

CHAPTER TWO

LITERATURE REVIEW

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The biosorption phenomena have opened up a new perspective in the treatment and recovery of heavy metals in dilute solutions. Although some promising types of biosorbents have already been identified, extensive screening of other biomass types are still taking place in both academic research laboratories as well as industrial research establishments (Volesky, 1990). One of the major challenges in the field of biosorption is the very interdisciplinary nature of these studies and the novelty of the field. Narrowing one's research scope to the choice of metal(s) to be studied in any biosorption will determine the size, volume and complexity of the research.

In this chapter, the structure of algae as well as environmental conditions responsible for metal uptake will be reviewed. In particular, copper as the model ion will be reviewed since it is commonly encountered in effluents from metal finishing and electroplating industries. Mathematical modelling to predict breakthrough curves in a fixed bed reactor and various properties of immobilising agents such as mechanical strength, chemical stability, swellability and porosity will be discussed.

2.1 Copper Toxicity

Pollution from man-made sources can easily create local conditions of elevated metal presence, which could lead to disastrous effects on animals as well as humans. This is especially true in the presence of indiscriminate discharges of heavy metal wastes from industries into the environment without proper treatment. High concentration of such metals can cause toxicity symptoms or even death. It is therefore important that effluents containing heavy metals be regulated by legislation to meet strict environmental standards because these toxic metals will eventually end up in the food chains (Volesky, 1990).

Copper is one example of a heavy metal that can be toxic if excessive amounts are present in the environment. Belonging to Group IIB of the periodic table, copper can occur in two major categories; the dispersed that forms in ordinary rocks, sediments and soils while the concentrated forms in mineral deposits. Both are transported by rivers to ocean either in soluble form or as solid load form associated with particulate or organic matter.

It is widely used as an industrial metal especially in the manufacturing of various alloys. It is also used in metal plating and in the production of copper wire. Other important uses include heat exchanger and piping. At high concentration, copper salts may serve as a pesticide (Trollop and Evans, 1976; Anderson *et al.*, 1978; Ferguson and Bubela, 1974; Melhuus *et al.*, 1978; Preston *et al.*, 1972).

According to Salmon and Wright (1971), absorption of excess copper by humans may result in "Wilson's disease", a condition in which copper is deposited in the brain, skin, liver, pancreas and myocardium. Accidental ingestion of food or beverages contaminated by copper may result in gastrointestinal disturbances with children suffering from diarrhoea coupled with weight loss. Although copper is an essential metal for human growth especially in the formation of haemoglobin with iron, in high concentrations it can be dangerous.

2.2 Algal Biomass

Algae are mainly considered to be all photosynthetic plants, with chlorophyll a and accessory pigments. This main feature and their mode of sexual reproduction as well as their morphology distinguish them from the fungi, which are dependent on non-photochemical reactions for their energy.

Contrary to popular belief, algae do not have cellulose as a component of their cell wall structure (Kuyucak and Volesky, 1990). They can be categorised as marine species, estuarine species, freshwater and subterrestrial plants. They can be further divided into unicellular microalgae or multicellular macroalgae. The earliest attempts at algal classification were based on their colour and three main groups were recognised. They are the green, brown and red species. Nevertheless, according to Kuyucak (1990), current systems recognise between four and nine divisions of the algal kingdom that have been organised according to the cell wall composition, differences in pigmentation, the type of metabolic and reserve products such as starch and lipids or the presence of motile cell features such as flagella and other morphological features.

2.2.1 The Structure of Algae

The cell interior of algae is made up of cytoplasmic materials such as nucleic acids, lipids, reserve products (storage products) and pigments. Reserve products, mainly in the form of glucose polymers may vary from species to species. Studies by Lee, (1980) revealed that alcohol mannitol and polysaccharide laminarin are typical for the brown algae; floridean starch is common in the red algae and amylose is associated with the green algae. Oil is found in the diatoms, the yellow-green and the golden-brown algae while tannins are stored in the cytoplasm of the brown algae.

In distinguishing principal classes of algae, pigments play a very important role (Kuyucak and Volesky, 1990). Almost all algae contain chlorophylls and a predominant accessory pigment. The nucleus represents a prominent feature in the algal cell except for the blue-green algae, also known as cyanobacteria which lack an organised nucleus, having their genetic material spread throughout the cell very much like a bacteria.

2.2.2 Algal Cell Wall

According to Hawker *et al.*, (1971) and Dodge (1973), the algal cell wall is similar to the fungal cell wall. It is protected by a true cell wall which is rigid as in bacterial cells. However, its chemical and structural characteristics different from bacterial cells. The algal cell wall is composed of a multilayered microfibrillar framework, consisting of cellulose and interspersed with amorphous material in a embedding matrix (Figueira *et al.*, 2000). Silica or carbonate sometimes encrusts the cell wall and more than ten layers can be found in certain algal cell walls, demonstrating their complexity. The algal cell wall layers are oriented either in parallel or they maybe structured randomly.

The cell wall constituent also plays an important role especially in metal uptake and binding. Substances such as algin, fucoidin, polygalactose-sulfate esters are found in the cell wall. Table 2.1 lists some of these cell wall constituents. The cell walls of brown algae contain alginic acid, fucoidin and cellulose while agar and carrageenan occur in the cell walls of red algae along with xylans, pectin and cellulose. In most green algae, the outer part of the cell wall consists mainly of pectic substances and of cellulose while golden-brown algae may bear silicified scales or calcareous bodies on their cell walls which are made up largely of pectic materials.

