

Review article

A review of spectroscopic methods for characterizing microbial transformations of minerals

Gill G. Geesey^{a,b,*}, Andrew L. Neal^c, Peter A. Suci^a, Brent M. Peyton^d

^aDepartment of Microbiology and Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717-3520, USA

^bBiotechnology Department, Idaho National Engineering and Environmental Laboratory, Idaho Falls, ID 83415-2203, USA

^cDepartment of Microbiology, Montana State University, Bozeman, MT 59717-3520, USA

^dDepartment of Chemical Engineering, Center for Multiphase Environmental Research, Washington State University, Pullman, WA 99164-2710, USA

Received 17 September 2001; received in revised form 28 February 2002; accepted 28 February 2002

Abstract

Over the past decade, advances in surface-sensitive spectroscopic techniques have provided the opportunity to identify many new microbiologically mediated biogeochemical processes. Although a number of surface spectroscopic techniques require samples to be dehydrated, which precludes real-time measurement of biotransformations and generate solid phase artifacts, some now offer the opportunity to either isolate a hydrated sample within an ultrahigh vacuum during analysis or utilize sources of radiation that efficiently penetrate hydrated specimens. Other nondestructive surface spectroscopic techniques permit determination of the influence of microbiological processes on the kinetics and thermodynamics of geochemical reactions. The ability to perform surface chemical analyses at micrometer and nanometer scales has led to the realization that bacterial cell surfaces are active sites of mineral nucleation and propagation, resulting in the formation of both stable and transient small-scale surface chemical heterogeneities. Some surface spectroscopic instrumentation is now being modified for use in the field to permit researchers to evaluate mineral biotransformations under in situ conditions. Surface spectroscopic techniques are thus offering a variety of opportunities to yield new information on the way in which microorganisms have influenced geochemical processes on Earth over the last 4 billion years.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Biogeochemistry; Spectroscopic techniques; Microorganisms

1. Introduction

Biogeochemistry is a discipline of great importance to our understanding of Earth's evolution and the

potential for bioremediation of metal-contaminated and radionuclide-contaminated subsurface sites. Recent advances in this area have been largely due to the application of surface-sensitive spectroscopic techniques to studies on microbe–mineral interactions and more specifically the role of microbial activities on metal, radionuclide and mineral oxidation state that in turn influences their solubility and reactivity toward other components in surface sediments, soils and subsurface environments. These techniques provide

* Corresponding author. Department of Microbiology and Center for Biofilm Engineering, Montana State University, Bozeman, MT 59717-3520, USA. Tel.: +1-208-526-2233; fax: +1-208-526-0828.

E-mail address: gill_g@erc.montana.edu (G.G. Geesey).

useful molecular and atomic information at single cell or microcolony scale. Some spectroscopic techniques such as Raman imaging can be used to obtain chemical maps of a mineral surface under fully hydrated conditions important for preservation of the integrity of biological systems involved in mineral transformations. This review describes advances in our understanding of inorganic mineral transformations by microorganisms in nature and under controlled laboratory conditions that are based on surface spectroscopy. The review also identifies features and limitations of the different spectroscopic techniques that are relevant to studies on microbe–mineral interactions.

2. Background

Biogeochemical research in recent years has changed the way we view life on Earth and in particular has expanded greatly our views on microbial ecology and diversity. These advances have, in part, resulted from our need to identify novel approaches to contain and detoxify hazardous waste that is buried in subsurface environments. The discovery in the 1980s of a subsurface biosphere containing viable and potentially active microbial populations spawned a large number of investigations to evaluate their value in contaminant detoxification, immobilization and stabilization. We are now at a point where interdisciplinary research is essential, and fields of science that previously have not been engaged in common activities must come together to solve these challenging problems.

Historically, mineral transformations involving microorganisms were evaluated from a priori knowledge of the solid phase mineralogy, and measurements of solution phase chemistry were used to infer microbiological modification of the solid phase. For example, dissimilatory iron reduction is typically assessed by the Ferrozine assay (Stookey, 1970; Viollier et al., 2000), which is used to quantify the amount of Fe(II) in solution (Kazem and Lovley, 2000; Roden et al., 2000). When insoluble products are formed during mineral biotransformation, these products are solubilized prior to solution phase analysis (Roden and Zachara, 1996; Fredrickson et al., 2000). Solution phase assays have also been used to evaluate biotransformations of mine-

erals containing other elements such as Cr(VI), Mn(IV), and U(VI) (Francis et al., 1994; Kazem and Lovley, 2000; Wielinga et al., 2001).

Recently, microbiological studies have utilized solid phase minerals synthesized in the laboratory for better control over the mineral structure of the starting material. Synthetic iron oxides, including goethite (α -FeOOH), lepidocrocite (γ -FeOOH), ferrihydrite ($\text{Fe}_5\text{OH}_8 \cdot 4\text{H}_2\text{O}$) and hematite (α -Fe₂O₃) have been prepared for assessment of metal reduction by microorganisms (Cornell and Schwertmann, 1996). The preparation of amorphous MnO₂ has been described by Lovley and Phillips (1988), while the production of birnessite (δ -MnO₂) films has been reported by Brulé et al. (1980). Because these preparations have been well characterized and appear to be similar to naturally occurring minerals, they are convenient to use in controlled laboratory studies.

The approach described above was used by Roden et al. (2000) to compare the rate and extent of bacterial reductive dissolution of a synthetic crystalline Fe(III) oxide-coated sand under batch culture and continuous-flow column reactor culture conditions. In this study, X-ray diffraction (XRD) was used to characterize the goethite-like mineral structure of the Fe oxide coating before solubilization by the Fe(III)-reducing bacterium *Shewanella putrefaciens* strain CN32. The results demonstrated the importance of advective removal of Fe(II) from the vicinity of the bacterial cell surface on the extent of Fe reduction and biomass production.

Generally, solution phase analysis yields limited information on the nature of the inorganic solids formed in the presence of microorganisms or the inorganic material adsorbed to surface-associated microbial biomass. Knowledge and control of the aqueous phase chemistry and hydrodynamics and application of mathematical models can overcome this limitation of solution phase analysis to quantify sorption of dissolved metal ions to a solid phase. By employing a bioreactor to control hydrodynamics, an aqueous phase with defined chemistry, and a chemical equilibrium model (MINEQL), Nelson et al. (1995) simulated the influence of the surface-associated microbial population on Pb distribution between the aqueous phase and the biofilm components on iron oxide and glass surfaces. The results indicated that only 10–20% of the Pb adsorbed to biofilm were

associated with cells and that the bulk of Pb adsorption sites in the biofilm were associated with extracellular polymeric material. In this case, solid phase analysis would have provided independent evidence to support the model predictions of Pb distribution between cell and extracellular polymeric components.

