Review

Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment

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Abstract

Biosorption may be simply defined as *the removal of substances from solution by biological material*. Such substances can be organic and inorganic, and in gaseous, soluble or insoluble forms. Biosorption is a physico-chemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface complexation and precipitation. Biosorption is a property of both living and dead organisms (and their components) and has been heralded as a promising biotechnology for pollutant removal from solution, and/or pollutant recovery, for a number of years, because of its efficiency, simplicity, analogous operation to conventional ion exchange technology, and availability of biomass. Most biosorption studies have carried out on microbial systems, chiefly bacteria, microalgae and fungi, and with toxic metals and radionuclides, including actinides like uranium and thorium. However, practically all biological material has an affinity for metal species and a considerable amount of other research exists with macroalgae (seaweeds) as well as plant and animal biomass, waste organic sludges, and many other wastes or derived bio-products. While most biosorption research concerns metals and related substances, including radionuclides, the term is now applied to particulates and all manner of organic substances as well. However, despite continuing dramatic increases in published research on biosorption, there has been little or no exploitation in an industrial context. This article critically reviews aspects of biosorption research regarding the benefits, disadvantages, and future potential of biosorption as an industrial process, the rationale, scope and scientific value of biosorption research, and the significance of biosorption in other waste treatment processes and in the environment.
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Keywords: biosorption; bioremediation; pollutants; toxic metals; radionuclides; organic wastes; dyes; bacteria; fungi; algae; biosorbent; adsorption

INTRODUCTION

Contamination and redistribution of toxic metals, metalloids, radionuclides in the environment as well as introduction of a plethora of organic pollutants necessitates ever increasing standards of pollutant detection and treatment. The deleterious effects of organic and inorganic pollutants on ecosystems and on human health are well known and much expenditure is devoted to industrial treatment methods to prevent or limit discharges. Apart from physical and chemical methods of treatment, biological methods have been in place for many years such as standard sewage and water purification treatments as well as auxiliary reed bed and wetlands approaches. Fundamental to these biotreatment processes are the activities of microorganisms upon which degradation of organic pollutants and transformations of inorganic pollutants, e.g. phosphate, nitrate, and metals, depends. The remarkable properties of microorganisms in the transformation and detoxification of organic and inorganic pollutants is well known and many processes have received attention in the general area of environmental biotechnology and microbiology.[1–7] Microorganisms are capable of the decomposition of a wide range of organic substances, natural and anthropogenic in origin, as well as effecting changes in the speciation and mobility of metal and radionuclide and other inorganic species by oxido-reductive and other transformations.[8–13] Many of these activities are a consequence

of the metabolic properties of living organisms, of which bacteria and fungi are the most important in the context of this article. However, biosorption is a physico-chemical process, simply defined as *the removal of substances from solution by biological material* (but see later), is a property of both living and dead organisms (and their components) and has been heralded as a promising biotechnology for pollutant removal from solution, and/or pollutant recovery, for a number of years because of its simplicity, analogous operation to conventional ion exchange technology, apparent efficiency and availability of biomass and waste bio-products.[2,11,14–23] It is probably true that most biosorption studies have been and continue to be carried out on microbial systems, chiefly bacteria, microalgae and fungi, and with toxic metals and radionuclides, particularly actinides and lanthanides.[24–27] However, practically all biological material has an affinity for metal species and a depth of other research exists with macroalgae (seaweeds) as well as plant and animal biomass and derived products (e.g. chitosan). While most

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Table 1. The top ten most cited articles in the ISI Web of Science database for 'All Years' (1970–2008) with 'Biosorption' in the topic (out of a total of 2824 articles appearing : database searched 7.4.08). Note that early biosorption articles only appeared late 1970s/early 1980s

1. Volesky B, Holan ZR (1995). Biosorption of heavy-metals. *Biotechnology Progress* **11**, 235–250. Times cited: 501.
2. Gadd GM (1993) Interactions of fungi with toxic metals. *New Phytologist* **124**, 25–60. Times cited: 336.
3. Veglio F, Beolchini F (1997). Removal of metals by biosorption : a review. *Hydrometallurgy* **44**, 301–316. Times cited: 242.
4. Fourest E, Rouz JC (1992). Heavy-metal biosorption by fungal mycelial by-products - mechanisms and influence of pH. *Applied Microbiology and Biotechnology* **37**, 399–403. Times cited: 237.
5. Tsezos M, Volesky B (1981). Biosorption of uranium and thorium. *Biotechnology and Bioengineering* **23**, 583–604. Times cited: 227.
6. Holan ZR, Volesky B, Prasetyo I (1993). Biosorption of cadmium by biomass of marine-algae. *Biotechnology and Bioengineering* **41**, 819–825. Times cited: 217.
7. Tsezos M, Volesky B (1982). The mechanism of uranium biosorption by *Rhizopus arrhizus*. *Biotechnology and Bioengineering* **24**, 385–401. Times cited: 208.
8. Kratochvil D, Volesky B (1998). Advances in the biosorption of heavy metals *Trends in Biotechnology* **16,** 291–300. Times cited: 205.
9. Gadd GM, White C (1993). Microbial treatment of metal pollution - a working biotechnology. *Trends in Biotechnology* **11,** 353–359. Times cited: 173.
10. Fourest E, Volesky B (1996). Contribution of sulfonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. *Environmental Science and Technology* **30**, 277–282. Times cited: 169.
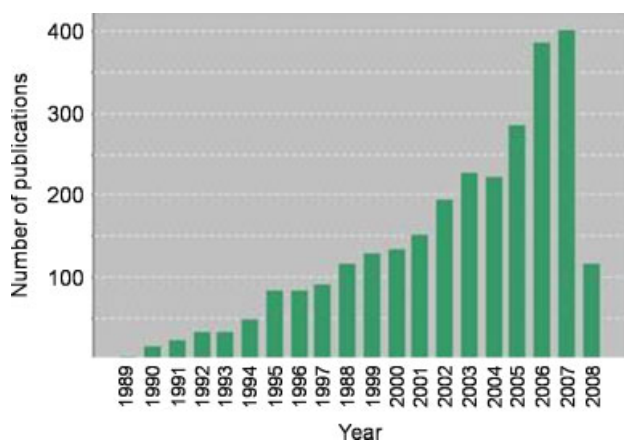

Figure 1. Numbers of papers appearing with 'biosorption' in the topic as listed in the ISI Web of Science database for 'All Years' (1970–2008) (out of a total of 2824 articles appearing: database searched 7.4.08). Note that early biosorption articles only appeared late 1970s/early 1980s. The small number of papers pre-1989 means they cannot be adequately shown on the *x*-axis.

biosorption research concerns metals and related substances (Table 1), unsurprising in view of the nature of adsorption and ion exchange mechanisms, the term is now applied to particulates and all kinds of organic substances. However, despite continuing dramatic increases in published research on biosorption (Figs 1 and 2), there has been little or no exploitation in an industrial context.
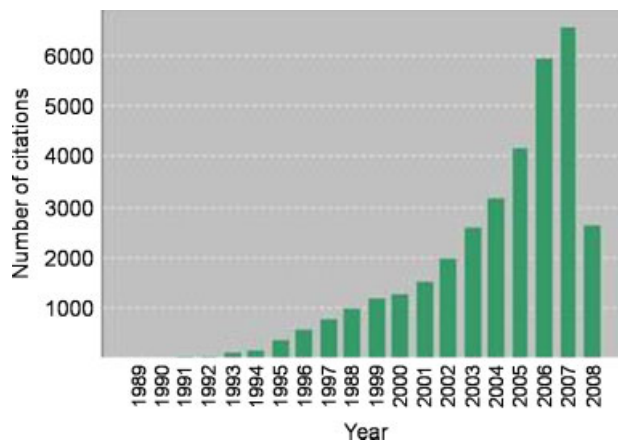

Figure 2. Numbers of citations of papers appearing with 'biosorption' in the topic as listed in the ISI Web of Science database 'All Years' (1970–2008) (out of a total of 2824 articles appearing: database searched 7.4.08; sum of the times cited = 34,572; average citations per item = 12.24; h-index = 70; approx. 654 articles have no citations to date). Note that early biosorption articles only appeared late 1970s/early 1980s: citations pre-1989 are not shown on the *x*-axis.

