorption properties of the non-living biomaterials towards an industrial scale than the living biomaterials.

2.3 Achievements over the past decade

During the 1970s, the increasing awareness and concern about the environment motivated research for new efficient technologies that would be capable of treating, inexpensively, waste waters polluted by metals with emphasis on radionuclides. This search brought biosorption to the foreground of scientific interest as a potential basis for the design of novel wastewater treatment processes. At that time, concepts of classical Chemical Engineering were also brought into this research effort. The equilibrium and the kinetics of biosorption started being investigated in a systematic way, utilizing tools from the field of adsorption such as activated carbon adsorption [Yang, 1987; Tsezos and Volesky, 1981].

The work on biosorption continued to expand and in the early 1980s, the first patents appeared claiming the application of specific microbial biomass types as biosorbents for the treatment of contaminated wastewaters. At first, the biomass was proposed to be used in its native form. Very quickly, techniques for immobilising microbial biomass were also developed, tested, patented and

proposed [Tsezos and Noh, 1981; Brierley *et al.*, 1987; 1988, 1990, 1991; Tsezos *et al.*, 1987; Garnham,1997]. These patents made use of immobilization approaches such as biomass encapsulation [Tsezos and Noh, 1987] or biomass chemical processing, hardening and then granulation [Tsezos, 1981]. The immobilization of the microbial biomass was shown to be an indispensable requirement for any potential technology development based on biosorption. At the same time, the immobilization of the microbial biomass offered to biosorption-based technologies the use of well known and well developed traditional chemical engineering reactor configurations, such as up flow or down flow packed bed reactors, fluidized bed reactors, etc.

Pilot installations and a few commercial scale units were constructed in the USA [Full scale AMT biosorption units:] and in Canada [Tsezos, 1981. The pilot plants confirmed the applicability of biosorption as the basis for a metals sequestering/recovery process, especially in the case of uranium where it was tested in order to be combined with in situ bioleaching giving rise to an integrated biotechnology-based uranium production scheme [Tsezos, 1981]. These pilot plants also helped to realize the limitations of the industrial application of biosorption. The first issue that emerged out of this experience was the requirement for a reliable supply of waste microbial biomass of the type that would be suitable for each one of the intended biosorption applications. Fermentation industry was reluctant or unable to secure a steady supply of waste microbial biomass as the inexpensive raw material that would be used for the production of the new biosorbents, taking advantage of the economy of scale. The cost for producing the required biomass for the sole purpose of transforming this biomass into biosorbents was shown to be too expensive. Logistical problems having to do with the immobilized biomass distribution, regeneration and re-use made the above issues even more complex. Furthermore, the negative effect of solution matrix co-ions on the immobilized microbial biomass targeted metal's uptake capacity upon recycling and re-use made matters even more difficult [Tsezos and Noh, 1987].

Three attempts to commercialize immobilized biomass biosorption in the fields of waste water treatment [Brierley , 1987, 1988, 1990, 1991; Tsezos and Noh, 1987; Tsezos, 1981] and metal value recovery [Garnham, 1997] finally did not manage to succeed. As of today, two more attempts to market two different types of immobilized microbial biomass, one by BV SORBEX [Wase and Forster, 1997] and the other by the US Bureau of Mines [Wase and Foster, 1997] are not known to have made a successful commercial application in the market.

Due to the difficulty in immobilizing the biomass, particularly for heavy metal wastewater disposal, the attention has been drawn to the use of sulphate-reducing bacteria (SRB) technology. Most of SRB are neutrophiles, with maximum growth obtained in the pH range 6-8. Some isolates can grow in moderately acidic conditions such as mine and surface waters where the pH is in the range 3-4. The biotechnology potential of SRB stems from the insolubility of the sulphides of many environmentally significant toxic metals and the ability to use a wide range of carbon substrates. Provided that the SRB technology can be engineered so that the degradation of the organic compound can be coupled to the production of hydrogen sulphide, the effective precipitate agent for the heavy metals. It therefore would be an ideal treatment process for biological removal of heavy metals and degradation of organic pollutants avoiding the extra cost to produce and immobilize the biomass solely for the heavy metal effluent treatment. The commercialisation of the technology lies in the provision of an appropriate reactor configuration that not only meets the water treatment process criteria but also protects the biological and metabolic considerations of the SRB.

An accurate knowledge of biosorption mechanisms and their main influencing factors is essential to the optimisation of the operating conditions both in uptake and regeneration phases (Pagnanelli *et al.*, 2000). By the beginning of the 1990s, the research work in the field of biosorption had focused again on the better elucidation and understanding of biosorption fundamentals, such as

the competing ions effect and selectivity (Figueira *et al.*, 1997) rather than on the biosorption process design.

2.4 Mechanism of Adsorption

There are two principal modes of adsorption of molecules on surfaces, namely physical adsorption (physisorption) and chemical adsorption (chemisorption). The basis of distinction is the nature of the bonding between the molecule and the surface.

Physical Adsorption: the only bonding is by weak Van der Waals - type forces. There is no significant redistribution of electron density either in the molecule or at the substrate surface.

Chemical Adsorption: a chemical bond, involving substantial rearrangement of electron density, is formed between the adsorbate and substrate. The nature of this bond may lie anywhere between the extremes of virtually complete ionic or complete covalent character. There is a possibility that the molecules can decay.

Four interaction forces are important:

- London-Forces (molecular forces between two anti - polar substance)
- Keesom-Forces (forces from the interaction between permanent dipoles)
- Debey-Forces (by induced dipoles)
- Chemical interaction forces (charge-transfer-interaction)

Table 2.2: Typical Characteristics of Adsorption Processes:

Aspect	Chemisorption	Physisorption
Temperature Range (adsorption takes place)	Virtually unlimited (but a given molecule may effectively adsorb only over a small range)	Near or below the condensation point of the gas (e.g. Xe < 100 K, CO_2 < 200 K)
Adsorption Enthalpy	Wide range (related to the chemical bond strength) typically 40 – 800 kJ mol^{-1}	Related to factors like molecular mass and polarity but typically 5-40 kJ mol^{-1} (i.e. ~ heat of liquefaction)
Crystallographic Specificity (variation between different surface planes of the same crystal)	Marked variation between crystal planes	Virtually independent of surface atomic geometry
Nature of Adsorption	Often dissociative, may be irreversible	Non-dissociative, Reversible
Saturation Uptake	Limited to one monolayer	Multilayer uptake possible
Kinetics of Adsorption	Very variable - often an activated process	Fast - since it is a non-activated process

To investigate whether Chemisorption or Physisorption takes place, we need to look at the adsorption enthalpy, which is the initial information required. The most definitive method for establishing the formation of a chemical bond between the adsorbing molecule and the substrate (i.e. chemisorption) is to use an appropriate spectroscopic technique; for example IR (Infra-red) to observe the vibrational frequency of the substrate/adsorbate bond; or UPS to monitor the intensity and energy shifts in the valence orbitals of the adsorbate and substrate.

Biosorption of metals is not based on only one mechanism. It consists of several mechanisms that quantitatively and qualitatively differ according to the type of biomass, its origin and it's processing. Biosorption involves a combination of active and passive transport mechanisms starting with the diffusion of metal ion to the surface of the microbial cell. Once the metal ion has diffused to the cell surface, it will bind to sites on the cell surface, which exhibits some chemical affinity for the metal. This step contains a number of passive accumulation processes and may include adsorption, ion exchange, coordination, complexation, chelation and microprecipitation.

Adsorption and desorption studies invariably yield information on the mechanism of metal biosorption: how is the metal bound within the biosorbent. This knowledge is essential for the understanding of the biosorption process, and it serves as a basis for quantitative stoichiometric considerations, which constitute the foundation for the mathematical modelling of the process.

A number of different metal-binding mechanisms have been postulated to be active in biosorption including ion exchange, complexation, coordination, chelation and microprecipitation. There are also possible redox reactions taking place in the biosorbent. Due to the complexity of the biomaterials used it is quite possible that at least some of these mechanisms are acting simultaneously to varying degrees depending on the biosorbent and the solution environment.

Generally, metal ion adsorption processes are fast, reversible, and not a limiting factor in bioremoval kinetics when dealing with dispersed cells. Biosorption is often followed by a slower metal binding process in which additional metal ions are bound, often, irreversibly. This slow phase of metal up-take can be due to a number of mechanisms, including covalent bonding, surface precipitation, redox reactions, crystallization on the cell surface or,

most often, diffusion into the cell interior and binding to proteins and other intracellular sites.

Recent studies with fungal biomass and seaweed in particular have indicated a dominant role of ion exchange metal binding. Indeed, the biomass materials offer numerous molecular groups which are known to offer ion exchange sites: carboxyl, sulfate, phosphate, amine, could be the main ones. The typical dependence of metal uptake on pH pointed to the weakly acidic carboxyl groups R-COOH (pK_a in the range of 3.5-5.5) of algal and fungal cell-wall constitutes as the potable sites of ion exchange [Kratochvil and Volesky,1998]. As early as 1979, the behaviour of acid-washed peat was described as being similar to that of polycarbonoxylic acid. The significant role of R-COOH groups of peptidoglycan in metal sequestration by Gram-positive bacteria was also pointed out. Conductometric and potentionmetric titrations with seaweed biomass of sargassum fluitans revealed the weakly acidic character of the metal-binding sites in this brown marine alga. This demonstrated that a good correlation existed between the degree of blocking of –COOH groups by esterification in fungal seaweed biomass and the corresponding decreases in metal uptake by these biomass types. The contribution of other functional groups present in the cells and cell walls of alga and fungi, such as the strongly acidic sulfate groups ($R\text{-}OSO_3^-$) of fucoidan and carrageenan in seaweeds, and the amino groups of chitin ($R_2\text{-}NH$) and chitosan ($R\text{-}NH_2$) in fungi has also been examined [Kratochvil and Volesky,1998].

In the light of recent results, biosorbents can be viewed as natural ion-exchange materials that primarily contain weakly acidic and basic groups. It follows from the theory of acid-base equilibria that, in the pH range 2.5-5, the binding of heavy-metal cations is determined primarily by the state of dissociation of the weakly acidic groups.

When the metal - biomass interaction mechanism(s) are reasonably understood, it opens the possibilities of:

- Optimizing the biosorption process on a molecular level;
- Manipulating the biosorption properties of biomass when it is growing;
- Developing economically attractive analogous sorbent materials;
- Simplifying and effectively guiding the screening process;
- 'Activating' biomaterials' low-level biosorbent behavior.
- Simple and economically feasible pretreatment procedures for suitable biomaterials may be devised based on better understanding of the metal biosorbent mechanism(s).

2.5 Use of the Marine Alga and Peat Moss as Biosorbents

The first major challenge for the biosorption field was to select the most promising types of biomass from an extremely large pool of readily available and inexpensive biomaterials. Among the most promising types of biosorbents studied are peat moss (Ferguson *et al.*, 1989; Breuer and Melzer, 1990; Jeffers *et al.*, 1989; Zümriye, 1997, Brown *et al.*, 2000), fresh water algae (Crist *et al.*, 1981; Ozer *et al.*, 1994), and marine algae (Holan *et al.*, 1993; Chong and Volesky, 1995; Fourest and Volesky, 1996; Matheickal and Yu, 1996; Matheickal *et al.*, 1997).

