MINERALOGICAL BIOSIGNATURES IN BIOFILMS: A LABORATORY STUDY OF THEIR FORMATION AND CHARACTERISTICS

Final Report

JPL Task 1046

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A. OBJECTIVES

Overall Goal: To characterize mineralogical biosignatures of bacteria that interact with Fe-bearing biominerals in the context of life-detection studies on Mars.

Specific Objectives of This Study

- To explore the formation of bacterial-mineral aggregates in biofilms growing on Fe substrates.
- To investigate whether distinct types of minerals are distinguishable in a mixed-species biofilm, and whether these types are traceable to the metabolic type of the species that caused their formation.

B. PROGRESS AND RESULTS

A biofilm in its simplest form is a consortium of microbial cells within a gel-like matrix of extracellular polymers (EPS) formed by the cells. At its most complex, it is a multi-species entity best described as a microbial community. It consists not only of living and dead cells, but parts of cells, extracellular polymers, metabolic by-products, and minerals. The degree of mineralization can vary markedly, and where the proportion of mineral is high in relation to the organic material, the entity is often known as a microbialite (Douglas and Beveridge, 1998). Microbialites, over geological time, become lithified and pass into the geological record as structures referred to as stromatolites, within which the microbial cells can be preserved as microfossils. The formation of microfossils has been studied, especially for silicious and carbonaceous mineralogical matrices. However, the formation of microfossils in metal oxide deposits has been little studied. Yet, in metal oxides, microbial communities also exist and mediate the deposition of minerals. Can this activity lead to the deposition of mineralogical biosignatures which will persist in the geological record of Earth and possibly Mars?

We were interested in finding out if the metabolic influence of bacteria could be reflected in the types of minerals deposited in a dual-species biofilm in the laboratory. The resulting data was compared to that from natural biofilms formed on similar surfaces (steel) immersed in natural marine water.

The main premise of our study was that not only the elemental composition of a mineral but also its form could serve as a biosignature. One of the most well-studied biogenic mineral classes is

that of Fe-containing minerals, especially the sulfides and the oxides/oxy-hydroxides, a class of minerals which also appears to be widespread on Mars. In order to test this hypothesis, and also to gain some insight into the phenomenon of mineralization in mixed-species microbial biofilms or microbial communities, we grew two types of bacteria as biofilms on steel, first separately and then together. These were *Desulfovibrio desulfuricans* G20, a sulfate-reducing bacterium, and *Shewanella oneidensis* MR1, a Fe-reducing bacterial species. Both are natural inhabitants of soils and groundwaters.

Since Fe is known to be plentiful in the Martian regoltih, it is important to add knowledge of Fe biominerals -- their formation, morphology, and depositional patterns -- to our expanding database of mineralogical biosignatures in order to help guide future efforts in extraterrestrial "bioprospecting."

One of the best systems for studying this is that of steel corrosion. Natural passivation of the steel surface results in the oxidation of Fe metal to Fe^{3+}-containing oxides, which are present as a ferric-oxide coating on the steel surface, generally as magnetite (Fe_3O_4) and haematite (Fe_2O_3). The ferric oxyhydroxides lepidocrocite ($\gamma FeO(OH)$) and ferrihydrite ($Fe(OH)_3$) have also been identified in these protective layers on carbon steel (Little et al., 1997) and thus serve as analogues for naturally present minerals.

When grown separately, each type of bacterium produced characteristic types of minerals, reflective of the metabolically-induced alterations in geochemical conditions brought about by the life processes of the bacteria. *Desulfovibrio desulfuricans* G20 is a sulfate-reducing bacterial (SRB) species. It gains energy by removing electrons from reduced organic molecules such as acetate and pyruvate, shunting them through an electron transport chain and using sulfate as the final electron acceptor according to the following general equation:

$$2(CH_2O) + SO_4^{2-} \longrightarrow H_2S + 2CO_2 + 2OH^-$$

H_2S quickly dissociates to $2H^+$ (protons) and S^{2-}(sulfide). The sulfide has a strong affinity for metal ions and, in our biofilms, complexed with Fe^{2+} released from the steel through bacterially-induced electrochemical processes. However, not enough Fe was released to complex all the available sulfur. As a result, some of the sulfide was partially re-oxidized, leading to the formation of colloidal sulfur spheres (Douglas and Douglas, 2000) in addition to the more abundant Fe-S precipitates formed (Figure 1 A,B,C). The hydroxyl ions released by the reaction likely associated with protons to form water.

