# REVIEW

## Biosorption of Heavy Metals

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Only within the past decade has the potential of metal biosorption by biomass materials been well established. For economic reasons, of particular interest are abundant biomass types generated as a waste byproduct of large-scale industrial fermentations or certain metal-binding algae found in large quantities in the sea. These biomass types serve as a basis for newly developed metal biosorption processes foreseen particularly as a very competitive means for the detoxification of metal-bearing industrial effluents. The assessment of the metal-binding capacity of some new biosorbents is discussed. Lead and cadmium, for instance, have been effectively removed from very dilute solutions by the dried biomass of some ubiquitous species of brown marine algae such as *Ascophyllum* and *Sargassum*, which accumulate more than 30% of biomass dry weight in the metal. Mycelia of the industrial steroid-transforming fungi *Rhizopus* and *Absidia* are excellent biosorbents for lead, cadmium, copper, zinc, and uranium and also bind other heavy metals up to 25% of the biomass dry weight. Biosorption isotherm curves, derived from equilibrium batch sorption experiments, are used in the evaluation of metal uptake by different biosorbents. Further studies are focusing on the assessment of biosorbent performance in dynamic continuous-flow sorption systems. In the course of this work, new methodologies are being developed that are aimed at mathematical modeling of biosorption systems and their effective optimization. Elucidation of mechanisms active in metal biosorption is essential for successful exploitation of the phenomenon and for regeneration of biosorbent materials in multiple reuse cycles. The complex nature of biosorbent materials makes this task particularly challenging. Discussion focuses on the composition of marine algae polysaccharide structures, which seem instrumental in metal uptake and binding. The state of the art in the field of biosorption is reviewed in this article, with many references to recent reviews and key individual contributions.

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### Introduction

Metallic species mobilized and released into the environment by the technological activities of humans tend to persist indefinitely, circulating and eventually accumulating throughout the food chain, thus posing a serious threat to the environment, animals, and humans. It is essential to realize that the metal is only "removed" from solution when it is appropriately immobilized. The procedure of metal removal from aqueous solutions often leads to effective metal concentration. Apart from the rather slow natural process of metal mineralization, the ultimate removal is attained only when the metal becomes concentrated to the point that it can be either returned to the process or resold. This aspect of the operation deals with the potential recovery of the metal, which ideally should go hand in hand with the removal aspect, making the overall process an ultimately effective procedure for controlling the utilization of metals by humans in their technological processes.

Apart from dispersed sources of dissolved and/or leached metals, large proportions of heavy metal species are released into the enviroment from industrial wastewaters through inefficiencies built into the technological activities used directly in the processing of metals or

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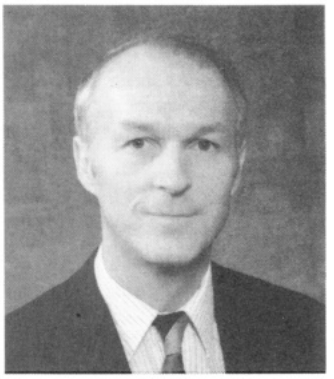

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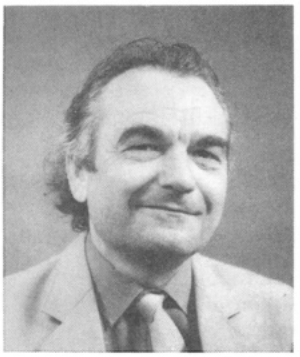


**Zdenek Richard Holan** recently concluded a 3-year stint as a research associate in Professor Volesky's research laboratory at McGill University (Montreal, Canada). Prior to 1989, he was a senior research at the Microbiological Institute of the Czech Academy of Sciences (Prague, Czech Republic), where he specialized in microbial cell wall polysaccharides and their biogenesis, structures, purification, and applications. His unique achievements in this field earned him the Czechoslovak Academy of Sciences Prize in 1983. He received his basic Dipl. Ing. degree in agricultural chemistry from the Institute of Agriculture in Prague in 1965. On several occassions he was a visiting scientist in Germany, Russia, Latvia, and the Pasteur Institute in France, always engaged in bio-polysaccharide research.

through other routes. Virtually any industrial activity using metals has a metal disposal problem. In addition, there are numerous technology-related activities that result in the release of metallic species. Industrially based metal-bearing discharges can be considered as point-source emissions, which in turn offer the possibility of feasible process-based remedial actions. Sorption and/or complexation of dissolved metals based on the chemical activity of microbial biomass, known as biosorption, provides the foundation for the new biosorption technology for metal removal and recovery, which offers promise as an alternative for potentially economically attractive treatment particularly suited for a wide variety of industrial metal-bearing point-source effluent discharges.

**Biosorbent Materials.** The work of Adams and Holmes (1935) represented not only the threshold in ion-exchange chemistry but also an early attempt at biosorption. They described the removal of Ca and Mg ions by tannin resin, black wattle bark (*Acacia mollissima*), which were treated directly so that the condensation product was fixed on the woody fibers (Adams and Holmes, 1935). The condensation product of *m*-phenylenediamine and formaldehyde was used for anion sorption. Strong biosorbent behavior of certain types of microbial toward metallic ions is a function of the chemical makeup of the microbial cells of which it consists. It is necessary to emphasize that this type of active biomass consists of dead and metabolically inactive cells. This aspect is particularly important when it comes to the process application, whereby new biosorbents represent "chemicals" capable of sequestering a relatively large amount of the metal (Volesky, 1990a,b).

Some types of biosorbents could be broad range, binding and collecting the majority of heavy metals with no specific priority, while others can even be specific for certain types of metals (Hosea et al., 1986; Volesky and Kuyucak, 1988). Table 1 summarizes most of the more consistent attempts to identify metal-sorbing biomass types in the microbial world. The results of these studies vary widely because of the different criteria used by the authors in searching for suitable material. Some used easily available biomass types, others specially isolated strains, and some processed the raw biomass to a certain degree to improve its biosorption application properties. In the absence of uniform methodology, results have been reported in different units and in many different ways, often making quantitative comparison impossible. However, a general conclusion can be drawn that there are potent biosorbent materials among easily available biomass types from all three groups: algae, fungi, and bacteria, the former two perhaps giving broader choices.

The source of the raw materials for the new family of biosorbents conveniently is a waste material, as is the case in using byproduct biomass from large-scale fermentation processes. In particular, some waste mycelia available in large quantities indicate an interesting potential of these biomass types in the collection and removal of heavy metals (Nemec et al., 1977; Niu et al., 1993; Paknikar et al., 1993). While some of this work has been reviewed recently (Siegel et al., 1990; Volesky, 1990a,b), fungi such as *Mucor* sp. (Mueler et al., 1992) and *Rhizopus* sp. (Fourest and Roux, 1992; Lewis and Kiff, 1988; Luef et al., 1991; Tsezos and Volesky, 1981; Volesky and Tsezos, 1981; Zhou and Kiff, 1991) continue to attract interest for their high metal uptake capacity. A different type of fungal biomass discarded from rubber plantations has also been of interest (Venkobachar, 1990). Application-oriented work on metal biosorption by waste bacterial biomass from *Bacillus subtilis* has not reached expectations in its application (Brierley, J. A., 1990; Brierley and Vance, 1988; Brierley et al., 1986).

Another inexpensive source of biomass, where it is produced in copious quantities, is in the oceans as seaweeds, representing many different types of marine macroalgae (Kuyucak and Volesky, 1990). However, most of even the recent contributions studying the uptake of toxic metals by live marine and, to a lesser extent, freshwater algae focused on toxicological aspects, metal

accumulation, and pollution indicators by living, metabolically active biomass. Focus on the technological aspects of metal removal by algal biomass has been rare (Bedell and Darnall, 1990; Darnall, 1991; Holan, et al., 1993; Kuyucak and Volesky, 1990; Volesky and Kuyucak, 1988).

Abundant natural materials, particularly of cellulosic nature, have been suggested as potential biosorbents for heavy metals. However, little work has been done in that respect. While the properties of sawdust have been rather superficially tested in a few recent studies (Aval, 1991; Bryant et al., 1992; Chan et al., 1992), modified cellulose has also been examined (Shukla and Sakhardande, 1992; Svoboda et al., 1992), as well as bark (Khangan et al., 1992; Shukla and Pandey, 1990) and modified starch (Wang et al., 1991; Zhang et al., 1990).

The meaningful search for metal-sorbent biomaterials would be expedited greatly if the phenomenon of metal biosorption were better understood. That knowledge could serve as a guide in the search for potentially high-sorbing materials in the natural domain. However, with the state of the art being in its infancy as it is, tedious experimental screening of selected readily available types of biomass is still the basis for discovering new biosorbents. Considering the number of candidate biomass types and the number of metals of interest, all multiplied by the number of variable experimental or process parameters, the scope of the task of prospecting for new and potentially feasible metal biosorbents is rather large.

Biosorption of metals is not based on only one mechanism. It consists of several mechanisms that quantitatively and qualitatively differ according to the species used, the origin of the biomass, and its processing. Metal sequestration follows complex mechanisms, mainly ion exchange, chelation, adsorption by physical forces; and ion entrapment in inter- and intrafibrillar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes. There are several chemical groups that could attract and sequester the metals in biomass: acetamido groups of chitin, structural polysaccharides of fungi, amino and phosphate groups in nucleic acids, amino, amido, sulfhydryl, and carboxyl groups in proteins, hydroxyls in polysaccharides, and mainly carboxyls and sulfates in the polysaccharides of marine algae that belong to the divisions Phaeophyta, Rhodophyta, and Chlorophyta. However, it should be stressed that the presence of some functional group does not guarantee their accessibility for sorption, perhaps due to steric, conformational, or other barriers.