2.5.1 Algae as Metal Biosorbents

In general, the mechanism of biosorption is based on a number of metal-binding processes taking place with components of the algae cell wall. The algae cell walls can reversibly biosorb metals, and thus function in a similar way to an ion-exchange resin. Thus, the biosorption mechanism can be considered as being dependent on the composition of the algal cell wall. Algal cell walls can be made up with further polysaccharides: mannan, xylan, alginic acid, chitin, ect. These components, along with the proteins present, can provide acid binding sites such as amino, amine, hydroxyl, imidiazole, phosphate and sulphate groups (Crist *et al.*, 1981). The biosorption mechanism has been described as not involving van der Waals' forces at the cellulose

network of the cell walls, thus both ionic charge and covalent bonding are involved in the metal biosorption process. It is thought that the proteins and polysaccharides are the major components responsible for the biosorption. Covalent bonding could be expected with amino and carboxyl groups and ionic charge bonding with carboxyl and sulphate groups associated with these components. Studies with the micro alga *Scenedesmus obliquus* indicated that the cell wall behaved like a weak acidic cation exchanger containing various cell wall ligands with different exchange capacities.

Detailed studies of the metal-binding capacity of the OM of the *E.coli* showed that the OM (outer membrane of the cell wall) exhibited certain selectivity, which depended on the metal suitability for various physicochemical roles. For instance, Ca^{2+} stabilised the LPS (lipopolysaccharde) and Mg^{2+} formed part of complexes in LPS and protein. The interaction between soluble metallic ions and the OM followed three physical principles, which regulate cation selectivity of biological membranes:

- Binding is dependent on the free energy difference between the site bound cation and cation-water interaction;
- Free energy of interaction originates from electrostatic forces;
- The principal electrostatic forces were Coulomb forces.

Metal binding occurs though a passive mechanism, which involves electrostatic interaction between the negatively charged groups in the wall and the metallic cation. Most metal binding occurs after initial metal complexation and neutralisation of the chemically active site. Binding to the cell walls might proceed through at least a two-step mechanism, for *Bacillus subtilis*: the first step is the stoichiometric interaction of metal with reactive chemical groups, followed by a second stage in which those same sites nucleate the deposition of more metal as a chemical precipitate, which results in the development of fine-grained minerals. The initial interaction is the “adsorption” phase, but metal retention ability by bacterial walls goes further than their adsorption capacity, since bacterial surface are favourable interfaces for mineral

nucleation. Some metals have greater tendency to form precipitates than other. In systems, which include living cells, it is possible that some active uptake or non-specific cation transport system could take place. While the overall metal removal process can be considered as a “sorption” process, metals may in fact be retained by one or more of the above mechanisms. This complication may contribute to the frequent inability of adsorption isotherms to describe experimental data. Adsorption equations may be useful for describing bacterium-metal interaction with certain metals, but this approach may not be adequate when precipitation of metals occurs (Mullen *et al.*, 1989).

The intrinsic composition and structural organisation of the cell envelope (in all its variations, such as cell walls, capsules, S-layer and sheaths) provide bacterial cell surfaces with a high density of negative charge, and result in a great metal-binding capacity. Cell surfaces also provide favorable interfaces for mineral formation by facilitating heterogeneous nucleation processes.

2.5.2 Peat Moss as a Low cost Biosorbent

The basic component of peat moss before biomodification is seen to be structurally stable. It comprises of aromatic rings with three-carbon side chains, arranged so that the material whose overall integrity is difficult to modify results. Peat, in contrast, has lost many of the three-carbon side-chains, and has become much more condensed. This change allows the development of charged groups, permitting good chelation, and it is the basic structure which can make peat such a good general adsorbent, although in this connection Bloom and McBride (1979) suggest that while ions seem to be located at carboxylate sites, chelation mechanism or sites of greatly different acid strength are not necessarily involved. In addition to chelation, cation exchange with various phenolic hydroxyl, heterocyclic and carboxyl groups has been invoked (Martin, 1991), as has hydrogen bonding and anion-cation bonds (Couillard, 1994). In spite of this confusion, it is fairly clear that chemical adsorption can occur in one way or another through the polar functional groups of lignin, which include alcohols, aldehydes, ketones, acids, phenolic

hydroxides and ethers as potential providers of chemical bonding (Adler and Lundquist, 1963). The peat particles are surface-active and have a high cation exchange capacity (90-150 meq/100 g dry matter) (Stewart, 1977). Indeed, Szalay (1969) has reported that a great number of cationic elements are sorbed, and that electrochemically equivalent quantities are sorbed from cations of different valencies by given peat preparations.

Even then there is confusion, for, as Bencheikh-Lehocine (1989) points out, while the main removal mechanism for zinc at acid to neutral pH values was found to be adsorption, at pH values in the alkaline range other processes considerably enhanced zinc removal. Because of the fairly polar character of peat, the specific adsorption for dissolved solids such as transition metals and polar organic molecules is very high. According to Asplund *et al.* (1972), peat has high specific surface area (> 200 m^2/g) and is extremely porous (95%). These two characteristics of peat, its polar and porous nature, clearly help in adsorption.

In this connection, Poots and McKay (1979) have reported the specific surface area of a peat moss using dye solution. The specifc surface area depend both on the chemical nature of the solution and adsorbent and also on the molecular dimensions of the solute. A value of 27 m^2/g was obtained for nitrogen, 11.8 m^2/g for acid dye, and 100 m^2/g for basic dye (Poots and McKay, 1979). This indicates the presence of negatively charged molecules associated with the polar functional groups of peat as well as exchange adsorption occurring with hydrogen ions. As in many natural materials, the natural capacity of peat to retain cations is related to the pH of the solution. At pH values above 8.5, peat itself is not stable. At low pH, below 3.0, most metals will be leached from peat. Between these values, most metals are adsorbed fairly efficiently (Coupal and Lalancette, 1976).

2.5.3 Low-Cost Adsorbents in Continuous Processes

It should be noted that that there are inherent difficulties in comparing the metal ion investigations carried out on peat, and to a lesser extent lignite. The main problems are the heterogeneity of peat, variable chemical and physical properties of different peat and lignite types.

There are several mechanisms by which metal ions may be removed from a solution and attached to a sorbent particle surface. This is due largely to the complex chemistry of the available surfaces of many of the less traditional sorbents. The mechanism may be due to ion exchange, physical sorption, chemisorption, chemical reaction, lone pair electron sharing or donating plus a number of other mechanistic processes.

(a) Peat structure and properties

Peat is enormously variable in composition. It varies from material so slightly decomposed that plants can be readily identified from bits of leaves and roots and stems, to soil so highly decompressed that it seems to be structureless thick mud (Kadlec and Keoleian, 1986; Kadlec and Rathburn, 1983). Consequently, peat is a rather complex material containing lignin and cellulose as major constituents (Souci, 1938). These constituents, especially lignin, bear polar functional groups such as alcohol's, aldehydes, ketones, carboxylic acids, phenolic hydroxides and ethers which can be involved in chemical bonding (Alder and Lindquist, 1963). Because of the very polar character of this material, the specific sorption potential for dissolved solids, such as metals and polar organic molecules, is quite high. These properties have consequently lead to the examination of the potential of peat as an agent for the purification of wastewater's contaminated with dissolved metals (Lalancette, 1974).

(b) Lignite structure and properties

Macroscopically, lignite is dark brown or black in colour when moist, turning light brown when dry. The mechanical strength of lignite is generally poor; consolidation increases as coalification proceeds. Its density lies in the range

1.0-1.35 g/cm^3. Lignites are amorphous and fibrous or woody in texture, the 'as mined' material processing numerous water-filled pores and capillaries. This feature means that the lignites have high moisture contents (30-70%). Pore diameters vary and include micropores (Pope, 1984). As a consequence of their highly porous nature lignites possess high surface areas, typically 100-200 m^2/g (Gan *et al.*, 1972).

As with peat, the organic constituents consist of complex polymeric materials formed during the coalification process. Geologically young coals, such as lignates, often contain plant materials which have undergone little change. For example, substantial properties of cellulose are present because of its high resistance to fungal attack (Wilson *et al.*, 1987). Lignates are, consequently, less reduced than more mature coals and, therefore, contain more. The polymeric matrix is composed of polycyclic aromatic ring systems joined by straight and branched chain aliphatic groups, which retain varying numbers of functional groups, depending on origin and mode of formation. Typically, there are carboxylic, carbonyl phenolic, hydroxyl groups, and ether linkages. These functionalities impart enhanced reactivity to the lignates and make them suitable to bond with polar organic and inorganic materials.

(c) Mechanism of metal sorption

The nature of metal binding in soil organic matter, including peat and lignite, has been extensively investigated but a common viewpoint on the exact mechanism of sorption has yet to be reached. Additionally, the comparison of results is very difficult because pre-treatment methods vary among authors, and the type of peat (and to lesser extent, lignite) investigated will have a significant effect on ion-exchange capacity (Tummavuoti and Aho, 1980); this view contradicts the statement by Gosset *et al.* (1986).

Clymo (1963) proposed that there is a good correlation between the content of unesterifed polyuronic acids in the cell wall of sphagnum peat and the cation exchange capacity. De Mumbrum and Jackson (1956) proposed that the

sorption of copper and zinc ions occur by the formation of complexes with carbonyl and nitrile groups in peat. Kashirtseva (1960) proposed that the presence of humic acids in peat primarily responsible for its ability to sorb metals. Furthermore, many workers have implicated carboxylic acid (COOH) groups in the reaction of divalent metals with humic acids (Schnitzer, 1978; Schnitzer and Khan, 1972; Van Dijk, 1971; Vinkler *et al.* 1976; Boyd *et al.*, 1981). They support the general view that the reaction of metal ions, such as Cu and Fe, with humic acid is one chelate ring formation involving adjacent aromatic carboxylate COOH and phenolic OH groups or, less predominantly, two adjacent COOH groups which participate in ion-exchange reaction by binding metal ions with the release of H^+ ions. Others believe that there is no direct evidence for chelation: NMR studies (Deczky and Langford, 1978) and an ESR study (Alberts *et al.*, 1976) have shown that the Mn^{2+} ion does not form an inner spere and is bound electrostatically. This was supported by Bloom and McBride (1979) who, after extensive investigation with acid metal ions, with the exception of Cu^{2+}, largely as hydrated ions. The binding of copper appears to involve the exchange of one or two aquo ligants by carboxylate oxygens. Thus, neither chelation by adjacent functional groups nor heterogeneity with respect to acidity constants can be postulated to explain the binding of metal ions by peat and humic acid.

Ong and Swanson (1966) carried out studies to challenge the theory that humic acids were primarily responsible for metal binding in peat and lignite. Their investigation centred on the sorption of copper by peat and lignite. They found that humic acids extracted from peat and lignite is able to complex copper in solution. IR studies indicated that the complex involved was probably the carboxylic group and could be considered a chelating complex similar to that found in soil organic matter, thereby agreeing that the humic acid may have been, at least in part, responsible for the sorption of copper. However, the sorption of copper by peat and lignite from which the humic acid had been removed, did not decrease, as expected, but actually increased. Accordingly, the sorption processes could not be solely explained by the formation of humic

acid complexes. The increased copper sorption explained because of the increased surface area in the organic matter that is exposed for metal sorption. Thus, humic acid in their soluble form is responsible for the fixation of metals, but in the solid form have quite different properties an can play only a very minor part in sorption process. They concluded that the sorption of metal ions by peat and lignite can be considered as an attraction between the negatively charged surface and a positively charged metal ion, i.e. sorption. It was also noted that sorption capacity decreases as the degree of metamorphism increases (resulting from compaction which reduces surface area). The order of capacity is peat > lignite > coal.

This theory is supported by more recent work. Bencheikh-Lehocine (1989) set out to determine whether sorption or ion exchange was responsible for zinc removal from peat at low pH. The theory is that sorption is an irreversible process whereas ion exchange is reversible, and this was the key to differentiating between the two processes. The effect of increasing sodium concentrations on the sorption of zinc in batch experiments was found to be negligible. It was then concluded that at low pH the process is irreversible and must be sorption of a strong type, encountered usually in chemisorption processes. In contrast, at moderate to high pH mechanisms other than that of sorption must be involved.

