



Interactions of bacteria with metals in the aquatic environment

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CHAPTER 2

Interactions of Bacteria with Metals in the
Aquatic Environment

Philip J. Bremer and Gill G. Geesey

TABLE OF CONTENTS

I. Introduction	42
II. Survival of Bacteria in the Presence of Elevated Metal Concentrations	43
A. Occurrence of Metal-Resistant Bacteria in Aquatic Environments	43
B. Mechanisms that Enable Bacteria to Survive Elevated Metal Concentrations	44
1. Extracellular Metal Complexation by Bacterial Exopolysaccharides	44
2. Extracellular Complexation by Compounds Other than Polysaccharides	46
3. Extracellular Precipitation and Crystallization	46
4. Alteration of Transport Mechanisms and Intracellular Reactions	47
5. Metal Transformation	47
C. Regulation of Metal Resistance	48
III. Influence of Bacteria on the Distribution of Metals in the Environment	49
A. Geomicrobial Processes	49
B. Accumulation of Metals by Bacteria	50
C. Economic Implications of Bacteria and Metal Interactions	51
1. Biomining	51

2. Recovery of Metals from Waste Streams	53
3. Corrosion	53
D. Role of Bacteria in the Passage of Metals to Foodchains	55
IV. Summary	57
References.....	57

I. INTRODUCTION

Historically, metals have been discharged into bodies of water on the assumption that they would either be diluted to nontoxic levels or form nonbiologically active complexes in the sediments. In the early sixties, this was dramatically and tragically proven to be a false assumption when bacteria, present in the sediments of Minamata Bay (Japan), methylated inorganic mercury, which had been released into the bay in effluents from a factory using mercuric sulphate catalysts in acetaldehyde production. The methylated mercury accumulated in fish and shellfish which were eaten by the local inhabitants. As a consequence, by 1975, 115 people had died and many were left paralyzed. This incident focused attention on the deleterious effects that can occur when heavy metals are released into the aquatic environment.¹⁻³

It is now known that when metals enter bodies of water, a number of complex events occur, with the result that the metals are either deposited to the sediments or remain in the water column either in suspension or in solution. The metals may also find their way into the biota.⁴ Partitioning is controlled by numerous physical, chemical, and biological characteristics of the ecosystem. These include the speciation and concentration of the metal, the composition of the effluent and natural waters with regard to organic and inorganic ligands, salinity, pH, E_h , temperature, microbial activity, and the nature of the biota.⁵

Of the various metal complexing agents in aquatic systems, microorganisms and their constituent polymers are among the most efficient scavengers of metallic ions. For this reason, it is important to develop a better understanding of the reactivity between metals and natural microbial populations.

Metals enter aquatic habitats from a number of natural and anthropogenic sources. Natural sources include metals in the earth's crust entering via: runoff following rain, land erosion, volcanic activity; or wind-blown dust.⁶ The quantity of some metals entering from natural sources is now exceeded in certain areas by that entering due to man's activities. These discharges can create point sources of elevated metal concentrations in the sediments and water column around the discharge point and for varying distances away from it.⁷

II. SURVIVAL OF BACTERIA IN THE PRESENCE OF ELEVATED METAL CONCENTRATIONS

A. Occurrence of Metal-Resistant Bacteria in Aquatic Environments

Bacteria containing multiple resistance to heavy metals have been isolated from a wide variety of environments,⁸⁻¹² including heavy metal-polluted and nonpolluted freshwater environments.^{13,14} That the ability of bacteria to grow in the presence of relatively high metal concentrations is found in a wide range of microbial species, including those isolated from unpolluted sites,⁹ suggests that resistance to high metal concentrations is not necessarily a result of genetic change but may result from intrinsic properties of the bacterium, such as the production of copious quantities of extracellular polymeric substances (EPS).¹⁵⁻¹⁷ Thus, the ability of a microorganism to survive and reproduce in a metal-contaminated environment is dependent, to varying extents, on both genetic and physiological processes.¹⁷

A number of studies have focused on the association of plasmids with heavy metal resistance in bacteria isolated from polluted and unpolluted environments.^{8-11,14,15,18} It is considered that plasmids play an important ecological role in natural bacterial populations. Bacteria, supplied with additional and transferable plasmid-encoded properties gain selective advantage over other organisms that lack the useful plasmid-encoded traits. Transfer of plasmids to other taxa accelerates horizontal evolution by spreading useful properties within bacterial communities. Transfer may also provide the genetic prerequisites for bacterial life in extreme environments, such as in metal-polluted aquatic habitats.¹⁰

It has been reported that bacteria isolated from polluted environments have a higher frequency of plasmids than those isolated from similar unpolluted sites;¹⁸ however, other studies report no differences in plasmid frequency in bacteria isolated from polluted or nonpolluted sites.¹⁴ It is, however, recognized that metal-resistant strains isolated from environmental or clinical sources generally have the genes conferring metal resistance on plasmids or on transposons, or at least the genetic determinants are homologous to those found on plasmids.⁹ Chromosomal mutations to heavy metal resistance can be produced in the laboratory but do not generally occur in nature.⁹

Research on the properties of plasmids has, up to recently, been conducted mainly on plasmids associated with bacteria isolated from clinical environments.^{9,14} Genetic information on natural bacterial assemblages from different environments is rather limited. To enable researchers to fully understand the role of transferable genetic elements in the survival of bacteria in metal-polluted environments, more information is required on (1) plasmid distribution within the environment, (2) plasmid stability in nature, (3) transfer mechanisms, and (4) the mechanisms of metal resistance imparted to the bacteria by the acquisition of a particular plasmid.