**Biosorption by Industrial Biomass Types.** The bulk of earlier work on biosorption indicated that uranium is a metal particularly susceptible to biosorption uptake and probably most is known about this particular type of biosorption. Uranium uptakes exceeding 200 mg of U/g for *Rhizopus arrhizus* have been noted (Treen-Sears, 1981; Tsezos, 1980). Even *Saccharomyces cerevisiae*, which is an otherwise mediocre metal biosorbent, sequesters uranium in the largest quantities. Although nonliving yeast cells accumulated more uranium than live cells (Volesky et al., 1993), phosphatase enzyme activity has been associated with the slower but copious additional deposition of uranyl phosphate microcrystals observed in the phosphatase-overproducing strain of *Citrobacter* sp. (Macaskie and Dean, 1988). Good evidence now exists showing that the biomass of filamentous fungi of the order Mucorales represents a good biosorbent material for a wide range of heavy metals. The metal-binding sites are predominantly associated with the cell wall structure of these molds (Remacle, 1990). Both *R. arrhizus* and *Rhizopus nigricans* contain chitin and chitosan in their cell walls, which have been reported to play a role in the sequestration of uranyl ions from solution (Muzzarelli, 1972; Tsezos, 1980). Although chitin and chitosan have been reported to remove a number of metal ions from solution, among these are titanium, zirconium, hafnium, mercury, copper, and uranium (Muzzarelli, 1972), they do not seem to be the major metal-binding compounds active in the complex sequestration of metals by biosorption.

Despite earlier optimistic reports on *Pencillium* biosorption (Jilek et al., 1975), the mycelium seems to be sorbing well only uranium and to a certain degree lead (Niu et al., 1993). Also, common types of *Aspergillus* have never performed very well. Lead seems to follow uranium in its propensity for being biosorbed. The fungal biomass of *Absidia orchidis* exhibited an excellent uptake of lead (Holan and Volesky, 1995). There are several reasons why this fungus, frequently used in transhydroxylation reactions of methylpyridines (Skryabin and Koshcheenko, 1987) or steroids (Gai et al., 1981), could bind heavy metals: (1) The presence of ionic groups capable of sequestering metals. There is glucuronic acid in linear, (1→4)-linked β-D-glucuronans (Tsuchihashi et al., 1983) and phosphates as key constituents of the cell wall (Campos-Tahaki et al., 1983). (2) The presence of *cis*-oriented hydroxyl groups in α-D-mannans (Yamada et al., 1982) capable of forming chelate complexes with metals. (3) The presence of SH groups, which may be more abundant in steroid-transforming fungi with elevated levels of cytochrome $P_{450}$, performing the transhydroxylation reactions. The formation of strong covalent bonds between lead and SH groups is well-known. (4) *p*-Toluenesulfonic acid is known to be used in the process of the microbial hydroxylation of steroids (Gai et al., 1981). When incorporated, it could enhance metal uptake. (5) Chitin and glucan belong to the main structural polysaccharides in fungi. The possibility of metal uptake by these compounds might be due to not only the acetamido groups of chitin but also the entrapment of metals in the inter- and intrafibrillar capilarities in both of these biopolymers.

Species of *Penicillium* do not show such diversity. Structural polysaccharides consist mainly of glucan and chitin (Edwards and Ho, 1988; Grisaro et al., 1968) and of glycans containing glucose, galactose, mannose, and sometimes organic acids, e.g., malonic acid. Phosphate groups are also present in abundance as part of galactans, not only in cell walls but also in exocellular polysaccharides (Preston et al., 1969). According to the structural analysis, the cell wall D-glucuronans from *A. orchidis* resembled those of *Rhizopus*. The differences mentioned earlier could partially explain the differences in lead sorption by the two types of fungal biomass, but not the cadmium uptake, which was higher for the *P. chrysogenum*. In general, significant differences in the biosorbent mechanisms of metal deposition in different types of microbial biomass can easily be postulated (Volesky, 1990a,b) but remain to be studied.

**Biosorption by Seaweed Materials.** Some seaweeds collected from the ocean have indicated impressive biosorption of metals (Kuyucak and Volesky, 1990). Brown algae in particular are suited for binding metallic ions, probably due to their polysaccharide content (Percival and McDowell, 1967). According to some results (Crist et al., 1988), the biosorption of heavy metals has two phases: a fast (<4 s) surface reaction and much slower metal uptake (2 h). The first phase is attributed to surface adsorption, mainly based on anion exchange with the participation of the carboxyl groups of uronic

**Table 1. Biosorbent Uptake of Metals by Microbial Biomass**

| metal | biomass type | biomass class | metal uptake[a] (mg/g) | reference |
|---|---|---|---|---|
| Ag | (freshwater alga)[b] | biosorbent | 86–94 | Brierley and Vance, 1988; Brierley et al., 1986 |
| | (fungal biomass)[b] | biosorbent | 65 | Brierley et al., 1986 |
| | *Rhizopus arrhizus* | fungus | 54 | Tobin et al., 1984 |
| | *Streptomyces noursei* | filament. bacter. | 38.4 | Mattuschka et al., 1993 |
| | *Saccharomyces cerevisiae* | yeast | 4.7 | Brady and Duncan, 1993 |
| Au | *Sargassum natans* | brown alga | 400 | Volesky and Kuyucak, 1988 |
| | *Aspergillus niger* | fungus | 176 | Kuyucak and Volesky, 1988 |
| | | | 15 | Gee and Dudeney, 1988 |
| | *Rhizopus arrhizus* | fungus | 164 | Kuyucak and Volesky, 1988 |
| | *Palmaria tevera* | marine alga | 164 | Kuyucak and Volesky, 1988 |
| | *Palmaria palmata* | marine alga | 124 | Kuyucak and Volesky, 1988 |
| | *Chlorella pyrenoidosa* | freshwater alga | 98 | Darnall et al., 1988 |
| | *Cyanidium caldarium* | alga | 84 | Darnall et al., 1988 |
| | *Chlorella vulgaris* | freshwater alga | 80 | Gee and Dudeney, 1988 |
| | *Bacillus subtilis* | bacter. cell walls prep. | 79 | Beveridge, 1986 |
| | *Chondrus crispus* | marine alga | 76 | Kuyucak and Volesky, 1988 |
| | *Bacillus subtilis* | bacterium | 70 | Gee and Dudeney, 1988 |
| | *Spirulina platensis* | freshwater alga | 71 | Darnall, et al., 1988 |
| | | | 58 | Gee and Dudeney, 1988 |
| | *(Bacillus licheniformis)* | bacter. cell walls prep. | 59 | Beveridge, 1986 |
| | *Rhodymenia palmata* | marine alga | 40 | Darnall et al., 1988 |
| | *Ascophyllum nodosum* | brown marine alga | 24 | Kuyucak and Volesky, 1988 |
| | (freshwater alga) | biosorbent | 27–127 | Brierley and Vance, 1988 |
| Cd | *Ascophyllum nodosum* | brown marine alga | 215 | Holan et al., 1993 |
| | *Sargassum natans* | brown marine alga | 135 | Holan et al., 1993 |
| | (fungal biomass)[b] | biosorbent | 135 | Brierley et al., 1986 |
| | *(B. subtilis)*[b] | biosorbent | 101 | Brierley et al., 1986 |
| | *Fucus vesiculosus* | brown marine alga | 73 | Holan et al., 1993 |
| | *Candida tropicalis* | yeast | 60 | Mattuschka et al., 1993 |
| | *Penicillium chrysogenum* | fungus | 56 | Holan and Volesky, 1995 |
| | | fungus | 11 | Niu et al., 1993 |
| | | | (0.8)[d] | Paknikar et al., 1993 |
| | *Rhizopus arrhizus* | fungus | 30 | Tobin et al., 1984 |
| | *Saccharomyces cerevisiae* | yeast | 20–40 | Volesky et al., 1993 |
| | | | 11.8 | Brady and Duncan, 1993 |
| | *Rhizopus arrhizus* | fungus | 27 | Fourest and Roux, 1992 |
| | *Rhizopus nigricans* | fungus | 19 | Holan and Volesky, 1995 |
| | *(Penicillium chrysogenum)*[b] | fungus | 20 | Nemec et al., 1977 |
| | *Pencillium spinulosum* | fungus | 0.4 | Townsley et al., 1986 |
| Co | *Ascophyllum nodosum* | brown marine alga | 100 | Kuyucak and Volesky, 1989a |
| | *Saccharomyces cerevisiae* | yeast | 4.7 | Brady and Duncan, 1993 |
| Cr | *Bacillus* biomass | bacterium | 118 ($Cr^{3+}$) | Brierley and Brierley, 1993 |
| | | | 60 ($Cr^{6+}$) | Brierley and Brierley, 1993 |
| | *Rhizopus arrhizus* | fungus | 31 | Tobin et al., 1984 |
| | *Candida tropicalis* | yeast | 4.6 | Mattuschka et al., 1993 |
| | *Streptomyces nouresei* | filament. bacter. | 1.8 | Mattuschka et al., 1993 |
| | *Penicillium chrysogenum* | fungus | (0.33)[d] | Paknikar et al., 1993 |
| Cu | *(Bacillus subtilis)*[b] | biosorbent | 152 | Beveridge, 1986; Brierley et al., 1986; Brierley and Brierley, 1993 |
| | *(Bacillus subtillis)* | bacter. cell walls prep. | 146 | Beveridge, 1986 |
| | *Candida tropicalis* | yeast | 80 | Mattuschka et al., 1993 |
| | (fungal biomass)[b] | biosorbent | 76 | Beveridge, 1986; Brierley et al., 1986 |
| | manganese-oxidizing bacteria | (MK-2) | 50 | Stuetz et al., 1993 |
| | *(Bacillus licheniformis)* | bacter. cell walls prep. | 32 | Beveridge, 1986 |
| | *Cladosporium resinae* | fungus | 18 | Gadd et al., 1988 |
| | *Rhizopus arrhizus* | fungus | 16 | Tobin et al., 1984 |
| | *Saccharomyces cerevisiae* | yeast | 17–40 | Volesky and May-Phillips, 1995 |
| | | | 10 | Mattuschka et al., 1993 |
| | | | 6.3 | Brady and Duncan, 1993 |
| | *Pichia guilliermondii* | yeast | 11 | Mattuschka et al., 1993 |
| | *Scenedesmus obliquus* | freshwater alga | 10 | Mattuschka et al., 1993 |
| | *Rhizopus arrhizus* | fungus | 10 | Gadd et al., 1988 |
| | *Penicillium chrysogenum* | fungus | 9 | Niu et al., 1993 |
| | *Streptomyces noursei* | filament. bacter. | 5 | Mattuschka et al., 1993 |
| | *Bacillus* sp. | bacterium | 5 | Cotoras et al., 1993 |
| | *Penicillium spinulosum* | fungus | 0.4–2 | Townsley et al., 1986 |
| | *Aspergillus niger* | fungus | 1.7 | Townsley et al., 1986 |
| | *Trichoderma viride* | fungus | 1.2 | Townsley et al., 1986 |
| | *Penicillium chrysogenum* | fungus | (0.75)[d] | Paknikar et al., 1993 |
| Fe | *(Bacillus subtillis)* | bacter. cell walls prep. | 201 | Beveridge, 1986 |
| | *Bacillus* biomass | bacterium | 107 | Brierley and Brierley, 1993 |
| | *(Bacillus licheniformis)* | bacter. cell walls prep. | 45 | Beveridge, 1986 |
| Hg | Rhizopus arrhizus | fungus | 54 | Tobin et al., 1984 |
| | *(Penicillium chrysogenum)*[b] | fungus | 20 | Nemec et al., 1977 |
| Mn | *(Bacillus subtillis)* | bacter. cell walls prep. | 44 | Beveridge, 1986 |
| | *(Bacillus licheniformis)* | bacter. cell walls prep. | 38 | Beveridge, 1986 |
| | *Rhizopus arrhizus* | fungus | 12 | Tobin et al., 1984 |

