Chapter Three

CHAPTER THREE

BIOSORPTION EQUILIBRIA: SINGLE AND BINARY COMPONENT STUDIES

3.1 Introduction

As alluded to in Chapter One, metal biosorption by algae is an emerging technology in the industrial wastewater treatment sector. In this process, biosorption equilibria are important physico-chemical aspects.

Industrial effluents usually contain more than one metal in solution. There are also a whole host of other components. This may pose problems for their removal by biosorption because it is known that the adsorption of each metal in a multicomponent mixture may be affected not only by their relative absorptive affinities but also by the relative concentrations of the metals too. De Carvalho *et al.* (1995) demonstrated that the presence of a secondary metal in the adsorption system always lowers the adsorption capacity of the primary one due to metal-metal competition. There may also be multimetal competitive interactions in solution with the sorbent material (Chong and Volesky, 1995).

The purpose of the present studies was firstly, to study the uptake of copper and lead by the biomass of *S. baccularia* within a single component system. Secondly, to assess the selectivity of the biomass towards copper and lead when both were present simultaneously in a binary system. A binary metal adsorption system may be viewed as an initial step towards studying multimetal adsorption systems which closely depict the composition of many industrial effluents.

Samuel (1996) had studied the biosorption of cadmium and copper by seven species of marine algae. The results showed that the brown algae *S. baccularia and S. siliquosum* had impressive copper and cadmium adsorption capacities. However there were crucial environmental parameters that had to be optimised. These include the pH of the metal solution and the biomass concentration of the algae (Kuyucak and Volesky, 1989a; Volesky, 1990; Leusch *et al.*, 1995).

The pH of metal solutions is critical because of its effect on the formation of metal-biosorbent complexes. It not only affects the ionisation of the functional groups responsible for metal binding on the cell wall surface, but also regulates the solution chemistry affecting the chemical speciation of metal ions. This in turn determines the mobility and ability of the ions to adsorb onto the biomass surface (Treen-Sears *et al.*, 1984; Kuyucak and Volesky, 1989a; Zhou and Kiff, 1991). Samuel (1996) investigated the effect of pH values on the adsorption of copper and cadmium by *S. baccularia* and found that metal adsorption increases with increasing initial solution pH. Maximum adsorption was at an initial pH of 5.0.

Furthermore, the use of buffers to control pH was found to affect the biosorption process. Cadmium biosorption was significantly lowered in a buffered system although the final pH of the test solution was maintained at pH 5.0 (Samuel, 1996). Similarly, Harris and Ramelow (1990) had earlier shown that the sodium acetate-acetic acid buffer lowered metal adsorption. They found that when the buffer concentration exceeded 0.1M, the biosorption of copper by *Chlorella vulgaris* and *Scendesmus quadricauda* was significantly reduced. It was postulated that the formation of metal-acetate complexes which are unable to adsorb onto the surface of the biomass, was the reason for the reduction in metal adsorption. Being a ligand acetate chelates metal ions. Metal acetate complexes are formed and no free metal ions are available for adsorption onto the biomass.

The present studies were conducted to evaluate copper and lead biosorption by the alga *S. baccularia* over a range of differing metal concentrations and to assess the selectivity of the biomass of *S. baccularia* towards copper and lead when both metals were present simultaneously. The Langmuir adsorption isotherm was applied to characterise the adsorption process. The other experimental parameters used were as determined by Samuel (1996). An initial pH of 5.0 was used. A buffer system was not employed in this study due to possible interference in the biosorption process by buffer ions. A particle size range of 500- 710 μm was used in all experiments.

3.2 Adsorption Isotherm

To measure the adsorption capacity and characterise the removal of heavy metals by adsorbents, adsorption isotherms have been used by many researchers (Fourest and Roux, 1992; Avery and Tobin, 1993; Chang and Hong, 1994; Cho *et al.*, 1994). They provide useful information on adsorption systems such as the maximum adsorption capacity of an adsorbent.

It has been stated that "the quantity of adsorbate that can be taken up by an adsorbent is a function of both the characteristics and concentration of adsorbate and the temperature". Generally, the amount of material adsorbed is determined as a function of the concentration at a constant temperature, and the resulting function is called an adsorption isotherm (Metcalf and Eddy, 1991). Various isotherms such as Freundlich, Langmuir and Brunauer, Emmet, and Teller (BET) have been developed to describe experimental isotherm data. Of these, the most commonly used adsorption isotherms are the Langmuir and Freundlich isotherms (Smith, 1981; Chang and Hong, 1994). These models have been widely used to analyse data for water and wastewater treatment applications (Patterson, 1977; Kutsal and Aksu, 1988). Furthermore, the Langmuir adsorption isotherm has also been successfully applied to evaluate metal biosorption to algae (Crist *et al.*, 1994; Holan and Volesky, 1994; Ozer *et al.*, 1994). The Langmuir adsorption isotherm is defined as :

$$q_{eq} \quad = \quad (q_{max}\, k\, C_{eq})/(1 + kC_{eq}) \qquad (1)$$

where

q_{eq} = solid phase equilibrium metal concentration (mmol/g biomass)

C_{eq} = liquid phase equilibrium metal concentration (mmol/L)

q_{max} = maximum metal adsorption capacity (mmol/g biomass)

k = Langmuir equilibrium constant (L/mmol or mM^{-1})

Maximum metal adsorption capacity (q_{max}) can be estimated with this model. The constant k is related to the energy of adsorption (Langmuir, 1918). The amount of metal adsorbed on the biomass surface in a batch system is equal to the solid phase equilibrium metal concentration and may be calculated using the mass balance below:

$$q_{eq} = V(C_i - C_{eq})/ M \qquad (2)$$

where

V = volume of the test solution (L)

M = weight of the biomass (g)

C_i = initial metal concentration (mmol/L)

C_{eq} = final equilibrium metal concentration (mmol/L)

The Langmuir adsorption isotherm (Langmuir, 1918) was developed by assuming that :

- Adsorption is limited to a monolayer;
- There is no transmigration of the adsorbed species within the plane of the surface; and
- The adsorption energy of all the sites is identical and independent of the presence of adsorbed species on neighbouring sites.