This article seeks to critically review the field of biosorption research to provide understanding of the rationale, benefits and drawbacks of biosorption as an industrial process, the value of biosorption research, and future prospects.

WHAT IS BIOSORPTION?

Biosorption is rather difficult to define because many mechanisms may contribute to the overall process depending on the substance to be sorbed, the biosorbent used, environmental factors and the presence or absence of metabolic processes in the case of living organisms. The 'bio' prefix denotes the involvement of a biological entity, i.e. living organism, component or product produced or derived from a living organism, exactly as in other terms like biotechnology, bioengineering, and bioprocessing. Coupling of 'bio' to a physico-chemical expression like 'sorption' also denotes the involvement of living organisms but does not necessarily mean that the 'sorption' process is somehow different to sorption in abiotic systems. For example, bioprecipitation and bioleaching are terms often used to encompass, for example, metal sulfide precipitation from a metal-containing solution, and metal leaching from ores mediated by bacterial activities, respectively, although the chemistry of these process may be identical to reactions carried out in the absence of the bacteria with the requisite pure chemical reagents. However, as will be discussed, 'sorption' to biological material may not be as simple as might be perceived.

Sorption is a term used for both absorption and adsorption. These terms are often confused. Absorption is the incorporation of a substance in one state into another of a different state (e.g. liquids being absorbed by a solid or gases being absorbed by water), i.e. into a three-dimensional matrix. Adsorption is the physical adherence or bonding of ions and molecules onto the surface of another molecule, i.e. onto a two-dimensional surface. In this case, the material accumulated at the interface is the adsorbate and the solid surface is the adsorbent. If adsorption occurs and results in the formation of a stable molecular phase at the interface, this can be described as a surface complex. Most solids, including microorganisms, possess functional groups like

–SH, –OH, –COOH on their surfaces. Deprotonated ligands, e.g. –RCOO$^-$, behave as Lewis bases and adsorption of metal cations can be interpreted as competitive complex formation.[28] Two general kinds of surface complexes exist: inner- and outer-sphere surface complexes. An outer-sphere complex occurs when at least one water molecule of the hydration sphere of the adsorbate molecule is retained on adsorption. An inner-sphere complex occurs when an ion or molecule is bound directly to the adsorbent without a hydration sphere.[29] Adsorption is the most common form of sorption used in 'traditional' clean-up technologies but unless it is clear which process (absorption or adsorption) is operative, sorption is the preferred term, and can be used to describe any system where a sorbate (e.g. an atom, molecule, a molecular ion) interacts with a sorbent (i.e. a solid surface) resulting in an accumulation at the sorbate–sorbent interface.[30] If adsorption occurs and continues through the formation of a new three-dimensional surface species, this new species can be defined as a surface precipitate. A number of different systems clearly exist in the continuum from adsorption and precipitation.[30] It should be noted that precipitation can occur even in simple laboratory biosorption systems depending on the substance and chemical conditions used.[25]

Biosorption may be simply defined as *the removal of substances from solution by biological material*. Such substances can be organic and inorganic, and in soluble or insoluble forms. Biosorption is a physico-chemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface complexation and precipitation. It is a property of living and dead biomass (as well as excreted and derived products): metabolic processes in living organisms may affect physico-chemical biosorption mechanisms, as well as pollutant bioavailability, chemical speciation and accumulation or transformation by metabolism-dependent properties. Some researchers include all biotic and abiotic mechanisms in effecting pollutant removal from solution under a 'biosorption' definition, especially when living cell systems are used, but this is not strictly accurate. The target substances for traditional adsorption/absorption processes are most organic contaminants and selected inorganic contaminants, such as toxic metals, from liquid and gas streams. Most biosorption research has concentrated on metals and related elements and several authors have emphasized this and defined biosorption as the removal of metal or metalloid species, compounds and particulates by biological material.[32] Several other definitions also exclusively refer to microbial material in view of the predominant focus of most biosorption-related research on microbe-related systems.[6] Clearly, in view of the wide variety of biosorbent materials used from all major Domains of Life, and the extension of biosorption research to include all manner of organic and inorganic substances, then only a simple, all-embracing definition such as that above may be necessary and appropriate. Thus, the term *biosorption can describe any system where a sorbate (e.g. an atom, molecule, a molecular ion) interacts with a biosorbent (i.e. a solid surface of a biological matrix) resulting in an accumulation at the sorbate–biosorbent interface, and therefore a reduction in the solution sorbate concentration*. Apart from the removal of organic substances, metal and radionuclide pollutants from contaminated matrices (which can include waste process streams, washes and volatiles, soil and other leachates, extracts, etc.) for environmental protection, biosorption also has application for subsequent recovery and use of precious metals, e.g. gold.[14,31]

WHICH SUBSTANCES ?

Most biosorption research has been carried out with metals and related elements, including actinides, lanthanides, metalloids, and various radioisotopes of these substances. As well as this, particulates and colloids have been studied as well as organometal(loid) and organic compounds, including dyes.[14,33,34] Such approaches may fall within the loose definition of 'biosorption' but clearly, a variety of mechanisms are involved in the removal of such diverse substances from solution.

Almost all metals in the Periodic Table have received considerable attention regarding their biosorption, except perhaps those that are highly mobile and do not associate much with biomass, and/or are of low toxicity, e.g. K^+, Mg^{2+}. The main reasons that determine the metal of interest may relate to its chemotoxicity and importance as a pollutant, whether it is a radionuclide, or whether it is a valuable element. This may result in differences in scale and approach, as well as goals of the research – environmental clean-up, health protection, recycling and/or recovery. Potential economic consequences may therefore vary also. Some of the most widespread metals studied are key environmental pollutants of major toxicity, e.g. lead, copper, mercury, cadmium, chromium and arsenic as well as radionuclides of Co, Sr, U, Th, etc. Even in such a short list there is a wide range of chemical properties, yet chemical speciation is ignored in many studies.[35] Among these elements, predominant chemical species may be cationic or anionic, exist as complexes, and exhibit a range of oxidation states. In many systems, even such common metals as Cu, Cd, and Zn, are hydroxylated, or complexed (e.g. to Cl) depending on the pH and medium composition. Many studies assume that such metals are entirely present as divalent cations: in many cases this will not be true.[28,35]

Many organic compounds released into the environment are degraded by natural microbial populations, and such biodegradation potential is the basis of many established and emerging treatment processes. However, in some cases, products of biodegradation may be hazardous, while some organic materials are extremely recalcitrant to biodegradation. Biosorption is again promoted as a potential biotechnology for removal of these and related organic substances from waste streams and effluents. Substances that have received attention include dyes, phenolic compounds, and pesticides.[34] Wastewaters containing dyes are very difficult to treat, since the dyes are recalcitrant molecules (particularly azo dyes), resistant to aerobic digestion, stable to oxidizing agents, and may be of low concentration. Common methods for removing dyes may be economically unfavourable and/or technically complicated. Because of the high costs, many of the physico-chemical methods for treating dyes in wastewater have not been widely used, with a combination of different processes often being used to achieve the desired water quality. Biosorption has been proposed as an effective decolourization method for dye-contaminated effluents.[36]

WHICH BIOSORBENTS?