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

Group	Location	PKa
Carboxyl	Protein C - terminal	3.5 - 4
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
Sulfonic acid	Cysteic acid	1.3
Phosphate	Serine as ester	2.0, 6.8
Phosphate	Polyol mono ester	0.9 - 2.1
Phosphate	Polysaccharide diester	1.5, 6.0
Hydroxyl	Tyrosine-phenolic	9.5 - 10.5
Hydroxyl	Saccharide-alcoholic	12 - 13.0
Sulfydryl	Cysteine	8.3
Amino	Protein N - terminal	7.5 - 8.0
Amino	Cytidine	4.11
Amino	Adenosine	3.45
Amino	Lysine	8.9, 10.5
Imidazole	Histidine	6 - 7
Imidazole	Guanosine	2.3
Imino	Peptide	13

Source: Hunt (1986)

The cell walls of algae are also porous, allowing molecules and ions to pass through freely. It is also interesting to note that the cell membrane of most algae is permeable to neutral molecules but not to ions. Studies conducted by Hope *et al.* (1975) revealed that the living cell of *Valonia*, a giant alga, was permeable to radon but impermeable to lead ions to which the dead cell was permeable. Furthermore, their finding indicates that cell viability may change the behaviour of the cell in removing metallic species from solutions.

In addition to the porosity of the algal cell wall structure, the algal cell constituent can provide an array of chemical ligands, binding functional groups to take up metallic ions. Differences in the cell constituents and in the composition and structure of the cell wall of widely different algal species can serve as a basis for selectively sequestering many different metallic ions.

2.2.3 Algal Uptake of Copper

Copper is a nutrient necessary for algal growth (Kuyucak and Volesky, 1990), but in high concentrations it is used widely as an algicide. In seawater it is present both in a particular form and as organic complexes. The cupric ion (Cu^{2+}) is the most toxic metallic form to flora and fauna and the speciation of the metal in freshwaters is dependent on ligand concentrations and pH.

There exists some linear relationships between the concentration of one element (trace elements such as copper, manganese, zinc, lead, cobalt and chromium in seawater) to another in many species of seaweeds. A typical case was the iron to aluminium content

in seaweeds. These elements which enter the ocean as particles were more concentrated in seaweeds than in seawater but Yamamoto and Ishibashi (1971) demonstrated that the concentration factor, CF [CF = metal in dried seaweed (mg/L) per dissolved metal in seawater (mg/L)] in the same seaweed varies inversely with the increase of residence time in the seawater. This implies a functioning live mechanism because metal that is taken up is excreted from the algal cell in time.

An earlier work by Hassall (1963) compared the uptake of copper by living and scalded cells. Copper uptake by scalded cells was observed to be more rapid than the living cells although the final concentration of copper remains the same in both studies. Later studies by Foster (1973) and Hall (1981) indicated that living algae can become copper tolerant and it was noted that a non-tolerant strain can accumulate five to ten times more copper than a tolerant strain.

McKnight and Morel (1979) investigated 21 species of marine and freshwater algae and their study revealed that the removal of toxic metals from solution via chelation exhibits a strong correlation between algal growth rate and free Cu^{2+} ion in solution. Jackson and Morgan (1977) also arrived at the same conclusion in their theoretical work about chemical equilibrium of cationic and anionic species in seawater. More recent work indicated a competitive relationship between cupric ion activity and silicic acid concentration that affected growth, as well as silicic acid and copper uptake in the marine diatom, *Thalassiosira pseudonana* (Rueter and Morel, 1981).

Cloutier-Martha and Brown (1980) found that a concentration as low as 0.37 mM of HgCl_2 was sufficient to change the levels and distribution of intracellular copper and zinc in algae. They suggested that metallothionein, a low molecular weight protein, maybe responsible in the storage and/or binding of heavy metals in the marine algae *Skeletonema costatum*.

In freshwater, copper is found as Cu^{2+} in various products of hydrolysis, in CuCO_3 (aq) and in organic complexes (McKnight and Morrel, 1979). Algal cell matter broken up by freeze drying, chopping and/or pressure extrusion was used and the CF was determined at 3.3×10^3 for *Chlorella* (Ferguson and Bubela, 1974). Metals recovered from solution by biosorption may end up deposited in bottom sediments enriched in metals. In the presence of copper, the photosynthetic rate decreased even in short term experiments. Perhaps copper may have penetrated into the cells and disrupted other metabolic activities. The simultaneous shedding of organic materials indicates that copper loosens the cell membrane, at least in the diatom *Nitzschia palea* (Crist *et al.*, 1981).

Hassett *et al.* (1979, 1981) developed a microplate technique to determine the conditions under which pure cultures of algae removed heavy metals from aqueous solutions. Cultures of 20 different species of algae such as green, blue-greens and diatoms, ages 11 and 44 days were investigated for the uptake of arsenic, cadmium, copper, mercury, nickel, lead and zinc at different pHs and metal concentrations.

Their results indicated that metal removal was fast, less than 3 hours. Young cultures were more efficient in accumulating the metal than the older ones. pH had little effect

on the accumulation of metals and the highest removal of copper was attained with *Gleotrichia*.

2.3 Environmental Factors Governing Algal Biomass Production

For optimal growth, algae require water, the correct solute density, the maintenance of optimum pH and temperature, coupled with adequate amounts of minerals, carbon dioxide, aeration and sunlight (Soeder, 1986). All are important but each should be examined from the standpoint of the environmental interrelationships that permit optimal algal biomass production.

