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BIOSORPTION OF METALS FROM DILUTE AQUEOUS SOLUTIONS

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ABSTRACT

Dilute aqueous solutions, generated or used by industry, can contain a variety of different metal ions. Various processes are suitable for reclamation of toxic metals and among them, attention is paid here to biosorption. The ability of microorganisms to remove metal ions from solution is a well known phenomenon. Industrial applications of biosorption often make use of dead biomass, which does not require nutrients and can be exposed to environments of high toxicity. Experimental laboratory batch experiments are described for actinomycetes, fungi and for activated sludge, as the metal biosorbents, providing insight into cadmium biosorption. Non-living biomass showed greater binding capacities for cadmium (a priority pollutant) than living biomass. Engineering considerations are central in decisions concerning the commercial future of biosorption and a practical solution is needed for certain problems, such as the efficient separation of metal-loaded biomass.

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1. Introduction

Treatability studies have indicated that wastes often contain toxic and non-biodegradable matter (such as metals), which generally limits the implementation of a conventional biological treatment process. In-plant control programmes including waste segregation and process-specific pretreatments have been proposed[1].

The mining, mineral processing and metallurgical industries are known to generate annually billions of tons of wastes (which include gases, dusts, solutions and a variety of massive mineral materials such as tailings and slags). Some of the metal and mineral constituents of these wastes have potential economic value and their recovery can augment our primary mineral and metal resource supply base. The resource potential of wastes generated by the iron and steel, aluminum, clay, phosphate, copper, coal mining and processing, nonmetallic, and selected secondary metals industries have been identified[2]. Every operation in the materials cycle generates wastes, but the extent to which a material is truly waste depends upon many factors involved in the overall process.

The importance of pollution prevention by clean technologies instead of remediation has also been recognised[3]; industrial waste management foresees an evolution from an end-of-pipe treatment mentality to source reduction as the preferred option. The World Commission on Environment and Development has focussed on the concept of sustainable development[4]; this suits renewable resources, but its application to non-renewable resources, such as minerals, is less clear. Effluent permits are subject to modification in the light of best available technology which, however, might not be commercially proven technology.

The U.S. Environmental Protection Agency (EPA) is evaluating technologies for their ability to recover metals from sludges and wastewaters[5], since the disposal of hazardous wastes in or on the land is restricted. The EPA also offers many informational resources to aid in screening and selecting innovative technologies for waste site remediation[6]. Methods of processing mining wastes include: (i) mine fill, landfill and road ballast (widely exploited), (ii) building bricks, ceramic tiles, etc. (less generally discussed), and (iii) the recovery of valuable metals.

Environmental legislation, both existing and proposed, lays down stringent guidelines for the location, construction and operation of mills, tailings dams, evaporation ponds and related processing facilities[7]. The milling industry has to be ready to adopt state-of-the-art methods for disposing of tailings (not necessarily conventional) and pollution control. The industry has to be prepared in many cases to upgrade existing waste disposal facilities, to comply with regulations, and may also become more active in research and innovation. The detrimental effects of surface mining are more easily offset than those from mineral processing. Various other industries also produce metal-laden liquid wastes. For example, a recent NATO Advanced Study Institute[8] reported that in the Thessaloniki area, it has been estimated (by PERPA, Greece) that among the different solid wastes produced per year, there are 30,000 tons of arsenic sludges and 70,000 tons of roasted pyrite, which are finally disposed in the Chalkidiki mines, in the Stratoni area.

1.2. MINERAL PROCESSING AND THE ENVIRONMENT

The unique features of ore deposits and effluents must be quantified through careful test work; generally, zero discharge should be the target. Even if the conditions of a disposal permit are met satisfactorily by an operating plant, there are some risks. Many sludge ponds are largely inert, but typically may contain a minor component that is radioactive, toxic or hazardous. In addition, in certain parts of the world, for example the Rocky Mountains in Colorado, a natural leaching of ores has been reported[9]. This phenomenon led to the appearance of waters with relatively high concentrations of metallic ions.

Although most mineral wastes have had the bulk of valuable minerals recovered, many older stockpiled solid wastes contain mineral values that may be worthwhile extracting under new economic and technologic conditions. Thus, operating companies are usually reluctant to dispose of their wastes and prefer to stabilise them for subsequent possible retreatment[10].

The gradual depletion of pure and high grade ores has resulted in the exploitation of ores and concentrates of lower grade and/or higher impurity contents. The problem of fine mineral particles has become significant in mineral processing[11]. A good example is the Florida phosphate industry.

The scarcity of fresh water resources for the mining industry and the desire to reduce effluent discharges from mining and processing operations have led to emphasis on reusing process water. Experience in Swedish sulfide ore concentrators illustrate this development[12]. Water recycling (and not only from the point of view of pollution) is essential. However, the processing of complex sulfide ores (by flotation) is characterized by strict demands on the chemical composition of the water phase; usually, the introduction of reclaimed water is considerably more complicated than is the use of fresh water.

Based on mineral processing experience, several toxic metal anions and cations have been removed using as substrates or adsorbents different industrial and mining by-products, namely pyrite, red mud (from bauxite processing) and dolomite[13]. Their fine particle size range is well-suited to processes like adsorption. The transfer of this mineral processing know-how to environmental technology, taking advantage of the mineral fines, is an obvious step, and here, too, flotation has been proposed as a suitable separation process downstream.

Flotation is a well known selective separation process in mineral processing. The importance of froth flotation to the economy of the whole industrial world is enormous. In addition to minerals applications, flotation has been applied successfully also to water and wastewater treatment, usually as a solid/liquid separation technique. Following extensive pilot treatment studies, and investigations into treatment options covering a wide range of processes, mostly conventional, the world's largest dissolved-air flotation plant has been constructed for the Severn-Trent Water Authority at Birmingham (UK) for potable water production, with a maximum capacity of 450,000 m^3/day[14].

Another good example of the interrelation between mineral processing and the environmental damage is acid mine drainage, which results from the reaction of pyritic material with oxygen in the presence of water. This is generally regarded as the principal environmental problem caused by mining of sulfide ore deposits[15]. Oxidation of pyrite, which proceeds by electrochemical mechanisms[16], may also be accelerated by naturally occurring bacteria. So, nature has already taught us how to use microorganisms for separation (i.e. leaching).

Precipitation of metals constitutes the main applied method for the removal of metals from different metal-containing effluents such as plating wastes, metal treatment wastes, dilute leach solutions and hydrometallurgical residues. The metals are precipitated in the form of their hydroxides by pH adjustment, usually with lime, or coprecipitation by ferric hydroxide[17] Alternative conventional methods for removal of heavy metals include ion exchange, adsorption and precipitation in the form of their sulfides[18].

2. The Use of Microorganisms

The identification and characterisation of lithotrophic iron and sulfur oxidising bacteria opened up new possibilities for the mining and metallurgical industries. In 1947, *Thiobacillus ferrooxidans*, the micro-organism responsible for acid generation in coal mines (as mentioned above), was isolated and characterised. Many applications of this bacterium have been published since then, for example among others, by Garcia Frutos[19].

Developments in the bioprocessing of minerals have been excellently reviewed by Smith and Misra[20]. The application of genetic engineering techniques for the improvement of microbial performance has also been initiated (see, for example, Torma[21)]. Mining wastes and complex unused mineral resources can be economically treated by bacterially-assisted heap and dump leaching processes. This technology grew during the 1970's into a more structured discipline called biohydrometallurgy. Efforts were devoted to the identification of a variety of micro-organisms involved in naturally occurring leaching processes and to the elucidation of leaching mechanisms.

Current trends in biohydrometallurgy include environmental remediation scenarios that afford considerable opportunities for research. The ability of micro-organisms to remove metal ions from solution is a well known phenomenon; it is clear that living organisms have influenced steady state levels of elements in the atmosphere, the oceans and at the surface of the earth throughout geological time. A high degree of metal accumulation has been observed for many organisms. Marine algae have been used, for instance, to monitor marine pollution[22].

The uptake of metals by three strains of dried sea algae was investigated[23]. It appeared, from a practical standpoint, that seaweed may function as a good biosorbent and particularly *Sargassum spp.*, because of their strong pH-dependent binding of most metals.

The ready availability of waste *Saccharomyces cerevisiae* biomass from fermentation industries has attracted much interest in this material as a practical sorbent. Granular biosorbent biomass produced by treating the yeast with hot alkali[24] offers certain advantages. This biosorbent was effective in simultaneous accumulation of zinc, chromium and copper from electroplating wastewater. It was less efficient in removing chromium from tannery wastewater, possibly owing to the presence of chromium-binding organics such as proteins, bacteria, or tannins in solution.

Biologically-induced separations offer considerable potential because of their low cost and relatively benign characteristics. The ability of microorganisms to oxidize and reduce various species should not be overlooked, as these properties can be exploited to either liberate or precipitate metals[25].

It is true of course that wastes are often more economically managed by transporting them off-site, than by utilising separation technologies to purify and recycle.

2.1. THE PROCESS

The aforementioned are no more than a few application examples of the biosorption process. Generally, metal recovery or removal from solution may involve the following pathways :

1. The binding of metal cations to cell surfaces, or within the cell wall, where microprecipitation may enhance uptake.
2. Translocation of the metal into the cell, possibly by active (metabolic energy-dependent) transport; the active uptake or concentration of metal by living microbial cells is often termed bioaccumulation.
3. The formation of metal-containing precipitates, by reaction with extracellular polymers or microbially produced anions such as sulfide or phosphate ("bioprecipitation").
4. The volatilization of the metal by biotransformation - see Hughes and Poole[26].

Before examining the process of element uptake by cells, it is necessary to consider some examples of biochemical metal selectivity evolved by living organisms. The mechanisms of microbial resistance to heavy metals include: the development of energy-driven efflux pumps that keep intracellular levels of toxic elements low; enzymic oxidation or reduction, which can extracellularly or intracellularly convert a more toxic species of an element to a less toxic one; biosynthesis of intracellular or extracellular polymers that serve as traps for the removal of metal ions from solution; biomethylation and transport through cell membranes by diffusion controlled processes; the binding of metal ions to cell surfaces; and the precipitation of insoluble metal complexes (metal sulfides, oxides) at cell surfaces[27].

Biological cells fall into two categories. Prokaryotic cells (or prokaryotes) do not contain a membrane-enclosed nucleus and usually exist as small, relatively simple unicellular organisms[28]. Eukaryotic cells (or eukaryotes) make up the other major class of cell types; they are defined as cells which possess a membrane-enclosed nucleus. These cells are generally 1,000 to 10,000 times greater in volume than prokaryotes. Some exist as unicellular micro-organisms (yeasts, for example), but eukaryotic cells are the constituents of nearly all multicellular organisms.