Since all biological material has an affinity for metals, and indeed other pollutants, the kinds of biomass potentially available for biosorption purposes are enormous. All kinds of microbial, plant and animal biomass, and derived products, have received investigation in a variety of forms, and in relation to a variety of substances.[14,33] A common rationale for such studies is to identify highly-efficient biosorbents that are cost-effective, i.e. cheap. These would, in theory, provide new opportunities for

pollution control, element recovery and recycling. A flaw in this approach is that biomass composition does not vary significantly between different species of the same genus or order. For example, cell wall structure and composition (the main site of metal/radionuclide biosorption) is similar throughout all Gram-positive bacteria.[37] Similarly, all Gram-negative bacteria have the same basic cell structure;[37,38] main fungal orders are similarly uniform in wall structure and composition, with some known variations due to varying content of chitin, glucans, etc.[39] Plant and algal material similarly shows considerable uniformity, albeit with some differences between major genera.[40] Since so many representative organisms have already been studied, there seems little justification in examining yet more different bacterial, fungal and algal species for remarkable new properties. There also seems little justification for examining systems which could never be applied in an industrial context, e.g. pathogenic bacteria and fungi, nutritionally-fastidious extremophiles, rare or endangered plants, macroalgae, macrofungi and lichens, examples of which are found widely in the literature. Perhaps research should employ those biomass types that are efficient, cheap, easy to grow or harvest and concentration be given to biomass modifications and/or alteration of bioreactor configuration and physico-chemical conditions to enhance biosorption. A biosorbent can be considered low cost if it requires little processing, is abundant in nature, or is a by-product or waste material from another industry.[41]

A wide range of microbial biomass types have been investigated in biosorption studies, including mixed organism/biomass systems.[42] These include archaea, bacteria,[43–48] cyanobacteria,[49–55] algae[56–61] (including macroalgae, i.e. seaweeds[40,62–69]) and fungi, the latter including filamentous forms[17,26,70–78] as well as unicellular yeasts,[20,79,80] fruiting bodies (mushrooms, brackets, etc.) and lichens.[81,82] Peptidoglycan carboxyl groups are the main binding site for metal cations in Gram-positive bacterial cell walls with phosphate groups contributing significantly in Gram-negative species.[83–85] Other bacterial metal-binding components include proteinaceous S-layers, and sheaths largely composed of polymeric materials including proteins and polysaccharides. Cyanobacteria (formerly known as blue-green algae) have cell walls similar to Gram-negative bacteria. Thus, a major cyanobacterial cell wall biosorptive component is peptidoglycan, with some species also producing sheaths as well as copious mucilaginous polysaccharide (extracellular polymeric substances, EPS). Archaeal cell walls are of diverse composition and, depending on the genus, may include pseudomurein (which resembles peptidoglycan), sulfonated polysaccharide and glycoprotein as major components providing anionic sites such as carboxyl and sulphate groups. There is some variation in the composition of algal cell walls, the only common component across algal divisions being cellulose.[40] Other algal components include other polysaccharides like mannan, alginic acid, xylans, as well as proteins. These provide binding sites such as amino, amine, hydroxyl, imidiazole, phosphate and sulphate groups.[86] Fungal cell walls are complex macromolecular structures predominantly consisting of chitins, glucans, mannans and proteins, but also containing other polysaccharides, lipids and pigments, e.g. melanin.[87–89] This variety of structural components ensures many different functional groups are able to bind metal ions to varying degrees.[32,41] Chitin is a very important structural component of fungal cell walls and is an effective biosorbent for metals and radionuclides, as are chitosan and other chitin derivatives.[17] In *Rhizopus arrhizus*, U biosorption involves coordination to the amine N of chitin, adsorption in the cell wall chitin structure and further

precipitation of hydroxylated derivatives.[90] Chitosan is of low cost compared with commercial activated carbon (chitosan is derived by deacetylation of chitin, the most abundant aminopolysaccharide in nature) and strongly complexes pollutants, especially metals. However, industrial production of chitosan generates large quantities of concentrated effluent containing polluting bases and degradation products while conversion to chitosan at high temperature with strong alkali can cause variability of product properties and increase the processing costs which appears to limit industrial acceptance. Since chitin is a dominant component of fungal cell walls, a fermentation approach to cultivate fungi for subsequent chitosan preparation has been proposed[36] although the economics of this do not appear favourable and extraction procedures would still result in noxious wastes. Chitosan and its grafted and cross-linked derivatives have also been assessed for dye removal from aqueous solutions.[36,91] Fungal phenolic polymers and melanins possess many potential metal-binding sites with oxygen-containing groups including carboxyl, phenolic and alcoholic hydroxyl, carbonyl and methoxyl groups being particularly important.[87,92–94] Fungal biomass has also received attention as biosorbent materials for metal-contaminated aqueous solutions, because of the ease with which they are grown and the availability of fungal biomass as an industrial waste product, e.g. *A. niger* (citric acid production) and *S. cerevisiae* (brewing).[32,95]

Many microorganisms from all the major groups can produce EPS, largely polysaccharide, and such capsules, slimes and sheaths can be an important biosorptive component in living cell systems, especially biofilms, depending on the nature of the polysaccharide and associated components.[96–98] Extracellular polymers are intimately involved in Cd biosorption by activated sludges.[99] EPS can also adsorb or entrap particulate matter such as precipitated metal sulfides and oxides.[100,101] Biofilms are capable of binding significant quantities of metals under natural conditions, and serve as matrices for precipitation of insoluble mineral phases. Many other kinds of excreted metal-binding metabolites can be produced by microorganisms.[6,102,103]

Many kinds of macroalgae (seaweeds), plant materials (leaves, bark, sawdust), animal materials (hair, crustaceans) have also been studied.[104–107] A common rationale is that 'waste' biomass will provide an economic advantage. A variety of sludges arise from sewage treatment and other waste processing applications and these have also been investigated for biosorption properties,[108–114] although metal sorbing properties may sometimes be low.[15] A variety of bacterial and fungal biomass types arise from many industrial fermentations and the food, brewing and distilling industries and these also receive continued study.[115–118] However, 'waste' will still incur treatment and transport costs while, if a commercial biosorption process was developed using a 'waste', it would soon be found that 'waste' costs would rapidly rise, i.e. it would cease to be a waste! If biomass is to be grown specifically for biosorption applications, then cheap substrates would be preferable, just as in other industrial fermentations. Renewable biomass harvested from the environment, or cultured in 'farms', is also another rationale and the harvesting and use of natural seaweeds, which may lend themselves to aquaculture, has also received support.[15]

Chemical modification of biomass may create derivatives with altered metal binding abilities and affinities.[6,119,120] *Aspergillus niger* mycelium was modified by introducing additional carboxy or ethyldiamino groups which increased metal biosorption.[121] Eukaryotic metallothioneins and other metal binding peptides have been expressed in *E. coli* as fusions to membrane or

membrane-associated proteins such as LamB, an outer membrane protein. Such *in vivo* expression of metallothioneins provides a means of designing biomass with specific metal-binding properties.[122–127] This approach would seem to be an expensive solution to most biosorption contexts unless there was a highly-specific high value recovery process. However, there appears to be little commercial development of this biotechnology to date.

The use of freely suspended microbial biomass has disadvantages that include small particle size, low mechanical strength and difficulty in separating biomass and effluent. However, the use of immobilized biomass particles in packed- or fluidized-bed reactors minimizes these disadvantages.[14,15,22,79,94,128] Immobilized, living biomass has primarily taken the form of biofilms on supports prepared from a range of inert materials. These have been used in a variety of bioreactor configurations, including rotating biological contactors, fixed-bed reactors, trickle filters, fluidized beds and air-lift bioreactors.[11] In addition to the use of biofilms, living or dead biomass of all microbial groups has been immobilized by encapsulation or cross-linking. Supports include agar, cellulose, alginates, cross-linked ethyl acrylate–ethylene glycol dimethylacrylate, polyacrylamide, silica gel and the cross-linking reagents toluene diisocyanate and glutaraldehyde.[57,79,94] The biomass may be used in its 'natural state', or modified, for example, by alkali treatment, to improve biosorption efficiency. In order to use conventional reactor technology in larger systems, immobilized biomass particles should have properties that are similar to those of other commercial adsorbents (for example, in size (0.5–1.5 mm), particle strength and chemical resistance).[14,15] Diffusion into particles may present a problem, and high porosity and hydrophilicity with a maximum amount of biomass and minimal amounts of binding agent are also required. A number of examples have been described in the literature.[11,57,79,94,115,116]

Biomass used for biosorption may be living or dead. While the use of dead biomass or derived products may be easier by reducing complexity, the influence of metabolic processes on sorption is often unappreciated, particularly where there is scant biological input to the problem. Microorganisms degrade organic pollutants and can sorb, transport, complex and transform metals, metalloids and radionuclides and many different processes may contribute to the overall removal process. These may be of value in systems where additional benefits will result from metabolic activity, e.g. biodegradation of organic substances. Pollution treatments where metabolic processes are highly important include sewage treatment, biofilm reactors for pollutants, anaerobic digestion, soil and water bioremediation processes, phytoremediation, reed bed and wetlands biotechnologies, among others. In such processes, many of established commercial use, biosorption is a component of the overall removal process (see later).

