Microbial Transformations of Minerals and Metals: Recent Advances in Geomicrobiology Derived from Synchrotron-Based X-Ray Spectroscopy and X-Ray Microscopy

Alexis Templeton and Emily Knowles

Department of Geological Sciences, University of Colorado, Boulder, Colorado 80309-0399; email: alexis.templeton@colorado.edu, emily.knowles@colorado.edu

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biofilm, XANES, EXAFS, STXM, X-ray microprobe

Abstract

The field of geomicrobiology has embraced efforts to define and quantify the role of microbial organisms in low-temperature geochemical processes. However, any mechanistic understanding of the interactions between microbial organisms and minerals or metals requires analytical tools with the appropriate chemical sensitivity and spatial resolution. In the past several years, increasing application of nanometer-, micron-, and bulk-scale synchrotron-based X-ray techniques has provided new insights regarding the feedbacks among microbial growth, mineral dissolution, redox transformations, and biomineralization processes. In this review, recent findings derived from X-ray absorption spectroscopy, X-ray microprobe mapping, and X-ray microscopy studies are integrated to provide a new view of the dynamic biogeochemistry occurring at the microbe-mineral interface.

INTRODUCTION

The rapidly maturing field of geomicrobiology seeks to unravel how microbial organisms act as agents of geochemical change in modern and ancient systems. In turn, fundamental questions arise regarding how the geochemical characteristics of a given environment shape the structure and activities of the microbial communities it hosts. Defining the basis and evolution of these feedbacks inherently requires interdisciplinary collaboration across the Earth sciences and the fields of environmental microbiology, evolutionary biology, chemistry, biochemistry, materials science, and environmental engineering.

From the Earth sciences perspective, there is a growing recognition that microbial organisms have been intimately and quantitatively involved in elemental cycling and mineralogical transformations over geological time scales. There exists a long-standing appreciation of the key roles microbial organisms play in the global carbon cycle by harnessing light energy as primary photosynthetic organisms or mineralizing products of photosynthesis as a carbon and energy source for growth. Increased attention is now being focused on the ubiquitous activity of microbial consortia in the production and consumption of methane in diverse geological environments, given the importance of methane as a potent greenhouse gas and potential source of energy. For the inorganic world, one of the largest outstanding questions is the degree to which microbial organisms participate in mineral weathering reactions, and thereby impact the rates and extent of mineral dissolution, chemical exchange with aqueous solutions, and the stability, structure, and composition of the new minerals that may form. Meanwhile, it remains an intriguing challenge to identify the numerous and diverse microbial organisms that rely on the chemical energy available where redox disequilibria exist in geological systems, either at the interfaces of reduced minerals and oxidizing solutions or in hydrothermal environments where soluble forms of reduced species, such as H_2, sulfide, and Fe^{2+}, are delivered into the near-surface environment.

The microbe-mineral interface serves as a starting point for interrogating the role of microbial organisms in large-scale geochemical transformations. For example, mineral weathering reactions can be promoted inadvertently from the release of metabolic byproducts, such as inorganic and organic acids and ligands, when microbial organisms attach to rock and mineral surfaces (e.g., Welch & Ullman 1993, Barker et al. 1997, Liermann et al. 2000). Some of the most intensively studied examples of microbial organisms driving the dissolution of minerals include pyrite oxidation, in which the mineral serves as a solid phase source of electrons (e.g., Edwards et al. 2000), and Fe(III)-oxide reduction, in which the mineral serves as a sink for electrons in their metabolism (e.g., Lovley & Phillips 1986, Fredrickson et al. 1998). In both of these examples, the "eating" of minerals, such as sulfides, results in high levels of acid generation and the release of potentially toxic metals. The "breathing" of rocks and minerals may also serve to liberate trace and toxic metals that are sequestered within their primary structures or on their surfaces (e.g., Zachara et al. 2001).

Microbial organisms are simultaneously responsible for "biologically-induced mineralization" (Lowenstam 1981) by releasing metabolic byproducts that are relatively insoluble. A few common examples include the precipitation of reduced forms of nanoparticulate uranium (e.g., uraninite or UO_2) (e.g., Suzuki et al. 2002), transition metal sulfides (e.g., orpiment or As_2S_3) (e.g., Newman et al. 1997), or oxidized forms of Fe (e.g., ferric hydroxides) or Mn (e.g., MnO_2) (Emerson & Moyer 1997, Tebo et al. 1997). Such biominerals are formed both intracellularly (in the cytoplasm and periplasm of a cell) and extracellularly, either on the cell surface or nucleated on filaments, sheaths, and extruded polymers. Microbial organisms can also explicitly control the formation of mineral products, such as intracellular accumulations of magnetite (Fe_3O_4) or sulfur. It is increasingly accepted that biogenic mineral byproducts may be quantitatively significant in geological deposits and subsurface sediments (e.g., Sakaguchi et al. 1993, Labrenz et al. 2000, Fortin & Langley 2005).

Given the enormous environmental implications of microbial mineral transformations, it has become increasingly necessary to elucidate the factors that control the size, structure, and composition of minerals that are prone to microbially induced precipitation, dissolution, and/or electron transfer. By determining how microbial organisms directly and indirectly affect elemental speciation (e.g., by altering redox states or the complexation of metals), numerous biogeochemical cycles can be more predictively assessed. However, the mineral phases rapidly produced and destroyed by microorganisms are often the smallest and most reactive particles and are only preserved after significant modification. This has led to one of the greatest challenges in geobiology, which is to definitively identify minerals as biologically versus abiotically formed through a combination of chemical, structural, and morphological criteria. For geobiologists and astrobiologists seeking to understand the chemical fingerprints and mineralogical products that may preserve a record of some of the earliest metabolisms of life on Earth and elsewhere (such as Mars and Europa), detailed insights into microbial mineral transformations will be required.

Challenges in Interrogating the Microbe-Mineral Interface

To unravel the mechanisms of metal cycling and biomineralization reactions in past and present environments, we must increasingly view the microbial world through the lens of biofilms. The vast majority of microbial organisms are attached to surfaces such as rocks and minerals rather than free-floating in solution. Aggregations of bacterial cells and exopolysaccharides are interconnected to each other and the underlying substrate within three-dimensional, hydrated structures. The resulting microbial biofilms are incredibly complex and dynamic systems characterized by small-scale variability in ion diffusion, metabolic state, oxygen availability, and pH and redox gradients. Microorganisms interact with each other, the microdomain solution chemistry, and the geological substrates to which they are attached in fundamentally different ways than when observed as isolated organisms in solution.

The realization that microbes operate as consortia of organisms rather than as single cells requires attention to be focused on the microenvironments in which chemical transformations occur. In environmental systems, many of the critical molecular-scale processes occur at biofilm-mineral interfaces, where chemical exchanges, electron-transfer reactions, and mineral nucleation processes are localized. However, one of the barriers to interrogating chemical dynamics at these interfaces is that we cannot directly "see" where these reactions occur, but can only infer them from changes in the bulk chemistry of the system. A significant challenge to studying interfacial chemical processes at the micron, nanometer, and angstrom levels has been the lack of appropriate tools. Fortunately, rapid developments in the application of synchrotron-based techniques have generated new methods capable of characterizing the distribution and speciation of major and trace elements at the surfaces of geological and biological materials. These molecular tools can also detect dynamic chemical transformations that occur between microbial biofilms and the adjacent minerals, which enables the study of integrated systems.

The goal of this review is to explore new discoveries and insights in the field of geomicrobiology that have recently been gleaned from the development and application of synchrotron-based X-ray spectroscopy and microscopy. The experimental approaches are described only sufficiently to understand the scientific significance, and a series of detailed references for each of the techniques used is provided herein.

Why Use Synchrotron Radiation?

Synchrotron radiation (SR) is electromagnetic radiation emitted tangentially from high-energy electrons accelerated in a circular orbit (e.g., in a storage ring at a synchrotron facility). The X-rays

produced are highly intense, collimated, polarized, and tunable. Key aspects of the synchrotron-based techniques are their high chemical sensitivity (subppm level), high spatial resolution (<50 nm to mm scale), and element specificity. Powerful approaches have been developed that permit the characterization of the chemical states and structures of almost every element in the periodic table. Incredibly heterogeneous materials, such as sediments or rocks that contain both biological and mineralogical components, can be interrogated. Often the materials can remain hydrated and minimally prepared, and the samples can be analyzed either as a bulk sample, from which averaged chemical information is obtained, or in a spatially resolved fashion, where microbial cells and by-products can be distinguished from the matrices in which they reside. The typically nondestructive nature of the measurements permits the samples to be structurally or biologically characterized subsequently using complementary microscopic techniques such as scanning and transmission electron microscopy (SEM and TEM, respectively) or conventional optical or fluorescence microscopy.