B. Mechanisms that Enable Bacteria to Survive Elevated Metal Concentrations

Metal resistance in microorganisms can occur by a variety of mechanisms that decrease either the toxicity of the metal or its accessibility to the cell. Such processes include physical sequestration, exclusion, and complexation or modification/detoxification of the metal.

1. Extracellular Metal Complexation by Bacterial Exopolysaccharides

Among the mechanisms employed by bacteria to survive in high metal concentrations is the production of large amounts of high molecular weight substances exhibiting a range of solubilities in association with metals. These substances may detoxify metals due to their complexing or chelating properties, or they may effectively form a barrier around the cell, which limits the access of the metal ions to vulnerable sites within the cell. The production of an envelope structure or capsule around the cell is considered to be a ubiquitous feature of bacteria isolated from aquatic environments. The capsule, which is usually a polysaccharide with a repeating sequence of two to six sugar subunits,¹⁹ is anchored to the bacterial outer membrane. In some instances, the oligosaccharide side chain of lipopolysaccharide in the outer membrane is believed to contribute to the capsular structure.²⁰ Protein components and other products of cell metabolism excreted by bacteria and trapped in the exopolymer matrix may also contribute to the overall chemical properties of the capsule.^{21,22}

Exopolymers exhibit a variety of associations with the cell surface. They may form a firmly bound capsule, which often enables the cell to establish a stable orientation with respect to its environment. Cell-bound exopolymers may extend from 0.1 to 10 μm from the cell surface into the surrounding environment, creating a buffer zone between the surface of the cell and the external environment.^{23,24}

In many instances, the capsular polymers maintain a more transient association with the cell and take the form of what is commonly referred to as "slime". Under these circumstances, a portion, or indeed, the bulk of the exopolymer sloughs into the surrounding environment. The network of polymers that make up the capsule or slime forms a colloid or gel phase, depending on the nature of the surrounding environment. The water enclosed by the capsule generally contributes greater than 99% of the capsule weight. Aggregation of the exopolymers may occur under certain conditions, resulting in the formation of visible flocs. This tendency for bacterial exopolymers to form a colloid or gel phase around the cell, which often results in biofilm or floc formation, suggests that these biomolecules possess properties that are different from those of other naturally occurring polymers.²⁵

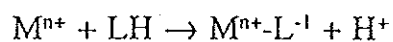
That bacterial polysaccharides play a role in protecting bacteria from the toxic effects of metal ions has been suspected for a number of years.²⁶ Studies indicated that bacterial survival rate in the presence of metals was significantly

greater for mucoid cells as opposed to nonmucoid variants.^{26,27} Indirect evidence also suggests that exopolysaccharides play a role in the protection of bacteria from metal ions; for example, it has been shown that the production of capsule and slime exopolymers is enhanced in the presence of metal ions.²⁸⁻³¹ Furthermore, there is evidence that the presence of elevated metal concentrations in the environment influences both the chemical composition of the EPS produced by the bacteria^{32,33} and its settling/floccing properties.^{30,34} Adsorption of metal ions by polymers has been suggested to enhance the protective capabilities of bacterial polymers by increasing their resistance to decomposition.³⁵⁻³⁸ The increased persistence in the environment is considered to be due to an inactivation of bacterial enzymes by the heavy metals.³⁹

Although different classes of biological molecules may be associated with bacterial exopolymers, the metal-binding reactions that will be considered here are restricted to those involving the polysaccharide component. Metals are electron acceptors. The most effective donor group associated with acidic capsules and slime polysaccharides is the carboxyl residue. Lone electron pairs on the carboxyl groups interact with the charge-compensating metal ions. Weak electron donors are also present on acidic and neutral polysaccharides in the form of oxygen atoms associated with the ether bond and hydroxyl residues on the sugar subunits.^{40,41} Pyruvate also contains free carboxyl groups which are free to react with positive-charged molecules such as Cu ions. Smith et al.⁴² have developed high performance liquid chromatography (HPLC) methods to quantify ketal-linked pyruvates present on exopolymers, and work is proceeding in our laboratory to determine the importance of these groups in metal binding.

On the basis of Rendleman's⁴³ interpretation of ion interactions with polysaccharides, metal binding by uncharged polysaccharides occurs as a result of coordination between the metal cation and oxyanion and hydroxyl groups on the donor molecule. The affinity exhibited by uncharged polysaccharides generally decreases with increasing ionic radius of metals.