In summary, solution phase analyses provide information on total and reduced metal and radionuclide concentrations, are amenable to determination of rates of biotransformation of chemical species that reside external to the cell, and permit assessment of sorption and precipitation phenomena. Restricting analyses to the solution phase, however, limits the type of information one can obtain on the chemical transformations that occur at mineral or other solid surfaces where biological oxidation or reduction may lead to the formation of surface complexes undetectable in solution.

3. Solid phase mineralogical and chemical analysis

Analysis of the solid phase on which microbially induced biotransformations occur offers the opportunity to obtain a wealth of biogeochemical information that cannot be acquired through solution phase analysis. Many solid phase-associated biogeochemical processes can be evaluated by surface spectroscopic analysis. Ideally, reactors used for biotransformation studies should accommodate both solution phase and solid phase analyses. Wielinga et al. (2001) used a stirred-flow reactor to recover both the solid and the solution phases to determine the reduction rates of Cr(VI) in the presence of iron (hydr)-oxides and dissimilatory iron-reducing bacteria (DIRB). A filter at the top of the reactor retained the biomass and insoluble inorganic material for subsequent solids analysis while allowing the liquid phase and soluble components such as Cr(VI) and Fe(II) in the reactor effluent to be recovered for solution phase assays.

3.1. X-ray diffraction

X-ray diffraction has been used for many years to determine the symmetry of crystals and to measure lattice parameters to high accuracy and has been used extensively to identify geological material through crystal structure. In fact, generation of interpretable

XRD patterns can only be obtained for material displaying a crystalline structure (Table 1). The diffraction pattern obtained from a sample is then compared with that of known minerals provided by databases such as the International Center for Diffraction Data for sample identification or by theoretical interpretation. The depth of penetration of the X-rays varies with X-ray source, sampling angle, and the type of mineral. Using a CuK_α source at a 30° sampling angle, the penetration depth for hematite is approximately $3\text{ }\mu\text{m}$, whereas with a MoK_α source at a 90° sampling angle, the penetration depth for alumina is approximately $675\text{ }\mu\text{m}$. Slurries of solid phase material exposed to cultures of microorganisms are typically recovered and subjected to analysis by powder XRD after drying on a glass slide in the appropriate gas phase. The distance between crystalline layers (D -spacing) obtained from X-ray diffractograms has been used to identify uraninite (UO_2) as a product of microbiological uranyl acetate and metaschoepite reduction (Fredrickson et al., 2000). This approach cannot be used to identify uraninite as a product of solid phase U(VI) reduction, however. XRD was also used to demonstrate that siderite (FeCO_3) is a product of magnetite (Fe_3O_4) reduction by Fe-reducing bacteria in bicarbonate-buffered medium (Dong et al., 2000), whereas Brown et al. (1997) used powder XRD to demonstrate that hematite is a product of magnetite biotransformation. Palladium-loaded microbial biomass, after washing with organic solvents and air drying, was evaluated by XRD to demonstrate the feasibility of elemental Pd recovery using sulfate-reducing bacteria (Lloyd et al., 1998). Neal et al. (2001) employed thin-film XRD, where the precipitate is observed as a surficial film, to identify a hexagonal pyrrhotite (Fe_7S_8) structure associated with ferrous sulfides formed by sulfate-reducing bacterial biofilms on hematite. In other studies, precipitates of iron sulfides formed in the presence of sulfate-reducing bacteria yielded powder XRD patterns that lacked reflections indicative of crystalline iron sulfide phases (Herbert et al., 1998).

The lack of well-defined crystalline phases among precipitates formed as a result of microbial activity often limits the utility of XRD in characterizing inorganic reaction products. Also, the large beam size associated with XRD requires relatively large quantities of material to produce a pattern. Precipitates formed in liquid microbial cultures are typically

Table 1
Features of different surface analytical techniques used to investigate microbe–mineral interactions

Technique	Elements	Detection limit	Spatial resolution	Penetration depth	Aqueous phase	Oxidation state	Coordination chemistry	Spatial information	Structural information	Limitations	Features
XRD	Those forming crystals	2%	100 nm	3–675 μm	No	Yes	Inorganic	No	Yes	Crystalline phases only	Mineralogy characterization
Synchrotron-XRD	Fe minerals to date		100 nm		Yes	Yes	Inorganic	Yes	Yes	Crystalline phases only	Mineral transformation reactions in situ
SEM-EDS	Atomic no. > 8	10^{-15} g (0.1–1%)	0.3 μm	1 μm	No	No	No	Yes	Limited	Limited chemical/mineral information	Spatial relationships between elements, microbes and solid phase; quantitative analysis
TEM-EDS	Atomic no. > 8	10^{-15} g (0.1–1%)	10–100 nm	Thin section (10–100 nm)	No	No	No	Yes	Limited	Same as SEM-EDS	High-resolution spatial relationships between elements, microbes and solid phase
HR-TEM electron diffraction	Those forming crystal phases	10^{-16} g	100 nm	Thin section (10–100 nm)	No	No	No	Yes	Yes	Crystalline phases only	Structure of nanometer-size crystals; spatial relationships between crystals and microbial structures
EF-TEM	Atomic no. > 1		10 nm	Thin section (10–100 nm)	No	No	No	Yes	Yes	Elemental imaging	Identification of amorphous and crystalline phases; spatial relationships between inorganic phase and microbial cell structures
EELS	Atomic no. > 1	10^{-20} g; (300 atoms in case of phosphorus)	10 nm	Thin section (10–50 nm)	Yes	Yes	Yes	Yes	No	Thin specimen required; requires knowledge of atomic and solid-state physics, electron optics and electronics	Identification of oxidation states of metals associated with hydrated microbial cells

XPS	most	Tens of micrometers	Tens of Angstroms	No	Yes	Yes	No	No	Interference from surface contamination	Surface sensitive, oxidation state, organic and inorganic compounds
Raman	Those involved in bonds yielding large Stoke's shift	10 μm	70 μm in water; near surface	Yes	Yes	Yes	Yes	Limited	Dissolved solutes must be in 1–5% concentration range	Spatial information on dissolved and precipitated organic and inorganic material; nondestructive, real-time sampling of biological material
XANES	Hard X-rays: metals, actinides; Soft X-rays: C, O, N, Si	Tens of nanometers	Tens of micrometers	Yes	Yes	No	Yes	Yes	Beamline accessibility	Information on average local structure; oxidation state of a metal or radionuclide
EXAFS	Same as XANES	Tens of nanometers	Tens of micrometers	Yes	No	Yes	Yes	Yes	Beamline accessibility	Information on average short-range order around an element in non crystalline material
X-PEEM	Metals, actinides, C, O, N, Si	Tens of nanometers	Thin section (tens of micrometers)	Yes	Yes	No	Yes	No	Not yet applied to microbe–metal interactions	Evaluation at specific depths
Mössbauer	Fe, Ni, Zn, Ge, Kr, Rb, Ru, Sn, Sb, Te, I, Cs, La, Hf, W, Re, Os, Ir, Pt, Au, Hg, Tl, Ra, Th, Pa, Np, U, Pu, Am, Cm		Surface atoms, 10–20 μm	No	Yes	Yes	Yes	Yes	Not applicable to some elements or minerals of interest	Resolution of different Fe minerals when present in same sample

spread over a surface to dry before XRD interrogation in order to accommodate the limited depth of penetration of the electron beam into the sample, thus disrupting spatial relationships between the micro-organisms and the different mineral phases that are present. Samples containing complex mixtures of crystalline phases or of a predominant amorphous nature are difficult to interpret. However, XRD can provide positive identification of those mineral phases that display crystalline structure.

