
Heavy metals removal from industrial wastewaters by biosorption

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Abstract: The use of biological materials for removing metals and, possibly, recovering them from contaminated wastewaters has emerged as a potential alternative method to conventional treatment techniques. The ability of microorganisms to separate metal ions is a well-known phenomenon. Various experimental data are presented for different metals and biomass types, and are reviewed and critically commented in comparison with the literature. Dead biomass is usually obtained from fermentation wastes or by-products. Focus of this paper constitutes, among others, the kinetics of the biosorption process, being still quite ambiguous. Bioaccumulation of metals is also discussed in comparison with biosorption, the latter is more effective.

Keywords: toxic metals; uptake; removal; sorption; equilibrium; bioaccumulation; biosorbent; separation; flotation.

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1 Introduction

Despite overall higher technological and safety levels, the number of industrial accidents continues to rise – and human-caused disasters cause generally more fatalities and economic losses than natural disasters. Maybe, a suitable example of mining accidents is that of Baia Mare in northwestern Romania, in January 2000: a dam wall failed at a mine tailings reclamation facility spilling 100,000 m³ of polluted wastewater into the Tisa river and then the Danube, eventually entering the Black Sea, by which time it had become significantly diluted; the spill devastated large numbers of plant and wildlife species in the river systems, according to UNEP.

On the other hand, municipal sewage treatment plants are not designed and equipped for handling toxic wastes. Metals and their toxicity persist even in the sludges and by-products of these plants. Heavy metals need to best be removed at the source in an especially pre-treatment step, which must be cheap, because it often deals with large volumes of effluents. The conventional as well as the new revealing approaches (membrane filtration and hybrid processes), with their advantages and disadvantages, for heavy metals removal are listed in Table 1. It is not within the framework of this text to discuss these techniques but better technologies are more costly and often not feasible. When metals are dissolved in huge volumes at relatively low concentrations, these methods become generally ineffective (e.g., less than 100 mg/L) (Patterson, 1985). The research is for efficient and particularly cost-effective remedies (Volesky, 2001; Blöcher et al., 2003).

Table 1 Metal removal technologies

<i>Method</i>	<i>Disadvantages</i>	<i>Advantages</i>
Chemical precipitation	For high concentrations Difficult separations Generates sludges	Simple Cheap
Chemical oxidation/reduction	Chemicals required	Mineralisation
Electrochemical	For high concentrations Expensive	Metal recovery
Ion exchange	Sensitive to particles Expensive	Effective Metal recovery
Evaporation	Expensive Generates sludges	Pure effluent
Reverse osmosis	High pressures Membrane scaling Expensive	Pure effluent
Hybrid methods (flotation-filtration)	Further research	Low operating costs High membrane fluxes

It was only in the 1990s that a new scientific area developed that could help to remove metals: sorption by employing biomasses (biosorbents).

1.1 The use of biosorbents

The economy of environmental remediation dictates that the biomass must come from nature or even has to be a waste material. Microorganisms, including actinomycete, cyanobacteria, and other bacteria, algae, fungi and yeasts, have the ability to accumulate heavy metals. Additionally, agricultural wastes, among other kinds of biomass, have been tested for metal sorption. Biosorption is usually termed as the process that uses non-living biomass (i.e., metabolism-independent, passive removal of metals and often, includes bioprecipitation) as adsorbent material, in contrast to bioaccumulation (i.e., metabolism-dependent), which refers to the application of living microorganisms (Gadd, 1990; Zouboulis et al., 1997).

Metabolism-dependent intracellular uptake of metal ions may be a slower process than biosorption. It is inhibited by low temperatures, the absence of an energy source and metabolic inhibitors. Many metals are essential for growth and metabolism and organisms possess transport systems for their accumulation. Non-essential metals may be taken via such systems.

Generally, biosorbents could be classified into the following categories:

- *Bacteria*. The walls of bacteria are efficient metal chelators though a wide spectrum of uptake capacities may be exhibited. Metal binding may be at least a two-stage process first involving interaction between metal ions and reactive groups followed by inorganic deposition of increased amounts of metal. The carboxyl groups of glutamic acid of peptidoglycan are the major site of metal deposition. In some

bacteria, metabolism-independent biosorption may be the most significant proportion of total uptake. Although biosorption is independent of metabolism, it is possible that a metabolism-dependent microenvironment may enhance metal deposition.

- *Algae*. Metabolism-independent accumulation of metals is often rapid and usually completes in 5–10 min. In common with other microbial groups, many potential binding sites occur in algae cell walls, which include polysaccharides, cellulose, uronic acid and proteins.
- *Fungi and yeasts*. Metabolism-independent binding of metal ions to fungal and yeast cell walls is usually rapid and large amounts may be bound. The biosorption capacity of dead biomass may be greater, equivalent to, or less than that of living cells and this may depend on the killing process used. As in other microbes, a variety of ligands may be involved including carboxyl, amino, phosphate, hydroxyl and sulphhydryl groups. In certain fungi, and especially yeasts, greater amounts of metals may be taken up by transport than by biosorption. However, for many filamentous fungi, it appears that general biosorption accounts for a major portion of metal uptake.
- *Agricultural wastes*. In this case, a variety of by-products are incorporated, e.g., wool, olive cake, sawdust, pine needles, almond, and coal. Either protein-based animal fibres, or cellulose-based plants fibres bear many amino, carboxylic or hydroxyl functional groups, which play a major role in metal binding.