Cell walls of prokaryotes and eukaryotes contain different polysaccharides as basic building blocks. The ion exchange properties of certain natural polysaccharides have been studied and it is well established that bivalent metal ions exchange with counter ions of anionic polysaccharides, such as alginic acid; ion exchange is probably involved to a large degree in metal sequestering by algal biomass[29].

Microbial biomass in its natural form consists of small particles of low density, low mechanical strength and low rigidity. Biomass is generally available in the form of individual cells or cell aggregates. The form depends on the characteristics of the biomass strain and the culture conditions.

Harvested biomass has the form of a dense slurry of living cells with considerable carry-over of growth medium residuals. Present industrial applications of biosorption make use of dead biomass, which does not require nutrients and can be exposed to environments of high toxicity that cannot support life, and several methods are available for inactivating microbial biomass. Immobilized microbial biomass can be produced in the form of particles of desirable size, mechanical strength and rigidity while maintaining the native properties of the biomass. However, the age of the microbial culture providing the biomass may affect the metal uptake capacity of the microbial biomass[30]. Commercial biosorbents have appeared on the market, for instance, by Advanced Mineral Technologies (AMT)[31].

Biosorption may well be a viable solution to metal ion removal. Remote acid mine drainage waters and small toxic seeps from hard-rock mining districts, for instance, were treated at the ex-U.S. Bureau of Mines[32]. Porous polymeric beads were developed, containing immobilized dead biological materials for extracting metal contaminants from wastewaters, having good handling characteristics.

One of the major obstacles encountered in the commercialization of biochemical products is their effective separation and purification, since it can represent up to 60% of the total production costs. For this reason, group-specific ligands have been effectively introduced into a number of bioseparation processes[33] in order to further improve them, especially for the cases where cost is not considered as a significant factor (difference from the case of treatment metal wastes).

3. Bioaccumulation

Microorganisms and microbial products can be highly efficient bioaccumulators of soluble and particulate forms of metals, especially from dilute external concentrations, and microbe-related technologies may provide an alternative or adjunct to conventional techniques of metal removal/recovery - (for a comprehensive review, see Gadd(34)). There are indications that microbe-based processes are often more economical than existing treatments, and some processes are in commercial operation in the mining and metallurgical industries (see also in the following). An outline scheme is presented in Figure 1[34]. Such processes are of industrial interest because as well as the removal of potentially hazardous heavy metals and radionuclides from industrial effluents and waste waters, microbial biomass or derived products can lead to the recovery of valuable elements, such as gold and silver, after appropriate treatment of the loaded biomass.

A variety of mechanisms exist for microbial metal and radionuclide accumulation from solution; these range from purely physico-chemical interactions, such as adsorption to cell walls and other constituents, to mechanisms dependent on metabolism, such as transport, internal compartmentalisation and extracellular precipitation by excreted metabolites. Uptake

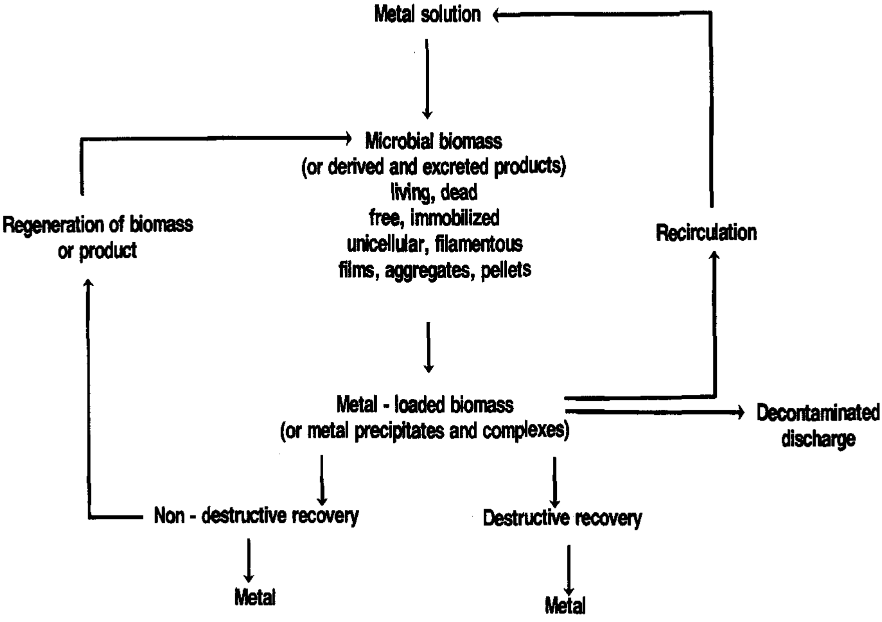

FIGURE 1
Scheme of the basic principles involved in metal removal from aqueous solution by microbial biomass. Reprinted from the paper by Gadd[34].

capacities and mechanisms may vary widely in different microorganisms, although common features do exist.

Active uptake systems can take up both essential and non-essential toxic metal ions and thus are of interest. Also since they are not equilibrium processes, countercurrent contacting processes would not be required. Each of the mechanisms for resistance to toxicity (as described above) requires inputs of cellular energy, either for active transport into living cells or for growth of microbes subsequently involved in passive uptake, and as such represents a non-equilibrium component for the distribution of elements at the Earth's surface[27].

Microbial metal uptake can often be divided into two main phases: the first, which can also occur in dead cells, is metabolism-independent binding or adsorption to cell walls and other external surfaces and is generally rapid. The second, slower phase is metabolism-dependent transport across the cell membrane. The algae *Chlorella vulgaris* and *Chlamydomonas sp.* (the latter less effectively) were shown to accumulate lead after 5 or 7 days exposure to Pb(II), within the cells as well as in the surface structures. However, with only 20 min exposure to lead, the accumulation was almost exclusively in cell walls[35]. The process depended also on the nutrient medium in which the algae were cultured.

Immobilized microalgal biomass has been used in a commercial bioremoval process and appears to hold promise[36]. Nevertheless, the quoted prices for two currently commercially available algae, *Chlorella* and *Spirulina*, are the highest among those for a variety of biomass products. It was concluded further that the immobilization processes currently used are far from optimal, which is a general problem in many other fields of biotechnology. The superiority, if any, of microalgal over bacterial and fungal biomass for bioremoval has not been established, as there are very few comparative or comparable studies. Interesting discussion by Volesky and Holan[37] has been focused on the composition of marine algal polysaccharide structures, which seemed instrumental in metal uptake and binding.

The removal of cadmium from industrial wastewaters using biosorption is of particular interest, due to its high toxicity. Gram-negative bacteria immobilize cadmium mainly in the cell envelope; it was suggested that phosphoryl and carboxyl groups of lipopolysaccharides may play a significant role in cadmium entrapment - see Brierley *et al.*[38]. Gram-positive and Gram-negative strains may display differential affinity of metal biosorption in their cell components. The so-called "Gram reaction" refers to the response of bacteria (i.e. prokaryotes) to a rapid staining test involving a complex of the dye crystal violet with iodine; bacteria retaining the color after the process are called Gram-positive[28]. This indicates basic differences in cell-wall structure, and many characteristics of bacteria correlate very well with this major division of bacterial groups.

Bacterial extracellular polymers, the most important of which are polysaccharides, have been studied for the capture and the subsequent removal of toxic metal ions from aqueous solutions. The flocculation step in the process has been examined with respect to metal uptake rate. Some of the results, from repeated flocculation of a solution containing copper, are presented in Figure 2[39]. Biomass, including a large quantity of adherent extracellular polysaccharide, was produced at a moderate cost from glucose using the organism *Zoogloea ramigera* 115. The copper concentration decreased from 0.525 to below 0.01 g/L after three successive flocculation steps. The flocs produced were rigid and porous, thereby simplifying the elimination of water.

Xanthomonas campestris, another Gram-negative bacterium, also produces an extracellular polysaccharide, xanthan, widely used in several biotechnological applications. Strains of *X. campestris* have been developed which can use whey as substrate, a by-product of dairy industries. In this way, biomass cells and xanthan can be produced on a large scale. A biosorption study of cadmium by living *X. campestris*, an important industrial microorganism[40], showed promising results, some of which are presented as Figure 3, under various

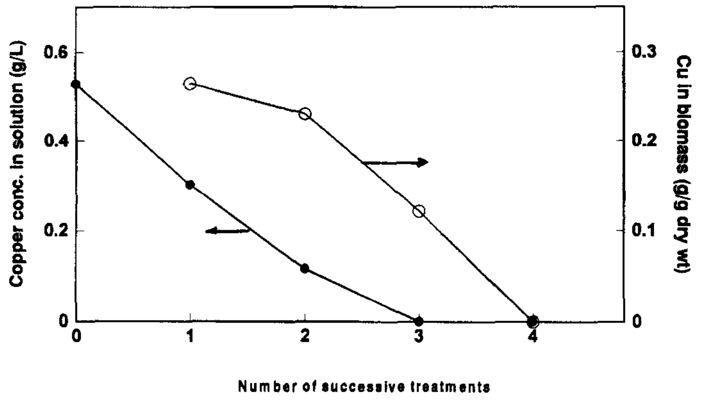

FIGURE 2
Effect of repeated flocculation on a copper solution; the biomass concentration was 0.83 g dry weight/L and the equilibration pH was 5.5. Reprinted with permission[39]; copyright 1984, J. Wiley & Sons.

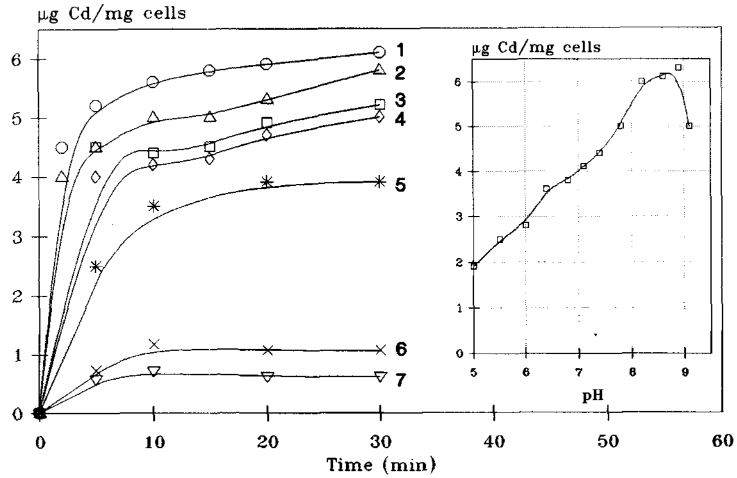

FIGURE 3
Kinetics of cadmium uptake by *X. campestris* cells (initial Cd concentration 5 mg/mL), at various conditions: (1) 0.25 M sucrose at pH 8.3, (2) 10 mM tris - 0.25 M sucrose at pH 8.3, (3) water at pH 8.3, (4) 10 mM tris at pH 8.3, (5) 10 mM bis-tris at pH 7, (6) 50 mM tris - 50 mM NaCl at pH 7, and (7) 10 mM tris - 0.25 M sucrose at pH 7. Inset: Cadmium biosorption at different pH values (10 mM bis-tris at pH 5-7 and 10 mM tris at pH 7-9.1). Reprinted with permission[40]; copyright 1994, SCI.

