

Metal sorption and mineral precipitation by bacteria in two Amazonian river systems: Rio Solimões and Rio Negro, Brazil

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ABSTRACT

The surfaces of epiphytic and episammic bacteria from the solute-rich waters of the Rio Solimões, Brazil, interacted with available cations in solution and provided major sites for iron deposition. Once bound to the bacteria, the iron served as nucleation sites for the formation and growth of fine-grained authigenic mineral phases. Because of progressive mineralization, the precipitates exhibited a wide range of morphologies, from amorphous "gel-like" to crystalline structures. Complete encrustation of some bacterial cells was observed. The most abundant mineral phase associated with the bacteria was a complex (Fe,Al) silicate with a variable composition. Energy-dispersive X-ray spectroscopy suggested that the gel-like structures were similar in composition to a chamositic clay, whereas the crystalline phases were increasingly kaolinitic. Bacteria collected from the solute-deficient waters of the Rio Negro remained conspicuously unmineralized, suggesting that metal sorption and biomineralization largely reflect the availability of dissolved metals in the water column. In a solute-rich river system, the fate of the metal-loaded bacteria has profound implications for the transfer of metals from the hydrosphere to the sediment. Through diagenesis, the bound metals may either be recycled to the overlying water column or become immobilized as stable mineral phases. If the latter occurs, microorganisms will have played an important role in metal deposition, low-temperature clay formation, and, invariably, mudstone diagenesis. This implies that in the geologic record, microorganisms may have been instrumental in the genesis of many sedimentary rocks of fluvial origin.

INTRODUCTION

Within most freshwater systems, the typical concentration of dissolved metals is extremely low. Microorganisms such as bacteria, however, have the ability to adsorb and concentrate metallic ions from solution. Experimental work by Beveridge (1978), Beveridge and Murray (1976), and Mullen et al. (1989) has demonstrated that substantial quantities of several metals can be bound and accumulated by bacterial cells at their surfaces. The fixation of metallic ions to bacteria arises through interaction with the anionic surfaces of the cell wall at circumneutral pH (Beveridge and Fyfe, 1985). Constituent carboxyl and phosphoryl groups interact passively with available cations and have been shown to be major sites for metal deposition (Beveridge and Murray, 1980; Ferris and Beveridge, 1984). In addition, some cells may have an extracellular sheath or capsule composed of acidic polysaccharides whose molecular components are similarly reactive and consequently accumulate metals around the cell (Ferris and Beveridge, 1985). Once bound to the bacteria, the metals seem to serve as nucleation sites for the formation and growth of authigenic mineral phases. Bacterial cells promote phosphate mineralization in laboratory simulations of sediment diagenesis (Beveridge et al., 1983) and the precipitation of both metal

sulfides and complex (Fe,Al) silicates in natural environments (Ferris et al., 1987).

The ability of microorganisms to undergo chemical exchanges with their aqueous environment, involving both metal sorption and authigenic mineral precipitation, has largely been overlooked as an important factor in determining the chemistry of world rivers. The paucity of information on this topic is particularly apparent in the Amazon Basin where research has been limited. We have previously described the complexation of various dissolved metals to both unicellular (Konhauser et al., 1992) and filamentous eucaryotic algae (Konhauser and

Fyfe, 1993) in the surface waters of Amazonian rivers. This study extends our earlier work by investigating the role of bacteria in both solute-rich and solute-deficient river systems.

In this study we have examined bacterial communities on various solid surfaces from two major river systems in the Amazon Basin, Brazil (Fig. 1). The Rio Solimões and the Rio Negro combine (near the city of Manaus) to form the Lower Amazon, which in volume of discharge is the world's largest river (Gibbs, 1967). The Rio Solimões, also known as the Upper Amazon, is rich in dissolved solutes (relative to other Amazonian rivers, Konhauser, 1993) and is extremely turbid, owing to the high concentration of suspended sediment. In contrast, the Rio Negro, volumetrically the largest tributary of the Amazon, is an extremely infertile river (Konhauser, 1993) characterized by low suspended sediment and tea-colored, acidic waters rich in dissolved humic material (Ertel et al., 1986).

