Fundamental Concepts and Mechanisms in the Metal Biosorption Technology for the Treatment of Industrial Wastewaters

Asma Saeed*, Asia Aslam and Muhammed Iqbal

Environment Biotechnology Group, Biotechnology and Food Research Centre, PCSIR Laboratories Complex,
Lahore-54600, Pakistan

Abstract. The review deals with the need to treat industrial effluents before their discharge into open water bodies. The technical and economical problems associated with conventional water treatment procedures have been pointed out. Biosorption, as an alternative technology for metal remediation of wastewaters is discussed. A detailed description of the theoretical basis, fundamental concepts and mechanisms involved in the adsorption of metals has been given. The validity of biosorption data as determined by the fit on the mathematical models of Freundlich and Langmuir adsorption isotherms has been described. The ion exchange mechanism, its differences with the Langmuir adsorption isotherms mechanism, and the advantages of one mechanism over the other in understanding the concepts of the biosorption process have been considered.

Keywords: toxic metal remediation, effluent treatment, metal biosorption, Langmuir/Freundlich adsorption isotherms, ion exchange, biosorption technology

The ecological impact of heavy metals. Enhanced industrial activity during recent decades has led to the discharge of unprecedented volumes of wastewaters. As a result, environment is being heavily polluted with pesticides, fertilizers, metals, and miscellaneous toxic materials (Travieso *et al*., 1999). Of the various activities associated with environmental pollution, mining operations, ore-processing and smelting, urbanization, metal-plating, tanneries, and agriculture-related pursuits are directly related to metal pollution (Pagnanelli *et al*., 2002; Puranik and Paknikar, 1997). Unlike most organic wastes and the microbial load in aquatic bodies, furthermore, metal contaminants are not biodegradable, tending to accumulate in living organisms, becoming a permanent burden on the ecosystem (Bailey *et al*., 1999). Their presence in the environment, even at low concentrations, has therefore the potential of becoming a cause of toxicity to humans and other forms of life (Gupta *et al*., 2001; Volesky, 2001). Industrial effluents and drinking water loaded with metals is thus a serious public health problem (Chiron *et al*., 2003).

Although several metals have a physiological role as prosthetic groups in metalloproteins, and as constituents of enzymes, coenzymes and cofactors in metabolic pathways (Lehninger *et al*., 1993), their presence in excessive amounts in the food chain leads to toxicity symptoms, to disorders in the cellular functions, and eventually death (Volesky, 1990). Overabundance of the essential trace elements and particularly their substitution by non-essential ones, such as Cd, Ni, Ag, can

also cause toxicity symptoms. Furthermore, when a metal with no known biological function competes with, or replaces a functional metal, toxicity results (Hughes and Poole, 1989).

In addition to the direct entry of toxic metals into the human body, such as through inhalation of metallic dust or ingestion of food contacted with metalware, food chain in the ecosystem is the major indirect contributor. Metals assimilated by microbes and plants, tend to accumulate as they move through the food chain (Yetis *et al*., 1998; Matheickal and Yu, 1996), which leads to food chain enrichment in metal- polluted environments. The levels of certain metals are frequently higher in older individuals than in juveniles, which is in accordance with the general principle of bioaccumulation. From the ecotoxicological point of view, metals such as Cd, Hg, and Pb, the so-called family of 'big three', are highly toxic and are included in the 'Red List' of priority pollutants published by the Department of the Environment, UK, and in 'List I' of EEC Dangerous Substances Directive (Volesky and Schiewer, 1999; Aderhold *et al*., 1996). The same directive lists Cu, Ni, Sn and Cr in 'List II', which contains pollutants of less toxicity. These metals, nevertheless, create environmental pollution problems since, although not necessarily toxic at low concentrations, they accumulate in the food chain and upon entry into human body are not excreted. Therefore, from the environmental and human safety points of view, it is of the utmost importance that metal contaminants are removed from industrial wastewaters before their release into natural water bodies or open landscapes. The environmental impact due to their toxicity has led to the enforcement of strin-

*Author for correspondence; E-mail: asmadr@wol.net.pk

gent standards for the maximum allowable limits of their discharge by different countries. Authorities enforcing these standards further require the treatment procedures to be environment friendly (Aderhold *et al*., 1996).

Conventional procedures for the removal of metals from wastewaters. Technologies for the treatment of metalliferous wastewaters principally aim at removing the metals to a minimum level of concentration that meets the legislative conditions of their safe discharge. These technologies are operated under the so-called 'best practicable environmental options' governed by such factors as safety of operation, cost-effectiveness, reliability, least generation of secondary/end-product wastes, flexibility of operation under variable conditions of pH, temperature, flow-rate and volume, and environmental friendly disposal of the recovered metal wastes. There is no single process that would satisfy all or most of these desirables.

Removal of metals from wastewaters has been conventionally done by several physical and chemical procedures (Pagnanelli *et al*., 2001), which include chemical precipitation, coagulation, reduction, osmosis and reverse osmosis, membrane separation technologies (ultrafiltration and electrodialysis), evaporative recovery, ion exchange, lime precipitation, solvent extraction, electrode dialysis, and electrolysis (Chong *et al*., 2000; Aksu *et al*., 1998; Yetis *et al*., 1998; Kapoor and Viraraghavan, 1995; Wilde and Benemann, 1993). However, their applications are often restricted because of practicability techno-economic considerations (Jansson-Charrier *et al*., 1995).

One of the conventional procedures of metals removal from industrial wastewaters involves their chemical precipitation, usually by lime, followed by settling of the metal precipitates and coagulation in a pond and/or clarifier (Kratochvil and Volesky, 1998). Lime precipitation, when used for the reduction of metal ions to very low concentrations, is either inefficient or very expensive (Wilde and Benemann, 1993). Precipitation processes, furthermore, have other disadvantages, such as the generation of chemical sludge (Krishnan *et al*., 1992). Disposal and handling of the resultant toxic sludge is not only expensive but also creates other environmental problems.

Technologies alternative to chemical precipitation and coagulation include reverse osmosis, electrodialysis, ion exchange, and passive treatment using engineered wetlands (Butter *et al*., 1998). These methods primarily result in the transformation of the dissolved metals into a more concentrated and manageable form prior to final disposal, usually to landfills. However, metals removed by these procedures continue to

remain in the ecosystem representing a further long-term environmental hazard. The usual non-resistance of resins to thermal and osmotic shock, deleterious effects of their oxidation by chemicals, and interference in metal removal in the presence of Ca and Mg ions are the principal disadvantages encountered during the ion exchange procedure (Aderhold *et al*., 1996; Kuyucak, 1990). Ion exchange resins, additionally, are not always selective enough to allow an effective recovery of metals present in industrial effluents (Kratochvil *et al*., 1997). Application of the ion exchange process is also rather expensive due to the cost of synthetic ion exchange resins. Electrolysis, being energy intensive, is a cost prohibitive technology (Atkinson *et al*., 1998). The membrane process tends to be hindered by the problems of limited flow-rates, instability of the membrane in salt and acid conditions, and fouling by inorganic and organic species (Aderhold *et al*., 1996). High operational costs, process complexity and low removal efficiency of membrane processes have limited their use in metal removal (Kapoor and Viraraghavan, 1995). Other methods, such as osmosis and reverse osmosis, dialysis and electrodialysis, and evaporation, are used in very specialized applications having prohibitive operational costs (Aderhold *et al*., 1996). Due to economics of dealing with large volumes of liquids and solvent losses, solvent extraction is limited to streams containing more than 1 g/l of the targetted metal (Kratochvil *et al*., 1997).