The general metal-molecule interaction is an acid-base reaction:



where the acid is represented by H^{+} , the metal ion by M^{n+} , and the base by L^{-1} . The binding of Cu ions to the capsule of a freshwater sediment bacterium (FRI) was shown to result in the displacement of protons that caused a shift in the pK_a of the capsule from 4.90 to 4.05.⁴⁴ An observed decrease in the conditional stability constant for the Cu-capsule complex with decreased pH also suggests that there was competition between Cu ions and protons at the site of metal binding. Although no uronic acids were detected in the capsular material, the pK_a value suggests the presence of carboxyl groups, possibly in the form of ketal-linked pyruvate residues.⁴⁵

The interaction between copper ions and carboxyl groups on acidic polysaccharides is believed to depend on several factors: the nature of the component

sugars and their relative distribution in the chain, the magnitude of the overall electrostatic field, and the ratios of copper to polymer and copper to simple supporting electrolyte.⁴⁶

For further information on the binding of metal ions by exopolymers we direct the reader to Geesey and Jang,²⁵ Geesey et al.,⁴⁷ Jang et al.,⁴⁸ and Jang et al.⁴⁹

2. Extracellular Complexation by Compounds Other than Polysaccharides

Extracellular complexation occurs when microorganisms produce metabolic products that are excreted, and whose presence near or around the cell results in the immobilization of the metal. For example, citric acid is an efficient metal chelator, and not only has it been reported to protect the bacteria from the toxic effect of free metal ions, it also renders the citric acid resistant to microbial degradation. This suggests that not only do metals have a toxic effect on microbes, but they can also influence microbial decomposition of organic compounds.⁵⁰ These observations corroborate the results of Lasik and Gordiyenko³⁹, which suggests that the binding of metals by polysaccharides increases the time the polymer-metal complex survives in the environment.

Iron is an essential element. Many microorganisms release various iron-binding molecules, called siderophores, which scavenge iron from the environment.^{51,52} Iron limitation can increase the extracellular production of siderophores.⁵³ In *Anabaena* species, these can function as strong copper complexing agents. It is, therefore, conceivable that in some circumstances, siderophore excretion may impart protection from metal toxicity.¹⁷

3. Extracellular Precipitation and Crystallization

Many bacteria mediate reactions or produce metabolites that result in the crystallization and precipitation of metals on microbial cell surfaces. For example, sulphate-reducing bacteria are involved in the formation of sulphide deposits which contain large amounts of metals. Sulphide formation thus leads to metal removal from solution, and this is associated with tolerance in a variety of microbes. Metal-tolerant strains of *Klebsiella aerogenes* precipitate Pb, Hg, or Cd as insoluble sulphide granules on outer surfaces of cells.⁵⁴ Reactions of metal ions, including Hg²⁺, Cd²⁺, Cu²⁺, and Zn²⁺, with H₂S produced by *Clostridium cochlearium* also resulted in the formation of insoluble metal sulfides.⁵⁵ Protection of *Desulfovibrio* species from copper and zinc was correlated with increased H₂S production and formation of mineral deposits such as covellite (CuS) and sphalerite (ZnS).^{12,56}

Bacterially produced H₂S has been shown to be capable of decreasing the toxicity of chromium by reducing Cr(VI) to the less toxic Cr(III) ions.⁵⁷ It has been noted that, in some cases, sulfide-producing organisms can protect sensitive organisms from the toxic effects of metals. When *Desulfovibrio desulfuricans* was grown in mixed culture with a metal-sensitive strain of

Pseudomonas aeruginosa, the latter organism could tolerate higher concentrations of mercurials than it could in pure culture. Results indicated that the H_2S produced by the sulfate reducer protected the pseudomonad.⁵⁸

4. Alteration of Transport Mechanisms and Intracellular Reactions

A number of metals can be accumulated intracellularly by bacteria.¹⁷ As a relationship between metal transport into microbial cells and metal toxicity is often observed,¹⁷ any mechanism that results in a decrease in the passage of metals into the cell can constitute a resistance mechanism; such mechanisms include decreased transport across the cell wall or the occurrence of metal efflux systems.

For example, the absence of outer membrane proteins (Omp) b and c in *E. coli* K12 and Omp b in *E. coli* 13/r conferred resistance on these cells to elevated copper concentrations by presumably preventing the entry of Cu^{2+} into the cells. Similarly, a reduction in the amount of Omp F resulted in increased resistance in *E. coli* strains.^{59,60} The presence, absence, or expression level of a particular protein may also increase resistance, as is the case with an *E. coli* strain that has been shown to be resistant to arsenic due to reduced uptake mediated by a change in the cells ATPase efflux system.⁹

Once inside the cell, metal ions may be compartmentalized and/or converted to more innocuous forms. Such processes can be effective detoxification mechanisms, and microbes expressing them may be able to accumulate metals to high intracellular concentrations. Examples of this type of mechanism include the formation of polyphosphate metal-sequestering compounds or the synthesis of intracellular metal-binding proteins, such as metallothioneins, which function in detoxification as well as in the storage and regulation of intracellular metal ion concentrations. Gadd¹⁷ has suggested that such mechanisms may be temporary and precede other means of expulsion of accumulated metals from the cells.