Unfortunately, it is the toxicity of some elements that does not allow investigation of bioaccumulation in the presence of high metal concentrations. In fact, little information is available about this kind of mechanism. Heavy metal transport across microbial cell membranes may be mediated by the same mechanism used to convey metabolically essential ions, such as potassium, magnesium and sodium (Veglio and Beolchini, 1997).

The use of dead biomass or derived products eliminates the problem of toxicity, not only from dissolved metals but also from adverse operating conditions, and the economic component of maintenance including nutrient supply. However, living cells may exhibit a wider variety of mechanisms for metal accumulation such as transport, and extracellular complex formation.

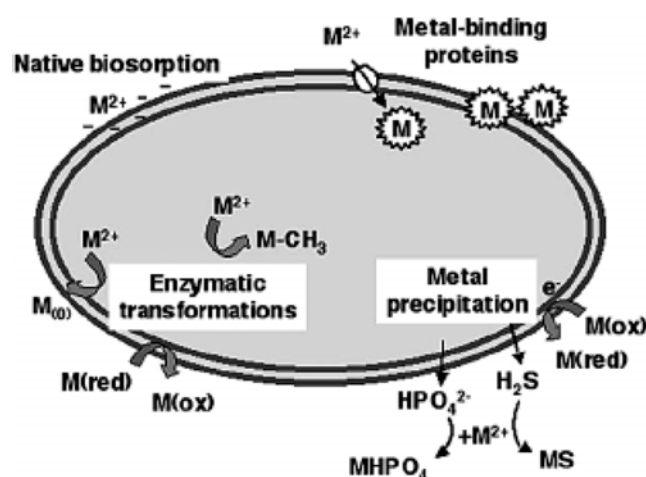
Various metal-resistant bacteria are known today that are well adapted to the harsh environments typically created by extreme anthropogenic situations or biotopes. In general, metal removal or recovery from solution may involve the following pathways (Hughes and Poole, 1989):

- the binding of metal cations to cell surfaces, or within the cell wall, where microprecipitation may enhance uptake
- translocation of the metal into the cell, possibly by active (metabolic energy-dependent) transport
- the formation of metal-containing precipitates, by reaction with extracellular polymers or microbially produced anions such as sulphide or phosphate
- the volatilisation of the metal by biotransformation – as presented in Figure 1.

The fundamental characteristics of biosorption and bioaccumulation of metals are given as shown in Table 2. Whereas the use of living organisms is often successful in the

treatment of toxic organic contaminants, living organisms in conventional biological treatment systems have been rarely useful in the treatment of solutions containing heavy metal ions as it is also shown in the following. Once the metal ion concentration becomes too high or sufficient metal ions are absorbed by the microorganism, the organism's metabolism is disrupted, thus causing the organism to die. This disadvantage does not exist if non-living organisms (biomass) or biological materials derived from microorganisms are used to adsorb metal ions from solution/effluents.

Figure 1 General schematic representation of metal uptake processes



Source: Valls and de Lorenzo (2002)

Table 2 Characteristics of metal biosorption and bioaccumulation

Feature	Biosorption	Bioaccumulation
Metal affinity	High under favourable conditions	Toxicity will affect metal uptake by living cells, but in some instances high metal accumulation
Rate of metal uptake	Usually rapid, a few seconds for outer cell wall accumulation	Usually slower than biosorption
Selectivity	Variety of ligands involved, hence poor	Better than biosorption, but less than some chemical technologies
Temperature tolerance	Within a modest range	Inhibited by low temperatures
Versatility	Metal uptake may be affected by anions or other molecules	Requires an energy source
	Extent of metal uptake usually pH dependent	Dependent on plasma membrane ATP-ase activity.
		Frequently accompanied by efflux of another metal

Source: Eccles (1999)

1.2 *Metal recovery*

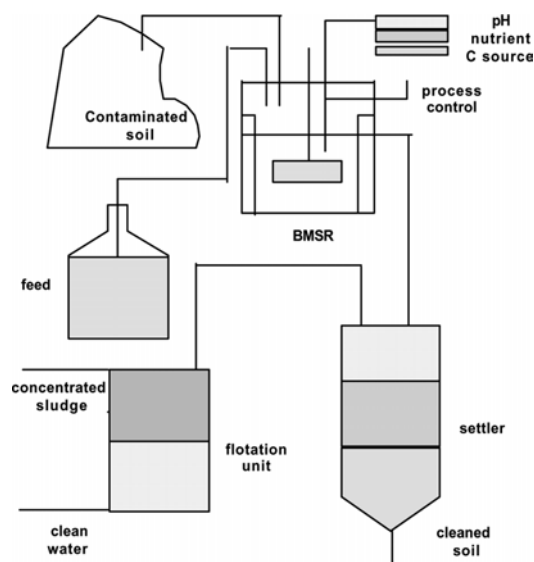
Technological applications of microbial metal accumulation may depend on the ease of metal recovery either for subsequent reclamation or for further containment of toxic waste. Non-destructive recovery may also be required for regeneration of the biomass for re-use in multiple biosorption–desorption cycles. Destructive recovery may be accomplished by pyrometallurgical treatment of the biomass or dissolution in strong acids or alkalis. The methodology of metal recovery depends on the ease of removal from the biomass. If cheap, waste biomass was used to take up valuable metals, then the economics of destructive recovery may be satisfactory. The choice of a recovery process also depends on the mechanism of accumulation. Metabolism-independent biosorption is often reversible and lends itself to non-destructive desorption whereas metabolism-dependent intracellular accumulation is often irreversible, necessitating destructive recovery (Gadd, 1990).