It may be noted that all the conventional metal treatment procedures are cost-prohibitive, often inefficient when used for the removal of metals to low concentrations, complicated in operation for practical applications, and may create yet another problem of the disposal of chemical sludge/solid waste containing the removed metals causing damage to groundwater reservoirs (Aksu *et al.,* 1998; Sandau *et al*., 1996). These concerns have led to an interest in the development of alternate effective technologies that have the capacity to reduce heavy metal concentrations to environmentally acceptable levels at affordable costs (Aksu *et al*., 1998; Atkinson *et al*., 1998).

Biosorption as the alternative metal remediation technology. The practical limitations associated with the conventional methods have led to the search of alternative water treatment procedures. Innovative methods of metal removal including biosorption (Volesky, 1990), sorption onto purified biopolymers (Jang *et al*., 1995), adsorptive filtration using coated sands (Benjamin *et al*., 1996), and adsorption on magnetic iron oxides (Chen *et al*., 1991), have been investigated in an ongoing effort to efficiently and economically remove metals contained in industrial wastewaters. Increased attention, however, is being paid to biological methods for metal remediation, termed

biosorption or bioaccumulation. Several biological materials have been extensively investigated for their ability to sequester metals through the process of biosorption, as an alternative technology (Pagnanelli *et al.*, 2003). Included in the range of biological materials used for this purpose are marine macroalgae (seaweeds) and freshwater algae (Akhtar *et al.*, 2003; Tien, 2002; Chong *et al.*, 2000; Davis *et al.*, 2000; Yu *et al.*, 1999), fungi (Baldrian, 2003; Veglio' and Beolchini, 1997), bacteria and cyanobacteria (Karana *et al.*, 1999), and miscellaneous categories of bioindustrial and agricultural wastes (Saeed and Iqbal, 2003; Iqbal and Saeed, 2002; Saeed *et al.*, 2002; Bailey *et al.*, 1999). Since most of the investigated biological materials have shown the ability to effectively remove metals from aqueous solutions and are either wastes or can be easily obtained, the biosorption procedure has been suggested to be cost-effective and a more efficient alternative to the existing effluent treatment techniques (Saeed *et al.*, 2005a, 2005b; Saeed and Iqbal, 2003; Schiewer and Volesky, 2000; Vieira and Volesky, 2000). Biosorptive removal of toxic metals is especially suited as a 'polishing' water-treatment step, after the application of conventional procedures, because it is possible to bring down their initial concentrations of 1 - 100 mg/l to the final equilibrium concentration of < 0.01 - 0.1 mg/l (Volesky and Schiewer, 1999).

Biosorption may be simply defined as the removal of metals or metalloid species, compounds and particulates from aqueous solutions by biological materials (Gadd, 1990). Virtually all biological materials, both living and dead, accumulate metal ions from aqueous solutions. A distinction, however, needs to be made between the two types of processes, namely, biosorption and bioaccumulation, when dealing with the removal of metals by biomass. The expression, 'metal biosorption', typically applies to the passive accumulation of metals by biological materials, which is not a metabolically mediated process (Schiewer and Volesky, 2000). 'Bioaccumulation', on the other hand, refers to a metabolically active process. Living microorganisms are thus capable of accumulating metal ions by two well-defined processes, namely, biosorption as an energy independent binding of metal ions to cell wall, and bioaccumulation as an energy-dependent process of metal uptake into cells (Karana *et al.*, 1999). Biosorption is considered to be better than chemical precipitation in terms of the ability to adjust to changes in pH and metal concentrations and is better than ion exchange and reverse osmosis in terms of no sensitivity to the presence of suspended solids, organic materials and the presence of other metals (Wilde and Benemann, 1993). Some comparative advantages of metal biosorption over the conventional procedures are (Aksu, 1998; Kratochvil and Volesky, 1998):

- mosty biomaterials used for metal biosorption are cheap and/or freely available
- high efficiency of metal removal from diluted aqueous solutions
- regeneration of the biosorbent biomass for repeated use
- ability to treat large volumes of wastewaters due to the rapidity of operation kinetics
- high selectivity in terms of removal and recovery of specific metals
- ability to handle multimetals and mixed waste effluents
- high efficiency to reduce residual metals to levels below 1 ppb in many cases
- less need for additional costly process reagents and supplementary procedures, which typically cause disposal and space problems
- operation over a wide range of physicochemical conditions, such as pH, temperature, and presence of other ions, including the commonly present alkali metal ions (Ca^{+2}, Na^+ and Mg^{+2})
- relatively low capital investment and low operational costs
- greatly improved recovery of bound heavy metals from the biomass
- substantial reduction in the large volumes of hazardous wastes

Mechanisms involved in biosorption. Biosorption has been attributed to a number of different sequestering mechanisms, such as microprecipitation, ion exchange, adsorption, complexation, coordination, or chelation (Volesky and Schiewer, 1999; Greene and Darnall, 1990). However, a combination of several mechanisms, each functioning independently, contribute to the overall metal uptake.

(a) Microprecipitation. Metal removal by precipitation may be either dependant or independent of cell metabolism. In living microbial cells, this process frequently appears to be an active self-defence mechanism against the adverse effects of toxic metals (Veglio' and Beolchini, 1997). Microprecipitation is the deposition of electrically neutral materials (metal or metal salt) at the surface of the biomass, and does not necessarily involve a bond between the biomass and the deposited layer (Volesky and Schiewer, 1999). The process of microprecipitation is facilitated by initial metal ion binding to the reactive sites present on the biomass, which therefore, serve as the nucleation sites for further precipitation (Mayers and Beveridge, 1989). This process is not limited to monolayer metal adsorption or just the saturation of active sorption sites. Biomass is thus able to accumulate metals, which may be-

come several times its own dry weight (Macaskie *et al*., 1992). Microprecipitation is based on interactions between the solute and the solvent, which occurs when the local solubility is exceeded.