X-ray fluorescence (XRF): when a core electron is ejected from an atom during an X-ray absorption event, secondary fluorescence X-rays of a well-defined or characteristic energy may be emitted when outer electrons fill the inner core hole

Microbe-mineral samples can be analyzed in several dimensions using a variety of experimental geometries and configurations. The most common measurements involve determining the average oxidation state and chemical form of an element of interest throughout a bulk sample using X-ray absorption spectroscopy techniques such as X-ray absorption near edge structure spectroscopy (XANES) or extended X-ray absorption fine structure spectroscopy (EXAFS) (see **Table 1**). It is then possible to choose a smaller region of a sample to investigate in a spatially resolved fashion to identify internal variations in elemental distributions (e.g., locations of hot spots versus regions devoid of Fe), elemental coassociations (e.g., colocalization of Fe with Mn, Ca, P, or As), elemental oxidation state (e.g., Fe^{2+} versus Fe^{3+}), elemental species (e.g., $Fe^{II}CO_3$ versus Fe^{II}-polymer), or localized mineralogy (e.g., Fe_3O_4 associated with quartz and kaolinite). Such information is obtained by creating X-ray fluorescence (XRF) elemental maps or transmission elemental maps using a highly focused X-ray beam, and then conducting microscale X-ray absorption spectroscopy measurements on small sample volumes. A three-dimensional analysis of complex samples can be obtained using tomographic methods (see Sutton et al. 2002), although these techniques have not yet received wide use. Instead, analyses suited to the vertical or z-dimension rely on surface-sensitive synchrotron-based techniques, such as long-period X-ray standing waves, total-reflection X-ray fluorescence, and grazing-angle X-ray absorption spectroscopy and X-ray diffraction, which can probe the contact zone of the microbe-mineral interface at the angstrom to nanometer scale.

The design and application of synchrotron-based X-ray microscopy and spectroscopy techniques are relatively specialized and require significant optimization depending on the facility used, the characteristics of the source X-rays, the monochromators, the focusing optics and detectors available, the nature of the sample to be interrogated, and the spatial and chemical resolution desired. **Table 1** is provided as a brief reference for the techniques that are discussed throughout this review. Many of the specific details required for data collection and analysis can be found in the thorough reviews presented in "Applications of Synchrotron Radiation to Low-Temperature Geochemistry and Environmental Science," published by the Geochemical Society (Fenter et al. 2002).

RECENT ADVANCES IN GEOMICROBIOLOGY

The most wide-reaching advance in the field of geomicrobiology has derived from the growing recognition that microbial organisms have been intimately involved in the global cycling of soluble and insoluble forms of iron. The environmental significance of Fe^{2+} as an electron donor and Fe^{3+} as an electron acceptor in microbial growth, and the importance of Fe biominerals as repositories for trace elements, provide a critical link between environmental microbiology and environmental

geochemistry. Numerous recent reviews summarize our rapidly developing view of how microbial organisms transfer electrons to and from mineral surfaces, using Fe as the most extensively studied geomicrobiological system (see Kappler & Straub 2005, Weber et al. 2006, Lovley 2008). Synchrotron-based studies have also elegantly demonstrated how coupled biotic-abiotic chemical transformations must be studied to understand the stability and transformations of Fe minerals (e.g., Hansel et al. 2003). To explore fundamentally related processes, we use the following sections of this review to integrate the recent findings from five other areas of geomicrobiological research that have increasingly relied on synchrotron-based spectroscopic and microscopic tools to unravel microbial transformations of minerals and metals.

K-edge: the binding energy that photons must exceed to ionize electrons from the K (e.g., 1s) shell

Sorption to Microbial Surfaces

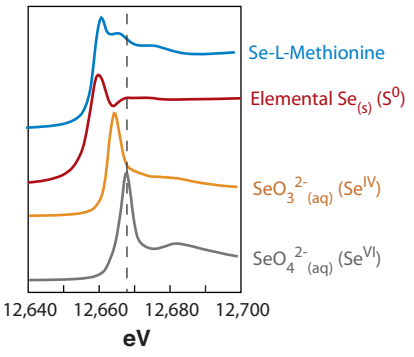
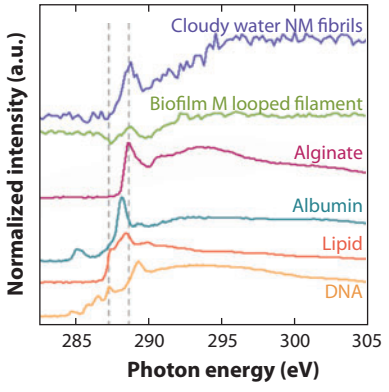
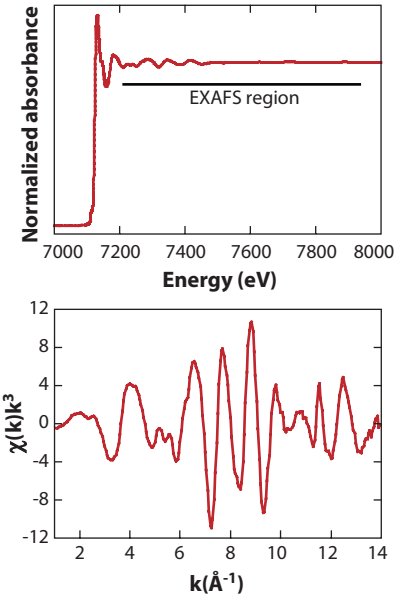
The concentration and speciation of metals, nutrients, and trace elements in rocks, soils, and aquatic environments can be dramatically influenced by sorption reactions at cell surfaces. Natural biofilms sequester several metallic and alkaline elements. For example, biofilms often contain K^+, Ca^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, and Pb^{2+} at concentrations 2 to 4 orders of magnitude greater than those measured in the surrounding bulk fluid (Brown et al. 1994, Ferris et al. 2000). The affinity of bacterial surfaces for aqueous cations is due in part to the low isoelectric points of the surfaces, resulting in a negative surface charge over a wide pH range. However, the structure and charge density of bacterial cell walls can vary greatly (Beveridge & Murray 1980).

There appears to be a continuum between metal ion sorption and precipitation reactions (Warren & Ferris 1998) in which bacteria or acidic mucopolysaccharides provide nucleation sites for the formation of metal oxides, carbonates, phosphates, silicates, sulfides, and organometallic complexes (Degens & Ittekkot 1982, Beveridge et al. 1983, Ferris et al. 1987, McLean & Beveridge 1990, Thompson & Ferris 1990, Schultze-Lam et al. 1992, Southam et al. 1995, Urrutia & Beveridge 1995, Fortin & Beveridge 1997). The mechanism of metal ion precipitation is assumed to follow multiple stages: Nucleation sites are first formed by the stoichiometric sorption of metal ions to functional groups on the cell walls, followed by the association of appropriate counter ions (e.g., CO_3^{2-}, PO_4^{3-}) and then additional metal ion deposition (Urrutia & Beveridge 1993).

Considerable macroscopic and molecular-level research has been directed toward understanding the modes and mechanisms of metal ion sorption reactions on bacterial surfaces. Numerous recent studies (e.g., Fein et al. 1997, Yee & Fein 2003) have used surface complexation modeling to quantify the site densities, deprotonation constants, and metal-binding constants of the functional groups present on bacterial surfaces. The carboxyl and phosphoryl sites are thought to dominate metal binding at low to neutral pH, and hydroxyl and amino groups have been invoked for metal complexation above pH 8. However, the surface complexation modeling can only tentatively infer the chemical identity of sorption sites from their acidity constants, and the modeling is also only sensitive to the dominant sites involved in metal binding.

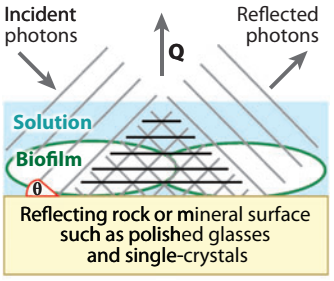
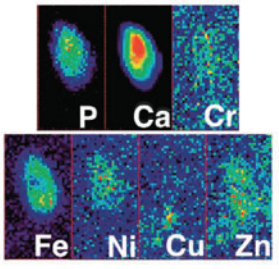
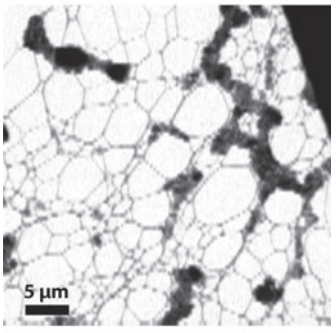
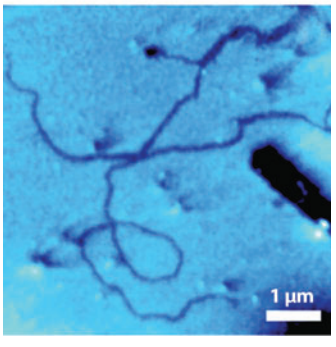
X-ray absorption spectroscopy can be used to independently determine the modes of metal ion binding to specific functional groups on bacterial surfaces. Metal-bacteria studies have primarily been conducted for Zn^{2+}, Pb^{2+}, Cd^{2+}, and UO_2^{2+}. To date, most spectroscopic studies have been in agreement with the surface complexation modeling (Kelly et al. 2002, Boyanov et al. 2003). Using Zn^{2+} as an example, metal K-edge EXAFS have identified the same dominant metal-binding sites on several different types of bacterial surfaces. Guine et al. (2006) found that Zn^{2+} binding at the surfaces of several bacterial species was dominated by phosphoester groups at all Zn^{2+} loadings. Similar data showing primary Zn association with phosphoryl groups were previously observed for *Pseudomonas putida* biofilms by Toner et al. (2005b) and for fungal cell walls by Sarret et al. (1998). Guine et al. conducted experiments across a broad range of Zn^{2+} loadings, and the resulting spectra