BIOSORPTION MODELS AND ISOTHERMS

Most biosorption studies employ simple closed batch systems although most envisaged industrial applications would employ some kind of flow-through or continuous process. A wide variety of biosorption systems have been used, often meaning comparisons are difficult between different studies. A whole sorption isotherm[15] is considered to be the most appropriate approach for assessing biosorbent capacity. Many such studies use a simple defined system and a single metal: biosorption is often examined under such different parameters as varying biomass density, pH, metal concentration, presence of competing cations, etc. Flow and other

continuous systems are more complex, but many column studies use 'breakthrough curves' to assess sorbent efficiency. Many other kinds of bioreactors are possible, with the biosorbent also being utilized in a variety of forms.

Equilibrium sorption studies provide some basic information on a given system. The sorbent is allowed to accumulate the sorbate to equilibrium: the equilibrium value of sorbate uptake (q_e) by the biosorbents is plotted against the equilibrium (final) sorbate concentration (C). Such equilibrium sorption isotherms can be used to compare different biosorbents, as well as compare the affinities of different substances for the same biosorbents. In simple terms:

$$q_e = V(C_i - C)/S$$

V is the volume (L) of solution contacted with the sorbent; C_i and C are initial and equilibrium (final) concentrations of the sorbate (mg L^{-1}); S is the amount of biosorbent usually expressed as dry weight. In this case, q_e is expressed as weight per unit dry weight. For example, if C_i and C were in mg L^{-1} and S in g, then q_e would be in mg (g dry wt)$^{-1}$. However, this form of units is unsatisfactory since it does not provide useful information on molecular stoichiometries between, for example, sorbate molecules and binding sites, and can mislead over the relative sorption efficiencies for different metals. For example, 100 mg L^{-1} of Cu or Cd equates to concentrations of 1.57 mM and 0.89 mM, respectively (1.57 µmol mL^{-1} and 0.89 µmol mL^{-1}, respectively). Sorption values of 10 mg (g dry wt)$^{-1}$ for Cu or Cd give 157 and 89 µmol (g dry wt)$^{-1}$ respectively. Identical uptake values in weight terms may be very different uptake values in molar terms. The use of molar terms, e.g. µmol (g dry wt)$^{-1}$, nmol (mg dry wt)$^{-1}$ etc., in any comparative and mechanistic studies should be favoured. There are some other means of expressing sorbate uptake, such as per volume or per wet (fresh) weight but these should be avoided because of serious inherent errors. Additionally, expression of a concentration per unit biomass is sometimes encountered, e.g. mM (g dry wt)$^{-1}$ which is meaningless.

While batch equilibrium sorption studies can provide useful information on relative biosorbent efficiencies and important physico-chemical factors that affect biosorption, they usually provide no information on mechanisms. Other potential drawbacks include equilibrium uptake values not being attained (e.g. if insufficient incubation time is allowed or where sorbate concentrations are low and biomass concentrations high), the use of unrealistic high sorbate concentrations compared with an industrial or environmental context, complete removal of sorbate from solution which may occur over a wide concentration range for a given biomass concentration and affect calculations, possible changes in solution chemistry, and nucleation, deposition and precipitation phenomena. Despite this, and probably because of their relatively simple nature, a great many batch sorption studies are published in the literature for all kinds of biomass, metals and other substances. Unfortunately, most contain little novelty or represent anything like a significant contribution to the field.

Batch studies often precede continuous dynamic studies and the most effective approach is usually considered to be a flow-through fixed-bed bioreactor, with efficacy being characterized by means of break-through points that occur when column contents become saturated with the sorbate.[14,15,18,33,129–131]

A variety of models have been used to characterize biosorption.[72,77,130–139] These range from simple single-component models, of which the Langmuir and Freundlich models are probably the most widely used, to complex multi-component
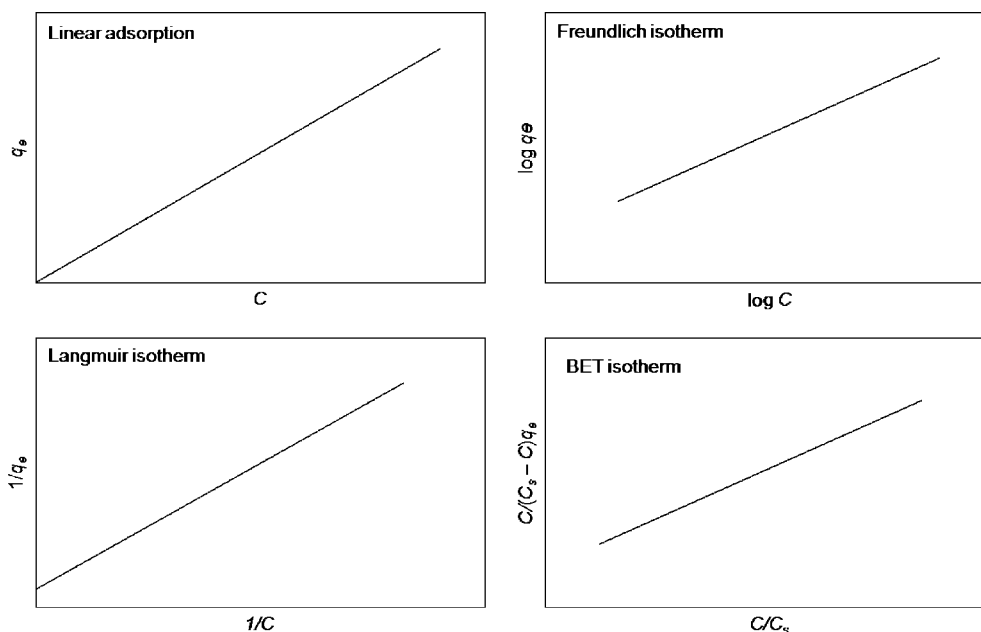
Figure 3. Graphical representation of four common adsorption isotherms used in biosorption studies: linear, adsorption, Freundlich, Langmuir and Brunuaer–Emmett–Teller (BET) isotherms.

models, some derived from Langmuir/Freundlich models.[140,141] While such interpretations have some use in comparing different metal–biosorbent systems (although untransformed data may provide the same conclusions), these models are based on assumptions that are quite simplistic for biological systems. They were originally derived for adsorption of gases in monolayers to activated carbon and some of the assumptions, such as all binding sites having the same affinity, do not often apply to biosorbents. Cell walls, and other biomass components, have multiple binding sites such as carboxyl, hydroxyl, carbonyl, thiol, amine, and phosphate, etc. These can have different affinities for sorbate species, and can be dramatically affected by changes in pH and solution chemistry. Some models which reflect multilayer adsorption such as the Brunauer–Emmett–Teller (BET) isotherm can also be used[72] although these are also usually derived from simple non-biological systems. Many biosorption models have now been described: only the most common will be described here (Fig. 3).[34,72,130,131,142,143]

The Freundlich isotherm defines adsorption to heterogeneous surfaces, i.e. surfaces possessing adsorption sites of varying affinities. Langmuir and Freundlich models are widely used in the biosorption literature but, apart from the drawbacks mentioned, they can only be applied at a constant pH value. In an unbuffered system, pH changes may result where ion exchange leads to H^+ displacement by binding cations: in this case, it is possible to apply amended models.[40] The Freundlich isotherm equation is:

$$q_e = KC^\beta$$

where q_e is the equilibrium value of sorbate uptake by the sorbent, C is the equilibrium sorbate concentration, K is an affinity parameter and β is a dimensionless heterogeneity parameter: the smaller the value of β, the greater the heterogeneity.[144] The Freundlich equation reduces to a linear adsorption isotherm when $\beta = 1$. Although strictly valid for metal adsorption at low aqueous concentrations,[29] it is often used over a wide range of concentrations. Data are usually fitted to the logarithmic form of

the equation:

$$\log q_e = \log K + \beta \log C$$

which should give a straight line by plotting $\log q_e$ versus $\log C$ of slope β and an intercept of $\log K$ for $C = 1$ ($\log C = 0$) (Fig. 3).