3.2. *In situ* time-resolved X-ray diffraction using synchrotron X-ray source

Recently, the use of synchrotron radiation to enhance the brilliance of X-rays has made it possible to preserve spatial relationships and follow mineral transformation reactions *in situ* (Table 1). Anoxic and oxic reactions during abiotic pyrite formation were followed for the first time in a Fe-S system using *in situ* time-resolved X-ray diffraction by Cahill et al. (2000). In one set of experiments, the incident X-ray beam was reduced to 1.0×0.5 mm, and the X-rays diffracted from the sample were collected on a translating image plate system. The reaction cell consisted of a 0.7-mm horizontally mounted quartz capillary mounted on a Huber four-circle goniometer. A full diffraction pattern was collected from a portion of the imaging plate, the plate was translated to expose a fresh area of the sample, and a new diffraction pattern was collected. With this translating image plate system, time resolution for these experiments was 5 min or less. Using this approach, it was demonstrated that mackinawite (Fe_{1+x}S) is the only Fe-S phase to occur during heating to 120 °C in the presence of H_2S under anoxic conditions. However, with the controlled introduction of small quantities of oxygen, mackinawite rapidly transformed to greigite (Fe_3S_4) and then to pyrite (FeS_2) with goethite ($\alpha\text{-FeOOH}$) present throughout the heating period. Typically, XRD of solid samples at a synchrotron source can detect less than 1.0% by weight of a secondary phase. However, with interference from water and the steel sample cell of the translating image plate system, the detection limit was estimated to be closer to 5.0%. It may be possible to reconfigure the reaction chamber in order to incorporate a biological component in studies of nucleation reactions of FeS and other minerals.

3.3. Mössbauer spectroscopy

Application of Mössbauer spectroscopy to investigate chemical and physical phenomena of surfaces began in 1964 with studies involving iron and tin. Mössbauer spectroscopy is based on nuclear transformations involving recoilless gamma resonance fluorescence (radiation, absorption, scattering), most successfully in isotopes having long-lived low-lying excited nuclear energy states, ^{57}Fe being of particular utility. The Mössbauer effect can be detected by monitoring radiation, which is resonantly reemitted by the absorber after resonance absorption of the incident γ -rays. Although Mössbauer spectroscopy has focused primarily on ^{57}Fe and Sn using ^{57}Co and ^{119}Sn radiation sources, it can be applied to a wide range of elements (Table 1). Mössbauer spectroscopy provides information on a broad range of chemical and physical phenomena at solid surfaces (Table 2). Chemical shifts and quadruple splitting in Mössbauer spectra of iron compounds can be used to identify those compounds and the Fe oxidation state. Mössbauer conversion electron spectroscopy has been used to determine qualitatively and quantitatively various iron oxides and oxyhydroxides like magnetite, hematite, wustite and $\alpha\text{-FeOOH}$, $\beta\text{-FeOOH}$ and $\gamma\text{-FeOOH}$, even when these different minerals coexist in the sample (Gutlick et al, 1978). ^{57}Fe Mössbauer spectroscopy can also yield depth-selective Mössbauer spectra over 10–20 μm by changing from one type of backscattered radiation to another (i.e., resonantly scattered γ -rays, L shell conversion electrons, K_α X-rays, and K_L Auger electrons). The conversion electrons and Auger electrons yield spectra of the <1000 atoms proximal to the surface of a sample, making it a

Table 2
Chemical and physical phenomena interrogated by Mössbauer spectroscopy (Gutlick et al., 1978)

Surface atoms
Electronic state and local symmetry
Magnetic properties
Intramolecular and intermolecular vibrations
Surface diffusion
Adsorption processes
Chemistry of surface compounds
Catalysis
Topochemical reactions
Boundary effects on internal atoms of a solid

useful technique to study oxide films. Mössbauer has been used to good effect in both laboratory and environmental samples to identify iron carbonate (siderite) and green rust formation in laboratory culture by *S. putrefaciens* strain CN32 (Kukkadapu et al., 2001) and in recent anoxic sediments from the Seine estuary (Boughriet et al., 1997).

3.4. High-resolution electron diffraction

High-resolution transmission electron microscopy (HR-TEM) has been used to obtain images and structural information of very small (nanometer-size) crystalline particles, including those associated with mineral-transforming bacteria (Fig. 1). At high magnification, lattice fringes can be resolved whose spacing is used to deduce crystal structure. Convergent beam diffraction (CBD) is accomplished using an incident-beam diameter of the order of 20 nm. Besides providing information on crystal symmetry, CBD can be used to measure small (0.1%) changes in lattice parameters due to atomic concentration gradients. Features of this technique are presented in Table 1. Watson et al. (2000) used the spacing of the lattice rings to deduce a greigite structure in nanometer-size crystals formed by sulfate-reducing bacteria in the presence of sulfate and iron. Similarly, Neal et al. (2001) used electron diffraction data to resolve a hexagonal structure associated with iron sulfides produced by sulfate-reducing bacteria on hematite, which

supported X-ray diffraction data consistent with a pyrrhotite phase.

Selected area electron diffraction (SAED) has been used to obtain electron diffraction patterns from crystals on the order of 0.1 μm in size. SAED was employed by Posfai et al. (1998) to characterize single crystal iron sulfides formed within magnetotactic bacteria. Donald and Southam (1999) used SAED to identify micrometer-sized cubic minerals associated with sulfate-reducing bacteria as [111] pyrite, having an observed d -spacing of 3.14 Å. Theoretically, one can use this technique to distinguish between an intracellular and a cell surface site of crystal initiation. However, it is often difficult to prepare sufficiently thin sections of sample without disrupting the organic–mineral interface.