Table 1

X-ray technique	Visual example
XANES spectroscopy (X-ray absorption near edge structure) Element sensitive tool where the element of interest absorbs a photon at or above a characteristic energy or absorption edge and a core electron is excited to unoccupied molecular orbitals or ejected from the atom (see Stohr 1992). XANES spectra typically extend ~30 to 100 eV above the absorption edge and can be analyzed by comparison to model spectra to determine the absorber's valence, molecular orbital states, and coordination chemistry. Right: Se K-edge XANES spectra of select model compounds.	
NEXAFS spectroscopy (Near edge X-ray absorption fine structure) Another term for XANES, often used in the context of soft X-ray spectroscopy, particularly at energies below 1 keV. NEXAFS is measured by fluorescence yield (FY) or total electron yield (TEY). For an extensive discussion of soft X-ray spectroscopy of C, N, P, and S functional group chemistry and macromolecular structure in organic molecules and biopolymers, see Myneni 2002. Right: C K-edge XANES of organic fibrils and model compounds. Figure reproduced with permission from Chan et al. 2004 (copyright AAAS, 2004).	
EXAFS spectroscopy (Extended X-ray absorption fine structure) The fine structure or modulations in an X-ray absorption spectrum up to ~800 eV above the absorption edge. The spectral oscillations are generated when an ejected photoelectron is backscattered by surrounding atoms (see Koningsberger & Prins 1988). EXAFS spectra can be analyzed to determine the local (<5 Å) atomic environment surrounding the absorbing atom (i.e. identity, number, and distances of neighboring atoms). EXAFS spectra can also be used to speciate numerous chemical forms of the absorber in any given sample (i.e. quantify fraction of the element in various adsorption complexes, solution complexes, and mineral forms) (see Brown & Sturchio 2002, Manceau et al. 2002). Right: Fe K-edge X-ray absorption spectrum and EXAFS spectrum.	

(*Continued*)

Table 1 *(Continued)*

X-ray technique	Visual example
XSW profiling (X-ray standing wave) Long-period (e.g. 100-10,000 Å) X-ray standing waves (XSW) are generated by the interference between incident and reflected X-rays at grazing incidence angles below the critical angle for total external reflection (see Bedzyk et al. 1989). Specular reflectivity techniques can be used to probe the vertical (z) position of elements above a highly polished mineral surface, either in a solution layer or within a thin (<10 μm) biofilm, by collecting fluorescence yield spectra as a function of incidence angle (see Trainor et al. 2005). Right: X-ray standing wave intensity field generated at a biofilm-mineral interface.	
XRF microprobe (X-ray fluorescence) Energy-resolved X-ray fluorescence (XRF) spectra are collected from a small spot when a focused X-ray beam impinges upon a sample. The size of the analyzed area depends upon the focusing optics and typically ranges from 0.1 to 10 microns in one dimension (see Sutton et al. 2002 and Kemner et al. 2005). By step-wise scanning the focused X-ray beam across a sample, individual fluorescence peaks obtained for each pixel can be quantitatively compared to create element specific maps. Spot-specific X-ray absorption spectroscopy (μ-XAS) and X-ray diffraction (μ-XRD) can also be collected as a separate experiment (see Manceau et al. 2002). Right: element-specific XRF intensity maps generated for a microbial cell. Figure reproduced with permission from Kemner et al. 2004 (copyright AAAS, 2004).	
STXM (Scanning transmission X-ray microscope) X-ray microscope that uses absorption contrast around soft X-ray absorption edges (e.g. C K-edge, Fe L-edge) using highly focused X-rays to obtain images and spectral data. Soft X-rays are focused to spot sizes as small as 30 nm using Fresnel zone-plate optics. Two-dimensional images are collected in transmission mode by rastering a thin, often still hydrated sample through the X-ray beam. Image stacks are then generated by remapping the area at incrementally increased incident X-ray energies (~0.1 eV steps). Maps of absorption contrast can be generated to quantitatively determine the distribution of the absorbing atom, and each pixel contains an absorption spectrum (i.e. NEXAFS spectra) for the element of interest (see Lawrence et al. 2003). Right: STXM image of a carbonate microbialite. Figure reproduced with permission from Benzerara et al. 2006 (copyright National Academy of Sciences, 2006).	
X-PEEM (X-ray photoemission electron microscope) A full-field imaging technique that combines photoemission electron microscopy and soft X-ray spectroscopy. Also used to detect, map and speciate low z elements with absorption edges below ~1 keV. Relies upon the detection of photoemission electrons emitted from the surface of flat samples analyzed in a UHV environment, where the material is often coated to prevent charging effects (see De Stasio et al. 2003). Image stacks are collected by rescanning the field of view at incremented energy steps through the absorption edges of elements of interest so that each pixel contains a full XANES spectrum (e.g. C K-edge XANES). Spatial resolution can be as good as 10 nm. Right: XPEEM image of mineralized bacterial filaments. Figure reproduced with permission from Chan et al. 2004 (copyright AAAS, 2004).	

could be fit using linear combinations of Zn complexed to simple and complex organics chosen to represent Zn-phosphoester, Zn-carboxyl, and Zn-sulfhydryl associations. Most intriguingly, these authors unexpectedly identified Zn-complexation by a small number of high-affinity sulfhydryl sites (with a local coordination similar to Zn-cysteine). Despite their reactivity, these surface sites would not be detected by modeling titration data because of their low abundance (e.g., <1% of total sites).

Metal K- and L-edge EXAFS spectra are typically collected as a function of metal loading or at varying pH to quantify the partitioning of the metals between chemically and structurally distinct bacterial surface sites. Typically, interpretation of EXAFS data collected for bulk bacteria-metal suspensions invokes relatively simplified structures for the sorption sites by broadly differentiating the sites as carboxyl, phosphoryl, sulfhydryl, or amino functional groups and does not provide any substantive macrostructural information. Because EXAFS spectroscopy is a probe of the local structural environment surrounding the absorbing atom, shell-by-shell fitting of EXAFS spectra enables determination of the identity, coordination number, and distance of neighboring atoms surrounding the metal ion that absorbs the X-ray (see Brown & Sturchio 2002). The spectra can also be fit by comparison with libraries of metal-organic and metal-inorganic species (**Figure 1**). Direct comparison with model compounds is often required to interpret the complex spectra that arise when metal ions not only sorb to the bacterial surface but also sorb to adjacent mineral surfaces or precipitate adjacent to the cell surface (e.g., Templeton et al. 2003a).

L-edge: the energy at which electrons are ionized from the L shell (i.e., 2s or 2p electrons). L-edges occur at lower X-ray energy than the K-edge of the same element

X-ray microprobe: beamline configuration that relies on focusing optics to produce small beam sizes (micron to submicron) to generate X-ray fluorescence maps and collect X-ray absorption spectra or X-ray diffraction data

Spectromicroscopy: spatially resolved combination of spectroscopy (often XANES) and microscopy to obtain elemental speciation from specific spots or pixels in an elemental map

Grazing incidence: grazing refers to an incident X-ray beam impinging on a sample at small angles (typically 0.1 to 1°)

Metal Ion Distributions in Biofilms

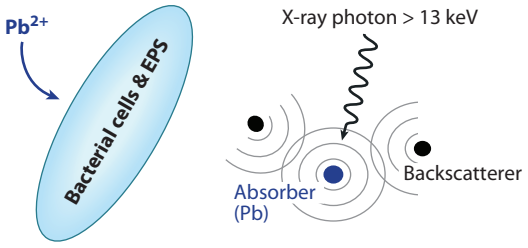
Our ability to interrogate the chemical reactions and gradients that occur at the nanometer to micron scale within biofilm-mineral microenvironments, where large variations in pH, redox state, or electrostatic interactions occur, remains an enormous challenge. Three recent developments in SR-based microscopy and spectroscopy have significantly improved the spatial scales and sensitivity by which chemical species can be resolved within microbial biofilms: long-period X-ray standing waves (XSW), X-ray microprobe (XRF mapping coupled with spectroscopy), and scanning transmission X-ray microscopy (STXM).

