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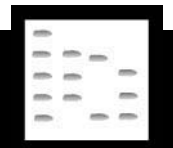
METAL UPTAKE ON MICROORGANISMS AND BIOMATERIALS: ION EXCHANGE



H. Eccles, British Nuclear Fuels, Preston, UK

Introduction

Microorganisms and biomaterials can be used for the removal/recovery of metals from process liquors and liquid wastes. The mechanisms involved in capturing metals from solution by microorganisms and related materials are now commonly referred to as biosorption. Biosorption has been defined as the removal of metals or metalloid species, compounds and particulates from solution by biological material. It is one of the fields in environmental biotechnology, which is itself a small, but growing, component of the biotechnology industry (**Figure 1**).

The use of microorganisms to treat waste liquors on a commercial scale dates back to the end of the nineteenth century when the first communal sewage plants in Berlin, Hamburg, Munich, Paris and other major cities came into operation. In the intervening 100 years the use of microorganisms and plants to protect the environment has developed into a multi-billion dollar (US$) industry. However, the foundations of this environmental biotechnology industry are still with the treatment of municipal/domestic effluents.

Although nature has demonstrated some subtle and intricate mechanisms for selectively controlling the mobility of pollutants in the environment, the conversion of this science to technology and to application has been very disappointing. In explaining this lack of application, it is important that these mechanisms

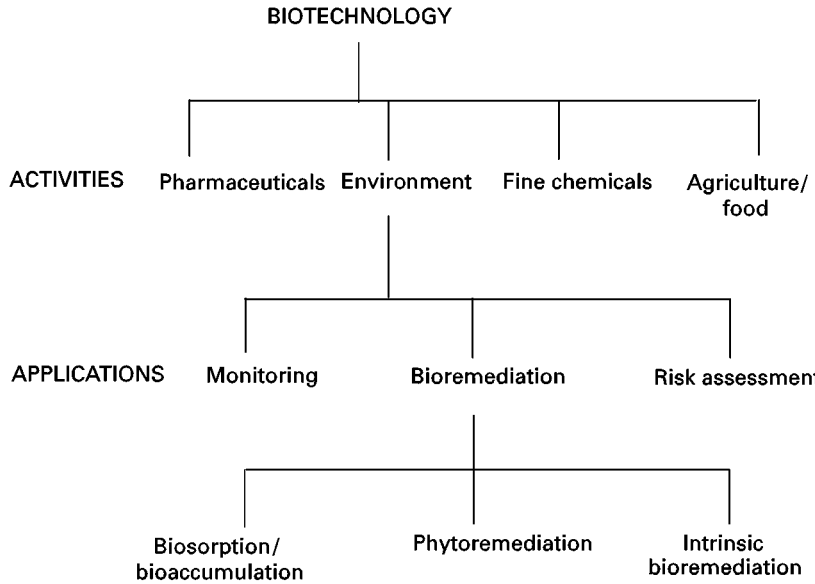


Figure 1 Biotechnology activities and applications. (This is an original developed by H Eccles.)

are appreciated; factors that influence their effectiveness need to be addressed to engineer robust and reliable processes.

Microorganisms and Biomaterials for Metal Removal

The biological systems studied for metal removal/recovery range from living microorganisms and dead cell systems to other biomaterials such as peat, coconut shells and eggshell membrane. All these systems have at least one common characteristic, namely their absorptive surfaces, and (in the case of living microorganisms within the cell structure) have at least one chemical moeity (functional group) that has an affinity for metal(s). This affinity can be accomplished by active and passive mechanisms that may act singly or in combination. The mechanisms can be microbiologically dependent, for example active transport across the cell membrane, or physicochemical and chemical-dominated reactions such as adsorption, cation exchange, complexation, chelation and precipitation. Essentially, active transport is a function of living cells, such as bacteria, algae and fungi. The remainder of the mechanisms are passive and may occur with living or dead cells or with other biomass.