MR1, the Fe-reducing bacterial species, uses Fe^{3+} in the oxide minerals on the steel surface as an electron acceptor for its electron transport chain, making the presence of oxidized Fe essential for energy production by the cells. This activity results in the formation of ferrous oxide minerals which are deposited as microcrystallites on the cell surfaces. This was seen in our samples (Figure 1, C and D) and served as an identifying feature of the MR1 cells in the mixed biofilm setting. The banana-shaped cells of G20 and their associated granular Fe-S and spherical S precipitates were also clearly distinguishable in the mixed biofilm. Thus we had morphological and chemical evidence for metabolic diversity as reflected in the characteristics of the minerals associated with the bacteria (Figure 2 A,B).

Environmental Scanning Electron Microscope (ESEM) images of natural marine biofilms growing on steel showed how complex a natural biofilm can be (Figure 2 C,D). The images show chains of cells of various shapes, covered in EPS and associated with minerals of similar appearance of to those in the laboratory biofilms. Energy-Dispersive X-ray Spectrometer (EDS)-analysis of these minerals showed that they were composed of essentially the same elements as those in the laboratory biofilms (data not shown).

C. SIGNIFICANCE OF RESULTS

This task developed a laboratory system for studying the effects of bacterial biofilms upon metal surfaces. In addition, we learned that specific mineral types formed by bacteria can be distinguished from one another and attributed to the metabolic type of bacterium which mediated their formation. Lastly, this study makes a contribution to our growing biosignature database, of which inorganic biosignatures are a vital part, as they have the greatest chance of maintaining a recognizable form over geological time periods.

D. FINANCIAL STATUS

The total funding for this task was $56,300 all of which has been expended.

E. PERSONNEL

No other personnel were involved.

F. PUBLICATIONS

[1] A.K. Lee, M. Buehler, and D.K. Newman. (in review) "Influence of a dual-species biofilm on the corrosion of mild steel" Journal of Corrosion Science.

[2] M. Buehler, A.K. Lee, and D.K. Newman. (in review) "A new electrochemical model used to analyze microbial corrosion" Journal of Corrosion Science.

G. REFERENCES

[1] B.J. Little, P.A. Wagner, and Z. Lewandowski. 1997. “Spatial relationships between bacteria and minerals. *Reviews in Mineralogy* 35:123-160.

[2] S. Douglas and D.D. Douglas. 2000. “ESEM studies of colloidal sulfur deposition in a natural microbial community from a cold sulfide spring near Ancaster, Ontario, Canada.” Geomicrobiology Journal 17:275-289.

[3] S. Douglas and T.J. Beveridge. 1998. “Mineral formation by bacteria in natural microbial communities.” FEMS Microbial Ecology FEMS Microbiology Ecology 26:79-88.