2.6 The Future

How microbial biomass sequesters metals and what are the requirements for a potential process design is becoming clearer. However, to produce an equivocal market success of the biosorption technology has not been achieved. Biosorption is not yet a proven technology from the point view of industrialization. The question one usually asks is that what is the future of biosorption as a potential technology base? There does exist a future. Biosorption is a process with some unique characteristics. It can effectively sequester dissolved metals out of dilute complex solutions with high efficiency

and quickly (rapid intrinsic kinetics). These characteristics make biosorption an ideal candidate for the treatment of high volume low concentration complex wastewaters. It is desirable to develop the hybrid technologies, which make use of a combination of the biosorption with various well-developed processes, in order to find its largest application in the detxification of metal-bearing wastewaters.

Hybrid technologies can be intra-biotechnological, that is to say, they make use of various biotechnology-based processes in their flow sheet as, for example, biosorption, bioreduction, and bioprecipitation. They could also be described as inter-technological, as they can integrate into their flow sheets biotechnology-based processes along with other non-biotechnology based processes as, for example, chemical precipitation, electrochemical processes, etc. Either type of hybrid technologies can make successful use of biosorption as one of the implemented processes benefiting from the advantages of biosorption (Tsezos M., 2001).

The combination of biosorption along with metabolically mediated processes as, for example, bioreduction (e.g., $Cr^{6+} \longrightarrow Cr^{3+}$, $Se^{4+} \longrightarrow Se^{0}$, etc.) and bioprecipitation is also possible inside novel reactor designs. It can even make use of combinations of biological and chemical processes into effective hybrid processing schemes inside new single reactor designs. It should be borne in mind that metabolically active organisms can be produced inside the reactors, thus overcoming the problem of the reliable, appropriately controlled biomass supply at a specific site. Such reactor systems have been proposed and are being tried successfully at pilot stage. More such ideas are needed. The fundamental research must also be continued into better understanding of the mechanism of biosorption on what drives the selectivity of biosorptive and bioaccumulatory processes. In the process of these new studies, there also appears to be a need to follow the well-tried and documented methodologies for the study of the biosorptive phenomena.

2.7 Conclusion

Research over the past decades has provided a better understanding of passive metal biosorption by certain high-performance types of biomass, however, due to the complex, heterogeneous composition and structural property of these biomaterials as well as the wide chemical spectrum of heavy metals, the fundamental knowledge about the whole family of very cheap biosorbent materials required to industrialize the biosorption process is still not completed, the effective configuration to apply best-performance biomaterial proved at the laboratory scale to the industry field is still limited at this stage.

Ultimately, there is a need to develop and propose to the market reliable, robust, simple and effective process designs in order to arrive at a success in commercialization of the biosorption process. This lies in the hybrid technology, combing the new biosorption process with the well-proved treatment process or reactor configuration.

3. Experimental Work

3.1 Materials

The stock metal solutions were made up of sulphates of the metal diluted with distilled water to the required concentration of 1000ppm. Analytical grades of HCl and NaOH (from Merck) were used to adjust the pH.

The ion exchange resin Amberlite IR120, which is a strong gel type acidic cation exchange resin was used to familiarize us with the adsorption process.

The sample of seaweed was obtained from a farm in Cape Town; the sawdust was obtained from Mondi Timber in Paarl; the tobacco dust was obtained from the British American Tobacco Company in Paarl, and peat moss was collected from the Eerste River. All samples were air dried, except for peat moss.

3.2 Preparation of the Materials

3.2.1 Sawdust

The biomaterial was ground and sieved to 1-2mm using a porcelain ball mill. A 500g sample of the biomaterial was washed with 5 liters distilled water for 30 minutes using an overhead stirrer. The water was discharged and the fine particles screened with a 300μm screen. Thereafter, the sample was washed with 5 liters of a 0.1M HCl solution for 1 hour followed by washing with distilled water. The process was repeated 8 to 10 times until the pH of the supernatant was approximately 5. The sample was dried in an oven between 60^0C and 70^0C until a constant weight was reached.

3.2.2 Tobacco Dust

The biomaterial was washed with distilled water with mild stirring till the supernatant was clear to remove any soluble organic substances. The cleaned tobacco dust, having a particle size in the range of 0.3mm-1.0 mm, was subsequently treated with 0.1 M HCl at 1:5 (w/v) for 30 minutes followed by

rinsing with distilled water. The biomaterial was again treated with 0.1M HCl at 1:5(w/v) for 30 minutes, which was followed by washing with distilled water constantly until the solution reached a pH of 4.5. The biomaterial was dried in the oven at 105 ^{0}C for 24 hours and stored in desiccators for subsequent use.

3.2.3 Peat Moss

The biomaterial was ground and sieved to 1-2mm using a porcelain ball mill. Samples of the biomaterial were thoroughly washed to remove all soil and dirt. The water was discharged and the fine particles were screened using a 300μm screen. Thereafter, the samples were washed with a 1M HCl solution at 5 times its volume using an overhead stirrer for 30 minutes. The samples were then washed with 10 times its volume of distilled water for 30 minutes. Thereafter, the samples were washed with 1M NaOH at 5 times its volume for 30 minutes followed by washing using distilled water at 10 times its volume for 30 minutes. The samples were dried in an oven at 70^{0}C for 24 hrs or until a constant weight was obtained. The samples were stored in a sealed, dry container for subsequent use.

3.2.4 Seaweed

A farm in Cape Town supplied the raw biomaterial, *Ecklonia maxima*, in the ground form. The harvested algae sample was air dried in sunlight before grinding. Three size fractions of the biomaterial were used in the experiments, viz. 0.075 mm, 0.800 mm and 1.200 mm. Activation of the biomaterial was carried out as follows: The biomaterial samples were soaked in 0.2 M $CaCl_2$ solution for 24 h under slow stirring. The solution pH was kept constant at 5.0 using 0.1 M of NaOH. The calcium treated biomaterial was washed several times with distilled water to remove excess calcium. These activated samples were then dried at 100°C for 24 hours and stored for later use.

3.3 Methodology

3.3.1 Kinetics Tests

Batch kinetic tests were performed using an overhead stirrer with a baffle attachment at a speed of 300rpm for sawdust, 600rpm for peat moss, and 800-1000rpm for tobacco dust and for seaweed. The biomaterial load was approximately 1g/1000ml metal solution.

3.3.2 Equilibrium Tests

Batch adsorption tests were performed using a roller system with 1-liter screw capped glass bottles under ambient temperature. The speed of the roller was 58-60rpm and the contact time was 24 hours. The final equilibrium samples were measured for solution pH, filtered (Schleider & Schuell filter paper), and analysed for ion content. The loading of the sorbents on the biomaterial was determined from the difference of metal ion concentrations in the initial and final solutions.

The metal ion content was measured using an atomic absorption spectrophotometer (AA) and ICP spectrometry (Varian Liberty Series II Sequential ICP AES)

Figure 3.1:Batch adsorption tests taking place in glass bottles on a roller system

3.3.3 Desorption Tests

Desorption tests were conducted using a roller system with 1-liter screw capped glass bottles under ambient temperature. After 24 hours of contacting, the reaction suspension was filtered and the filter cake washed using distilled water to remove any un-adsorbed ions trapped in the macrostructure of the biomass. The washed solids were oven-dried overnight at a temperature of 105^0C. The biomass was then suspended in HCl solution with magnetic stirring. After the required contact time, the reaction mixture was filtered, and the filtrate analysed for heavy metal content.

3.3.4 Column Tests

Column Tests were performed using seaweed biomass. The system consists of a single column, 50cm high, with an internal diameter of 2.5cm. The column was packed with seaweed to the required height (initial height used = 15cm). A Millipore peristaltic pump was used to feed the heavy metal solution from the bottom of the column at a flow rate of 19-20 ml/min. The working volume for the column was 250 ml, which is equivalent to a residence time of 12.5 to 13.1 minutes. The outlet solution of the column was periodically collected and analysed to measure the residual heavy metal concentration.

Figure 3.2: Column setup for adsorption and regeneration experiments with seaweed

Owing to the swelling nature of the seaweed with initial contact with the metal solution, it was necessary to keep the seaweed loose during the initial contact. This was achieved by tilting the column to an angle of 45^0 until the column was nearly filled and then bringing the column into an upright position.

The desorption tests were performed with the same column setup, using HCl as the solution. The HCl was passed down through the column, periodic samples were collected at the base of the column and analysed for heavy metal content.

3.4 Characterization of the Biomaterials

In order to have a basis for comparison between all the biomaterials, standardization tests were performed. These tests included lignin tests for sawdust, seaweed, peat moss and tobacco dust as well as chemical analysis for all four biomaterials. Scanning Electron Micron (SEM) photographs were also taken for the biomaterials to compare the surface structure for each.

3.4.1 Lignin Tests

The Klason method was used for determining lignin content of the biomaterials. This method defines lignin as a biomaterial constituent, which is insoluble in 72% sulphuric acid (H_2SO_4).

Preparation Of Sulphuric Acid.

665ml of 95% concentrated sulphuric acid was poured into 300ml-distilled water, cooled and the solution made up to a volume of 1000ml. The strength of the acid was adjusted to 24N by titration with an alkali. The solution was cooled in a refrigerator to 15^0C.

Lignin Content Test.

A 1g sample of the biomaterial was placed in a 100ml beaker in a water bath. The water bath temperature was 2^0C. 15ml of the acid was gradually added to the beaker, using a dropper while stirring. The beaker was kept in the bath at 2^0C until dispersion was complete. The beaker was covered with a watch glass and the water temperature increased to 20^0C. The beaker was left in the bath for 2hrs.

300ml distilled water was added to an Erlenmeyer flask. The acid/biomaterial solution was transferred to the flask. The solution was diluted to 3% concentration sulphuric acid (total volume). The solution was brought to boiling point and allowed to boil for 4 hours. Distilled water was frequently added to maintain the volume. The lignin (insoluble material) precipitated out.

A filtering crucible was tarred and weighed. The solution was decanted, taking care not to stir up the precipitate. The lignin was transferred to the crucible using hot water and a rod. The lignin was dried in an oven at 105^0C until a constant weight was reached. The decanter was cooled and weighed.

Correction For Ash Content.

An empty porcelain crucible was covered and heated in a furnace at 600^0C for 15 minutes. The crucible was placed in a desiccator, cooled for 45 minutes and weighed to the nearest 1mg. The lignin was transferred to the crucible and placed in the furnace at 100^0C with the cover removed. The temperature was gradually raised to 600^0C so that the material became carbonized without actually flaming. The material was allowed to ignite at 600^0C for 4hrs, to burn away any carbon. The crucible was covered, cooled in a dessicator and weighed to the nearest 0.1mg

Determination of lignin content.