Passive uptake mechanisms generally occur at the cell wall level (see **Table 1**) and the biomasses involved are generally tolerant to their environment. In their interactions with metals, dead cell systems and biomaterials behave as surrogate ion exchange materials. Ion exchange is a proven commercial technology that is widely used for the removal of metals from various liquors. Where major differences occur between biosorption and conventional ion exchange is when living microorganisms are involved, which results in the participation of other metal-removal mechanisms.

The basis for these differences and also for variations in metal-accumulation abilities is partly the result of cell surface characteristics. Microbial cell walls are complex and are normally charged. Certain cell wall properties, such as the type of polar groups present and the charge within the cell wall macromolecules, may influence the metal capacity and selectivity. The types of macromolecules present in cell walls for bacteria, fungi and yeast are shown in **Table 2**.

Table 1 Cell wall metal functional groups

Cell wall functional group	*Metal affinity*
Carboxyl	Ca, Mg, Cu, Zn
Imidazole	Cu, Pb
Sulfhydryl	Zn
Amino	Co, Ni, Cu
Phosphate	Ca, Mg, Fe, U
Sulfate	Ba, Ca, Sr
Thioether	Cu(I)
Amide	Cu, Co, Ni, Fe
Hydroxyl	Ca, Pb, Cu, Sr, Ba, Ni, Co, Zn

Table 2 Cell wall macromolecules

Cell wall macromolecules	*Microorganism*
Polysaccharides, proteins, lipids, peptidoglycan	Bacteria
Mannan polysaccharides, chitin, galactosamine, proteins, lipids	Algae and fungi

Classification of bacteria, algae and fungi is based on aspects other than cell wall composition. Living organisms can be divided into two groups, prokaryotes and eukaryotes, based on cell structure. Bacteria belong to the prokaryote group, while algae and fungi belong to the eukaryotes. Further simple distinctions are that bacteria lack a nucleus and membrane-bound organelles and most bacteria have cell walls. Bacteria are without question the oldest and simplest organisms. Eukaryotic organisms have both a nucleus and membrane-bound organelles. Fungi are distinct from algae in that they have filamentous growth, lack chlorophyll and motile cells, and have chitin-rich cell walls (**Figure 2**).

Although these simple descriptions may be helpful in understanding the different behaviour of bacteria, fungi and algae with metals, they are by no means rigorous biological definitions, and the purist microbiologist may find them too superficial. It is probable, however, that the more rigorous and sophisticated explanations have contributed to the restrictive use of biotechnology in the environmental arena. This point will be discussed in more detail later.

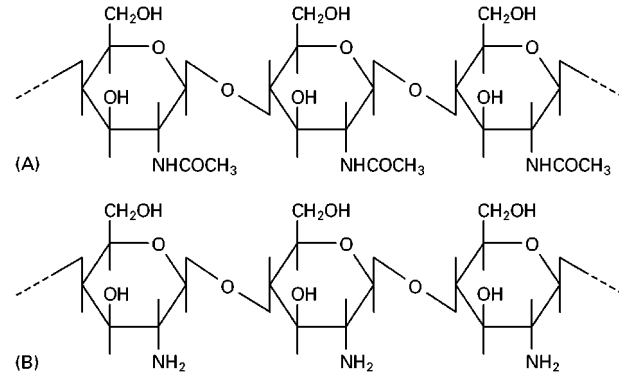


Figure 2 Chemical structure of (A) chitin and (B) chitosan. (Hardman DJ, McEldowney S and Waite S (eds), *Pollution: Ecology and Biotreatment* (1993), p. 284, Addison Wesley Longman Ltd, UK.)

The complex nature of cell walls bestows some unique abilities on living microorganisms and to a lesser extent dead cell systems and biomaterials. Equally this complexity confers some serious drawbacks, as modelling of metal-removal mechanisms and predicting process efficiencies are extremely difficult when more than one metal functional group is available.

Notwithstanding these reservations, numerous microorganisms and biomaterials have been evaluated for a diversity of metals by many scientists worldwide. Some of the more definitive work is discussed in the following sections.