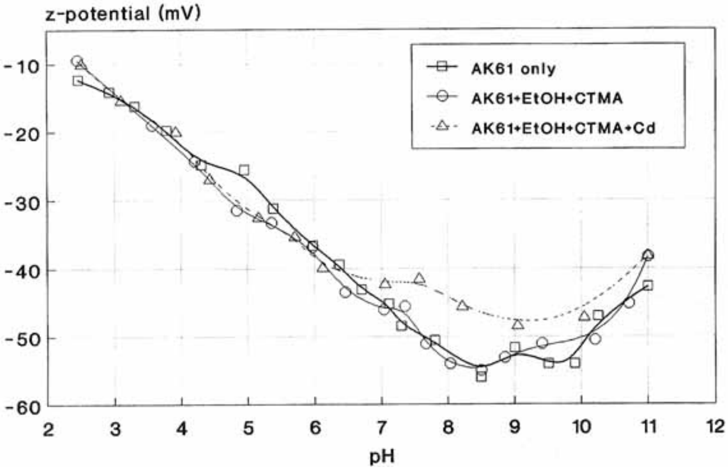

FIGURE 4
Zeta-potential measurements of non-living AK61 biomass against the solution pH; effect of ethanol or cetyl trimethyl ammonium bromide. Reprinted with permission[42]; copyright 1995, IAWQ.

experimental conditions (as shown). It was proved to be a fast and effective process. The sorption capacity, best in the pH range of 8-9, was more than 6 Ëg Cd/mg biomass. A comparison was also attempted with three other bacterial species, namely *Escherichia coli* (Gram-negative, which did not found to produce extracellular polysaccharide, *Bacillus subtilis* (Gram-positive, which did not produce also extracellular polysaccharides) and *Leuconostoc mesenteroides* (Gram-positive, which produces dextran, an uncharged extracellular polysaccharide). It was concluded that *X. campestris* and xanthan could potentially be used as Cd biosorbents for the detoxification of aqueous solutions. In the same Figure, the influence of time on metal removal is also shown. Kinetic studies indicated that mass transfer plays an important role in the biosorption rate[41].

Electrokinetic measurements were carried out, with an apparatus of Rank Brothers, UK (Mark II), to investigate the mechanism of cadmium biosorption[42]. The principle and details have been presented previously[43]; electrophoretic mobility values were converted to zeta-potential by the Smoluchowski equation. Figure 4 presents the results with biomass of a *Streptomyces* strain AK61. No substantial change of the surface charge of the non-living biomass was observed when biomass was examined alone or in the presence of ethanol (used as a frother) and the cationic surfactant (collector). It was further found that both the living

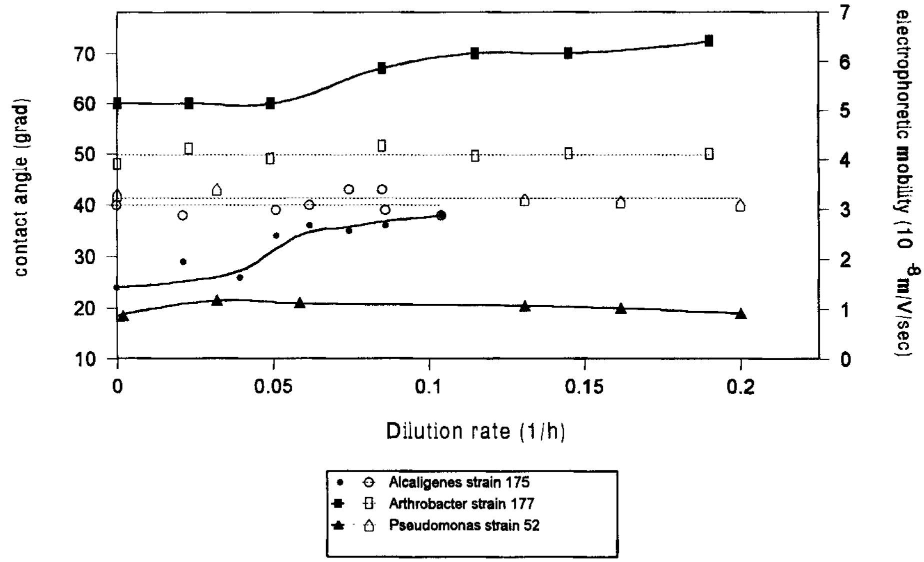

FIGURE 5

Cell surface hydrophobicity (——) determined by the water contact angle method and cell electrophoretic mobility (······) as a function of dilution rate. Reprinted with permission[45]; copyright 1987, Amer. Soc. Microbiol.

bacteria examined (*Streptomyces* strains JL322 and AK61) had almost the same maximum binding capacity of around 23 mg Cd/g biomass. The maximum binding capacities of non-living JL322 and AK61 biomass, 33.7 and 46.9 mg Cd/g biomass respectively, were observed to be higher than those of living bacteria (the strains were kindly supplied from the culture collection of the Microbiology Dept., Univ. Newcastle).

Microbial walls are anionic owing to the presence of carboxyl, hydroxyl, phosphoryl and other negatively charged sites. Cationic metal ions rapidly bind to these sites by an energy-independent reaction. The Gram-positive bacteria (including those of the *Streptomyces* genus mentioned above) are particularly suited for metals removal; the processes involved in metal binding by this group of bacteria have been extensively reviewed[44].

The electrophoretic mobility and hydrophobicity (expressed as contact angles) of many bacteria have been measured[45]. The necessary values required for adhesion of a microorganism to glass and other surfaces were determined; of course, if the particles are oppositely charged there is no barrier to adhesion. The influence of bacterial growth rate on surface properties was measured in a chemostat, as presented in Figure 5. Hydrophobicity increased with increasing dilution rate (higher specific growth rate), whereas electrophoretic mobility did not change markedly. If a microorganism has a hydrophobic surface and adheres to a mineral species it should promote aggregation of the minerals and flocculation of an aqueous suspension of the minerals, such as does a conventional flocculant and/or surfactant. The adsorption of *Mycobacterium phlei* (a hydrophobic bacterium) onto a mineral was reported and found to be a good flocculant for fine hematite[46].

4. Engineering Aspects

Biosorption consists of accumulation by predominantly metabolism- independent interactions, such as adsorptive or ion exchange processes. Sorption isotherm experiments have been conducted, in order to find the maximum binding capacity of the living cells and of non-living biomass. Comparison such as those presented in Figure 6 indicate that killed biomass exhibit greater binding capacities for cadmium than living material. The removal of the metal was nearly 100%, the residual cadmium was below 0.1 mg/L, over a wide solution pH range.

Langmuir isotherms were shown to describe the process well. The quantity q_e (amount of metal sorbed per unit mass of biosorbent) was plotted against C (concentration of free soluble metal at equilibrium). The respective equation is generally written as:

$$q_e = b X_m C/(1 + b C)$$

where X_m and b are empirical constants, characterizing the biomass-metal interactions and which are calculated from the plots. The sorption equation is usually linearized (reciprocal Langmuir plot)

$$(1/q_e) = (1/X_m) + (1/b X_m)(1/C)$$

or, alternatively -

$$(C/q_e) = (1/b X_m) + C (1/X_m)$$

The basic assumptions explicit in development of the Langmuir equilibrium model for adsorption are not met in most adsorption systems relevant to water and waste treatment (for example, the existence of a true ligand monolayer, or that the sorption energies are uniform over all sites)[47]. However, the corresponding Langmuir equation has been found particularly useful for correlating equilibrium data for such systems, as long as its restrictions are

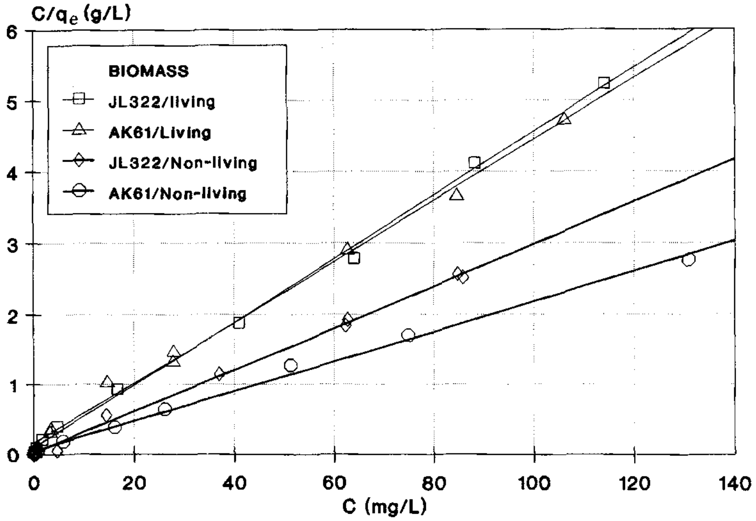

FIGURE 6
Langmuir sorption isotherms of cadmium by living cells and non-living biomass ([JL322]=0.405 g/L, [AK61]=0.605 g/L, C: concentration of free metal at equilibrium, q_e: amount of metal adsorbed per unit mass of biosorbent. Reprinted with permission[42]; copyright 1995, IAWQ.

recognized. Such deviations from the Langmuir model do not negate its value for quantification and mathematical comparison of observed equilibrium relationships.

The correlation of high cell wall content of particular types of phosphate-containing polymers with high cadmium binding capacity and affinity was said to provide a method for rationally searching for improved strains[48]. New isolates from cadmium-contaminated soil, in the surroundings of a pyrometallurgical plant in the North-East of England, showed no advantage in terms of binding capacity; however some strains exhibited a much higher affinity for Cd than did a commercial *Streptomyces clavuligerus* biomass. The ability to isolate strains with high affinity for Cd might be important in situations where high concentrations of other bivalent cations are also present.

Consistent with previous observations, the strains with highest wall teichoic acid content were those that exhibited the highest capacity for Cd binding[48]. In general, strains having the greatest resistance to cadmium toxicity exhibited the lowest Cd-binding capacities and low affinities for Cd (low Langmuir constants). All the strains with high affinities were susceptible to cadmium toxicity. Thus, a degree of selection for high capacity or high affinity could be made on the basis of cadmium resistance.