Percentage lignin content was determined by:

$$\%lignin = \frac{(W_L - W_A)}{W_T}$$

where W_L = weight of the lignin in mg

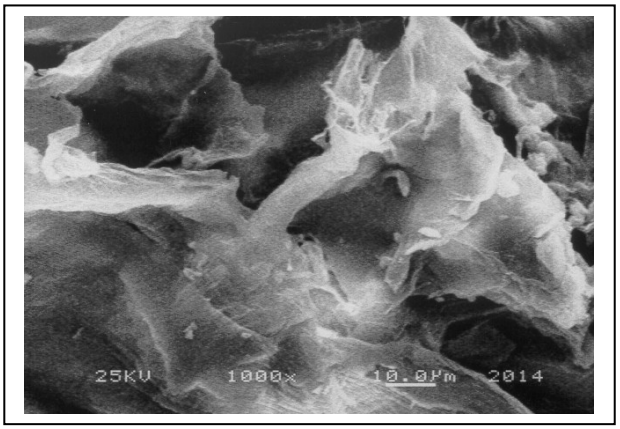
W_A = weight of the ash in mg

W_T = weight of test specimen in mg

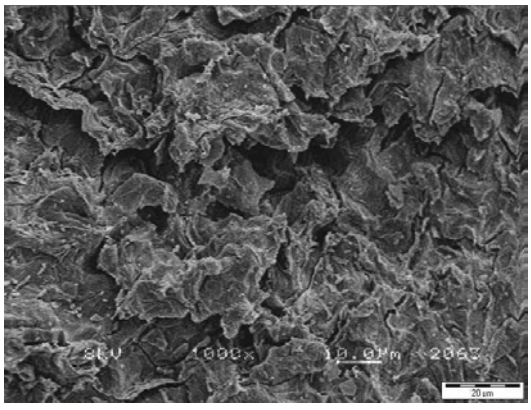
The lignin content was determined to the nearest 0.1%.

3.4.2 Scanning Electron Microscope Photographs

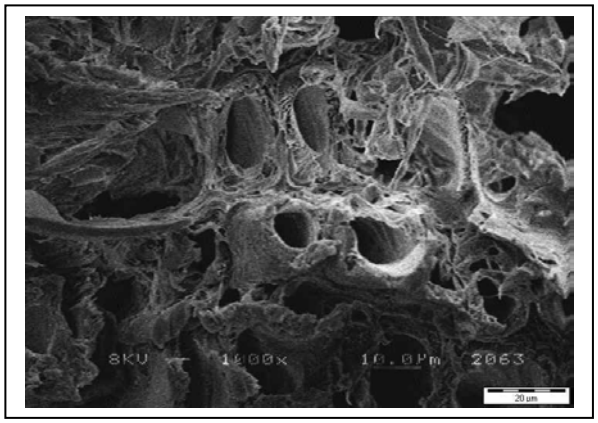
SEM of the biomaterial was obtained using a Topcon ABT-60 scanning electron microscope to study the morphological microstructures of the biomaterial in terms of surface roughness and porosity.

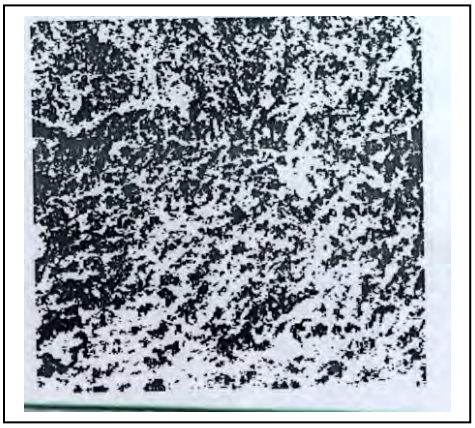


(a) Tobacco Dust



(b) Seaweed





(c) Sawdust (d) Peat Moss

Figure 3.3: Microstructure of the biomaterals

All four photographs clearly show the ruffled surface of the biomaterials. This indicates a porous medium, which is advantageous to the adsorption process, as there is a greater surface area available for adsorption.

3.4.3 Chemical Analysis

A detailed chemical analysis is given in Appendix A.

Because of the heterogeneous structure and composition of the biomaterials, the chemical elemental analysis was expected to show the chemical constitutes which could influence the heavy metal adsorptive capacity of the biomaterial.

4. Results And Discussions

The biosorption process involves both a solid phase (sorbent) and a liquid phase (water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to the high affinity of the sorbent for the sorbate species, the sorbate is attracted to the sorbent and bound by a number of different mechanisms. This process continues until equilibrium is achieved between the dissolved and solid-bound sorbate. Sorption equilibrium and kinetics are therefore the important aspects of the sorption process used to evaluate the metal-binding capacities of the biomass and the suitability of the biosorption process in heavy metal removal.

Solution pH is presumed to be an important factor influencing the heavy metal distribution in the liquid phase and the interactions between the sorbate and the sorbent.

The biosorbing characteristics of the biomass for the individual heavy metal can be obtained by performing the sorption experiment in a single-ion sorption system. The interaction of the heavy metals for the biomass can be studied using a multiple-ion sorption system.

4.1 Characterisation of Biomaterials Based on Lignin Content

It is believed that lignin and humic acid in peat moss is directly involved in the chemical bonding of heavy metals during the biosorption process. Literature states that there is a direct relationship between the lignin or lignin-like content of a biomaterial and the affinity a biomaterial has for heavy metal adsorption. The higher the lignin content, the higher the affinity for heavy metal adsoption. Table 3 summarizes the results of the lignin tests for each of the four biosorbents. The lignin results listed below were averaged based on three trials each.

Table 4.1: Lignin Content of Biomaterials

	Seaweed	Peat Moss	Sawdust	Tobacco Dust
Lignin %	40	70	55	28

All four biomaterials indicate high lignin content with peat moss the highest and tobacco dust the lowest. On this basis, a good metal ion adsorption capacity should be expected from peat moss, sawdust and seaweed.

The physicochemical analysis of seaweed, tobacco dust and sawdust are given in Appendix 1. Most of the organic compounds were volatile solids. Contamination with metal ions could indicate the propensity of biomaterials to accumulate these metal ions. The compositions of soluble NO_3-N and NH_4-N could suggest metal leachning or chelating properties of biomaterial through N containing functional groups. The results in Appendix 1 do not show any definite relationships based on the volatile solid content. Based on the NO_3-N and NH_4-N compositions and metal ion contents of these biomaterials, it is still difficult to assess the heavy-metal ion adsorption capacity of these biomaterials. Various parameters could interactively affect the biomaterials to respond to heavy metal containing environment.

4.2 Kinetics Tests

The kinetic experiments were conducted in a multiple-ion sorption system for tobacco dust, where the dynamic biosorption selectivity of the tobacco dust biomass for the heavy metals can be obtained. The experiments were started at two different initial solution pH conditions with the same biomass load (1.0 g/l) and heavy metal doses (2.4 mg/l each). The changes of the solution pH during the sorption process shown in Figure 4.1 indicates that the solution pH of the sorption system decreased steadily during the first 370 min, then stabilized throughout the sorption process. This implied a possible proton-metal ion-exchange process accompanied with the sorption process.

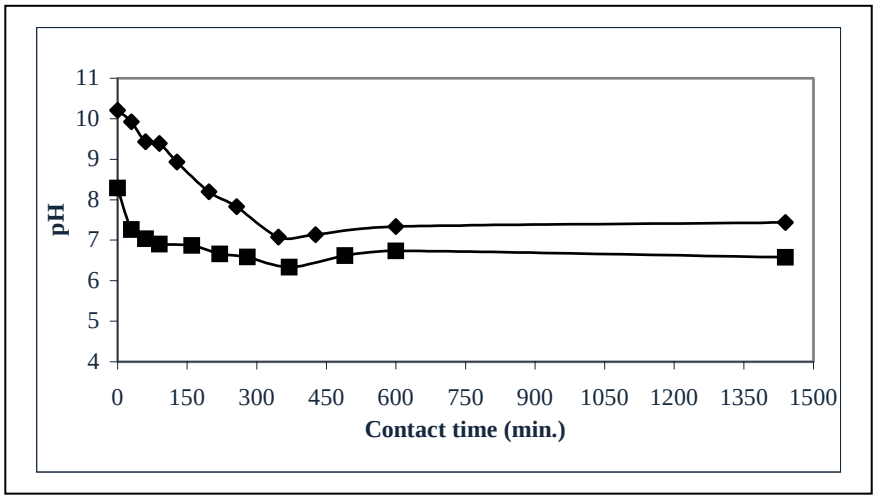


Figure 4.1: Influence of pH (two different initial values) on the removal of heavy metal solution consisting of 2.4 mg/L of each of Cd, Cu, Ni, Pb and Zn from Tobacco Dust (1g/L)

As can be seen in Figure 4.2 almost all the heavy metals achieved their sorption equilibria within approximately 3 hours at equilibrium pH 7.44. Sorption equilibrium was reached within 1.5 hours at equilibrium pH 6.34. The biosorption selectivity are approximately in the order: Cu (Zn)>Cd>Pb>Ni.

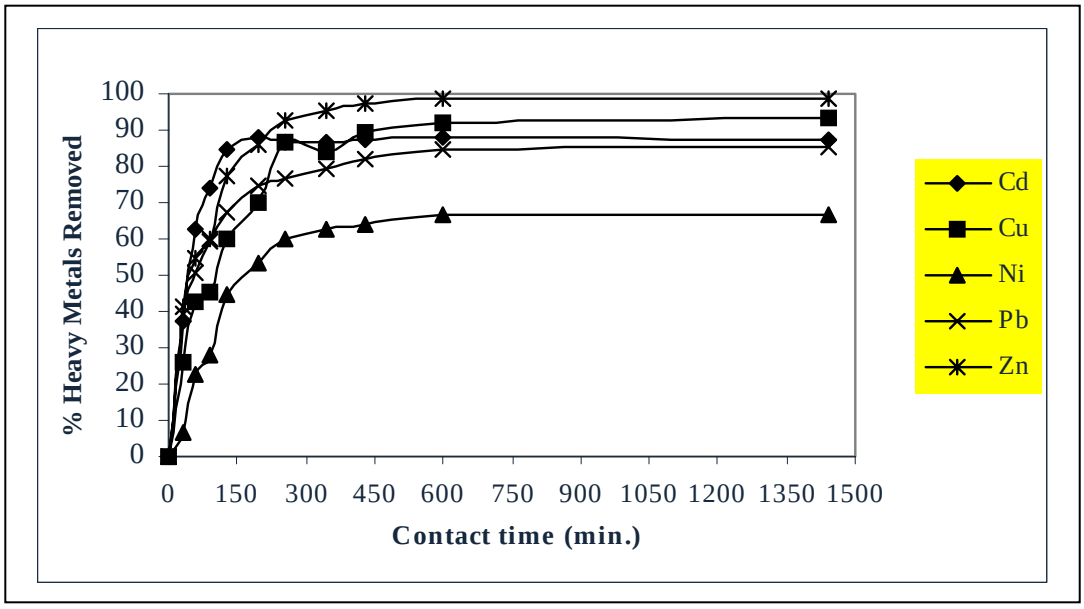


Figure 4.2: Kinetics of heavy metal adsorption on tobacco dust

A mixture of Cu, Pb, Ni and Cd was used for kinetics tests using peat moss. The equilibrium for Pb was achieved within approximately 60 minutes, while the equilibria for Cu, Ni and Cd were achieved within approximately 6 hours. The peat moss initially adsorbed all the metals in the solution. When the capacity of the peat moss was approached, metals with a higher affinity appeared to displace metals with lower affinities. The adsorption capacities for peat moss are in the sequence Pb>Ni>Cu>Cd, and the adsorption kinetics are in the same sequence as indicated by Figure 4.3.