**Table 1 (Continued)**

| metal | biomass type | biomass class | metal uptake[a] (mg/g) | reference |
|---|---|---|---|---|
| Ni | *Fucus vesiculosus* | brown marine alga | 40 | Holan and Volesky, 1994 |
| | *Ascophyllum nodosum* | brown marine alga | 30 | Holan and Volesky, 1994 |
| | *Sargassum natans* | brown marine alga | 24–44 | Holan and Volesky, 1994 |
| | *(Bacillus licheniformis)* | bacter. cell walls prep. | 29 | Beveridge, 1986 |
| | *Candida tropicalis* | yeast | 20 | Mattuschka et al., 1993 |
| | *Rhizopus arrhizus* | fungus | 18 | Fourest and Roux, 1992 |
| | manganese-oxidizing bacteria | (MK-2) | 16 | Stuetz et al., 1993 |
| | *(Bacillus subtillis)* | bacter. cell walls prep. | 6 | Beveridge, 1986 |
| | *Rhizopus nigricans* | fungus | 5 | Holan and Volesky, 1995 |
| | *Absidia orchidis* | fungus | 5 | Holan and Volesky, 1995 |
| Pb | *(Bacillus subtilis)*[b] | biosorbent | 601 | Brierley et al., 1986 |
| | *(fungal biomass)*[b] | biosorbent | 373 | Brierley et al., 1986 |
| | *Absidia orchidis* | fungus | 351 | Holan and Volesky, 1995 |
| | *Fucus vesiculosus* | brown marine alga | 220–370 | Holan and Volesky, 1994 |
| | *Ascophyllum nodosum* | brown marine alga | 270–360 | Holan and Volesky, 1994 |
| | *Sargassum natans* | brown marine alga | 220–270 | Holan and Volesky, 1994 |
| | *(Bacillus subtilis)*[b] | biosorbent | 189 | Brierley and Brierley, 1993 |
| | *Penicillium chrysogenum* | fungus | 122 | Niu et al., 1993 |
| | | | 93 | Holan and Volesky, 1994 |
| | | | (0.98)[d] | Paknikar et al., 1993 |
| | *Rhizopus nigricans* | fungus | 166 | Holan and Volesky, 1995 |
| | *Streptomyces longwoodensis* | yeast | 100 | Friis and Myers-Keith, 1986 |
| | *Rhizopus arrhizus* | fungus | 91 | Tobin et al., 1984 |
| | | | 55 | Fourest and Roux, 1992; Holan and Volesky, 1995 |
| | *Streptomyces noursei* | filament. bacter. | 55 | Mattuschka et al., 1993 |
| | *(Penicillium chrysogenum)*[b] | fungus | 25 | Nemec et al., 1977 |
| | (*Citrobacter* sp.)[c] | bacterium | (4000) | Macaskie, 1990 |
| Pd | (freshwater alga)[b] | biosorbent | 436 | Brierley and Vance, 1988 |
| | (fungal biomass)[b] | biosorbent | 65 | Brierley et al., 1986 |
| Pt | (freshwater alga)[b] | biosorbent | 53 | Brierley and Vance, 1988 |
| U | *Streptomyces longwoodensis* | filament. bacter. | 440 | Friis and Myers-Keith, 1986 |
| | *Rhizopus arrhizus* | fungus | 220 | Volesky and Tsezos, 1981 |
| | | | 195 | Tobin et al., 1984 |
| | *(Penicillium chrysogenum)*[b] | fungus | 25 | Nemec et al., 1977 |
| | *Saccharomyces cerevisiae* | yeast | 55–140 | Volesky and May-Phillips, 1995 |
| | *Bacillus* sp. | bacterium | 38 | Cotoras et al., 1993 |
| | *Chaetomium distortium* | fungus | 27[d] | Khalid et al., 1993a |
| | *Trichoderma harzianum* | fungus | (26)[d] | Khalid et al., 1993a,b |
| | *(Penicillium chrysogenum)*[b] | fungus | 25 | Nemec et al., 1977 |
| | *Alternaria tenulis* | fungus | (24)[d] | Khalid et al., 1993a |
| | *Fusarium* sp. | fungus | (24)[d] | Khalid et al., 1993a |
| | *Saccharomyces cerevisiae* | yeast | (24)[d] | Khalid et al., 1993a |
| | *Aspergillus amsta* | fungus | (22)[d] | Khalid et al., 1993a |
| | *Penicillium herguei* | fungus | (20)[d] | Khalid et al., 1993a |
| | *Rhizopus* sp. | fungus | (16)[d] | Khalid et al., 1993a |
| | *Zybgorenchus macrocarpus* | fungus | (13)[d] | Khalid et al., 1993a |
| | *Aspergillus niger* | fungus | (12)[d] | Khalid et al., 1993a |
| | (*Citrobacter* sp.)[c] | bacterium | (8000) | Macaskie, 1992 |
| Th | *Rhizopus arrhizus* | fungus | 160 | Tsezos and Volesky, 1981 |
| | | | 93 | Gadd et al., 1988 |
| | *Saccharomyces cerevisiae* | yeast | 70 | Gadd et al., 1988 |
| Zn | *(Bacillus subtilis)*[b] | biosorbent | 137 | Brierley et al., 1986 |
| | (fungal biomass)[b] | biosorbent | 98 | Brierley et al., 1986 |
| | manganese-oxidizing bacteria | (MK-2) | 39 | Stuetz et al., 1993 |
| | *Saccharomyces cerevisiae* | yeast | 14–40 | Volesky and May-Phillips, 1995 |
| | *Candida tropicalis* | yeast | 30 | Mattuschka et al., 1993 |
| | *Rhizopus arrhizus* | fungus | 20 | Tobin et al., 1984 |
| | | | 14 | Fourest and Roux, 1992 |
| | *Penicillium chrysogenum* | fungus | 6.5 | Niu et al., 1993 |
| | | | (0.67)[d] | Paknikar et al., 1993 |
| | *Bacillus* sp. | bacterium | 3.4 | Cotoras et al., 1993 |
| | *Penicillium spinulosum* | fungus | 0.2 | Townsley et al., 1986 |

[a] Metal uptake as reported not necessarily at maximum, recalculation sometimes necessary. Residual concentrations for appropriate comparison often not given. [b] Biomass not necessarily in its natural state. Its preprocessing sometimes is not specified. [c] Surface-immobilized biomass enzymatically active with a phosphate substrate. Assessment of its dry weight is not possible. Metal microprecipitation apparently present. [d] Incomplete or inappropriate basis for conversion given in the original paper.

acids. The second phase represents the diffusion of ions into the cell structures. Crist et al. (1990) demonstrated that copper was adsorbed not only by ion exchange but also by additional covalent bonding with the carboxyl groups of *Vaucheria* pectins. Biosorbed copper was also found to be bound by chelation between the *cis*-oriented hydroxyls at C2 and C3 of yeast α-mannans, which formed an insoluble complex and helped in their isolation (Peat et al., 1961). It is possible that similar chelation could occur between the *cis*-oriented hydroxyls at C2 and C3 in the D-mannuronic and L-guluronic acids of algal polysaccharides. The ion exchange in algal biomass is not so simple. The chemical composition exhibits at least two groups capable of ion exchange: carboxyls of uronic acids and sulfates of carrageenans, xylans, and galactans.

Although carboxyl groups have been identified as

possibly the main metal-sequestering sites (Majidi et al., 1990), they are not likely to be the only strongly active sites. It is interesting to note that, after the esterification of carboxyl groups in algal biomass, the sorption of copper dropped, but the binding of $Au^{3+}$ increased (Gardea-Torresday et al., 1990). The biomass of *Sargassum* species offers excellent biosorbent properties, whereby even very selective and previously patented sequestering of gold is possible (Kuyucak and Volesky, 1989b,d,f; Volesky and Kuyucak, 1988). *Ascophyllum*, reported to bind effectively at least cobalt (Kuyucak and Volesky, 1989a,c,e), cadmium (Holan, et al., 1993), and lead (Holan and Volesky, 1994), contains a unique mixture of polysaccharides offering a variety of active groups capable of effectively binding metallic ions.

Alginates, mainly composed of (1→4)-linked β-D-mannopyranosyl uronate and its C5 epimer α-L-gulopyranosyl uronate, are members of a glucuronan family. Both uronic acids occur in varying ratios and differing quantities in samples of polysaccharides taken from different algal species or strains. These parameters can also differ according to the age, season, and origin of the alga (Haug et al., 1974; Haug and Smidsord, 1965).