(b) Ion exchange. Cell walls contain different polysaccharides as the basic building blocks. Studies on the ion exchange properties of certain neutral polysaccharides have established that divalent metal ions exchange with counter ions of polysaccharides (Volesky, 1990). There is a substantial evidence to suggest that ion exchange is the main mechanism of metal biosorption (Figueira *et al*., 2000). The polyhydroxy polyphenol groups of pine bark tannin are the active species in the adsorption process during which ion exchange takes place as metal cations displace adjacent phenolic hydroxyl groups forming a chelate (Bailey *et al*., 1999; Vázquez *et al*., 1994). The uptake of divalent metals by algal biomass (Schiewer *et al*., 1995), and by filamentous fungi (Fourest and Roux, 1994), is accompanied by the release of ionic species from the biosorbents. Biosorption of metals on fungi occurs as a result of ionic interactions and complex formation between the metal ions and functional groups present on the fungal surface (Mashitah *et al*., 1999; Kapoor and Viraraghavan, 1997). In marine algae, alginic acid and fucoidan occur in the cell walls and as extracellular polysaccharides. Both of these molecules contain hydroxyl and carboxyl as the functional metal binding groups. These are both acidic in nature and the binding of metals from solutions is characterized by the liberation of protons (Kratochvil *et al*., 1997; Crist *et al*., 1994a; 1994b). It appears reasonable to conclude that in most cases ion exchange, rather than sorption to free sites, is the relevant overall mechanism for the binding of metal ions in the biosorption process (Volesky and Schiewer, 1999). Since the overall charge of the biomass particle has to be neutral, any binding of one cation must be accompanied by either a stoichiometric release of other cations or by the binding of anions.

(c) Adsorption. Adsorption is a surface phenomenon, which is defined as the concentration of a substance at the interface between two immiscible phases, and larger the surface area, the greater will be adsorption (Bajpai, 1998). Effective biosorbent materials, therefore, must have a large surface area with a well-developed and well-defined inframicrocrystalline structure (Al-Duri, 1996a; 1996b). Any selectivity exhibited by the biosorbent is determined by the nature of this structure. A donor-acceptor complexation mechanism is involved in the adsorption process. Atoms of the surface functional groups (on the biosorbent) donate electrons to the sorbate species (metal cations). The nature of the sorbent-sorbate bond is determined by the position of functional groups on the biosorbent surface. The metal adsorption reaction may be

of two types (Al-Duri, 1996a):

(i) physical adsorption (physisorption), in which type physical bonds are formed, the process is reversible and non-specific, having low heat of adsorption, and involves no transfer of electrons although some polarization of the sorbate may occur;

(ii) chemical adsorption (chemisorption), in which type ionic or covalent bonds are formed between the sorbent and the sorbate, the process is highly specific in nature in which considerable transfer of energy is involved, and the process is irreversible.

The nature of binding forces involved in the adsorption process may be either physical or chemical. Physical forces may be either electrostatic or London-van der Waals forces (Myers, 1991). In the resultant physical bond, electrons stay in their original systems and thus the adsorption process is reversible. Chemical forces on the other hand extend over very short distances and involve sharing of electrons in the formation of covalent bonds. The binding of metal ions to biologically inactive materials occurs by various mechanisms, including covalent or electrostatic bonding (Greene and Darnall, 1990). In any given adsorption system, therefore, both physisorption and chemisorption are expected to occur, though in most cases physical adsorption is the dominant process (Al-Duri, 1996b). The adsorption of metals on the microbial cell wall surface is a physical process as reported for thorium and uranium biosorption by the chitinous cell wall of *Rhizopus arrhizus* (Tsezos and Volesky, 1982a; 1982b). The biosorption of Cu by the bacterium *Zoogloea ramigera* and the alga *Chlorella vulgaris* was due predominantly to electrostatic interactions (Aksu *et al*., 1992).

(d) Complexation, coordination and chelation. Complexation plays an important role in both metal-ligand and sorbate-sorbent interactions (Volesky and Schiewer, 1999). A complex, also referred to as a coordinate compound, is a polyatomic molecule that consists of one or more central atoms (usually metal cations) surrounded by ligands (atoms or groups of atoms, which are usually negative or neutrally charged) that are attached to it. Complexes can be neutral, positively or negatively charged. The number of coordinating atoms in the ligands that are directly attached to the central atom is called the coordination number, which can be larger than the valence of the central atom. If one ligand is attached to the central atom through two or more coordinating atoms, then the complex is called a chelate (Cahn and Dermer, 1979). Complexing of metal ions with ligands or functional groups present on cell wall surface in the biosorption process may occur in living or dead cells and/or their derivatives (Bolton and Gorby, 1995).

This phenomenon is comparable with chemical exchange process (Chang and Hong, 1994), and the process requires neither an active membrane transport mechanism nor metabolic energy controlled by non-directed physicochemical reaction (Gadd, 1992). Metal binding to the cell walls of *Saccharomyces cerevisiae* has been attributed to the metal coordination with amino, carboxyl and hydroxyl groups (Brady and Duncan, 1994). It has been reported that in the accumulation of Ca, Mg, Cd, Zn Cu and Hg ions by *Pseudomonas syringae*, complexation was the only mechanism involved (Cabral, 1992). The uptake of thorium and uranium by *Rhizopus arrhizus* was, in part, due to the formation of complexes between chitin of the fungal cell wall and metals (Tsezos and Volesky, 1982a; 1982b). Chelating agents synthesized by microbes, called siderophores, have been reported to naturally bind iron and gallium (Gascoyne *et al*., 1991), or in the sequestering of several metals when siderophores were artificially modified (Brierely *et al*., 1989).

(e)Bioaccumulation/intracellular accumulation. Bioaccumulation occurs in living cells/organisms. It is an active mode of uptake and the process is dependent on the metabolic activities of the cells/organisms (Volesky, 1990). The process is affected by the presence of metal ions. Since active transport of metals across the cell membrane is involved through metabolic activity, the metal uptake can occur only in living cells. In addition to a well-developed metal transfer system, specific metal binding proteins and peptides are involved (Mehra and Winge, 1991; Brierley, 1990). For example, the small cysteinrich polypeptides, metallothioneins, are involved in the bioaccumulation of Cu and Zn in fungi. Living algal cells usually have an extracellular polysaccharide protective cover. This is involved in metal bioaccumulation through microprecipitation or complexation (Brierley *et al*.,1989). Bioaccumulation of metals in living cells occurs through a number of definite phases (Veglio' and Beolchini, 1997; Brierley, 1990). During the first phase, metal biosorption is very rapid in which ion exchange, adsorption and complexation occur around the extracellular polysaccharides, cell wall and cell membrane. The rapid phase is followed by a slower lag phase associated with intracellular accumulation. Though this phase is slow, its involvement is significant in certain cases, such as Cu removal by *Streptomyces pilosus* biomass occurs through internal accumulation (Golab *et al*., 1995).

The theoretical bases of biosorption. Biosorption efficiency is usually reported in terms of binding as milligrams or millimoles of the metal bound per gram of the biomass at equilibrium. The biosorption equilibrium, however, depends on different operational parameters. These are useful values for providing the theoretical bases for constructing mathematical models of the biosorption process under given conditions. The theoretical basis of biosorption in most of the studies reported in literature is provided by fits to absorption isotherms and ion exchange models.

