Chapter Two

CHAPTER TWO

BIOSORPTION OF HEAVY METALS: REVIEW OF THE LITERATURE

2.1 Introduction

The need for economical and effective methods for removing heavy metals from wastewater has led to advances in biotechnological research. The main thrust of the research has been the use of bacteria, fungi and algae as biosorbents (Strandberg *et al.*, 1981; Brown and Lester, 1982; McHardy and George, 1990). Biosorption is the ability of biological organisms. both living and non-living, to passively bind metal species dissolved in solution onto the cell surface. The accumulation of heavy metals by these organisms including algae, has been studied extensively for bio-monitoring or bio-remediation purposes. This technology and its potential for the treatment of wastewater and environmental pollution has been highlighted recently (Volesky, 1990). So innovative was the idea and so encouraging were the results that it even attracted the attention of Newsweek (Begley and Waldeop, 1989).

The mechanism by which these biological systems takes up heavy metals, is in two stages. The first is by `physical adsorption' or `biosorption' onto the surface of the cell wall. This involves a passive, rapid and reversible reaction. The second is `chemisorption or `bioaccumulation'. This is a metabolically active process by which, the heavy metals are taken into the cells. The dominant process is biosorption which accounts for 90% of the total metal uptake and occurs within 5 to 40 minutes. The remaining 10% is attributed to bioaccumulation and the process occurs over hours or days (Fehrman and Pohl, 1993).

Algal ponds have been constructed for the treatment of sewage and tannery effluents with good results (Laubscher *et al.*, 1990). These algal ponds have the advantage of being grown easily and with little need for maintenance. McHardy and George (1990) used *Cladophora glomerata* in artificial freshwater channels and found the algae capable of concentrating zinc two to five thousand times. Chan *et al.*, (1991) used two species of *Chlorella* to remove copper and nickel from electroplating effluent. They found that while 68% of the copper was removed within 72 hours, the results for nickel was less encouraging; only 20% were removed over the same time. On the other hand, Brady *et al.*, (1994) found that the microalgal biomass of the *Scenedesmus, Selenastrum and Chlorella* species were 67 to 98 % efficient in accumulating copper, lead and chromium.

Despite these advantages there were limitations to the use of ***viable*** biomass for the removal of heavy metals from wastewaters. The main difficulty was to maintain algal growth in those polluted waters. The reason for this was that the pH of those waters did not encourage the survival of the species (Becker, 1983). The lack of organic substrates to maintain algal growth was another factor. Furthermore, the adsorption of heavy metals itself would affect the viability of the algae. It would necessitate the addition of complexing agents and the constant adjusting of the pH of the wastewaters. This involves cost and in some instances it would be impractical to do so.

However, the use of dead cell systems would do away with these disadvantages. It would make the operations less complicated and cut down costs. The disadvantage of course is that the process of active bioaccumulation would be sacrificed. Considering the fact that 90% of total metal uptake by algal biomass can be attributed to biosorption, it is a small price to pay. It has also been shown that dead cells are capable of accumulating heavy metals just as efficiently as their living counterpart (Sakaguchi *et al.*, 1979). Because of these advantages, the main thrust of research into the field of biosorption has now been the use of dead biomass.

2.2 Biosorption by dead biomass

Current research has indicated that there is an outstanding potential for biosorption of heavy metals by the dead biomass of some seaweeds namely *Sargassum, Eklonia* and *Ascophyllum.* These materials can accumulate up to 35% of their dry weight in heavy metals, sometimes even very selectively. Recovering the accumulated metals can be done from the highly concentrated wash solutions used to regenerate the biosorbent material for reuse (Volesky, 1998). Their main action is by surface binding and accounts for 90% of its efficacy. It has added advantages. Firstly, it can be used in wastewaters in which the growth of these biomass is not conducive.

Secondly and most importantly, it can be used repeatedly. The metal ions adsorbed can be removed easily with dilute acids. Moreover, the metal ions can be recovered. This is an advantage especially when one is dealing with precious metals (Harris and Ramelow, 1990).

Non-viable biomass function on the same principle as their active counterparts in the sequestration of heavy metals. The surface sequestering of the metal ions occurs because the metallic cations are attracted to the negatively charged parts of the cell wall surface. These anionic ligands belong to the phosphoryl, carboxyl, sulphydryl and hydroxyl groups of the membrane proteins. The adsorption process continues until an equilibrium is reached between the metal adsorbed and what is left in the solution. At this point all the binding sites are occupied and the biosorbent has reached its maximum capacity (Volesky, 1990).