The Langmuir isotherm was derived originally from studies on gas adsorption to activated carbon. This model contains a number of assumptions which include that (a) all binding sites possess an equal affinity for the adsorbate, (b) adsorption is limited to formation of a monolayer, and (c) the number of adsorbed species does not exceed the total number of surface sites, i.e there is a 1 : 1 stoichiometry between surface adsorption sites and adsorbate. It is likely that none of these assumptions apply in biological systems. The Langmuir adsorption isotherm is:

$$q_e = \frac{Q^0 KC}{1 + KC}$$

where Q^0 is the maximum adsorption of sorbate per unit mass sorbent (in forming a complete monolayer on the surface), K is an affinity parameter related to the bonding energy of the sorbate species to the surface, and other symbols are as previously described. The Langmuir isotherm assumes a finite number of uniform adsorption sites and the absence of lateral interactions between adsorbed species. These assumptions are clearly invalid for most complex systems including biological material. In many cases, the Langmuir isotherm is only able to describe adsorption at low sorbate concentrations. Two derivatives of the Langmuir equation are:

$$\frac{C}{q_e} = \frac{1}{Q^0 K} + \frac{C}{Q^0}$$

or

$$\frac{1}{q_e} = \frac{1}{Q^0} + \left[\frac{1}{Q^0 K}\right]\left[\frac{1}{C}\right]$$

Either of these forms may be used to linearize data that conforms to the Langmuir model (Fig. 3).

A multisite Langmuir adsorption isotherm allows for more than one type of binding site. The multisite Langmuir adsorption isotherm is:

$$q_e = \sum_{i=1}^{n} \frac{Q^0{}_i K_i C}{1 + K_i C}$$

where n is the number of types of surface sites. This isotherm may provide a better fit to metal adsorption data than the single Langmuir isotherm.

The BET represents isotherms with multilayer adsorption at the adsorbent surface and assumes that a Langmuir equation applies to each layer. A further assumption is that a given layer may not need to be completely formed before the next layer forms. The BET equation is:

$$q_e = \frac{BCQ^0}{(C_s - C)[1 + (B - 1)(C/C_s)]}$$

where C_s is the saturation concentration of the solute, B is a constant relating to the energy of interaction with the surface, and other symbols are as previously described. A plot of $C/(C_s - C)q_e$ against C/C_s gives a straight line for data conforming to the BET isotherm of slope $(B - 1)/BQ^0$ and intercept $1/BQ^0$ (Fig. 3).[72]

The Scatchard plot, used to describe protein–ligand binding, has also been used to describe metal biosorption. Binding affinity constants, K_{ads}, and maximal binding capacity, R_{tads}, can be determined from the intercept on the y axis and slope of the plot, respectively.[56,58,59,145] Curved Scatchard and reciprocal Langmuir plots are usually interpreted as reflecting surface heterogeneity of the biosorbent, including a mixture of ionic and covalent binding, and diverse chemical composition meaning a range of distinct binding sites with different affinities.[59,72,146]

Adsorption reactions of, for example, soils and minerals, are also described using adsorption isotherm equations, and these have been applied widely in biosorption studies. The simplest adsorption isotherm equation is a linear function written in terms of the distribution coefficient, K_d:

$$q_e = K_d C$$

where q_e is the amount of sorbate adsorbed per unit mass sorbent at equilibrium and C is the equilibrium solution concentration of the sorbate. Because of the linear assumption, the distribution coefficient may only be effective over a narrow sorbate concentration range.[147]

Fitting biosorption data to adsorption isotherm equations provides no information about the mechanisms, and should be considered simply as numerical relationships used to fit data. Experimental evidence is necessary before any chemical significance can be attributed to isotherm equation parameters. Further, these parameters are valid only for the chemical conditions under which the experiment was conducted. Use of these equations for prediction of metal adsorption behaviour under changing pH, ionic strength, and solution metal concentration is impossible.[147] Application of adsorption isotherms may also be inadequate when precipitation of metals occurs[148] although the Langmuir isotherm has sometimes been applied to such cases despite being theoretically invalid.

The criteria for choosing a isotherm or kinetic equation for biosorption data is mainly based on the goodness of curve fitting which is often evaluated by statistical analysis. However, good curve fitting in the sense of statistical evaluation may not necessarily imply that this curve fitting has true physical meaning, i.e. if a set of biosorption data is analyzed by different isotherm or kinetic equations, the best fit equation may not be the one reflecting the biosorption mechanism(s). It therefore seems that many isotherm and kinetic studies of biosorption are basically a simple mathematical exercise. It has been stated that selection of kinetic equations should be based on the mechanisms.[149] Consequently, to formulate a mathematical expression of biosorption, models with strong theoretical characteristics are needed rather than simple curve fitting.[142] In view of the previously discussed uncertainties about mechanisms, this may not be possible and the application of biosorption isotherms may remain dubious in many studies.

In some metal biosorption studies, the equilibrium constant has been defined as:

$$K_{eq} = \frac{q_e}{C}$$

where q_e and C are as described previously. This equation is incomplete or possibly invalid as the equilibrium law cannot be applied to a biosorption process unless the reaction stoichiometry is known.[142]

Surface complexation models provide molecular descriptions of metal adsorption using an equilibrium approach that defines surface species, chemical reactions, mass balances and charge balances. Such models can provide information on stoichiometry and reactivity of adsorbed species.[147] However, their use in describing ion adsorption by a variety of solids, particularly biological material, is rather limited. In order to use surface complexation models, the adsorption mechanism and types of surface complexes must be specified for all adsorbing metal ions. This may necessitate independent experimental determination of adsorption mechanisms using techniques including Raman and Fourier transform infrared (FTIR) spectroscopy, nuclear magnetic resonance spectroscopy (NMR), electron spin resonance (ESR) spectroscopy, and X-ray absorption spectroscopy (XAS), which includes X-ray absorption near-edge (XANES) and extended X-ray fine structure (EXAFS) spectroscopy, and X-ray reflectivity. Most of these techniques have been used but only in a small number of biosorption studies. Indirect experimental evidence for mechanisms may come from point of zero charge shifts, ionic strength effects and molecular modelling.[147] Surface complexation models have an advantage in that they have the potential to be predictive though this has not been widely achieved or applied in biosorption. However, in a related context, the application of surface complexation modelling or a linear programming approach to specific chemical and electrostatic interactions occurring at the solution–cell wall interface has related variations in surface properties with variations in metal affinity in order to predict metal mobilities in complex environmental systems.[150–154]

MECHANISM(S) OF BIOSORPTION

The imprecise definition of sorption perhaps gives a clue that the mechanism(s) involved in biosorption are often difficult to characterize, except perhaps in the simplest laboratory systems. Biological material is complex and a variety of mechanisms may be operative under given conditions. The variety of structural components present in biomass means that many functional groups are able to interact with metal species, e.g. carboxyl, phosphate, hydroxyl, amino, thiol, etc., to varying degrees and influenced by

physico-chemical factors. For biosorption, defined as a physico-chemical process independent of metabolism, such mechanisms as adsorption, ion exchange and complexation/coordination may be important and, in these cases, biosorption can be rapid and reversible with biomass properties analogous to conventional ion exchange resins. Ion exchange is the replacement of an ion in a solid phase in contact with a solution by another ion. More specifically, it is the replacement of an absorbed, readily-exchangeable ion by another.[29] Although a simple concept, in reality it can be a mechanistically highly complex process[28,155] depending on the system. Several other mechanisms may occur which may complicate sorption and/or desorption. Precipitation, where bound metal/radionuclide species can act as loci for subsequent deposition, can lead very high uptake capacities but this may inhibit desorption. Extensive precipitation of actinides such as uranium and thorium on non-living fungal biomass is a good example of precipitation arising from hydrolysis product formation.[25] It is likely that the various mechanisms involved in biosorption can operate simultaneously to varying degrees.