A semispeculative model of the structure of the cell walls of brown algae has been proposed recently (Kloareg et al., 1986): cellulose chains form the rigid structural network in which four other biopolymers (alginates, xylofucoglucans, xylofucoglycuronans, and homofucans) are embedded. Similarities of the fucose-containing polysaccharides of the cell walls of several genera of brown algae, as well as the variations in the sugar constituents of algal polysaccharides, were also described (Mabeau et al., 1990; Nishida et al., 1990). Although the algal species of *A. nodosum* and *F. vesiculosus* examined recently (Holan et al., 1993) for their metal-sequestering ability differ taxonomically, their biomass features two common moieties. (1) Sulfate esters in the cellular polysaccharides, including (a) fucoidans (after removal of alginates) in the division Phaeophyta with $OSO_3^-$ groups at carbons C2 or C3 or containing disulfate esters at C2 and C3 in α-(1→4)-linked L-fucopyransoyl residues (sulfate esters were described in 21 species, including *Fucus vesiculosus*, *Sargassum thrunbergii*, and *Padina arborescens* (Nishida et al., 1990); (b) carrageenans, agars, porphyrans, furcellarans, and funorans, etc., in the division *Rhodophyta*—they are sulfated galactans at C2, C4, or C6 (disulfate esters at C2 and C6 were identified in λ-carrageenan) with different proportions of (1→3)- and (1→4)-D,L-linked galactosyl residues (Stevenson and Furneaux, 1991); (c) sulfate esters at C2 and C6 of β-(1→3)-galactans are present in *Codium taylori* (division *Chlorophyta*) (Chapman, 1978a); (d) sulfate esters of xylans (in green algae, e.g., *Ulva lactuca*); and (e) a complex of hetero-polysaccharide sulfates is present in green algae, where the esterification of L-arabinose, D-galactose, and possibly D-xylose and L-rhamnose was observed but not fully investigated. (2) The presence of polyuronides that are represented by galacturonic, glucuronic, guluronic, and mannuronic acids in all three divisions mentioned earlier.

The carboxyl groups, present in abundance in the four above-mentioned uronic acids, together with sulfate groups could be considered the ligands mainly responsible for the bulk of metal sorption (Crist et al., 1992).

**Choice of Metals.** The appropriate selection of metals for biosorption studies is extremely important since the experimental volume increases exponentially with each additional metallic species considered for investigation. Depending on the angle of interest and the impact of different metals, they could be divided into four major categories: (1) toxic heavy metals, (2) strategic metals, (3) precious metals, and (4) radionuclides. In terms of environmental threat, it is mainly categories 1 and 4 that are of interest for removal from the environment and/or the point-source effluent discharges.

When considering the environmental impact of mobilized metals, the "big three", mercury, lead, and cadmium, are in the limelight (Volesky, 1990a,b). Less so is perhaps mercury, which is being displaced from industrial processes by the introduction of new technologies. With relatively well-defined point sources of lead contamination, the major spread of lead in the environment has been curbed by the introduction of unleaded gasoline. While the uses of mercury and lead are limited and do not mark an increasing trend, cadmium is toxic and ubiquitous in its distribution throughout the world. With the uses of cadmium on the rise, it represents a recognized great potential hazard to humans and the environment.

Apart from toxicological criteria, the interest in specific metals may also be based on how representative their behavior may be in terms of the eventual generalization of results of studying their biosorbent uptake. The toxicity and intriguing solution chemistry of elements such as Cr, As, and Se make them interesting. A few more widely used metals, Ni and V among them, as well as common metals such as zinc and copper are worth studying. Zinc is an essential element for enzyme activators in humans, but it is also toxic at levels of 100–500 mg/day. Copper is not toxic to the extent of zinc, but its extensive use and increasing levels in the environment are cause for concern. While hexavalent chromium is extremely toxic, it occurs as an anion with properties that are correspondingly different from those of the usual metal cations. However, it can easily be chemically, or even biochemically (Paknikar and Bhide, 1993; Reischl et al., 1993), reduced to its trivalent state, making it more amenable to removal (Siegel et al., 1986).

A special case is that of radioactive isotopes originating from both upstream and downstream operations of the nuclear industry, from the utilization of fission reaction fuel rods, and from military and reprocessing operations. Some of the radioisotopes present may require individual control procedures. The hazard level depends on the half-life of the isotope. Uranium mill tailings are a source of low-level radiation, 0.1–1.0 mR/h. Radium-226 is very important because it is the most hazardous of all isotopes found in the tailings. Relatively little work has been done on the biosorption of radium (Tsezos and Matar, 1986) and thorium (Tsezos and Volesky, 1981). The uptake of strontium and several other radionuclides by microbial cells was noted mainly in toxicologically oriented studies conducted for different purposes. Among all the metals, there are numerous indications from previous work that uranium tends to be biosorbed exceptionally well (Macaskie and Dean, 1985, 1988; Tsezos and Volesky, 1981; Volesky et al., 1983). This feature of the metal undoubtedly is related to its large atomic weight and ionic radius (Tobin et al., 1984). However, naturally occurring uranium is not particularly a problem in the environment and interest in the element has subsided, resulting in a glut of uranium on the world markets. The early work done with uranium, and to a lesser extent with thorium (Tsezos and Volesky, 1981, 1982a,b), was driven by the interest shown in the metal by the nuclear industry, which is currently stagnant.

Strategic and precious metals, while not necessarily posing environmental threats, are the prime targets for

concentration and effective recovery that could use sorption processes in concentrating the metallic species of interest from dilute solutions.

An important consideration in studying biosorption is the solution chemistry of metals, particularly as it relates to their hydration and hydrolysis reactions. The large charge-to-size ratio of cations results in an increase in hydration energy if no reaction beyond the coordination of water molecules to the cation occurs:

$$M^+ + nH_2O \rightarrow [M(H_2O)_n]^+$$

The hydrolysis reactions proceed when the acidity (charge-to-size ratio) of the cation is so great that it causes a rupture of the H−O bonds with ionization of the hydrate to yield hydronium ions:

$$Al^{3+} + 6H_2O \xrightarrow{H_2O} [Al(H_2O)_6]^{3+} \rightarrow H_3O^+ + [Al(H_2O)_5OH]^{2+}$$

Cations that hydrolyse are those that are either small (e.g., $Be^{2+}$) and/or highly charged (e.g., $Fe^{3+}$, $Sn^{4+}$).

The existence of multinuclear hydrolysis products is a rather general phenomenon. The hydrolyzed species can be considered to dimerize by a condensation process. The dimer can undergo an additional hydrolytic reaction, providing additional hydroxo groups, which in turn could form more bridges. The sequence of both reactions usually results in the formation of colloidal hydroxo polymers and, ultimately, in the formation of precipitates. The formation of mononuclear and polynuclear complexes is, of course, concentration dependent. The reactions mentioned also change the chemical composition of metal ion solutions during their "aging".

In the presence of water, metal or metaloid oxides are generally covered with surface hydroxyl groups so that hydroxylated oxide particles could be, to a certain degree, understood as polymeric oxo acids or oxo bases. The charge of the metal or metaloid hydrated oxides is pH dependent, with the proton transfers at the amphoteric surface. The surface chemistry of metal oxides and hydroxides should be taken into account.

Biosorption of heavy metals usually leads to the acidification of solutions. As the pH may have to be controlled, neutralization with sodium or ammonium hydroxides is favorable to the formation of metal hydroxides, and neutralization with amines leads to the formation of amine−metal complexes.

Study of the sorption of metals that tend to form insoluble (micro)precipitates becomes more complicated due to the fact that the collection of the metal species is not due to the stright sequestration mechanism. In the case of lead in particular, its solution chemistry is more complex. The presence or formation of insoluble $Pb(NO_3)_2Pb(OH)_2$ and $Pb(NO_3)_25Pb(OH)_2$ complexes can result in a distrotion of the sorption results. The danger of microprecipitation starts at pH values above 5.0 for lead and at pH 6.7 for nickel (Britton, 1943). The formation of amphoretic lead hydroxides causes a problem with pH adjustment in the study of sorption systems. Metal ions are coordinated by water molecules, which causes a hydrolytic reaction:

$$M^{n+} + H_2O \rightarrow (MOH)^{(n-1)+} + H^+$$

or more realistically

$$M(H_2O)_x^{n+} \rightarrow [M(H_2O)_{(x-1)}(OH)]^{(n-1)+} + H^+$$

The dissociation of amphoteric hydroxides (which are weak acids) points to the fact that the higher the number of covalent M−O bonds, the more acidic the hydrogen atoms in the hydrated ion, and the following neutralization of protons results in further release of protons. Moreover, the hydroxide ion has the ability to form bridges between metal ions. The most common example is the formation of hydroxo bridges at an early stage in the precipitation of hydrous metal oxides:

When the solution becomes uncharged, the resulting pH represents an isoelectronic point with the lowest solubility of metal ions. While this situation is very favorable for the sorbent deposition of metals, it may make the study of the binding sites more difficult. Another favorable feature of the above-mentioned equation is the fact that the addition of water (or other neutral groups) does not alter the valency, but increases the molecular mass of the complex formed, thus favoring sorbate deposition. While microprecipitation occurring in the sorption system can complicate study of the sorption, from the process application point of view, it may desirably augment the metal immobilization, thus increasing the apparent overall uptake capacity of biosorbent materials.

An example using uranium, which was shown to biosorb so well in many investigations, may be given. The distribution curves available in the literature for the $UO_2^{2+}$ hydrolysis products suggest that, for low total $U^{6+}$ concentrations and for pH values below 5, $UO_2^{2+}$ continues to be one of the predominant uranium species in solution. The following equations most probably describe $U^{6+}$ hydrolysis in noncomplexing medium:

$$UO_2^{2+} + H_2O \overset{K_1}{\rightleftharpoons} UO_2(OH)^+ + H^+ \qquad (\log K_1 = -5.7)$$

$$2UO_2^{2+} + 2H_2O \overset{K_2}{\rightleftharpoons} (UO_2)_3(OH)_2^{2+} + 2H^+ \qquad (\log K_2 = -5.62)$$

$$3UO_2^{2+} + 5H_2O \overset{K_3}{\rightleftharpoons} (UO_2)_3(OH)_2^{+} + 5H^+ \qquad (\log K_3 = -15.63)$$

**Assessment of Sorption Performance.** Despite the well-established and simple foundations of the sorption process, there seems to be a great deal of confusion concerning the evaluation of experimental results on biosorption as they have been reported throughout recent literature by authors from different backgrounds. Examination and preliminary testing of a solid−liquid sorption system are usually based on two types of investigations: (a) equilibrium batch sorption tests and (b) dynamic continuous-flow sorption studies.