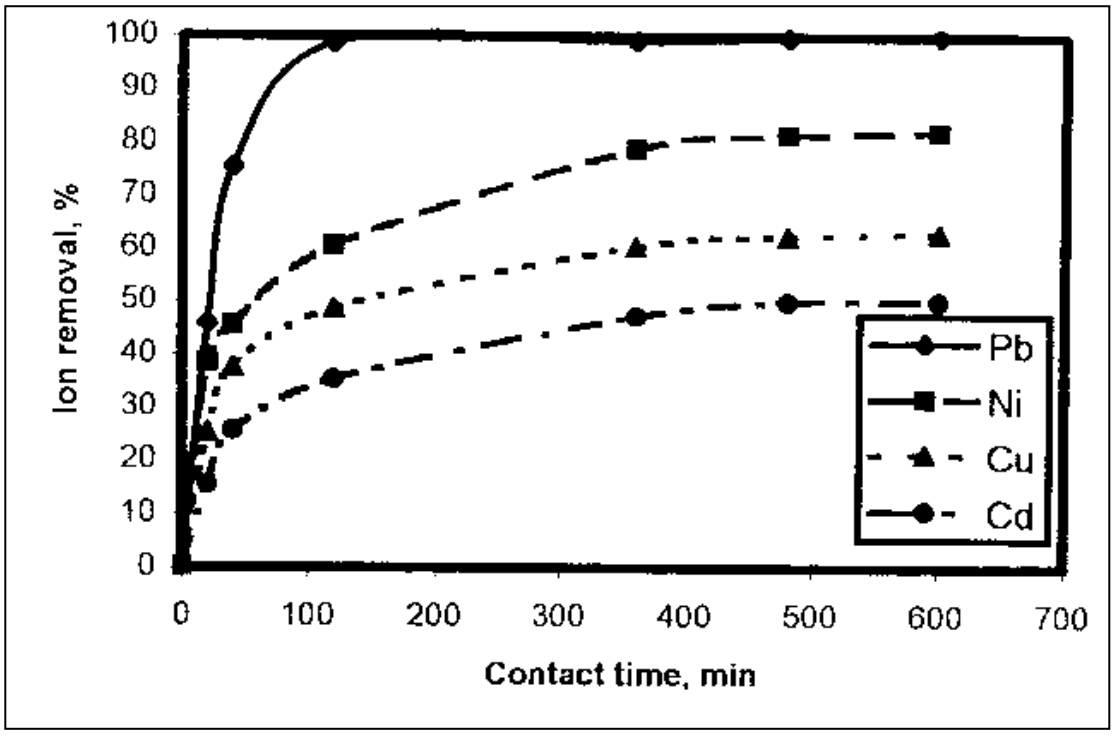


Figure 4.3 Kinetics of heavy metal adsorption with peat moss

Kinetic experiments for sawdust were conducted in a single-ion sorption system. The experiments were started at an initial pH between 6 and 7 with the same biomass load (1.0g/l) and heavy metal doses (20mg/l). The pH dropped during the initial stages and then stabilized throughout the sorption process (Figure 4.4), which implies a possible proton-metal ion-exchange process accompanied with the sorption process.

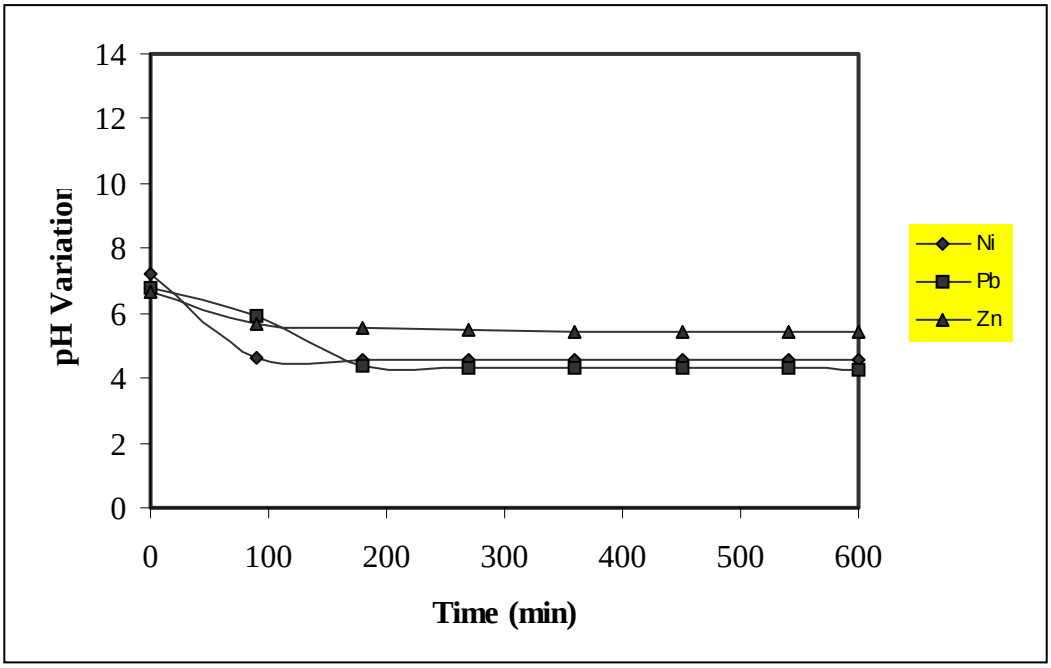


Figure 4.4: Variation of pH during adsorption of heavy metals with sawdust

From Figure 4.5 it can be seen that the heavy metals had not reached sorption equilibrium after 600 minutes and that the heavy metal recovery ranged from 35% for Copper to 80% for Nickel. The experiments were repeated using untreated, oven-dried sawdust as indicated through literature (Junter et.al., 2000). These experiments yielded slightly better results with the heavy metal recovery ranging from 41% for Cu to 89% for Ni.

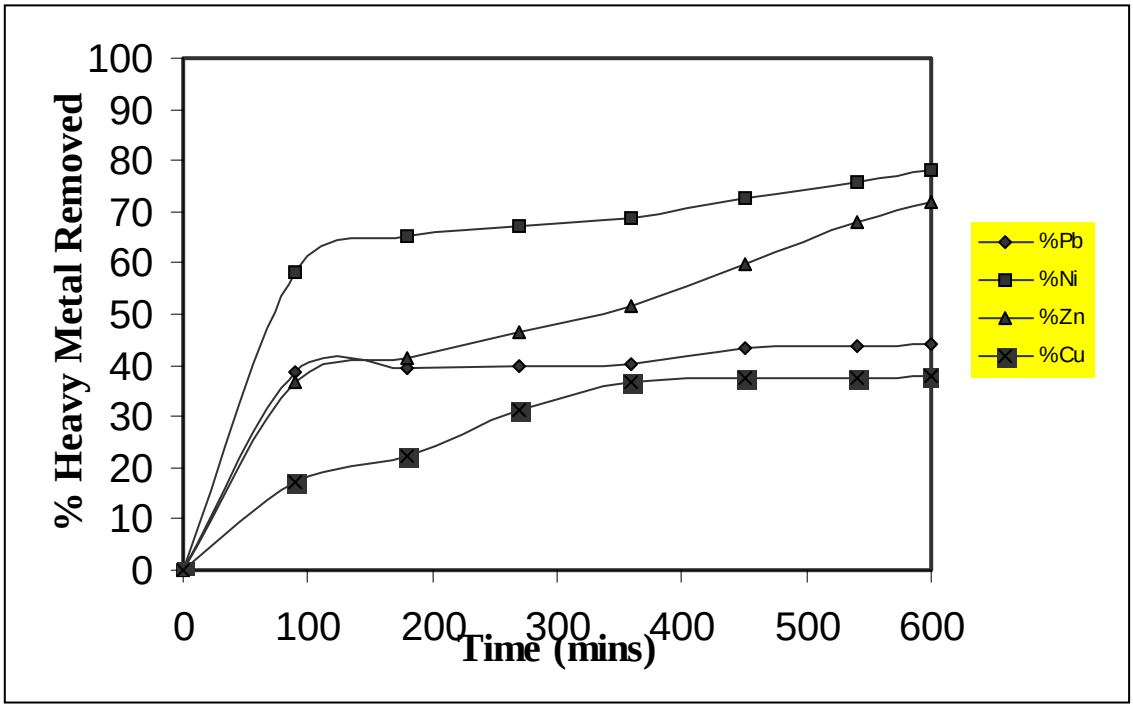


Figure 4.5: Kinetics of heavy metal adsorption with sawdust

Kinetic experiments were performed for seaweed using a single-ion sorption system. The two heavy metals concentrated on were Copper and lead for three varying sizes of seaweed. The alga (seaweed) dosage was 1 g, and the initial concentrations for Cu^{2+} and Pb^{2+} solutions were around 100 ppm, respectively. The biosorption kinetic result is shown in Figure 4.6. As can be seen the equilibria for the adsorption of Cu^{2+} and Pb^{2+} on the 0.075 mm size fraction of alga was achieved within only 10 minutes and the equilibria the adsorption of Cu^{2+} and Pb^{2+} on the 0.800 and 1.200 mm size fractions of alga were achieved within 1 h. The high rate of adsorption of heavy metals on the marine alga has also been observed in kinetic studies conducted by Kuyucak and Volesky (1989). Slower diffusion rate for Cu^{2+} and Pb^{2+} onto the interior ion exchange sites in the coarse alga particles may contribute to the slower adsorption rate for coarse particles.

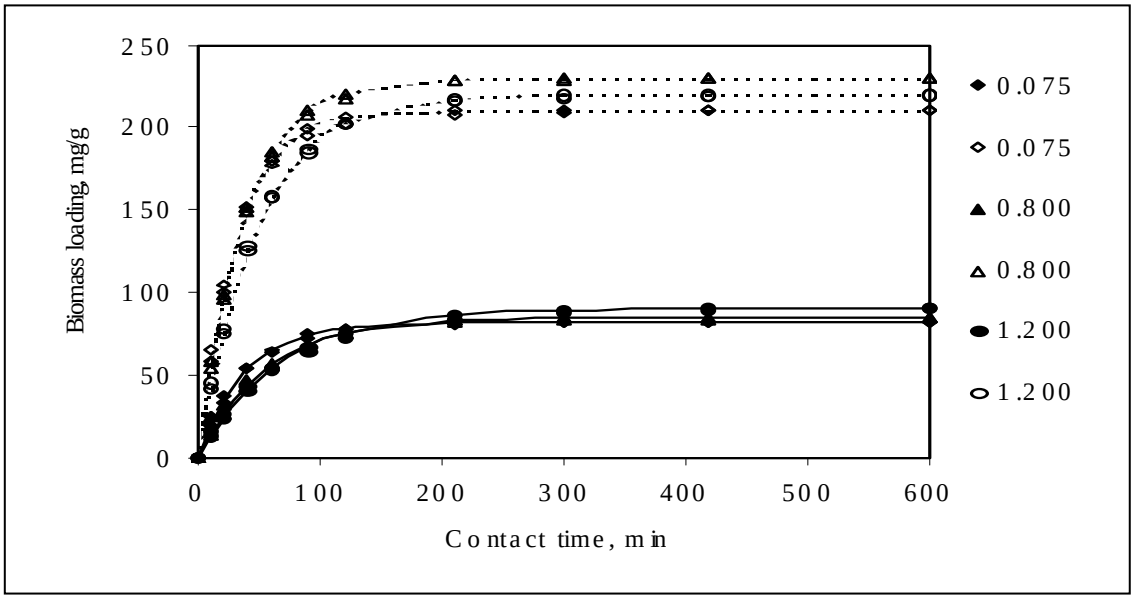


Figure 4.6: Kinetics of heavy metal adsorption on seaweed particles of different sizes.

4.3 Equilibrium Tests

Equilibrium tests were performed for both single-ion sorption systems as well as multi-ion sorption systems using tobacco dust. The equilibrium sorption uptake (capacity) from the single-ion sorption isotherms can be used to compare biosorptive capacity of the tobacco biomass for different heavy metal ions. The single-metal ion's sorption isotherms of the tobacco biomass are presented in Figure 4.7. It can be seen that the sorption capacities for Pb, Cu, Cd, Zn, Ni of the tobacco dust biomass is about 39.6, 36.0, 29.6, 25.1 and 24.5 mg/g biomass sorbent, respectively.

The potential impact from the various combinations of the heavy metals on the biosorption capacity of the biomass can be obtained with the multiple-ion sorption system. The multiple-ion sorption isotherms are given in Figure 4.8.