1.3 *Biosorption process operation*

The most effective mode of a sorption process should undoubtedly be based on a fixed-bed reactor/contacter configuration. The sorption bed has to be porous to allow the liquid to flow through with minimum resistance but allowing the maximum mass transfer into particles. The particles thus should be as small as practical for the reasonable pressure drop across the bed. Because microbial biomass is generally of small particle size and low mechanical strength, it should be granulated. The latter generally causes loss of activity as well as limitations in mass transfer (Volesky, 2001).

Flotation, a relatively simple process capable of removing flocs from aqueous suspensions, could be used as an alternative to fixed-bed bioreactor configuration. It exploits the natural or induced hydrophobicity of the flocs when a stream of gas bubbles is introduced into the free suspension of biomass. The hydrophobic material adheres to bubble surfaces and floats to the top of the liquid phase, forming a froth layer rich in solute. Surface-active chemicals (surfactants), termed collectors, are often used to artificially induce hydrophobicity, in some cases selectively, so as to remove from solution only the desired or undesired substances (Zouboulis et al., 2002). Flotation has been recently investigated for the separation of *Alcaligenes eutrophus* CH34 strain in the presence of cadmium and zinc, as a part of a soil remediation concept, termed Bio Metal Sludge Reactor (Diels, 1997). Developing at VITO Belgium the BMSR concept, it was found out that the presence of CH34 bacteria could improve the settling of soil (see Figure 2). The soils treated with the bacteria showed in about 1 h very good sedimentation, whereas CH34 remained in the suspension. This behaviour was used to separate soil from bacteria, as the latter could be recovered from the suspension via the flotation process (Lazaridis et al., 2005).

In this work, the removal of cadmium metal ions from aqueous solutions by bioaccumulation and biosorption onto a variety of biomasses was investigated (in some cases in the presence of zinc and copper ions). Kinetic and thermodynamic analysis was also performed. The solid–liquid separation was succeeded by dispersed-air flotation.

Figure 2 The bio metal sludge reactor

2 Experimental

2.1 Bioaccumulation

Alcaligenes eutrophus CH34: Tris-minimal medium (pH = 7) was used for the growth of CH34 strain and cells were grown at 30°C. As carbon source 0.2% sodium gluconate or 0.2% sodium acetate was tested. 100 mL of an overnight preculture (with an $OD_{600} = 0.8-1$) was added to 1000 mL fresh minimal medium containing the carbon source, NH_4Cl and Na_2HPO_4 . The cultures were incubated further in the presence of $CdCl_2$ or $ZnCl_2$ with aeration by shaking at 30°C. Samples were taken at time intervals and the cells were harvested by centrifugation. *Ralstonia metallidurans*, formerly known as *Alcaligenes eutrophus* and thereafter as *Ralstonia eutropha*, is a β -Proteobacterium colonising industrial sediments, soils or wastes with a high content of heavy metals (Mergeay et al., 2003).

2.2 Biosorption

For the biosorption batchwise experiments, various biomass types were tested usually on aqueous solution of metals commonly found in industrial wastewaters (i.e., a simulating approach):

Aeromonas caviae biomass was used for the removal of cadmium ions in a stirred batch reactor. It is a Gram-negative bacterium isolated from raw water wells near Thessaloniki; then grown in the laboratory at 29°C in a rotating shaker for 24 h in a liquid medium containing: yeast extract (0.5% w/v), tryptone (1% w/v), NaCl (0.5% w/v) and $FeSO_4 \cdot 7H_2O$ (0.2 g/L). The produced biomass was separated by centrifugation at 3000 rpm, washed several times by a solution of NaCl (0.9% w/v), sterilised and stored as a slurry.

Penicillium chrysogenum fungal waste biomass, with trade name Mycan, kindly supplied by Synpac Ltd. (UK).

Two mixtures of commonly existing metals were examined during biosorption experiments:

- solution A: Cd 1.5, Zn 7.5 and Cu 30 mg/L (prepared from nitrate salts)
- solution B: Zn 50, Cu 10, Ni 2, Ca 100 and Na 100 mg/L.

The contact and mixing time for biosorption was kept constant at 15 min. Non-living and unmodified biomass was applied. The biomass samples, harvested mainly in the form of cell aggregates, were properly inactivated by autoclaving and washed extensively with portions of deionised water for the removal of different soluble residuals. The practice of biomass modification (by common surfactants, polyelectrolytes, etc.) has been attempted in several applications, as for instance for arsenates removal (Loukidou et al., 2003), in a case where the employed biomass showed originally a relative low affinity for metallic anions. The pH of the solution was kept almost constant around seven.

2.3 Flotation

Four biomasses were tested:

Streptomyces rimosus: Actinomycetes bacterium kindly supplied by Pfizer Pharmaceuticals, resulting from tetracycline production.

Saccharomyces carlsbergensis: Yeast kindly supplied by Federation Brewery (Gateshead, UK).

Grape stalks: Biomass kindly supplied by the vineyard Adegba Cooperativa da Arruda dos Vinhos (Portugal).

Penicillium chrysogenum. The aqueous mixture contained Zn 50, Cu 10, Ni 2, Ca 100 and Na 100 mg/L. The presence of the heavy metals in the solution was also found to alter the electrokinetic properties of biomass. More information concerning biomass types was already given (Zouboulis et al., 1999).

2.4 Metal analysis

The concentrations of the metal in the supernatant solutions were determined using an Atomic Absorption Spectrophotometer (AAS). The amount of metal adsorbed was estimated from the difference between the amount added and the amount remaining in the solution.