Ion exchange versus adsorption models were discussed by Crist *et al.*[49] for metal sorption on algae *Rhizoclonium* and *Vaucheria* and a reassessment was made of the Scatchard plots - i.e. of (sorbed amount)/(solution concentration) vs (sorbed amount). It was pointed out that with such plots, which are often used in microbiology, misinterpretations can occur at low metal concentrations in an ion exchange system. A curved Scatchard plot observed in biosorption studies should therefore viewed with caution, since it may indicate that the chemical process is one of ion exchange, not simple adsorption.

The logarithmic equation of Freundlich has also been applied to studies of sorption with biomass. This equation has the following form[50] :

$$q_e = K\ C^{1/n}$$

where K and n are constants.

Process flow diagrams currently in use for biosorption have been presented[51]. These processes include the following treatment units: (a) packed bed reactor, (b) fluidized bed reactor, (c) rotating disc reactor, (d) sludge blanket bioreactor, and (e) artificial wetlands/ stream meanders.

Two typical examples, commonly used for sorption, are presented in Figure 7. A two-stage countercurrent biosorption process was further proposed by Volesky[29], being more efficient in the continuous flow mode of operation.

Metals removal from water has been accomplished using biologically complex or "intact" ecosystems[38]. Study of these systems is difficult owing to the complex array of interactions among the biota present (algae, bacteria, fungi, higher plants and animals) and distribution of the biota (sediment-associated, surface-associated and water-suspended). The physical setting for living-system metal-removal processes may either be natural or man-made: the natural-setting systems incorporate existing landforms such as ponds, streams, bogs, or marshy areas. The man-made systems employ purpose-built ditches and ponds with or without an underlying containment of clay, plastic sheeting or other suitable material.

Engineering considerations are central in decisions concerning the commercial future of biosorption. A kinetic mass transfer model was developed for the description of biosorption of metal ions by immobilized biomass from aqueous solution in a batch reactor[52]. Elsewhere, a method for removal of metals from solution using suspended plant cells, including *Datura innoxia*, was patented by Jackson *et al.*[53]; this scheme implies a CSTR (continuous-stirred-tank-reactor) type equipment for the solution-biosorbent contact.

Tsezos[30] argued that the main difficulty, in the later case, lies in the rapid and efficient separation of biomass from the reaction mixture after contact. It was shown that flotation may provide an answer to this problem. Sorptive flotation has been recently reviewed[54]. Both

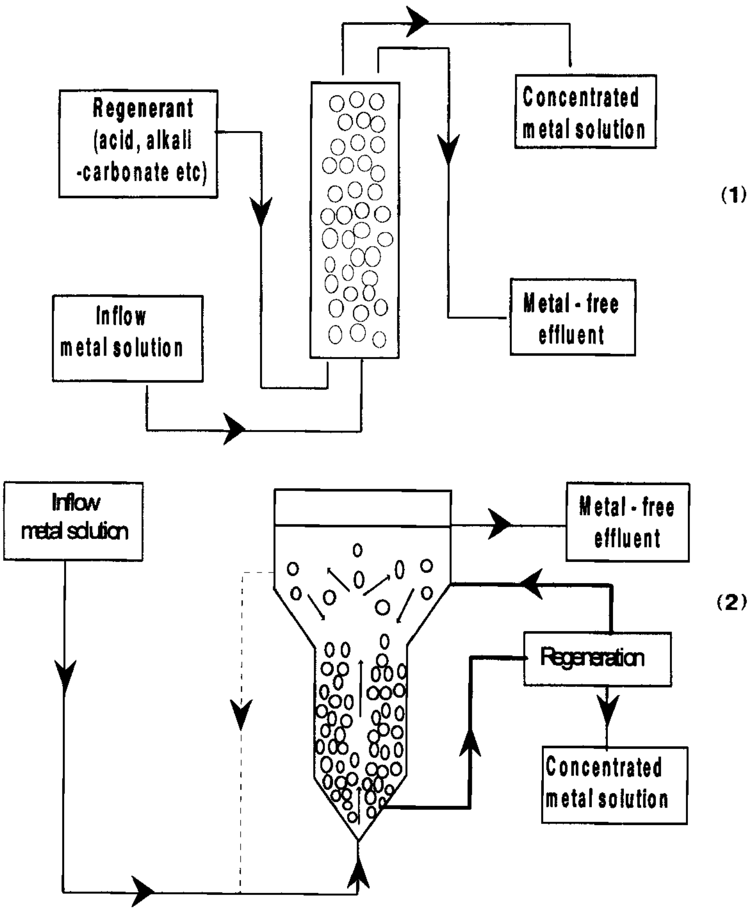

FIGURE 7
Simplified diagrams of two common biotechnological processes currently in use for alleviating toxic metal pollution by biosorption (immobilized biomass). (1) **Packed-bed reactor** (Regeneration: in-situ, Scale: up to 20 kg biomass, but modular construction is possible, Commercial applications: AMT-Bioclaim™, Algasorb, BIO-FIX, (2) **Fluidized-bed reactor** (current applications use non-living material, Regeneration: separate cycle, but can use pulsed removal, Scale: 80-90 Kg biomass, Commercial application: AMT-Bioclaim™). Key: oooo biomass particles, ____ Inflow, outflows, ---- Recirculation, — Biomass regeneration. Reprinted from the paper by Gadd and White[51].

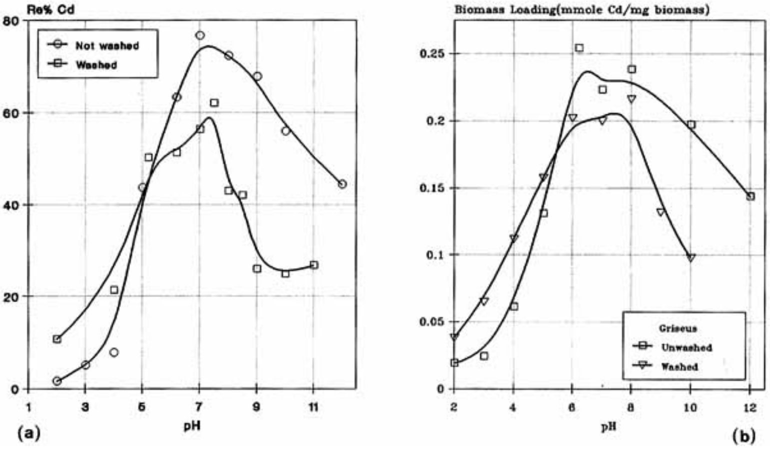

FIGURE 8

Cadmium biosorption by *S. griseus* (an actinomyces) versus pH; comparison of washed and unwashed samples: (a) Results expressed as cadmium removal (%), obtained following also dissolved-air flotation. Reprinted with permission[56]; copyright 1994, Marcel Dekker. (b) Biosorption loading of *S. griseus* , results following dispersed-air flotation. Reprinted with permission[57]; copyright 1994, J. Wiley & Sons.

dissolved-air and dispersed-air flotation have been applied successfully. The difference between the two flotation techniques is mainly in bubbles size and hence, hydrodynamics in the flotation cell[55].

Various surfactants have been used as flotation collectors for microorganisms; in this case, cetyl trimethyl-ammonium bromide (a cationic surfactant) was applied. Biosorption results versus solution pH are given in Figure 8 (the samples were collected following flotation, as metal-loaded biomass)[56]. A comparison between washed and unwashed *Streptomyces griseus* samples was made. The main difference between them appeared at a pH of about 7.5. Cadmium removal was higher with the unwashed samples, due possibly to its coprecipitation with insolubles. Foreign organic and inorganic substances may accompany the microorganisms and these substances may function as surfactants or modifiers. The solution pH was generally a crucial parameter in biosorption (also in the subsequent flotation). The influence of metal speciation in solutions certainly requires attention.

Selected measurements (under the applied flotation conditions) for zeta-potential of *S. griseus* surface have been presented in the literature[57] (to be discussed below). It was *concluded that the theories of adsorption mechanisms of metals on inorganic colloid particles* can be extended to cellular surfaces. The contact time for biosorption was 900 s, with a low energy input. The dilute aqueous solution had a concentration of 10 mg/L Cd and dead biomass in dispersion was usually added to 1 g (dry weight)/L. The effects of the concentrations of both components were investigated. Cadmium concentration was varied up to 150 mg/L and biomass addition up to 3.5 g/L. The unwashed biomass contained culture-medium residues (approximately 30%). The metal biosorption was carried out in appropriate... beakers (500 mL - 1 L) and mixed with a stirrer at a low rate (i.e. CSTR type contact). Satisfactory metal removals started from about the solution pH of 5, reaching usually almost 100%, under optimum conditions. Waste biomass from the antibiotic fermentation industry had considerable potential in biosorption.

The *Streptomyces* biomass "particles" were in the fine particle size range, around 20-150 μm (given here as a typical sieving scale). Flotation is based on surface activity, thus some surface tension measurements of the solution were conducted during biosorption. In the acidic pH region, up to neutral, the surface tension increased with the addition of cadmium (and 0.25% v/v ethanol) up to about 60 mN/m.

The removal of the same metal ion from aqueous solution, by precipitate flotation as cadmium hydroxide (i.e. with pH adjustment) and without the presence of any biosorbent, was also investigated for comparison[58]. Promising results were obtained in the pH range between 10 and 11 approximately, applying sodium dodecylsulfate as collector. Comparing biosorption by *Streptomyces* with ion flotation (using the term in the broad sense[17]), the former gave a pH limit shifted to lower pH, showing an advantage of biosorption.

Flotation was also proposed by Diels[59], in a research workshop, as a part of a bio metal slurry reactor (termed BMSR) for the decontamination of sandy soils from heavy metals; *Alkaligenes eutrophus* CH34 bacterium was applied in the well mixed reactor.

Often the prediction of sorption equilibria is complicated by the presence of several sorbed ions. A model case for two ion exchange sites and three ions was compared to the experimental data for the sorption of Cd, Cu and Zn by protonated *Sargassum fluitans* (marine algae) in systems containing two metal ions and protons at different pH values using a computer program (i.e. MINEQL+)[60]. The model predicted a similar trend to that observed in the experimental data. An example is given in Figure 9, which demonstrates the sorption behavior of a Cu-Zn system at changing pH values for a constant total concentration (solid + liquid phase) of each metal.