Dead Cell Systems and Biomaterials

Dead cell systems can be derived from living cells by subjecting them to a physical or chemical method to terminate the living cell metabolic activity. As growth conditions can confer some metal affinity characteristics on living cells, it is prudent to explain briefly the growth phase and the various parameters that may be controlled to achieve the desired goal. The more important growth phase conditions that can confer metal adsorption characteristics are:

1. time of cell harvesting;
2. composition of nutrient medium.

The growth of bacterial populations is normally limited either by the exhaustion of available nutrients or by the accumulation of toxic products of metabolism. This is particularly true for batch growth conditions. As a consequence, the rate of growth declines after the exponential phase and growth eventually stops. At this point a culture is defined as being in the stationary phase (**Figure 3**). The transition between the exponential phase and the stationary phase involves a period of unbalanced growth during which the various cellular components are synthesized at unequal rates. Consequently, cells in the stationary phase have a chemical composition that is different from that of the cells in the exponential phase. In general, cells in the stationary phase are small relative to cells in the exponential phase and they are more resistant to adverse physical and chemical agents.

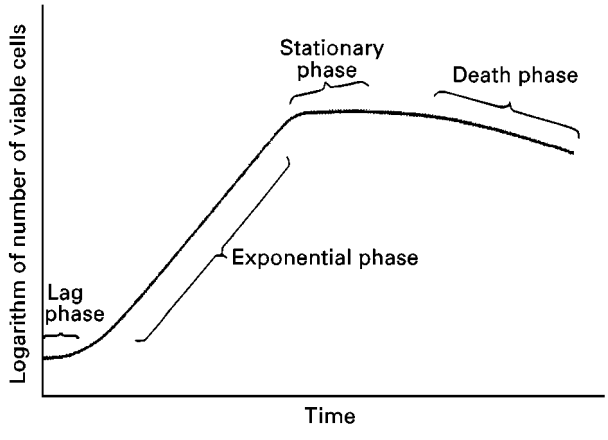


Figure 3 Generalized growth curve of a bacterial culture. Stanier RY, Ingraham JL, Wheelis ML and Painter PR (eds) *General Microbiology* (1986), 5th edition, p. 185, Prentice-Hall Inc, NJ.)

Bacterial cells held in a nongrowing state eventually die, largely due to the depletion of the cellular reserves of energy. When cells are transferred from a culture in the stationary phase to a fresh medium of the same composition they undergo a change of chemical composition before they are capable of initiating growth. This period of change is called the lag phase.

Culture age affects the biosorption properties of microorganisms. For example, younger cells (12 h growth) of *Saccharomyces cerevisiae* removed approximately five times more uranium than older cells (24 h-growth).

The surface charge of living cells can vary both their age and with the nature and composition of the growth medium. With respect to metal adsorption, surface charge will have a predominant, if not the prime, influence. The cell wall surface charge is itself strongly affected by the pH value of the growth medium. This pH influence is due to the different ionogenic groups at the cell surface being susceptible to protonation/deprotonation reactions. Experimental evidence involving bacterial cells shows that carboxyl groups are present in excess over amino groups, and thus they dominate electrokinetic behaviour.

Physical methods that have been used to kill living cells include vacuum and freeze drying, boiling, autoclaving and mechanical disruption such as ultrasonics. Chemical methods include contacting the cells with various organic and inorganic compounds. The main aim of controlling the growth conditions coupled with the appropriate killing stage is to produce a biomaterial that has metal affinity properties superior to those of the parent living cell. The advantages of dead cells over living cells are:

- the metal removal process is largely independent of toxicity limitations;
- there are no requirements for growth media and nutrients;
- biosorbed metals can be eluted and the dead cells re-used;
- there is an ample and ready supply of some biomasses (dead cells);
- pretreatment of the biomass can enhance the metal biosorptive characteristics;
- the process is simpler and akin to ion exchange;

Table 3 Pretreatment methods to improve metal selectivity

Biomass type	*Pretreatment method*	*Metal studied*
Penicillium digitalum	0.1 mol L^{-1} sodium hydroxide	Ni, Cu, Zn, Cd, Pb
	Contacted with dimethyl sulfoxide for 90 min	U
	Trichloroacetic acid	U
Saccharomyces cerevisiae	10% (v/v) nitric acid, 30 min boiling	Cu
	10% (w/v) formaldehyde	Cd, Zn
	Detergent	Th
Aspergillus niger	Freeze-dried	U
	5% KOH	Cu, Cd, Zn, Co, Ni

- disposal of spent and/or excess nutrient media or surplus cells does not present a problem;
- the shelf-life of the dead biomass is nearly infinite.

