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MICROBIAL PRECIPITATION OF SIDERITE AND PROTOFERRIHYDRITE IN A BIOFILM

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ABSTRACT

The specimens from a microbial biofilm formed on the wall of an excavated granite vault in a deep underground laboratory were examined by ^{57}Fe Mössbauer spectroscopy. In the aerobic face of the biofilm, iron was found in a form of protoferrihydrite, whereas in the anaerobic face at the rock–biofilm boundary, it was found as very fine particles of siderite, typically 2–3 nm in size. The same iron compounds were formed in aerobically and anaerobically cultured samples of the biofilm. To the best of our knowledge, this is the first clear experimental evidence of biogeochemically formed siderite and ferrihydrite in the natural environment. Finding microbial consortia capable of precipitation of Fe^{2+} and Fe^{3+} in close proximity may have a bearing on the development of early forms of life and, in particular, on the deposition of banded iron-formations.

Keywords: biofilm, biomineralization, ^{57}Fe Mössbauer spectroscopy, protoferrihydrite, siderite, banded iron-formations.

SOMMAIRE

Nous avons examiné par spectroscopie ^{57}Fe de Mössbauer une biopellicule microbienne prélevée de la paroi d'une gallerie souterraine profonde servant de laboratoire et creusée dans un massif granitique. Dans la partie aérobie de la pellicule, le fer se trouve sous forme de protoferrihydrite, tandis que dans sa partie anaérobie, à l'interface avec le roc, nous décelons des particules infimes de sidérite, typiquement d'une taille de 2 à 3 nm. Les mêmes composés de fer ont été formés dans des cultures aérobies et anaérobies de la biopellicule microbienne. A notre avis, il s'agit de la première mise en évidence de la formation biogéochimique de la sidérite et de la ferrihydrite dans un milieu naturel. La découverte de colonies microbiennes susceptibles de précipiter de façon contiguë des composés de Fe^{2+} et de Fe^{3+} pourrait bien avoir un lien avec le développement de formes précoces de la vie sur terre et, tout particulièrement, la déposition de formations de fer rubanées.

(Traduit par la Rédaction)

Mots-clés: biopellicule, biominéralisation, spectroscopie ^{57}Fe de Mössbauer, protoferrihydrite, sidérite, formations de fer rubanées.

INTRODUCTION

Biofilms are part of a microbial strategy for survival in nutrient-poor groundwaters, such as those of the Canadian Shield. In this environment, bacteria preferentially adhere to solid surfaces, where nutrients tend to concentrate. As more bacteria accumulate, a biofilm is formed; this biofilm matures with the growth of microcolonies and the secretion of extracellular polymers. The internal concentration-gradients of nutrients and waste products influence the diversity and spatial distribution of the metabolic activity of the microbial population, producing structured physiological relationships, which contain a number of micro-environments with different fields of stability. Biofilms are considered to provide the chemical conditions and the nucleation sites necessary for mineral formation. Microbial cell-walls and the associated exopolymers are negatively charged, allowing the bacteria to sorb and bind metal ions from the surrounding media (Beveridge 1989). The metal sorption in some cases is so great that precipitates are formed, which in time may develop into sedimentary formations. The precipitation of calcite and gypsum in a fresh-water lake has been shown to be influenced by a symmetrical paracrystalline surface-array of the extracellular