METHODS

Benthic microorganisms, growing in dense mats on various substrates below the water surface, were collected upstream of Manaus on the Rio Negro (and some of its tributaries) and on the Rio Solimões. Sections of biofilms were scraped (with a sterile scalpel) off both filamentous algae (freshwater seaweed), which were attached to submerged twigs, and surface sediment, which was collected from the top several centime-

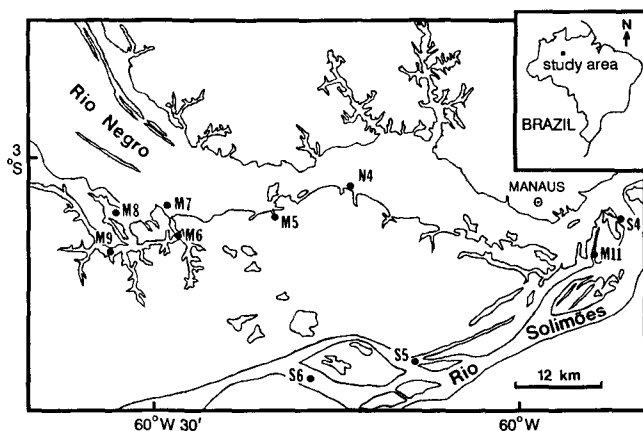


Figure 1. Location of study area and sample sites (large black dots).

tres of the river bottom by divers, at depths of ~5 m below the water surface. Immediately after the biofilms were removed from the water column and sediment-water interface, portions were placed in 5 mL metal-free plastic tubes containing aqueous 2.0% (vol/vol) glutaraldehyde, a fixative for electron microscopy.

The biofilms were prepared for thin sectioning by washing in a solution of 0.05M N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) buffer (Sigma Chemical Company, St. Louis) adjusted to pH 7.2 to remove excess glutaraldehyde, dehydrating through a graded acetone series, and em-

bedding in epoxy resin (Epon 812, CanEM, Guelph). Thin sections, ~60 nm in thickness, were obtained with a Reichert-Jung Ultracut E ultramicrotome and mounted on Formvar and carbon-coated 200-mesh copper grids. To increase the electron contrast of cytoplasmic material inside intact cells, some thin sections were stained with uranyl acetate and lead citrate before imaging in the electron microscope.

Electron microscopy was done at 100 keV in a Philips EM400T transmission electron microscope-scanning transmission electron microscope (TEM-STEM) equipped with a Link Analytical energy-dispersive X-ray

spectrometer (EDS). Analysis was conducted with electron-beam spot sizes of 400 nm and a beam current of 0.1 μ A. Spectra were obtained by collecting counts for 100 s (livetime). Crystalline mineral phases were determined by using selected-area electron diffraction (SAED) with a camera length of 800 mm. Amorphous phases were identified by EDS spot analyses run with the Link quantification program to determine stoichiometric ratios.

RESULTS

TEM analyses indicated that the biofilms growing on filamentous algae and surface sediment (from both river systems) served as substrates for epiphytic (growing on plants and microorganisms) and episammic (growing on sediment) bacterial communities, respectively. Epiphytic bacterial cells from the Rio Solimões showed abundant mineralization, ranging from Fe-rich capsules to fine-grained (<500 nm) authigenic mineral precipitates. Many of the sample cells exhibited capsules, which varied greatly in their proportions; some encapsulated only an individual cell (Fig. 2A), but other microorganisms produced so much extracellular material that several cells became encapsulated to produce a "microcolony" (Costerton et al., 1981). EDS analyses indicated that these surfaces had sequestered significant amounts of Fe from solution, with lesser amounts of K, Ti, Mn, and Ni (Fig. 3). The accumulation of trace metals such as Mn and Ni, which are found in extremely low aqueous concentrations (Table 1), suggests a selective accumulation by the cell wall. Conversely, Ca, which is the most abundant dissolved cation, is not complexed in detectable amounts, implying that transition metals are chelated more efficiently (Ferris et al., 1989b).