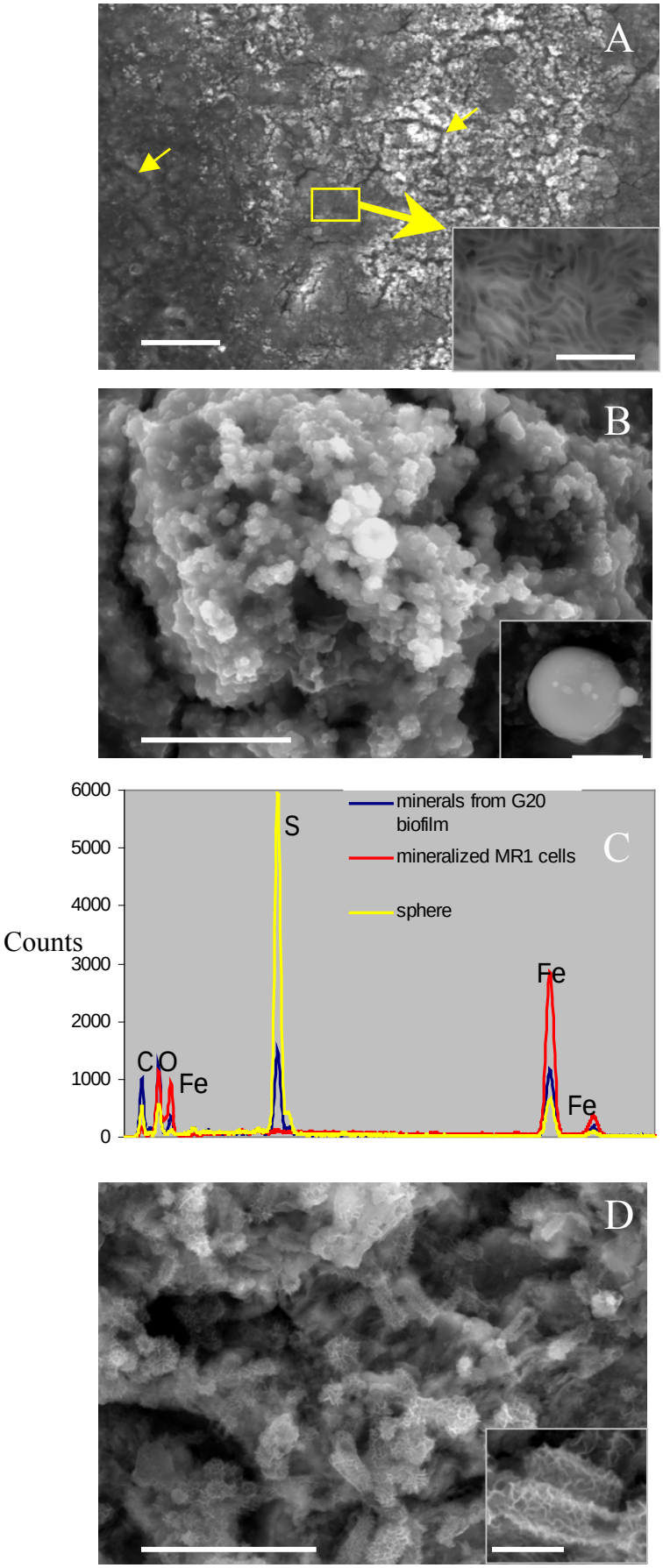

Figure 1: (A and B) ESEM images showing details of the *Desulfovibrio desulfuricans* G20 biofilm on steel and the minerals formed by the organisms. Image **(A)** gives an overview of the biofilm structure, showing the cell-EPS matrix (darker areas) and the mineral precipitates (light areas). Channels in the biofilm (arrows) are also visible and these are important for transfer of liquids/nutrients throughout the biofilm. The inset at bottom right gives a close up view of the closely packed, banana-shaped cells. Scale bars = 50 µm (main image) and 5 µm (inset). Image **(B)** gives a detailed view of the two types of mineral precipitates found in association with the G20 biofilms. The more prevalent clumped granular precipitate is composed of Fe and S in almost equal proportions and may coat individual cells, although it generally seems to be present as an amorphous form embedded within the EPS matrix. The spheres (inset) are composed of elemental sulfur and are not found in close association with cells, implying that they are abiotically precipitated. Scale bars = 10 µm (main image) and 2 µm (inset).
(C) Spectrum from Energy-Dispersive X-ray Spectrometer (EDS) showing the three main mineral types formed by the cells in the laboratory system. *Desulfovibrio desulfuricans* G20 formed Fe- and S-containing minerals (blue line) as a result of their release of H_2S and its reaction with Fe^{2+} released from the steel by bacterially-induced corrosion (see the main text for details). In contrast, *Shewanella oneidensis* MR1 needs to interact directly with the steel substrate in order to release the Fe^{2+} needed for cellular energy. As a consequence, the cells themselves are covered by mineral precipitates (ESEM image, **D**) which consist of Fe oxides (red line in EDS spectrum). In the G20 culture, the yellow line corresponds to analysis of the spheres found in association with the G20 culture. Bars = 10 µm (main image D) and 1 µm (inset).