The two widely accepted and easily linearized equilibrium adsorption isotherm models for single solute systems used in the literature are the following:

$$\text{Langmuir:} \quad q = bC_f q_{max}/(1 + bC_f)$$

$$\text{Freundlich:} \quad q = kC_f^{1/n}$$

where $q$ is the uptake of the solute (metal), $q_{max}$ is the maximum uptake, $C_f$ is the equilibrium (final) concentration of solute in the solution, $b$ and $n$ are constants

related to the energy of adsorption (or "affinity"), and $k$ is a constant. Both models, while capable of describing many biosorption isotherms, can hardly have a meaningful physical interpretation in biosorption. The results cannot be extrapolated, and no predictive conclusions can be drawn for systems operating under different conditions. These simple basic models also do not incorporate the effects of any external variable environmental factors. Moreover, biosorption isotherms may exhibit an irregular pattern due to the complex nature of both the sorbent material and its varied multiple active sites, as well as the complex solution chemistry of some metallic compounds. This, however, rarely has been recognized by students of the phenomenon, who often force smooth (bio)sorption isotherm curves through scattered experimental points or use the preceding simplistic sorption models to fit to those data points (Gadd et al., 1988; Holan et al., 1993; Khalid et al., 1993a,b).

The basic evaluation of sorption systems relies on the classical sorption isotherm derived from equilibrium batch contact experiments carried out under controlled environmental conditions. A quantitative comparison of two different sorption systems can only be done at the same equilibrium (final, residual) concentration. Any other comparison carries an inherent error and can only serve as a qualitative comparison, often used for quick-screening purposes. For example, an often-used criterion, the percent of metal removed, does not indicate the concentration range. Even if all experimental parameters are given, this criterion can only result in a qualitative, and relative comparison (better or worse performance) that is adequate only for material screening purposes. Any figures given are essentially misleading because they lead to inadvertent and erroneous comparative calculations. The presence of other ions in solution can complicate the evaluation of the sorption system to a large degree, depending on the way the new solute species interact with the sorbent and with the original one. Knowledge of these aspects may not be readily available. Appropriate and meaningful evaluation of a sorbent system with three or more metallic ions becomes very complicated, if not impossible for all practical purposes.

Evaluation of equilibrium sorption performance needs to be supplemented by process-oriented studies of its kinetics and eventually by dynamic continuous-flow tests. The rate of the sorption metal uptake, together with the hydrodynamic parameters, determines the size of the contact equipment. Reaction engineering (Levenspiel, 1972) concepts apply for the experimental approach, leading to expression of the values of key process parameters used for comparative, process design, and scale-up purposes.

Biosorption, just like conventional sorption processes, involves inherently very fast sorption reaction mechanisms based predominantly on chemisorption. The kinetics of such reactions is actually so fast that the experimental determination of metal uptake rates may represent a challenging problem requiring specially designed equipment (Tsezos and Volesky, 1982a,b; Tsezos, 1981). There are indications that, in many biosorption systems, most of the metal biosorption occurs in a matter of 5–15 min after solid–liquid contact (Kuyucak and Volesky, 1989a,b), followed in some cases by residual and much slower additional metal deposition (Tsezos and Volesky, 1981), perhaps indicating a different secondary metal-binding mechanism.

For application purposes, the high rates of biosorption are very advantageous. Because of the fast kinetics of the sorption reactions, the overall sorption rate is more often controlled by mass transfer of the sorbed solute (metal) to the active reaction site. While the intraparticle diffusion normally appears to be the overall rate-controlling step, the particle external film diffusion also plays a role, which depends on the hydraulic flow regime (turbulence and/or back-mixing) in the reactor system. The most widely used contacting device for sorption processes is the fixed-bed reactor configuration and its modifications. The principles and methodology of deriving and evaluating the key fixed-bed sorption process parameters have been dealt with extensively in the chemical engineering literature (Ruthven, 1984; Weber and Crittenden, 1975).

## Example of Current Results

**Cd, Pb, Cu, and Zn Equilibrium Biosorption Uptake.** Different types of microbial biomass were selected from among the industrially used fungi and marine algae for their ready availability and biosorbent potential to bind heavy metals. Industrial samples of dried (28 °C) *Pencillium chrysogenum* were supplied by the courtesy of Hindustan Antibiotics, Ltd. (Pimpri, Pune, India) and Sechuan Pharmaceutical Company (Chengdu, People's Republic of China). The samples of brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* were harvested from the Atlantic Ocean (Nova Scotia) in October, dried at 60 °C, and ground. A brown alga, *Sargassum natans*, was supplied sun-dried by the University of Puerto Rico. Normally, the crude biomass materials were not purified or otherwise treated for biosorption unless specifically noted. Some of the biomass was reinforced by cross-linking using modified procedures described in earlier work (Holan et al., 1993; Jilek et al., 1971; Stamberg et al., 1977).

Only a cross section of selected original experimental data will be presented in order to illustrate the types of investigation and results obtained in studying a variety of different biomass types for their biosorbent potential. From comparison of biosorption isotherm results derived from equilibrium batch experiments, conclusions can be drawn concerning the level of biosorption exhibited by different materials. Since different biosorbents have to be compared at the same residual equilibrium concentrations, a certain choice must be made concerning those concentrations. Usually it has been made on the basis of the selection of a low (e.g., 10 mg/L) and a high (e.g., 200 mg/L) residual metal concentration in the solution for comparison purposes. The values of $q_{max}$ were calculated by fitting the Langmuir sorption model to the experimental data.

Sample results of cadmium uptake tests for different types of biomass and for recommended ion-exchange resins are compared in Table 2. The preliminary screening data, organized as shown in Table 2, enable a quick and simple comparison of the sorbent results. The highest metal biosorbent uptake of 170 mg of Cd/g at the equilibrium final concentration of 200 mg/L was observed for native *A. nodosum* biomass, followed by *Sargassum natans* with 106 mg of Cd/g. In comparison, the commercial ion-exchange resins (e.g., Duolite GT-75) did not perform as well. A relatively fast uptake rate was observed, whereby 73% of cadmium was sequestered from the solution within the first 3 min. The presence of cadmium in the biomass was confirmed by X-ray energy dispersion analysis.

The algal biomass types examined in this work for their lead accumulation have been selected because of earlier indications of their high binding capacities for cadmium (Holan et al., 1993). The lead biosorption results are

**Table 2. Experimental and Calculated Cadmium Uptake by Different Types of Sorbent Materials**

| sorbent type | experimental | | Langmuir parameters[a] | | | | difference[b] | | |
|---|---|---|---|---|---|---|---|---|---|
| | $q_{10}$ (mg/g) | $q_{200}$ (mg/g) | $q_{10}$ (mg/g) | $q_{200}$ (mg/g) | $q_{max}$ (mg/g) | $b$ ($\times 10^2$) | $q_{10}$ (%) | $q_{200}$ (%) | $R^2$ [d] |
| *Sargassum natans* at pH 3.5 | 24 | 110 | 23 | 106 | 132 | 2.09 | 4 | 4 | 0.99 |
| *Fucus vesiculosus* at pH 3.5 | 3 | 65 | 3 | 54 | 73 | 0.21 | 0 | 20 | 0.75 |
| *A. nodosum* at pH 2 | 2 | 23 | 2 | 20 | 38 | 0.56 | 0 | 15 | 0.85 |
| *A. nodosum* at pH 3.5 | 30 | 114 | 29 | 112 | 133 | 2.78 | 3 | 2 | 0.99 |
| *A. nodosum* at pH 4.9 | 31 | 172 | 33 | 170 | 215 | 1.86 | −6 | 2 | 0.99 |
| *A. nodosum*, cross-linked with | | | | | | | | | |
| formaldehyde[e] | 29 | 111 | 29 | 108 | 126 | 2.99 | 0 | 3 | 0.99 |
| formaldehyde[f] | 28 | 125 | 31 | 125 | 149 | 2.66 | −10 | 0 | 0.99 |
| formaldehyde−urea | 24 | 104 | 26 | 100 | 117 | 2.90 | −8 | 4 | 0.99 |
| divinyl sulfone | 43 | 117 | 45 | 117 | 128 | 5.39 | −4 | 0 | 0.99 |
| glutaraldehyde | 21 | 109 | 18 | 104 | 138 | 1.52 | 17 | 5 | 0.99 |
| entrapped in silica gel | 2 | 18 | 3 | 18 | 26 | 1.22 | −33 | 0 | 0.98 |
| Duolite GT-73 | 28 | 63 | 26 | 62 | 67 | 6.43 | 8 | 2 | 0.97 |
| Amberlite IRA-400 | 2 | 29 | 3 | 27 | 56 | 0.46 | −33 | 6 | 0.67[c] |

[a] $q_{10}$ and $q_{200}$ metal uptake at the residual concentrations of 10 and 200 mg/L, respectively. [b] $(q_{exp} - q_{cal})100/q_{calcd}$. [c] Significant at $P < 0.05$. [d] Significant at $P < 0.01$. [e] Sorption of cadmium from the solution of $3CdSO_4 \cdot 8H_2O$. [f] Sorption of cadmium from the solution of $Cd(CH_3COO)_2$ (according to Holan and Volesky (1993)).