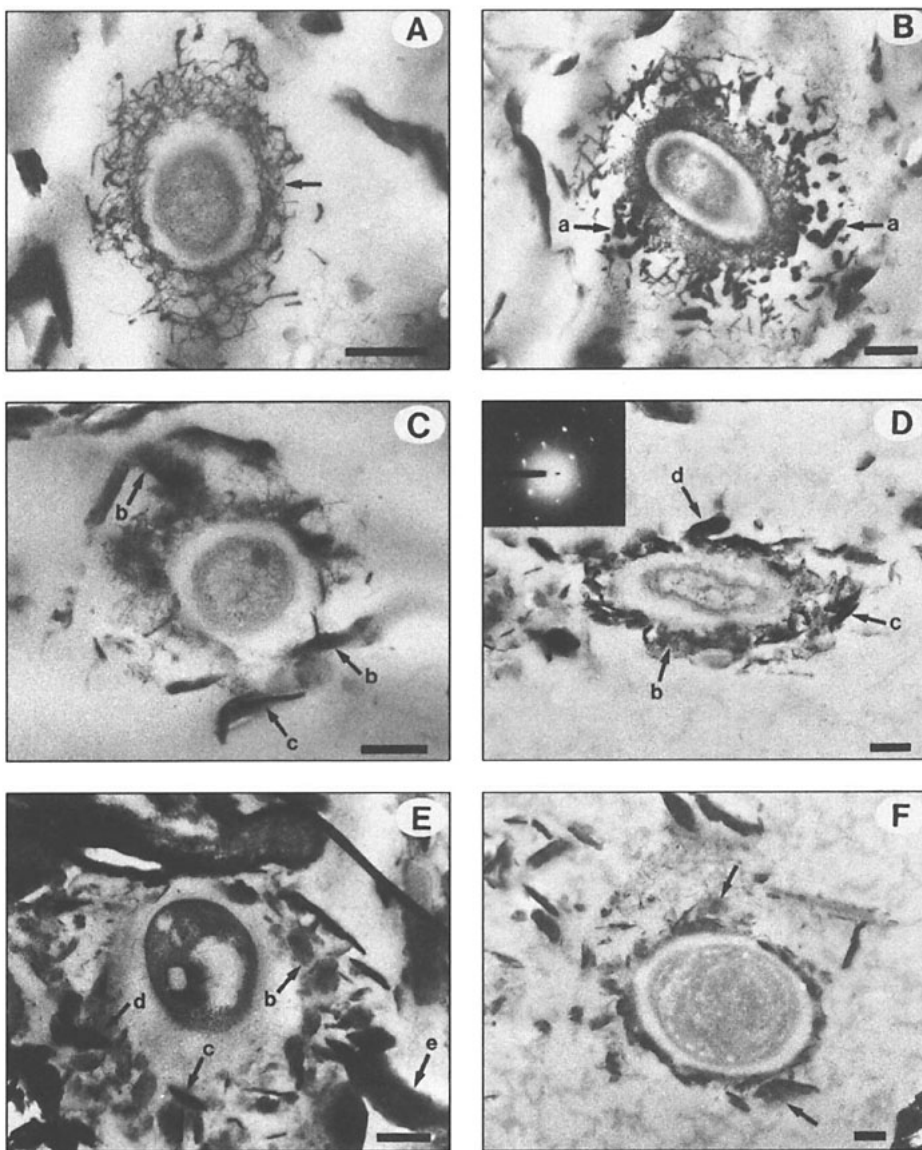


Figure 2. Transmission electron micrographs of bacteria (stained) from Rio Solimões. Scale bars represent 400 nm. A: Epiphytic bacterial cell with Fe-mineralized capsule (arrow). B: Fe-mineralized capsule with formation of dense Fe-rich aggregates (a). C: Partially encrusted cell with gel-like phase (b) and transition phase (c). D: Completely encrusted cell with gel-like phase (b), transition phase (c), and crystalline phase (d) with SAED pattern. E: Bacterial cell in sediment with mineralized capsule, gel-like phase (b), transition phase (c), crystalline phase (d), and large, highly siliceous grains (e). F: Epiphytic cyanobacterial cell with gel-like phase on outer cell wall (arrows).