3.5. Energy-dispersive X-ray spectroscopy (EDS)

Energy-dispersive X-ray spectroscopy is used in conjunction with electron microscopy to identify elemental composition, relative abundance and distribution within a sample at high spatial resolution (Table 1). EDS does not distinguish oxidation state of elements, which is important in demonstrating the biotransformation of a mineral. When used with a scanning electron microscope (SEM), the level of resolution is on the order of 1 μm and when used with a transmission electron microscope (TEM) can achieve a resolution on the order of 0.1 μm . The EDS

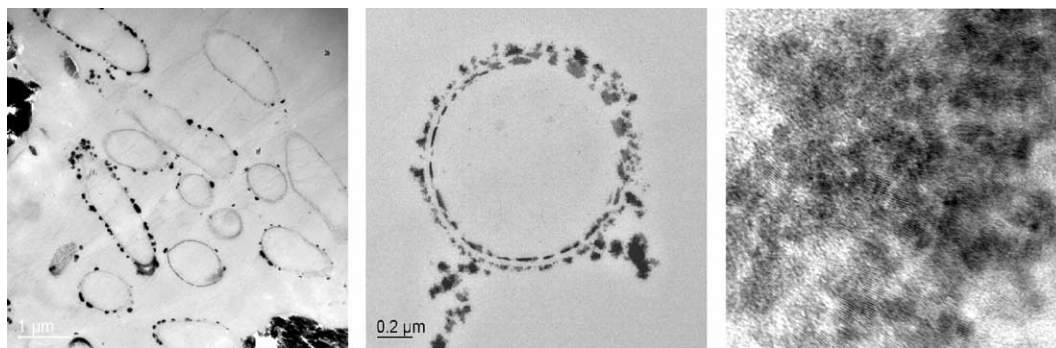


Fig. 1. TEM low (left) and high (center) magnification image of a thin section of the metal-reducing bacterium *S. algae* strain BrY incubated in an anaerobic solution containing 100 mM uranyl acetate. This unstained preparation clearly reveals the presence of uranium precipitates on the cell surface and within the periplasmic region of this Gram-negative bacterium. Extracellular precipitates resulting from the reduction of dissolved U(VI) to highly insoluble U(IV) exhibit d -spacing of 3.0 Å, as shown by high-resolution lattice fringe imaging, and are consistent with the reduced uranium oxide uraninite (right). Courtesy of Yuri Gorby, Bruce Weilinga and Alice Dohnalkova.

energy-dispersive detector partitions and quantifies X-rays emitted from the specimen when probed with the electron beam. Element discrimination is based on the fact that each element emits X-rays of a characteristic energy. Elements lighter than boron cannot be detected using standard EDS, and many elements must be at levels 100 ppm or greater to produce a detectable signal. Because the electrons bombarding a sample at typical energies of ~ 15 keV can penetrate on the order of $1\ \mu\text{m}$ into the specimen, the emitted X-rays provide information on elemental composition of the bulk material. Before analysis under high vacuum, samples must be dehydrated. Spatial relationships between biological and mineral components may be disrupted during dehydration, even when steps are taken to stabilize biological structures through chemical fixation.

Donald and Southam (1999) employed SEM-EDS to determine the extent of conversion of FeS to FeS₂ at the surface of cells of sulfate-reducing bacteria based on relative abundance of Fe and S at these locations. Lloyd et al. (2001) used TEM-EDS to demonstrate that the products of Cr(VI) reduction and Te(IV) reduction were associated with cells of sulfate-reducing bacteria. Lloyd et al. (2000) used TEM-EDS to demonstrate the precipitation of Tc at the periphery of Tc-reducing cells of *Geobacter sulfurreducens*. McLean and Beveridge (2001) and McLean et al. (2000) successfully imaged a Cr-reducing pseudomonad by TEM-EDS without fixation or staining due to the electron density contributed by the reduced Cr species adsorbed to the cell. Thus, EDS has become a valuable means of resolving spatial relationships between microbial cells and specific elements associated with mineral phases.

3.6. Energy-filtering transmission electron microscopy (EF-TEM)

Energy-filtering transmission electron microscopy is a powerful new method for detecting and identifying inorganic precipitates formed in the presence of microorganisms. An electron energy filter is used to filter out all inelastically scattered electrons in the precipitate, thus eliminating chromatic aberration from the final image. A two-dimensional elemental image is produced from thin specimens with nanometer resolution in less than 1 min (Table 1). The

method can be used for chemical characterization of both crystalline and amorphous materials that nucleate at the surface of a bacterial cell. The technique has been successfully used to characterize secondary phases in steels (Warbichler et al., 1998). Barker and Banfield (1998) employed EF-TEM to resolve discrete zones of biogeochemical weathering of silicate mineral assemblages containing lichen communities.

3.7. Electron energy loss spectroscopy (EELS)

Electron energy loss spectroscopy is one of the many techniques that can be used to determine structure and/or chemical composition of a solid (Table 1). EELS provides information about chemical, crystallographic, and electronic structure of an element. EELS compares the differences in fine structure of absorption edges (or white lines) of metals in standards of known oxidation state to determine the oxidation state of metal atoms at high spatial resolution. Absorption edges, such as the $L_{2,3}$ absorption edge, are produced by excited electron dipole transitions between core $2p_{3/2}$ and core $2p_{1/2}$ spin-orbit split levels to unoccupied $3d$ levels. Spectra of different $3d^n$ configurations differ and can be used to identify valance state. The minimum detectable concentration or mass fraction is 60 ppm for Ca in a carbon matrix using this technique (Egerton, 1986). In recent years, electron microscopists have used EELS as a method of light-element microanalysis, for which purpose a simple magnetic-prism spectrometer mounted beneath a conventional TEM with scanning transmission electron microscopy facilities is sufficient. Daulton et al. (in press) used EELS to determine the oxidation state of Cr associated with metal encrusted cells of *Shewanella oneidensis* strain DSP10 under hydrated conditions.

3.8. X-ray photoelectron spectroscopy (XPS)

Of the spectroscopic techniques included here, X-ray photoelectron spectroscopy is one of the more established techniques, applicable to both mineral (Hochella 1988) and microbial (Rouxhet and Genet 1991; van der Mei et al., 2000) surfaces (Table 1). XPS exploits the unique energies of photoelectrons liberated from atoms of different elements within a sample exposed to stimulating X-rays. Any electron

with a binding energy lower than that of the stimulating radiation (1486.6 eV for an AlK_{α} source, 1256.6 eV for an MgK_{α} source) may be ejected from a sample. Analysis of the ejected photoelectrons provides elemental and chemical state information. XPS generally probes a few tens of angstroms and is therefore considered a *near-surface* probe. This “surface sensitivity” may be problematic when studying mineral surfaces and associated microbial cells because the chemistry of interest may be buried under other material. At low resolution, XPS provides data similar to EDS (see above) describing elemental abundance in a sample. High-resolution spectra yield information about the valence state of elements present.