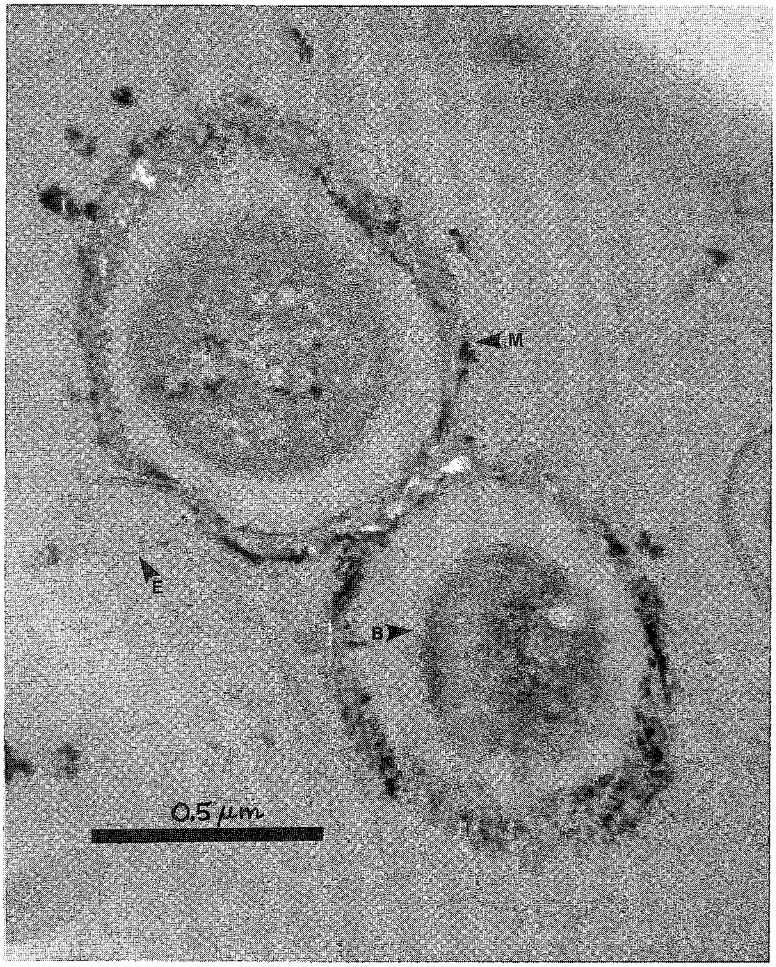

FIG. 1. Electron micrograph of the thin section (bar: 0.5 μm) showing biofilm bacteria (B) with extensive metal precipitates (M) at the extracellular matrix (E). The specimen was viewed in a Philips EM400T at 100 kV equipped with an energy-dispersion X-ray spectrometer (Link Analytical, U.K.). The energy-dispersion spectroscopy (EDS) was conducted using spot sizes of the electron beam of 2.0 μm or less, and counts were collected for 300 s (live time). Analysis by EDS indicates that iron and silicon are the main elements concentrated in the biofilm.

matrix (Schultze-Lam *et al.* 1992). Metals and inorganic compounds, notably iron and sulfate, also may be exploited by bacteria as sources of energy for growth.

EXPERIMENTAL

We have examined a biofilm that formed spontaneously in AECL's Underground Research Laboratory (URL), situated in the Lac du Bonnet Batholith, within the tectonic Superior Province, in Manitoba. A large borehole, drilled on the 420-m level through the granite floor, became fractured by stress-release during excavation. The biofilm occurred shortly after the hole was drilled, and developed rapidly (four to six weeks), eventually covering the whole of the wet surface, about 20% of the total area, up to a thickness of 10 mm. Samples for analysis by Mössbauer spectroscopy and X-ray diffraction were obtained both from the biofilm, which was rapidly freeze-dried to prevent alteration of the iron minerals, and from laboratory cultures grown using ferric ammonium citrate as substrate. No attempt was made to identify the organisms. Aerobically, the bioreactors produced a brown-colored sludge, whereas anaerobically a black-colored sludge was obtained. There was no coloration or sludge production in sterile reactors, confirming that these were biologically mediated products.

Electron micrographs of thin sections of the biofilm fixed in glutaraldehyde (Fig. 1), combined with energy-dispersion X-ray spectroscopy (EDS), indicated that iron is embedded in a thin electron-dense layer surrounding individual bacteria, in similar fashion to that described for the deposition of gypsum and calcite (Schultze-Lam *et al.* 1992). The X-ray diffractograms of aerobic brown samples indicated only two very broad diffraction-maxima, at the positions consistent with those previously reported for poorly crystalline hydrated ferric oxyhydroxide, protoferrihydrite ($\sim Fe_5HO_8 \cdot 4H_2O$) (Murad & Johnston 1984). The diffractograms of anaerobic specimens show distinct lines of siderite (Fig. 2). At room temperature, the Mössbauer spectrum of this specimen (Fig. 3A) indicates the presence of ferric iron, Fe^{3+}, represented by a quadrupole-split doublet with the isomer shift δ = 0.35 mm/s, and quadrupole splitting, Δ = 0.8 mm/s, consistent with the spectral parameters of protoferrihydrite. A thermal scanning of the resonant absorption of the 14.4 keV γ-ray showed a gradual onset of magnetic order between 30 and 10 K. The spectra taken at 4.2 K (Figs. 4A, B) show a broadened sextet described by a hyperfine field of 45 T with a spread of 2 T, typical for extremely poorly crystallized specimens of protoferrihydrite (Murad 1988, Pankhurst & Pollard 1992), and similar to that observed, for instance, in deep-sea iron–manganese nodules (Hrynkiewicz *et al.* 1970) and in a "ferric gel" deposited near freshwater springs (Coey & Readman 1973). Protoferrihydrite forms spherically shaped particles 2–7 nm in diameter having a defect hematite structure, and it can age directly to hematite by loss of water and coalescence of small spherical particles to larger