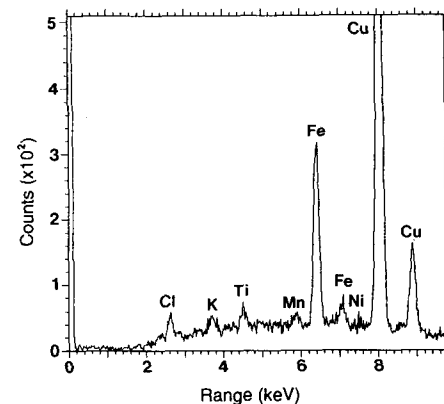


Figure 3. EDS spectrum of mineralized capsule from epiphytic bacterial cell from Rio Solimões. Cu peaks are from supporting grid; Cl peak is due to contamination from epoxy resin.

TABLE 1. AVERAGE CONCENTRATION OF DISSOLVED METALS IN AMAZONIAN RIVERS

| | Rio Negro | Rio Solimões |
|------------------|-----------|--------------|
| SiO ₂ | 4000.0 | 11500.0 |
| Aluminum | 181.3 | 1017.6 |
| Iron | 385.0 | 1796.3 |
| Magnesium | 128.5 | 1262.5 |
| Potassium | 463.8 | 1400.0 |
| Calcium | 391.3 | 8825.0 |
| Titanium | 0.7 | 7.4 |
| Manganese | 9.6 | 50.1 |
| Nickel | 0.3 | 0.7 |
| Copper | 0.3 | 2.4 |
| TDS | 3500.0 | 20000.0 |
| pH | 5.0 | 6.6 |

Note: Values (μg/l) from Konhauser (1993). TDS = total dissolved inorganic solids.

In the same samples, other epiphytic bacterial cells showed the nucleation of small (~100 nm in diameter), dense aggregates on the outer surfaces of the capsule (Fig. 2B). Predominantly Fe-rich, these aggregates suggested an early stage of mineralization within the metal-loaded capsule, as represented in Figure 2A.

Progressive mineralization, leading to the partial (Fig. 2C) and complete (Fig. 2D) encrustation of some bacterial cells, was also observed. The authigenic grains exhibited a wide range of morphologies, from amorphous "gel-like" to crystalline structures. EDS analyses indicated that the most abundant mineral phase associated with epiphytic bacteria (in the Rio Solimões) was a complex (Fe,Al) silicate with a variable composition. With the exception of K, no other metals were detected in association with the authigenic mineral phases. A ternary plot of Fe, Si, and Al (on an atomic percent basis), with the position of various clay minerals labeled (Fig. 4), shows that the gel-like structures were similar in composition to a chamositic clay [(Fe₅Al)(Si₃Al)O₁₀(OH)₂], whereas the crystalline phases were increasingly kaolinitic [Al₄(Si₄O₁₀)(OH)₄]. Intermediate compositions, corresponding to the transition from a low-order state to a high-order state, were also identified. SAED patterns generated on the samples with good crystallinity indicated a hexagonal crystal habit (i.e., normal to *c* axis) with *d*-spacings of 2.56 and 4.58 Å. The 001 *d*-spacings for the crystalline material were not detectable; therefore, a precise mineralogical identification was not possible.