2.3.1 Temperature

Temperature effects on algal biomass growth and activity may be explained in terms of two primary factors:

- (1) The effect of temperature on the synthesis and structure of cell components such as genetic factors
- (2) The temperature coefficients of reaction rates which depend on the reaction activation energies. This is dependent on other chemical and physical factors

Also, in all probability, according to Pirt (1975) these primary factors give rise to many secondary effects which are basically physiological in nature. It is probably the secondary physiological effects which have the greatest impact on algal biomass production although, to some extent, this effect may be counteracted by special environmental conditions discussed below.

All algae species have a temperature range within which most cells will survive and within which an optimum temperature usually exists. Until the optimum temperature of a particular species is reached, the effect of temperature on growth is to provide an exponential increase in the biomass yield (Richmond, 1986). Conversely, at 10 to 25°C below the optimum growth temperature, the growth rate of most algal species approaches zero (Pirt, 1975) but temperature increase by as little as 2 to 3°C will result in a pronounced inhibitory effect on the growth of most algae (Payer *et al.*, 1980).

For most algae, temperature has its greatest effect on the dark respiratory metabolism. Thus, for some strains, high night time temperatures might lead to a decline in biomass production due to high respiratory-mediated utilization of stored food reserves. In general, however, strains of algae that grow or have been adapted to grow, at elevated temperatures will show a greater biomass productivity than those strain grown at lower temperatures (Richmond, 1986; Sorokin, 1959).

Also, in some cases, the overall nutritional status of the algae will affect the response of the cells to temperature. In addition, culture temperatures often have profound effects on chemical composition of the algal cells (Goldman, 1977; Sato and Murata, 1980). Results obtained by Verity (1981) have indicated that elevated temperatures, combined with certain types of chemical stress, tend to increase the biosorptive properties of certain freshwater microalgae.

2.3.2 Effects of Light

Light is the second major limiting environmental factor that affects the growth of algal biomass. If all other growth factors, including temperature is maintained at optimal condition, then the yearly yield of algal biomass should be determined solely by the diurnal and seasonal light flux (Richmond, 1986; Bedell, 1985).

An interrelationship exists between light and temperature. By increasing the growth temperature for algal biomass, saturation light intensity for photosynthesis will also increase. However, for practical purposes of growing algal biomass, the outdoor light problems are twofold:

- (1) Either there is not enough light due to geographical location or cloud cover
- (2) There is so much light under certain conditions that the light damages the algal cells.

Photoinhibition and photoinactivation cause an impairment of photosynthetic processes which causes decreased algal growth yield or sometimes death of the entire biomass (Richmond, 1986). Photoinhibition occurs because supraoptimal intensities of light cause a destruction of the photosynthetic pigments as a result of oxygen and light dependent bleaching of cells (Abeliovich, 1972; Powles, 1984).

Bedell (1990) also states that insufficient light usually causes a decrease in algal biomass yields due to lower photosynthetic rates, but this seldom causes cell damage unless the culture is so thick that some of the cells are never exposed to light. On the other hand, photooxidation and photoinhibition resulting from the high light intensities create far worse problems due to injured cells. Nonagitated algal cells at the surface of

a pond maybe exposed to very intense solar irradiation. Therefore, these surface algal cells are the ones most likely to be damaged by intense solar irradiation since the irradiation only penetrates a fraction of the pond profile. Thus, by regulating the pond depth, the population density and the rate of pond mixing, a useful means exists by which to modify the effects of these extreme solar fluctuations.

2.3.3 Aeration

Dense monocultures of algae that are undergoing very intense photosynthetic activity in the sunlight, under otherwise optimum conditions are capable of producing dissolved oxygen contents of over 35mg of O₂ per liter and up to 400% saturation (Richmond, 1983). Unless removed, such oxygen levels can become toxic to the algae by promoting photooxidation reactions. Therefore, vigorous aeration is needed to disperse such an oxygen build-up. Vigorous aeration also assists in the mixing of the suspension and helps to provide the 'proper' light to dark ratio for the given species. Vigorous aeration also assist in providing CO₂ to the algae.

2.3.4 Carbon Dioxide

All photosynthetic algae require CO₂. For optimal photosynthesis in most media, most algae require more CO₂ than is found in the air. The amount needed varies with the species grown. For example, for most blue-green algae (cyanobacteria), the optimal level is usually reached by aerating with approximately 1% CO₂ in air. For eukaryotic algae, it is 5%. Sechbach *et al.*, (1970) reported that the only algae able to grow in 100% CO₂ is the acidophilic red algae, *Cyanidium caldarium*.

2.3.5 pH

Each algal species has its own optimum pH requirement. Since pH has a marked effect on the dissociation of various salts and complexes (including trace metal compounds), for each alga a pH rise may lead to a trace element deficiency or a pH decrease may lead to growth inhibition or toxicity (Richmond, 1986). Thus, most algal species have been found to grow best when the pH is more neutral, somewhere between pH 5 and pH 8.

2.3.6 Mineral Nutrition and Salinity

According to Aaronson (1970), approximately 16 elements are required by all living organisms, some in major concentrations, others in trace quantities. Providing the mineral nutrition that is optimal for the growth of each algal species often requires some unique ratio of not only the preceding 16 elements but as many as 11 additional ones.