5. Metal Transformation

The ability of bacteria to alter the chemical state of a metal not only gives them a potentially important, but little understood, role in the biogeochemical cycling and the bioavailability of many elements, but may also constitute mechanisms of tolerance.^{17,61}

Metals cannot be broken down into other products, but may, as a result of biological action, undergo changes in valence and/or interconversion between organic and inorganic compounds.

Mercury has received considerable attention because of its high toxicity and has become an excellent model chemical for the study of biotransformation of metal salts. The complete detoxification of mercury involves the reduction of the inorganic (Hg^{2+}) or organomercurial ($CH_3-Hg^+[R-Hg^+]$) form to the less toxic elemental mercury (Hg^0). Mercuric reductase, the enzyme encoded by

the *merA* gene, is involved in the conversion of Hg^{2+} to Hg^0 , and the organomercurial lyase enzyme encoded by the *merB* gene is involved in the cleavage of the $\text{H}_3\text{C-Hg}$ bond with subsequent release of Hg^{2+} .⁶² These enzymes have frequently been reported to be plasmid encoded.⁶³

Other detoxifying enzymes include arsenite oxidase, which catalyzes As^{3+} to As^{6+} , and chromate reductase, which catalyzes Cr^{6+} to Cr^{3+} ; both of these reactions result in an end product which is considerably less toxic to the cell, and, hence, may be considered as an effective detoxification mechanism.^{17,64,65}

In summary, the ability of microorganisms to survive in sediments containing high concentrations of metals allows no simple explanation. This is in large part due to the multiplicity of interactions that can occur between microbial cells, metal ions, and the environment. It has been estimated that for a given environment, traditional microbiological culture techniques isolate less than 10% of the microbiological populations. Given this constraint, it is highly likely that many mechanisms of metal resistance occur, about which we know very little. As our understanding of the mechanisms used by bacteria to survive in metal-polluted environments increases, so will our knowledge of how we can best take advantage of bacterial reactions for the benefit of man and the environment.

C. Regulation of Metal Resistance

In order to gain an understanding of cellular regulation of metals, it is informative to study the regulation of both an essential and a toxic metal. Mercury salts, for example, are toxic to all living organisms and have no known beneficial function. Copper is an essential element involved in redox reactions and is a cofactor for a number of enzyme reactions; at high concentrations, however, it can cause cell damage through modification of the active sites of cellular enzymes and the peroxidation of membranes. Bacteria, therefore, have to utilize different approaches in regulating the entry of these two elements into the cell.

Similarities exist between the biological processes to protect against excess copper or mercury in terms of binding proteins and the transport of the metals. Both metals are taken into the cell, and either detoxified, sequestered, or exported. Differences occur in cell regulation of these metals, as copper requires a two-way control, while mercuric ion resistance is only required to deal with toxicity by responding to mercuric ions at any concentration and is, therefore, a one-way control system.⁶⁶

Resistance to mercuric ions, while mainly inducible, can also be constitutive. In contrast, resistance to copper must be inducible, as over-expression of the resistance proteins would result in available cellular copper concentrations decreasing to levels that inhibited cell metabolism. Using an *E. coli* strain isolated from a high copper environment, Lee et al.⁶⁶ determined that the level of expression of copper resistance as a function of metal concentration was linear, whereas a threshold response was found with increasing concentrations

of mercury. The linear response to increasing copper concentrations was consistent with the necessity for homeostatic control of this type of resistance, as the level of resistance must correlate with the level of environmental copper. With mercury, however, a threshold response is expected because the homeostatic level of mercury is close to zero, so over-expression of the response is not an immediate problem.⁶⁶ In the *E. coli* strain mentioned above, the copper resistance was coded on a plasmid (pRJ1004),⁶⁷ and four genes have been identified in the resistance determinant *pco*.⁶⁶

III. INFLUENCE OF BACTERIA ON THE DISTRIBUTION OF METALS IN THE ENVIRONMENT

A. Geomicrobial Processes

Processes carried out by microbes present in aquatic environments can result in the formation, concentration, dispersion, alteration, or fractionation of metals.

Bacteria can cause localized accumulation of metals by (1) binding the metals either intracellularly or extracellularly, as covered above in Section II.B., or by (2) altering the microenvironment surrounding the cell so that the metals precipitate or form insoluble complexes near or around the cells. The net effect of these localized precipitations is the manifold local increase in the accumulation of metals around or associated with the bacterial cell.

Iron sulfides such as pyrite, iron oxides such as ochre, or manganese oxides such as vernadite and psilomelane may be generated authigenically by microbes.⁶⁸ Bacteria can reduce ferric oxide or manganese dioxide to soluble compounds. Microbes may act selectively on a mixture of inorganic compounds by promoting selective chemical change of one or a few compounds of the mixture, causing a selective concentration or dispersion — for example, in the oxidation of arsenopyrite by *Thiobacillus ferrooxidans* or in the preferential reduction of Mn(IV) over Fe(III) in ferromanganese nodules.⁶⁹ Microbes can alter rock structure and transform primary minerals into secondary minerals, as in the conversion of orthoclase to kaolinite.⁶⁹

Microbes can affect the solubility and availability of metals by promoting either the oxidation or reduction of an element; such processes may be carried out within the bacteria enzymatically or indirectly (nonenzymatically) through interaction of the metal with metabolic products.