The XSW technique is a spatially sensitive spectroscopic approach suitable for vertically profiling metal ion distributions in thin (<10 μm) microbial biofilms that have formed on single-crystal surfaces of metal oxides such as Al_2O_3 or Fe_2O_3 or on highly polished slabs of naturally occurring minerals and glasses such as sulfides and quenched basalts. X-rays impinge on the sample at grazing-incidence angles, where total external reflection of the X-rays occurs. Below a critical angle that is dependent on the substrate and X-ray energy, there is little penetration of the X-rays into the reflecting material (i.e., mineral surface); thus this technique provides chemical

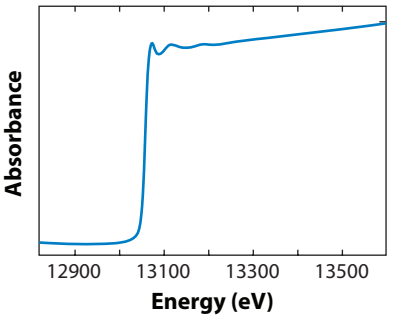
Figure 1

Example of EXAFS spectroscopy data collection and analysis. When an element (e.g., Pb^{2+}) is taken up by a microbial organism, element-specific EXAFS spectra can be collected to determine speciation. Energy is scanned through the absorption edge for several hundred eV, and the absorption spectrum is converted into an EXAFS spectrum. This EXAFS data can be treated as a chemical fingerprint of all the major chemical species present (>5%) and can be fit by adding the EXAFS spectra of model compounds using a linear-combination fitting routine. In this example, the EXAFS spectrum of Pb in a biofilm comprises two components, Pb^{2+} sorbed to the bacterial surface and Pb^{2+} crystallized in $Pb_5(PO_4)_3OH$. EXAFS spectra can also be directly fit using conventional methods such as nonlinear least-squares fitting procedures to analyze shell-by-shell contributions to the total EXAFS spectrum and Fourier transform. In this example, the model spectrum of Pb sorbed to a bacterial surface comprises a Pb-O shell and a more distant Pb-C shell that are interpreted to represent Pb-carboyxl group interactions (see Templeton et al. 2003a).

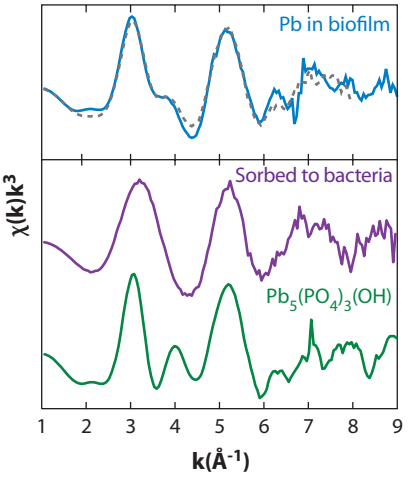
X-ray absorption spectroscopy of metal-bacteria interactions
(e.g. Pb in biofilm)



Pb X-ray absorption spectrum (XANES & EXAFS)

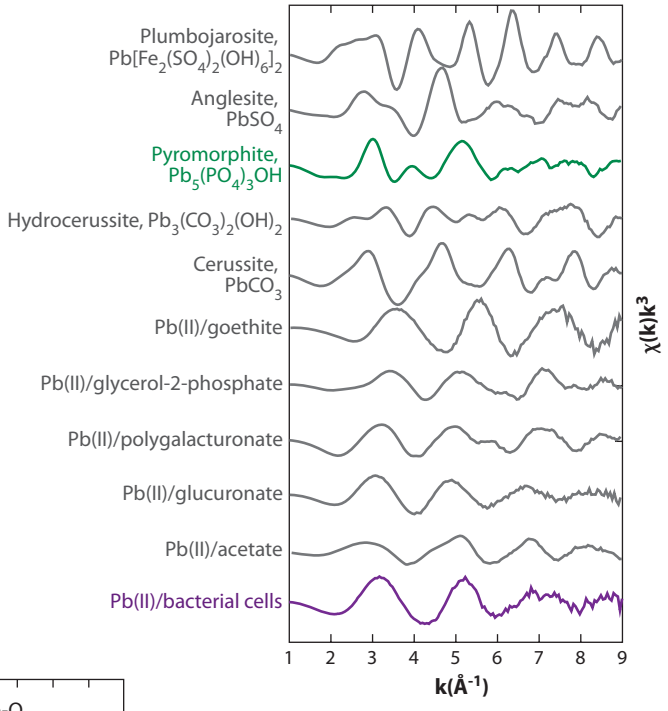


Linear combination fitting of EXAFS spectra
(Pb in biofilm = two species)

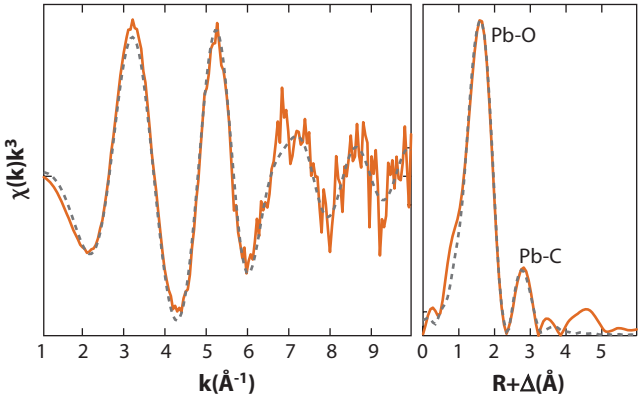


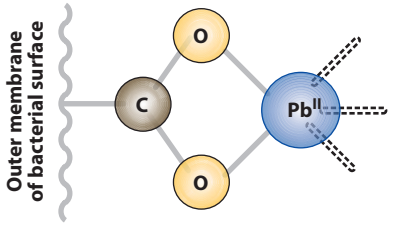
Pb in biofilm spectrum (—) and fit (- -)
by combining:
55% of Pb sorbed to cells spectrum
45% of Pb in $Pb_5(PO_4)_3OH$ spectrum

Pb EXAFS model compound library
(showing 11 out of 24 compounds)



Conventional EXAFS fitting
(For single spectrum of Pb sorbed to bacterial cells)





information for ions residing nanometers above a mineral-water or mineral-biofilm interface (see Waychunas 2002).

In an XSW experiment, the X-ray reflectivity and fluorescence yield of the element of interest is measured over a small range of grazing angles (**Table 1**, **Figure 2**). For example, Pb fluorescence yield profiles were collected as a function of X-ray incidence angle to quantitatively determine the partitioning of Pb within biofilms formed on Fe- and Al-oxide surfaces (Templeton et al. 2001). At low Pb concentrations, all profiles indicated that Pb(II) is preferentially sorbed to the mineral surfaces rather than the biofilms. Thus, this approach demonstrated that the formation of a biofilm will not block the highly reactive sites on the mineral surfaces, although the biofilms

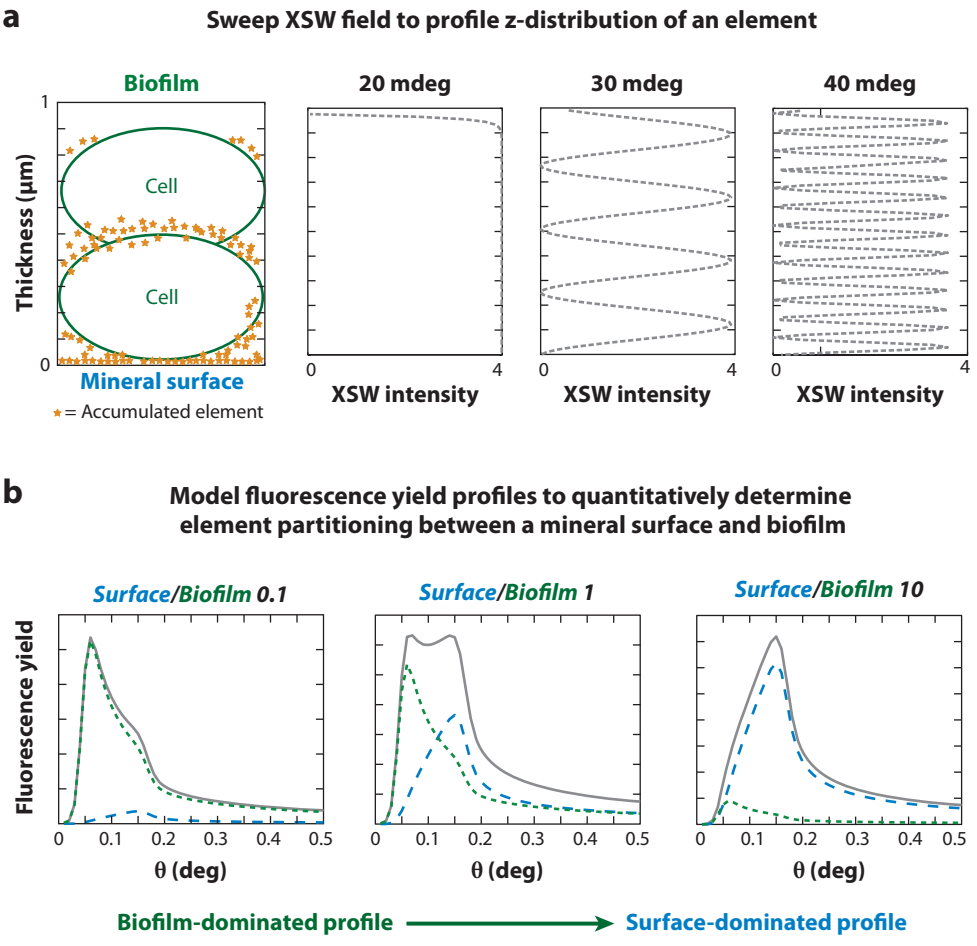


Figure 2

Conceptual diagram for the long-period X-ray standing wave (XSW) method to vertically profile element distributions within microbial biofilms. (*a*) If a monolayer of cells or thin (<10 micron) biofilm forms on a polished mineral surface, the z position of an element of interest can be determined by systematically varying the X-ray incidence angle through the critical angle for total-external reflection of the X-rays. As the incidence angle is increased [e.g., 20, 30, 40 millidegrees (mdeg)], the XSW intensity profile will compress, shift in toward the surface, and excite fluorescence from the atoms it intersects. (*b*) The resulting fluorescence yield profiles can be quantitatively modeled to determine the partitioning of the element between mineral surface sites and the overlying biofilm (surface/biofilm ratio) (see Trainor et al. 2005).

do form a quantitatively important sink for Pb(II) at micromolar Pb concentrations owing to the large number of available surface functional groups.