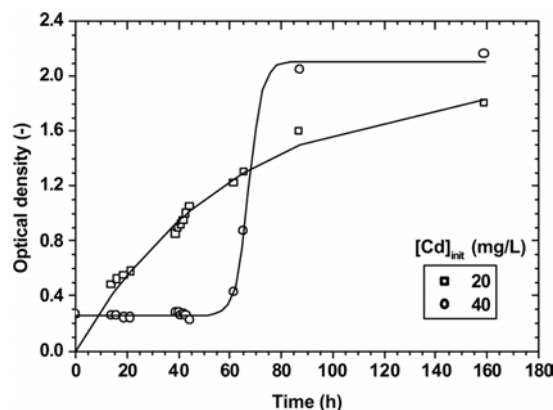
3 Results and discussion

3.1 Bioaccumulation

The type strain CH34 carries two large plasmids bearing a variety of genes for metal resistance. So, it has been used as a model system to investigate bacterial interactions with metal ions (Taghavi et al., 1997). *Ralstonia metallidurans* was found to be able to

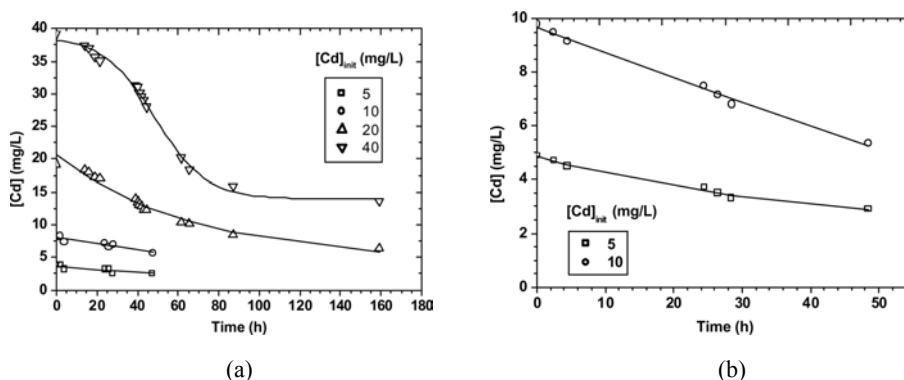
sorb heavy metals from aqueous solution in a quite appreciable degree (see Figure 3); the percentage removal varied between 50% and 75%. The bioaccumulation rate was a function of the initial metal concentration in the feed.

Figure 3 Effect of metal (cadmium) on the growth of bacterium CH34



However, the behaviour of the microorganism was rather problematic in higher concentrations. For this reason, most of this work was limited to low metal concentrations (of 20 mg/L and 40 mg/L initially). Using the living organism, the recorded replicability of results was also not promising, as small variations in the experimental conditions (solution pH, temperature, contact time, mixing speed, etc.) produced significant changes in the uptake efficiency. The change in the source of carbon, in this case, did not affect the process much (Figure 4).

Figure 4 Metal (cadmium) uptake by bacterium CH34 in presence of: (a) gluconate and (b) acetate



The effects of substrates and growth conditions present special interest: the biosorptive capacity, for instance, was greater when the cell walls were richer in S, C, N or P (Engl and Kunz, 1995). Elsewhere (Kujan et al., 1995), the ability of yeast to remove cadmium from the culture nutrient substrate was said to be highly dependent on the carbon source; when glucose was used, the capacity was ten times higher than the case of xylose.

A sorption capacity for cadmium ions of around 110 mg/g was recorded for non-living *Bacillus licheniformis* compared with only 73 for the same but living organism; for *B. laterosporus*, the capacity was 130 mg/g for the non-living biomass compared with 80 for the living, while the differences for chromates sorption were less (Zouboulis et al., 2004). Similar results were also published in the literature (Srinath et al., 2002; Kaduková and Virčíková, 2005). In the latter, it was found by SEM and TEM images that copper significantly damaged the surface of living algal cells, which resulted in partial loss of cell-binding abilities and release of the accumulated metal back into solution.

The highly toxic and variable conditions encountered in many waste and process waters, sometimes taking extreme values, usually preclude the use of living systems and dictate the application of non-living ones for metals removal. Anyway, live or dead biomass was proved to acquire, more or less, rather equal biosorptive capacities (Brieley et al., 1989). Nevertheless, the use of dead biomass eliminates the problem of toxicity and the economic aspects of nutrient supply and culture maintenance. Additionally, potential microbiological hazards were not foreseen for the case of dead biomass utilisation.

Kefala et al. (1999) examined two specific strains of Gram-positive *Actinomycetes*, applying living microorganisms for cadmium removal, as well as non-living bacterial biomass for comparison; non-living biomass exhibited higher metal uptake. Other potential advantages of the non-living biomass for metals removal are certainly that they can be easily applied, using the already-existing treatment technologies, and are eliminated in such a way: the problem of metal toxicity, the possible adverse operating conditions, and the economic burden of culture maintenance and nutrient supply for the living cells (Gadd, 1990).

On biomass surfaces, several chemical groups may be present that could attract and subsequently sequester metals from the surrounding aqueous medium. Attention should also be paid to the possible influence of pH on these chemical groups. Nevertheless, the presence of particular functional groups does not necessarily guarantee their accessibility as sorption sites owing to the co-existence possibly of steric, conformational or other types of barriers (Zouboulis and Matis, 1998).