If the required metal affinity characteristics have not been achieved at the harvesting stage and by the process used to kill the living cells, the dead biomass can be treated to enhance the key metal biosorptive properties such as metal capacity, metal selectivity and rate of uptake.

In **Table 3** are reported some of the pretreatment methods employed and their effect on metal removal. Alkali treatment of fungal biomass has been shown to increase significantly the metal sorption capacity of *Aspergillus*, *Mucor* and *Penicillium*, and deacetylation of chitin in the cell wall to form chitosan–glucan complexes results in higher affinity for metal ions.

In what follows dead cell systems and biomaterials will be regarded as being equivalent, but any specific subtle differences will be highlighted. The biomasses that have attracted most attention are those that are readily available in significant quantities, with or without pretreatment to enhance their metal uptake capability. To date these materials have included: peat, lignates, seaweed waste (dealginated seaweed), yeast from brewing operations, algal and fungal biomasses and activated carbon from a variety of sources.

The last material is presently used commercially in the treatment of water for the removal of nonmetallic species; numerous papers have been published on the uses of activated carbon. In evaluating the above materials one feature regularly considered has been their particle size and the implications to scale-up. In their behaviour to metals these materials resemble ion exchange resins, which are invariably packed into columns. Thus laboratory studies have included the immobilization of biomaterials into particles of appropriate shape and size. Spherical particles 1–3 mm in diameter appear to be acceptable.

As the metal-removal processes are passive and of a chemical/physicochemical nature, the parameters considered are like those for ion exchange, namely pH, temperature, competing cations and metal speciation. These parameters are equally important to metal removal by living cell systems. Without doubt, pH is by far the most dominant influence on metal uptake. The optimal pH values for a variety of metals and biomaterials are presented in **Table 4**.

The efficiency of metal removal by the biomaterial as a function of pH will be related to: (1) the functional group involved and (2) metal speciation chemistry. At low pH values, i.e. about 3 or less,

Table 4 Optimal pH values for metal uptake by various biomaterials/microorganisms

Biomaterial / microorganism	*Optimal pH value for metal removal*	*Metal(s)*
Peat	1.5	Cr(VI)
	5.0	Cu(II)
	7.0	Ni(II)
Seaweed	4.0	Au
	5.0–6.0	Cu, Zn, Pb, Cd, Cr, Ni, Fe
S. cerevisiae	1.5	Mo(V)
	3.0–4.0	U(VI)
Penicillium digitatum	7.0	Cd
Bacillus subtilis	4.1	U
Chlorella salina	4.0	Tc(VII)
	8.0	Co

metal(s) uptake will be comparatively low as the metal(s) will be in competition with the hydrogen ion and most functional groups with an exchangeable hydrogen ion will be undissociated. On increasing the pH, competition with the hydrogen ion diminishes, dissociation of the hydrogen of the functional group increases and the speciation of the metal(s) changes from being a hydrated cation to hydroxy metal species thus:

$$M(H_2O)_4^{2+} \xrightarrow[\text{increasing pH}]{} M(H_2O)_3(OH)^+ + H^+$$

This progressive displacement of the inner sphere of water molecules with increasing pH results in the metal ion becoming more amenable to adsorption by the biomaterial.

Temperature increases within a modest range, i.e. 20°C to 60°C, have little or no effect, either thermodynamically or kinetically, on metal removal for most biomaterial and metal systems. In general, optimal metal uptake will occur at 25°C for most dead cell systems and at a similar value (25–30°C) for biomaterials.