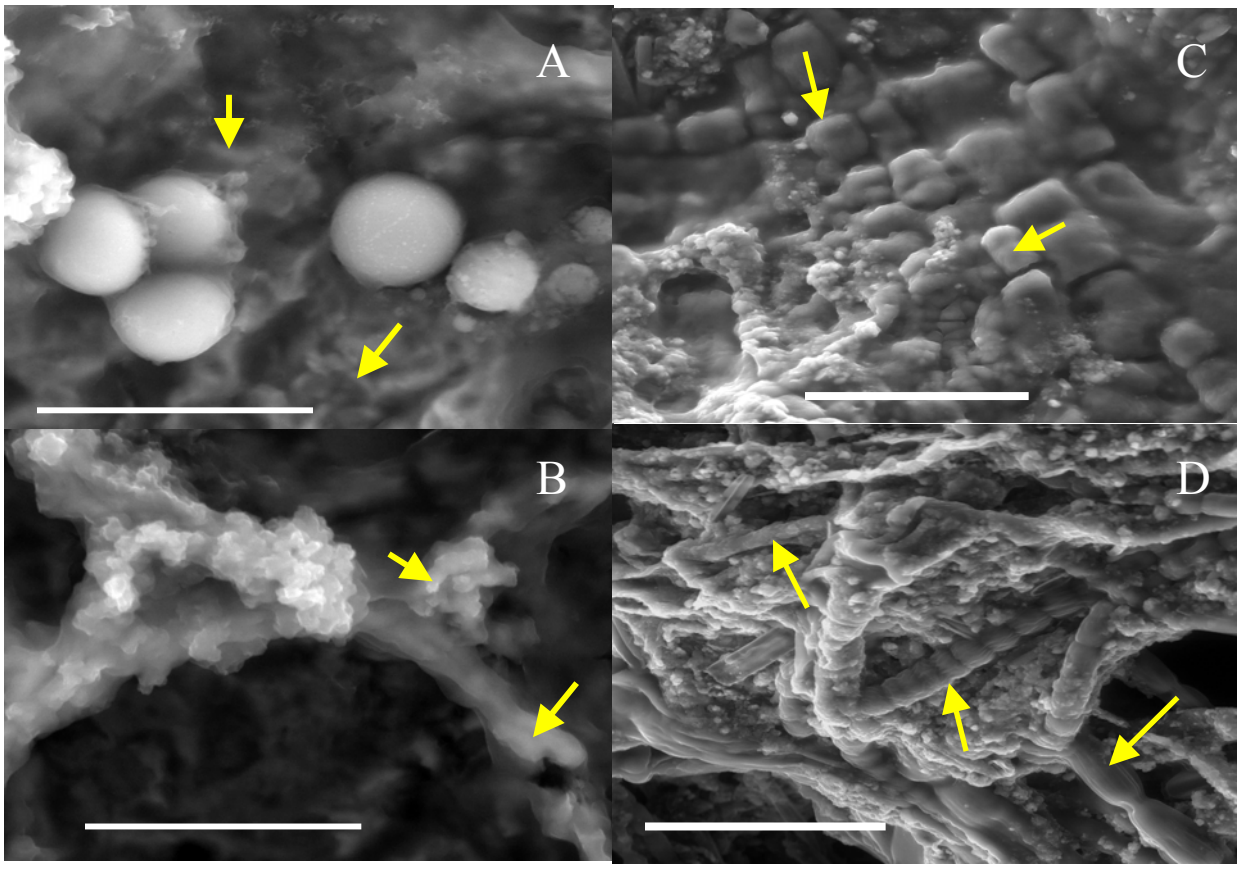

Figure 2: (A) ESEM image of dual-species biofilm. When both types of bacteria were grown together, they each expressed their characteristic mineralization in the biofilm, making the presence of these two different species recognizable and distinguishable. The granular FeS precipitates and colloidal sulfur spheres typical of the sulfate-reducing bacterial (G20) biofilms are visible. The arrows point to cells which have the characteristic banana shape of *Desulfovibrio desulfuricans* G20. Bar = 10 µm. In the lower image **(B)** of another area in the same biofilm, Fe oxide-coated cells of *Shewanella oneidensis* MR1 (arrows) are enclosed within EPS. Bar = 10 µm.

(C) and **(D)** These ESEM images show natural marine biofilms on steel. There is a significant variety of microbial types present, as shown by the diversity of shape and size of the cells (arrows). The cells are often accompanied by and coated in mineral grains (lighter, granular particles in the images). In addition, the cells are coated by a layer of extracellular polymeric substance (EPS) which helps them trap nutrients, avoid desiccation, and avoid predation. EPS is a general feature of biofilms and is very similar in composition and characteristics to the mucus we produce ourselves. The EPS layer is also a very important structural component of a microbial community such as a biofilm but can only be visualized by special electron microscopic techniques (i.e., environmental scanning electron microscopy). Most of the material seen in these images would have been lost during sample preparation for conventional electron microscopy. Thus, JPL's ESEM facility represents a core capability for obtaining realistic insights into the spatial organization of natural microbial communities.