To compound the problem of algal mineral nutrition, all of the environmental factors discussed above plays a role in determining the optimum mineral elemental requirement needed by any given monoalgal species that is grown outdoor. In other words, physical conditions such as temperature, light, mineral nutrient and vitamins are factors that will influence the optimum mineral nutrition.

From the above, it can be seen that environmental interrelationships needs to be carefully examined. In recent years, it has become apparent that two or more limiting factors might be operating simultaneously to affect the growth rate of algae (Bader, 1978; Young and King, 1980; Zhou and Kiff, 1991). Therefore, most culture media for

algae differ significantly from what would be required naturally because the concentrations of all mineral nutrient elements required for an algal medium usually far exceed those levels required in nature by the same algae (Richmond, 1986).

For the most part, the algal biomass produced for use as biosorbents maybe grown in that medium that has been established as being appropriate for the particular algae desired. Nevertheless, defined media are expensive and contribute towards making the algal biomass product expensive. Since the production of biosorbent algal polymer is an industrial process, it is possible that non-human food (technical grade) production of algal biomass maybe sufficient for those algae grown for use as biosorbents.

According to Bedell (1990), research efforts now in progress indicate that certain variations in either the algal macro or micronutrients may have a profound impact on the production of both the types and the amounts of the different moieties on the algal cell walls that are involved in the biosorption of heavy metals. Although 'food grade' quality may not be needed, the practical application of these nutrient level studies maybe affected more by its ultimate effects on algal production costs and biomass yields than by percentage of increase in metal ion biosorption by the algae.

While some species of algae can tolerate only milimolar amounts of salt, algae as a group exhibit an extremely wide range of tolerance to various salts in their surroundings, some surviving in saturated brine (Richmond, 1986). This observation implies that many algae may be adapted and acclimatised to various levels of salt or types of salts.

Regarding adaptation to sodium chloride or table salt, the algae maybe divided into two general groups, the halotolerant and the halophilic. The former have physiological response mechanisms that permits their existence in a certain saline medium while the latter requires high salt levels in their medium for growth (Richmond, 1986). Salt adaptation is being investigated not only with the purpose of modifying the biosorptive cell wall moieties on the algae, but to make the most desirable species less subject to contamination when grown outdoors.

2.3.7 Maintenance of Monoalgal Cultures Outdoors

According to Bedell (1990), most contaminating species of any type cannot compete very well when placed into an environment that is maintained optimally for a specific and desired species. This is true when the optimal conditions for a desired species are beyond the ordinary. Conversely, moderate temperatures, neutral pH and low salinity generally tend to promote the growth of a wide variety of organisms (De Pauw *et al.*, 1984). Thus, optimum growth conditions maintained for a given species favour its rapid growth at the expense of other and usually competing species.

2.4 Factors Affecting Biosorption

There are many factors that influence both the rate and magnitude of biosorption, underscoring the difficulty in developing predictive models that would apply to all complex heavy metal wastewater. The reason for the variations in metal binding among algae depends on the fact that different species of algae have different cell wall compositions (Bold *et al.*, 1980). Hunt (1986) pointed out that different cell wall compositions would result in different metal adsorptive moieties being present on the cell walls, which are discussed below.

2.4.1 pH Dependence

According to Bedell and Darnall (1990), metal ion biosorption by specific algae is dependent upon pH, on the presence of competing ions. Temperature and pH coupled with the presence of competing ions has the greatest influence on the biosorption of algae if temperature is held constant at 25°C. A study conducted by Darnall *et al.* (1986) and Greene *et al.* (1987) on the effects of pH on the biosorption of dried *Chlorella vulgaris* can be categorized into three major groups (Greene and Darnall, 1990):

- 1) One group of metal ions that bound to *C. vulgaris* rather independently of pH values between 2 and 7 included Hg(II), Au(III), AuCl₄⁻, Ag(I), Pd(II) and Au(I)thiomalate. According to the general coordination chemistry of metal ions, this behaviour is consistent, with the binding being more covalent in nature rather than ionic. Gold (Au), silver (Ag) and palladium (Pd) are classified as soft according to Pearson (1973). Soft metal ions are capable of undergoing covalent binding to softer ligands, such as amine and sulfhydryl groups. The binding interaction between soft

(polarizable) metal ions and soft ligands generally are affected minimally by ionic interactions and pH in contrast to interactions between hard(nonpolarizable) metal ions and hard ligands.

- 2) A second group of metal ions was found to bind more strongly to the dried *C. vulgaris* as the pH increased from 2 to 5. This group of metal ions consisted of those ions intermediate between "soft" and "hard" metal cations, including Cu(II), Ni(II), Zn(II), Co(II), Pb(II), Cr(II), Cd(II), U(VI), Co(III), and the hard metal ions including Be(II) and Al(III). The biosorbent pH-dependent behaviour resembled the binding of these metal ions to those cation exchange resins that contained primarily amine or carboxylate groups. When the active metal binding sites, such as amine or carboxylate groups also binds protons, a pH dependence of metal cation binding generally occurs. This suggests that metal ions and protons compete for the same binding sites.
- 3) The third group of metal ions was found to bind more strongly to *C. vulgaris* at pH 2 than 5. Included in this group were mainly the oxoanions MoO_4^{2-} , SeO_4^{2-} and CrO_4^{2-} , plus other anionic metal complexes, including $\text{Au}(\text{CN})_2^-$ and PtCl_4^{2-} . At low pH, the increased binding of these metal ions was consistent with electrostatic binding to positively charged groups such as amines or imidazoles. Anions would be expected to interact more strongly with the cells as the concentration of positive charges increases due to protonation of groups at low pH values. Also, as the pH is decreased below the isoelectric pH, the overall net charge on the cell wall promotes an easier access of anions to positively charged binding sites. In support of this concept, the isoelectric point for many algal species has been determined to lie between 3 and 4 (Crist *et al.*, 1981).