When a metal ion in solution collides with the biosorbent surface, a number of processes are likely to occur. These are summarised as follows (Allen and Brown, 1995):

(i) The metal ion may rebound from the surface;

(ii) The metal ion may be adsorbed and the biosorbent may preferentially concentrate specific metal ion species from the solution onto their surfaces via two types of adsorption processes. These are:

- physical adsorption which is associated with weak forces of physical attraction, for instance, the van der Waals' forces and
- chemical adsorption or chemisorption which is associated with the exchange of electrons and the formation of a chemical bond between the adsorbed metal ion and the biosorbent surface; and

(iii) A reaction may take place between the incoming ion and the chemical species (functional groups) present on the biosorbent surface - the phenomenon of ion-exchange.

When the concentration of a metal in the bulk solution is in dynamic balance with that of the interface, an adsorption equilibrium is established. At this point of equilibrium there is a distinct distribution of metal ions between the liquid (reaction solution) and solid phases (biosorbent surface).

An isotherm plot is obtained when the metal adsorbed by the biosorbent is plotted against the residual metal concentration. This isotherm is a functional expression for the variation of adsorption with metal ion concentration in the bulk solution at constant temperature. The relationship between metal adsorption and residual metal is typically hyperbolic (Volesky, 1987).

The shape of the adsorption isotherm is a matter of interest. An isotherm steep from the origin is highly desirable as it indicates a high affinity of the biosorbent for the adsorbed metal ions. A biosorbent of this nature would be efficient at metal uptake at very low metal ion concentrations. Another important feature of the biosorbent is its maximum adsorption capacity. The maximum adsorption capacity is a measure of the performance of the biosorbent at high residual concentrations.

The Langmuir adsorption isotherm was used in this study to determine and compare the maximum adsorption capacities of copper and lead by the non-living biomass of *S. baccularia* at a pH of 5.0. Single component and binary component equilibrium adsorption studies were conducted in a series of batch experiments. The results were quantified using the Langmuir model.

The Langmuir isotherm parameters which best fit the experimental equilibrium data obtained in this study were determined by a non-linear regression analysis using a commercial software package. The isotherm parameters determined in this study were used to compare the equilibrium behaviour of copper and lead in single and binary component equilibrium situations.

3.3 Materials and methods

3.3.1 Materials

- Fresh samples of the marine alga *S. baccularia* (Plate 2.1) were collected from the Cape Rachado beach, Port Dickson on the west coast of Peninsular Malaysia. The location of this site is shown in Appendix III.
- Analytical grade nitric acid and sodium hydroxide were obtained from Fluka, Switzerland.
- Copper (II) and lead (II) salts i.e. $Cu(N_2O_6).3H_2O$ and $Pb(N_2O_6)$ were obtained from Fluka, Switzerland.
- Inductively-coupled plasma (ICP) standard solutions were obtained from Fluka, Switzerland.
- Ultra-pure water was obtained after filtration, distillation and de-ionisation by a water purification system (Elgastat UHQ II (England)).

3.3.2 Methods

3.3.2.1 Preparation of *S. baccularia*

The freshly collected algal biomass was thoroughly washed twice with distilled water and then with ultra-pure water to remove adhering sand and epiphytes. The biomass samples were then blotted dry with absorbent paper and air dried overnight on bandage gauze placed over silica gel in a closed chamber. The samples were dried further in an oven at 45^0C to constant weight. Completely dried samples were ground in a blender and sieved to a particle size range of 500-710 μm using a Standard Testing Sieve (W.S Tyler Inc., USA). The biomass samples were then stored in capped glass containers with sachets of silica gel (silica gel wrapped in bandage gauze) at room temperature. The sachets of silica gel were regularly replaced and served to keep the biomass at a constant weight.

Care was taken to ensure that no metallic containers were used in cleaning, storage or handling of the algal samples. The only exceptions were the stainless steel blades and sieves that were involved in the grinding and sieving process.

All the glassware and working containers were washed thoroughly with detergent and then rinsed with distilled water. They were next soaked overnight in 10% nitric acid and then rinsed thrice with distilled water. This was done to eliminate any metal contamination.

3.3.2.2 Preparation of metal solutions

Preparation of copper solutions

Stock solutions of copper (500 mg/L) were prepared by dissolving $CuN_2O_6.3H_2O$ in distilled de-ionised water. All working metal solutions were prepared fresh daily. Copper solutions ranging from 0.8 to 6.3 mmol/L (50 to 400 mg/L) were prepared from the stock solutions by diluting the stock solutions in distilled de-ionised water.

Preparation of lead solutions

Stock solutions of lead (II) (500 mg/L) were prepared by dissolving PbN_2O_6 in distilled de-ionised water. All working metal solutions were prepared fresh daily. Lead solutions ranging from 0.2 to 7.2 mmol/L (50 to 1500 mg/L) were prepared from the stock solutions by diluting the stock solutions in distilled de-ionised water.