(a) Adsorption isotherms. The biosorption efficiency of various biosorbents for different metals is usually reported in terms of how well the observations fit the adsorption isotherms equations (Pagnanelli *et al*., 2003; Reddad *et al*., 2002; Sag *et al*., 2001). Adsorption and microprecipitation are the terms used to describe accumulation of electrically neutral material, which does not involve the release of stoichiometric amount of previously bound ions. The difference between adsorption and microprecipitation is that the affinity between sorbent and the metal sorbate in the former case, while limited solubility in the latter case, represent the main driving forces (Schiewer and Volesky, 2000). Biosorption is a specific example of adsorption, which is essentially the binding of chemical species to biopolymers (Simmons *et al*., 1995). The mechanism and kinetics of biosorption is discussed in terms of adsorption isotherms, which is a mathematical expression representing the variation of adsorption with concentration of the solute at equilibrium, at constant temperature. There are several types of adsorption isotherms (Bajpai, 1998), but more relevant for the understanding of the metal biosorption process are the Freundlich and Langmuir equations, which predict metal uptake as a function of the concentration of that metal present in the solution (Crist *et al*., 1992; Chen *et al*., 1990; Tsezos and Deutschmann, 1990).

(i) The Freundlich adsorption isotherms. The Freundlich equation for adsorption isotherms is purely an empirical model, having no theoretical basis, and while its validity extends to nonuniformity of the adsorption surfaces, its application is limited to low solute concentrations (Bajpai, 1998; Moore, 1972). The Freundlich isotherms are based on the following equation:

$$\frac{x}{m} = K C_{eq}^{1/n} \quad , \text{ or}$$

$$\log[\frac{x}{m}] = \log K + \frac{1}{n} \log \frac{C_{eq}}{n} \quad , \text{ or}$$

$$\log q_{eq} = \log K + \frac{1}{n} \log C_{eq} \tag{1}$$

This is mathematically an equation of straight line

where:

x = mass of solute adsorbed

m = mass of adsorbent

K= Freundlich constant for the nature and capacity of the adsorbent (the measure of sorption capacity)

$\frac{1}{n}$ = Freundlich constant for the adsorbability of the solute (the sorption intensity)

C_{eq} = equilibrium concentration of the solute

q_{eq} = solute adsorbed per unit mass of adsorbent

The Freundlich equation can be experimentally tested by plotting $\log [\frac{x}{m}]$ against $\log C_{eq}$. A straight line confirms that the adsorption isotherm validly fits the Freundlich equation. From this plot (Fig.1) can be calculated the Freundlich constant as: slope of straight line = $\tan \theta = \frac{1}{n}$. As the value of C_{eq} increases, the sorption sites approach saturation or limiting value. The Freundlich isotherms can be interpreted as sorption to sites with the affinity distribution, whereby the sites with higher affinity for the metal become occupied first (Stumm, 1992; Smith, 1981).

(ii) Langmuir adsorption isotherms. The Langmuir isotherms model is based on the following assumptions (Bajpai, 1998; Langmuir, 1918):

- the solid surface contains a fixed number of adsorption sites

- each site can hold one adsorbed molecule in a monolayer

- there is no interaction between molecules on different sites

- adsorption of a molecule (metal) at an unoccupied site and desorption from an occupied site does not depend on whether or not the neighbouring sites are occupied

- the surface atoms of the adsorbent have residual negative force due to unshared electrons, thus forming a sort of covalent bond on coming in contact with the positively charged solutes, which is sufficiently strong to prevent motion of the sorbate along the surface

- the adsorbent surface is energetically uniform

- adsorption is a dynamic process such that at equilibrium the rate of adsorption is equal to the rate of desorption
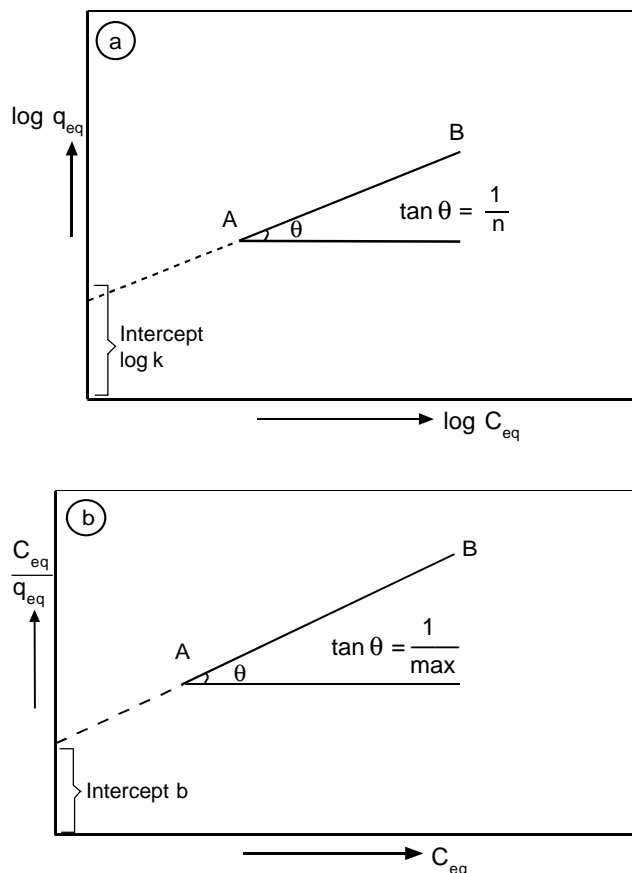
The Langmuir model, based on the above assumptions for metal sorption is:

$$\frac{C_{eq}}{q_{eq}} = \frac{1}{b \times q_{max}} + \frac{C_{eq}}{q_{max}} \qquad (2)$$

This is mathematically an equation of straight line

where:

C_{eq} = equilibrium concentration of the solute

q_{eq} = solute adsorbed per unit mass of the adsorbent

Fig. 1. **(a)** Freundlich adsorption isotherm, and **(b)** Langmuir adsorption isotherm (adapted from Bajpai, 1998); C_{eq} = equilibrium concentration of the solute; q_{eq} = solute adsorbed per unit mass of the adsorbent; the Freundlich constants, $[\frac{1}{n}]$ and K, respectively, for the adsorbability of the solute (the sorption intensity) and for the nature and capacity of the adsorbent (the measure of sorption capacity); the Langmuir constants b and q_{max}, respectively, for the ratio of the adsorption rate to the desorption rates and the maximum sorbate uptake.

q_{max} = maximum metal uptake

b = Langmuir constant

The Langmuir equation can be experimentally tested by plotting $\frac{C_{eq}}{q_{eq}}$ against C_{eq}. A straight line confirms that the adsorption isotherm validly fits the Langmuir equation. The Langmuir constant, b, is the ratio of the adsorption rate to the desorption rate, which is related to energy of adsorption through the Arrhenius equation (Chong and Volesky, 1995). The value of b can be calculated from the intercept of the straight line of Langmuir isotherm based on experimental data (Fig. 1). The value of q_{max}, the other Langmuir constant, can be calculated from the slope as:

slope of straight line $= \tan \theta = \dfrac{1}{q_{max}}$

The consideration behind the Langmuir isotherms is that sorption is a chemically equilibrated phenomenon (Langmuir, 1918). The basic assumption is that the forces exerted by chemically unsaturated surface atoms do not extend further than the diameter of one sorbed molecule. This is the basis for biosorption to occur in a monolayer when considered within the scope of Langmuir adsorption isotherms. The rate of adsorption, therefore, is proportional to the following (Volesky and Schiewer, 1999):