Enzymatically, bacteria can oxidize manganous manganese (Mn^{2+}) (*Metallogenium* spp. *Hyphomicrobium* spp.) and/or iron (*Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*) to yield some energy for the cell. Aerobic and anaerobic reduction of Cr(VI) by bacteria (*Aeromonas dechromatica*, *Flavobacterium devorans*, *Arthrobacter* spp.) have also been demonstrated, and while it is unclear if it is an enzymatic reduction in all species, in some cases it has been shown to be involved in respiration

processes. Cr(VI) reduction to Cr(III) is beneficial ecologically because Cr(III) is less toxic than Cr(VI). Furthermore, Cr(III) tends to precipitate as a hydroxo compound around neutrality, which is the pH range around which all known Cr(VI) reducers operate.⁶⁹

Indirect oxidation or reduction results from the fact that many heterotrophic bacteria, whether aerobic, facultative, or anaerobic, form significant quantities of organic acids (e.g., oxalic, citric, and gluconic acids) and/or CO₂ from the catabolism of complex molecules. Some of the CO₂ hydrolyzes to form carbonic acid in aqueous solution. Some chemolithotrophs and photolithotrophs form significant amounts of sulfuric or nitric acids, depending on the substrate they use as their source of energy and/or reducing power. Other heterotrophs, when growing at the expense of nitrogenous carbon and energy sources such as proteins or peptides, generate ammonia, which forms NH₄OH in aqueous solution. The acids and bases that are produced affect the redox potential of the environment and/or pH, which have a strong effect on metal solubility and mobility.³ Furthermore, the utilization of organic acids present in the environment may alter the pH to such a degree that previously unfavorable reactions can now proceed. For example, the bacterial utilization of hydroxycarboxylic acids results in a rise in pH in the surrounding environment, which favors the oxidation of Mn(II).⁶⁹

Bacterial utilization of substrates, such as oxalate, citrate, humic acids, and tannins may make available metals that were previously insoluble. Bacterial break down of substrates has been reported to result in the release of free ferrous iron, which then autooxidizes to ferric iron.⁷⁰ The production of ferric iron from the oxidation of ferrous iron at pH values above 5 usually leads to precipitation of the iron. However, the presence of chelating agents, such as humic substances or citrate, can prevent precipitation, which results in the unchelated ferric iron hydrolyzing at higher pH values and forming compounds such as ferric hydroxide. The latter is relatively insoluble and will tend to settle out of suspension or crystallize and dehydrate, forming FeOOH, goethite (Fe₂O₃·H₂O), or hematite (Fe₂O₃).⁶⁹

In summary, bacteria play an important, if little understood, role in the cycling and transformation of metals within the aquatic environment. Bacteria or reactions mediated by bacterial products have the potential to make a metal more or less available to other aquatic organisms and to alter the toxicity of the metal to the biota. It is only with a full understanding of the reactions mediated by bacteria that predictions regarding the impact of pollutants on an environment can be assessed. Such predictions have serious implications for the environment and for the well-being of mankind.

B. Accumulation of Metals by Bacteria

The accumulation of metals by bacteria can occur by either passive or active mechanisms. In active accumulation, metal transformations or microbe-metal interactions are carried out by living, metabolically active cells. In passive

accumulation, metals are transformed by physical-chemical actions not necessarily requiring participation by living cells.⁷¹

A number of the mechanisms involved in metal accumulation have been discussed in the preceding section on the tolerance of bacteria to metals. In summary, active metal accumulation often depends on the expenditure of energy and includes such processes as: (1) precipitation, (2) intracellular accumulation and complexation, (3) oxidation and reduction, and (4) methylation and demethylation. Passive immobilization of metals occurs when (1) a solubilized metal is chelated by a substance produced and excreted by a microbial cell, or (2) a metal binds to a cell surface by physical-chemical reactions.⁷¹ Microbial cell walls are anionic due to the presence of carboxyl, hydroxyl, phosphoryl, and other negatively charged groups. Cationic metals rapidly bind to these sites by an energy-independent reaction.⁵⁶ Cell-surface binding of metals by bacteria is discussed in Chapter 3 of this book.

In studying the kinetics of metal accumulation by microorganisms, it has become apparent that the above processes can be divided in terms of rate of accumulation into two main types. The first involves a rapid nonspecific binding of the metal to cell surfaces, slime layers, extracellular matrices, etc., whereas the second, slower process involves metabolism-dependent intracellular uptake or modification of the metal.⁵⁸ It appears as if most heavy metals can be adsorbed onto the surface of both living and dead microbial cells.