**Table 3. Experimental and Calculated Lead Uptake by Different Types of Sorbent Materials at pH 3.5**

| sorbent type | experimental | | Langmuir parameters[a] | | | | difference[b] | |
|---|---|---|---|---|---|---|---|---|
| | $q_{10}$ (mg/g) | $q_{200}$ (mg/g) | $q_{10}$ (mg/g) | $q_{200}$ (mg/g) | $q_{max}$ (mg/g) | $b$ ($\times 10^2$) | $q_{10}$ (%) | $q_{200}$ (%) |
| Phaeophyta (Order Fucales) | | | | | | | | |
| *Fucus vesiculosus*[c] | 95 | 174 | 53 | 197 | 229 | 3.03 | 79.2 | −12.0 |
| *Fucus vesiculosus*[d] | 11 | 131 | 11 | 131 | 301 | 3.87 | 0 | 0 |
| *Fucus vesiculosus*[e] | 63 | 250 | 36 | 250 | 363 | 1.10 | 75 | 0 |
| *Ascophyllum nodosum*[f] | 37 | 251 | 37 | 251 | 359 | 1.17 | 0 | 0 |
| *Ascophyllum nodosum*[c] | 112 | 201 | 46 | 219 | 272 | 2.03 | 143 | −8.2 |
| *Ascophyllum nodosum*[g] | 41 | 211 | 41 | 213 | 273 | 1.79 | 0 | −0.9 |
| *Ascophyllum nodosum*[h] | 29 | 189 | 29 | 191 | 271 | 1.18 | 0 | −1.0 |
| *Ascophyllum nodosum*[i] | 48 | 149 | 48 | 156 | 177 | 3.67 | 0 | −4.5 |
| *Sargassum fluitans*[c] | 47 | 216 | 47 | 215 | 266 | 2.14 | 0 | 0.4 |
| *Sargassum fluitans*[g] | 88 | 193 | 109 | 193 | 202 | 12.82 | −19 | 0 |
| *Sargassum natans*[c] | 52 | 211 | 52 | 213 | 253 | 2.61 | 0 | −0.9 |
| *Sargassum vulgare*[c] | 20 | 149 | 19 | 148 | 228 | 0.93 | 5.2 | 0.7 |
| *Padina gymnospora*[c] (Dictyotales) | 22 | 59 | 22 | 59 | 65 | 5.26 | 0 | 0 |
| *Padina gymnospora*[j] | 4.3 | 23 | 4 | 22 | 31 | 1.28 | 7.5 | 4.5 |
| Rhodophyta (Order Gigartinales) | | | | | | | | |
| *Chondrus crispus*[k] | 20 | 142 | 20 | 143 | 209 | 1.07 | 0 | −0.7 |
| *Chondrus crispus*[c] | 3 | 65 | 3 | 48 | 195 | 0.16 | 0 | 35.4 |
| *Galaxaura marginata*[j] (Nemalionales) | 83 | 252 | 75 | 255 | 317 | 2.05 | 10.7 | −1.2 |
| *Galaxaura marginata*[c] | 1 | 11 | 1 | 12 | 25 | 0.44 | 0 | 8.3 |
| *Palmaria palmata*[c,l] (Palmariales) | | | | | | | | |
| Chlorophyta (Order Codiales) | | | | | | | | |
| *Codium taylori*[c] | 9 | 130 | 10 | 130 | 376 | 0.26 | −10 | 0 |
| urea−formaldehyde complex | 2 | 21 | 2 | 21 | 42 | 0.49 | 0 | 0 |
| Amberlite IR-120 (wet) | 138 | 283 | 138 | 283 | 299 | 8.49 | 0 | 0 |
| (dry) | 264 | 350 | 70 | 351 | 444 | 1.87 | 277 | −0.3 |
| Duolite GT-73 (wet) | 28 | 114 | 28 | 114 | 136 | 2.63 | 0 | 0 |
| (dry) | 80 | 234 | 80 | 252 | 284 | 3.93 | 0 | −7.1 |

[a] $q_{10}$ and $q_{200}$ metal uptake at the residual concentrations of 10 and 200 mg/L, respectively. [b] $(q_{exp} - q_{cal})100/q_{calcd}$. [c] Native biomass material. [d] Cross-linked with formaldehyde and HCl. [e] Cross-linked with buffered formaldehyde (pH 2). [f] Cross-linked with diethenyl sulfone. [g] Cross-linked with glutaraldehyde. [h] Cross-linked with formaldehyde and acetic acid. [i] Cross-linked with formaldehyde and urea. [j] After Removal of $CaCO_3$ (aragonite). [k] Cross-linked with 1-chloro-2,3-epoxypropane. [l] $q = 13.5$ mg/g at $C_f = 243$ mg/L.

summarized in Table 3. Brown algae *Sargassum natans* and *Ascophyllum nodosum* can be easily identified for their good biosorption of lead, exceeding approximately 40 and 210 mg of Pb/g for the low and high residual concentrations of lead in the solution, respectively. Ion-exchange resin IRA-400 tested under the same conditions, however, demonstrated better performance at low residual concentrations.

**Two- and Three-Metal Sorption Systems.** Among all the metals of interest that could be considered, for practical reasons the choice focused on three: Cd, Cu, and Zn. The sorption performance evaluation, conventionally carried out for a single metal cation in solution, becomes more complex when two or more metals are considered. The effect of other metallic ions (or anionic co-ions) on the performance of one-metal biosorption has been assessed in earlier studies by showing the difference from the original (one-metal) sorption when a known concentration of "contaminants" was introduced. An example of these results is seen in Figure 1, showing how little an effect of the co-ions there was on the original uptake of gold by *Sagassum natans* biomass (Kuyucak and Volesky, 1989b). On the contrary, Figure 2 shows that there was a significant negative effect of Zn and particularly of Cu on the biosorbent uptake of Cd by reinforced biomass of *Ascophyllum nodosum*.

It is essential to emphasize that the respective concentrations of co-ions in Figures 1 and 2 mean their *initial* concentrations in the solution. This approach offers only very superficial information on the actual sorption process, whose true equilibrium relationships are reflected in the isotherm where the *final (residual)*

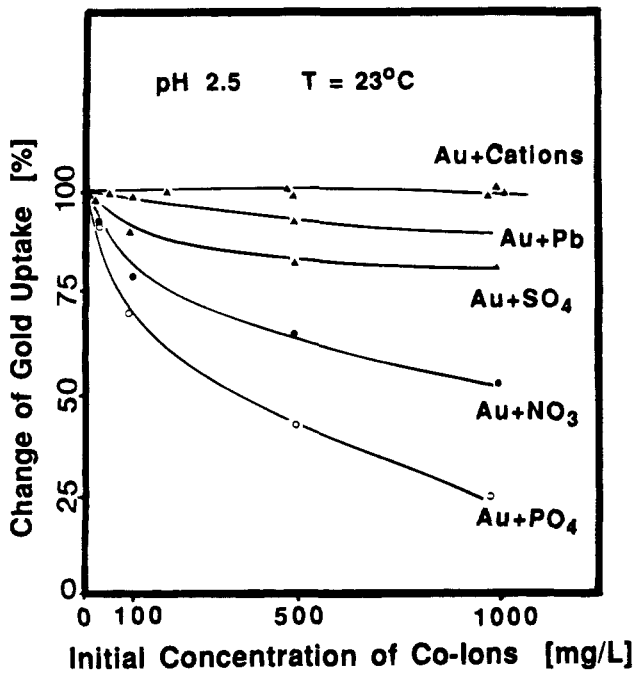



**Figure 1.** Effect of co-ion presence on the Au uptake by biomass of *Sargassum natans*. The equilibrium maximum Au uptake $q_{max} = 420$ mg/g at the optimum pH 2.5 was considered as 100% (Kuyucak and Volesky, 1989b).

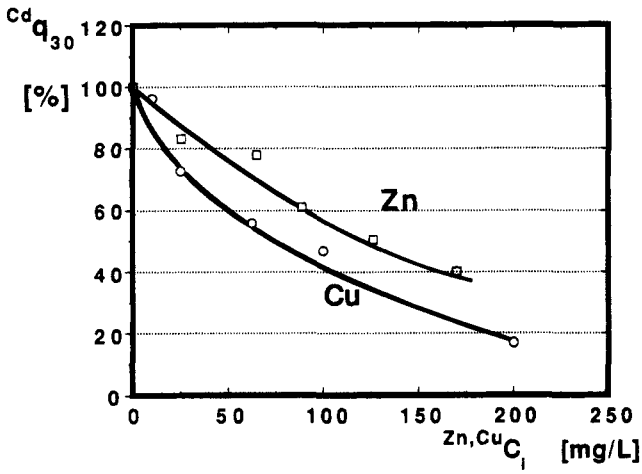



**Figure 2.** Effect of Zn or Cu pesence on Cd uptake by the reinforced *Ascophyllum nodosum* biosorbent. Expressed as a function of initial concentrations ($C_i$) of Zn and Cd, respectively (unpublished results courtesy of K.-H. Chong, McGill University). The basic equilibrium Cd uptake $^{Cd}q_{30} = 38.5$ mg/g (at the equilibrium residual Cd concentration of $C_f = 30$ mg of Cd/L) at pH 4.5 was considered as 100%. Equilibrium sorption contact: 100 mg of biosorbent; 50 mL of solution. (□) % Cd uptake in the presence of Zn; (○) % Cd uptake in the presence of Cu.

concentration of the sorbate in the solution is the one that really matters. A better approach to the evaluation of the multi-ion sorption phenomenon is to use the relationship seen as an example in Figure 3, reflecting the respective equilibrium-based effects of Zn and Cu on the biosorbent uptake of Cd by a reinforced *A. nodosum* biosorbent. Figure 4 reveals, however, that the total cumulative equilibrium metal uptake for the two metals present in the solution for each of the two-metal sorption experiments was greater when compared to a single-metal sorption performance for Cd only.

A two-metal system can be evaluated graphically by using three-dimensional plotting of the sorption isotherm surface, whereby the second metal residual concentration

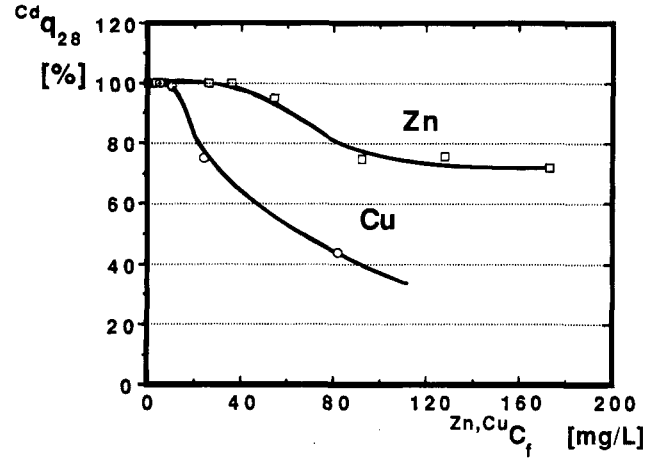



**Figure 3.** Effect of Zn or Cu presence on the total metal uptake by the reinforced *Ascophyllum nodosum* biosorbent. Expressed as a function of equilibrium final concentrations ($C_f$) of Zn and Cd, respectively (unpublished results courtesy of K.-H. Chong, McGill University). The basic equilibrium Cd uptake $^{Cd}q_{28} = 32$ mg/g (at the equilibrium residual Cd concentration of $C_f = 28$ mg of Cd/L) at pH 4.5 was considered as 100%. Equilibrium sorption contact: 100 mg of biosorbent; 50 mL of solution. (□) % Cd uptake in the presence of Zn; (○) % Cd uptake in the presence of Cu.