The XSW experiments can be coupled to energy scans (e.g., XANES at a fixed incident angle) to clearly differentiate between the speciation of the metals within the biofilm versus the mineral surface. When profiling the partitioning of selenate [Se(VI)] within *Burkholderia cepacia* biofilms, Templeton et al. (2003b) unexpectedly found that *B. cepacia* will rapidly reduce Se(VI) to selenite [Se(IV)] and elemental selenium [Se(0)], and the selenite intermediate rapidly diffuses through the biofilm to preferentially bind to the underlying mineral surface. The rapid stratification of Se redox-species would not have been observed, nor predicted, using bulk spectroscopic techniques or studying biofilms or mineral surfaces alone.

The most widely used method to determine the lateral (x-y) distribution of metal ions within cell clusters at the micron to submicron scale is hard X-ray microprobe mapping. Full X-ray fluorescence spectra can be collected to simultaneously map several elements. Each specific element map is made by integrating the fluorescence intensity associated with individual, energy-resolved peaks in each pixel. The size of individual spots or pixels ranges from 0.1 to 10 microns and depends on the energy of the incident X-rays and the focusing optics used (e.g., capillaries, Kirkpatrick-Baez mirrors, or Fresnel zone-plates). After the X-ray fluorescence mapping is completed, it is then possible to return to the x-y position of any given pixel and scan in energy to collect a spot-specific XANES spectrum to determine the oxidation state or local coordination of an element of interest (**Figure 3**). X-ray absorption spectroscopy can provide a powerful complement to the fluorescence maps by identifying changes in redox state or element speciation in hot spots of metal accumulation or across spatial transitions such as a microbe-mineral interface.

Submicron XRF mapping is necessary to identify intracellular distributions of elements. For example, Kemner et al. (2004) made elemental maps of individual *Pseudomonas fluorescens* cells (e.g., P, S, Cl, K, Ca, Cr, Fe, Ni, Cu, and Zn) using a beam size of 150 microns and demonstrated enhanced resistance of the cells to Cr(VI) when attached to surfaces. More recently, Makarova et al. (2007) used XRF mapping of Fe and Mn (as well as several other elements) in *Deinococcus radiodurans* and *Deinococcus geothermalis* cells to explore mechanisms of radiation and desiccation resistance. The maps indicated that both *Deinococcus* strains accumulate exceptionally high levels of intracellular Mn(II), resulting in high Mn/Fe ratios relative to microorganisms that are significantly more sensitive to ionizing radiation. Daly et al. (2007) also used cellular XRF mapping and Mn K-edge XANES spectroscopy to demonstrate that *D. radiodurans* accumulates high intracellular levels of Mn(II) as an oxidative protection mechanism.

Larger beam sizes are required to create sufficiently large maps to explore chemical heterogeneity across extensive biofilms (i.e., using pixel sizes of 1 to 2 microns to cover areas hundreds of microns across). For example, in bulk Pb L_{III} EXAFS spectroscopic studies, Templeton et al. (2003a) determined that *Burkholderia cepacia* both sorbed and biomineralized Pb, forming a highly insoluble Pb-hydroxyapatite mineral, pyromorphite [$Pb_5(PO_4)_3(OH)$], as a major sink for Pb. However, it was necessary to use X-ray fluorescence mapping, spot-specific Pb L_{III} EXAFS, high-resolution TEM, and selected-area diffraction measurements to chemically image *B. cepacia* biofilms and show that discrete nanocrystalline clusters of pyromorphite were formed in the periplasm of only ~10% of the biofilm cells. This mineral was undersaturated in the bulk solutions and yet was microbially precipitated in defined microenvironments as a detoxification mechanism.

The majority of macromolecular structures in microbial biofilms contain light elements (e.g., C, N, O, S, P) that are not easily detected and cannot be spectroscopically interrogated using hard X-rays. However, recent advances in soft X-ray microscopy, coupled with soft X-ray spectroscopy, are providing new insights into microbe-mineral interfaces. Studies by Chan et al. (2004) and De Stasio et al. (2005) clearly demonstrated that photoelectron XANES coupled with X-ray

Hard X-rays: X-rays with energies greater than ~4 keV, typically used to probe elements close to Ca and higher Z number in the periodic table

Soft X-rays: X-rays with energies lower than ~4 keV, used to probe K-edges for elements with low atomic numbers and L-edges of transition elements

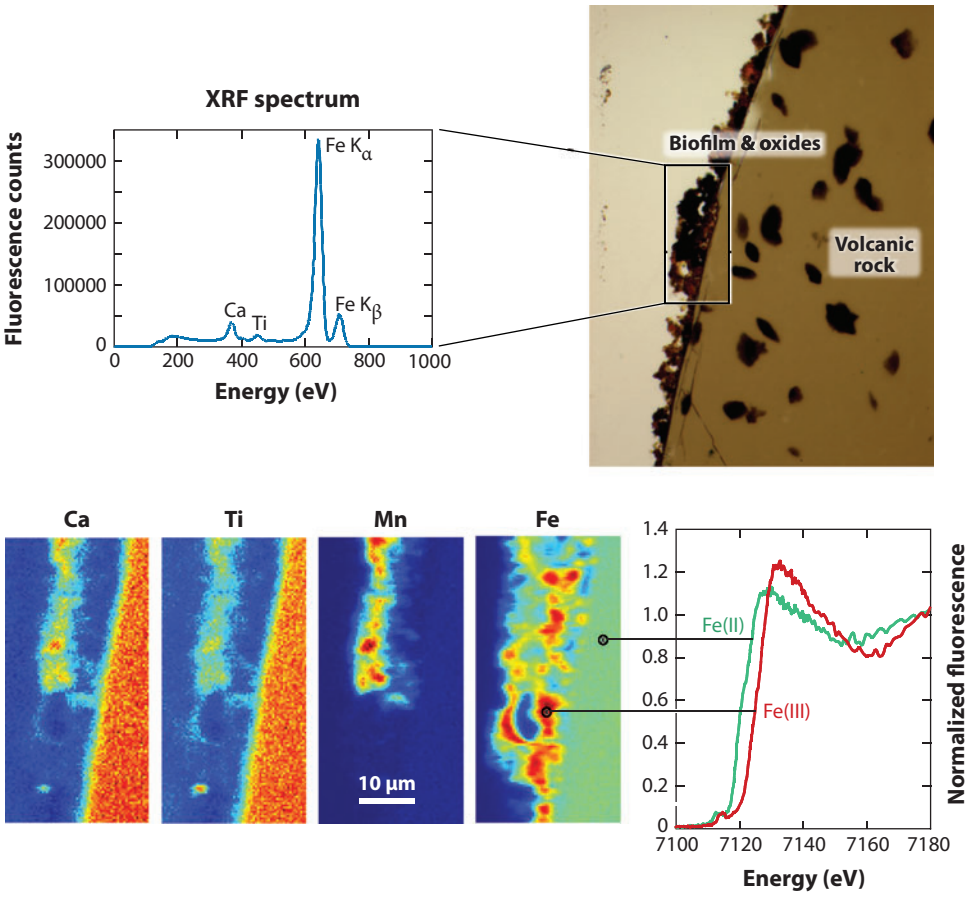


Figure 3

Example of hard X-ray microprobe mapping coupled with X-ray absorption spectroscopy. X-rays at 7.3 KeV are focused to a 1 × 1 micron spot using Kirkpatrick-Baez mirrors and rastered across a volcanic glass that has been colonized by microbial organisms on the seafloor. Full X-ray fluorescence spectra are collected for each pixel to quantify the amounts of Fe, Ca, Ti, and Mn present. Element-specific maps can be made to show hot spots of accumulation (e.g., Mn enrichment in a crust of biominerals). X-ray absorption spectra can then be collected from a specific spot, which can be used to determine the valence state or speciation of the element of interest (e.g., Fe^{2+} versus Fe^{3+} ratios derived from spatially resolved Fe K-edge XANES). (Previously unpublished data collected at BL 2-3 at the Stanford Synchrotron Radiation Laboratory by Templeton and Knowles.)

photoemission electron microscopy (XPEEM) is a unique method for unraveling the initial steps of mineral nucleation and templating on microbially produced polymers such as polysaccharide-rich filaments. For example, Chan et al. (2004) used this technique to identify acidic polysaccharide fibrils that templated the formation of akaganeite (β-FeOOH) pseudosingle-crystal cores (identified by TEM) surrounded by ferrihydrite.