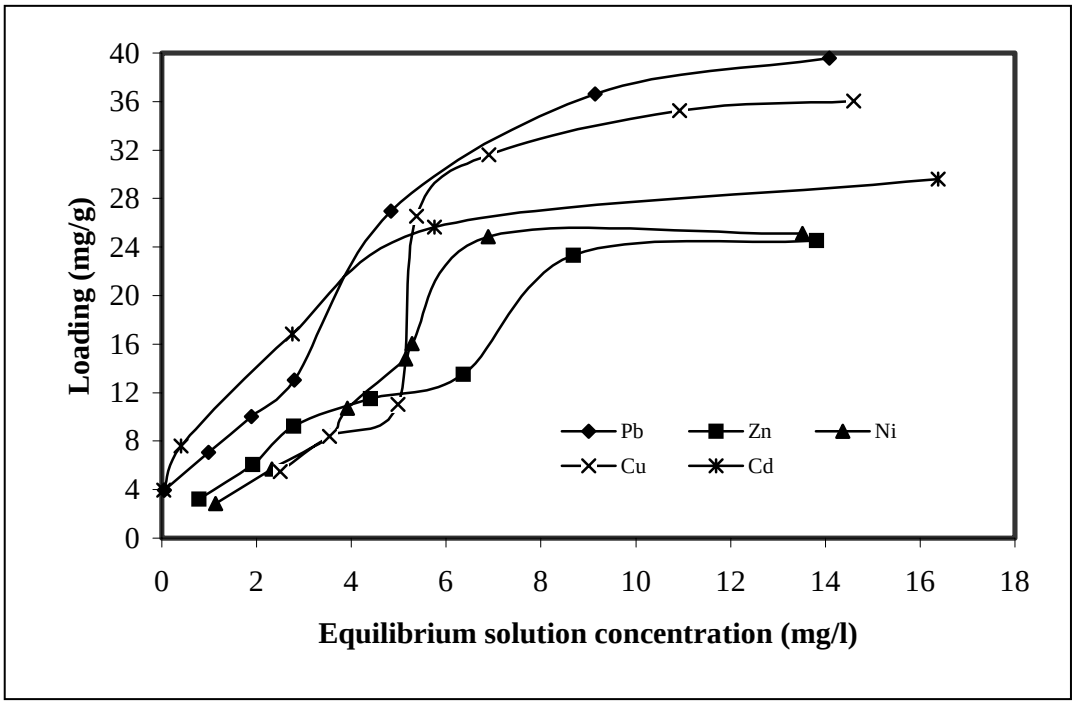


Figure 4.7: Relationship between load and equilibrium concentrations of single heavy metal ions in solution with tobacco dust

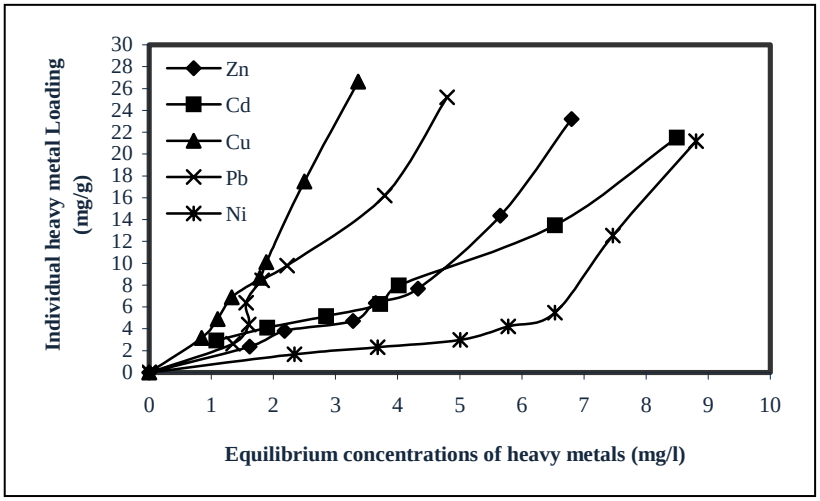


Figure 4.8: Relationship between initial loading and equilibrium concentration of multiple heavy metals in solution with tobacco dust.

In contrast, the sorption isotherms of these metals in the multiple-ion sorption system appear quite different from those in single-ion sorption system. This indicated that the sorption models of the heavy metals in the multiple-ion

sorption system are different from those in the single-ion sorption system. In addition, from Figure 4.8, it can be seen that the selectivity of the metal binding capacities of the tobacco dust biomass is in the order of Cu>Pb>Zn>Cd>Ni in the multiple-ion sorption system, instead of the order of Pb>Cu>Cd>Ni (Zn) which was deduced from the single-ion sorption system in Figure 4.7.

The heavy metals Cd and Pb were focused on when investigating the heavy metal adsorption using peat moss. The concentration range was approximately 4ppm and the dosage was 0.1g. As can be seen from Figure 4.9, the ion removal increased with solution pH for both heavy metals but decreased once a pH of 10 was reached. A possible explanation is that at low pH values, H^+ completed with the metal ions for adsorption, resulting in less metal being removed. At higher pH values, the metal ions formed precipitates with ^-OH, which are easily absorbed onto the surface of peat moss. As the heavy metals –hydroxide precipitates are amphoteric, they tend to form anionic complexes at high pH values. These complexes are rejected by the negatively charged peat moss particles, owing to the electrostatic repulsion, which explains the lower metal removal observed at pH values above approximately 10.

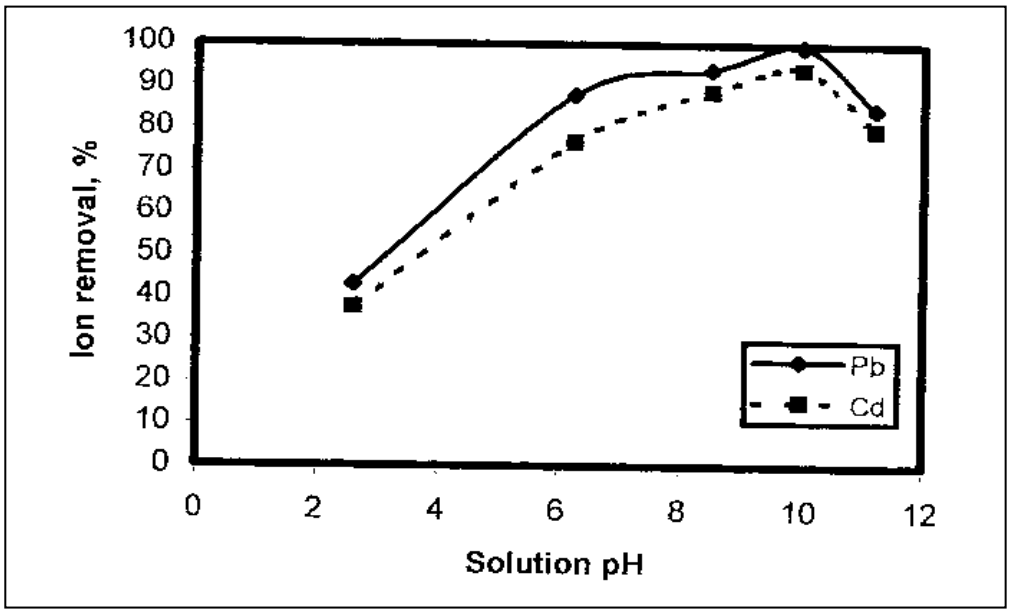


Figure 4.9: Influence of pH on the removal of heavy metals from peat moss

Figure 4.10 indicates the sorption capacities of peat moss. It clearly indicates that peat moss has a higher sorption capacity and higher sorption intensity for Pb than for Cu.

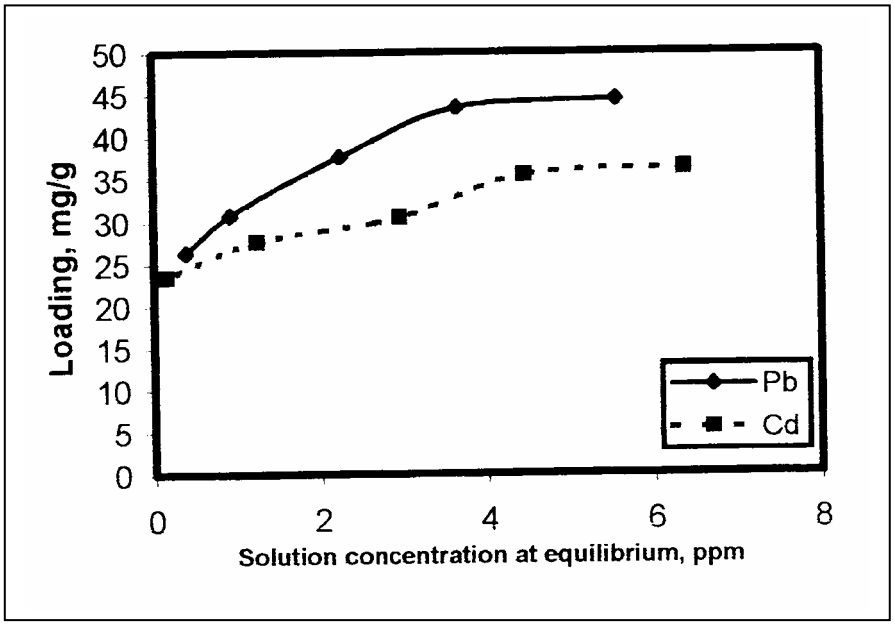


Figure 4.10: Relationship between loading and equilibrium concentration for peat moss

The heavy metals studied with the seaweed were Pb and Cu. As can be seen from Figure 4.11, a pH lower than 4 resulted in lower levels of biosorption. Optimal pH values were achieved in the pH range 5.8 to 8.5, which agrees with previous observation by Fourest and Volesky (1997).

Figure 4.12 shows various size fractions of seaweed adsorbing Pb. As can be seen, the activated seaweed had a higher Pb adsorption capacity than the seaweed not activated, especially as far as the fine size fraction is concerned. The Pb adsorption capacity of the seaweed increased with an increase in the larger pariticle size, but activation had a progressively smaller influence on adsorption in the larger size fractions. The Pb saturation capacity of the was larger size fraction was between 227 – 243 mg/g.