The concentration of metal accumulated by the bacterial cell is dependent on the bacterium under study, the speciation of the metal, the composition of the culture medium, and growth conditions.^{72,58} Rather than examine single cases in detail, we have decided to cite a few of the very many references available on metal accumulation by bacteria. Metals reported to be accumulated by bacteria include: Cd⁷³, Cr⁷⁴, Cu⁷⁵, Ge⁷⁶, Mo⁷⁷, Ni⁷⁸, Pb^{79,80}, and U.⁸¹

C. Economic Implications of Bacteria and Metal Interactions

I. Biomining

Microbiological bioleaching is a process of extracting metals from sulfide ores with low metal concentrations. Currently, microbiological leaching of metals from ore is practiced in dump and underground uranium and copper-leaching operations.^{82,83} The bioleaching of refractory precious metal ores in which gold and silver are finely disseminated in sulfide minerals such as pyrite and arsenopyrite is becoming of greater interest as the price of these metals increases and the availability of simple, free-milling ores decreases.⁸⁴

In the general microbial-leaching process, low-grade ore is dumped in a large pile (the leach dump), and a dilute sulfuric acid solution (pH around 2) is percolated down through the pile. The liquid coming out of the bottom of the pile, rich in minerals, is collected and transported to a precipitation plant where the metal is reprecipitated and purified. The liquid is then pumped back to the

top of the pile, and the cycle is repeated. As needed, more acid is added to maintain the low pH.

The principal reactions catalyzed by bacteria are the direct oxidation of sulfide minerals and the indirect dispersion or solubilization of metal sulfides and oxides by ferric iron and/or sulfuric acid.⁸⁵ To illustrate these processes, examples are taken from Brock et al.⁸⁶ of the oxidation of two copper minerals. Microbial leaching is especially useful for copper ores because the copper sulfate, formed during the oxidation of the copper sulfide ores, is very water soluble.

The first mechanism involves the direct oxidation of copper by bacteria, such as *Thiobacillus ferrooxidans*, so that the monovalent copper in chalcocite (Cu_2S) is oxidized to divalent copper, thus removing some of the copper in the soluble form (Cu^{2+}) and forming the mineral covellite (CuS); in this reaction there is no change in the valence of sulfide. The bacteria apparently utilize the reaction Cu^+ to Cu^{2+} as a source of energy; however, it is not possible to rule out iron as a prime oxidizing agent for reduced copper.⁸⁵

A second mechanism, and probably the most important in most mining operations, involves an indirect oxidation of the copper ore with ferric iron that were formed by the bacterial oxidation of ferrous iron. In most ore, pyrite is present and the oxidation of this pyrite leads to the formation of ferric iron. Reaction of the copper sulfide with ferric iron results in the solubilization of the copper and the formation of ferrous iron. In the presence of oxygen, at the pH values (1.5 to 3.0) involved, *T. ferrooxidans* reoxidizes the ferrous iron back to the ferric form, so that it can oxidize more copper sulfide. Thus, the process is kept going indirectly by the action of the bacterium on iron.⁸⁷

Next to copper, the most important bioleaching process involves uranium. In most ores, the uranium occurs as a mixture of minerals containing the uranium in either the tetravalent (an insoluble oxide) or the hexavalent form containing the soluble uranyl ion (UO_2^{2+}).^{86,88} Bacterial leaching of uranium also occurs via an indirect mechanism. Bacterially generated Fe^{3+} oxidizes tetravalent uranium to hexavalent uranium. The Fe^{2+} so formed is then reoxidized to the Fe^{3+} by *T. ferrooxidans*. Most uranium contains associated pyrite, which serves as a source of Fe^{3+} . It is also possible to add ferric iron to trigger the process. Once the initial reaction has occurred, the iron can continue to cycle between the oxidized and the reduced form as the uranium is oxidized and solubilized. The soluble uranyl ion formed in the process is removed from the leach solution by means of organic solvent extraction, which does not involve bacteria. *Thiobacillus ferrooxidans* can directly oxidize reduced compounds of uranium (uranous sulfate, and UO_2) without the involvement of the $\text{Fe}^{3+}/\text{Fe}^{2+}$ couple as the electron carrier; however, this reaction is not significant during dump or heap leaching due to the abundance of pyrrhotite and pyrite present within most ores.^{86,88}

Thiobacillus ferrooxidans appears to be the most dominant organism in the oxidation of mineral sulfides in most acidic environments if the temperature is below 40°C .⁸⁹ Other bacteria implicated in at least some steps in the bioleaching

of sulfide minerals include: *Leptospirillum ferrooxidans*, which can grow on and degrade pyrite; *T. thiooxidans*, which can influence the leaching of minerals, e.g., zinc sulfides and cadmium sulfide, but cannot directly attack the lattice crystal structure of minerals or oxidize iron;⁸⁵ and *Sulfobacillus thermosulfidooxidans*, which has been isolated from geothermal mineral-enriched areas. *Sulfobacillus thermosulfidooxidans* will grow autotrophically on iron but requires a source of reduced sulfur.⁸⁹

While the bacteria do not appear to have to attach to the mineral surface for leaching to occur, transmission electron microscopy studies have revealed that the bacteria will selectively attach to the surfaces that act as energy sources, such as, CuFeS_2 and FeS_2 .⁹⁰

2. Recovery of Metals from Waste Streams

Most of the current research on metal accumulation by microorganisms has focused on the use of microorganisms as a viable commercial alternative to costly and often ineffective physical-chemical technologies for the treatment of vast quantities of waste water containing low concentrations of soluble and particulate metals.^{91,92} Applications of these bioabsorbents include: (1) removal of metals from aqueous, industrial effluents for pollution control; (2) remediation of contaminated surface waters, groundwaters, and lagoons; and (3) treatment of industrial process streams for resource recovery.⁷¹

The first generation of biological-based products for metal removal from waste and process streams is currently in use. The systems utilize either dead or living biomass. The advantage to utilizing a living system is that it is essentially a renewable resource; however, they have proven to be difficult to regulate and are susceptible to fluctuations in the loading of the metal and to the presence of other toxic compounds that may be present in the waste-water stream.

