# **Imaging Reduced Zones Created by Sulfate-Reducing Bacteria at Mineral Surfaces**

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## Introduction

Along with nitrate- and iron-reducing bacteria, sulfate-reducing bacteria (SRB) are considered an important class of bacteria in bioremediation schemes that involve reduction or precipitation of contaminants. Our work has focused on determining how SRBs interact with mineral surfaces. In this paper, we demonstrate our ability to map reductive capacity located at mineral surfaces and generated by SRB metabolic activity.1

## **Methods and Materials**

We developed a technique for mapping reducing zones on mineral surfaces. Our approach was to use the reduction of soluble Ag(I) to produce an insoluble precipitate at the surface where Fe(II) or S(-II) is present. The location of the precipitated Ag, as well as other elements of interest, is then determined by x-ray microscopy. Unfinished rock thin sections were treated with the modified lactate-C medium inoculated with Desulfovibrio desulfuricans under anoxic conditions. After 5-7 days, the thin sections were washed free of medium with deoxygenated deionized (DODI) water and then reacted for several minutes with a 10 mM solution of AgF/HF prepared from AgF<sub>2</sub>. The thin sections were washed gently again with DODI water and allowed to air dry under anoxic conditions. X-ray microscopy was performed at the PNC-CAT beamline (20-ID) at the Advanced Photon Source using Kirkpatrick-Baez focusing mirrors, 25.6-keV incident radiation, and a 13-element Ge detector to detect Ag, Rb, and Fe K-shell fluorescence. The spot resolution was slightly less than 2 µm. Mapping was performed on a 5-µm grid spacing and each point on the grid was counted for 1 s.

## Results

A number of mineral thin sections containing a variety of mineral types were analyzed to locate reducing zones at the surface. In many images (not shown), distinct features associated with the location of Ag on the thin-section surface were noted. In some, a clear alignment with mineralogy was noted, whereas in others, no particular distribution with respect to mineralogy was seen. Physical features such as cracks, fissures, and scratches seemed to have high levels of Ag. As shown in Fig. 1, the boundary region between two mineral types, one containing high levels of Fe (riebeckite) and the other having essentially no Fe (plagioclase and/or quartz) yielded the highest levels of Ag. In this specimen, scratches from grinding were also seen to attract the Ag, and broad swathes of Ag were seen on the low-Fe-mineral surface.

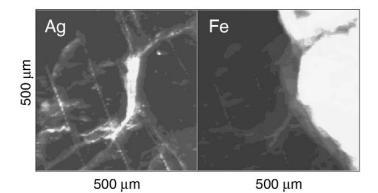


FIG. 1. Location of Ag and Fe on a rock thin section surface as determined by x-ray microscopy. Ag marks the reducing zones where microbially generated Fe(II) or S(-II) are present.

#### Discussion

This work clearly demonstrates the heterogeneous micronscale distribution of reductive capacity on the surface of minerals treated with SRBs. A slight correlation with Fe was seen in most specimens, suggesting that Fe(II) dissolved by reductive processes may have sorbed to other mineral surfaces or precipitated as FeS as a result of interaction with S(-II) plumes generated by SRBs. We note that micaceous samples also sorb Ag(I) in the interlayer region (Amonette et al., 2000) and thus care must be taken in the interpretation of these images. In this sample, no mica is expected, and the locations of Ag and SRB colonies/biofilms are presumed to be similar.

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#### Reference

<sup>1</sup> J.E. Amonette, C.K. Russell, S.M. Heald, K.A. Carosino, N.L. Robinson, and J.T. Ho, PNNL-13456/UC-400, pp. 2-13 to 2-14. (2001).