The application of STXM to hydrated biofilm samples may prove to be one of the most important advances in quantitatively imaging complex microbe-mineral associations with high spatial resolution and chemical sensitivity. Gilbert et al. (1999) provided one of the first intriguing tests of soft X-ray microscopy to image the initial formation of *Ps. putida* DMP-1 biofilms, but suggested that the method could be used only on immature biofilms <10 microns thick, similar

to the XSW approaches discussed above. STXM techniques are very effective so long as a thin sample (e.g., biofilm grown directly on a silicon nitride window) or highly dilute microbe-mineral dispersion (<1 μl sandwiched between SiN windows) is created to achieve an optimum absorption density. Contrast images are obtained from the X-ray absorption maps generated at a series of sequential energies around the absorption edge of an element of interest using near edge X-ray absorption fine structure spectroscopy (NEXAFS) as a probe (Lawrence et al. 2003). It is possible to create quantitative chemical maps within hydrated biofilms with ~40 nm resolution. If sequential images or line scans are collected at low energies across the C K-edge, maps of key organic macromolecules such as proteins, carbohydrates, lipids, and nucleic acids can be produced.

Absorption edge: a sharp increase in an X-ray absorption spectrum that occurs at an energy where the incident photon energy exceeds the binding energy of a K, L, or M electron

Currently, STXM can be used to characterize biological and inorganic particulates using the K-edges of low Z elements (e.g., B to Si) and the L-edges of heavier biological elements (e.g., P, S), as well as major alkaline earth elements (e.g., K, Ca), first row transition metals (e.g., Cr, Mn, Fe, Ni, Cu, Zn), and metalloids such as As and Se. Therefore, STXM is not restricted to imaging biomolecules. When biofilm images are collected at the C K-edge, inorganic components will show up as dark spots of high contrast (i.e., high absorption). If these inorganic components are somewhat chemically defined (i.e., known to be made up of Al, Fe, or Mn minerals), then complementary STXM measurements can be made at the Al K-edge, Fe L-edge, or Mn L-edge to identify the mineral phases and metal oxidation state. For example, Yoon et al. (2004) used C 1s spectra to determine the distribution of proteins and extracellular polysaccharides (EPS) in *Caulobacter cresentus* biofilms and then obtained Al K-edge image stacks to determine the distribution of mineral colloids and to identify which Al-mineral phases were present in the biofilm. Similarly, Toner et al. (2005a) used STXM to define the distribution of *Ps. putida* biofilms and then detected the enzymatic oxidation of Mn(II) to Mn(III) and Mn(IV) as a function of time.

Dynes et al. (2006) significantly increased the sophistication of the STXM approach by mapping the biomolecule (e.g., cells and EPS) and oxide components within a bacterial-algal biofilm with C K-edges and O K-edges. They then tested methods for quantifying the distribution of Fe(II) versus Fe(III) within these biofilms, using image sequences collected across the Fe L-edge. Maps of Mn biominerals and associated Ni^{2+} could also be correlated with the data, resulting in a high-resolution chemical image of a complex natural biofilm.

Microbial Mn Oxidation and Biomineralization

Manganese is an environmentally abundant transition element that rapidly cycles between reduced and oxidized forms in dynamic biogeochemical systems. Mn(II) is soluble, highly mobile, and initially released into the environment from hydrothermal fluids or weathering of primary silicates and carbonates. However, upon oxidation to Mn(III) or Mn(IV), manganese precipitates as a series of chemically and structurally diverse oxides.

The initial oxidation and mineralization of Mn is considered to be microbially controlled, because numerous isolated microorganisms have been shown to catalyze Mn(II) oxidation by up to five orders of magnitude relative to abiotic conditions. As a result, Mn oxides commonly form within hydrothermal plume particles, reaction rims on the surfaces of weathered mafic rocks (e.g., basalt), layers within ferromanganese nodules, coatings on sedimentary rocks such as desert varnish, and as ubiquitous surface coatings on minerals in subsurface sediments and aquifer materials. Because the properties of the oxides (e.g., structure, surface area, and reactivity) are critically important in the sorption and ion exchange of major cations and trace element species from aqueous solutions, there has been a growing interest in determining the mechanisms whereby bacteria oxidize Mn and characterizing the biominerals that form.

The Mn-oxide mineralogy in natural samples is often hard to characterize due to the complex mixtures of small, disordered, and often relatively amorphous minerals. Synchrotron radiation scattering and spectroscopic techniques have provided unique and detailed insights into the biological oxidation and precipitation of Mn by several model organisms (e.g., *Bacillus* sp. SG-1 and *Ps. putida* MnB1) and are well suited to characterizing environmental samples, setting the stage for direct comparison between model systems and diverse natural materials.

The most significant advances in unraveling the differences between biological oxidation of Mn and successive stages in the (bio)mineralization process have been derived from careful synchrotron-based X-ray diffraction and Mn XANES and EXAFS measurements (e.g., Bargar et al. 2000, 2005; Webb et al. 2005b). In these studies, *Bacillus* sp. SG-1 spores were incubated in the presence of Mn over several days at varying concentrations of Mn(II) in solutions that mimicked seawater versus freshwater and were analyzed in a time-resolved fashion. First, Bargar et al. (2000) used Mn K-edge XANES to determine that the first stable oxidation product formed by *Bacillus* SG-1 was a Mn(IV)-rather than Mn(III)-bearing phase. They accomplished this by collecting Mn K-edge XANES spectra as a function of time as spores were incubated with Mn(II). However, it has since been revealed that Mn-oxidizing *Bacillus* sp. oxidize Mn(II) to Mn(IV) through a rapid series of enzymatic one-electron transfers (Webb et al. 2005a). This was independently confirmed for the Mn-oxidizing bacterium *Ps. putida* MnB1 by Toner et al. (2005a), who combined STXM and NEXAFS approaches to map the progressive oxidation of Mn(II) within hydrated biofilms. Mn L-edge XANES spectra extracted from image stacks of Mn-rich portions of the biofilms indicated that both Mn(III) and Mn(IV) oxides accumulated over time.

Enzymatic Mn oxidation will rapidly proceed all the way to the formation of Mn(IV), which is released to solution concurrent with hydrolysis, forming structurally disordered, nanoparticulate, and poorly crystalline oxides. This is supported by quantitative fitting of Mn XANES spectra, which indicate that the initial Mn bio-oxides generated by both *Bacillus* sp. SG-1 and *Ps. putida* MnB1 typically exhibit a high average oxidation state (~3.7–4). These precursor or biogenic oxides are aggregates of small (<100 nm) particles only a few layers thick with a local structure similar to δ-MnO_2 or a poorly ordered hexagonal birnessite (Villalobos et al. 2003, Bargar et al. 2005) that has most recently been labeled a "disordered nanoparticulate MnO_2" (Webb et al. 2005b).

These oxides are so highly reactive that they immediately transform to more metastable phases and may never be detected in environmental systems (**Figure 4**). It appears that biogenic Mn oxides are highly labile, sensitive to changes in thermodynamic stability, and should continue to convert in response to dynamic changes in the environmental conditions. Thus, ultimately the solution chemistry [e.g., presence of divalent cations, concentration of Mn(II)] will exert the primary control over the stable Mn mineralogy and average oxidation state detected in natural systems.

Microbial Sorption and Biomineralization of U

Uranium is found as a trace element in most rocks and soils and occurs naturally in groundwaters as a result of weathering processes. In oxidizing environments, uranium is typically found in the highly soluble hexavalent form as the uranyl ion (UO_2^{2+}) and carbonate complexes. In reducing environments, U is prone to reduction and precipitates as uranium oxide minerals such as uraninite (UO_2) and triuranium octaoxide (U_3O_8) in the highly insoluble U(IV) form. In the past 70 years, uranium mining and processing for nuclear fuel rods and weapons production have dramatically increased the levels of uranium in many aquatic systems. The contamination of groundwater is a serious environmental health concern, and recent remediation strategies have increasingly focused on in situ bioremediation. Bioremediation strategies tend to focus on the microbial sorption and

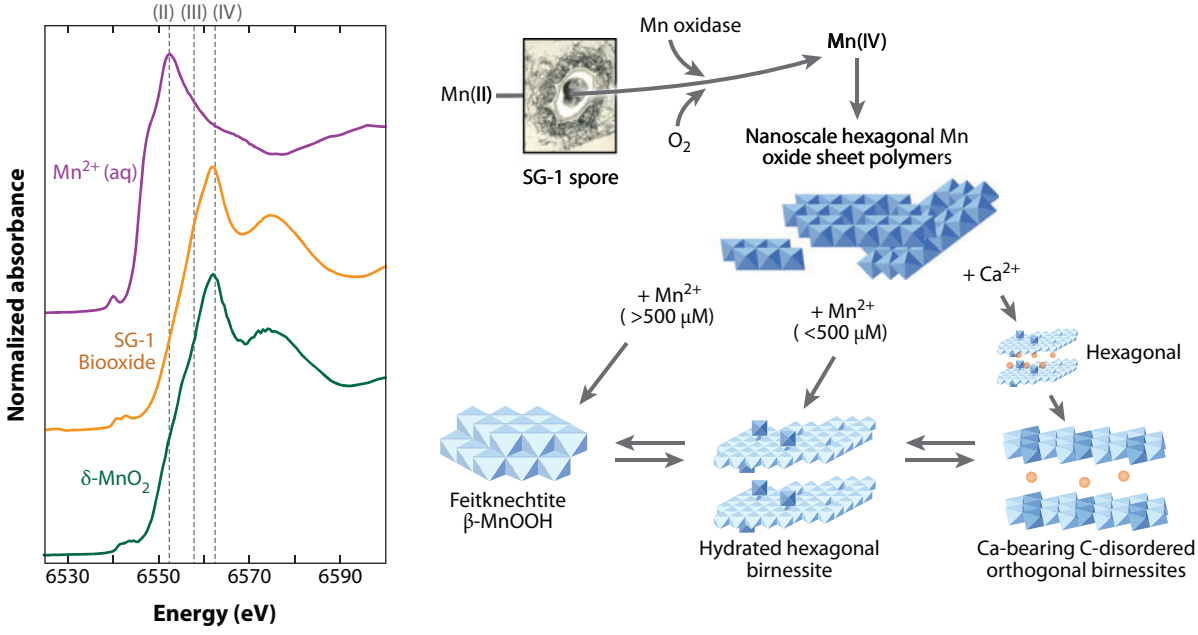


Figure 4

Mn K-edge XANES for Mn(II) reacted with spores of *Bacillus* sp. SG-1. Over time, Mn(II) is biologically oxidized to Mn(IV), forming nanoparticulate hexagonal Mn(IV)-oxide minerals. These biogenic oxides will then transform to a variety of Mn-oxide structures, such as Ca-bearing orthogonal birnessites, hydrated hexagonal birnessites, or feitkneichtite, depending on environmental conditions such as Ca^{2+} concentration or Mn^{2+} concentration. (Conceptual figure prepared with John Bargar to synthesize ideas presented in Bargar et al. 2005 and Webb et al. 2005b.)