Variable (Fe,Al) silicates were also evident in the bacterial communities on the surface sediment. Commonly, large (1.5 μm) mineral grains were identified in association with episammic bacterial cells (Fig. 2E) or their remains (some of the lysed cells may also have originated in the overlying water

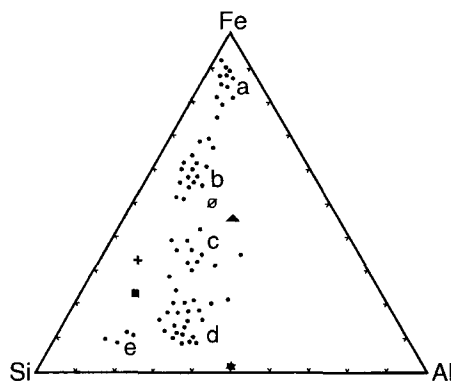


Figure 4. Distribution of Fe, Al, and Si (on an atomic percent basis) in (a) dense aggregates, (b) gel-like phases, (c) transition phases, (d) crystalline phases in epiphytic bacteria, and (e) siliceous grains in episammic bacteria. Fe, Al, and Si contents are indicated for several "ideal" clay minerals, including chamosite (slashed circle), kaolinite (star), nontronite (plus sign), glauconite (square), and berthierine (triangle).

column). EDS analyses indicated that many of these grains were extremely siliceous in composition (Fig. 4). Although some cells have detrital material (e.g., kaolinite) adsorbed on their outer surfaces, the siliceous grains shown in Figure 2E appear to be authigenic, because they differ mineralogically from the suspended sediment of the Rio Solimões, composed of quartz, kaolinite, feldspars, smectite, illite, and chlorite (Konhauser, 1993).

The precipitation of (Fe,Al) silicates was not restricted to cells with encapsulating material. Colonies of epiphytic cyanobacteria (identified by their intracytoplasmic membranes), which were found attached to a larger eucaryotic algal cell, showed minerals precipitated directly on the outer surface of the cell wall (Fig. 2F). Similar to that observed with the epiphytic bacteria, a complete range of (Fe,Si,Al) compositions and morphologies was identified; grain sizes were typically larger (1 μm). This may reflect a greater efficiency of mineral precipitation, because cations do not have to diffuse through a capsule.

In contrast to the prolific mineralization by bacteria in the Rio Solimões, epiphytic and episammic cells (with or without capsules) collected from the Rio Negro remained unmineralized (Konhauser, 1993). Also, compared to the Rio Solimões, the abundance of bacteria on biological and/or abiological substrates was much less.

DISCUSSION

The role of bacteria in forming authigenic minerals involves a complex interaction between metals in solution with the reactive components of the cell. The anionically

charged cell wall and the encompassing layers clearly serve as special microenvironments for the deposition of Fe and other soluble cationic species. Ferric iron, which exhibits unstable aqueous chemistries, was bound in significant amounts, and previous studies indicate that such binding may be sufficient to induce transformations to the insoluble hydroxide form (e.g., [Fe(OH)₃]) (Ferris et al., 1989a). This corresponds closely to the dense, Fe-rich aggregates formed on the outer surfaces of the epiphytic bacteria sampled from the Rio Solimões.

Through progressive mineralization, these Fe-rich aggregates served as nucleation sites for the precipitation and growth of the complex (Fe,Al) silicates. It is likely that the initial (Fe,Al) silicate phases may have been precipitated directly by the reaction of dissolved Si and Al to the bound metallic cations, a process that inhibits the conversion of the Fe hydroxides (e.g., ferrihydrite) to more stable Fe oxide forms (e.g., goethite) (Carlson and Schwertmann, 1981). Continued aggregation of these hydrous precursors resulted in the formation of low-order, "gel-like" phases (Ferris et al., 1987), which are characterized by large surface areas with a high adsorptive affinity for additional metal cations (Wada, 1981).