Solutions for pH adjustment

Solutions of 0.5 M nitric acid and 0.5 M sodium hydroxide were prepared for pH adjustments. The pH was measured using a calibrated pH meter (Corning, UK). The initial pH of metal solutions prepared was fixed at 5.0.

3.3.2.3 Determination of adsorption isotherms

Batch experiments were performed to determine the equilibrium isotherms of copper and lead at an initial pH of 5.0. The batch adsorption experiments were performed by suspending 0.1 g of *S. baccularia* biomass of particle size ranging from 500-710 μm in 50 mL of metal solutions. These experiments were carried out in 100 mL Erlenmeyer flasks and agitated in a rotary shaker (ISV-1-V, Kuhner, Switzerland) at 150 rpm for a period of 40 hours under a constant temperature of

25 ^{0}C. Samuel (1996) had found that the time taken to attain equilibrium for the biosorption of copper and cadmium by *S. baccularia* was approximately 30 minutes. Hence, a contact period of 40 hours was more than sufficient for the copper and lead biosorption systems to reach equilibrium. Metal free and biomass free controls were run simultaneously with the batch experiments (Appendix VI).

At the end of the experiments, the slurry was filtered with cellulose acetate membrane filters of 0.45 µm (Sartorius, Germany) with a 20 mL syringe (B.Braun, Germany). The filtrate was then diluted appropriately with distilled de-ionised water and analysed for its metal content. The experiments were conducted in duplicates.

The residual metal concentration in the contact solutions (the filtrate) was determined by Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) with a Baird ICP 2000 (Baird, Switzerland) instrument.

3.3.2.4 Binary metal experiments

3.3.2.4.1 Copper biosorption in the presence of lead

Preparation of stock solutions

Stock solutions of 500 mg/L (7.9 mmol/L) of copper and 500 mg/L (2.4 mmol/L) of lead were prepared as described earlier. A stock solution of 2000 mg/L (9.7mmol/L) of lead was prepared by dissolving $Pb(N_2O_6)$ in distilled de-ionised water.

Experimental design

The experimental design required a fixed concentration of copper ions. It was decided to fix this at 50 mg/L (0.786 mmol/L) for all the batch reaction flasks containing copper. Metal solutions containing combinations of copper and lead in molar ratios of copper to lead ranging from 1:0, 1:1, 1:2, 1:5 and 1:10 were prepared. This was done by appropriately diluting the copper and lead stock solutions with distilled water so that these molar ratios were maintained in 5 separate reaction flasks. A biomass of 0.05 g of *S. baccularia* was incubated in solutions containing the varying molar ratios of copper and lead in a series of batch experiments. The residual metal concentrations remaining in solution after biosorption onto the biomass were analysed.

Solutions of 0.5 M nitric acid or 0.5 M sodium hydroxide were used for pH adjustments. pH measurements were done as in the previous experiments. The initial pH of the solutions was fixed at 5.0.

3.3.2.4.2 Lead biosorption in the presence of copper

Preparation of stock solutions

Stock solutions of 500 mg/L (7.9 mmol/L) of copper and 500 mg/L (2.4 mmol/L) of lead were prepared as described earlier.

Experimental design

The experimental design required a fixed concentration of lead ions. It was decided to fix this at 50 mg/L (0.24mmol/L) for all the batch reaction flasks containing lead.

Metal solutions containing combinations of lead and copper in molar ratios of lead to copper ranging from 1:0, 1:1, 1:2, 1:5 and 1:10 were prepared. This was done by appropriately diluting the lead and copper stock solutions with distilled deionised water so that these molar ratios were maintained in five separate reaction flasks. The biomass (0.05 g) of *S. baccularia* was incubated in solutions containing the varying molar ratios of lead and copper in a series of batch experiments. The residual metal concentrations remaining in solution after biosorption onto the biomass were analysed.

Solutions of 0.5 M nitric acid or 0.5 M sodium hydroxide were used for pH adjustments. The pH was measured as in the earlier experiments. The initial pH of the solutions was fixed at 5.0.

3.4 Results

3.4.1 Determination of adsorption isotherms

Adsorption isotherms are plots of solid phase equilibrium metal concentration (q_{eq}) against the respective liquid phase equilibrium metal concentration (C_{eq}). The Langmuir adsorption isotherm was used to describe the copper and lead single component systems.

Figure 3.1 shows the experimental data for copper biosorption onto *S. baccularia* at pH 5.0. The Langmuir model was fitted to the experimental data by a non-linear regression analysis. The maximum adsorption capacity (q_{max}) and the Langmuir constant (k) were estimated as 1.19 mmol/g or 75.6 mg/g and 10.33 mM^{-1} respectively. These estimates were used to plot the solid line in Figure 3.1. The coefficient of correlation r was 0.945, indicating that the fit was good and that the experimental data corresponded closely to the Langmuir adsorption model.

As a result of the biosorption process, the initial pH of the reaction solution may change. For copper biosorption, the final pH recorded was generally lowered after the biosorption process, from an initial pH of 5.0 to a range of 4.1-5.6 (Figure 3.2). The only exception was when the initial copper concentration was the lowest (0.78 mmol/L). In this case the final pH actually rose to 5.6.

Similarly for lead uptake, the Langmuir model was fitted to the experimental data. The maximum adsorption capacity (q_{max}) and the Langmuir constant (k) were estimated as 1.32 mmol/g or 273.5 mg/g and 85.42 mM^{-1} respectively. These estimates were used to plot the solid line in Figure 3.3. There was a strong coefficient of correlation r of 0.988, indicating that the fit was good and that the experimental data corresponded closely to the Langmuir adsorption model.