This type of analysis originates mainly from the inorganic sorbents area and several variations of the basic surface complexation approach have been developed[61]. The fundamental concepts upon which all the models are based are the same:

a) Sorption on oxides takes place at specific coordination sites;

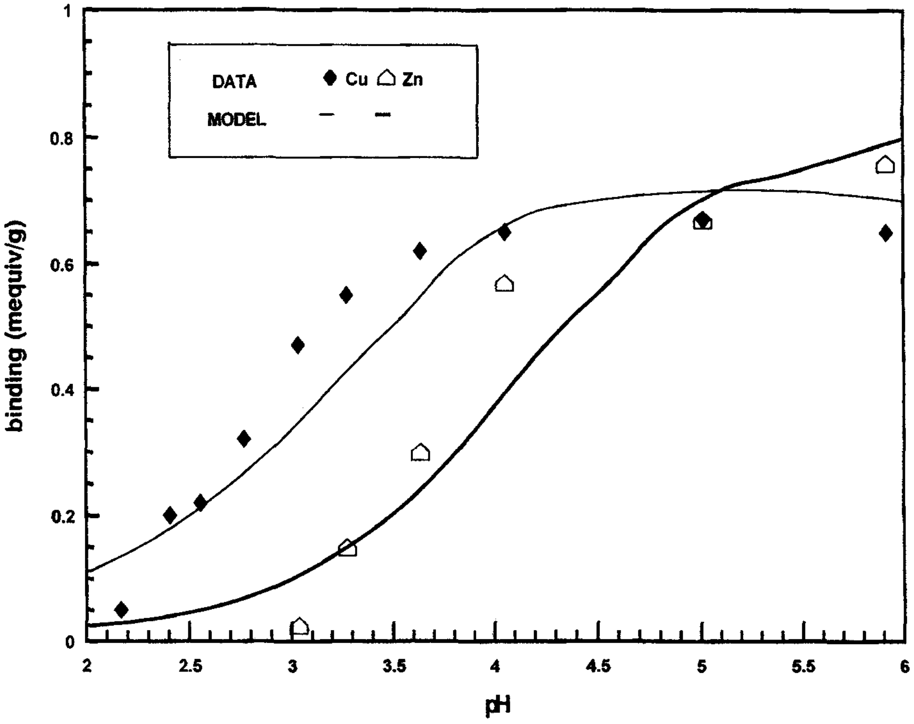

FIGURE 9
Titration of *Sargassum fluitans* biomass in the simultaneous presence of Cu and Zn at constant total concentrations of each metal: biosorption binding data and computer programme model predictions. Reprinted with permission[60]. Copyright 1996, Amer. Chem. Soc.

b) *sorption reactions on oxides can be described quantitatively via mass action law* equations;

c) surface charge results from the surface reactions themselves, and

d) the effect of surface charge on sorption can be taken from the electrical double layer theory applied to mass law constants for surface reactions.

Nevertheless, the oxide surface, which is known to exhibit an amphoteric behavior (due to surface ionization reactions), is considered rather "homogeneous" in comparison with that of biosorbents. Therefore, the surface cation complexation of the former is quite straight forward involving the formation of bonds with surface oxygen atoms and the release of protons from the surface; in case of higher cation concentrations in the solution, surface precipitation is also possible.

4.1. ECONOMICS

Biosorption processes are generally very case-specific and highly depend on the properties of the biosorbent in both affinity and capacity for heavy metals of interest in the multi-ion environment of real wastewaters. Only recently some microbial treatment systems have attained greater operational sophistication, an important aspect for greater efficiency[34].

Comparative costs for biosorptive processes with ion exchange and chemical precipitation, using either lime or caustic soda, were predicted by Eccles[62]. For example, the Biofix process[33] was compared with chemical precipitation for treating acid mine drainage: it was predicted that the capital cost of the former was $3.37m with an annual operating cost of $1.26m. These figures ultimately equated to drainage water treatment costs of $1.4 per 1000 US gallons. For conventional lime treatment, it was calculated that the corresponding cost would be $1.5.

In another example[62], for the AMT-Bioclaim process, which uses granules of about 0.1 mm size, based on selected microbial biomass (*Bacillus subtilis*)[31], applied as a polishing step, it was predicted that it could reduce the cost per gallon of treated waste water by over 50% when compared with a chemical precipitation process. The number of times the metal recovery agent could be regenerated was a key assumption in the economic analysis; the life expectancy of the biosorbent was 25 cycles. As the metal concentrations rose above 100 mg/L so did the operating costs. It was also predicted that this secondary waste treatment was significantly less expensive, able to reduce capital costs by 20%, operating costs by 36% and total treatment costs per gallon by 28%, than that of the ion exchange system.

It was proposed[62] that these costs, although indicative, were sufficiently accurate to justify further evaluation of bioprocesses. Cost could be one of several good reasons for selecting biosorption, as in many instances biomass will be regarded as an industrial waste and therefore, considerably cheaper than an ion exchange resin (typically $1-2/kg).

5. Biosorption

5.1. ACTINOMYCETES

The metal binding properties of Gram-positive bacteria are largely due to specific anionic polymers (for example, peptidoglycan, teichoic or teichuronic acids), in the cell wall structure. Due to this high fixed anion content, they exhibit high sorption capacities, important for the industrial application of these bacteria as biosorbents. Another important advantage of these bacteria, including the actinomycetes, is the ease with which their wall properties can be controlled[44]

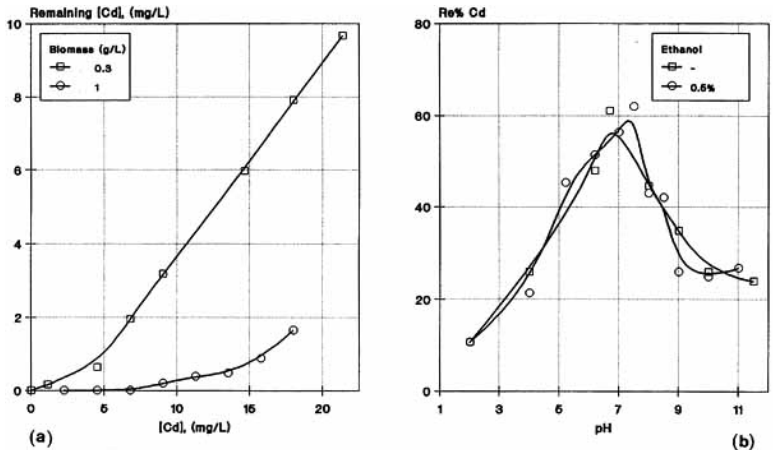

FIGURE 10

(a) Remaining cadmium following its biosorption by washed *S. griseus*, at two different biomass concentrations: influence of initial feed cadmium concentration (pH 6.2). (b) Cadmium biosorption with the solution pH, in presence and absence of ethanol (the later used as flotation collector). Reprinted with permission[56]; copyright 1994, Marcel Dekker.

Some experimental data for camium biosorption by actinomycete bacteria of the genus *Streptomyces* are presented below. Two biomass concentrations were tested for cadmium biosorption; the residual Cd concentrations in solution are shown in Figure 10. High Cd removals were obtained[56]. The initial biomass concentration, as expected, is important. No influence on biosorption by *S. griseus* was observed due to the presence of ethanol (being harmless to the environment, at such concentration), which was used later as a frother mainly during dispersed-air flotation.

Some batchwise results obtained with *Streptomyces clavuligerus* are presented in Figure 11a[63]; this biomass was an industrial by-product (kindly provided by SmithKline Beecham, UK). *S. clavuligerus* is a branched filamentous actinomycete that is used for the production of clavulanic acid (ß-lactamase inhibitor). Unwashed samples again showed higher loading, due to the presence of culture medium residues. The removal process was effective at pH values above approximately 5; the preliminary stage of biosorption was quite fast. The values found for metal removal were considered promising, particularly when compared with other sorbents, like activated carbon, etc.[57].

The effect of foreign ions was also examined (Figure 11b) by the addition of a salt, sodium sulfate, in various concentrations. Ionic strength was found to be detrimental for

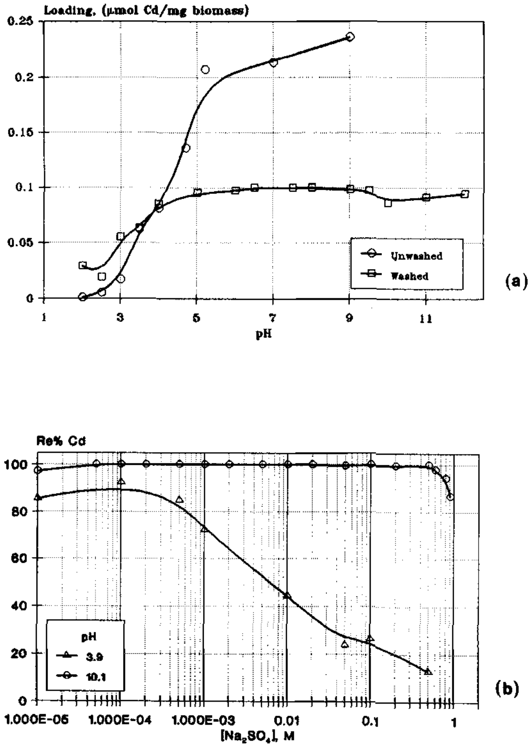

Figure 11

(a) Cadmium biosorption by *S. clavuligerus* biomass, expressed as loading, (b) Influence of ionic strength (adjusted by foreign salt addition) on cadmium removal, at solution pH values of: (i) 3.9 and (ii) 10.1. Reprinted with permission[63]; copyright 1994, Elsevier.

biosorption, mainly at a pH of 3.9, which anyway was not the optimal for operation[63]. Sodium sulfate was later applied as a suitable and effective eluant.

All microorganisms are likely to be electrically charged in an aqueous environment. The addition of multivalent ions, under some conditions, was shown to reverse the surface charge of microorganisms from negative to positive[57]; foreign ions are known to affect the surface potential of particles. Much of the work in this area was performed in an attempt to elucidate the role of various substances, such as antibiotics, on the behavior and condition of pathogenic bacteria and viruses.

In Figure 12a the influence of a quaternary ammonium surfactant, on Cd biosorption, is presented. This compound is a flotation collector used to improve biomass recoveries at higher ionic strengths. At high surfactant concentrations, a deterioration in Cd removal was apparent.

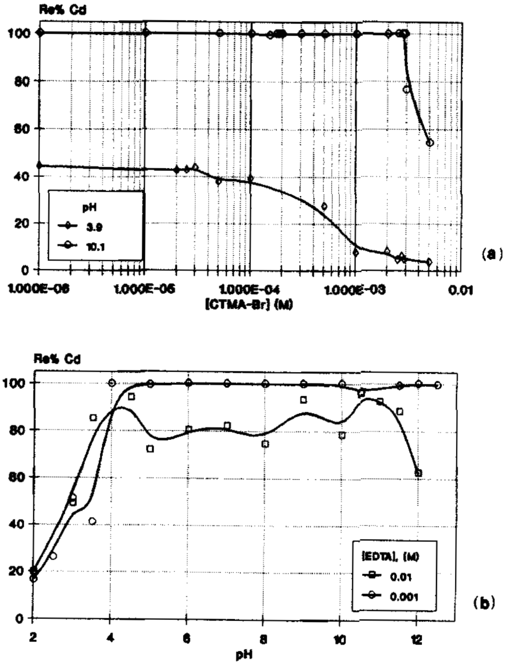

FIGURE 12
(a) Effect of cetyl trimethyl ammonium bromide concentration (a flotation collector) on Cd biosorption at 0.01 M Na_2SO_4, at the same pH values as in Fig. 11b. (b) Influence of the eluant applied in a second biosorption cycle. *S. clavuligerus* previously eluted by EDTA of concentration: (i) 0.001 M and (ii) 0.01 M; the biomass contained 5.14 mg Cd/g of biomass). Reprinted with permission[63]; copyright 1994, Elsevier.