2.4.2 Metal Ion Competition

In addition to pH, at a constant temperature, the presence of competing ions also plays a very large role in metal ion biosorption by algae. Metal ions normally found in most hard waters do not compete strongly for the algal sites which bind transition metal ions. Thus, the one important advantage that algae cells have over commercial ion-exchange resins is that they bind both Ca(II) and Mg(II) only very weakly. This is noted in *C. pyrenoidosa*, *C. vulgaris*, *Cyanidium caldarium* and *Spirulina platensis* (Greene, 1987). This fact is important because attempts to use commercial ion-exchange resins for the removal of heavy metal ions from hard waters have shown that Ca(II) or Mg(II) may saturate the resins and interfere with the binding of the heavy metal ions.

During most algal biosorption, metal ions often compete with each other for algal surface binding sites (Nakajima *et al.*, 1982; Crist *et al.*, 1981). For example, Greene (1987) found that *C. vulgaris* was sequentially exposed to a solution containing an equimolar concentration mixture of nine metal ions at pH 5. His results showed the following order of selectivity in the biosorption of the nine different metal ions:

$$\text{Al (III)} = \text{Ag (I)} \gg \text{Cu (II)} > \text{Cd (II)} \geq \text{Ni (II)} \geq \text{Pb (II)} > \text{Zn (II)} = \text{Co (III)} \geq \text{Cr (III)}$$

The binding of Ag (I) and Al (III) in the experiment described above was essentially unaffected by the presence of all other metal ions. In contrast, the binding of the other metal ions was decreased in multi component metal ion mixtures. One explanation is that there are distinct classes of binding sites on the algae, which have a preference for the binding of either very hard or very soft metal ions. The hardest metal ion in the medium was Al (III) and the softest was Ag (I). The others were borderline soft or

hard. Therefore, it would be expected that Ag (I) and Al (III) should not compete with each other since they would be expected to bind soft and hard sites while the others would due to their similarities in co-ordination chemistry (Greene and Darnall, 1990).

Hg (II), like the Ag (I) ion, bound to *C. vulgaris* rather independently of pH in the 2 to 5 range. It is predicted that since Hg (II) is also a soft metal, competition for binding sites would be expected. By exposing the algal cells repeatedly to equal volumes of fresh 0.1mM solutions of either Ag (I) or Hg (II) at pH 5 or to a solution containing equimolar concentration of the metal ions, it was demonstrated that the algal cells have a higher binding capacity for Hg (II) than Ag (I) and that the presence of Hg (II) drastically inhibited the binding of Ag (I) to the alga (Greene, 1990). These results indicate that while Hg (II) is the more strongly bound of the two ions, both Hg (II) and Ag(I) compete for binding sites on *C. vulgaris*. Other experiments have shown that the addition of Hg (II) to algae results in the displacement of Ag (I) from the binding sites.

2.4.3 Temperature

Temperature changes will affect a number of factors, which are important in metal ion biosorption. Some of these factors include:

- 1) The stability of the metal ion species initially placed in solution
- 2) The presence of competing ligands
- 3) The autoprotolysis constant of water
- 4) The stability of the algal-metal complex (depending on the biosorption sites)
- 5) The effect of temperature on the algal cell wall configuration
- 6) The ionisation of chemical moieties on the cell wall

Uranium biosorption by polyacrylamide immobilized *C. regularis* was determined to be an overall endothermic process since increased binding occurred as the temperature was increased (Darnall *et al.*, 1986). The binding of Cu (II), Zn (II), Cd (II), Cr (III), Pb (II) and Au (III) to both free and immobilised, nonviable *C. pyrenoidosa* and *S. platensis* was endothermic (Greene *et al.*, 1990). Ni (II) binding to these algae had an enthalpy nearly zero. Binding capacities for Cu (II) and Au (III) in columns containing silica-immobilized *C. pyrenoidosa* increased by nearly an order of magnitude as the temperature changed from 0 to 60°C (Greene, 1990).

Later work by Greene (1991) utilising silica immobilized *C. pyrenoidosa* to examine Cu (II) binding in a batch reactor system, suggest that the temperature dependence of Cu (II) binding was not due to irreversible changes in the morphology or chemical composition of the algal cells but was a direct result of net endothermic binding interactions. Generally, the formation of co-ordination complexes between transitional

metal cations and carboxylate ligands is endothermic, whereas amine ligands complex formation is exothermic. The observed endothermic nature of the of the algal biosorption of Cu (II) is consistent with metal carboxylate interactions. However, a binding site provided by a combination of amine and carboxylate ligands with the overall enthalpy dominated by the carboxylate ligand cannot be ruled out. Cr (VI) binding to silica immobilized *C. vulgaris* showed an increased reduction to Cr (III) as the temperature was increased from 25 to 55⁰C. Since the conversion of Cr (VI) to the less toxic Cr (III) is a useful detoxification procedure, this result may have practical significance.