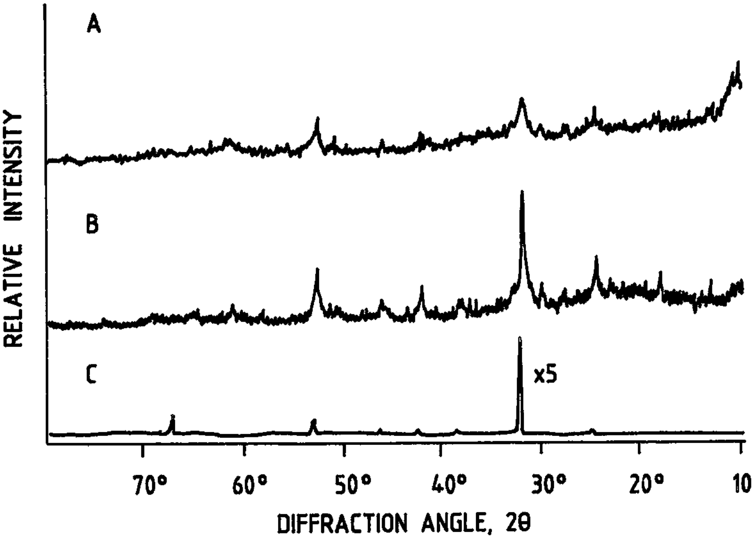

FIG. 2. Cu$K\alpha_1$ X-ray diffractograms of (A) freeze-dried biofilm, (B) cultured black specimen, and (C) natural siderite. The reference specimen consists of crystals of siderite from the Michipicoten mine, Ontario, kindly given to us by Dr. J.A. Mandarino of the Royal Ontario Museum, Toronto, Ontario (ROM no. 11824).

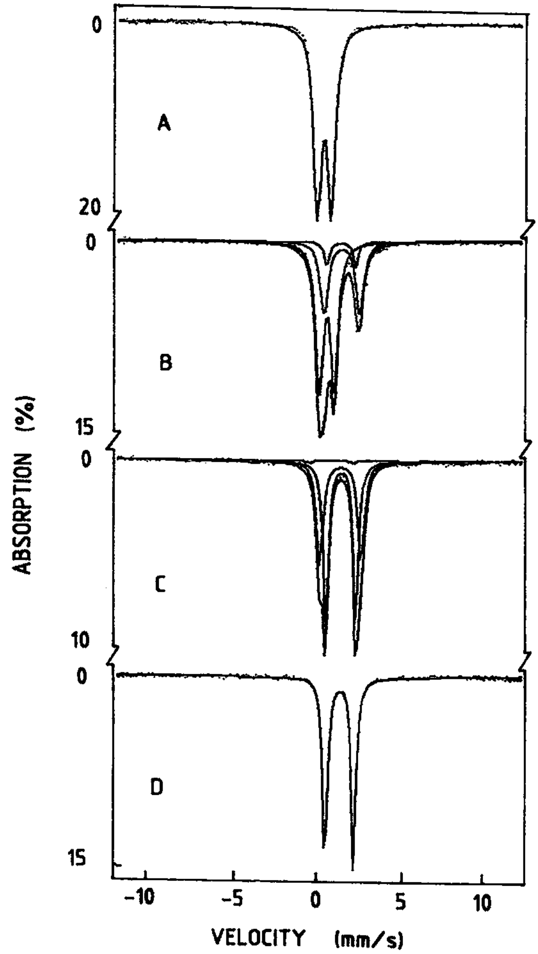

FIG. 3. Mössbauer spectra of 14.4 keV γ-rays of ^{57}Fe taken at 295 K using a 30 mCi ^{57}Co source in Rh matrix and a Kr–CO_2 proportional counter. (A) The aerobic (brown) face of the biofilm shows a pattern typical of protoferrihydrite; (B) the anaerobic (black) face of the biofilm shows both protoferrihydrite and siderite; (C) the cultured (black) sludge shows mostly siderite; (D) reference siderite taken from the banded iron-formations of the Michipicoten mine.