reduction of U(VI) to U(IV), the same process that may have originally formed many uraninite mineral deposits (e.g., Lovley et al. 1991).

Synchrotron-based X-ray spectroscopy techniques have elucidated the mechanisms of adsorption of uranyl ions and other soluble uranium species to cell surfaces. Using EXAFS, several different studies have shown that at low pH uranium binds primarily to surface phosphoryl groups, but with increasing pH more complexes form with carboxyl groups (e.g., Kelly et al. 2001, Panak et al. 2002, Nedelkova et al. 2007). The spectra also reveal the coordination of uranyl ions with the functional groups on the cell surfaces, indicating monodentate phosphate complexes and bidentate carboxyl bonds (Kelly et al. 2002, Kemner et al. 2005, Merroun et al. 2005). Sorption of U(IV) species to biogenic metal-oxide minerals is also critically important. EXAFS spectroscopy has been used to determine the coordination chemistry of U complexes associated with biomineralized manganese (Webb et al. 2006) and iron oxides (e.g., O'Loughlin et al. 2003, Jeon et al. 2004, Sani et al. 2004, Kemner et al. 2005) and to determine how sorbed U complexes can inhibit microbial reduction of the associated mineral phases (Brooks et al. 2003, Kelly et al. 2007).

It is feasible to promote uranium immobilization by precipitation of U(VI) by bacterial biofilms (Beyenal et al. 2004) and microbial mats (Bender et al. 2000). Microbial metabolic processes can reduce uranium either by directly using U(VI) as an electron acceptor, or by releasing metabolic by-products that promote reduction. Fredrickson et al. (2000) demonstrated that dissimilatory metal-reducing bacteria such as *Shewanella putrefasciens* CN-32 can utilize both direct and indirect mechanisms of U reduction. More recently, Marshall et al. (2006) were able to show that the

reduction of hexavalent uranium by *Shewanella oneidensis* MR-1 is dependent on electron transfer from c-type chromosomes in the outer membrane. Numerous studies have provided various electron donors to batch reactors or uranium-contaminated field sites to effectively stimulate direct enzymatic bioreduction (e.g., Suzuki et al. 2003, Wu et al. 2007, Kelly et al. 2008, Komlos et al. 2008). XANES was used in all of these studies to confirm the microbial reduction of hexavalent to tetravalent uranium.

The application of EXAFS spectroscopy has also been used to characterize the mineral precipitates formed from biological uranium reduction, often in combination with other methods such as TEM and energy-dispersive X-ray analysis. A number of studies have identified the microbially induced precipitates as nanoparticles (e.g., Suzuki et al. 2002, Kemner et al. 2005, Marshall et al. 2006) on the order of less than one to a few nm depending on the rate of formation (Senko et al. 2007). EXAFS spectra show that these biomineralized nanoparticles tend to be composed of uranyl phosphates, primarily in the meta-autunite mineral group. Whether these precipitates form on the cell surface associated with organic phosphates or in the surrounding medium as a result of inorganic phosphates released by the cells appears to be dependent on pH (Francis et al. 2004, Fomina et al. 2007, Nedelkova et al. 2007).

Microbial Transformations of Sulfur in Modern Systems and Microfossils

The application of synchrotron-based soft X-ray spectroscopy to the sulfur K-edge has been used in a number of studies on biological sulfur speciation and biomineralization. The global sulfur cycle is complicated due to the rapid transformations of S among numerous organic species, inorganic species, and oxidation states. Thus, microbial S metabolisms, as well as the assimilation of sulfur in proteins and their later degradation, are important biotic controls on the sulfur speciation in many environments.

Many of the physical and chemical details regarding microbial S accumulation have remained elusive. It has long been recognized that many sulfur-oxidizing organisms, such as purple and green sulfur bacteria (both anoxygenic phototrophs), take up sulfur and store it internally in microscopically visible globules. Although it is clear that the sulfur is imported in the reduced form, usually elemental sulfur, the chemical structure of the incorporated sulfur has been a long-standing source of debate. Detailed analyses of the XANES spectra of bacterial sulfur globules have led to controversy. In a recent environmental study of the sulfur found in microbial mats in caves, Engel et al. (2007) used XANES to show that the dominant form is cyclooctasulfur (S_8). They concluded that this could be either a biosignature of bacteria that preferentially take up and oxidize S_8 or a sign that the polymeric form of sulfur is preferred, thus leaving cyclooctasulfur to accumulate. These findings are supported by a previous XANES study by Pickering et al. (2001), which shows that the structure of the sulfur in the globules of many different strains best fits that of cyclooctasulfur. However, Prange et al. (1999, 2002) show that the sulfur in globules varies significantly across different bacterial groups. Although the sulfur in the globules of *Beggiatoa alba* and *Thiomargarita namibiensis* indeed consists of S_8, those of *Acidithiobacillus ferrooxidans* are made of polythionates, and the globules in various purple and green sulfur bacteria consist mainly of polymeric sulfur chains. Lee et al. (2007) also found sulfur chains in the globules of *Thermoanaerobacter sulfurigignens* and *Thermoanaerobacterium thermosulfurigenes* using XANES analysis and identified chain end groups made up of organics. Similarly, in 2007, Franz et al. showed that the purple sulfur bacterium *Allochromatium vinosum* takes up only elemental sulfur in the polymeric form. The contrasting results from these various studies arise from differences in data collection and spectral interpretation and clearly demonstrate the need for further studies of the oxidation states and transformations of sulfur species by microorganisms.

Another key metabolic pathway involving the transformation of sulfur is the disproportionation of thiosulfate ($S_2O_3^{2-}$) in anoxic sediments. Bacterial disproportionation of thiosulfate involves splitting the two sulfur atoms to produce sulfide (e.g., sulfide HS^-) and sulfate (SO_4^{2-}). Historically, it was assumed that there was no net change in the −2 and +6 oxidation states of the two sulfur atoms, suggesting that microbial energy conservation could not occur. In one of the earlier studies using synchrotron-based X-ray spectroscopy for the S K-edge, Vairavamurthy et al. (1993) were able to show that the two sulfur atoms in thiosulfate are in the −1 and +5 oxidation states. These results show that the microbial disproportionation of thiosulfate must be a redox reaction, involving the transfer of electrons, and thus can form the basis for microbial metabolism.

An important aspect in understanding the role of microorganisms in the sulfur cycle is determining whether S transformations are localized inside cells, on cell surfaces, or completely removed from the organisms as secondary reactions. In a study of hydrothermal sediments collected from the Mid-Atlantic Ridge, López-García et al. (2003) isolated several filamentous epsilon-Proteobacteria that metabolized sulfur (both sulfur oxidizers and reducers). López-García et al. then generated S oxidation-state maps to determine the spatial distributions of three different sulfur species along the filaments and demonstrated that S was being metabolized in situ within the filaments.

Similar techniques have been applied to putative microfossils in order to confirm their biogenicity. The small sizes of microorganisms and their lack of hard bodies make fossilization unlikely. The best indications that microorganisms were once present in a geological sample usually come from chemical fingerprints such as biominerals. There have been various mineralized structures discovered in both recent and ancient deposits that strongly resemble microbial filaments or coccoid cells, but their biogenicity cannot be confirmed from morphology alone. In these cases, the in situ biomineralization resulting from metabolisms such as sulfate reduction (which often leads to metal-sulfide precipitation on the exterior of cells) plays an important role in preservation of the cells, and chemical analyses of such biominerals are key to determining whether or not they are true microfossils. Synchrotron-based X-ray spectroscopy techniques such as micro-XANES of sulfur K-edges are ideal for nondestructively analyzing the mineralogy of these putative microfossils.