2.5 Reactor Configuration

Most metal ion sequestering processes using nonviable algae usually involve either a batch or a column configuration. Batch configuration systems involve the mixing of free or immobilized nonliving algal cells with the metal ion solution, and following an equilibrium time, the cells are removed from the metal ion solution by settling, filtration or centrifugation. Column configuration systems permit the metal ion solution to be pumped through a column packed with immobilized algal cells. For reasons that are similar to those used to describe ion exchange equilibria, algal reactors employing a column configuration offer greater metal binding capacity and higher purity and are more readily adapted to automation and continuous flow than batch reactors (Bedell and Darnall, 1990; Volesky, 1990).

Free algal cells are not suited for use as a column packing since the cells tend to clump together and excessive hydrostatic pressures are required in order to generate suitable flow rates. Furthermore, since the algal cells are inherently fragile, high pressures may cause a disintegration of the free biomass. The fragility problem has been alleviated by the use of algae immobilized in a suitable porous matrix. Polyacrylamide, calcium alginate and silica are among the porous matrices that have been used to immobilize nonviable algae and to permit their use in packed columns for metal ion recovery (Tan, 1999; Aksu *et al.*, 1998; Greene, 1990; Darnall *et al.*, 1986; Greene *et al.*, 1987; Nakajima *et al.*, 1982).

2.5.1 Fixed Bed Reactor (FBR) for Biosorption

As mentioned earlier, column configuration offers more advantages than batch systems. For fixed bed reactors, there are several modes of flow. The fundamental types are down-flow and up-flow units with either a series or parallel arrangement as depicted in Figure 2.1. In the former, wastewater laden with heavy metals are allowed to trickle down the bed packed with biosorbents under the influence of gravity while the latter is influenced by pressure.

The usage of down-flow fixed bed reactors to remove wastewater containing heavy metals by biosorbents are well documented (Brady *et al.*, 1999; Aksu and Kutsal, 1998; Zhou *et al.*, 1998; Hu and Reeves, 1997; Spinti *et al.*, 1995; Wong *et al.*, 1992; Zhou and Kiff, 1991). Zhou *et al.* (1998) reported that adsorption with *Sargassum kjellmanianum*, a macroalga showed undiminished performance when applied to a fixed bed reactor in adsorption and desorption experiments. Similar results were reported by

Kratochvil *et al.* (1995) using both protonated and native biomass of *Sargassum fluitans* to remove copper in a fixed bed reactor.

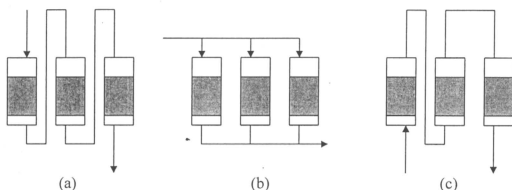


Figure 2.1: Modes of operation: (a) Down-flow in series fixed bed; (b) Down-flow in parallel fixed bed; (c) Up-flow in series expanded bed.

Source: Montgomery (1989)

Nevertheless, the application of biomass as biosorbents in its native form is rather limited. This is because native biomass exhibits very poor flow characteristics, often resulting in pressure drop and head loss when used in a fixed bed reactor. It also has a tendency to agglomerate, hence recovery of adsorbed heavy metal waste from the biomass will be difficult. Therefore, native biomass are immobilised to increase its mechanical strength, density and resistance to chemical and unfavourable physical conditions that often appear in a typical wastewater treatment facility.

2.6 Immobilisation of Algae

Biosorbent materials suffer from serious limitations with respect to their physical and chemical properties. Such limitations can restrict their range of application. Low molecular weight soluble metal adsorbents cannot be used other than as metal sequestrants in solutions unless they are first chemically modified. Although they

adsorb or complex metals, they cannot be readily recovered with their contained metal (Bauer *et al.*, 1996; Wu and Wisecarver, 1991;). Other materials, such as cell walls or whole microbial cells, suffer from outright chemical liability or greatly restricted use due to their physical properties and there are difficulties associated with the dispensing and retrieval of metal adsorbing biomass.

The general procedures for the chemical modification and immobilization of biosorbents such as whole cells, microbial polymers and low molecular weight organic molecules are analogous to those applied to the immobilization of enzymes. The principle advantages of employing such procedures are summarised in Table 2.2.

Table 2.2: Advantages for chemical modification (immobilization) of biosorbent materials

- 1) To permit recovery of a metal-laden adsorbent, e.g., for low molecular weight adsorbents
- 2) To permit biosorbent usage in a fixed or fluidized bed reactors, e.g., to overcome hydraulic deficiencies of microbial cells when used in columns
- 3) To enhance chemical and physical stability of biosorbents, e.g., extend the useful life of a biosorbent by lowering attrition process
- 4) To improve metal adsorption and desorption characteristics of a biosorbent, e.g., cross-linking of polymers to create rigid pores which promote better metal loading and elution
- 5) To alter or extend the range of metal selectivity of a biosorbent, e.g., electrophilic group addition to phenolic-type ligands to help prevent oxidation by adsorbed metal.