A second type of system that is showing great promise is the use of immobilized, dead, or nonmetabolizing cells, or cellular constituents in matrices that are granular in form and are both chemically and physically stable. Once the immobilized cells are saturated, they can be removed from the waste stream, stripped of products, and regenerated for further use.^{56,71} While the use of dead biomass or derived products eliminates problems of toxicity, nutrient supply, and maintenance of optimal growth conditions, living cells can exhibit a wider variety of mechanisms for metal accumulations, including transport and intracellular and extracellular precipitation as discussed above in Section II. B.

3. Corrosion

Corrosion is an electrochemical reaction generated by point heterogeneities in potential on a metal surface. Once a surface makes contact with an electrolyte, an electromotive force (EMF) is generated between two parts of the surface,

which results in the formation of a corrosion cell. One site on the metal surface acts as the anode, and its atoms ionize and become soluble. Simultaneously, electrons from the ionized metal atoms migrate to the less reactive point, which acts as the cathode. The surface seeks an equipotential state, and, as this state is reached, metallic dissolution of the anode follows and continues until the EMF decreases and corrosion stops. Outside forces, of course, can intervene, and it is these intervening forces which sustain or increase corrosion.⁹³

Bacteria on the surface of a metal can influence corrosion rates in a number of ways. Bacterial attachment and growth on a metal surface is usually very irregular; the bacteria form a film or consortium comprised of different organisms, which can frequently be several to hundreds of cells thick. The development of this so-called "biofilm" results in heterogeneities in microbial types and their distribution over the surface of the metal.⁹⁴ Bacterial activities within a biofilm can contribute to corrosion in many ways: (1) by the production of organic or inorganic acids; (2) bacteria can alter the E_h or electrode potential of a point on the metal surface by the production of acids or bases, or by differential binding of metals by the bacteria or their exopolymers, leading to the formation of oxygen-differential or metal-concentration cells; (3) bacteria can depolarize surfaces by oxidizing hydrogen; (4) sulfate reducers produce H_2S which, in itself, is corrosive.

The effect of bacteria on iron corrosion has been documented in two recent reviews.^{95,96} The remainder of this section will be devoted to bacterially induced corrosion of copper.

Recently, bacteria have been implicated in the pitting corrosion of copper in freshwater systems.^{97,98} While the exact mechanism of this corrosion process is unclear, exopolymers secreted by biofilm bacteria have been implicated. Studies carried out using copper-coated germanium (Ge) internal reflection elements (IRE) in a Fourier transform infrared (FT-IR) spectrophotometer indicated that isolated bacterial exopolymers adsorbed to copper thin films deposited on the surface of the IREs.^{99,100}

Results of several studies suggest that copper is oxidized by acidic polysaccharides. X-ray photoelectron spectroscopy (XPS) demonstrated that some of the copper deposited on IREs exposed to gum arabic and alginic acid was oxidized to Cu^{2+} , copper from thin films exposed to *Alteromonas colwelliana* exopolymer was oxidized to Cu^{1+} , while copper from thin films exposed to *Alteromonas (Pseudomonas) atlantica* exopolymer displayed little oxidation and remained as Cu^0 .^{101,102}

Auger depth profiles of copper thin films exposed to various acidic polysaccharides and exopolymers from biofilm bacteria verified FT-IR and atomic absorption spectroscopic results that copper had been removed from the surface. The overall rate of copper removal based on Auger depth profile results: alginic acid > gum arabic > *A. colwelliana* exopolymer > *A. atlantica* exopolymer was consistent with results obtained by ATR/FT-IR.

The interactions of bacteria isolated from corroded copper coupons on thin films of copper deposited onto Ge IRE were evaluated nondestructively in real

time by FT-IR. The films were stable in flowing or static, sterile culture medium. When exposed to and colonized by the bacterium CCI#8, which had been isolated from a pit on a corroded copper coupon, the copper thin film corroded. Corrosion was enhanced under quiescent conditions. In conjunction with corrosion of the copper thin film, FT-IR spectra indicated that there was an increase in the concentration of polysaccharide material at the copper biofilm interface.⁹⁸

On the basis of the data presented above, deterioration of copper surfaces colonized by microbial biofilms is likely due to interactions between copper ions in equilibrium with metallic copper and the exopolysaccharides secreted by the adherent microorganisms. Acidic polysaccharides, including those secreted by biofilm-forming microorganisms, have been shown to possess high-affinity binding sites for copper ions.^{44,49} It has been proposed that the complexation of copper ions by the polysaccharides reduces the free metal ion concentration at the metal surface and promotes further ionization of metallic copper in order to establish equilibrium conditions.¹⁰³ A result of copper ion complexation by acidic polysaccharides from some biofilm bacteria is the liberation of hydrogen ions.⁴⁴ The resulting increase in acidity within the biofilm is likely to promote further dissolution of metallic copper.