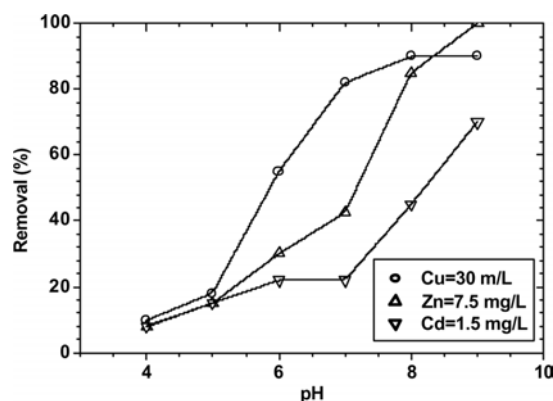
3.2 *Biosorption*

Speciation thermodynamic calculations that were conducted (using the Mineql + computer programme) under these conditions for the three metals (solution A) showed the following pH values of respective hydroxide precipitation: for copper approximately 6, for zinc 7.4 and for cadmium 8.6. The used biosorbent was a fermentation waste from a large-scale pharmaceutical industry, and the results are given in Figure 5. It is apparent that the observed metals removal by biosorption, resembling as pH-fronts, follows exactly the same order.

During a comparative study of biosorption with other commonly applied metal separation methods, such as precipitation, filtration, centrifugation and flotation, it was reported in terms of metal removal efficiency and applicability for more acidic pH values. The former treatment method (i.e., biosorption) was favoured. In conventional physico-chemical separation processes, the removal of metal cations is mainly due to their precipitation as hydroxides, because of pH alteration towards alkaline values. Figure 6 presents the comparison between biosorption removal of metals and typical

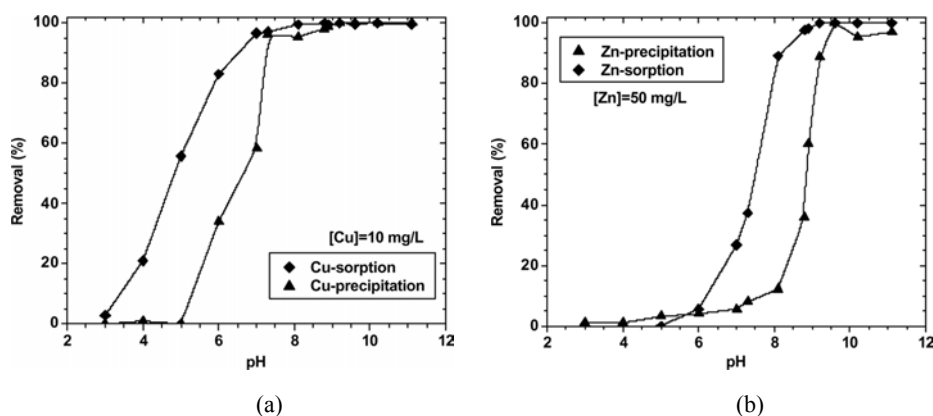
precipitation (solution B); the pH-front seems to have been moved towards the left, to lower pH values.

Figure 5 Selected results from the treatment of an aqueous dispersion, containing a mixture of metal ions and a fungal biomass



Source: Matis et al. (2002)

Figure 6 Comparative evaluation of biosorption and precipitation removals for aqueous solution containing a mixture of metals (a) copper and (b) zinc. Modified from the experimental results published by Zouboulis et al. (1999)



Two important sorption parameters were elsewhere calculated (Zouboulis et al., 2008) from results obtained from pilot-scale work, including biomass separation:

- the distribution coefficient, D [L/g]
- the loading capacity coefficient, q [$\mu\text{mol/g}$].

Two feed solutions of metal mixtures were examined separately, but also in several cases consequently, according to the decided two-stages countercurrent operational scheme: a 'lean' solution, simulating partially treated industrial wastewaters, and a 'strong' one, simulating raw wastewaters of plating industry. Also, two biosorbent types were tested: *Penicillium chrysogenum* and grape stalks biomass.

The aforementioned two-stage countercurrent, of the polluted aqueous system and the pre-treated biomass, flowsheet was proposed following extensive experiments, with each stage containing biosorption of metals and appropriate separation of loaded biomass, and these stages were followed by only one elution stage (Zouboulis et al., 2002). Maybe, the difficulty in biomass separation (harvesting) was advocated as one of the main disadvantages of biosorption, and for this reason, flotation has been thoroughly examined. These two processes of biosorption and flotation can be efficiently combined, forming the so-called ‘biosorptive flotation’ process.

3.3 Biosorption kinetics

Engineering considerations are central in decisions concerning the commercial future of biosorption, hence the study of relative kinetics and modelling is of outmost importance (Ho et al., 2000). Table 3 presents a list of applied kinetic equations. Among them belongs the Elovich equation, known as many others from the kinetics of chemisorption of gases on solids, but used by Cheung et al. (2001) for cadmium-contaminated water and bone char as the adsorbent. Adsorption on solids from a liquid phase is perhaps a different process than adsorption from a gas phase where traditionally the remaining bulk concentration dictates the kinetics.