Despite potential problems arising from the surface sensitivity of XPS, the technique is used to good effect in probing bacterial–metal interactions. Association of Fe with exopolymers of *Desulfovibrio indonensis* (Beech et al., 1999) and a *Burkholderia* sp. (Johansson and Saastamoinen, 1999) has been identified. Depletion of Fe at the surface of hornblende by an *Arthrobacter* species was detected using XPS by comparing Fe/Si ratios of samples exposed to bacteria to ratios of unexposed samples (Kalinowski et al., 2000). Francis et al. (1994) employed a curve fitting algorithm to the peaks in the U4f core region of the spectrum to demonstrate reduction of U(VI) by a *Clostridium* sp. Curve fitting of peaks in the Fe2p regions of XPS spectra of precipitates produced during growth of *Desulfovibrio* spp. consisted of mixed valence Fe(II)/Fe(III) sulfides that resembled greigite and pyrrhotite (Herbert et al., 1998; Neal et al., 2001).

3.9. Raman spectroscopy

Raman spectroscopy, like infrared spectroscopy, probes quantum state transitions associated with molecular vibrations but relies upon scattering of the incident light rather than absorption (as is the case with infrared). An organic or inorganic molecule, which has an inducible dipole moment, has the potential to produce a Raman spectrum having unique albeit complex features that permit positive identification. Raman has been used to probe the surfaces of metal sulfides exposed to microbial populations known to be important in pyrite dissolution at the Richmond Mine at Iron Mountain in northern Cal-

ifornia (Edwards et al., 2000). Comparison of Raman spectra of the surface of a single pyrite crystal exposed to the microbial populations with the spectra of a pyrite surface exposed to sterile acid solution under otherwise similar conditions revealed three peaks at 470, 217, and 151 cm^{-1} when the microorganisms were present, which were not present under abiotic conditions. The peaks were characteristic of orthorhombic (S_8^0) sulfur. Calibration of the Raman data indicated that the sulfur layer was on the order of several hundred angstroms thick if distributed uniformly across the surface.

Raman spectroscopy was also employed to compare the species of the sulfur deposited on marcasite (FeS_2) and arsenopyrite (AsFeS_2) mineral surfaces in the presence of a sulfur-oxidizing bacterial isolate and in the presence of sterile acid solution. In contrast to pyrite, the surfaces of these other minerals accumulated elemental sulfur (S_8^0) under abiotic conditions but not in the presence of the bacterial isolate (Edwards et al., 2000). These results encouraged reconsideration of a widely accepted mechanism of sulfide mineral dissolution involving sequential oxidation of surface sulfur atoms to form thiosulfate anion, which is then liberated along with Fe^{2+} ions into the bulk aqueous solution (Singer and Stumm, 1968).

The laser beam can be highly focused using a microprobe to obtain Raman spectra at high spatial resolution. Cooney et al. (1999) used this approach to probe the complex geochemistry of the Martian meteorite ALH84001. Raman spectroscopy was used to look for evidence of biochemical signatures of the submicroscopic structures associated with the meteorite. Raman imaging was employed by Boughriet et al. (1997) to identify FeS and pyrite formation in anaerobic sediments from the Seine estuary. By combining Raman microprobe and confocal microscopy, Pasteris et al. (2001) showed that S_8^0 was precipitated intracellularly in the bacterial endosymbionts of the clam *Calypptogena*.

Shortcomings in the sensitivity of Raman spectroscopy are driving the development of instruments with deep ultraviolet (UV) lasers with excitation wavelengths between 224.3 and 248.6 nm. Many organic molecules have electronic adsorption bands, which fall in this range, and coupling of these electronic and vibrational transitions leads to a significant increase in

spectral intensity termed resonance Raman scattering. Many of the molecules, in microbial cells for which resonance Raman is pertinent, have a ring structure, such as the amino acids tryptophan and tyrosine and the nucleic acids guanine, adenine and cytosine. Even though these molecules are found in all cells, resonance Raman spectra vary among species and over different phase of growth in cultures at cell densities between 10^8 and 10^{10} cells/ml (Wu et al., 2001). The utility of deep UV excitation is further emphasized by employing the native fluorescence arising from cellular components (again, amino acids and nucleic acids) to visualize cells as well as to collect resonance Raman spectra. Using this combination, single cells of *S. oneidensis* have been imaged in situ in real time on unpolished surfaces of calcite and in a Mars soil analogue (Storrie-Lombardi et al., 2001). Lasers, which excite in the deep UV, are of little utility in collecting spectra from inorganic molecules that are more typically excited with visible radiation such as the 514.4 and 676.4 nm lines from Ar⁺ and HeNe lasers, respectively.

Because Raman is relatively insensitive to water, high-quality spectra can be readily obtained for fully hydrated samples. Suci et al. (2001) used Raman microscopy to determine nondestructively in real time the concentration of an antimicrobial agent at different locations within a yeast biofilm growing on a germanium surface in the presence of a flowing aqueous phase. By combining native fluorescence imaging and resonance Raman spectroscopy using deep UV excitation for detection of biological constituents with nonresonance Raman spectroscopy using visible excitation for detection of the mineral phases, it should be possible to evaluate mineral transformations at the cellular level under hydrated conditions.

3.10. X-ray absorption spectroscopy

X-ray absorption spectroscopy has evolved as a means to study surface chemistry under fully hydrated conditions. In X-ray absorption spectroscopy, the intensity of a transmitted beam of X-rays is measured as a function of incident wavelength. A synchrotron is used to produce high-energy electrons, which are then captured by a bending device and manipulated to enhance the brilliance of generated X-rays. X-ray absorption edges occur at an incident energy close to

the binding energy of the core electrons of an atom. These absorption edges are analogous to the ionization edges seen in electron energy loss spectra. Fine structure is present in the near-edge region as well as in a region up to several hundred electron volts beyond the edge. Near-edge information is captured by X-ray absorption near-edge spectroscopy (XANES), while information at energies further from the edge is captured by extended X-ray absorption fine structure (EXAFS). Fine structure provides information on the immediate atomic environment and is used to determine interatomic distances relative to a known element even in the case of amorphous samples (Table 1). Synchrotron radiation offers the means to determine the number and identity of coordinating atoms and using model compounds bond angles around an element of interest. Such information is often critical for establishing mechanisms of biotransformations of a mineral by microorganisms.

Beamlines at synchrotron radiation facilities worldwide are becoming increasingly available to evaluate oxidation state and coordination chemistry of atoms of metals, minerals and radionuclides transformed by microorganisms. The high-energy (hard) X-rays from synchrotron sources can penetrate samples that are tens of micrometers in thickness while maintained in a fully hydrated environment. For example, the density and distribution of elements such as chromium and potassium have been obtained in a fully hydrated environment at the resolution of a single bacterial cell using synchrotron X-ray absorption spectroscopy (Fig. 2). Alternatively, low-energy (soft) X-ray absorption techniques are more sensitive to the lighter physiological elements such as C, O, N and Si, but progress in this area has been slow due to specimen charging under X-ray illumination. For detailed reviews on the experimental aspects of X-ray absorption spectroscopy and geological applications of synchrotron radiation, the reader is referred to Meitzner (1998) and Henderson et al. (1995), respectively.