Cadmium desorption from loaded biomass was achieved either by a complexing agent (such as citric acid or ethylenediaminetetraacetate) or by another specific chemical compound. Biomass re-flotation was also found possible, permitting in this way subsequent biomass recycling. Metal desorption tests of the (floated) biomass were carried out in bench scale (Figure 12b). The higher concentration of EDTA (0.01 M) slightly depressed biosorption *in a subsequent cycle*. EDTA, a powerful hexadentate chelating ligand, is often used as a sequestering agent in processes where the concentrations of dissolved, free metal ions have to be controlled. Its main uses are in industrial cleaning, household detergents, photographic industry, pharmacy, textile and paper manufacturing; thus, it occurs widely in wastewater streams. Recently, there has been some concern about the potential of EDTA to remobilize toxic heavy metals out of sewage sludges and sediments[64].

A filter aid, celite (i.e. calcined diatomaceous earth), present in the industrial biomass material, was found to be compatible with the developed process[65]. When biomass is applied to biosorption as an industrial solid waste, it often contains filter aids (known also as body addition); the latter are used by industry to improve the permeability of the filter bed and achieve better separation characteristics.

5.2. FUNGI

Fungi can accumulate metal and radionuclide species by physico-chemical and biological mechanisms including extracellular binding by metabolites and biopolymers, binding to specific polypeptides and metabolism-dependent accumulation - see Tobin *et al.*[66]. However, to date the most promising approach to metal removal by fungi is biosorption. The fungal cell wall is thought to have two main components: interwoven skeletal framework microfibrils, usually of chitin, embedded in an amorphous layer of proteins and various polysaccharides[29]. Sorption capacity variations between different biosorbent types could be related to their acidity; pH neutralisation during the sorption reaction considerably enhanced zinc chelation. It has also been reported that prior treatment of mycellial wastes with NaOH increased their capacity for metal sorption[67].

The applicability of the hard-and-soft principle of acids and bases in predicting metal adsorption characteristics in a biological context was investigated for metabolism-independent uptake of metal ions by *Saccharomyces cerevisiae* (which has mannan-,-glucan as the main structural polymer)[68]. The results showed good agreement with the principle with respect to both the nature of bonding and preferred ligand binding of the metals examined.

Figure 13 presents some of the results for strontium (a hard metal). The metal uptake data were transformed and fitted to Scatchard and Langmuir plots and the resultant graphs were non-linear. Data which do not conform to the Langmuir model are generally taken to indicate multilayer adsorption; however, it has been suggested that nonconformity may also indicate adsorption behavior that cannot be accounted for by ionic or covalent bonding alone[68].

Filamentous fungi are used in fermentation industries to produce varied metabolites such as enzymes, flavourings and antibiotics. These industrial by-products are able to chelate various heavy metals. The main chemical groups in biomass which are able to partake in biosorption are electronegative groups such as hydroxyl or sulphydryl groups, anionic groups such as carboxyl or phosphate groups and nitrogen-containing groups such as amino groups.

Preliminary cadmium biosorption results with two fungi are presented in Figure 14; the samples (industrial fermentation by-products) were generously supplied by Roux (Grenoble,

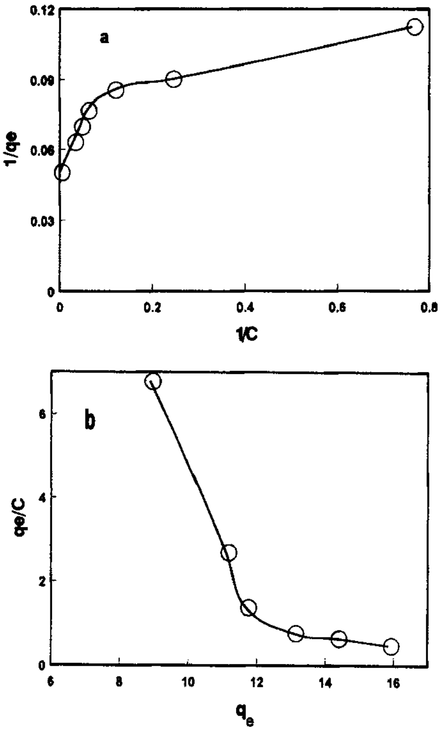

FIGURE 13

Reciprocal Langmuir (a) and Scatchard (b) plots of Sr^{2+} adsorption by *S. cerevisiae*; where q_e is in mmol g^{-1} (dry wt.) and C in mmol. Reprinted with permission[68]; copyright 1987, Amer. Soc. Microbiol.

France). A reduction in removal by biosorption at the alkaline pH (particularly, with *Rhizopus*) was observed[69]. This research is currently ongoing.

5.3. SEWAGE SLUDGE

Early work focused on metal ions in biological wastewater treatment processes and mainly emphasized the toxicity of metals to bacteria and inhibition of organic matter reduction. Since the discovery that the activated sludge treatment process could remove metals from wastewater much progress in research has been made on the immobilization of metals by waste biomass. The microbiology of activated sludge was reviewed by Kasan[70].

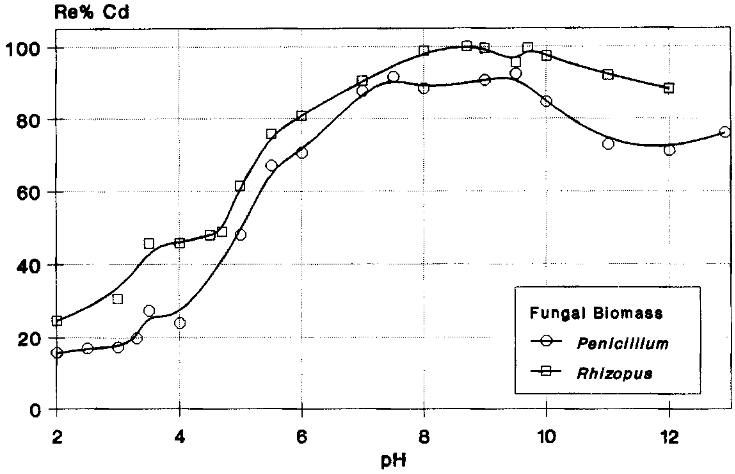

FIGURE 14
Cadmium biosorption by fungi, *Penicillium chrysogenum and Rhizopus arrhizus;* effect of solution pH. Reprinted with permission[69]; copyright 1993, Sci. Technol. Letters.

A zinc-cadmium binary mixture has been examined and some of the results are shown in Figure 15[71]. The variation in initial metal concentrations (as in actual cases of effluents) is stressed, because it is a component of the mathematical expression for metal percent removal. The competition for chelation between the metal ions at cell wall adsorption sites is evident. As might be expected, increasing biosorbent concentration produced increased metal removal; while at 1 g sewage sludge/L, by increasing the initial zinc content, a decrease in percentage removal has been noted. The influence of pH was also crucial; biosorption of metals occurs above pH 5, as also mentioned above.

5.3.1. Sorption Equilibria

The Langmuir type equation was used for describing equilibrium conditions for sorption, with correlation coefficients of the order of 0.99 (application of the Freundlich isotherm gave poorer correlation)[71]. The model can provide the parameters, X_m and b, with which to quantitatively compare the adsorption behavior in this adsorbate-biosorbent system. The value of X_m measures the binding capacity of the sorbent surface and that of b quantifies the relative strength of the binding interactions. These empirical constants were calculated for

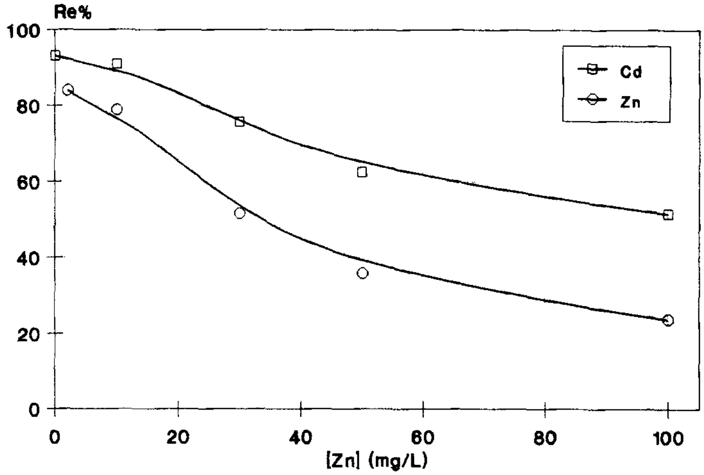

FIGURE 15
Biosorption by sewage activated sludge from a zinc/cadmium mixture: influence of Zn initial concentration ([Cd]=10 mg/L, [SS]=1 g/L, pH natural). Reprinted with permission[71]; copyright 1996, Marcel Dekker.

single-component metal solutions for the used (unwashed) activated-sludge biosorbent and found to be:

Cadmium:	X_m = 36.54 mg/g,	b = 0.57 L/mg
Zinc:	X_m = 25.63 mg/g,	b = 0.28 L/mg
Nickel:	X_m = 21.66 mg/g,	b = 0.05 L/mg

This yields the following order of binding capacity (but also of binding strength): Cd > Zn > Ni. However, previous based on copper equilibrium constants[70], it has been reported that sludges with high binding strengths for particular metal ions do not necessarily possess increased capacities for the respective metal ion.

Heavy metals present in sewage sludges can be leached by acid addition, by microbial leaching using iron oxidizing bacteria or sulfur oxidizing bacteria[72]. The advantages and disadvantages of these three processes have been evaluated in laboratory scale metal leaching experiments. Acid addition, which was less time consuming but needed a large amount of acid, was found more attractive at high plant capacities, as shown by cost estimations (in Canadian $) - see Figure 16. The sulfur oxidation process was the most attractive at low plant capacities and high solids concentrations.