Metal affinity by many dead cell systems measured using single metal systems has been shown to comply with the Irving–Williams series. Most dead cell systems exhibit selectivity for heavier metals, such as Zn, Cu and Ni, over the lighter alkali metal ions. The selectivity of dead cell systems and biomaterials for a particular metal is highly dependent on the pH value of the solution under investigation and the functional group involved in the metal adsorption process. The determination of metal selectivity for dead cell systems and biomaterials is complicated in that more than one functional group may be involved in the biosorption process. These interactions (biosorption processes) may operate independently or may be interrelated. For example, Tsezas and Volesky indicated that three mechanisms are operative in uranyl ion biosorption by *Rhizopus arrihizus*. Two of the mechanisms occur simultaneously and rapidly (< 60 s to equilibrium). In the first process, uranyl ions coordinate with the amino nitrogen of the cell wall chitin, facilitating the second process in which the complexation sites acted as nucleation points for deposition of additional uranium. These two mechanisms accounted for 66% of the total uptake capacity (0.05 mmol U per g cell dry weight). The third process is considerably slower, only reaching equilibrium after 30 min, and involves the precipitation of uranyl hydroxide within the cell wall microcrystalline chitin.

Although most of the available literature is concerned with single metal experiments, a limited number of studies have involved binary metal systems or multimetal solutions. These studies demonstrate that copper generally has the highest binding capacity and exerts the largest competing effect.

The presence of anions in the metal solution under consideration will have an impact on metal biosorption, depending on the concentration of the anion and its metal-complexing ability. The more common anions, such as nitrate, chloride and sulfate, rarely affect the biosorption of metals when in near stoichiometric concentrations to the metal. Increasing their concentration will affect the biosorption (lower it), but this will depend on the ability of the metal to form anionic species. When the anion species such as $EDTA^{2-}$, phosphate, citrate, etc., is capable of coordination/complexation with the metal, the metal capacity will be strongly reduced.

Living Cell Systems

Living cells systems offer some unique and subtle metal-accumulation mechanisms not possible with dead cell systems, biomaterials or conventional ion exchange resins. These active metal-accumulation mechanisms, in addition to the passive ones, are generally coupled with the metabolic activity of the algal, bacterial, fungal living cells.

Many organisms have developed detoxification mechanisms to overcome the detrimental effects of metals. For the purpose of this article two mechanisms will be taken as predominant, namely bioaccumulation and bioreduction. Although these mechanisms are generally encompassed by the term biotransformation, this terminology is far more appropriate to the treatment of organic pollutants using microorganisms, as these pollutants can be biodegraded to bengin metabolic end products such as carbon dioxide and water. Metals are persistent and their toxicity is infinite. Microorganisms can only affect their physical and/or chemical state and transform them into more immobile forms that are less bioavailable to plants and other higher organisms. In achieving the biotransformation of metals, microorganisms are behaving as minute chemical factories generating chemicals, for example hydrogen sulfide, alkali and inorganic phosphate, or providing electrons from interconnected redox processes. These processes are summarized in **Table 5**. One process in particular will now be described in more detail.

In the natural environment the major mechanism for bacterial metal precipitation is through the formation of hydrogen sulfide and the immobilization of metal cations as metal sulfides. The bacteria involved in this process are the sulfate-reducing bacteria including members of the genera *Desulphovibrio*

Table 5 Metal bioaccumulation and bioreduction processes

Process	*Metal-removal mechanism*	*Microorganism*
Bioaccumulation	Sulfide precipitation	Sulfate-reducing bacteria
	Phosphate precipitation	*Citrobacter* sp.
	Hydroxide precipitation	*Alcaligenes eutrophus*
Bioreduction	Hydroxide/oxide precipitation	*Shewanella alga*
	Sulfide precipitation	Sulfate-reducing bacteria

and *Desulphotomaculum*. Sulfate-reducing bacteria (SRB) are widely distributed in anaerobic environments such as sediments and bogs. Sulfide production by sulfate-reducing bacteria is a consequence of their energy-generating processes. They couple the reduction of oxidized forms of sulfur, e.g. sulfates and sulfur, with the oxidation of reduced carbon in the form of simple organic molecules such as lactose or ethanol. The optimal chemical environment for effective sulfate reduction to sulfide is a pH value between 5.5 and 6.5 with a negative redox value of about 200–400 mV.