As a result of the biosorption process, the initial pH of the reaction solution may change. In all the 6 reaction flasks where the initial lead concentrations ranged from 0.26 to1.59 mM there was an increase in the final pH from 5.0 to a range of 6.0 -

7.0 (Figure 3.4). However, in those flasks with higher initial lead concentrations (2.1 - 7.1 mM) a drop in the pH from pH 5.0 to a range of 4.1-4.8 was observed.

3.4.2 Binary metal experiments

The study revealed that increasing concentrations of lead suppress the uptake of copper by *S. baccularia* (Table 3.1 and Figure 3.5). The percentage reduction in copper uptake (Table 3.1) was calculated by deducting the value of q_{eq} of copper at a specific initial molar ratio of Cu:Pb from the value of q_{eq} of copper at the initial molar ratio of Cu:Pb = 1:0, dividing this by the value of qeq of copper at the initial molar ratio of Cu:Pb = 1:0 and multiplying by 100. For example in the one metal system, the copper uptake was 0.76 mmol Cu/g of biomass. As lead ions increased to 10 times, copper uptake decreased to 0.04 mmol Cu/g, an average of 5.3% of the original value i.e. a 94.7 % reduction in copper uptake. Even when initial numbers of lead ions were twice as much as copper ions (Cu:Pb=1:2), there was a 72% reduction in copper uptake.

Table 3.1 Reduction (%) in Cu uptake with increasing initial molar ratios of Cu:Pb.

Cu:Pb Molar ratios	q_{eq} of Cu (mmol/g)	Reduction (%) in Cu uptake
1:0	0.76	-
1:1	0.47	38.2%
1:2	0.21	72.4%
1:5	0.04	94.7%
1:10	0.04	94.7%

The initial pH of the reaction solution may change as a result of the biosorption process. In all the 5 reaction flasks there was a decrease in the final pH from 5.0 to a range of 4.1-4.9 (Figure 3.6).

The study also revealed that increasing concentrations of copper have no effect on the uptake of lead by *S. baccularia* (Table 3.2 and Figure 3.7). Copper did not seem to inhibit nor did it enhance the adsorption of lead onto *S. baccularia* when both metals were present simultaneously.

Table 3.2 Percentage (%) reduction in Pb uptake with increasing initial molar ratios of Pb:Cu.

Pb:Cu (Molar ratios)	q_{eq} of Pb (mmol/g)	Reduction (%) in Pb uptake
1:0	0.25	None
1:1	0.25	None
1:2	0.25	None
1:5	0.25	None
1:10	0.25	None

As a result of the biosorption process, the initial pH of the reaction solution may change (Figure 3.8). In the first three reaction flasks i.e. Pb:Cu = 1:0, 1:1 and 1:2, there was an increase in the final pH to 6.4, 5.9 and 5.4, respectively. For the remaining two flasks at higher initial copper ion concentrations the final pH decreased to 4.5 and 4.3, respectively.

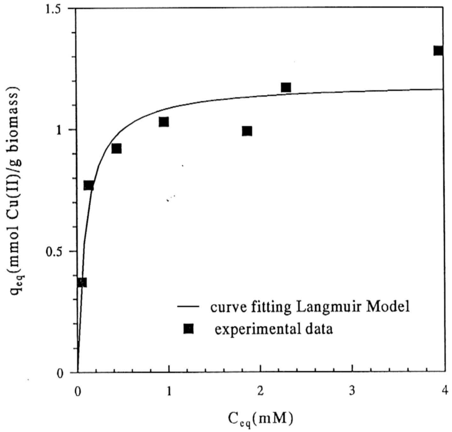

Figure 3.1: Copper(II) adsorption isotherm for *Sargassum baccularia* at 25°C and pH 5.0.

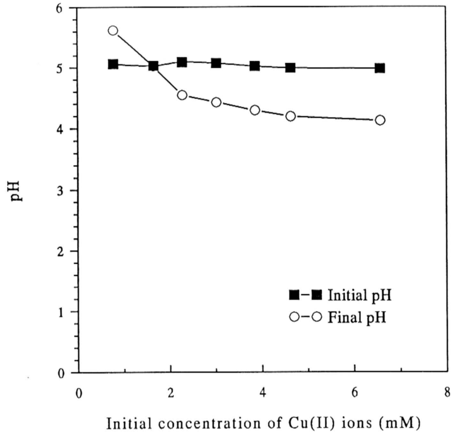

Figure 3.2: pH change during copper(II) biosorption.

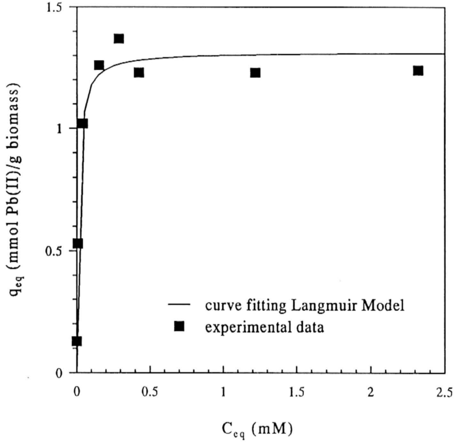

Figure 3.3: Lead(II) adsorption isotherm for *Sargassum baccularia* at 25 °C and pH 5.0.

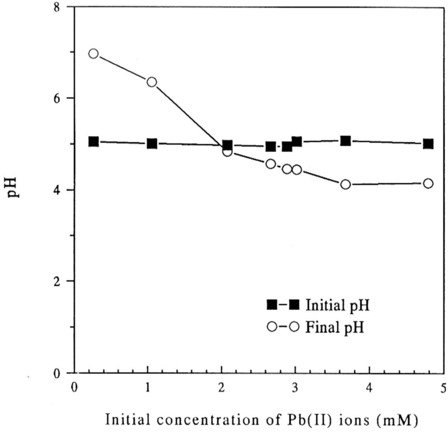

Figure 3.4: pH change during lead(II) biosorption.