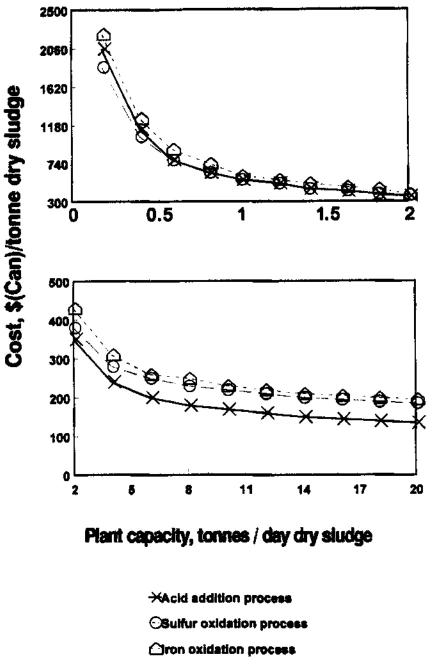

Figure 16

Cost of metal leaching from a sludge with solids concentration of 20 gl^{-1} using the three processes. Reprinted with permission[72]; copyright 1994, Publications Division Selper Ltd.

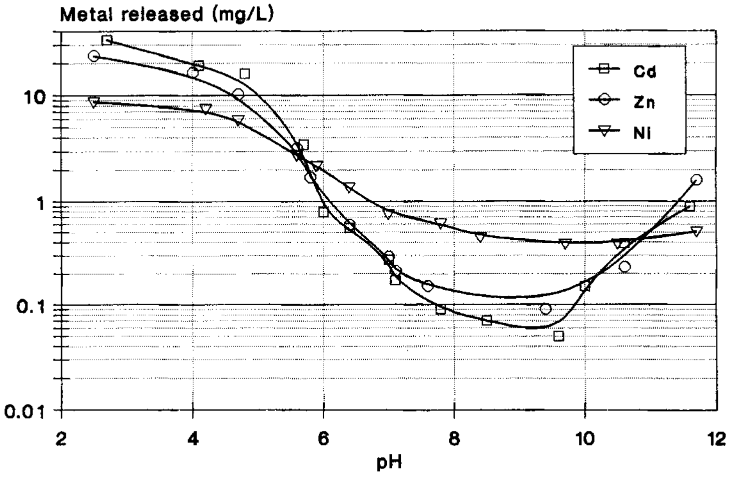

FIGURE 17

Desorption study of metals from loaded sludge biomass, as a function of dispersion pH (at a semi-logarithmic scale). Reprinted with permission[71]; copyright 1996, Marcel Dekker.

The elution of metals from the separated biomass (following biosorption) is of importance for a number of reasons, such as the stability of the loaded sludge and the possibility of pollution prevention. Relative results are presented in Figure 17. At acidic pH values, at an approximate pH of 5.5 (and also in the alkaline region), significant desorption of all the metals appeared, which has to be taken into account, for instance, in subsequent disposal. It is possible that this easy biomass elution by pH adjustment could lead on one hand to metals recovery (in a concentrated solution) and on the other, to biosorbent reuse in another cycle of metals removal. The biomass was observed to be rather unaffected by this procedure.

Sorption of metal ions onto a bacterial surface may be conceptually regarded as the formation of a surface-metal complex, according to Solari *et al.*[71] :

$$S + M \longleftrightarrow SM$$

with a conditional equilibrium constant K_a (L/g), which incorporates both X_m and b constants, providing a measure for the overall affinity, given by:

$$K_a = [SM] / [M] \{S\}$$

where M denotes free metal species, S unoccupied surface sites, SM sludge-metal surface complexes, [ ] is the concentration in mol/L and { } concentration in g/L. The lower removal results obtained in this work for nickel could be promising from a selectivity point of view.

A selectivity factor, f, can be defined[71] (when considering two metals, A and B) as

$$f = \frac{(C_A / C_B)_{biosorbed}}{(C_A / C_B)_{remaining}}$$

in order to describe the effectiveness of biosorption as a separation process, similar to the one used in ion exchange or solvent extraction processes. For the system Zn/Cd, where some selective separation was apparent, the value of f reached around 40 in the pH range 8 to 9. Selectivity is of interest specifically if recovery of metals is sought from a mixture of metal ions, which most of the practical cases of wastes are. This could be applied in conditions, as illustrated in Figure 18 (at pH 5-6), where a differentiation between the metals is apparent. Selectivity is generally a problem with biosorption systems, but this may be minimized depending on the relative concentrations of the various metals present[34].

The results of metals removal by precipitation, in absence of any biosorbent, is also shown in Figure 18 for comparison. Due to metal speciation in dilute aqueous solutions, removals as hydroxide at pH values of approximately 9 or higher (depending on the conditions) were found, which illustrates the advantage of biosorption.

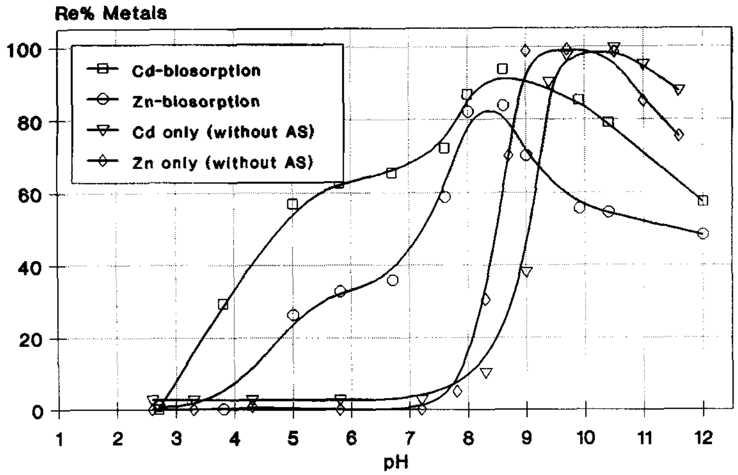

FIGURE 18

As in Figure 15; effect of pH on metals removal, in presence and absence of sewage sludge. Reprinted with permission[71]; copyright 1996, Marcel Dekker.

Figure 19 presents additional results. Poor removals of nickel were observed due to stronger affinity of this metal for soluble biopolymers than for biomass, so that these soluble complexes of nickel were unavailable for uptake by activated sludge. For comparison, metal precipitation as a function of solution pH in the absence of sludge is also shown. Langmuir adsorption isotherms give a good fit of the metals removal data.

A countercurrent operation mode with live activated sludge biosorbent has been reported[73], but it has been pointed out[26] that the inactivation of cells in activated sludge has very little effect on the uptake of a range of metals, confirming the process to be largely a passive one. Therefore, sewage sludge could be applied as a non-living system, avoiding many problems.

5.4. A MULTI-STAGE PROCESS

A complete process has been proposed for the removal of aqueous cadmium, at low concentrations, from industrial effluents and other metal contaminated waters[74]. The process would contain the following steps:

I) Contacting biomass of microbial origin with the metal-containing waste stream, so that

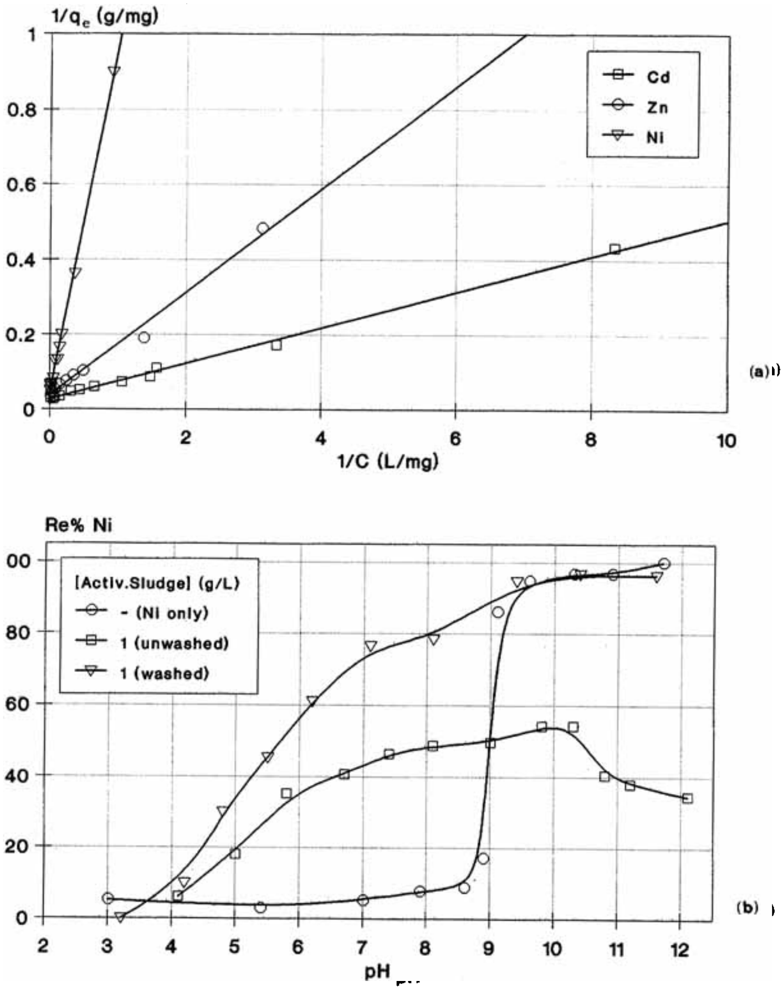

FIGURE 19

(a) Sorption of cadmium, zinc and nickel onto dead (unwashed) activated sludge: Langmuir isotherms (pH natural, temperature 293 K). (b) Effect of dispersion pH on sorption of nickel (initial concentration 10 mg/L) by activated sludge: comparison between washed and unwashed biosorbent. Reprinted with permission[71]; copyright 1996, Marcel Dekker.

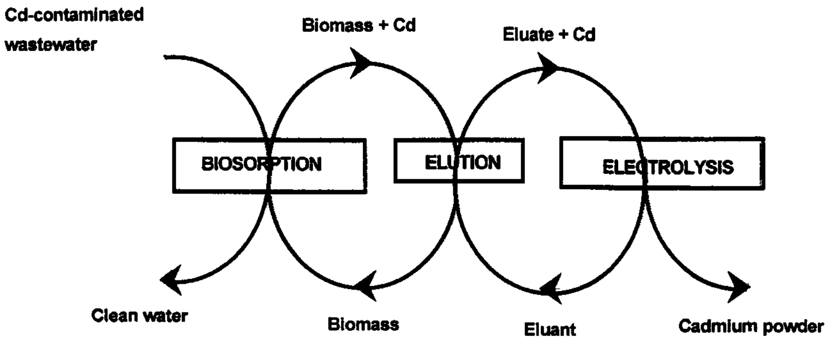

FIGURE 20
Conceptual flow diagram of total process showing recycle routes for biomass and eluant; the S/L separation stage of the metal-loaded biomass is not shown. The resulting metal from the electrolysis step and clean water may be recycled for industrial use. Reprinted from the paper by Butter *et al.*[74].

biosorption of the metal ions can occur. A stirred tank contactor, where the biosorbent was suspended in the liquid, can be applied for surface binding.

ii) Separation (possibly by flotation) of the biomass from the aqueous phase, which can then be discharged.

iii) Elution of the biosorbed cadmium ions from the biomass, at a high concentration and recovery of cadmium electrochemically using a rotating cathode cell, in the form of metal (end product).

iv) Recycling of biomass and eluant, wherever possible.