One of the underpinning features for the effectiveness of a SRB metal-removal process is the extremely low solubility product value for metal sulfides, as reported in **Table 6**. Other process advantages are:

- removal of metal anions such as chromate with initially the reduction of Cr(VI) to Cr(III) by the biogenic hydrogen sulfide;
- the ability of the microorganisms to nucleate the sulfide precipitation and thus assisting in the coagulation of metal sulfide particles;
- maintaining (slightly increasing) chemical neutrality of the process sulfate liquor due to the carbonate formed from oxidation of lactose or ethanol, or other suitable carbon sources, and sulfide from reduction of sulfate;
- the ability to remediate inorganic pollutants, for example toxic heavy metals and sulfate, as well as organic pollutants.

Table 6 Solubility product values for metal sulfides and hydroxides at 25°C

Metal	*Hydroxide*	*Sulfide*
Ag	2×10^{-8}	1.6×10^{-49}
Cu(II)	1×10^{-20}	8.5×10^{-45}
Zn	1×10^{-17}	1.2×10^{-23}
Ni(II)	1×10^{-15}	1.4×10^{-24}
Co(II)	1×10^{-15}	3.0×10^{-26}
Fe(II)	1×10^{-15}	3.7×10^{-19}
Cd(II)	1×10^{-14}	3.6×10^{-19}

These advantages may have been behind the development by Shell Research Ltd. of a biological process in preference to chemical ones for the removal of zinc, cadmium and sulfate from contaminated groundwater accumulating below a zinc smelter site. This process is the only biological metal-removal system employing specifically SRB operating on a commercial scale. The reasons for this unique situation is addressed in the next section.

The active metal biotransformation processes that rely on the precipitation of the offending metal(s) are not selective. The microorganisms require carefully controlled conditions both for their growth and to maintain enzyme activity, which are usually pH values ranging from 5.0 to 7.0, slightly aerobic 0 to $+200$ mV or anaerobic -200 mV to -400 mV.

Metals capable of forming insoluble hydroxides/oxides, phosphates or sulfides will precipitate effectively under these conditions. Consequently, competing cations that form such insoluble materials are only a problem in that they consume the precipitant, which in turn generally requires more carbon substrate. Interfering anion conditions will apply, similar to those already discussed above.

Although dead and living cell systems have been shown to have some unique capabilities for metal removal, the transfer to commercial technology has been very limited. The reasons for this will now be discussed.

Engineering and Process Considerations

Although dead cell systems, biomaterials and living cells have some distinct advantages compared with conventional ion exchange resins in the removal of metals from aqueous waste liquors, the number of recorded pilot-plant and commercial processes is extremely small, as illustrated in **Table 7**.

It would appear that even the rapid metal uptake by dead cell systems (less than 60 s for outer wall adsorption) combined with potentially cheap biosorbents and greater versatility of living cell systems such as SRB, capable of accommodating more than one

Table 7 Biological metal-removal pilot plants and commercial facilities

Process	*Biomaterial/ microorganism*	*Target metal(s)*	*Process conditions*
AMT-Bioclaim	*Bacillus subtilis*	Pb, Zn	Pilot plant (PP) Capacity 20 BV h^{-1} Columns 18 L BV^{-1}
Alga SORB	Algae	Cd, U, Pb, Hg	PP Capacity 10 BV h^{-1} Columns 0.4 L BV^{-1}
BIOFIX	Various biomasses	Ni, Zn, Mn, Cd	PP Capacity 30 BV h^{-1} Columns 14.3 L BV^{-1}
Shell Chemicals	Sulfate-reducing bacteria	Zn, Cd	PP Capacity 3 m^3 h^{-1} USAB 12 m^3 Commercial scale Capacity 300 m^3 h^{-1} USAB 1800 m^3

BV, bed volume; USAB, upflow anaerobic sludge blanket reactor.

pollutant such as sulfate and metals, are generally insufficient to convince potential users to install, or even consider, a biological process in preference to a chemical/physicochemical process.