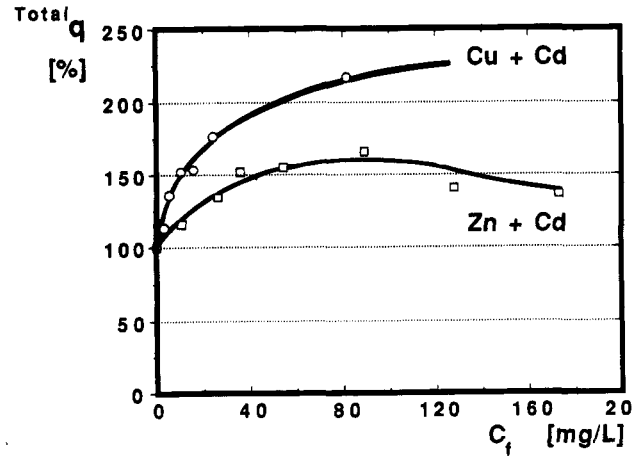



**Figure 4.** Effect of Zn or Cu pesence on the total metal uptake by the reinforced *Ascophyllum nodosum* biosorbent (unpublished results courtesy of K.-H. Chong, McGill University). The basic equilibrium Cd uptake $^{Cd}q_{28}{}^{90} = 32$ mg/g (at the equilibrium residual Cd concentration of $C_f = 28$ mg of Cd/L and the initial metal concentration of 90 mg/L) at pH 4.5 was considered as 100% Equilibrium sorption contact: 100 mg of biosorbent; 50 mL of solution. (□) % the total metal uptake for the (Cd + Zn) combination; (○) % of the total metal uptake for the (Cd + Cu) combination.

is also used and both metal uptake values are summed, representing the total uptake on the vertical axis. An example of the resulting 3D plot reflecting the equilibrium performance of the two-metal sorption by cross-linked *A. nodosum* biomass is presented in Figure 5 for the (Cd+Cu) system. The limitation of this approach is obvious because the effects of the third and subsequent metallic species on the sorption performance cannot be depicted graphically. Instead, a multiparameter mathematical approach has to be taken to describe, evaluate, and hopefully predict the performance of these complex multimetal sorption systems. This is the direction of current ongoing biosorption studies with multimetal systems.

**Dynamic Continuous-Flow Biosorption Tests.** There are two diametrically different systems that could be used for the assessment of continuous-flow sorption

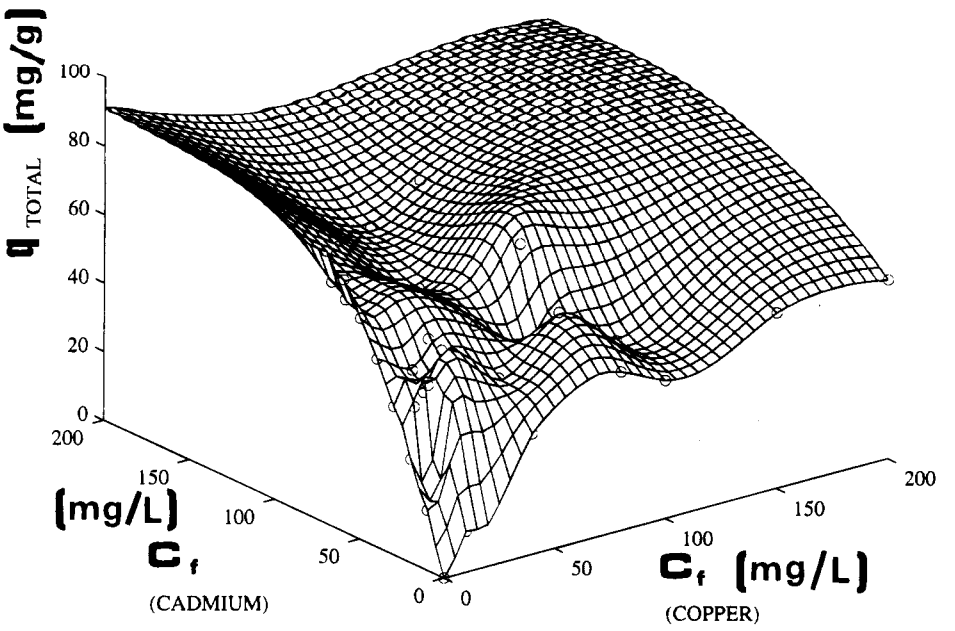



**Figure 5.** Three-dimensional plot of the two-metal equilibrium biosorption system (reinforced *Ascophyllum nodosum* biosorbent) containing Cd and Cu in solution (unpublished results courtesy of R. P. de Carvalho, K.-H. Chong, and V. Mathur, McGill University).

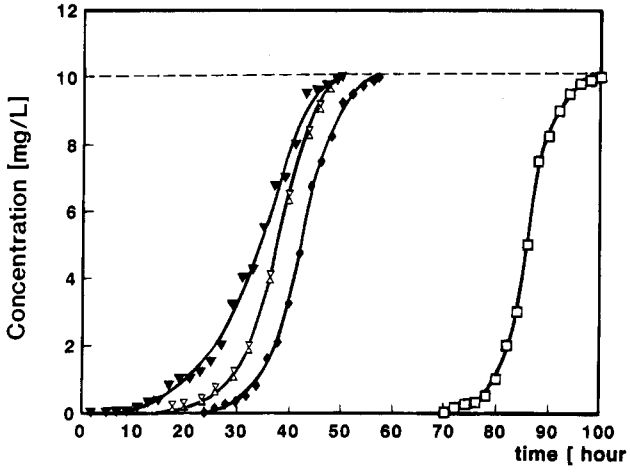



**Figure 6.** Continuous-flow fixed-bed column sorption of cadmium with reinforced *Ascophyllum nodosum* biosorbent (FCAN). Profiles of breakthrough curves for different sorption column feed flow rates (loadings): (□) 2.4 L $h^{-1}$ $cm^{-2}$; (◆) 4.8 L $h^{-1}$ $cm^{-2}$; (⧖) 7.2 L $h^{-1}$ $cm^{-2}$; (▼) 9.6 L $h^{-1}$ $cm^{-2}$ (Volesky and Prasetyo, 1994).

dynamics: the fixed-bed contactor (reactor) and the the continuous-flow stirred-tank reactor (CSTR). Both can exist in different configurations and modifications featuring various process pros and cons. Perhaps the most efficient use of reactor space is associated with the fixed-bed contactor arrangement. Very few biosorption studies have been reported with these systems for two practical reasons: the limited availability of granulated biosorbents and the proprietary nature of such process design-oriented tests.

A recent study (Prasetyo, 1992; Volesky and Prasetyo, 1994) of cadmium sorption in the continuous-flow fixed-bed arrangement using cross-linked, granulated *A. nodosum* biomass describes the procedures involved, resulting in valuable data that can serve as a basis for the scale-up of the system. The operational saturation metal uptake, the minimum theoretical bed depth, and the bed service time represent the key parameters characterizing biosorbent performance. The bed breakthrough exit concentration profiles resulting from these tests, which served for the derivation of the preceding process parameters, are presented in Figure 6. For the duration of the sorption test up to the breakthrough point, the exit concentration of cadmium was in the range below 5 ppb specified as a limit for potable water.

The calculated biosorbent use for the given operation of the fixed-bed contactor, removing the incoming concentration of cadmium at 10 mg/L, is given in Figure 7. Needless to say, there are several approaches to carrying out the dynamic tests of sorption columns, which have been discussed and demonstrated in the literature (Ramalho, 1983; Ruthven, 1984; Weber and Crittenden, 1975).

## Discussion

**Quantitative Evaluation of Multimetal Biosorption Systems.** While most of the studies on biosorption deal mainly with single-metal uptake, multimetal sorption results are rare. Recent results with binary mixtures containing two-metal combinations of Cd, Cu, and Zn (deCarvalho et al., 1995) demonstrate that a complete description of equilibrium sorption in a 3D plot could be very revealing. Further insight can be obtained from cuts through the 3D sorption surface by planes of constant concentration of one or the other metal. More work with multimetal biosorption systems is currently under way with the following dual purpose: to predict the biosorption performance of new materials and to gain more information on the metal biosorption mechanisms. While this line of work usually employs equilibrium methodology, the ultimate purpose is to develop an adequate, quantitative background for the application of biosorption in continuous-flow sorption processes. When fixed-bed sorption columns are considered for this purpose, very little guidance exists concerning the operating parameters for the column (e.g., optimal dimensions, flow rates) in laboratory studies, which should provide a solid basis for the equipment and process scale-up. This aspect applies to both metal uptake and its subsequent desorption, whereby the ultimate overall evaluation of the sorption process performance is provided by the metal concentration ratio, expressed as the metal concentration in the solution eluted from the column upon desorption to the original feed metal concentration.

In multiple cycles of sorption and desorption, where

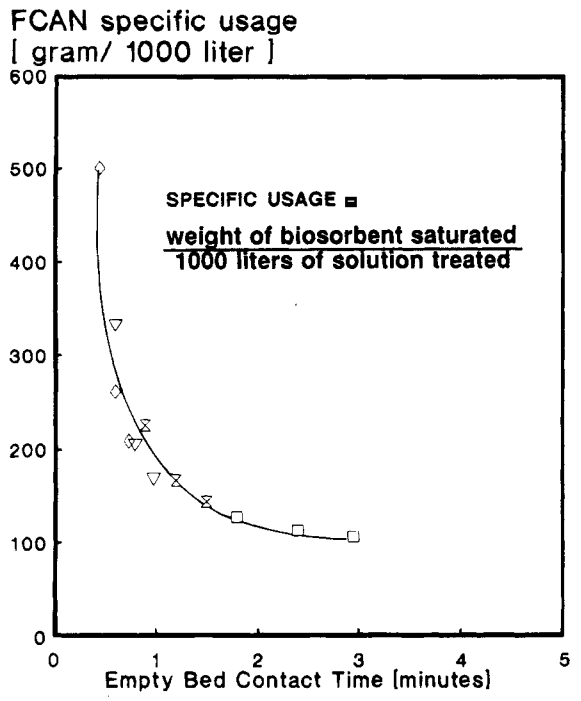



**Figure 7.** Effect of the sorption column liquid residence time on the specific usage of biosorbent FCAN derived from brown seaweed *Ascophyllum nodosum*. Flow rate: (□) 2.4 L $h^{-1}$ $cm^{-2}$ and (⧖) 2.4 L $h^{-1}$ $cm^{-2}$ at loadings (◇) 4.8 L $h^{-1}$ $cm^{-2}$ and (▽) 4.8 L $h^{-1}$ $cm^{-2}$ (Volesky and Prasetyo, 1994).

the biosorbent is being regenerated for reuse, the evaluation of the biosorbent deterioration has to be established, taking into account both its decreasing performance in terms of metal uptake as well as its physical deterioration, which may also be reflected in the increasing overall column pressure drop. Since most of these aspects form a special area of general sorption process know-how, very little useful background literature exists to provide specific quantitative methodologies and guidance for the evaluation of sorption column performance. No systematic studies have been performed with new biosorbent materials so far.