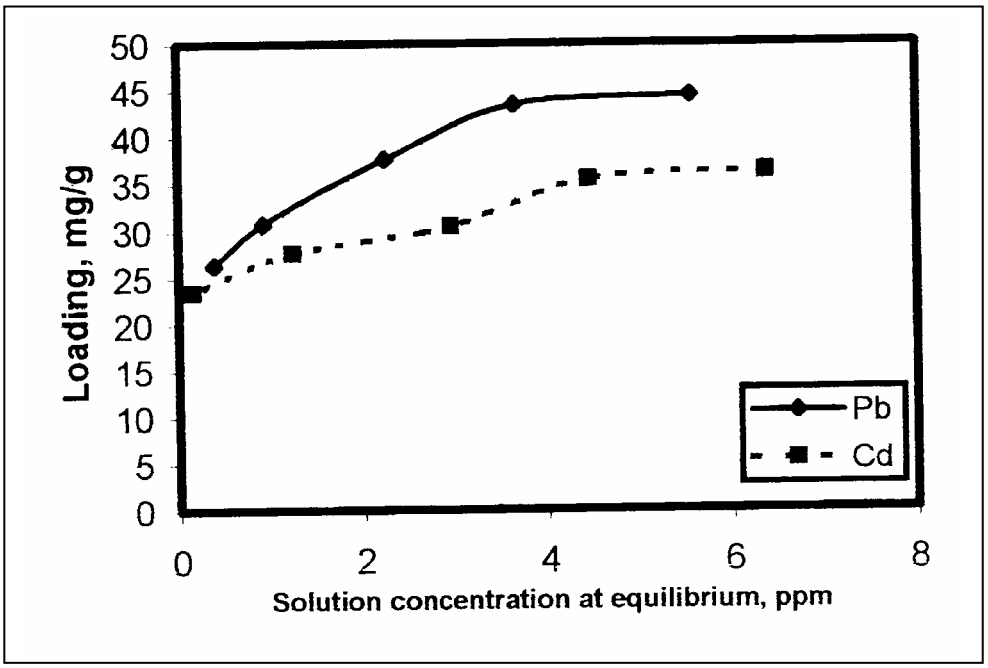


Figure 4.11: Influence of pH on the loading of heavy metals on seaweed

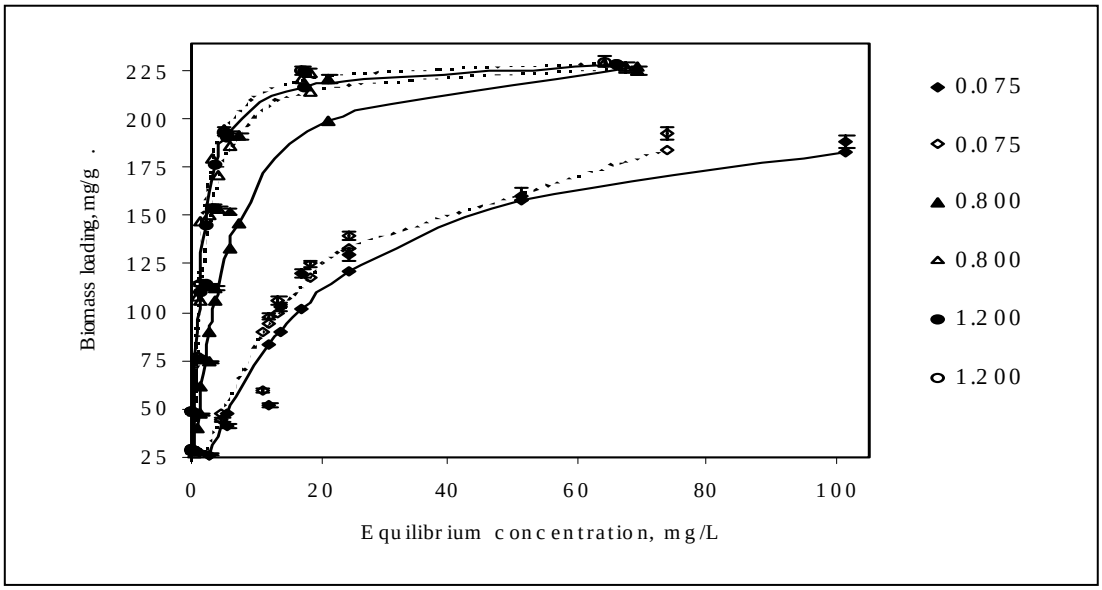


Figure 4.12: Sorption isotherms of Pb on seaweed

4.4 Desorption Tests

The desorption of heavy metals from the tobacco dust biomass under different HCl concentrations (desorption time 6 hours, twice the adsorption volume) are shown in Figure 4.13.

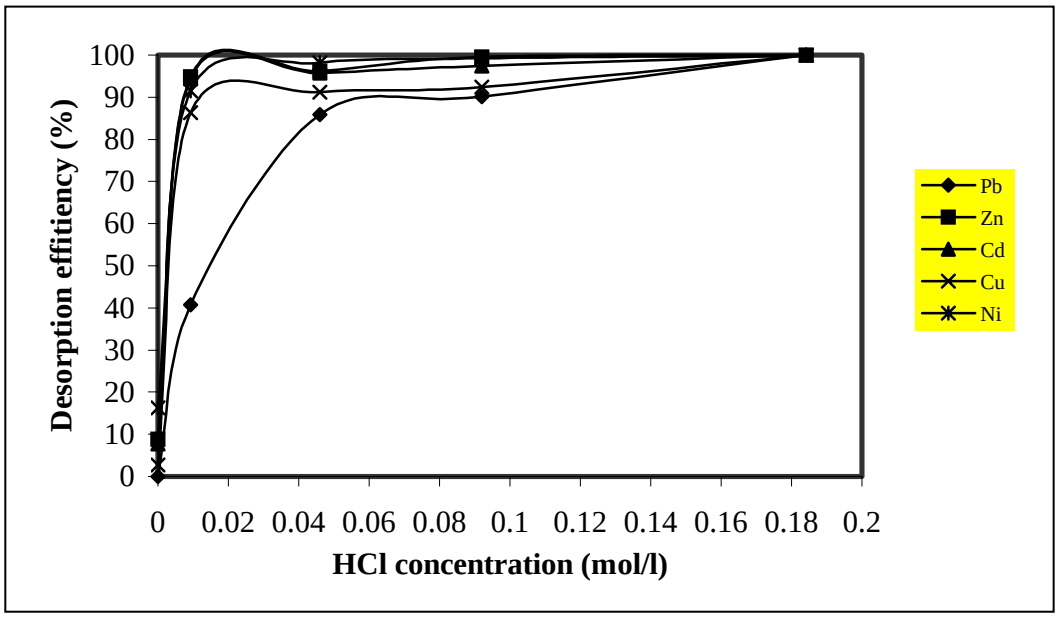


Figure 4.13 Desorption equilibria of heavy metals on tobacco dust

The results in Figure 4.13 indicate that almost all the heavy metals adsorbed on the tobacco dust biomass except Pb can be easily desorped into the solution by HCl solution with the concentration less than 0.02 mol/l.

The desorption of heavy metals with HCl solutions (pH<2.0) is a quick process, the desorption equilibria can be reached in less than 60 minutes. This suggested that H^+ has a higher competitive capacity then the heavy metals at low pH condition (pH <2.0).

It was also found that the desoprtion of heavy metals from the tobacco dust biomass by distilled water was very difficult, resulting in prolonged desorption periods of 24 hours or more.

The desorption efficiency of the heavy metals are in the order: Ni>Zn>Cd>Cu>Pb. This confirmed that the tobacco dust biomass has much

higher binding capacities for Pb and Cu than for Ni. The Ni, which was adsorbed on the tobacco dust biomass, can be partially desorbed using distilled water.

Desorption experiments were performed using 1g of peat moss loaded with 30.25mg of Cd and was subjected to varying concentrations of HCl solutions. The desorption of Cd increased with an increase in HCl concentration. At high HCl concentrations H^+ could replace Cd on the peat moss and elute Cd ions into the solution. In addition, Cd could form the anionic complex $CDCL_3$ with Cl at high HCl concentrations. The desortion of Cd from the loaded peat moss appeared to be incomplete, even at high HCl concentrations.

The seaweed biomaterial could be desorbed using a 2M sodium chloride solution. About 95% of the heavy metals could be removed using 8BV (bed volume) of eluant. The elution mechanism involved the ion exchange of Na with Cu and Cd ions, and the formation of anionic complexes between Cl and Cu ions and Pb and Cd ions. After prolonged elution, all the loaded metals could be removed.

4.5: Discussion of Results

From the batch adsorption tests with the four biomaterials, the following conclusions can be drawn:

- The four biomaterials all showed good metal binding capacities for heavy metals such as Pb, Cu and Cd.
- The heavy metal adsorption capacities for seaweed were high. For example, in the case of lead, it was in the range of 227 – 243mg Pb/g. The kinetics of the heavy metals adsorbed by the seaweed was rapid with equilibria occurring within 10 minutes for the 0.075mm particle size. This contrasts with the tobacco dust where sorption equilibria were only reached within 150 minutes, and the peat moss where sorption equilibria took almost 200 minutes.

- Considering the adsorption equilibrium and kinetics results, the activated seaweed had a significantly higher heavy metal saturation capacity and relatively high heavy metal adsorption efficiency. It performed the best among the four biomaterials, especially when high concentrations of heavy metal effluents had to be treated. Tobacco dust showed a comparable Cu adsorption capacity to activated seaweed and peat moss
- The saw dust, which had a higher lignin content that the seaweed and peat moss, and the highest content of volatile solids among these three biomaterials, did not show any obvious better adsorption properties than the other two biomaterials. In contrast, tobacco dust, which had the lowest lignin and volatile solid content among these three biomaterials, shoed a comparable or better adsorption capacity and efficiency than sawdust. This suggests that the biomaterials with higher portions of lignin and volatile solid compositions were not necessarily the better biosorbents. It was previously assumed that for lignocellulose biomaterial, it is especially the lignin and humic acid that are involved in the chemical binding of heavy metals during biosorption. For this reason, the relationship between the number of chemically active functional groups in the humic-like substances of the biomaterials, such as carboxyl and phenolic groups, and the biosorption capacity of lignocellullose biomaterials should be quantified. Without such a model, it would not be very useful to use lignin content as a reliable indicator of the adsorption capacity of biomaterials.
- Additionally, as mentioned before, it is also difficult to make assumptions about the adsorption capacity of biomaterials based only on the physicochemical analysis of the materials. However, it can be found in Appendix 1 that the content of metal ions, such as Ca and Mg and K, were normally higher in seaweed and tobacco dust than in sawdust. Since these metal ions are very common in natural terrestrial and aqueous environment, they might be useful as a common indicator to assess the natural metal accumulating capacity of the plant biomaterials. A higher content of these metal ions in the plant

biomaterials could indicate a better heavy metal adsorption capacity, especially if the biosorption of heavy metals is by means of ion exchange.

- In terms of desorption or regeneration, the metal-loaded tobacco dust could be easily regenerated with a dilute HCl solution (twice the adsorption volume), in contrast, the desorption of Cd from the loaded peat moss appeared to be incomplete, even at high HCl concentrations, while the seaweed biomaterial could be desorbed using 2M sodium chloride solution with a 8 BV (bed volume) of eluant. It could be concluded that the tobacco dust performed better with regard to desorption and regeneration efficiency than the other biomaterials.
- Based on the biosorption capacity, as determined by batch experiments, the seaweed proved to be the best among the four biomaterials and was selected as a promising biosorbent for column experimentation designed to give an indication of potential large-scale applications.

4.6 Column Tests

Batch column tests were conducted using seaweed biomass. A synthetic heavy metal solution comprising Pb, Ni, Cr, Cu and Zn with a total concentration of 100ppm was passed up the column at a flow rate of approximately 15 BV (bed volume).

As can be seen from above figure 4.14, 100% of the Pb and Cr were removed with approximately 95% of the Cu and 75% of Zn and Ni removed. Sorption equilibrium was reached within 10 minutes for all heavy metals. The Pb and Cr removal remains constant at 100% whereas the other heavy metals peak close to 90% and then decrease steadily afterwards. The decrease in Ni and Zn concentrations could be due to the displacement of these heavy metals with Pb and Cr. This shows us that the seaweed is very selective to Pb and Cr and to a lesser extent to Cu.