- the rate constant of the forward reaction, $^{BM}k_f$
- the number of free sites, $B = {}^tB - {}^Mq$
- the number of sorbate molecules hitting the surface per unit time, which is determined by the sorbate concentration [M]

where:

B = total number of binding sites

^Mq = sorbate bound per unit mass of the sorbent (binding or uptake)

M = initial concentration of sorbate

[M] = sorbate concentration at equilibrium

Under similar set of conditions, the rate of desorption is proportional to the following:

- the rate constant of backward reaction, $^{BM}k_b$
- the number of occupied sites, ^Mq

at equilibrium:

$$^{BM}k_f B[M] = {}^{BM}k_b {}^Mq \tag{3}$$

The equilibrium constant (^{BM}K), indicating affinity of the sorbent for the sorbate, may be calculated as the ratio of rate of adsorption and the rate of desorption, as:

$$^{BM}K = \dfrac{^{BM}k_f}{^{BM}k_b} \tag{4}$$

it follows, therefore:

$$^{BM}q = \dfrac{^tB \text{ x } {}^{BM}K[M]}{1 + {}^{BM}K[M]} \text{ (mmol/g)} \tag{5}$$

The following may be concluded from the foregoing equation (Schiewer and Volesky, 2000): (a) $^Mq_{max}$, maximum value of metal uptake, which is reached at high concentrations; (b) ^tB, the total number of binding sites, which correspond to q_{max}; (c) $^tB \, {}^{BM}K$, a value proportional to the slope in the origin of adsorption isotherms; and (d) it is desirable that both a high metal binding

capacity ^tB or $^Mq_{max}$, and a high affinity, ^{BM}K occur as a steep slope in the origin.

(b) Ion exchange. Ion exchange is another important metal ion binding mechanism in biosorption (Kratochvil and Volesky, 1998). Several workers have independently concluded this to be the case in the uptake of metals by some algae (Kratochvil *et al.*, 1995; Crist *et al.*, 1990), fungi (Fourest and Roux, 1994), and peat moss (Spinti *et al.*, 1995). The term ion exchange is used when the charge of the ions taken up equals the charge of ions released, so that charge neutrality of the particles is maintained, regardless of whether these ions are bound electrostatically or by complexation (Schiewer and Volesky, 2000). The driving force of ion exchange is mostly the attraction of the biosorbent for the metal biosorbate. The amounts of ions from the natural environment (Na^+, K^+, Ca^{2+}, Mg^{2+}, H^+) released during biosorption, balance the metal uptake. This has led to the development of ion exchange-based models, which have been used to interpret and finding a fit of data on biosorption equilibria (Kratochvil and Volesky, 1998; Kratochvil *et al.*, 1997). The model based on ion exchange mechanism assumes the existence of only one type of a binding site in the biomass reacting with ions from the solution. The removal of metals, based on ion exchange mechanism, is a reversible chemical reaction. The exchange sites are initially loaded with easily removable counter ions (usually H^+ or Na^+). In a perfect ion exchange system, the total number of occupied sites remains constant, called the ion exchange constant (Schiewer and Volesky, 2000). For two monovalent ions, the ion exchange constant (K) is:

$$K = \dfrac{q_A \text{ x } [B]}{q_B \text{ x } [A]} \tag{6}$$

where:

q_A and q_B = metal uptake at equilibrium (meq/g sorbate)

[A] and [B] = known concentration of metals A and B in solution

The value of K is, however, insufficient for the direct calculation of q_A and q_B from known concentrations of metals A and B in solution. It is possible, nevertheless, to use explicit isotherm equations, such as the Langmuir isotherms equation, which allows the calculation of q_A and q_B as a function of the ion concentrations in solution at equilibrium (Yu and Neretnieks, 1990). Consequently, ion exchange models have been introduced to fit and interpret data obtained from both ion exchange equilibrium and dynamic biosorption experiments (Kratochvil *et al.*, 1997). The ion exchange constant

may then be rewritten as ion exchange equilibrium constant (K_{AB}) as:

$$K_{AB} = \frac{q_A \times C_B}{q_B \times C_A} \qquad (7)$$

where:

C_A and C_B = concentrations of metal species A and B in liquid at equilibrium (meq/l)

The value of equilibrium constant K_{AB} can be determined from the slope of the graph of experimental values of q_A / q_B versus C_A / C_B (Kratochvil and Volesky, 1998). The overall binding capacity, Q (meq/g of dry biomass), is given by the density of the functional groups in the sorbent and can be expressed as:

$$Q = q_A + q_B \qquad (8)$$

The total normality of the solution C_o is given by:

$$C_o = C_A + C_B \qquad (9)$$

From the foregoing it follows that adequate use of the Langmuir sorption isotherms is not precluded by the appropriate ion exchange models with equilibrium constants taking into account the reversibility of the ion exchange reactions (Volesky and Schiewer, 1999; Crist *et al*., 1992). The basis for this may be derived from the assumption that the ion that is displaced into the solution can compete with the sorbed metal ion for the binding sites (Crist *et al*., 1994a; Crist *et al*., 1981; Haug and Smidsrod, 1970). A comparison of the Langmuir and ion exchange equations, which for simplicity of consideration assumes a monovalent metal ion M^+ replaces H^+ from a biosorbent site B, may be made from the following equations (Volesky and Schiewer, 1999):

Langumir adsorption isotherms:

$$B + M \leftrightarrow BM \qquad (10)$$
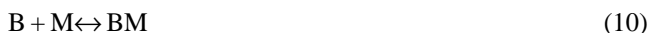
$$^{BM}K_{Langmuir} = \frac{BM[H]}{BH[M]} \quad [B] = [BH] + [BM] \qquad (11)$$

Ion exchange:

$$BH + M \leftrightarrow BM + H \qquad (12)$$

$$^{BM}K_{Langmuir} = \frac{BM}{B[M]} \quad [B] = [B] + [BM] \qquad (13)$$

therefore:

$$^{BM}K_{Langmuir} = \frac{^{BM}K_{Ion\ exchange}}{H} \qquad (14)$$

The major difference between the Langmuir and ion exchange mechanisms is that the former assumes that all sites are initially free and does not consider any reverse reaction of a displaced ion, in this case a proton, with the site; whereas the latter assumes that all sites to which metal ions are sorbed are initially occupied, that is the number of free sites stays constant. Since most metals in effluents are divalent, two monovalent binding sites are needed for their binding, which need to be accounted for, as 1:2 stoichiometry. The following two different reaction mechanisms may be considered:

$$2B + M^{++} \leftrightarrow B_2M \qquad (15)$$
$$2B + M^{++} \leftrightarrow B + M_{0.5} \qquad (16)$$