3.11. X-ray absorption near-edge structure

X-ray absorption near-edge structure, typically within 40 eV of the absorption edge, is sensitive to both electronic and molecular (or cluster) structure, providing information on the average local structure and to some extent oxidation state of a metal or

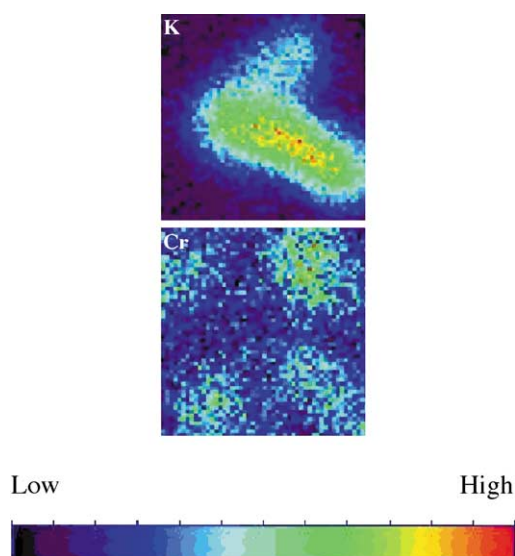


Fig. 2. Potassium (top) and chromium (bottom) elemental maps obtained by synchrotron X-ray absorption spectroscopy at sector 2 at the Advanced Photon Source at Argonne National Laboratory showing spatial relationship between a hydrated bacterial cell (*P. fluorescens*), as defined by potassium density, and chromium precipitated on the outside of the cell. Each image is $25\ \mu\text{m}^2$. Courtesy of B. Lai, Z. Cai, J. Maser, P. Ilinski, D. Legnini, W. Rodrigues, W. Yun, S.T. Pratt and K.M. Kemner (Argonne National Laboratory), M.A. Schneegurt and C.S. Culpa, Jr. (University of Notre Dame) and K.H. Nealson (California Institute of Technology).

radionuclide (Table 1). In general, near-edge absorption features represent transitions to unoccupied electronic levels successively higher in energy, with modulations due to selection rules, as well as multiple scattering phenomena (Durham, 1988). A linear relationship has been shown to exist between the fraction of an element in a solid and relative XANES energy edge (Bertsch et al., 1994). A shift to higher energy with respect to the XANES energy edge indicates an increase in the average oxidation state of an element.

Samples of biological material for XANES or EXAFS analysis may be inserted in a chamber sealed with Mylar film. Alternatively, a small amount of solid sample can be placed in the well of a Lucite plate and sealed with Kapton (polyimide) tape before exposure to the radiation source (Fredrickson et al., 2000). Some chambers have been modified to control the composition of the gas phase to achieve anaerobic conditions.

Francis et al. (1994) utilized XANES to reproduce XPS results indicating the reduction of U(VI) by anaerobic cultures of a *Clostridium* sp. Whereas the XPS results suggested a U reduction product with a valence of 4^+ , XANES data suggested a more reduced oxidation state. Because XANES can probe to greater depths in the sample than is possible with XPS, comparison of results obtained by these two spectroscopic techniques may offer a means to distinguish the oxidation state of an element residing at different locations on or within a bacterial cell.

XANES was used to characterize the solid phase of mineral suspensions in U(VI)-containing aqueous media inoculated with dissimilatory metal-reducing bacteria (Fredrickson et al., 2000). U- L_3 edge XANES revealed that microbiologically reduced solids were predominantly in the U(IV) oxidation state. XANES was also used by these authors to demonstrate microbial reduction of U(VI) in crystalline metaschoepite to a tetravalent form of U.

XANES was used to study the structural properties of strongly magnetic iron sulfide produced in the presence of sulfate-reducing bacteria (Watson et al., 2000). By comparing the Fe K-edge spectrum of this biologically derived material to that of several iron sulfide model compounds, these investigators suggested the magnetic iron sulfide material most closely resembled that of mackinawite, in which the Fe is tetrahedrally coordinated to four S atoms.

Wielinga et al. (2001) employed Cr K-edge XANES to determine valence state of Cr associated with the solid phases produced in a stirred-flow reactor containing iron (hydr)-oxides and dissimilatory iron-reducing bacteria. XANES analysis verified that all of the Cr present was in the reduced Cr(III) state and offered preliminary structural data indicating that the reduced Cr was associated with Fe hydroxides. Bargar et al. (2000) used in situ XANES to characterize the intermediates and products of Mn(II) oxidation by spores of a marine *Bacillus* sp. in a flow-through reaction cell described by Villinski et al. (1999). After agitating bacterial spores in an anoxic MnCl_2 solution for a period of time to allow adsorption of Mn(II) to the bacterial cells, the bacterial suspension was circulated through the reactor at the same time an air-equilibrated Mn(II) solution was introduced and X-ray absorption spectra were measured continuously throughout the subsequent oxida-

tion reaction. Only MnO_2 was detected during the oxidation reaction even though Mn(III)/(II/III) oxides were predicted to be more stable than MnO_2 under these conditions. This approach permits detection of short-lived (>10–60 min) intermediates that occur at abundances >5–10%.

3.12. Extended X-ray absorption fine structure

Extended X-ray absorption fine structure provides information on average short-range order around an element of interest and has been used to characterize interactions between atoms within solution phase metal–organic complexes (Table 1). EXAFS revealed that the U and Fe of a U/Fe/citrate ternary complex are coordinated through carboxylate and hydroxyl groups to the citric acid molecules with chelation of Fe to U occurring through a *m*-citrate bridge (Dodge and Francis, 1997). EXAFS can also be used to identify complexation reactions between metal ions and organic molecules, such as those produced by microorganisms proposed for in situ mobilization and immobilization of radionuclide contaminants in subsurface environments. The reader is referred to a recent review by Charnock (1995) for additional information on the biological applications of EXAFS spectroscopy.

EXAFS is well suited to structural analysis of noncrystalline materials, providing short-range order information in the absence of X-ray diffraction patterns. Using this approach, Suzuki et al. (2001) showed that the local structures around ferric ions in ferric oxyhydroxides are disordered, although the atomic local structures of Fe–O behave as local structural units. Because the reaction products of microbiologically influenced inorganic precipitation reactions at mineral surfaces often have limited crystallinity, EXAFS offers a means of characterizing the nature of these products. Lloyd et al. (2000) employed EXAFS to verify microbiological reduction of technetium (VII) as the pertechnetate ion (TcO_4^-) to Tc(IV) as $\text{TcO}_2 \cdot \text{H}_2\text{O}$. Technetium reduction occurred indirectly via biogenic magnetite formation and directly via a hydrogen-dependent mechanism. EXAFS has also been used in conjunction with XANES to characterize bacterially derived magnetic iron sulfides (Watson et al., 2000). In this study, EXAFS fitting parameters supported XANES data,

suggesting that Fe is mainly tetrahedrally coordinated and likely to be in the form of mackinawite.