Acid production by bacteria has been considered one of the possible mechanisms of microbially-enhanced corrosion of metals.¹⁰⁴ Dissolved low-molecular weight acids, such as acetic acid, have received the greatest attention in this regard.¹⁰⁵ Acidic polysaccharides, however, possess properties that make them particularly important agents of metal corrosion. The interligand distance of ionizable groups on polysaccharides, such as alginic acid and exopolysaccharide of *A. atlantica*, range from 4 to 8 Å.¹⁰⁶ Therefore, the concentration of acidic groups associated with exopolysaccharides immobilized at or near the metal surface is likely to be considerably greater than that achieved by diffusible low-molecular weight acids excreted by some bacteria.

Acidic exopolysaccharides appear to be one of the most common metabolic products of surface-associated bacterial populations. Their widespread existence appears to stem from their participation in the adhesion of biofilm microorganisms to surfaces.¹⁵ The results presented above suggest that acidic exopolysaccharides may also play an important role in copper corrosion.

D. The Role of Bacteria in the Passage of Metals to Foodchains

As discussed in previous sections, microorganisms not only have the ability to survive in sediments containing high concentrations of metals, but they also have the ability to accumulate metals. This raises the question of what the effect is on higher organisms, and whether they ingest bacteria which have accumulated metals. Bacteria or their extracellular secretions form the base of many foodchains.¹⁰⁷⁻¹⁰⁹ Evidence that metals concentrated by bacteria can be passed up the foodchain has been reported from a study of a freshwater habitat.

A bacterium of the genus *Sphaerotilus* was determined to concentrate a variety of metals. Elevated metal concentrations were found in tubificid worms after ingestion of these bacteria.¹¹⁰ Furthermore, ingestion of the tubificid worms by tropical fish led to increased metal concentrations being detected in the fish tissue after 4 d.¹¹¹ Further evidence proving that bacteria can play a role in incorporating metals into foodchains has come from a number of studies which have investigated the mobilization and transformation of mercury and its entry to foodchains.¹¹²⁻¹¹⁴

The realization that bacterial epiphytes can be major contributors to the metal concentration of plants growing in polluted freshwater environments is further evidence that bacteria may play a role in the passage of metals through foodchains. The removal of the epiphytes from the aquatic plant *Alisma plantago-aquatica* resulted in a reduction in the plants Cr level of 25 to 50%.¹¹⁵ The authors postulated that animals grazing in such epiphytes in polluted environments are consuming bacteria with elevated metal concentrations. These results were supported by a study in the marine environment of the periwinkle *Melarapha cincta* grazing on the surface of the sea lettuce *Ulva*. The *Melarapha* consumed *Leucothrix*-like organisms, and elevated metal concentrations in the bacteria resulted in elevated metal concentrations in the periwinkle.¹¹⁶ Similarly, periphytic bacteria found on the carapace and gills of crabs collected from near a tannery-effluent outlet were shown to be able to concentrate Cr. It was speculated that ingestion of the crabs and their associated bacteria could contribute to the passage of Cr through the foodchain.¹¹⁷

By virtue of their physical properties, bacterial exopolymers are capable of adsorbing and concentrating many metals, and this may facilitate the entry of the metal into the foodchain. It was reported that metals became more available to the sediment-feeding clam *Macoma balthica* when the metals were adsorbed to exopolymers, compared to when the metals were free in solution or bound to glass beads.¹¹⁸ In a similar study, the feeding of bacterial polysaccharides with bound Cr to two common marine organisms, a polychaete species and the mudsnail *Amphibola crenata*, resulted in an increase in their Cr concentration. For the polychaete, 61% of the total Cr was shown to be contributed from ingested Cr, and autoradiographic evidence following the use of radiolabeled ¹⁴C-labeled polysaccharide indicated that the polychaetes were indeed ingesting the polysaccharide.¹¹⁹

The information presented above indicates that bacteria can play a significant role in the transfer of metals to higher trophic levels and ultimately to man. These findings reinforce the concepts presented in the introduction of this chapter, that the ultimate fate of metals in aquatic environments is controlled by numerous physical, chemical, and biological characteristics of the environment. Bacteria, due to their unique ability to adapt to and survive in metal-polluted environments, play an integral role in the fate of metals deposited into aquatic environments.

IV. SUMMARY

The further study of the interactions that occur between bacteria, metals, and the environment will give us an insight into how to control bacterial/metal interactions for the benefit of man and the environment. Examples of the way in which we can utilize microbe/metal interactions for our benefit include the mining and recovery of semiprecious metals or the bioremediation of metal-contaminated sites. Other reactions of interest include those that are detrimental to man, such as microbially induced corrosion, or the bacterial methylation of inorganic mercury and the subsequent transfer of the methylated mercury to organisms in higher trophic levels and ultimately to man.

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