The formation of B_2M complex has been used to propose a modified Langmuir model (Jang *et al*., 1995). The equilibrium constant from the above equations may be described as below:

$$^{BM}K = \frac{[BM_{0.5}]^2}{[B]^2[M]} \qquad (17)$$

The formation of $BM_{0.5}$ provides the basis for formulating a multicomponent isotherm for binary metal solutions (Schiewer and Volesky, 1995). A binary exchange system containing divalent metal ions A and B may be described by the following ion exchange reaction (Kratochvil and Volesky, 1998):

$$A^{2+} + (B\text{-biomass}) \leftrightarrow B^{2+} + (A\text{-biomass}) \qquad (18)$$

A multicomponent Langmuir isotherm, in a metal biosorption system, assumes a 1:1 stoichiometry between metal ions and binding sites, in which all metals compete and bind to the same and all available binding sites (Chong and Volesky, 1995; Trujillo *et al*., 1991). Apart from an easier calculation of the metal uptake, a further advantage over the use of ion exchange constants is that the isotherm accounts not only for ion exchange but also for free binding sites that become occupied with increasing metal concentrations (Schiewer and Volesky, 2000). At low metal concentrations, when free sites may occur, the electrostatic attraction accounts for the occupation of these sites either by the covalent binding of ions or by electrostatic binding of cations, such as Na^+, in their vicinity.

Acknowledgement

References

Aderhold, D., Williams, C.J., Edyvean, R.G. J. 1996. The removal of heavy metal ions by seaweeds and their derivatives. *Bioresource Technol.* **58**: 1-6.

Akhtar, N., Saeed, A., Iqbal, M. 2003. *Chlorella sorokiniana* immobilized on the biomatrix of vegetable sponge of *Luffa*

cylindrica: a new system to remove cadmium from contaminated aqueous medium. *Bioresource Technol.* **88:** 163-165.

Aksu, Z. 1998. Biosorption of heavy metals by microalgae in batch and continuous systems. In: *Wastewater Treatment with Algae,* Y.-S. Wong, N.F.Y. Tam (eds.), pp. 37-53, Springer-Verlag and Landes Bioscience, New York, USA.

Aksu, Z., Egretli, G., Kutsal, T. 1998. A comparative study of Cu(II) biosorption on Ca-alginate agrose and immobilized *Chlorella vulgaris* in a packed bed column. *Process Biochem.* **33:** 393-400.

Aksu, Z., Sag, Y., Kutsal, T. 1992. The biosorption of copper (II) by *Chlorella vulgaris* and *Zoogloea ramigera. Environmental Technol.* **13:** 579-586.

Al-Duri, B. 1996a. Introduction to adsorption. In: *Use of Adsorbents for the Removal of Pollutants from Wastewaters,* G. McKay (ed.), pp. 1-5, CRC Press, Boca Raton, Florida, USA.

Al-Duri, B. 1996b. Adsorption modeling and mass transfer. In: *Use of Adsorbents for the Removal of Pollutants from Wastewaters,* G. McKay (ed.), pp. 133-173, CRC Press, Boca Raton, Florida, USA.

Atkinson, B.W., Bux, F., Kasan, H.C. 1998. Considerations for application of biosorption technology to remediate metal-contaminated industrial effluents. *Water S. A.* **24:** 129-135.

Bailey, S.E., Olin, T.J., Bricka, R.M., Adrian, D.D. 1999. A review of potentially low-cost sorbents for heavy metals. *Water Res.* **33:** 2469-2479.

Bajpai, D.N. 1998. *Advanced Physical Chemistry*, pp. 712-737, S. Chand and Company, New Delhi, India.

Baldrian, P. 2003. Interactions of heavy metals with white-rot fungi. *Enzyme Microbial Technol.* **32:** 78-91.

Benjamin, M.M., Sletten, R.S., Bailey, R.P., Bennet, T. 1996. Sorption and filtration of metals using iron-oxide-coated sand. *Water Res.* **30:** 2609-2620.

Bolton, H., Gorby, Y. A. 1995. An overview of the bioremediation of inorganic contaminants. In: *Bioremediation of Inorganics,* R.E. Hinchee, J.L. Means, D.R. Burris (eds.), pp. 1-16, Battele Press, Ohio, USA.

Brady, D., Duncan, J.R. 1994. Binding of heavy metals by the cell walls of *Saccharomyces cerevisiae. Enzyme Microbial Technol.* **16:** 633-638.

Brierley, C.L. 1990. Bioremediation of metal contaminated surface and groundwaters. *Geomicrobiol. Journal* **8:** 201-223.

Brierley, C.L., Brierley, J.A., Davidson, M.S. 1989. Applied microbial processes for metals recovery and removal from wastewater. In: *Metal Ions and Bacteria,* T.J. Beveridge, R. J. Doylec (eds.), pp. 359-382, John Wiley and Sons, NY,

USA.

Butter, T.J., Evison, L.M., Hancock, I.C., Holland, F.S., Matis, K.A., Philipson, A., Sheikh, A.I., Zouboulis, A.I. 1998. The removal and recovery of cadmium from dilute aqueous solutions by biosorption and electrolysis at laboratory scale. *Water Res.* **32:** 400-406.

Cabral, J.P.S.1992. Selective binding of metal ions to *Pseudomonas syringae* cells. *Microbios* **71:** 47-53.

Cahn, R.S., Dermer, O.L. 1979. *Introduction to Chemical Nomenclature*, Butterworths, London, UK.

Chang, J.-S., Hong, J. 1994. Biosorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* PU21 (Rip64). *Biotechnol. Bioeng.* **44:** 999-1006.

Chen, W.Y., Anderson, P.R., Holsen, T.M. 1991. Recovery and recycle of metals from wastewater with a magnetite-based adsorption process. *Res. J. Water Poll. Control Fed.* **63:** 958-968.

Chen, X.H., Gosset, T., Thevenot, D.R. 1990. Batch copper ion binding and exchange properties of peat. *Water Res.* **24:** 1463-1471.

Chiron, N., Guilet, R., Deydier, R. 2003. Adsorption of Cu(II) and Pb(II) onto a grafted silica: isotherms and kinetic models. *Water Res.* **37:** 3079-3086.

Chong, A.M.Y., Wong, Y.–S., Tam, N.F.Y. 2000. Performance of different microalgal species in removing nickel and zinc from industrial wastewater. *Chemosphere* **41:** 251-257.

Chong, K.H., Volesky, B.1995. Description of two-metal biosorption equilibria by Langmuir-type models. *Biotechnol. Bioeng.* **47:** 451-460.

Crist, D.R., Crist, R.H., Martin, J.R., Watson, J.R. 1994a. Ion exchange systems in proton-metal reactions with algal cell walls. *FEMS Microbiol. Rev.* **14:** 309-314.

Crist, R.H., Martin, J.R., Carr, D., Watson, J.R., Clarke, H.J., Crist, D.R. 1994b. Interaction of metals and protons with algae. 4. Ion exchange vs adsorption models and a reassessment of scatchard plots; ion exchange rates and equilibria compared with calcium alginate. *Environ. Sci. Technol.* **28:** 1859-1866.