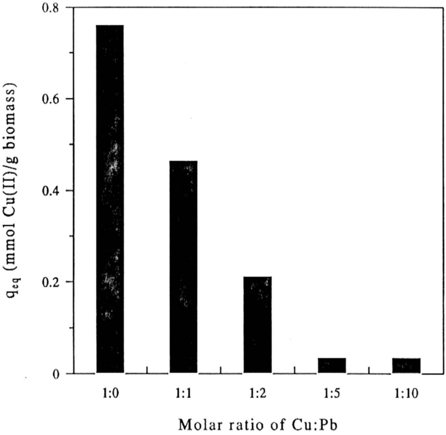

Figure 3.5: Copper(II) biosorption as a function of initial molar ratios of copper(II) to lead(II).

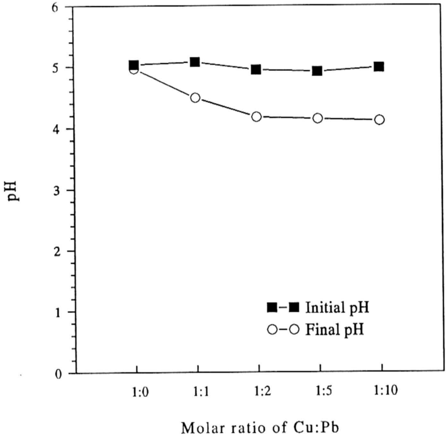

Figure 3.6: pH change during copper(II) biosorption as a function of initial molar ratios of copper(II) to lead(II).

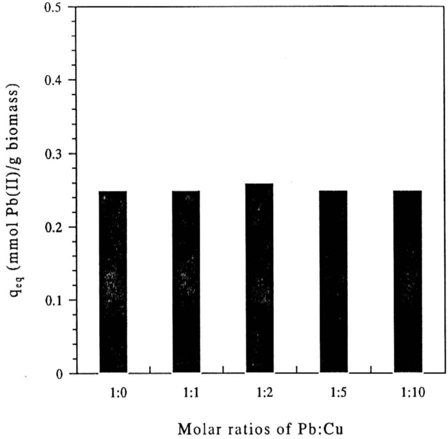

Figure 3.7: Lead(II) biosorption as a function of initial molar ratios of lead(II) to copper(II).

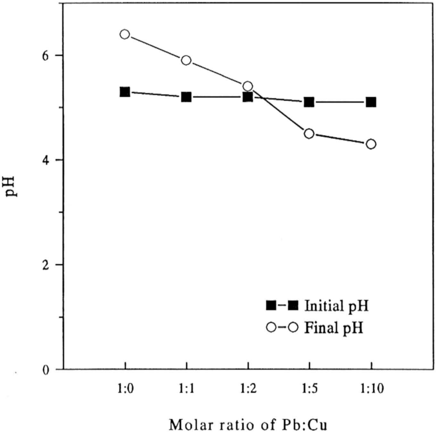

Figure 3.8: pH change during lead(II) biosorption as a function of initial molar ratios of lead(II) to copper(II).

3.5 Discussion

The present study set out, firstly to evaluate the uptake of copper and lead within a single component system and then to determine whether there is any interaction of one metal over the other in a binary metal system. The alga *S. bacculària* was used as the biosorbent. To explain the results some understanding of the biosorption process need to be fully understood.

Algal cell walls play a significant role in the intracellular uptake and surface adsorption of metals. The cell walls contain acidic polysaccharides and proteins which provide different functional groups that can bind to metal ions. Kuyucak and Volesky (1989b) revealed that the mechanism of biosorption was due to metals being bound by alginates present in the cell wall of marine algae. Cobalt uptake by *Ascophyllum nodosum* for instance was reduced when alginate was extracted, revealing the importance of alginates in metal biosorption.

The thallus of the brown algae (Phaeophycae), for instance, contains extracellular polyuronic acid (alginate), an acidic polysaccharide and sulfated glucurono-xylo-fucane (fucoidan), a sulphated polysaccharide, embedded in a fibrous cellulose skeleton (Kreger, 1962; Percival and McDowell, 1967; Fourest and Volesky, 1996). In brown algae, alginates are the major structural polysaccharide.

Alginates are copolymers of guluronic and mannuronic acids. One polymer segment consists of D-mannuronic acid units, another consists of L-guluronic acid units and the third consists of alternating D-mannuronic acid units and L-guluronic acid residues. These alginates perform like ion-exchange material. It is mainly due to ion exchange between the sea-water and the alginates present in the cell wall of the brown algae that the divalent metals calcium, magnesium and strontium are present in brown algae (Haug and Smidsrod, 1967; Myklestad and Haug, 1981)

In metal binding, the carboxyl groups of alginates play a critical role. For example, cobalt biosorption by *A. nodosum* was mainly due to interactions with the carboxyl groups via an ion-exchange mechanism (Kuyucak and Volesky, 1989b). The process involves an exchange of the bivalent ions with the counter ions of alginic acid:

$$2NaAlg + Me^{2+} \leftrightarrow Me(Alg)_2 + 2Na^+$$

where Na is the sodium ion, Alg represents alginate and Me denotes metal ions.