In a study of relatively recent microfossils, Foriel et al. (2004) analyzed mineralized filaments collected from a piece of an inactive chimney wall collected from the East Pacific Rise (EPR). The EPR is a location of active hydrothermal activity, with large inputs of reduced compounds such as hydrogen sulfide. Dense microbial mats and biofilms have been found along active chimney walls and seeps, often with sulfur-metabolizing organisms well represented. Foriel et al. used micro-XANES to quantify and map trace elements, C-H bonds, and sulfur speciation in the microfossils extracted and compared the data to actively mineralizing bacterial filaments collected from hydrothermal vents along the Mid-Atlantic Ridge (**Figure 5**). Both the modern and fossilized filaments showed heterogeneous distributions of sulfate, sulfite, and organic sulfur, which can be interpreted as a sign of sulfur metabolism.

Lemelle et al. (2008) were the first to apply synchrotron-based X-ray techniques to ancient microfossils. Using both scanning X-ray microscopy in fluorescence mode and XANES at the sulfur K-edge, Lemelle et al. analyzed Precambrian microfossils from the 700- to 800-million-year-old Draken Formation in Svalbard, Norway. These techniques enabled them to map sulfur within the microfossil cell walls, showing a likely endogenous origin. They were also able to identify the sulfur not only in the reduced state, but also more specifically as a part of heterocyclic organic thiopene-like compounds. Such data significantly strengthen arguments regarding the biogenicity of the microfossils.

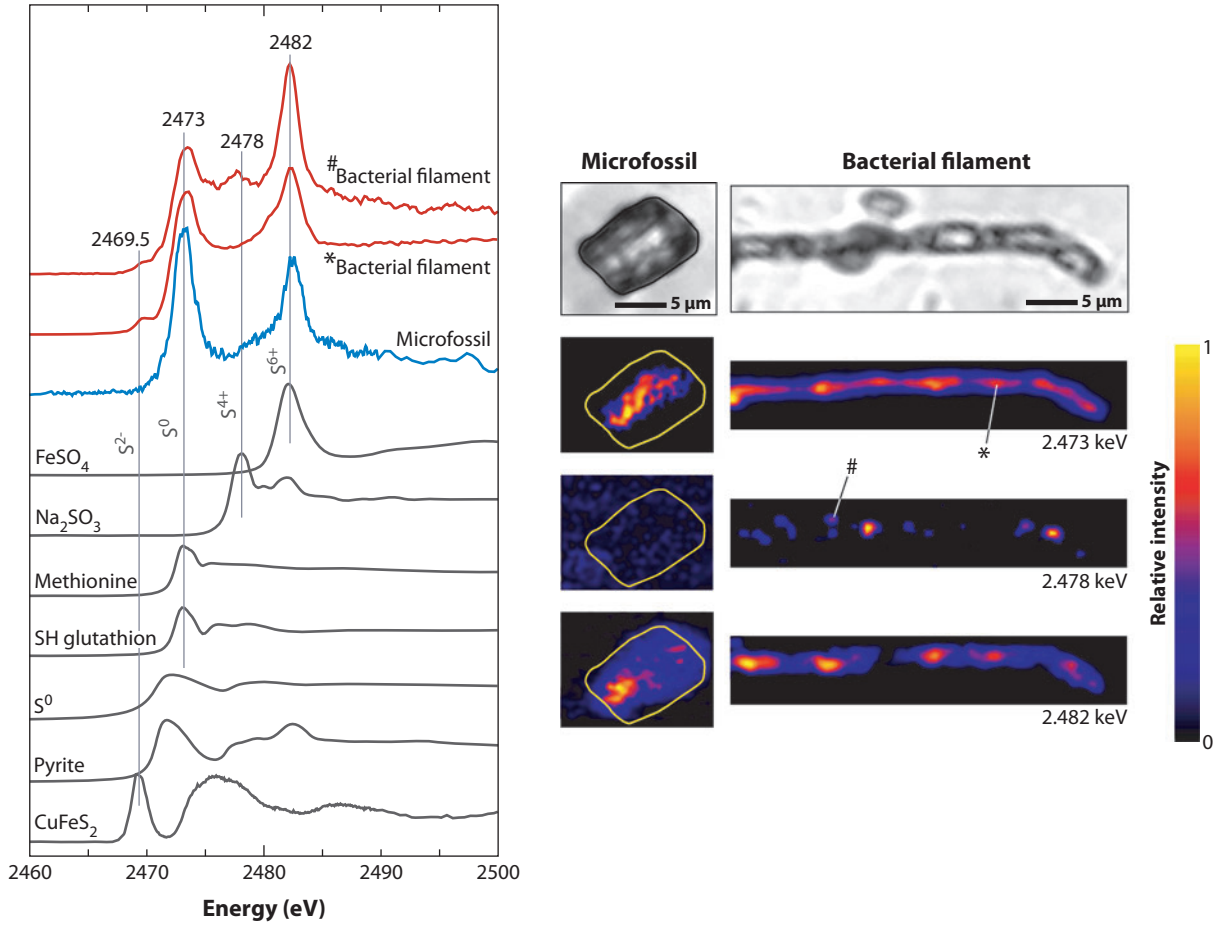


Figure 5

Example of S K-edge micro-XANES spectra collected for select model sulfur compounds, a bacterial filament from the Mid-Atlantic Ridge, and a microfossil from the East Pacific Rise. Three sulfur distribution maps were generated at 2.473 eV, 2.478 eV and 2.482 eV to identify sulfate, sulfite, and organic sulfur surrounding the filament and microfossil. (Figure reproduced with permission from Foriel et al. 2004; copyright Elsevier, 2004.)

CURRENT CHALLENGES AND NEW FRONTIERS

Although one of the most fundamental reasons to use synchrotron-based X-rays is the high brightness and brilliance of the beams produced, many studies of the microbe-mineral interface focus more than 10^{11} photons s^{-1} into a very small spot size or cross section. Thus, efforts to achieve high element sensitivity can result in significant localized damage, as commonly observed in other high-resolution techniques such as transmission electron microscopy. Beam-induced photoreduction of redox-sensitive elements and biominerals enmeshed in organic matrices can occur, and metabolic activity is typically halted for living organisms, although extensive viability tests have not been conducted. Therefore, the techniques must still be optimized and carefully monitored for experimental artifacts, and it is anticipated that cryogenic apparatus will be increasingly used. In addition, there is a need for greater availability of dedicated beamline scientists to help continue

technique development; initiate geomicrobiologists in the intricacies of sample preparation, data collection, and analysis; and to help coordinate the assembly of appropriate model-compound libraries needed to accurately deconvolute complex X-ray absorption spectra.

Fortunately, synchrotron-based X-ray spectroscopies and microscopies are becoming increasingly routine in geochemical and geomicrobiological studies, and we now anticipate increasing applications in the related field of astrobiology. Astrobiology also integrates many different disciplines, from the physical and life sciences to humanities and politics, with the common goal of seeking to better understand the nature and origin of life in the universe. The study of biosignatures is key in the search for both extraterrestrial life and ancient life on Earth and is ripe for new mechanistic and nanoscale approaches. Synchrotron-based methods have already been successfully applied in a number of biomineralization and biosignature studies on terrestrial rocks (e.g., Philippot et al. 2003; Benzerara et al. 2004, 2007; Foriel et al. 2004; De Stasio et al. 2005; Lemelle et al. 2008). Although there have been a limited number of studies in which these techniques were applied to extraterrestrial materials, the results have been intriguing.

The Alan Hills meteorite (ALH84001) received worldwide attention in 1996 when McKay et al. (1996) announced that it contained possible martian microfossils. These structures have since been the source of much debate, as their origins cannot yet be unequivocally proven as biogenic. One important discovery of McKay et al. was the presence of polycyclic aromatic hydrocarbons (PAHs) on the meteorite in association with carbonates. In 2000, Jacobsen et al. used STXM methods and carbon K-edge spectroscopy to confirm the results of McKay et al. and better constrain the locations of the organic carbon. Other studies have also discovered possible signs of life in meteorites, using soft X-ray spectroscopy to identify important biological compounds (e.g., Lemelle et al. 2003, 2004). Similar techniques have also been used to investigate organic matter in interplanetary dust particles, which has important implications for understanding the origin of life (Flynn et al. 2003a,b). The future application of high-resolution synchrotron-based X-ray imaging and spectroscopic techniques to samples returned from other planets and asteroids should prove to be an exciting endeavor.

SUMMARY POINTS

1. A quantitative and predictive understanding of the microbial controls on geochemical processes requires information on the chemical reactions that occur at the interfaces between microbial organisms and mineral surfaces.
2. Synchrotron radiation (SR) can be used to nondestructively probe the distribution and chemical state of elements across the periodic table in complex natural materials by providing X-rays with high brilliance and brightness that are tunable across a large energy range.
3. Hard X-ray microprobe, X-ray standing wave, and X-ray spectroscopic techniques have been successfully used to mechanistically understand the microbial sorption, oxidation/reduction, and biomineralization of numerous transition metals and heavy elements in biogeochemical systems.
4. Rapid advances in soft X-ray microscopy and spectroscopic techniques have led to powerful methods necessary for imaging complex biological structures and the minerals they template in their natural (e.g., hydrated) state.

5. Increased availability and training in these specialized techniques, community development of model compound libraries, and continued improvements in spatial resolution and sensitivity should enable SR techniques to become routine tools in modern microbe-mineral studies, analyses of microfossils, and future investigations of extraterrestrial materials.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affecting the objectivity of this review.

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