In considering process technologies for removal of metals from waste liquors, certain criteria need careful and critical appraisal. These process criteria and their implications to biological systems are briefly reviewed.

Robustness

Dead cell systems and other biomaterials exhibit similar robust chemical properties to conventional ion exchange resins. They lack equality, however, because of their mechanical properties. Living organisms are significantly less robust as it is crucial to maintain cell growth and/or metabolic activity, which requires a carefully controlled environment. It is however possible, by judicious design, to arrange the biological stage of the process remote from and/or independent of the metal removal stage.

Selectivity

The metal selectivity of dead cell systems and biomaterials can be significantly improved by a variety of chemical treatments, but obviously at a cost and in some instances to the detriment of other process considerations, e.g. chemical treatment may result in a lowering of the total metal affinity.

However, metal selectivity may not be a prime consideration as effluents often contain several offending metals, in which case living cell systems are more appropriate as they are less discriminatory.

Compatibility

By definition effluent treatment processes have to be compatible with upstream, and in some instances downstream, operations. In many circumstances the compatibility requirement is exacerbated as effluent processes are retrofitted. Chemicals used in the biological metal-removal processes such as regeneration liquors for dead cell/biomaterial systems and excess sulfide and/or carbon substrate in SRB processes will require careful examination, in particular when water from the treated effluent is to be recycled.

Reliability

Site effluent treatment systems operate continuously, virtually 365 days a year. Biological processes are equal in reliability to their chemical competitors but may require greater control to ensure that environments are maintained, in particular for living cell metal removal processes.

Reliability can be enhanced by the installation of duplicate facilities and/or buffer storage, but with a cost penalty.

Simplicity

Modern automation has allowed effluent treatment facilities to be left unattended for significant periods. The greater the simplicity of metal removal, the lower the automation requirements. Dead cell/biomaterial systems are no more complicated than ion exchange resin processes, but this is not the case for living cells. Ensuring the activity of appropriate enzymes requires added process considerations.

Predictability

At the outset, effluent treatment designers will have to be confident that appropriate scientific and engineering information is available to meet all eventualities, in particular maloperation of the facility and the environmental impact this may have.

When more than one metal-removal mechanism may be participating, either synergistically or antagonistically, such information may not be readily available. Even when this information is available, predictability becomes more complex and difficult. Dead cell/biomaterial systems may fall into this category.

Efficiency

The biological process should have high metal-accumulation capacity and accumulation should be sufficiently rapid and efficient to compete with those of conventional technologies. In order to be competitive, the process should remove at least 99% of the target metals. There is clear evidence that biological systems can compete in efficiency with existing technologies, some reaching a sorption capacity of greater than 200 mg per g dry weight of dead cell systems.

The rate of heavy metal accumulation by biosorbents also compares favourably with existing separation techniques.

Versatility

Since waste streams are highly variable, the efficiency of metal removal should ideally be unaffected by other waste stream constituents and should be relatively stable to variations in pH. This presents one of the biggest challenges in the development of liquid waste treatment. The effect of pH on metal removal is often significant and varies with the biomass and metals. The presence of inorganic and organic components other than the target metal or metals common in waste streams. Such components have often been found to alter the efficiency of sorption. There are several possible mechanisms for this effect, ranging from direct competition for the binding sites resulting in lower uptake of target metal(s), to organic pollutants forming soluble complexes with metal(s) and thus reducing removal efficiency.

Economics

The dead cells/biomaterials and living cells should be cheap to grow and/or harvest. Clearly it is economically desirable to utilize waste biomass or material from other processes since the production cost will be reduced. Waste biomass is produced from a number of industrial processes (**Table 8**) but the use of waste biomass should not, however, be at the expense of process efficiency.