Table 3 Some characteristic kinetic equations successfully applied in the literature

<i>Equation</i>	<i>Model</i>	<i>Reference</i>
Zero Order*	$q_t = q_0 + k_0 t$	Ho et al. (2000)
First order	$\ln q_t = \ln q_0 + k_1 t$	Ho et al. (2000)
Second order	$\frac{1}{q_t} = \frac{1}{q_0} + k_2 t$	Ho et al. (2000)
Ritchie**	$\frac{d\theta}{dt} = k_n (1 - \theta)^n$	Cheung et al. (2001)
Shrinking core (internal diffusion)	$1 - 3(1 - a)^{2/3} + 2(1 - a) = \frac{6D}{R_2 C^0} \int C dt$	Rao and Gupta (1982)
Parabolic diffusion	$q = a + k_d \sqrt{t}$	Ho et al. (2000)
Elovich (linearised)	$q_t = \frac{1}{\beta} [\ln(a\beta) + \ln(t + t_0)]$	Cheung et al. (2001)

*The amount loaded $q = q_0$ at $t = 0$; ** θ the surface coverage of biosorbent.

Perhaps, a strong argument in favour of searching for the actual mechanism is that if we find one that we think represents what truly occurs, extrapolation to new operating conditions is more safely done. Very precise and reproducible data should also be used, which in itself is quite a problem in biosorption, owing to bacteria growth, etc. To recall a good student’s handbook (Levenspiel, 1972) but from a period where the computers were just starting: there is no reason why we should not use the simplest and easiest-to-handle equation of satisfactory fit. If a number of alternative mechanisms fit the data equally

well, we must recognise perhaps that the model selected can only be considered to be one of the good fit, not one that represents reality.

The kinetic mechanism of metal sorption on biosorbent particles was investigated (Loukidou et al., 2004a, 2004b) putting more emphasis on samples collected at short times after the initiation of the process (where the major part of the adsorption occurs) and following the known concept of the rate controlling step, being certainly a simplifying approach. For the case of a non-porous biomass, transport of solute inside the 'particle' may be neglected, and it can be assumed that biosorption occurs mainly at the particle surface (cell wall); this idea can be effectively extended to cases of relatively large macropores where the metal ions may have a ready access to react with internal surface sites. For sufficiently high agitation speed in the reaction vessel, the bulk diffusion step can be safely ignored since then sorption onto sorbent particles is decoupled from mass transfer in the bulk mixture.

The finally chosen kinetic models were those that not only fit closely the data, but also represent reasonable sorption mechanisms. To compare measurements from various experiments, it was first necessary to introduce a dimensionless degree of conversion. A finite volume diffusion model with a time-dependent concentration at the surface of the biosorbent was found to closely fit the experimental data for the largest part of the process. The adsorption rate of metal ions onto *Aeromonas caviae* was found to be particularly sensitive to initial bulk concentration, biomass load and ionic strength of the solution. *A. caviae* is often present in groundwaters and exhibits particular tolerance to heavy metals (Miranda and Castillo, 1998).

A usually necessary and rather simple attempt is to try and differentiate between chemical kinetics and mass transfer. The diagram of the biosorbent capacity with the square root of contact time is often used (with k_d the diffusion coefficient); as referred by Ho et al. (2000), in their overview, this relationship was used as criterion if the rate-limiting step is intraparticle diffusion, in case of obtaining a straight line passing through the origin.

The respective calculations were conducted for the constants of the Elovich equation (Loukidou et al., 2006), showing also quite an acceptable fit based on the observed correlation coefficient (r^2) for cadmium biosorption; similar were the results for the case of chromates. Teng and Hsieh (1999) proposed that the constant a is related to the rate of chemisorption and the constant b is related to the surface coverage.

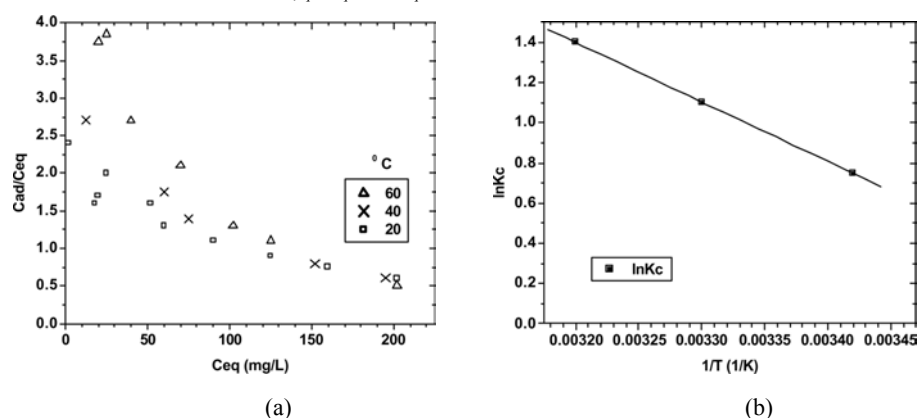
It is noted that other equations tried less effectively, i.e., 'worse'-fit models on the present system, were the following: zero, first order, Ritchie equation, parabolic diffusion, and the shrinking core model (see also Table 2). If one stopped searching up to this point, then the conclusion is apparent. Nevertheless, the suitability of a pseudo second-order chemical reaction for the biosorption of these ions was suggested, following the wide investigation (Loukidou et al., 2004b). In the latter equation, in essence, a numerically determined parameter was used in place of the equilibrium capacity, and in this treatment case, one bivalent ion was considered to occupy two binding sites.

To prove that a certain mechanism is followed, we must show that the family of curves representing the rate equation type of the favoured mechanism fits the data so much better than the other families that the others can be rejected. We should bear in mind that it is not good enough to select the mechanism that well fits, or even best fits the data. Differences in fit may be so slight as to be explainable entirely in terms of experimental error; in statistical terms, these differences may not be significant.