Assessing a suitable biosorbent for a particular metal involves examining the adsorption and desorption process of the metal. The point of equilibrium reached for these processes can be obtained from what we call biosorption isotherms, similar to that used for activated carbons. The isotherm, commonly referred to as the Langmuir curve, is usually expressed as a graphical plot of the metal uptake by the biosorbent (mmol metal/g dry weight) against the residual concentration of the metal in solution. The curve is usually hyperbolic.

Amongst others there are two important features for an efficient biosorbent. The first is its maximum adsorption capacity. This characterises its efficacy at high metal concentrations. The other is the rate of metal adsorption and release. A rapid uptake would require a shorter contact time with the metal ions and require a shallower bed for the biosorbent material when it is packed in a column. Others include its specificity for a particular heavy metal and the ability to desorb that metal so as to make it reusable. To summarise, it has generally been accepted that for a biosorbent to be efficient it must fulfil the following criteria (Volesky, 1990):

i. It should adsorb and release metals efficiently and rapidly.
ii. It must be economical to use.
iii. It must be reusable.
iv. Its particle size, shape and mechanical properties should be such that it can be used in continuous flow systems in a variety of configurations.
v. It can be easily removed from the solution.
vi. It should be metal specific in order to deal with the selected metal in the solution.
vii. It must be easily desorbed of its metal and be economically viable.

2.3 Algae as metal biosorbents

Algae are a genus of plants that are photosynthetic, non-vascular and contain chlorophyll. The group comprises more than 1800 genera and over 21000 species.

They may be unicellular, such as *Chlorella* or multicellular, such as seaweeds. The genus has three main divisions: *Phaeophyceae, Rhodophyceae and Chlorophyceae*.

The principal mechanism of metallic ion sequestration involves the formation of complexes between a metal ion and functional groups present on the surface or inside the porous structure of the biological material (Fourest and Volesky, 1997). Most algal species have unique metal binding properties. Both the living and non-living forms can reversibly bind metal ions from aqueous solutions. Different species exhibit different binding characteristics at a given pH. The main reason for this is in their different cell wall structures. The cell wall is a fibrillar lattice of cellulose derivatives, pectins and proteins. This structure provides for a large surface area for the rapid but reversible binding of cations. Table 2.1 lists the probable potential groups on the cell wall that are implicated for metal binding and their respective pKa levels (Segel, 1976).

These functional groups on the cell wall are capable of binding both cations and anions. While the amino groups, carboxyl groups, the imidazole of histidine and the nitrogen and oxygen of peptide bonds are involved in co-ordination metallic ions, the unprotonated carboxyl oxygen and sulphate form the sites for electrostatically bound metal ions (Crist *et al.*, 1981).

Table 2.1 Ionisable groups in biological polymers capable of participating in metal binding.

Group	Location	pK_a
Carboxyl	Protien C-terminal	3.5-4.0
Carboxyl	Beta aspartic	4-5
Carboxyl	Gamma glutamic	4-5
Carboxyl	Uronic acid	3-4.4
Carboxyl	N-Acetylneuraminic	2.6
Carboxyl	Lactate	3.8
Sulphonic acid	Cysteic acid	1.3
Phosphate	Serine as ester	6.8, 2.0
Phosphate	Polyol mono ester	0.9-2.1
Phosphate	Polysaccharide diester	1.5, 6.0
Hydroxyl	Tyrosine-phenolic	9.5-10.5
Hydroxyl	Saccharide-alcohol	12-13.0
Amino	Protein N-terminal	7.5-8.0
Amino	Cytidine	4.11
Amino	Adenosine	3.45
Amino	Lysine	8.9, 10.5
Imidazole	Guanosine	2.3
Imidazole	Histidine	6-7
Imino	Peptide	13

Cho *et al*. (1994) showed that nonviable cells of the microalga *Chlorella vulgaris* were capable of binding heavy metals such as cadmium and zinc. Harris and Ramelow (1990) investigated the metal binding properties of particulate material from *C. vulgaris* and *Scenedesmus quadricauda* and found similar binding patterns for silver, copper, cadmium and zinc. However, the adsorption rates were dependent on the pH. The efficacy for binding decreased from silver>copper>cadmium>zinc. Holan *et al.* (1993) showed that the non-living biomass of the brown algae *Sargassum natans, Fucus vesiculosus* and *Ascophyllum nodosum* demonstrated a high equilibrium adsorption of cadmium from aqueous solutions. *A. nodosum* showed the highest accumulation of cadmium, exceeding 100 mg/L at a pH of 3.5. The adsorption efficacy by these biomass was compared to a commercial ion exchange resin DUOLITE GT-73 and found to be superior.