Table 8 Sources of waste biomass for use in heavy metal removal

Waste biomass	*Source*
Activated sludge, digested anaerobic sludge	Wastewater treatment
Saccharomyces cerevisiae (yeast)	Brewing
Bacillus subtilis (Gram-positive bacterium)	Enzyme production
Penicillium chrysogenum (fungus)	Penicillin production

Realizing the Potential of Biosorption/Bioaccumulation of Metals

The understanding of the interactions of microorganisms with metals for a variety of applications such as health care, environmental protection and process technology (biocatalysis) has been pursued for nearly half a century. In this time numerous microorganisms have been isolated, characterized and evaluated for a diversity of metals. Notwithstanding this colossal effort, to date there are few installations, possibly no more than five major ones, that use microorganisms (excluding activated sludge processes) to remove and/or recover metals from waste waters/liquid wastes. Why is this? First, we need to consider what microorganisms are capable of – they are no different to their chemical counterparts, in that they cannot, for example, convert lead to gold or 'eat' plutonium! The perception of microorganism capabilities for dealing with metal pollutants is for the nonbiologists clouded by the great successes, reported worldwide, that these minute chemical factories have secured in dealing with oil spillages and land contaminated with a variety of organic pollutants. Without a doubt the microbial degradation of such pollutants is truly the 'green ticket', assuming of course that these pollutants are ultimately and quickly degraded to carbon dioxide and water.

One major reason hampering potential is the approach taken by microbiologists. In the past, and in many instances even today, screening for new microorganisms has been a major preoccupation and many person-years effort are expended in the laboratory with little thought as to how these microorganisms can/will be engineered into a technological process. The interaction between microbiologists and

workers in other scientific disciplines, in particular chemists, is now more strongly evident, largely because an array of scientific techniques is needed to characterize the microorganisms. The involvement of engineers is still lacking and consequently key questions are missed or omitted when considering the potential of microorganisms to treat complicated waste streams. Many engineers are surprised at the microbiologist's approach in tackling a seemingly new pollution problem. The technique of screening the polluted environment for thriving microorganisms is logical to the microbiologist, but curious to the engineer, who may not understand the subtleties of genera and strains.

It is this fusion of scientific and engineering approaches that is needed to enable bioremediation, and hence environmental biotechnology, to achieve its true potential.

Environmental legislation is now stringent and is likely to become even more so in the future. In this situation it is important not only that the process technology is understood, but also that the implications and consequences of perturbations to this technology can be accurately predicted. With environmental processes perturbations will undoubtedly arise, as to date there is no specification for effluents that is definitive.

Unfortunately, in the present commercial environment, the quest for scientific knowledge is too often perceived as no longer valuable or affordable. In this respect the success of biotechnology in other areas, e.g. pharamaceuticals, may well have a positive benefit to other markets, persuading nonscientists that knowledge and intellectual property is valuable and ignorance is unaffordable.

See also: **II/Ion Exchange:** Theory of Ion Exchange. **III/Biological Systems: Ion Exchange. Resins as Biosorbents: Ion Exchange.**

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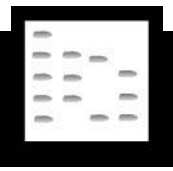
METALLOPROTEINS: CHROMATOGRAPHY



E. Parisi, CNR Institute of Protein Biochemistry and Enzymology, Naples, Italy

Classification and Characteristics of Metalloproteins

Metals are known to play essential roles in catalysis, macromolecular structure and membrane stabilization as well as hormonal and genetic regulation. Metals present at very low concentrations in tissues and biological fluids are termed oligoelements. Usually they do not occur in the biological matter as free ions, but as metal–protein complexes. The term metalloprotein is used to define a large group of proteins containing one or more atoms of metal bound to specific sites in the polypeptide chain. The binding sites on the protein are provided by histidine nitrogens, glutamate or aspartate oxygens and cysteine sulfurs; the metal ligand is usually represented by calcium, selenium, iron, zinc, copper and other heavy metals.

Metalloproteins can be divided into two groups: biologically active metalloproteins and proteins with