A clean technology was, in this way, developed for the treatment of wastewaters containing toxic metals and the basic idea is illustrated in Figure 20[74]. The biomass selected for this project consisted of dead bacteria of the genus *Streptomyces* grown as a branched, filamentous, flocculent biomass. Gram-positive bacteria, such as the actinomycetes, exhibit several features that make them of potential value for metal biosorption (see above).

5.5. BIOSORPTIVE FLOTATION

Flotation was proposed and investigated as a suitable solid/liquid separation technique downstream for metal-loaded biomass[56,57,63,65] in biosorption processes. No loss of ultrafine biomass particles was foreseen in, in contrast to what would be expected in sedimentation

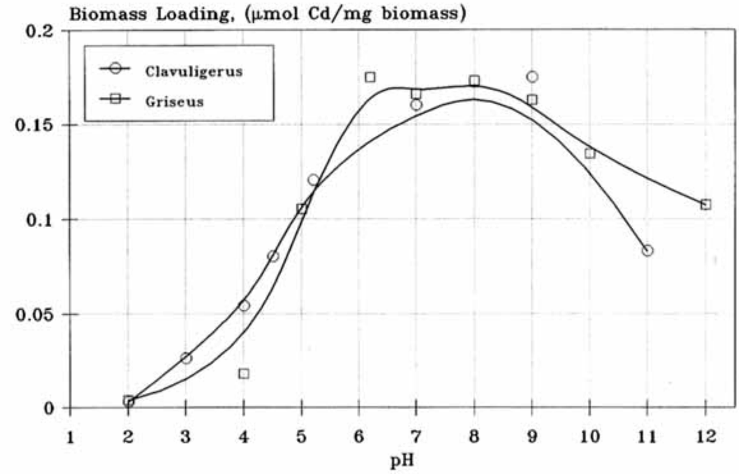

FIGURE 21

Cadmium loading of (unwashed) *Streptomyces* biomass against the pH; of two types (as shown) at 0.3 g/L initial concentration. Reprinted with permission[56]; copyright 1994, Marcel Dekker.

processes. It was further proposed that biosorption and flotation might operate in one very fast process, called biosorptive flotation[56].

Figure 21 presents some results on biomass loading of cadmium, with two types of streptomycetes, *S. griseus* (laboratory grown) and *S. clavuligerus* (a solid fermentation waste from a pharmaceutical industry). Flotation recoveries, denoted as Re, % (as also are the metal removals by biosorption), indicate the biomass harvesting efficiency by flotation.

Biosorption (starting from around pH5) was suggested to follow mainly an ion exchange mechanism between sodium ions from the biomass cell surface and cadmium ions from solution and vice versa, during elution. The surface charge of the microorganisms was measured by an electrokinetic method[57]- see Figure 22. It was observed that washed *S. griseus* had an isoelectric point of approximately pH 2.4 but increasing negative values of zeta-potential were observed at pH values above 5. The presence of toxic metals altered the electrokinetic properties of biomass. The behavior (not shown) of *S. clavuligerus* was quite similar. It is noted that the biomass solubility of the unwashed samples was of the order of 70%.

Various elution methods were examined, including the application of EDTA, as presented in Figure 23. During two hours contact at pH 4.5, over 80% of the cadmium was

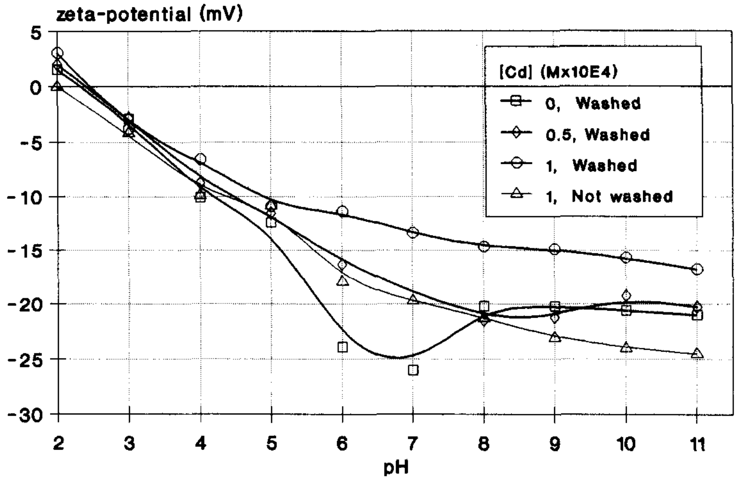

FIGURE 22
Zeta-potential measurements of washed *S. griseus* (in presence of 0.5% ethanol) against the pH of solution, with various cadmium concentrations and comparison with unwashed sample (in 10^{-4} M Cd). Reprinted with permission[57]; copyright 1994, J. Wiley & Sons.

desorbed from floated, metal-loaded biomass[63]. However, decreased cadmium removal was observed during recycling, possibly due to retention of EDTA which acted as a chelating agent and also inhibited separation. With increasing EDTA concentration in the desorption stage from 0.001 to 0.01 M, a further deterioration of cadmium removal was detected. The use of a cationic collector (cetyl trimethyl-ammonium bromide), at a certain concentration range, improved biomass re-flotation recoveries in a second cycle.

A laboratory pilot scale (40 L) continuous-flow experiment of five consecutive biosorption/flotation/elution cycles was recently reported[75]. Effective flotation of the loaded biomass was found, at the range of 96-99% (in all of the cycles). Metal (i.e. cadmium) biosorption declined from 96% in the 1st cycle to 25% in the 5th, possibly due to interference from the flotation cationic collector (a quaternary ammonium compound). However, in an improved experiment the use of dodecylamine instead (i.e. primary amine) was successful.

Sadowski *et al.*[76] have also used froth flotation experimentally to harvest microorganisms (i.e. *Streptomyces pilosus*) after lead accumulation. This biomass, laboratory grown, was found to accumulate Pb(II) from aqueous solution. The rate of accumulation was very fast and dead cells had a greater sorptive capacity than live cells.

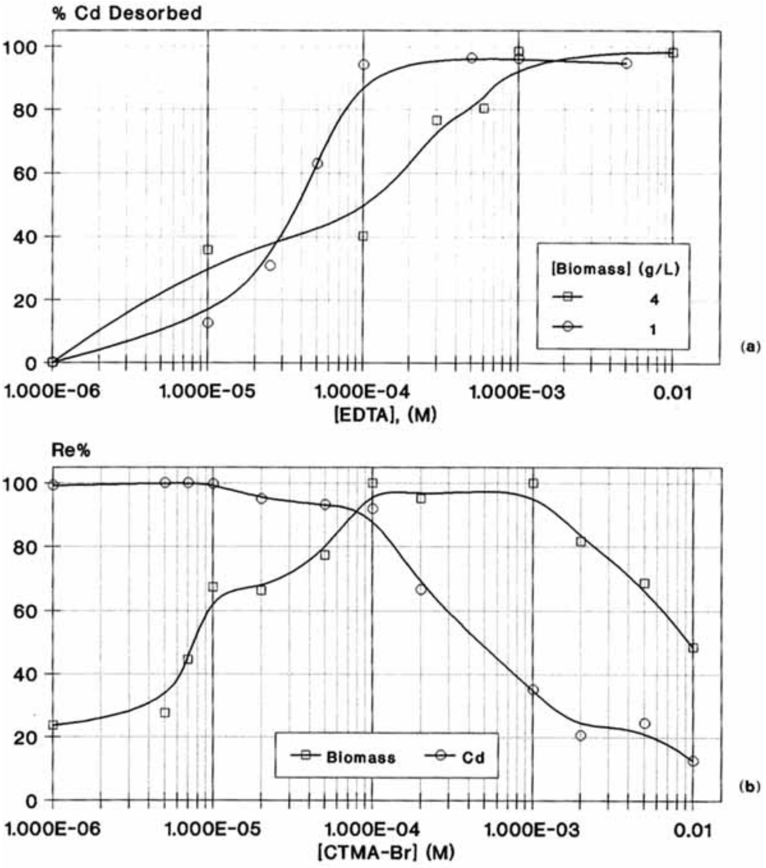

FIGURE 23

(a) Cadmium desorption by EDTA from floated biomass: effect of EDTA addition, at two different industrial waste biomass concentrations. (b) Reuse of biomass eluted with 0.001 M EDTA, at pH 5; effect of cationic collector during flotation, in various concentrations. Reprinted with permission[63]; copyright 1994, Elsevier.

6. Concluding Remarks

The initial concept, that very cheap biosorbents could be used in purifying industrial wastewater solutions by non-specifically decreasing their metal content, has been revised in the light of results from applied studies, which have shown not only that metal uptake capacities are high but also that uptake can be metal selective. Consequently, biosorbents are now also being considered as replacements for ion exchangers or other metal extraction and concentration operations in "upstream" metal recovery[29].

Activated (living) sludge is known for its biosorptive capability for toxic metals. The metal uptake depends mainly on surface binding mechanism rather than on metabolic activity. Hence, biosorption is largely passive. Conceptually, adsorption of metal ions onto a bacterial surface may be accepted as the formation of a surface-metal complex. Non-living, but sterilized, anaerobically digested activated sludge has been tested, in order to remove heavy metals from aqueous solutions. The availability of this sorbent material, cheap and in sufficient quantities (obtained from the central sewage treatment plant of the city) is obvious. Present methods of sludge disposal include incineration, landspreading and landfilling.

Thousands of tons of residual biomass are also produced each year in the fermentation industries, containing poorly biodegradable biopolymers[67]. This waste microbial biomass may well be an economic metal adsorbent and ion exchange material. Biomass is produced as a by-product in industrial fermentation and examples of such studies, given above, include *Penicillium chrysogenum* and *Rhizopus arrhizus*, *Streptomyces clavuligerus* and *Streptomyces griseus*.

Serious concerns about possible contamination of ground and surface waters by toxic metals, leached from abandoned disposal sites but, in certain cases, also from currently operating ones, have generated great efforts aimed at remediating contaminated soils. Bioremediation of abandoned polluted industrial sites requires development of new and viable technologies capable of removing low concentrations of toxic metals from high volumes of solid and/or liquid wastes. Biosorptive processes appear to have a great potential in environmental technology or recovery of precious and/or strategic metals.

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