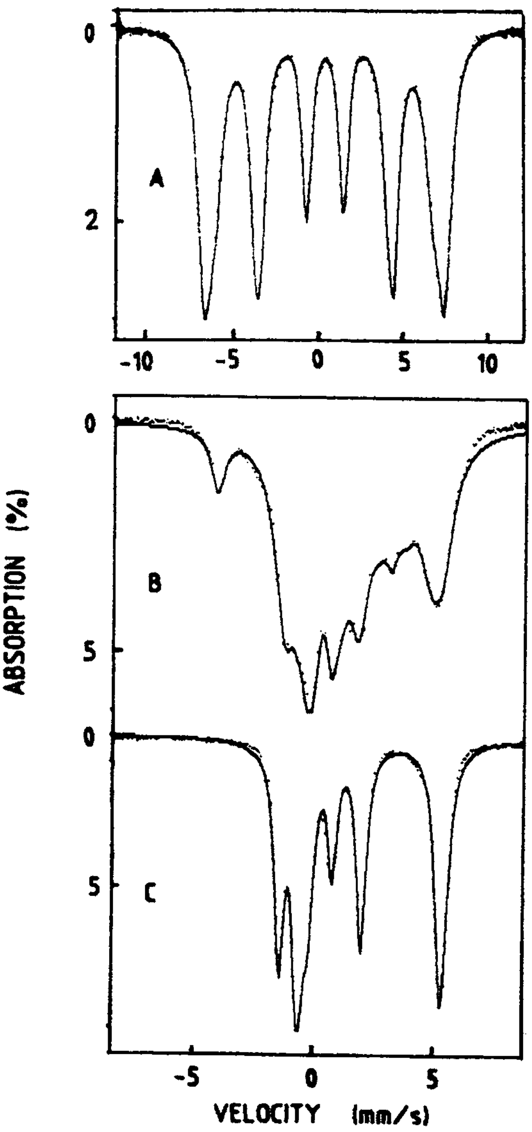

FIG. 4. Mössbauer spectra taken at 4.2 K. (A) Brown sludge, (B) black sludge, and (C) natural siderite.

hexagonal particles of hematite (Murad & Johnston 1984). Accordingly, we have observed a small signal, similar to that of fine-grained hematite, in several specimens left open to the moist air for a long period of time. The appearence of a small portion of fine hematite particles in some of our specimens could be also attributed to a loss of water and an increase in degree of crystallinity due to the process of freeze-drying, but no systematic studies have been performed by us to evaluate this possibility.

At 295 K, the Mössbauer spectra of the black fragments of the biofilm (Fig. 3B) and black sludge from the bioreactor (Fig. 3C) show two broad absorption lines with an isomer shift and a large quadrupole splitting characteristic of ferrous iron. The best fit was to two quadrupole doublets with almost equal intensities. The parameters of the first doublet (δ = 1.22 mm/s, Δ = 1.79 mm/s) match those of siderite, $FeCO_3$ (δ = 1.24 mm/s, Δ = 1.80 mm/s) (Ôno & Ito 1964), whereas those of the second doublet

(δ = 1.20 mm/s, Δ = 2.39 mm/s) indicate ionic Fe^{2+}; however, the host compound cannot be identified. At 4.2 K, the spectrum of the black sludge indicates a pattern of superposed broad lines that can be analyzed in terms of two components, one with parameters H = 166 kOe and Δ = +1.91 mm/s, which corresponds to the antiferromagnetic state of siderite (T_N = 38 K) (Ok 1969, Forester & Koon 1969), and the other, less intense, indicating a larger hyperfine magnetic splitting, H = 223 kOe, and a large negative quadrupole splitting, Δ = –3.62 mm/s (Fig. 4C). The origin of this second component is not clear, but as studies with other bacterial systems have shown that there is initiation of mineral formation by ionic adsorption of metal to a bacterial surface (Schultze-Lam *et al.* 1992), we tentatively attribute this component to ferrous ions held in a loose attachment by negatively charged biological polymers.