Crist, R.H., Martin, J.R., Guptill, P. W., Eslinger, J.M., Crist, D. R. 1990. Interactions of metals and protons with algae. 2. Ion exchange in adsorption and metal displacement by protons. *Environ. Sci. Technol.* **24:** 337-342.

Crist, R.H., Oberholser, K., McGarrity, J., Crist, D.R., Johnson, J.K., Brittsan, J.M. 1992. Interaction of metals and protons with algae. 3. Marine algae, with emphasis on lead and aluminum. *Environ. Sci. Technol.* **26:** 496-502.

Crist, R.H., Oberholser, K., Shank, N., Nguyen, M. 1981. Nature and bonding between metallic ions and algal cell walls. *Environ. Sci. Technol.* **15:** 1212-1217.

Davis, T.A., Volesky, B., Vierira, R.H.S.F. 2000. *Sargassum*

seaweed as biosorbent for heavy metals. *Water Res.* **34:** 4270-4278.

Figueira, M.M., Volesky, B., Ciminelli, V.S.T., Roddick, F.A. 2000. Biosorption of metals in brown seaweed biomass. *Water Res.* **34:** 196-204.

Fourest, E., Roux, J.C. 1994. Improvement of heavy metal biosorption by mycelial dead biomass (*Rhizopus arrhizus, Mucor miehei* and *Penicillium chrysogenum*): pH control and cationic activation. *FEMS Microbiol. Rev.* **14:** 325-332.

Gadd, G.M. 1992. Microbial control of heavy metal pollution. In: *Microbial Control of Pollution,* J. C. Fry, G. M. Gadd, R. A. Herbert, C. W. Jones, I. A. Watson-Craik (eds.), pp. 59-88, Cambridge University Press, Cambridge, UK.

Gadd, G. M. 1990. Biosorption. *Chem. Ind.* **13:** 421-426.

Gascoyne, D.J., Connor, J.A., Bull, A.T. 1991. Capacity of siderophore-producing alkalophilic bacteria to accumulate iron, gallium and aluminium. *Appl. Microbiol. Biotechnol.* **36:** 136-141.

Greene, B., Darnall, D.W. 1990. Microbial oxygenic photoautotrophs (cyanobacteria and algae) for metal ion binding. In: *Microbial Metal Recovery,* H.L. Ehrlich, C.L. Brierley, (eds.), pp. 277-302, McGraw-Hill Book Co., NY, USA.

Golab, Z., Breitenbach, M., Jezierski, A. 1995. Site of copper binding in *Streptomyces pilosus. Water, Air and Soil Poll.* **82:** 713-721.

Gupta, V. K., Gupta, M., Sharma, S. 2001. Process development for the removal of lead and chromium from aqueous solutions using red mud: an aluminum industry waste. *Water Res.* **35:** 1125-1134.

Haug, A., Smidsrod, O. 1970. Selectivity of some anionic polymers for divalent metal ions. *Acta Chem. Scand.* **24:** 843-854.

Hughes, M.N., Poole, R.K. 1989. *Metals and Microorganisms*, Chapman and Hall, London, UK.

Iqbal, M., Saeed, A. 2002. Removal of heavy metals from contaminated water by petiolar felt-sheath of palm. *Environment Technol.* **23:**1091-1098.

Jang, L.K., Nguyen, D., Geesey, G.G. 1995. Effect of pH on the absorption of Cu(II) by alginate gel. *Water Res.* **29:** 315-321.

Jansson-Charrier, M., Guibal, E., Surjous, R., Cloirec, P. Le. 1995. Continuous removal of uranium by biosorption onto chitosan: application to an industrial effluent. In: *Biohydrometallurgical Processing*, C. A. Jerez, T. Vargas, H. Toledo, J.V. Wiertz (eds.), pp. 267-276, University of Chile, Chile.

Kapoor, A., Viraraghavan, T. 1997. Heavy metal biosorption sited in *Aspergillus niger*. *Bioresource Technol.* **61:** 221-227.

Kapoor, A., Viraraghavan, T. 1995. Fungal biosorption: an alternative treatment option for heavy metal bearing wastewaters: a review. *Bioresource Technol.* **53:** 195-206.

Karana, R.R., Uma, L., Subramaniam, G., Mohan, P.M. 1999. Biosorption of toxic metal ions by alkali-extracted biomass of a marine cyanobacterium, *Phormidium valderianum* BDU 30501. *World J. Microbiol. Biotechnol.* **15:** 729-732.

Kratochvil, D., Fourest, E., Volesky, B. 1995. Biosorption of copper by *Sargassum fluitans* biomass in fixed bed column. *Biotechnol. Letters* **17:** 777-782.

Kratochvil, D., Volesky, B. 1998. Biosorption of Cu from ferruginous wastewater by algal biomass. *Water Res.* **32:** 2760-2768.

Kratochvil, D., Volesky, B., Demopoulus, G. 1997. Optimizing Cu removal/recovery in a biosorption column. *Water Res.* **31:** 2327-2339.

Krishnan, E. R., Utrecht, P. W., Patkar, A.N., Davis, J. S., Pour, S. G., Fourest, M. E. 1992. *Recovery of Metals from Sludges and Waste Waters,* Noyes Data Corporation, NJ, USA.

Kuyucak, K.N. 1990. Feasibility of biosorbents applications. In: *Biosorption of Heavy Metals,* B. Volesky (ed.), pp. 371-378, CRC Press, Boca Raton, Florida, USA.

Langmuir, I. 1918. The adsorption of gases on plane surfaces of glass and platinum. *J. Amer. Chem. Soc.* **40:** 1361-1403.

Lehninger, A.L., Nelson, D.L., Cox, M.M. 1993. *Principles of Biochemistry*, 2nd edition, Worth Publishers, NY, USA.

Macaskie, L.F., Empson, R.M., Cheetham, C.P.G., Skarmulis, A. J. 1992. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymically mediated growth of polycrystalline HU_2PO_4. *Science* **257:** 782-784.

Mashitah, M.D., Zulfadhly, Z., Bhatia, S. 1999. Binding mechanism of heavy metals biosorption by *Pycnoporus sanguineus. Art. Cells, Blood Subs. and Immob. Biotechnol.* **27:** 441-445.

Matheickal, J.T., Yu, Q. 1996. Biosorption of lead from aqueos solutions by marine alga *Ecklonia radiata. Water Sci. Technol.* **34:** 1-7.

Mayers, I.T., Beveridge, T.J. 1989. The sorption of metals to *Bacillus subtilis* walls from dilute solutions and simulated Hamilton Harbour (Lake Ontario) water. *Canad. J. Microbiol.* **35:** 764-770.

Mehra, R.K., Winge, D. R. 1991. Metal ion resistance in fungi: molecular mechanisms and their related expressions. *J. Cellular Biochem.* **45:** 30-40.

Moore, W.J. 1972. *Physical Chemistry,* Longman Scientific and Technical, Essex, UK.

Myers, D. 1991. *Surfaces, Interfaces, Colloids: Principles and Applications*, VCH, Weinheim, Germany.