Quite contradictory indications on the subject were pointed out, in conclusion: the scenario of an external surface chemical enhancement, dominating the very beginning of the process, being followed by external film diffusion, was rather proposed. A two-stage kinetic mechanism also looks to be quite possible, as it meets the fitting requirements and deserves attention on physical grounds (Matis et al., 2008).

Perhaps, to shed some more light on the whole questioning as far as kinetics is concerned, mention will be finally given to the respective calculations of thermodynamic parameters of this biosorption system, according to the known van't Hoff equation. The values of the apparent standard thermodynamic equilibrium constant (K_c°) of biosorption at different concentrations were found from the $C_{ad,eq}/C_{eq}$ vs. C_{eq} plot (see Figure 7(a)), to find the value of ΔG° , standard Gibbs free energy; this for cadmium at 20°C was found to be -1.7 kJ/mol, where the negative figure is because the process is spontaneous with high affinity of the metal to the biomass (Loukidou et al., 2005).

Figure 7 Calculation of thermodynamic properties for cadmium biosorption onto *A. caviae*: (a) $C_{ad,eq}/C_{eq}$ vs. C_{eq} plot and (b) Arrhenius plot



The standard enthalpy and entropy changes determined from the $\ln K_c^\circ$ vs. $1/T$ diagram were 14.5 kJ/mol and 0.06 kJ/mol K, respectively (see Figure 7(b)). The positive value of ΔH° suggests the endothermic nature of biosorption. The positive value of ΔS° confirms the increased randomness at the solid–solution interface during biosorption. Reasonably similar were the findings and also procedure of Aksu (2002). For the case of chromates and *A. caviae*, respectively, were found: at 20°C $\Delta G^\circ = -0.65$ kJ/mol, $\Delta H^\circ = 7.6$ kJ/mol and $\Delta S^\circ = 0.03$ kJ/mol K.

From initial calculations, the obtained activation energies were lower than 10 kJ/mol (being usually the accepted limit for the differentiation of chemisorption from physisorption). The value, in contradiction with the aforementioned, indicates rather a physical phenomenon than chemisorption. This perhaps is supported by the fact that the kinetic observations are similar both for the metal cation and the oxyanion adsorbate. Sorption of heterogeneous liquid–solid systems has been usually accepted to follow Arrhenius kinetics; however, to apply the model in such systems, the morphology of the reactive surface as well as the final biosorption capacity must not vary with temperature, conditions often overlooked.

Analysis of equilibrium data is important for developing an equation that can be used for design purposes. Classical adsorption models have been extensively used to describe the equilibrium established between adsorbed metal ions on the biomass (q_{eq}) and metal ions remaining in solution (C_{eq}) at a constant temperature (Ho et al., 2000). The adsorption isotherms obtained for Cd(II) and Cr(VI) ions uptake by *Aeromonas caviae* were found to follow at a satisfactory extent both the Freundlich and Langmuir predictions within the studied metal concentration range (Loukidou et al., 2004b). Yet, the correlation coefficients of the Langmuir curves were a little better. This observation implies that monolayer biosorption, as well as heterogeneous surface conditions, may co-exist under the applied experimental conditions. Hence, the overall sorption of metal ions on the biomass may involve more than one mechanism (such as ion exchange, surface complexation and electrostatic attraction).

The values of the Freundlich constants showed a relatively easy uptake of metal ions with high biosorptive capacity of *A. caviae*. In particular, the value of n (parameter in the integer), which is related to the distribution of bonded ions on the sorbent surface, is greater than unity, indicating that the ions are favourably adsorbed under all the examined experimental conditions. In general, the Freundlich equation satisfactorily describes experimental data over a wide range of values of θ (fraction of surface coverage with the adsorbed solute) and for systems that does follow the Langmuir isotherm. Even for a system, which does follow the Langmuir isotherm, over a range of surface coverage (i.e., in the Ritchie equation) between the extremes of $\theta = 0$ and $\theta = 1$, the latter isotherm is nearly equivalent with the case when θ is proportional to a fractional power of C_{eq} (Yiacoumi and Tien, 1995). Therefore, both isotherms can be used to model biosorption data from dilute aqueous solutions.

It is true that the ability of the different biosorbents to remove toxic metals varies depending on the type of microorganism and the applied conditions – see for instance Table 4, for the case of chromates sorption, compared with a maximum capacity spanning in the range ~70–285 mg/g for *A. caviae*.

Table 4 Comparison of different biosorbents based on their maximum capacity for chromates

Biomass	q_{max} (mg/g)	PH (-)	T (°C)	C_o (mg/L)	X (g/L)	Reference
<i>Zoogloera ramigera</i>	3	2	25	25–400		Nourbakhsh et al. (1994)
<i>Saccharomyces cerevisiae</i>	3	1–2	25	25–400		Nourbakhsh et al. (1994)
<i>S. equisimilis</i>	11.8	2	30	50	2	Goyal et al. (2003)
<i>Streptomyces noursei</i>	10.6	5.5	26	~	~	Mattuschka and Stranbe (1993)
<i>Saccharomyces cerevisiae</i>	4.4	2	30	50	2	Goyal et al. (2003)
<i>Rhizopus arrhizus</i>	4.5	1–2	25	25–400		Nourbakhsh et al. (1994)
<i>A. niger</i>	113	2	30	150	0.5	Goyal et al. (2003)
<i>Firms sylvestris</i>	201.81	1	25	50–300	1	Ucun et al. (2002)
<i>C. vulgaris</i>	3.5	1–2	25	25–400	~	Aksu (2001)
<i>Rhizopus nigricans</i>	47	2	30	100	0.5	Bai and Abraham (2001)

Our results (presented previously as tables) demonstrated that the biomass concentration strongly affected the amount of metal removed from aqueous solution. Moreover, as the biomass concentration rises, the maximum biosorption capacity drops, indicating poorer biomass utilisation. Elsewhere, it was suggested that the sorption of metals is more correctly described by more than one model (Smith, 1996). Kinetic analyses not only allow estimation of sorption rates but also lead to suitable rate expressions characteristic of possible reaction mechanisms.