3.13. Soft X-ray spectromicroscopy

Soft X-ray transmission microscopy allows high-resolution, through-sample observations of reactions in a wet environment at normal pressure. Originally developed for imaging wet biological material, this technique has expanded its applications to industrial chemistry and environmental science. Interactions between X-ray photons and electrons in the sample produce contrast, which can be displayed as a microscopic image. While phase contrast is used to some extent, photoelectric absorption is the primary mechanism for producing contrast. For each element, absorption varies with X-ray photon energy. Contrast depends primarily upon the difference between absorption by the solid and liquid phases in a sample. The spectroscopic capabilities of the soft X-ray microscope compare the image from a more absorbing region of an element of interest with that obtained from a less absorbing “edge” region. The instrument operates optimally (43 nm resolution) when imaging samples of up to 10 mm in thickness but has been used successfully for samples up to 50 mm in thickness. Details of the technique have been published by Meyer-Ilse (1999).

Spectroscopic capabilities in the soft X-ray range (1–50 nm) permit evaluation at specific depths, thus allowing characterization of reaction products at the interface of an oxide film and base metal in a fully hydrated aqueous environment (Kurtis et al., 2000). The corrosion of iron in water has been studied by this technique by sandwiching a mixture of iron filings and polystyrene beads between two silicon nitride windows mounted on a sample carrier. Iron distribution maps were produced by this approach that revealed locations of iron corrosion. Although this technique has potential to yield novel information on mineral–microbe interactions, to our knowledge, it has not yet been applied in this area.

3.14. X-ray photoelectron emission spectromicroscopy (X-PEEM)

X-ray photoelectron emission spectromicroscopy has developed into a tool to investigate magnetic

properties of materials, microchemistry of cells and tissues, bacteria and their metabolic by-products, and elements that are highly localized minerals (Table 1). X-PEEM can achieve lateral resolution of 20 nm while providing information on elemental composition through total yield XANES. Labrenz et al. (2000) used X-PEEM in conjunction with other spectroscopic techniques to identify nanometer-diameter sphalerite (ZnS) particles formed within biofilms of aerotolerant sulfate-reducing bacteria. X-PEEM confirmed the presence of sulfide and sulfate within the cell-associated mineral aggregate. X-PEEM was used successfully to interrogate the valence state of Mn in a 1-mm thick rock (De Stasio et al., 2000; 2001). X-PEEM has also been applied to wet samples confined in a sealed cell that can be installed in a ultrahigh vacuum system.

X-PEEM interrogation of dehydrated samples composed of lighter elements is restricted by sample charging. Gilbert et al. (2001) attempted to overcome the problem of charging in ultrathin samples of biogenic silica in a cyanobacterial mat under ultrahigh vacuum by inducing photoconductivity. In this case, they used near-UV laser illumination in conjunction with Si L-edge X-ray photoelectron emission spectroscopy to investigate Si mineral chemistry. While the electron yield from the Si was elevated by this approach, the image obtained was highly distorted and the internal structures were obscured. Sample charging thus remains a largely unresolved problem for X-PEEM analysis of most biological material.

4. Conclusions

Microbial transformations of minerals can be investigated by a variety of surface spectroscopic techniques. In addition to identifying and mapping the distribution of elements on a surface (EDS and soft X-ray TEM), some surface-sensitive spectroscopic techniques can resolve the oxidation state of an element (XPS, EELS and X-PEEM) and provide information on the identity of neighboring atoms and their orientation (XANES and EXAFS). That some of these techniques can obtain this information from hydrated samples offers the opportunity to characterize biological reactions involving mineral oxidation and reduction and the adsorption and precipitation processes accompanying

these redox reactions (X-PEEM, RS, EELS, XANES and EXAFS). These techniques will become increasingly useful for determining the range of biogeochemical transformations in which subsurface microorganisms participate and the conditions under which microorganisms may be exploited to remediate metal-contaminated and radionuclide-contaminated sites in subsurface environments.

Acknowledgements

The authors gratefully acknowledge financial support provided by the Natural and Accelerated Bioremediation Research Program (NABIR), Office of Biological and Environmental Research (grant nos. DE-FG03-98ER62630/A001 and DE-FG03-01ER63270) and Environmental Management Science Program (EMSP) (grant no. FG07-98ER62719), U.S. Department of Energy.

References

- Bargar, J.R., Tebo, B.M., Villinski, J.E., 2000. In situ characterization of Mn(II) oxidation by spores of the marine *Bacillus* sp. strain SG-1. *Geochim. Cosmochim. Acta* 64, 2775–2778.
- Barker, W.W., Banfield, J.F., 1998. Zones of chemical and physical interaction at interfaces between microbial communities and minerals: a model. *Geomicrobiol. J.* 15, 223–244.
- Beech, I.B., Zinkevich, V., Tapper, R., Gubner, R., Avci, R., 1999. Study of the interaction of sulfate-reducing bacteria exopolymers with iron using X-ray photoelectron spectroscopy and time-of-flight secondary ionization mass spectrometry. *J. Microbiol. Methods* 36, 3–10.
- Bertsch, P.M., Hunter, D.B., Sutton, S.R., Bajt, S., Rivers, M.L., 1994. In situ chemical speciation of uranium in soils and sediments by micro X-ray absorption spectroscopy. *Environ. Sci. Technol.* 28, 980–984.
- Boughriet, A., Figueiredo, R.S., Laureyns, J., Recourt, P., 1997. Identification of newly generated iron phases in recent anoxic sediments: ^{57}Fe Mössbauer and microRaman spectroscopic studies. *J. Chem. Soc., Faraday Trans. 93*, 3209–3215.
- Brown, D.A., Sherriff, B.L., Sawicki, J.A., 1997. Microbial transformation of magnetite to hematite. *Geochim. Cosmochim. Acta* 61, 3341–3348.
- Brulé, D.G., Brown, J.R., Bancroft, G.M., Fyfe, W.S., 1980. Cation adsorption by hydrous manganese dioxide: a semi-quantitative X-ray photoelectron spectroscopic (ESCA) study. *Chem. Geol.* 28, 331–339.
- Cahill, C.L., Benning, L.G., Barnes, H.L., Parise, J.B., 2000. In situ time resolved X-ray diffraction of iron sulfides during hydrothermal pyrite growth. *Chem. Geol.* 167, 53–63.