The size of the siderite crystallites was determined from the broadening of the XRD peaks (Figs. 2A, B) to be 2 to 3 nm, comparable in size to that of the iron precipitate around the bacteria. This small size of the particles suggests that the siderite is most likely attached to a surface, probably that of a bacterial polymer. The large broadening of the Mössbauer absorption lines in spectrum C in Figure 4, as compared to the reference spectrum of siderite (Fig. 4D), can be attributed partly to the small size of the crystallites and partly to a slow electron-spin – lattice relaxation (Ok 1969). We have also compared the temperature dependence of the Mössbauer spectra of the black sludge and siderite between 4.2 and 295 K. The collapse of the magnetic hyperfine splitting in the sludge was observed at about 30 K, a somewhat lower temperature than that in crystalline siderite. The temperature dependence of the quadrupole splitting for the siderite-like fraction of the sludge was found to be very similar to that of the reference specimen of siderite.

DISCUSSION

The discovery of siderite in the biofilm was unexpected, as most black iron precipitates in the natural environment have been thought until recently to be sulfides, produced from the bacterial reduction of sulfate. However, Coleman *et al.* (1993) and Roden & Lovley (1993) have recently shown that bacteria can enzymatically reduce Fe^{3+} directly to form siderite. The biofilm described here clearly demonstrates that a bacterial community is capable of mediating both the oxidation and the reduction of iron in close proximity, so that hematite and siderite can be biogeochemically formed and immobilized simultaneously. The deep groundwater of the Canadian Shield is reducing and contains iron as Fe^{2+}, which is oxidized in the open excavation of the URL by bacteria at the aerobic face of the biofilm; the Fe^{3+} produced is then either precipitated as protoferrihydrite maturing to hematite, or reduced, by other bacteria, at the anaerobic face next to the rock, back to Fe^{2+}; this, in turn, either binds to the electronegative biopolymers of the biofilm, or reacts with carbon dioxide (as HCO_3^-) from the fermentation of organic matter.

There has been speculation for many years on the mechanisms involved in the deposition of banded iron-formations (BIF) of the Archean (Cloud 1983). It has been suggested (LaBerge *et al.* 1987) that iron-bearing minerals, originating mainly as fine-grained biological ferric hydrate precipitates, mature to ferrihydrite and hematite, while at the same time the degradation of organic matter in anoxic sediments generates Fe^{2+}, which was precipitated as siderite, altering later to magnetite. These ferric and ferrous iron minerals were assumed to coexist in metastable equilibrium in sedimentary environments. Although an early study concluded that the reduction of Fe^{3+} was not enzymatic (Starky & Halvorsen 1927), there is now considerable evidence that in the natural environment, this reduction is generally bacterially mediated (Lovley 1991). Bacteria requiring a reduced form of iron for nutrition were first reported by S. Winogradsky [as referenced by Harder (1919, p. 11)], and the recent observation of oxidation of Fe^{2+} to Fe^{3+} by purple, non-sulfur bacteria (Widdel *et al.* 1993) has explained how Fe^{2+} could have been oxidized in the anoxic Archean.

Our investigation of the biofilm tempts us to suggest that the deposition of BIF was microbially mediated. The problem concerning Archean BIF has been to explain how both oxidized and reduced forms of iron could have been deposited in such narrow bands when their stability fields are so different. Our observation of an active biofilm shows that microbial consortia are capable of producing discrete microenvironments, enabling the formation of hematite and siderite to occur within very narrow spatial limits. The microbially mediated precipitation of the oxidized and reduced compounds of iron could thus have produced the correct stability-fields for their deposition in BIF. If similar microbial consortia were present during the Precambrian era, narrow oxidized beds intercalated with beds where the iron is reduced may have been laid down in a microbial mat in shallow seas. The existence of micro-organisms capable of immobilizing large quantities of iron and carbon dioxide could also have a bearing on climatic changes.

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