**Possible Effects of the Biosorbent Material Architecture.** It is generally agreed that it is the microbial cell walls that are mainly responsible for the metal biosorption behavior. Stereochemical differences in the polysaccharide structures of cell walls can make a significant difference in the acceptance of metallic ions by these structures, which can have a profound bearing on the biosorption performance of the natural materials containing them.

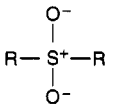
Indications of the advantages of morphologically different architecture of algal tissues in relation to increasing the metal biosorbent uptake could be seen in comparing the sorption performances of *F. vesiculosus* and *A. nodosum*. The thallus of the former consists of parenchymatous cells with apical meristems (Chapman, 1978b), instead of the predominantly filamentous architecture of the latter. The architecture of parenchymatous cells represents a greatly increased surface area that is available for sorption. Comparison of the density of cross-linked particles of *F. vesiculosus* and *A. nodosum* indicated a 34% higher density of the latter (Holan et al., 1993). This means that morphological differences existing within the same order can influence the sorption process. Also, the structure of alignates present can differ between young and old tissues, as well as between different parts of the same plant (Haug et al., 1974). The content of sulfate groups as well as uronic acids in the algal biomass examined differed not only between species but even seasonally and geographically within the same species (Percival and McDowell, 1967), with the variations being responsible for potentially differing sorption capabilities.

There was a conspicuous difference observed in our work with seaweed materials between the biosorbent uptakes of lead and nickel: the latter was 1 order of magnitude lower (Holan and Volesky, 1994). Several factors may be responsible for this difference. The lead and nickel sorptions follow the general rule that metal sorption increases with increasing valence and atomic number. The lower sorption of nickel than of lead could be related to the fact that nickel has a much lower selectivity coefficient for alginates than lead, which features the highest coefficient, reflected in the highest values of alginate gel shrinkage (Percival and McDowell, 1967). Nickel belongs to the intermediate metals with high affinity not only to ligands like phosphoryl, $SO_3^{2-}$, $RNH_2$, and $R_2NH$ but mainly to $COO^-$ groups, which it likely shares with lead. The *cis* position of sulfate esters at C3 and C4 of the branched fucose residue may be instrumental in the strong binding of lead to only one sugar residue. However, the same site could sequester nickel by an ion-exchange mechanism. Sulfate groups at other carbons could assist in forming covalent bonds between adjacent chains of fucoidans and other sulfated polysaccharides. This covalent bond (polysaccharide–$OSO_3$–Pb–$SO_3O$–polysaccharide) practically represents another type of cross-linking worthy of attention in studies of the metal-loaded biosorbent behavior during desorption. At the same time, it is interesting to note that the methodologies for quantitative evaluation of metal desorption have not been developed for routine use.

**Biosorbents for Sorption Column Applications.** Biomass of any kind cannot be used directly in the standard sorption process. Usually it is very soft, and without reinforcement and granulation it cannot be used in column operations. Early attempts were performed with the cross-linking of *Penicillium* sp. with a urea–formaldehyde mixture (Jilek et al., 1978; Stamberg et al., 1977; Votapek et al., 1976). Further attempts have been reported but are not necessarily well described (Brierley, J. A., 1990; Brierley et al., 1988; Greene and Darnall, 1990; Holan et al., 1993; Tsezos, 1990).

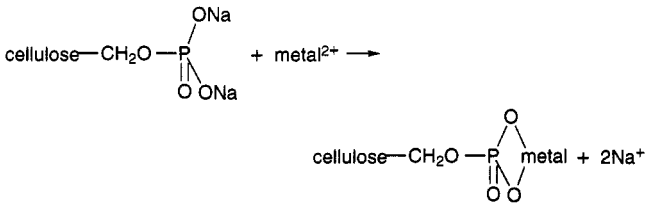
Cross-linking could be classified according to the formation of ethers or esters of the natural biopolysaccharides present in the biomass. Ethers could be formed via aldehydes (formaldehyde, dialdehydes, acrolein), *N*-hydroxymethyl compounds (triazine, ureas, amides, carbamates), activated vinyl compounds (diethenyl sulfone, acrylamides, vinyl ketones, and crotonates), epoxy and aziridinyl compounds (1-chloro-2,3-epoxypropane, diepoxides, dihalohydrins, triaziridinylphosphine oxide, and reaction products of ethylene imine with bis(chloroformate), acid chlorides of dicarboxylic acids, diisocyanates, etc.), bifunctional aliphatic chlorides, disulfonate esters of polymethylene diols, and bis(diazo)alkanes. Esters could be formed via acid anhydrides (phthalic, maleic, and succinic anhydrides), acid chlorides of dicarboxylic acids (from succinyl to sebacoyl), dicarboxylic acids, diisocyanates, and cross-linking of polysaccharide derivatives (including modification by introducing new reactive groups for new types of covalent bonds or the formation of new substrates that would be cross-linked with a known system): reactions with unsaturated compounds; reactions with mercapto groups; reactions with dialdehyde or otherwise modified polysaccharide with diamines, hydrazides, alkanedithiols, disulfides, etc.; reversible cross-links containing disulfide groups.

The higher sorption values observed with several cross-linked types of biomass revealed an additional advantage of cross-linking, because the majority of sulfate groups and uronic acids present in the cellular polysaccharides could be covalently attached to the structural polysaccharide network. The cross-linking of *A. nodosum* native biomass with diethenyl sulfone confirmed the possibility of increasing the sorption capacity of the biosorbent material by the incorporation of sulfone groups. Cross-linking of native biomass with diethenyl sulfone (Holan and Volesky, 1995) revealed the possibility of increasing the metal sorption capacity of native biomaterials by the incorporation of sulfone groups with two coordinate links:



Free aldehydic groups originating from dialdehydes used for cross-linking and incompletely anchored at one end only could, after the reduction of metal ions and the oxidation of aldehydic groups to carboxyl groups, also possibly increase the sorption capacity of a prepared biosorbent.

Modifications of the cellulosic materials tested (Holan and Volesky, 1994) demonstrated that processed natural materials, e.g., phosphated sawdust, could successfully compete with fungal biomass in sequestering metals. The mechanism of ion exchange apparently prevails:



This approach has already been used for example, for thorium recovery from monazite in the past (Head et al., 1959). The oxidation of sawdust to oxo and carboxy forms, as well as the introduction of carboxymethyl and sulfoethyl groups, was tested for illustrating biomass transformation into a better metal-sorbent material and for pointing to the eventual possibility of studying differnt metal-sequestering functional groups and metal uptake mechanisms.

There is a great deal of suitable cross-linking procedures that could be used in the reinforcement of any kind of biomass. However, there may be a limitation in the first step of cross-linking: alkaline or acidic conditions required for the reaction to proceed are usually very strong. While the durability of different cross-linked materials to extreme acidic and alkaline conditions has been studied only to a rather limited extent (Andrews et al., 1962), another limitation for cross-linked biosorbents may relate to the pH range in which they should eventually work. This could relate not only to the metal sorption cycle but also, perhaps particularly, to the desorption/regeneration cycles where more extreme pH process conditions are likely to occur.

Apart from successfully attaining appropriate rigidity and swelling characteristics of biosorbent particles, cross-linked biosorbents have to feature a well-controlled, uniform size and porosity in order to meet the process requirements relating to the micro- and macroscopic mass transfer characteristics of the material when used in the actual sorption process. These aspects still represent an outstanding research and development challenge, which is expanded by the variable nature of the individual raw biomass types already identified for their extremely exciting metal-sorbing capabilities.

## Conclusions

There has been steady progress in studying the biosorption of heavy metals, resulting in the identification of some biomass types that show very promising uptake of metallic ions. The focus is on several fungal strains generated as a byproduct of fermentation processes and several species of abundant brown marine algae. A better understanding of the biosorbent mechanisms responsible for heavy metal binding assists in the optimization of performance of the new biosorbent materials being prepared from these biomass types for process application in detoxifying metal-bearing industrial effluents.

While the equilibrium biosorption performance assessment uses batch tests, resulting in biosorption isotherm data, solutions with multiple metals have to be examined using advanced methodologies based on three-dimensional plotting and mathematical modeling of sorption performance. The current trends in this report point toward exploitation and suitable adaptation of existing mathematical models, as well as toward the synthesis of more advanced computer models reflecting the ligand–metal interaction on a molecular level and the process mass transfer and hydrodynamics on a macroscopic scale relating to the process equipment design and scale-up.

Work is currently in progress in several key academic laboratories that addresses the fundamental as well as applied aspects of the biosorption process, relating particularly to the better understanding of biosorption mechanisms, metal desorption and biosorbent regeneration, formulation of new biosorbent materials suitable for process application, and development of new methodologies to facilitate the quantitative process description, its performance prediction, and optimization. At the same time, commercial interest in the exploitation of new biosorption technology is on the rise.

## Acknowledgment

Original research on biosorption has been supported by a Canadian Natural Sciences and Engineering Research Council Operating Grant and is being carried out at McGill University in collaboration with BV Sorbex, Inc. The contributions of current and past members of the research group cited in this article are gratefully acknowledged.

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Accepted December 20, 1994.[⊗]

BP9400957

[⊗] Abstract published in *Advance ACS Abstracts,* February 15, 1995.