There are several ways of classifying metals according to their chemical properties.[35] In a biological context, relevant schemes consider ligand preferences, a property that can underlie biological activity, and is relevant to biosorption. Type A (hard acids) preferentially bind to oxygen-containing ligands (hard), while type B (soft acids) preferentially bind to S and N-containing ligands (soft) (Table 2). While such schemes may inform or explain some biosorptive phenomena, definitions are not absolute and there can be varying properties of metal species rather than absolute distinctions, as well as borderline categories. Some behaviour will be affected by metal concentration,[52] as well as the relative metal concentrations in mixtures where competitive effects may occur. The hard/soft scheme predicts that bonds formed between hard acids and hard ligands will be predominantly ionic whereas soft acid–ligand complexes are more covalent in character. However, this can vary depending on the nature of the biomass: Sr^{2+} binding to denatured yeast biomass was ionic but interaction with cell wall of living yeast exhibited increased covalent binding.[156] This could be due to the involvement of amine and sulphydryl functional groups that are active on cell surfaces of living cells but which may be denatured in dead biomass, where phosphate and carboxylate groups may be more significant.[52]

A further mechanistic complication is where living cell systems are used. Many biosorption researchers do not restrict themselves to dead biomass or purified products, and a variety of mechanisms may be exhibited, depending on growth and other conditions, that lead to metal accumulation. Metabolic activities such as respiration, nutrient uptake, and metabolite release will alter the microenvironment around the cells which, in turn, may affect adsorption, ion exchange, complexation and precipitation.[11]

Table 2. Classification of metal ions into type-A, transition metal, and type-B metal cations, and according to the hard and soft acid scheme, with ligand preferences and stability sequences (adapted from Stumm and Morgan[28]; Gadd[35])

Type-A metal cations	Transition metal cations	Type-B metal cations
Electron configuration of inert gas	1–9 outer shell electrons	Electron number corresponds to Ni^0, Pd^0 and Pt^0 (10 or 12 outer shell electrons)
	Not spherically symmetric	Low electronegativity
Low polarizability		High polarizability
'Hard spheres'		'Soft spheres'
(H^+), Li^+, Na^+, K^+, Be^{2+}	V^{2+}, Cr^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}	Cu^+, Ag^+, Au^+, Ga^+,
Mg^{2+}, Ca^{2+}, Sr^{2+}, Al^{3+}, Sc^{3+}, La^{3+}, Si^{4+}, Ti^{4+}, Zr^{4+}, Th^{4+}	Ni^{2+}, Cu^{2+}, Ti^{3+}, V^{3+}, Cr^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+}	Zn^{2+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Sn^{2+}, Tl^{3+}, Au^{3+}, In^{3+}, Bi^{3+}
Hard acids	Borderline	Soft acids
All type-A metal cations plus Cr^{3+}, Mn^{3+}, Fe^{3+}, Co^{3+}, UO^{2+}, VO^{2+}	All divalent transition metal cations plus Zn^{2+}, Pb^{2+}, Bi^{3+}, SO_2, NO^+, $B(CH_3)_3$	All type-B metal cations minus Zn^{2+}, Pb^{2+}, Bi^{3+}
In addition species such as		All metal atoms, bulk metals
BF_3, BCl_3, SO_3, RSO_2^+, RPO_2^+		I_2, Br_2, ICN, I^+, Br^+
CO_2, RCO^+, R_3C^+		
Preference for ligand atom		
$N \gg P$		$P \gg N$
$O \gg S$		$S \gg O$
$F \gg Cl$		$I \gg F$
Stability sequence		
Cations:	Cations	
Stability $\propto$ (charge/radius)	Irving–Williams series:	
	$Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+}$	
	$< Cu^{2+} > Zn^{2+}$	
Ligands		Ligands
$F > O > N = Cl > Br > I > S$		$S > I > Br > Cl = N > O > F$
$OH^- > RO^- > RCO_2-$		
$CO_3^{2-} \gg NO_3-$		
$PO_4^{3-} \gg SO_4^{2-} \gg ClO_4-$		

Extracellular polymeric materials are capable of binding and entrapment of metal and radionuclide species: such phenomena may be significant in biofilms.[157] A variety of oxido-reductive transformations that alter metal or radionuclide speciation can lead to enhanced deposition in and around cell walls, or even volatilization.[6,158] Living cells also possess a variety of specific and non-specific transport systems for intracellular accumulation.[94,128] Thus, depending on the system, multiple mechanisms may be involved in metal removal from solution and their dissection, modelling and analysis may be difficult. Pragmatically, of course, it may not be necessary to understand what mechanism(s) are operative if the prime research goal is to identify an efficient biosorbent system. However, living cell systems will need maintenance and optimization and a knowledge of the main metabolism-dependent processes in operation would be useful.

The diversity of chemical structure encountered in organic pollutants mean that molecular size, charge, solubility, hydrophobicity, and reactivity, all affect biosorption as well as the type of biosorbent and wastewater composition. There is a general lack of detailed understanding of mechanisms of organic substance biosorption although adsorption, complexation, and related phenomena can all be implicated. In addition, there may be permeation of biomass so that general absorption may also contribute.

Hydrophobic compounds, e.g. hydrocarbons, are, by definition, not readily soluble in water. However, these substances will associate with non-polar environments such as the surface of organic particles.[28] Such hydrophobic sorption clearly occurs when hydrophobic compounds encounter biomass in biosorption systems. Additionally, the lipophilic nature of hydrophobic compounds means that they can pass through membranes and be absorbed into the organic matrix. Absorption may be a significant component of biosorption in such cases.
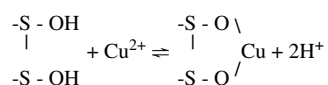
The mechanisms involved in dye biosorption onto chitosan are various and include surface adsorption, chemisorption, diffusion and adsorption-complexation. The most important steps are film diffusion, pore diffusion and chemical reactions like ion exchange and complexation.[36] Amine sites appear to be the main reactive groups for dyes, though hydroxyl groups might contribute, with intermolecular interactions of the dye molecules being most probable in chitosan–dye systems.[36]

Mechanisms of anion biosorption have been little studied, although this can be markedly affected by chemical conditions such as the pH. For example, anionic species like TcO_4^-, $PtCl_4^{3-}$, CrO_4^{2-}, SeO_4^{2-} and $Au(CN)_2^-$ exhibit increased biosorption at low pH values.[59,145]

Surface complex formation of cations may involve coordination of metal ions with oxygen donor atoms and proton release:

$$S-OH + Cu^{2+} \rightleftharpoons S-OCu^+ + H^+$$
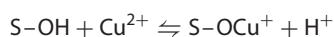
Bidentate surface complexes may also result:

$$
\begin{array}{l}
\text{-S - OH} \\
\;\;\;| \qquad\qquad + Cu^{2+} \rightleftharpoons \\
\text{-S - OH}
\end{array}
\qquad
\begin{array}{l}
\text{-S - O} \backslash \\
\;\;\;| \qquad Cu + 2H^+ \\
\text{-S - O} \diagup
\end{array}
$$

where S represents a surface site (S-S represents interconnected surface sites).

A cation can associate with a surface as an inner-sphere or outer-sphere complex depending whether a chemical (i.e. largely covalent) bond is formed between the metal and the electron donating oxygen ion in this case (inner-sphere complex) or if a cation approaches the surface negative groups to a critical distance but the cation and base are separated by at least one water molecule.

FACTORS AFFECTING BIOSORPTION

Many factors can affect biosorption. The type and nature of the biomass or derived product can be very important as discussed, including the nature of its application as, e.g. freely-suspended cells or biomass, immobilized preparations, living biofilms, etc. Physical and chemical treatments such as boiling, drying, autoclaving and mechanical disruption will all affect binding properties while chemical treatments such as alkali treatment often improve biosorption capacity, especially evident in some fungal systems because of deacetylation of chitin to form chitosan-glucan complexes with higher metal affinities.[20] Growth and nutrition of the biomass, and age can also influence biosorption due to changes in cell size, wall composition, extracellular product formation, etc. The surface area to volume ratio may be important for individual cells or particles, as well as the available surface area of immobilized biofilms. In addition, the biomass concentration may also affect biosorption efficiency with a reduction in sorption per unit weight occurring with increasing biomass concentration.[72] Apart from these, physico-chemical factors such as pH, the presence of other anions and cations, metal speciation, pollutant solubility and form, and temperature may also have an influence. With living cell systems, the provision of nutrients and optimal growth conditions is an obvious requirement.