Source: Holbein (1990)

2.6.1 Categories of Immobilisation Procedures

The principle types of immobilisation procedures as can be applied to metal adsorbents are shown diagrammatically in Figure 2.2. Holbein (1990) depicted a component biosorbent or ligand as a "tuning fork" since it has utility in describing certain principles of immobilisation and activities of biosorbents with respect to metal adsorption. To be generally useful, a metal adsorbent should have specific metal-adsorbing sites, that is, the active portion (the fork) which allows metal adsorption either specifically or non specifically, but in a reversible manner. That is to say, adsorbed metal as adsorbed under certain conditions of use such as pH and temperature should be releasable for recovery of metal and adsorbent for reuse under other elution conditions. This principle is important with respect to the method for immobilization as chemical modification at the binding site can destroy the metal binding activity or create interference by steric hindrance. The shaft of the tuning fork is depicted as representing a portion of the adsorbent away from the metal-binding site and to which chemical modifications can be effected without impairment of the metal-binding activity of the adsorbent.

Of the various immobilization approaches shown in Figure 2.2, adsorption, entrapment and cross-linking tend to be more suitable for high molecular weight adsorbents, namely whole cells or macromolecular cell components. These structures have either sufficient size or the presence of sufficient non-sensitive sites to allow immobilization by these procedures.

The immobilization of low molecular weight metal binding adsorbents represents a special category as these require association with carrier materials to provide finished

adsorbents of sufficient size and structural integrity for actual use. Due to the limited possibilities for stable adsorption, entrapment or cross-linking, covalent attachment of low molecular weight adsorbents to carrier materials represents the only effective means for their immobilization. Nevertheless, there is a multitude of carrier materials and chemistry for covalent attachment of a wide variety of different organic molecules available. This would enable the selection of appropriate carrier characteristics, length of attachment linkages, adsorbent loading density and provides reproducible systems for immobilisation which effectively scale-up for commercial scale synthesis of finished biosorbents.

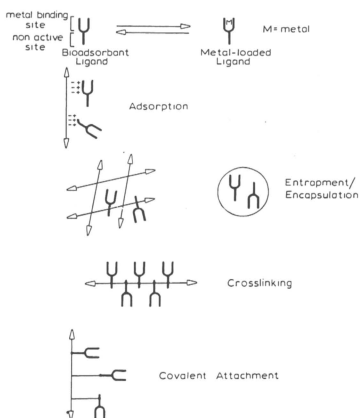


Figure 2.2: Categories of immobilisation procedures
Source: Holbein (1990)

2.6.2 Carrier Selection

The range of carrier materials that can be used for the immobilization of metal ligands are shown in Table 2.3. The selection of an appropriate carrier for use in preparing an immobilised metal adsorbing ligand relies on a number of factors. These are summarised in Table 2.4.

Table 2.3: Carrier materials suitable for immobilization of metal adsorbing ligands

Class	Type	Examples
Organic	Natural and modified natural	Dextran, cellulose, glucan, agar, alginate, cellulose acetate, carbon
	Synthetic	Polyacrylamide, polyester, nylon, polypropylene, polystyrene, polyfluoroolefin
Inorganic	Silicates	Controlled pore glass silica gel
	Aluminosilicates	Zeolites (natural and synthetic)

Source: Holbein (1990)

According to Holbein (1990), surface areas of various carriers cover a wide range with some synthetic resins having surface areas approximately $30\text{m}^2/\text{g}$, while some controlled pore glasses have surface areas of over $700\text{m}^2/\text{g}$. Pore sizes and total pore volumes also vary widely with different carriers. Some controlled pore glasses have mean pore diameters of approximately 20\AA with pore volumes of 0.43 mL/g , whereas some macroporous polystyrene resins have pore diameters of approximately 1000\AA and pore volumes of greater than 1 mL/g of material.

Table 2.4: Factors determining the selection of a carrier material for the immobilisation of a metal adsorbing ligand

Factors	Importance
Surface area	Area available for immobilisation
Effective pore size and pore volume	Ease of utilizing surface during preparation and final use
Particle size	Impacts on hydraulic characteristics in columns
Hydrolytic stability	Utility at high or low pH values
Swellability	Important when using columns
Abrasion resistance	Important in fluidized beds
Density and sedimentation rate	Important in fluidized beds
Ease of functionalization	Number of steps, chemicals, time, etc.
Cost	Of great commercial importance

Source: Holbein (1990)

Synthetic resin carrier materials such as macroporous polystyrene have better hydrolytic stability than do controlled pore glasses, the latter being quite sensitive to alkaline hydrolysis even when the surface is silanated for functionalization (Atkinson and Mavituna, 1983; Buchholz, 1979). Synthetic resin materials and natural organic polymers are however more prone to swelling and shrinking as a function of pH changes during use. The use of an adsorbent or biosorbent in fixed bed columns demands that attention be paid to swellability of the carrier material.

Attrition resistance becomes an important issue should the immobilized adsorbent be utilized in a fluidized bed with clear liquors (autogenous attrition) and especially if abrasive solids (exogenous attrition) are present in the material to be treated. Generally,

the best abrasion resistance is obtained with macroporous polystyrene carriers followed by controlled pore glasses, with the poorest attrition resistance being found with gel-type synthetic resins and natural polymers such as cellulose and dextran.

2.7 The Breakthrough Curve

The most important factor in treating heavy metal waste using a column configuration for either adsorption or ion exchange systems is the characterisation of the effluent profile as a function of throughput that is either volume or time. This profile, commonly referred to as the breakthrough curve, represents the specific combination of equilibrium and rate factors that control process performance in a particular application (Weber and Thaler, 1983).