Pagnanelli, F., Esposito, A., Toro, L., Veglio', F. 2003. Metal

speciation and pH effect on Pb, Cu, Zn and Cd biosorption onto *Sphaerotilus natans*: Langumir-type empirical model. *Water Res.* **37:** 627-633.

Pagnanelli, F., Esposito, A., Veglio', F. 2002. Multi-metallic modeling for biosorption of binary systems. *Water Res.* **36:** 4095 - 4105.

Pagnanelli, F., Trifoni, M., Beolchini, F., Esposito, A., Toro, L., Veglio', F. 2001. Equilibrium biosorption studies in single and multi-metal systems. *Process Biochem.* **37:** 115-124.

Puranik, P.R., Paknikar, K.M. 1997. Biosorption of lead and zinc from solutions using *Streptoverticillium cinnamoneum* waste biomass. *J. Biotechnol.* **55:** 113-124.

Reddad, Z., Gerente, C., Andres, Y., Cloirec, P. Le. 2002. Adsorption of several metal ions onto a low-cost biosorbent: kinetic and equilibrium studies. *Environ. Sci. Technol.* **36:** 2067-2073.

Saeed, A., Akhtar, M. W., Iqbal, M. 2005b. Affinity relationship of heavy metal biosorption by the husk of *Cicer arientinum* (chickpea var. black gram) with their atomic weights and structural features. *Fresenius Environ. Bull.* **14:** 219-223.

Saeed, A., Iqbal, M., Akhtar, M.W. 2005a. Removal and recovery of lead(II) from single and multimetal (Cd, Cu, Ni, Zn) solutions by crop milling waste (black gram husk). *J. Hazard. Mat.* **117:** 65-73.

Saeed, A., Iqbal, M. 2003. Bioremoval of cadmium from aqueous solution by black gram husk (*Cicer arientium*). *Water Res.* **37:** 3472-3480.

Saeed, A., Iqbal, M., Akhtar, M.W. 2002. Application of wood wastes for the sorption of heavy metals in contaminated aqueous medium. *Pak. J. Sci. Ind. Res*. **45:** 206-211.

Sag, Y., Akcael, B., Kutsal, T. 2001. Evaluation, interpretation, and representation of three-metal biosorption equilibria using a fungal biosorbent. *Process Biochem.* **37:** 35-50.

Sandau, E., Sandau, P., Pulz, O., Zimmermann, M. 1996. Heavy metal sorption by marine algae and algal by-products. *Acta Biotechnol.* **16:** 103-119.

Schiewer, S., Fourest, E., Chong, K.H., Volesky, B. 1995. Ion exchange in metal biosorption by dried seaweed: experiments and model predictions. In: *Biohydrometallurgical Processing: Proceedings of the International Biohydrometallurgy Symposium,* C.A. Jerez, T. Vargas, H. Toledo, J.V. Wiertz (eds.), pp. 219-228, University of Chile, Santiago, Chile.

Schiewer, S., Volesky, B. 2000. Biosorption process for heavy metal removal. In: *Environmental Microbe-Metal Interactions,* D. R. Lovley (ed.), pp. 329-362, ASM Press, Washington DC, USA.

Schiewer, S., Volesky, B. 1995. Modeling of the proton-metal ion exchange in biosorption. *Environ. Sci. Technol.* **29:** 3049-3058.

Simmons, P., Tobin, J.M., Singleton, I. 1995. Considerations on the use of commercially available yeast biomass for the treatment of metal-containing effluents. *J. Indust. Microbiol.* **14:** 240-246.

Smith, J.M. 1981. *Chemical Engineering Kinetics*, pp. 310-322, McGraw-Hill Book Co., NY, USA.

Spinti, M., Zhuang, H., Trujillo, E.M. 1995. Evaluation of immobilized biomass beads of removing heavy metals from wastewaters. *Water Environ. Res.* **67:** 943-952.

Stumm, W. 1992. *Chemistry of the Solid-Water Interface,* pp. 87-97, John Wiley and Sons, Inc., NY, USA.

Tien, C.–J. 2002. Biosorption of metal ions by freshwater algae with different surface characteristics. *Process Biochem.* **38:** 605-613.

Travieso, L., Canizares, R.O., Borja, R., Benitez, F., Dominguez, A.R., Dupeyron, R., Valiente, V. 1999. Heavy metal removal by microalgae. *Bull. Environ. Contam. Toxicol*. **62:** 144-151.

Trujillo, E.M., Jeffers, T.H., Ferguson, C., Stevenson, H.Q. 1991. Mathematically modeling the removal of heavy metals from wastewater using immobilized biomass. *Environ. Sci. Technol.* **25:** 1559-1565.

Tsezos, M., Deutschmann, A.A. 1990. An investigation of engineering parameters for the use of immobilized biomass particles in biosorption. *J. Chem. Technol. Biotechnol.* **48:** 29-39.

Tsezos, M., Volesky, B. 1982a. The mechanism of uranium biosorption by *Rhizopus arrhizus. Biotechnol. Bioeng.* **24:** 385-401.

Tsezos, M., Volesky, B. 1982b. The mechanism of thorium biosorption by *Rhizopus arrhizus. Biotechnol. Bioeng.* **24:** 955-969.

Vázquez, G., Antorrena, G., González, J., Doval, M.D. 1994. Adsorption of heavy metal ions by chemically modified *Pinus pinaster* bark. *Bioresource Technol.* **48:** 251-255.

Veglio', F., Beolchini, F. 1997. Removal of metals by biosorption: a review. *Hydrometallurgy* **44:** 301-316.

Vieira, R.H.S.F., Volesky, B. 2000. Biosorption: a solution to pollution? *Internat. Microbiol.* **3:** 17-24.

Volesky, B. 2001. Detoxification of metal bearing effluent: biosorption for the next century. *Hydrometallurgy* **59:** 203-206.

Volesky, B. 1990. Removal and recovery of heavy metals by biosorption. In: *Biosorption of Heavy Metals*, B. Volesky (ed.), pp. 7-43, CRC Press, Boca Raton, Florida, USA.

Volesky, B., Schiewer, S. 1999. Biosorption, metals. In: *Encyclopedia of Bioprocess Engineering,* M.C. Flickinger, S.W. Drew (eds.), pp. 433-453, John Wiley and Sons Pub-

lishers, NY, USA.

Wilde, E.W., Benemann, J.R. 1993. Bioremoval of heavy metals by the use of microalgae. *Biotechnol. Adv.* **11:** 781-812.

Yetis, Ü., Özcengiz, G., Dilek, F.B., Ergen, N., Erbay, A., Dölek, A. 1998. Heavy metal biosorption by white-rot fungi. *Water Sci. Technol.* **38:** 323-330.

Yu, J.-W., Neretnieks, I. 1990. Single-component and multi-component adsorption equilibria on activated carbon of methylcyclohexane, toluene and isobutyl methyl ketone. *Indust. Engg. Chem. Res.* **29:** 220-231.

Yu, Q., Matheickal, J.T., Yin, P., Kaewsarn, P. 1999. Heavy metal uptake capacities of common marine macroalgal biomass. *Water Res.* **33:** 1534-1537.