The carboxyl groups from alginate were also proved to be partially responsible for the chelation of copper and aluminium by algae (Gardea-Torresday *et al.*, 1990) but their contribution to the overall ion-exchange capacity may vary from one species to another. Studying *Sargassum fluitans*, Fourest and Volesky (1996) clearly demonstrated the predominance of carboxyl groups in alginic acid among the total acidic moieties contained in the *S. fluitans* biomass. Another study (Fourest and Volesky, 1997) showed that heavy metal binding capacities of four different brown seaweeds (*S. fluitans, A. nodosum, Fucus vesiculosus, and Laminaria japonica*) were directly proportional to their respective total carboxyl group content.

The sulphated polysaccharides in brown algae also selectively adsorb trace metal ions through an ion-exchange mechanism (Percival and McDowell, 1967) and thus play an important role in metal biosorption. Furthermore, it has been shown that the presence of both alginate and sulfonate groups are necessary to model and correctly predict the ion-exchange properties of the *Sargassum* biosorbent at different pH values (Schiewer and Volesky, 1995).

Yet another mechanism by which metal uptake occurs in algae, is through bond formation with the functional groups found in amino acids of the algal cell wall. Co-ordination bonds can be formed between metal ions such as copper with the imidazole of histidine and the nitrogen and oxygen of the peptide bond. The bond formed may be accompanied by a displacement of protons which is determined by the extent of acidification.

The nature and selectivity of the active sites on the algal cell wall for metal ions is dependent upon the complexing power of ligands. Metal ions compete with protons for sites on ligands. But at a fixed pH, a metal ion, in having to choose between two ligands, would complex to a ligand with a lower pKa (dissociation constant) value. Thus metal ions would bind more with carboxyl ligands on uronic acid (alginate) sites (pKa = 3.0 - 4.4) rather than with amino groups on protein N-terminal sites (pKa = 7.5 - 8.0) (Hunt, 1986; Segel, 1976).

The access to binding sites in biological molecules is regulated by pH as the proton directly competes with metal ions. A metal ion in biological systems has to frequently displace a proton from the binding site. The proton displacement reaction is appropriately written as:

$M + HL = ML + H$

(charges are omitted for convenience)

In this study, the initial pH of metal solutions was fixed at 5.0. The reason is that, at this pH value, most of the lead and copper ions exist as cations in solution and are available to participate in the biosorption process by displacing the protons from the binding sites. This may explain the subsequent drop in final pH measured in some of the experiments conducted.

Metal ions are capable of being electrostatically bonded to the unprotonated carboxyl oxygen and sulphate (Crist *et al.*, 1981). With infrared spectroscopy measurements of alginate and *S. fluitans* biomass, Fourest and Volesky (1996) showed that cadmium complexes with the carbonyl group (the unprotonated carboxyl oxygen) by dative co-ordination. Their results also revealed that the S=O and C–S–O bonds were not clearly and specifically detected on *Sargassum* spectra, thus confirming the low amount of sulfonate groups in the *Sargassum* biomass.

Futhermore their results indicated that sulphonate contribution to heavy metal binding is small but could be significant at low pH.

Thus it appears that most of the metal sequestering occurs via ion-exchange for metal biosorption in the non-living *S. baccularia* biomass. Other mechanisms also play a part. One such mechanism is the co-ordination bonding of the metal ions to amino acids which form part of the cell wall structure and electrostatic bonding to unprotonated functional groups on the cell wall.

The present study revealed that the maximum biosorption capacity of *S. baccularia* for lead was higher than that for copper (Table 3.3).

Table 3.3 Copper and lead adsorption constants for *S.baccularia* in single component systems.

Metal	Langmuir constants		
	k (mM^{-1})	q_{max} (mmol/g)	r
Copper	10.330	1.19	0.945
Lead	85.419	1.32	0.988

It has long been recognised that the chemical characteristics of the metal itself can influence its biosorption behaviour but the actual reasons for it have not been investigated until recently (Hughes and Poole, 1991; Avery and Tobin, 1993).

Gardea-Torresday *et al.* (1990) and Brady and Tobin (1995) considered the principle of hard and soft acids and bases (HSAB) as a qualitative means of predicting metal biosorption behaviour. Pearson (1963) had originally proposed the HSAB principle classifying metal cations (acids) into two groups: hard acids (Class A) and soft acids (Class B). Borderline metals are those that do not fit into either category. The hard-and-soft principle predicts that Class A metal ions form predominantly ionic bonds with non-polarisable (hard) ligands while Class B metal ions form soft metal-ligand complexes which are more covalent in nature.

Nieboer and Richardson (1980) extended the HSAB principle by proposing that for biological systems, the electronegativity (the charge and the ionic radius of metal ions) should be considered to determine their relative hard and soft behaviour. The metal ions were divided into three categories i.e. Class A, Class B and borderline ions (Appendix IV). The Class A (hard) metals have a preference for oxygen-containing (hard) ligands. In contrast, Class B (soft) metal ions seek out nitrogen and sulphur centres in biological systems and often become irreversibly bound (Nieboer and Richardson, 1980). They form more stable bonds with nitrogen or sulphur containing (soft) ligands. Class A metals prefer to bind with biological ligands in the order O>N>S while Class B prefer the opposite order: S>N>O. As for the borderline metal ions, they form an intermediate group and are able to form stable complexes with all categories of ligands. However, preferences do exist which reflect the degree of Class A or Class B character of the particular borderline metal and the relative availability of the different ligands in a system. In general, borderline metals have a higher affinity however for Class B metal binding atoms and ligands than for Class A metal binding donor atoms (Brady and Tobin, 1995).