According to Weber (1996, 1972) the breakthrough curve maybe visualised as the trace generated by a hypothetical mass transfer zone (MTZ) which is established and moves through the bed as a function of mass loading and eventual exhaustion of the adsorbent or biosorbent. The effluent breakthrough curve is the concentration /throughput pattern or profile corresponding to the bed discharge, the breakpoint is that point where the effluent concentration no longer meets the treatment objectives, and the point of practical saturation is that where the adsorption or exchange material is ready for regeneration and beyond which such regeneration is more difficult or less efficient.

This conceptualisation is useful for defining the boundaries of that region of the reactor in which the majority of removal occurs at any given time, a zone which moves through the bed at a given velocity and length for a particular application (Michaels, 1952). For

a given set of operating conditions, the velocity of the MTZ correlates the breakpoint with bed depth since the breakpoint occurs when the effective zone begins to exit the bed while the length of the overall zone represents the minimum contact period required to reach the treatment objectives since the objectives are met at the end of the zone. Figure 2.3 illustrates these points.

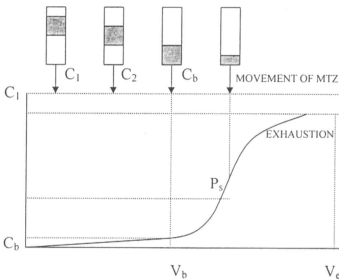


Figure 2.3: Typical breakthrough curve showing movement of MTZ
Source: Metcalf and Eddy (1992)

A breakthrough profile generally exhibits an S - curve characteristic, the actual position and shape of which depends on several factors. These generally include the physical and chemical properties of both the adsorbate and adsorbent, the influent concentration of the adsorbate, solution pH, particular rate limiting mechanism, nature of the equilibrium conditions, depth of column and velocity of flow. The relative effects of these factors are highly specific to a particular application, which constitutes one of the major difficulties for the design and scale-up of such processes.

2.8 Modelling Breakthrough Curve

The first step in developing a mathematical model for description or prediction of the dynamics of adsorption or ion exchange is to provide a reasonable representation of the associated rate phenomena. In general, the overall rate for adsorptive removal of a solute from solution by a porous adsorbent, biosorbent or exchange resin is controlled by a resistance to mass transport rather than by a phase change reaction. Figure 2.4(a) is a schematic representation of a porous adsorbent or exchange particle comprised of macropores and micropores surfaces and surrounded by a hydrodynamic boundary layer or film.

The macropores are defined as those pores large enough that diffusion rates are unhindered by the pore walls, while the micropores are those with radii or size comparable to the diffusing species so that diffusion rates are hindered by the pore walls (Weber and Thaler, 1983). This conceptual view translates into the series resistance rate model shown in Figure 2.4(b). The steps providing the greatest resistance to mass transport are assumed to control the overall rate of solute removal from solution.

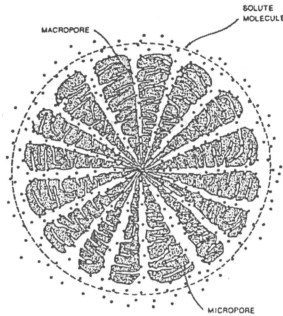


Figure 2.4(a): Schematic representation of a porous adsorbent or ion exchange particle
Source: Weber and Thaler (1983)

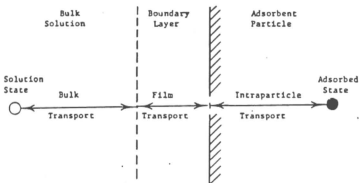


Figure 2.4(b): The series resistance rate model for adsorption and ion exchange
Source: Weber and Thaler (1983)

A second important aspect of any dynamic model is the thermodynamic principle of conservation of mass. Implementation of this principle results in a material balance for each component of interest within a particular control volume, that is for substance i in a reactor of volume V :

$$[\text{Net rate of accumulation}] = [\text{Flux In}] - [\text{Flux Out}] + [\text{Net Rate of Generation}] \quad (2.1a)$$

$$[\text{Net rate of depletion}] = [\text{Flux In}] - [\text{Flux Out}] - [\text{Removal by Reaction}] \quad (2.1b)$$

The above equation assumes that the component flux entering or leaving the control volume can include that of bulk flow, dispersive flow or molecular diffusion.

The normal procedure for applying the continuity principle is to consider a small but finite control volume that is representative of the overall systems. After the appropriate relationships are developed to describe the localized mass balance, the control volume is assumed to become infinitesimally small so as to generate the continuity relations on a differential scale. Since the usable form of the dynamic model requires integration of the differential equations, boundary conditions and initial conditions are also required to specify the constants of integration. Thus, the complete dynamic mathematical model must consist of partial differential equations for continuity of each component in each phase, as well as the specific boundary and initial conditions corresponding to the particular application. A solution method to provide the required integration is also needed.

Apart from models that require numerical analysis to transform a set of partial differential equations to ordinary differential equations, a two parameter model which has an analytical solution (Belter *et al.*, 1988) was also used to predict theoretical patterns of packed column. This model was used by Brady *et al.* (1999) to predict the breakthrough pattern of immobilized *Rhizopus arrhizus* and *Mucor miehei* in a fixed bed reactor. The model was tested on untried operating conditions and it was reported that the agreement between experimental and theoretical data were favourable.

The major advantage of the Two Parameter model lies in its simplicity. Unlike other complicated models that require numerical analysis, the Two Parameter model has a analytical solution which means that error arising from numerical analysis such as truncation errors and round off errors are not encountered.