In another study comparing the use of *A. nodosum* to commercially used ion exchange resins, the dead biomass was found to be as equally efficacious, the *A. nodosum* exhibiting a high affinity for cobalt with an adsorption capacity of 156 mg/g of dry weight (Kuyucak and Volesky, 1989a). Furthermore, using the brown alga *Ectocarpus siliculosis*, Winter *et al.* (1994) showed that its adsorption of cadmium was more effective than that by activated carbon, silica gel or siliceous earth.

2.4 Factors affecting the biosorption process

The efficacy of algal biomass as adsorbents is affected by certain bio-physical properties. They will be considered in turn.

2.4.1 pH of the solution

Solution pH appears to be a critical factor that can affect the formation of metal-biosorbent complexes. Changes in pH can change the speciation or the availability of the metallic ions in solution and alter the chemical state of the functional groups involved in the binding to the biomass. The crucial factor is the isoelectric point. At pH values above the isoelectric point of the algal biomass, which usually occurs at around pH 3.0, there is a net negative charge on the surface. Because of this, the anionic state of the surface ligands such as the carboxyl, phosphate, imidazole or amino groups promote adsorption. As the pH is lowered the net surface charge on the biomass will become positive, which, in turn, creates an electrostatic repulsion of the metal ions (Stumm and Morgan, 1970). Protons will then compete with the metal ions for the ligands and decrease the interaction between the metal ions and the algal cell wall.

Analysing the biosorption patterns of *A. nodusum* on cadmium, Holan *et al.* (1993) showed that the removal was more efficient with increasing pH. The highest adsorption was seen at pH 4.9 and lowest at pH 2. In changing the pH of the solution from 2.0 to 5.0, Greene and Darnall (1990) found that the biosorption of copper increased by non-living cells of *Chlorella vulgaris*. Studying the alginate properties of heavy metal (calcium, zinc, cadmium, copper and lead) biosorption by four different brown seaweeds (*Sargassum fluitans*, *A. nodosum*, *Fucus vesiculosus and Laminaria japonica*), Fourest and Volesky (1997) found that the maximum uptake was at pH 4.5.

2.4.2 Interference by coincidental ions and chelating agents

When more than one metal is present in solution, the biosorption by the biomass of one over the other may occur. Kuyucak and Volesky (1989b) found that the presence of copper, nickel, chromium and lead had a suppressing effect on cobalt uptake on the biomass. Studying cadmium, zinc and copper adsorption by *A. nodosum* in a two metal system, Chong and Volesky (1995) found considerable competitive and inhibitive behaviour of the metals present. More recently, Figueira *et al.* (1997) studied the behaviour of a two metal system involving cadmium and iron using the alga *Sargassum fluitans*. They found that the biosorption uptake of cadmium decreased significantly in the presence of iron. At an equilibrium iron concentration of 1.5 mmol/L, cadmium adsorption was significantly suppressed. Only 26% of the original value of cadmium was adsorbed. With increasing equilibrium concentrations of iron, the reduction in cadmium uptake was even more significantly reduced. An equilibrium iron concentration of 4.5 mM caused a greater reduction in cadmium uptake with only 50% of the cadmium being adsorbed.

In the same system, cadmium also interferes with iron uptake but its interference is much more pronounced and was observed at much higher ratios of Fe:Cd concentrations. A 55% reduction in iron uptake was observed at 1.5 mmol/L concentration of cadmium.

2.5 Biosorption of copper and lead by algal biomass

Existing research on the biosorption of copper and lead by algal biomass is still not well established. Because of this the present research study was carried out to investigate the biosorption of copper and lead by *S. baccularia* (Plate 2.1).

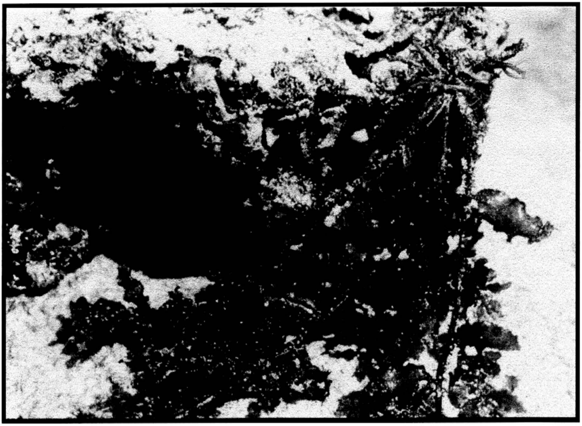

Plate 2.1 *Sargassum baccularia*