The metals in this study, copper and lead, are regarded as borderline metals (Nieboer and Richardson, 1980). Both metals would therefore prefer to bind to the Class B preferred soft ligands like sulphur and nitrogen. However, as borderline metals both copper and lead are expected to display some degree of ionic and covalent bonding at the algal cell wall. But the degree (ionic/covalent bonding ratios) to which this occurs will probably depend on the nature of the metal and the metal concentration.

The relative degree of covalent bonding is probably greater with the biosorption of lead than with copper as lead has greater soft character (Appendix V). Metal concentration has probably a comparatively small effect on relative ionic and covalent bonding because of the relatively high (millimolar range) metal concentrations used in this study. In a study with marine algae, Crist *et al.* (1992) showed that relatively high (millimolar range) concentrations had a comparatively small effect on bonding of metals to algae. But in their study with *Saccharomyces* *cerevisae* (yeast cells) Avery and Tobin (1993) demonstrated that at higher metal concentrations and at the micromolar range, relative covalent bonding increased with metal softness. The only exception was cadmium (II). They had studied the displacement of calcium (II), magnesium (II) and hydrogen ions by a selection of hard (strontium (II), manganese (II)), borderline (zinc (II) and copper (II)) and soft (cadmium (II) and telenium (II)) ions and observed that cation displacement behaviour was concentration dependent. They suggested that in terms of the hard and soft acid base principle, the capacity of the cell surface for covalent binding relative to ionic bonding increases with metal softness. Furthermore, the capacity of the cells for covalent binding may be the principal limiting factor for determining maximum levels of metal uptake.

Most of the lead and copper ions in this study would have bound to the biosorbent via an ion exchange mechanism. This mechanism has been shown to be the predominant sequestering mechanism in algae and seaweed based metal biosorption (Crist *et al.*, 1994; Fourest and Volesky, 1996; Schiewer *et al.*, 1995). The lead and copper ions also probably bind via co-ordination covalent bonding to the amino acid groups found in the *Sargassum* biosorbent. They may bind only to a lesser extent to the sulphur sites in the sulphonate groups as the sulphonate groups, stated earlier, are limited in the *Sargassum* biomass (Fourest and Volesky, 1996). Fourest and Volesky (1996) had worked with *S. fluitans*, but it is assumed here that the *S. baccularia* biomass, being a *Sargassum* species, would also be limited in sulphonate groups. Further studies using infrared spectroscopy investigations of the *S. baccularia* biomass are needed to confirm this hypothesis.

Perhaps, because lead has greater soft character, a greater ionic potential and smaller ionic radius, it is adsorbed more than copper by the *S. baccularia* biomass. However it is not possible to deduce whether *S. baccularia* contains a greater number of soft sites compared to hard sites since both metals studied are intermediate. It may have been possible to deduce this if one of the metals studied was a soft metal and the other a hard metal.

The result of the present study on the biosorption of copper and lead in the single component system is in agreement with that of other workers. Samuel (1996) used *S. baccularia* as biosorbent and found the maximum adsorption capacity for copper to be 0.96 mmol/g under similar experimental conditions. A study by Leusch *et al.* (1995) revealed that glutaraldehyde cross-linked *Sargassum fluitans* of particle size 841-1000 µm at pH 3.5, had a lead adsorption capacity of 277 mg/g (1.3 mmol/g) and a copper adsorption capacity of 75 mg/g (1.2 mmol/g). These results compare favourably to those in this study, i.e. 273.5 mg/g (1.32 mmol/g) and 75.6 mg/g (1.19 mmol/g) for lead and copper respectively. The same study by Leusch *et al.* (1995) showed that glutaraldehyde cross-linked *A. nodosum* had a lead adsorption capacity of 139 mg/g and a copper adsorption capacity of 44 mg/g. Lead biosorption per gram of biomass was again more than that of copper for both, *S. fluitans* and *A. nodosum* .

It has been recognised that different biomass types exhibit different capabilities in lead and copper uptake. There would be differences in the structures and composition of the biomass cell walls that would affect the differences in uptake. For example, using the alga *A. nodosum*, de Carvalho *et al.* (1995) demonstrated that the formaldehyde-cross-linked *A. nodosum* was capable of approximately 0.5 mmol/g of copper uptake, much lower than the 1.19 mmol/g obtained in this study for *S. baccularia*. In another study (Fourest and Volesky, 1996) the maximum biosorption capacity of the dry mass of *S. fluitans* for lead at pH 4.5 was close to 1.06 mmol/g. In comparison Ashkenazy *et al.* (1997) who had worked with acetone washed yeast biomass of *Saccharomyces uvarum* demonstrated lead uptake at pH 5.5 to be 0.24mmol/g.

It appears therefore, that even within a similar biomass type there are differences, although it is not marked. *S. baccularia* for instance, was found in this study to adsorb 1.19 mmol/g of copper and 1.32 mmol/g of lead from an aqueous solution at pH 5. Whereas in glutaraldehyde cross-linked *S. fluitans* at pH 3.5, copper biosorption was 1.2 mmol/g and lead biosorption was 1.3 mmol/g respectively. Copper and lead biosorption by these two closely similar biomasses (both are *Sargassum* species) happened to be equal. A point to note however, is that the experimental conditions of pH and biomass treatment were different for both the biomasses. Had these conditions been identical, the results may have been different. Finally the seasonal, interspecies and geographical variations may be factors to be considered in accounting for the difference.