The specific surface area of *Penicillium chrysogenum* was elsewhere calculated from the nitrogen adsorption curve as $9.7 \text{ m}^2/\text{g}$, much smaller than other typical inorganic adsorbents; the pore volume (calculated from the desorption curve) for this fungal biomass was found to be $0.016 \text{ cm}^3/\text{g}$ as previously presented in a conference (Matis et al., 2002). Taking into account that the specific surface area and the pore volume were small, the pore size distribution was not of any significant importance (which may affect the kinetics, too); noting as well the possible influence of the needed specific treatment of the said biosorbent, being of organic matter, for the above measurement. A table was presented (Schneider et al., 2001) with values for the surface areas of selected porous solid materials but measured by different techniques; owing to the varying range obtained, a question was also raised about the true surface areas of biomass.

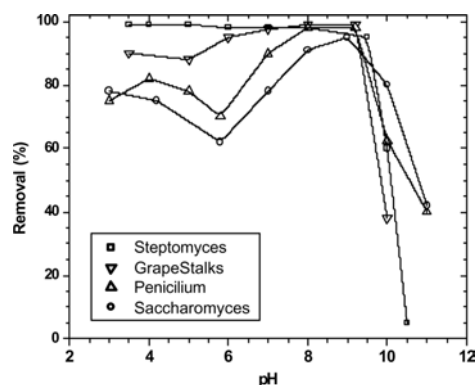
Therefore, abnormal temperature dependence of sorption rate was also found (Loukidou et al., 2004b), attributed to alterations of the surface morphology of biomass particles. The gross features of the biomass at 40°C and 60°C observed by SEM photographs were distinctly different from that at 20°C . No flakes or grooves were noticeable but instead the surface appears quite uneven and fragmented with prominent ridges emerging, here and there. Evidence has been provided, in conclusion, that this is a complex process.

The obtained information may have a practical value for technological applications, since modelling successfully replaces time- and material-consuming experiments, necessary for process equipment design.

3.4 Flotation

A comparison between the various types of biomass for their potential use as biosorbents was earlier carried out in the laboratory, as far as floatability is concerned, against the whole pH range – see Figure 8; conditions: biomass 1 g/L , dodecylamine as flotation collector $3 \times 10^{-4} \text{ M}$ (Zouboulis and Matis, 2005). Four types of biomass were tested previously loaded with metals. Improved recoveries of biomass by flotation were observed, some reaching almost 100%. Hence, biomass separation by flotation is effective and the optimum conditions have been determined for the different biomass systems examined. The bacterial cell surfaces are known to be mostly anionic, owing to the presence of respective ionised groups in the various cell wall polymers; this was also the reason that a cationic surfactant was applied for the separation of biomass by flotation.

Figure 8 A comparison of various biomass types examined as biosorbents, showing the obtained floatability as a function of pH change



Source: Zouboulis and Matis (2005)

The elution of the metal from the floated biomass is of great importance for a number of reasons, such as the stability of the metal-laden biomass and, possibly, pollution prevention. The used biomass can be treated (by simply pH modification) to desorb the abstracted metal and it was used again in flotation experiments, giving high floatabilities of biomass, up to the pH value of seven (Matis et al., 1994; Matis and Zouboulis, 1994).

It has also been recognised that immobilising biomass in a polymer matrix may improve the biomass performance and capacity, allowing simultaneously column operation; hence, there is no need for the downstream solid-liquid separation of metal-laden biomass from the treated solution. So, polyacrylonitrile (PAN) was tried as a convenient binding polymer of fungal biomass, known previously to be applied for a number of inorganic ion exchangers (Zouboulis et al., 2003). The physico-chemical properties, as mechanical strength, hydrophobicity, etc., can be modified by the degree of cross-linking of the polymer, the use of suitable co-polymers or by changing the composition and temperature of polymerisation.

In a relevant paper, trying to explain the disappointing lack of biosorption's application for real technological problems, it was stated that relatively small number of pilot-plant studies have been carried out so far, aiming to investigate the potential of microorganisms to remove metals from liquid wastes (Eccles, 1999). This author also reported that the use of a biological process with a commercially available adsorbent material (AMT-Bioclaim) was shown under specific experimental conditions to decrease the operation running costs by 36% and total cost by 28% compared with conventional techniques like ion exchange and chemical precipitation.

Concluding this, various arguments were raised earlier, concerning the kinetics of biosorption and mainly regarding the:

- influence of temperature
- rate-determining step(s)
- specific surface area and nature of biomasses
- type of isotherm followed
- maximum loading capacity.

Many biomass types are capable to remove heavy metals from aqueous solutions; they are not expensive material, can be easily recovered at the end of fermentation processes and are produced as commercial by-product in large quantities. The interactions between metal ions and biomass present potential applications for the remediation of metal-contaminated waters in various industries. The release of toxic metals into the environment would be restricted in this way, and so, the process could be used more extensively as an alternative but promising method to the conventional treatment techniques.

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