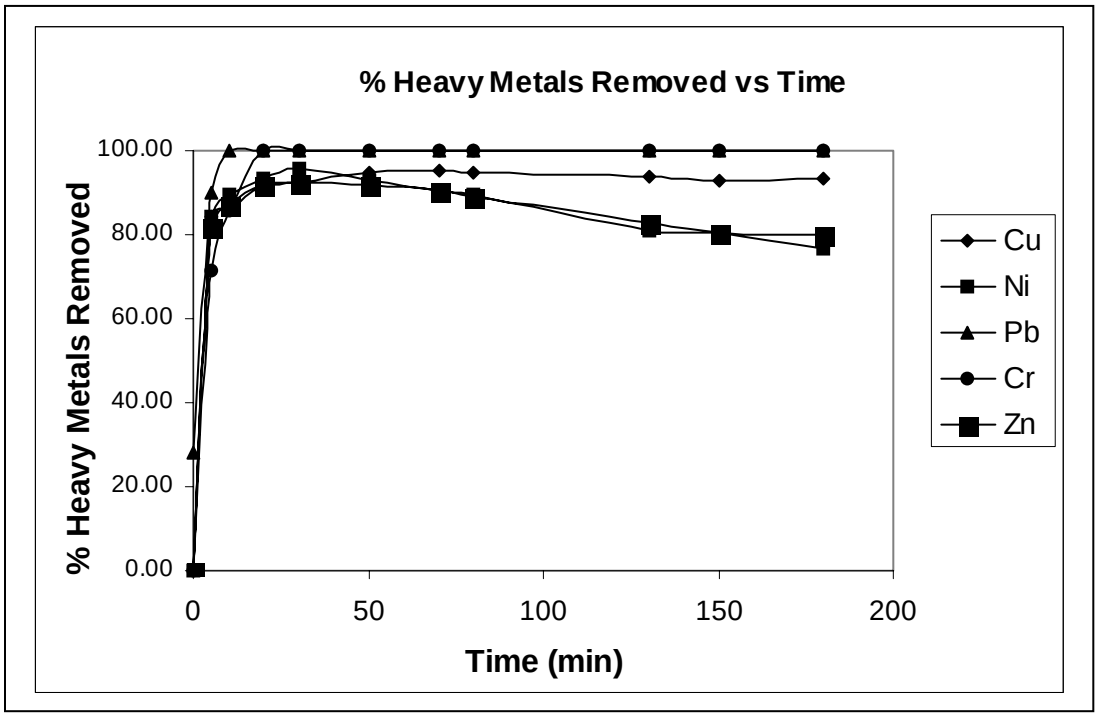


Figure 4.14: Sorption Isotherms of seaweed in the batch column

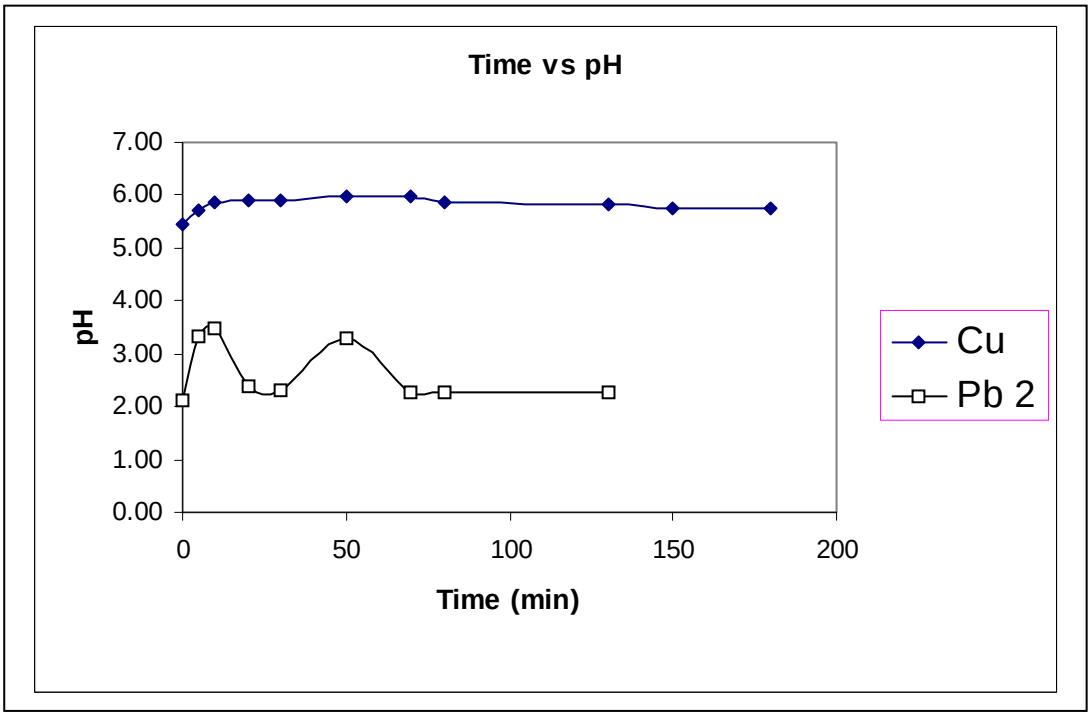


Figure 4.15: Variation of pH with time during the adsorption of Cu and Pb

Figure 4.15 shows the pH profile for Cu and Pb. The pH of lead was initially very low due to a solubility problem. The fluctuations are not indicative of the

process, but of outside factors. i.e. some of the Pb was absorbed by the metal sample canisters before analyses, and this affected the pH. Also we constantly had to add nitric acid to keep the Pb from precipitating. This is a problem still bearing some investigation at present. The pH profile for Cu is more representative of the process. It shows a slight increase in pH until sorption euqilibria is reached at which point the pH stabilizes. The optimum range for column operation would thus be at a pH of approximately 6.

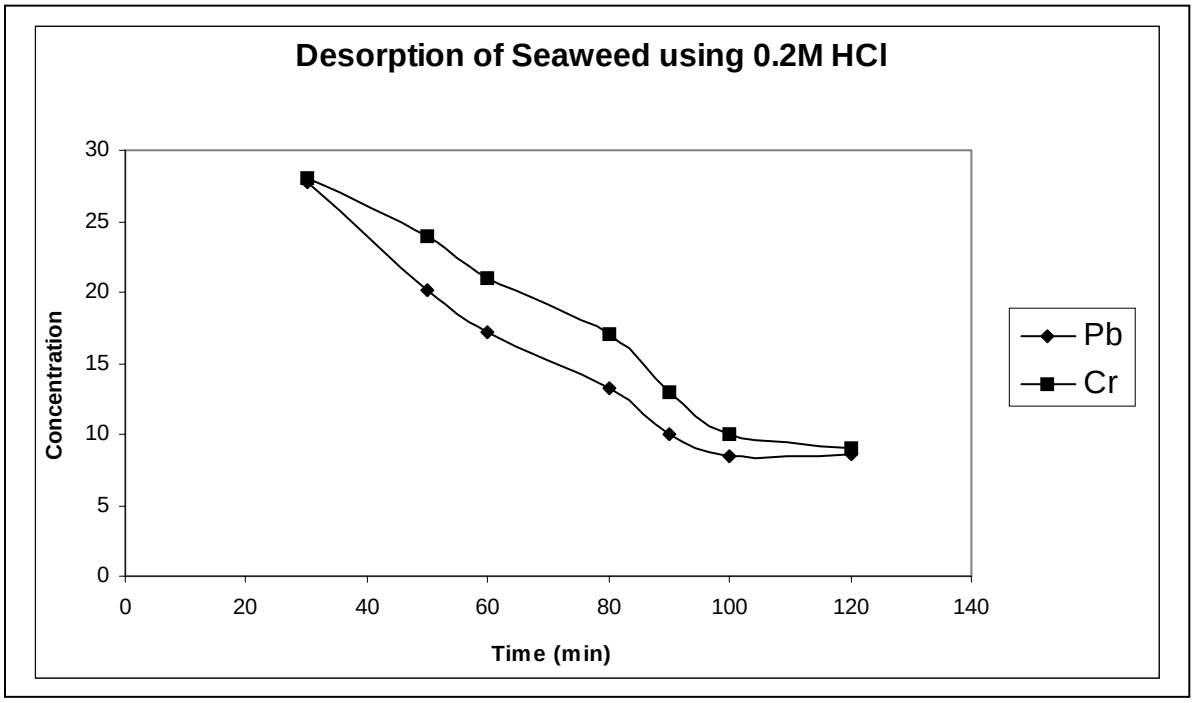


Figure 4.16: Desorption Pb and Cr from seaweed in a column

The desorption of Pb and Cr from the seaweed was achieved using a 2M HCl solution. The HCl solution was passed down through the column, the sorbate collected periodically at the bottom and analyzed for heavy metal content. We were only able to remove 95% of the Cr and Pb within 120 minutes. Initial heavy metal removal was fast, with more than 70% being removed within the first 20 minutes of operation.

In the batch columns sorption equilibria was reached within 10 minutes with 100% of the Pb and Cr being removed. It is however not vlear how many

times the seaweed can be reused after the initial desorption process or how effective it would be as a reusable biological ion-exchanger. What is clear from the research done is that seaweed has a great capacity for heavy metal removal; kinetics of the adsorption process is fast; we are able to remove the heavy metals once they have been adsorbed.

5. Economic Consideration of Biosorption

The batch test and column test in the laboratory both indicated that seaweed could be a promising biosorbent on an industrial scale for the treatment of heavy metal effluents. Besides a good understanding of the mechanisms of metal biosorption and the operational characteristics of the process, commercial success also depends on an inexpensive and reliable supply of a biomaterial, a suitable means of the immobilization of the biomaterials, as well as their regeneration and reuse. The cost for producing the required biomaterial for the sole purpose of bisorption, regeneration, and eventual disposal should also not be excessive.

Compared to chemical precipitants and flocculants, especially with the commercial resins, the seaweeds are naturally growing marine plants, which can be obtained at a very low cost, although a sustainable supply of the seaweeds in areas, which are a long distance from, the coast could be a problem. The physicochemical and biological characteristics of the seaweeds are very stable throughout the year and special treatment is not required to handle and to store the seaweeds. The immobilization of the seaweeds for large-scale biosorption can be realized using a packed-bed column configuration, which can be operated easily by following existing ion-exchanging column techniques. The cost to regenerate the metal-loaded biosorbents can be expected to be comparable or even less expensive than commercial ion-exchange columns, if the seaweed is provided in a suitable

form (particle size). In the light of the above considerations, the use of seaweed to treat effluent contaminated by heavy metals appears to hold promise as a cost effective alternative to conventional ion exchange technology.

6. Capacity Building

Capacity building formed an integral part of the research completed. A culture of research and development was promoted at the Cape Technikon, via interaction with the University of Stellenbosch and a sharing of resources and expertise.

At the Cape Technikon, three students from previously disadvantaged backgrounds completed full or partial qualifications based on the research performed, that is 1 Masters Degree (with title of thesis 'Biosorption of Heavy Metals From Aqueous Solutions'), 1 In-Service Trainee who gained practical experience to complete the requirements for obtaining a diploma, and 1 Btech student who completed his Research Dissertation Project based on the research performed.

7. Technology Transfer

The research conducted was presented as a full presentation at an international conference on Biohydrometallurgy in March 2002 (Esau et al;, 2002). A poster presentation was presented at the SAIMM (South African Institute for Mining and Metallurgy) in August 2002, where it received the poster prize for the conference. Presentations were also made at the Chemical Engineering Research and Development Conference. We are also in the process of preparing a publication based on the research for an international journal.

Appendix 1: Physicochemical Analysis of Biomaterial

Characteristics	Tobacco Dust	Sawdust	Seaweed
Total Solids (% TS)	94.20	-	-
Volatile Solids (% of TS)	71.87	98.08	78.59
Ash (% of TS)	28.03	1.02	21.41
C (%)	43	53	35.1
N (%)	2.37	0.262	1.681
NO_3-N (mg/kg)	308	67.0	242.0
NH_4-N (mg/kg)	761	14.0	22.5
K (%)	1.70	0.046	2.640
Ca (%)	4.29	0.023	1.790
Mg (%)	0.70	0.008	0.858
B (mg/kg)	26	1.592	99.483
Cu (mg/kg)	57	47.256	2.028
Fe (mg/kg)	0.57	113.143	117.132
Zn (mg/kg)	761	24.004	49.550
Na (mg/kg)	288	213.789	24362.859

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