The biosorption of lead and copper when both were present simultaneously, showed that lead significantly inhibited the uptake of copper over the range studied when it was present in increasing molar ratios compared to copper (Figure 3.5). On the other hand copper did not seem to inhibit the uptake of lead over the range of molar concentration ratios studied (Figure 3.7). Lead uptake was constant throughout. It has been argued that the biosorption of each metal in a binary-metal system is affected by the relative adsorptive affinities and the relative concentrations of the metals (Kuyucak and Volesky, 1989a; de Carvalho *et al.*, 1995; Samuel, 1996). In contrast, the results of this study showed that increasing molar ratios of copper did not inhibit lead uptake. Lead uptake remained constant. How can this be explained in the light of existing theories?

There may be many factors other than their relative concentrations that determine the new equilibria of binary metal systems. These include metal-metal and metal-algae interactions which occur at the binding sites of the biomass surface where the equilibrium occurs. Competition between the metals for binding sites is dependent on one or all of the following parameters: ionic radii, ionic potential, electronegativity, hydration capacity and system pH. The nature and selectivity of the binding sites will in turn influence the metal-algae interactions.

Lead uptake was not inhibited by increasing initial molar ratios of copper. In contrast lead suppressed the uptake of copper when its initial molar ratio was more than copper. This is probably because lead ions were preferentially adsorbed compared to copper ions. Based on the HSAB principle mentioned earlier, both lead and copper are intermediate (borderline) ions. Both would therefore compete for the same binding sites on the biomass. However because lead has greater soft character than copper it would occupy most of the sites and suppress the uptake of copper. Furthermore, lead ions have a smaller radius compared to copper ions. The hydrated radius of Pb (II) is 0.401nm while that of Cu (II) is 0.419nm (Budinova *et al.*, 1994). Lead ions are thus able to better penetrate the biomass surface and have greater access to more binding sites. Lead also has the higher ionic potential (3.3) compared to copper (2.8) (Budinova *et al.*, 1994). In addition lead is more electronegative than copper and thus more lead than copper would be adsorbed to positively charged sites. Fourest and Volesky (1997) demonstrated a similar pattern with three different brown seaweeds, *L. japonica, S. fluitans, and F. vesiculosus* biosorbents. These brown seaweeds selectively bound five metals at pH 4.5 in the order $Pb > Cu \geq Cd > Zn \geq Ca$. This observation was attributed to the differences in the electronegativity of the elements considered.

Other workers have also obtained similar results when they studied other metals in a binary component system. Figueira *et al.* (1997), using *S. fluitans* as biosorbent, showed that the presence of cadmium significantly affected the uptake of iron, reducing its binding capacity from 10% to 4.5%. De Carvalho *et al.* (1995), using the brown alga *A. nodosum* as biosorbent showed that although both copper and cadmium were capable of inhibiting each other, the inhibition by copper was the more dominant. The authors concluded that a secondary metal always lowers the biosorption capacity of the primary one due to metal-metal competition. Samuel (1996), working on copper and cadmium and using *S. baccularia* as biosorbent, demonstrated similar results. Similarly, Kuyucak and Volesky (1989a) showed that copper significantly suppressed cobalt biosorption by *A. nodosum* regardless of the initial concentrations of copper. The adsorption capacity of cobalt was reduced by approximately 40%. In the present study increasing initial molar concentrations of lead suppressed the biosorption of copper. Copper biosorption was reduced as much as 94.7%.

3.6 Conclusion

The results obtained show that although the alga *S. baccularia* was capable of adsorbing both copper and lead, it appears to be more efficient for lead under similar experimental conditions. When both metals were studied together within a binary system containing increasing molar concentrations of lead in the presence of a fixed molar concentration of copper, lead was found to suppress the uptake of copper. However, when increasing molar concentrations of copper was present with a fixed molar concentration of lead, copper neither suppressed nor enhanced the uptake of lead. Therefore the results confirm previous studies that show the *Sargassum* species as an effective biosorbent of heavy metals. But in a binary component system, depending on the types of metals present, biosorption of one may be affected by the other.

3.7 Recommendations and scope for further research

This study has confirmed that non-living biomass *S. baccularia* can serve as a basis for the development of potent biosorbent materials for the concentration and recovery of strategic or valuable heavy metals. Their role as new biosorbents may further be enhanced if they can be regenerated for multiple reuse. To achieve these goals the biosorption process must be more fully understood and optimised.

The results of this study revealed that there was inconsistent data in the subsequent changes of the final pH of the solutions with varying concentrations of copper and lead, both, in single and binary systems. In some experiments, a reduction in final pH was observed for certain concentrations of the metal ions. On the other hand, in others, there was an increase in final pH. Because changes in pH may affect the biosorption process, this has to be investigated and understood further. Perhaps it may be related to the characterisation of *S. baccularia.* This was not investigated. Infrared spectroscopy investigations are needed to characterise the biomass.

It is of interest to investigate the effect of extraneous anions, cations and chelating agents on the biosorption of copper and lead. This is because they are commonly present in industrial wastewater. Furthermore, to realise its full potential as a biosorbent, desorbing studies of copper and lead should be conducted to investigate the feasibility of reusing the biosorbent. Lastly, there is a need to investigate how effective the adsorbent properties of the biomass are with actual industrial wastewater. Only then can it have potential for practical application.

It is possible then, that as a result of these studies, the actual potential of *S baccularia* as a biosorbent may be realised. Furthermore, it can be a suitable alternative technology in the prevention and treatment of industrial heavy metal pollution. *S. baccularia* occurs naturally and is a renewable resource. It can be used in a process arrangement almost identical to that used in conventional, expensive and end-of-pipe treatment technologies. It can provide industries with a cost-effective and viable cleaner production option for managing heavy industry wastewater. It could mean cost-savings, greater productivity and eco-efficiency for industry and the environment in the long term. It will be a win-win-win situation for all, industry, government and the environment.