Of physico-chemical factors, pH is possibly the most important. Metal biosorption has frequently been shown to be strongly pH-dependent in almost all systems examined, including bacteria, cyanobacteria, algae, and fungi. Competition between cations and protons for binding sites means that biosorption of metals like Cu, Cd, Ni, Co and Zn is often reduced at low pH values.[159,160] Conversely for anionic metal species like TcO_4^-, $PtCl_4^{3-}$, CrO_4^{2-}, SeO_4^{2-} and $Au(CN)_2^-$, increased biosorption may be seen at lower pH values.[145] Biosorption of some metals may be pH-independent and some examples exist for, e.g. Ag^+, Hg^{2+} and $AuCl_4^-$, explained by the formation of covalent complexes with N and S-containing ligands.[59]

Competition will also occur between cations and such an effect can also depress biosorption of the metal of interest. Various selectivity series have been published which reflect such competition, e.g. Al^{3+}, Ag^+ > Cu^{2+} > Cd^{2+} > Ni^{2+} > Pb^{2+} > Zn^{2+}, Co^{2+} > Cr^{3+} for *Chlorella vulgaris*, and Cu > Sr^{2+} > Zn^{2+} > Mg^{2+} > Na^+ for *Vaucheria* sp.[59] In some cases, cations may increase biosorption of anionic species by enhancing binding of the negatively-charged anions.[57,58] In some cases, cation loading of biomass may enhance biosorption of another cation because of pH buffering effects. Calcium-saturated fungal biomass showed enhanced Zn biosorption, for example.[161] In many cases, certain anions have been found to have little effect on biosorption although there are also many contradictory studies. It is likely that some effects of anions may be indirect resulting from, e.g. pH changes, or the presence of the accompanying metal cation, but such influences may be difficult to resolve. Anions like CO_3^{2-} and PO_4^{2-} may clearly affect biosorption through the formation of insoluble metal precipitates. Chloride may influence biosorption through the formation of complexes, e.g. $CdCl_3^-$.[162]

21

Metal speciation in the system is another important factor. The extent of biosorption may vary depending on the speciation, which in turn influences the solubility and mobility of metal species.[35]

Over modest physiological-type ranges, temperature usually has little effect on biosorption, although high temperatures, e.g. 50 °C, may increase biosorption in some cases.[27] Low temperature will, however, affect living cell systems and any auxiliary metabolism-dependent processes that aid biosorption.

DESORPTION

Desorption of loaded biomass enables re-use of the biomass, and recovery and/or containment of sorbed materials, although it is desirable that the desorbing agent does not significantly damage or degrade the biomass.[26] In some cases, desorption treatments may improve further sorption capacities, although in other cases there may be a loss of efficiency of the biomass. For operation of continuous flow systems, columns in parallel arrangements may allow sorption and desorption processes to occur without significant interruption. A variety of substances have been used as metal/radionuclide desorbents including acids, alkalis, and complexing agents depending on the substance sorbed, process requirements and economic considerations. In addition, there may be a means of selective desorption, e.g. for certain metals. Combustion and subsequent recovery of metal/radionuclides from ash ('destructive recovery') may also be a possibility. Dye-laden biomass can be eluted and regenerated by some organic solvents such as methanol, ethanol, surfactants and NaOH.[34] Distilled deionized water and $CaCl_2$, and NaOH has been used to desorb phenolic compounds and pesticides.[34]

CRITICAL ASSESSMENT OF BIOSORPTION RESEARCH

There has been an explosion of biosorption-related research in recent years (Figs 1 and 2). However, it is doubtful whether such a dramatic rise in published output has significantly improved knowledge of the process, or aided any commercial exploitation, which so often is the prime rationale for such work. Most studies involve characterization of a chosen biomass type in sorbing a given substance from solution, and the effect of physico-chemical parameters in affecting biosorption. Most such studies involve metals, although an increasing number involve organic pollutants. Since the majority of elements in the Periodic Table are metals, the possible number of 'original' studies is probably beyond comprehension if coupled with the huge numbers of microbial species, strains, derived products, etc. The output of publications shows no sign of abating and will be increased by the continuing numbers of new journals, including those that are web-based. Simple analysis of literature databases shows that a small number of journals account for a high proportion of published articles (Table 3).

CRITICAL ASSESSMENT OF EXPLOITATION POTENTIAL

The apparent exploitation potential of biosorption is often cited in the literature and used as a basis for rationale for the work to be carried out. Biosorption is quoted as being a low cost treatment method, and especially applicable where a 'low tech' approach may be a suitable option. Other quoted advantages include low

Table 3. The top twenty publications for articles in the ISI Web of Science database for 'All Years' (1970–2008) with 'Biosorption' in the topic (out of a total of 2862 articles appearing: database searched 18.4.08: percentage values listed to two decimal places only)

Publication	Record Count	Percentage
Journal of Hazardous Materials	211	7.37
Bioresource Technology	175	6.11
Process Biochemistry	137	4.78
Water Research	132	4.61
Journal of Chemical Technology and Biotechnology	74	2.58
Chemosphere	70	2.44
Water Science and Technology	69	2.41
Separation Science and Technology	64	2.23
Environmental Science and Technology	57	1.99
Environmental Technology	57	1.99
Applied Microbiology and Biotechnology	47	1.64
Biotechnology Letters	47	1.64
World Journal of Microbiology and Biotechnology	47	1.64
Hydrometallurgy	42	1.46
Biochemical Engineering Journal	40	1.39
Biotechnology and Bioengineering	39	1.36
Applied Biochemistry and Biotechnology	36	1.25
Chemical Engineering Journal	36	1.25
Minerals Engineering	29	1.01
Separation and Purification Technology	29	1.01

operating costs, minimization of the volume of chemical and/or biological sludge to be handled and high efficiency. As well as detoxification of pollutant metals, the recovery of precious metals such as gold, palladium and platinum is also a potential area for exploitation. There is rather less information published on such elements and some chemical differences may occur from other 'base' metals. Biosorption of base metal cations is pH dependent and usually takes place in the range pH 3–7. Gold and platinum group metals are routinely present in solution in anionic form, and as well as other anionic metal species, e.g. CrO_4^{2-}, they are bound most strongly at low pH or exhibit pH independent binding.[74]

Although several dead biomass-based systems have been evaluated at pilot scale, none have been significantly commercialized despite the impression given in many papers and reviews on the subject from many countries (Table 4). Biosorptive processes have been regarded simply as pseudo-ion-exchange processes where the metal/radionuclide species is exchanged for a counterion attached to the biomass.[163] Biosorption, in contrast, may involve more than one functional group on the biomass, and is often non-selective meaning that application to metal mixtures (a common occurrence in waste streams) would be problematic. As ion-exchange resins can be synthesized to have only one metal-binding functional group of high affinity, they are much more predictable for a given metal ion, and are more suitable for selective recovery of target substances. The lack of specificity and lower robustness of biomass-based systems compared with ion exchange resins are often cited as major reasons limiting biosorption commercialization.[163] Suspended biomass is not effective and durable in repeated long-term application, and also makes post-separation of suspended biomass from the treated effluent difficult.[142] Immobilized and/or granular biomass preparations

Table 4. Top 30 countries publishing papers with 'biosorption' in the topic as listed in the ISI Web of Science database for 'All Years' (1970–2008) (out of a total of 2824 articles appearing: database searched 7.4.08: percentage values listed to two decimal places only)

Country	Number of articles	Percentage of total
India	365	12.92
Turkey	330	11.68
China	271	9.59
USA	227	8.03
Canada	180	6.37
South Korea	142	5.02
France	121	4.28
Brazil	107	3.78
England	105	3.71
Spain	100	3.54
Japan	96	3.39
Taiwan	69	2.44
Germany	65	2.30
Greece	61	2.16
Italy	61	2.16
Poland	50	1.77
Portugal	50	1.77
Australia	45	1.59
Pakistan	45	1.59
Singapore	42	1.48
Egypt	38	1.34
South Africa	36	1.27
Czech Republic	35	1.23
Malaysia	35	1.23
Scotland	35	1.23
Thailand	34	1.20
Mexico	30	1.06
Iran	29	1.02
Northern Ireland	29	1.02
Ireland	27	0.95

may overcome the robustness and separation issue, but still do not overcome the specificity problem. It should also be noted that (bio)sorption technology transfers the sorbate from one medium to another, and so also raises questions regarding the safe disposal of loaded biosorbent, sorbate recovery, and regeneration or replacement of the biosorbent.