Because they lack a regular crystal structure, these hydrous compounds are unstable and will, over time, dehydrate and convert to more stable crystalline forms. Ferris et al. (1987) found that an increasing incorporation of Fe (in a metal-contaminated lake sediment) accompanied the conversion of the low-order (Fe,Al) silicates into a crystalline form of chamosite. In our study area, the hydrous chamosite-like clay appeared especially reactive to silicic acid, and continued adsorption of Si accompanied the solid-state transformation from the low-order phase to the siliceous, crystalline phase. Eventually this process led to the complete encrustation of the bacterial cell within a mineral matrix rich in Fe + Si. In our opinion these crystalline phases represent single mineral compositions, not intimate mixtures of chamosite and kaolinite. Although these mineralized grains likely do not represent intercalated clays, a certain amount of elemental zoning is expected, with the Fe initially bound as ferrihydrite on the cell wall and Si and Al bound to the ferrihydrite, outward from the cell. Such zoning, however, is not detectable by EDS owing to the small size of the mineral precipitates (<500 nm).

With the final stages of mineral formation inorganically driven, the preferential accumulation of Si may reflect the higher concentration of dissolved Si to dissolved Fe (Table 1), as well as the different behavior of

these two elements in the aqueous system. In addition, the water analyses indicate that the Rio Solimões is close to quartz saturation (6 ppm at 25 °C; Fournier, 1960) and, therefore, at the low end for clay formation in terms of silica activity. This finding suggests that other factors, such as redox and pH conditions proximal to the bacteria, could influence the authigenic mineral reactions.

We were interested to discover that all bacterial populations examined in the Rio Solimões, regardless of their physiology, consistently formed identical mineral phases. This implies that the biomineralization was a surface process associated with the anionic nature of bacterial cell walls (Beveridge and Fyfe, 1985). Although all of the bacteria from our sampling area were capable of serving as passive nucleation sites for (Fe,Al) silicate precipitation, bacteria from the Rio Negro showed a conspicuous absence of mineralization. This discrepancy further suggests that the differences in mineral accumulation exhibited by bacterial cells in the Rio Solimões and the Rio Negro must reflect differences in the physical and chemical conditions of their riverine environments. The Rio Solimões is a fertile river, rich in suspended sediments and dissolved inorganic solutes (Table 1). As a result, the bacteria had an abundant supply of metals in solution to complex with and to accumulate. Conversely, because of the low levels of dissolved metals in the Rio Negro (Table 1), the bacteria were presumably able to bind only enough metals to fulfill physiological requirements, not the large quantities of metals necessary to form the authigenic mineral phases. These findings indicate that both metal sorption and biomineralization reflect the availability of dissolved solutes in the water column.

The absence of mineralization by bacteria in the Rio Negro may also be partially due to competition, for the few available cations, with the high concentration of dissolved organic carbon (DOC) in the river (Ertel et al., 1986). With higher DOC than total dissolved inorganic solids (TDS), sufficient reactive sites exist for the adsorption of both major cations and trace metals (Konhauser, 1993). In addition, organic compounds (e.g., humic acid) substantially perturb the interactions of Al ions with silicic acid and thus further inhibit the formation of aluminosilicate phases (Huang, 1991).

In a solute-rich river system such as the Rio Solimões, the fate of the metal-loaded bacteria may have profound implications for the transfer of metals from the hydrosphere to the sediment (Beveridge et al., 1983). Given that planktonic bacterial populations

in the Rio Solimões are typically on the order of 1 to 4 × 10⁶ cells/mL (Wissmar et al., 1981), in addition to the unknown benthic populations, it is not difficult to imagine that these microorganisms could effectively cleanse the water of dilute metals and partition them into the sediments (Beveridge and Fyfe, 1985). As the epiphytic cells lyse and settle to the sediment-water interface, or as the episammic cells lyse in situ, the organo-metallic complexes may undergo a series of microbial or chemical transformations. Through diagenesis, the bound metals may either be recycled to the overlying water column or become immobilized as stable mineral phases. If the latter occurs, it is reasonable to expect that during diagenesis in an oxic environment, microdeposits of (Fe,Al) silicates would develop. From a geological standpoint then, it is clear that microorganisms, such as bacteria, play an important role in metal deposition, low-temperature clay formation, and, invariably, mudstone diagenesis. This implies that in the rock record, microorganisms may have been instrumental in the genesis of many sedimentary rocks of fluvial origin.

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