COMPETING TECHNOLOGIES

Common procedures for removing metal ions from aqueous streams include chemical precipitation, ion exchange, oxidation/reduction methods, solid/liquid separation, reverse osmosis and solvent extraction (Table 5).[163] Adsorption, using granular activated carbon (GAC) has also been examined but is expensive and may not be efficient for all metals. Reverse osmosis is a process in which contaminated water is forced through a semi-permeable membrane through which the water can pass but not the contaminating solutes. One disadvantage of this method is that it is expensive. Electrodialysis separates ionic components through semi-permeable ion-selective membranes. Application of an electrical potential between two electrodes causes migration of cations and anions towards respective electrodes. With alternate spacing of cation- and anion-permeable membranes, cells of

concentrated and dilute salts are formed. The main disadvantage of this method is the formation of metal hydroxides, which clog the membrane. Ultrafiltration involves pressure-driven membrane filtration for the removal of, for example, toxic metals, but the main disadvantage of this process is again the generation of sludge. Ion-exchange is often the method of choice, especially in the nuclear industry, and is where metal/radionuclide species from dilute solutions are exchanged with ions held by electrostatic forces on a suitable ion exchange resin. Some disadvantages include high cost and only partial removal of certain ions. However, unlike biomass-based biosorption systems, they have the capacity to be highly selective. Chemical precipitation is achieved by the addition of coagulants such as alum, lime, iron salts and other organic polymers, with a large amount of sludge containing toxic compounds being produced. Solvent (or liquid) extraction depends upon the selective dissolving of one or more constituents of the contaminated solution into a suitable immiscible liquid solvent. The simplest and cheapest method of removing most metals from solution is to increase the pH, converting the soluble metal into an insoluble form (i.e. hydroxide). Precipitation by adjusting the pH is, however, not selective and precipitation by alkali addition (usually lime) produces large quantities of solid sludge for disposal. However, precipitation processes can be highly efficient.[163] The performance characteristics of some heavy-metal-separation technologies are presented in Table 1. The kind of process used will depend on the substances to be treated, and the target effluent concentrations. Overall process costs, both operational and capital, will be influenced by several criteria, such as versatility and simplicity. Some processes, e.g. precipitation and ion exchange, have been incorporated into many well-established industrial processes and demonstrated on a large scale, are predictable and well understood.[163]

Some of the above disadvantages like incomplete metal removal, high reagent and energy requirements, generation of toxic sludge or other waste products that require careful disposal have often been used as the basis for arguments supporting a cost-effective biological approach (Table 5).[2,14,163] Biosorption as an alternative or adjunct biotechnology has often been proposed in this context, yet, ironically, has probably had the least success in exploitation.

BIOSORPTION IN THE ENVIRONMENT

In natural systems, bioavailability of nutrients, including essential metals, and pollutants is determined by interactions with environmental components. In terrestrial and aquatic ecosystems, such components include clay and other minerals, humic substances, soil colloidal materials, biogenic debris and exudates, and living organisms. Sorption is one of the most important reactions that influences bioavailability, and therefore biosorption must also have a role to play within the spectrum of sorptive interactions with environmental components.[164] These will include influencing distribution of substances between aqueous solution and particulate matter (including microorganisms) and their transport through environmental compartments and ultimate fate, e.g. settling in ocean sediments. Sorption of metals to cells is likely to play a critical role in all microbe–metal–mineral interactions. Interactions with specific groups on the surface of the cell may also enhance or inhibit metal transport and, thus, metal transformations and biomineralization processes.[165]

In natural systems, sorption–desorption processes can occur over wide time scales, ranging from milliseconds to years, with

Table 5.   Performance characteristics of toxic metal removal and recovery technologies. (GAC, granulated activated carbon) (Adapted from Eccles[163])

Technology	Performance characteristics				
	pH change	Metal selectivity	Influence of suspended solids	Tolerance to organic molecules	Working level for appropriate metal (mg l^{-1})
Adsorption (e.g. GAC)	Limited tolerance	Moderate	Intolerant	Can be poisoned	<10
Electrochemical	Tolerant	Moderate	Can be engineered to be tolerant	Can be accommodated	>10
Ion Exchange	Limited tolerance	Selective	Intolerant	Can be poisoned	<100
Membrane	Limited tolerance	Moderate	Intolerant	Intolerant	>10
Precipitation					
- Hydroxide	Tolerant	Non-selective	Tolerant	Tolerant	>10
- Sulphide	Limited tolerance	Limited selectivity	Tolerant	Tolerant	>10
Solvent extraction	Some systems tolerant	Selective	Intolerant	Intolerant	>100

lower reaction rates being due to such factors as diffusion into micropores, the existence of sites with low reactivity and surface nucleation-precipitation.[30] This is often seen in soils, although just as in simple laboratory biosorption systems, biphasic sorption kinetics may be observed with an initial 'rapid' phase being followed by a slower phase occurring over longer time periods, progressing towards equilibrium.

The major biosphere compartments, e.g. soils, oceans, contain material with high surface area to volume ratios and, of course, microorganisms like bacteria have the highest surface area : volume ratios of any living organism. Microorganisms are major components of the soil environment while biogenic particles dominate detrital phases in the oceans.[28] In fact, several studies have shown that microbial cells, on a specific unit area basis, can exhibit higher sorption values for metals than, e.g. clay minerals which are one of the most important metal-sorbing components in soils.[54,166]

It may be concluded that biosorption phenomena have a more significant role in metal/radionuclide speciation, bioavailability and mobility in aquatic and terrestrial environments than has previously been supposed.[85,167–169] Further, it should be emphasized that accompanying nucleation and precipitation can lead to biomineral formation.[1,164]

BIOSORPTION IN ESTABLISHED WASTE TREAT-MENT PROCESSES AND BIOREMEDIATION

Biosorptive processes may be a component of varying significance in any form of primary or secondary biological treatment process for aqueous waters and process streams including domestic, municipal and industrial wastes, and in some circumstances, solid wastes. Sewage treatment, activated sludge plants, biofilters, biofilm reactors, fixed and suspended film systems, lagoon treatments, stream meanders, nitrification and denitrification treatments, biological phosphate removal processes, wetlands and reed-bed technologies, composting, in situ and ex situ bioremediation processes all rely on the activities of microorganisms to break down organic substances. Many wastes contain metals as well as organics and therefore biosorption of metals and related materials may also play a part in the overall process. The significance of such a role is difficult to establish but it may be significant in some cases: the problem of metal-laden sewage disposal is well known. In biological treatment processes, a clear advantage is that metabolic activities contribute to the degrada-

tion of organic materials. Some examples have been described where organic and inorganic transformations are closely linked to biosorptive removal, e.g. rotating biological contactors to treat dilute metal-containing mine waste streams.[11]

CONCLUSIONS
Biosorption is a ubiquitous property of living or dead biomass and derived products, and is undoubtedly an important process in the environment, and in several conventional waste treatment processes. It has been proposed as a cheap and effective biotechnology for many years, yet has had extremely limited industrial exploitation to date, even as an addition to conventional pollutant treatment approaches in hybrid technologies. Biosorption is frequently compared with ion exchange technology[11,22,163] and often stated to provide a cheaper alternative.[22] However, as mentioned previously, specificity is a problem while biosorbents also exhibit a shorter life cycle.[22] Common suggestions for future research directions include identification of better and more selective biosorbents, more development of biosorption models and identification of biosorption mechanisms, and further assessments of market size, and costs of development. After so many years of biosorption research, it is debatable whether any more efforts in these directions will result in significant developments or novel contributions to understanding. Despite the apparent advantages of biosorption, it is ironic that many established and novel biotreatment methods for pollutants rely on living cell systems.[11] It is possible that biosorption may be combined with such systems in the future,[16] although at present this seems unlikely. The development of specific metal-binding molecules and/or engineered highly-specific biosorbents was heralded as a promising research direction,[11] although there seems to have been little progress in industrial application. More recently, biosorption has been proposed for the purification and recovery of high-value proteins, steroids, pharmaceuticals, etc. by identifying a system that is highly selective for a given substance.[22] This seems speculative in view of the general non-specificity of biosorption for both inorganic and organic substances, although the application of monoclonal antibodies for selective protein sorption has been suggested as one example.[22] While it can be concluded that the rationale for many biosorption studies is rather weak, especially if based on commercial development and application, the importance of biosorption in the environment and conventional biotreatment processes perhaps suggests further research should be directed in these areas.

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