- Charnock, J.M., 1995. Biological applications of EXAFS spectroscopy. *Radiat. Phys. Chem.* 45, 385–391.
- Cooney, T.F., Scott, E.R.D., Krot, A.N., Sharma, S.K., Yamaguchi, A., 1999. Vibrational spectroscopic study of minerals in the Martian meteorite ALH84001. *Am. Mineral.* 84, 1569–1576.
- Cornell, R.M., Schwertmann, U., 1996. *The Iron Oxides: Structure, Properties, Reactions, Occurrences and Uses*. VCH, New York, NY.
- Daulton, T.L., Little, B.J., Lowe, K., Jones-Meeham, J., 2001. In situ environmental cell—transmission electron microscopy study of microbial reduction of chromium (VI) using electron energy loss spectroscopy. *Microsc. Microanal.* 7, 470–485.
- De Stasio, G., Gilbert, B., Nelson, T., Hansen, R., Wallace, J., Mercanti, D., Capozzi, M., Baudat, P.A., Perfetti, P., Margaritondo, G., Tonner, B.P., 2000. Feasibility tests of transmission X-ray photoelectron emission microscopy (X-PEEM) of wet samples. *Rev. Sci. Instrum.* 71, 11–14.
- De Stasio, G., Gilbert, B., Frazer, B.H., Neelson, K.H., Conrad, P.G., Livi, V., Labrenz, M., Banfield, J.F., 2001. The multidisciplinary of spectromicroscopy: from geomicrobiology to archaeology. *J. Electron Spectrosc. Relat. Phenom.* 114–116, 997–1003.
- Dodge, C.J., Francis, A.J., 1997. Biotransformation of binary and ternary citric acid complexes of iron and uranium. *Environ. Sci. Technol.* 37, 3062–3067.
- Donald, R., Southam, G., 1999. Low temperature anaerobic bacterial diagenesis of ferrous monosulfide to pyrite. *Geochim. Cosmochim. Acta* 63, 2019–2023.
- Dong, H., Fredrickson, J.K., Kennedy, D.W., Zachara, J.M., Kukkadapu, R.K., Onstott, T.C., 2000. Mineral transformation associated with the microbial reduction of magnetite. *Chem. Geol.* 169, 299–318.
- Durham, P.J., 1988. Theory of XANES. In: Koningsberger, D.C., Prins, R. (Eds.), *X-ray Absorption: Principles, Applications, Techniques of EXAFS, SEXAFS and XANES*. Wiley, New York, pp. 53–84.
- Edwards, K.J., Bond, P.L., Druschel, G.K., McGuire, M.M., Hamers, R.J., Banfield, J.F., 2000. Geochemical and biological aspects of sulfide mineral dissolution: lessons from Iron Mountain, California. *Chem. Geol.* 169, 383–397.
- Egerton, R.F., 1986. *Electron Energy-Loss Spectroscopy in the Electron Microscope*. Plenum, New York, NY, pp. 1–25.
- Francis, A.J., Dodge, C.J., Lu, F., Halada, G.P., Clayton, C.R., 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp. *Environ. Sci. Technol.* 28, 636–639.
- Fredrickson, J.K., Zachara, J.M., Kennedy, D., Duff, M.C., Gorby, Y.A., 2000. Reduction of U(VI) in goethite (α -FeOOH) suspensions by a dissimilatory metal-reducing bacterium. *Geochim. Cosmochim. Acta* 64, 3085–3098.
- Gilbert, B., Margaritondo, G., Douglas, S., Neelson, K.H., Egerton, R.F., Rempfer, G.F., De Stasio, G., 2001. XANES microspectroscopy of biominerals with photoconductive charge compensation. *J. Electron Spectrosc. Relat. Phenom.* 114–116, 1005–1011.
- Gutlick, P., Link, R., Trautwein, A., 1978. *Mössbauer Spectroscopy and Transition Metal Chemistry*. Springer-Verlag, New York, NY, pp. 238–243.
- Henderson, C.M.B., Cressey, G., Redfern, S.A.T., 1995. Geological applications of synchrotron radiation. *Radiat. Phys. Chem.* 45, 459–481.
- Herbert Jr., R.B., Benner, S.G., Pratt, A.R., Blowes, D.W. 1998. Surface chemistry and morphology of poorly crystalline iron sulfides precipitated in media containing sulfate-reducing bacteria. *Chem. Geol.* 144, 87–97.
- Hochella, M.F., 1988. Auger electron and X-ray photoelectron spectroscopies. *Rev. Miner.* 18, 573–637.
- Johansson, L.-S., Saastamoinen, T., 1999. Investigating early stages of biocorrosion with XPS: AISI 304 stainless steel exposed to *Burkholderia* species. *Appl. Surf. Sci.* 144–145, 244–248.
- Kalinowski, B.E., Liermann, L.J., Brantley, S.L., Barnes, A., Pantang, C.G., 2000. X-ray photoelectron evidence for bacteria-enhanced dissolution of hornblende. *Geochim. Cosmochim. Acta* 64, 1331–1343.
- Kasem, K., Lovley, D.R., 2000. Reduction of Fe(III), Mn(IV), and toxic metals at 100 °C by *Pyrobaculum islandicum*. *Appl. Environ. Microbiol.* 66, 1050–1056.
- Kukkadapu, R.K., Zachara, J.M., Smith, S.C., Fredrickson, J.K., Liu, C., 2001. Dissimilatory bacterial reduction of Al-substituted goethite in subsurface sediments. *Geochim. Cosmochim. Acta* 65, 2913–2924.
- Kurtis, K.E., Meyer-Ilse, W., Monteiro, P.J.M., 2000. Soft X-ray spectromicroscopy for in situ study of corrosion. *Corros. Sci.* 42, 1327–1336.
- Labrenz, M., Druschel, G.K., Thomsen-Ebert, T., Gilbert, B., Welch, S.A., Kemner, K.M., Logan, G.A., Summons, R.E., De Stasio, G., Bond, P.L., Lai, B., Kelly, S.D., Banfield, J.F., 2000. Formation of sphalerite (ZnS) deposits in natural biofilms of sulfate-reducing bacteria. *Science* 290, 1744–1747.
- Lloyd, J.R., Yong, P., Macaskie, L.E., 1998. Enzymatic recovery of elemental palladium by using sulfate-reducing bacteria. *Appl. Environ. Microbiol.* 64, 4607–4609.
- Lloyd, J.R., Sole, V.A., Van Praagh, C.V.G., Lovley, D.R., 2000. Direct and Fe(II)-mediated reduction of technetium by Fe(II)-reducing bacteria. *Appl. Environ. Microbiol.* 66, 3743–3749.
- Lloyd, J.R., Mabbett, A.N., Williams, D.R., Macaskie, L.E., 2001. Metal reduction by sulfate-reducing bacteria: physiological diversity and metal specificity. *Hydrometallurgy* 59, 327–337.
- Lovley, D.R., Phillips, E.J.P., 1988. Novel mode of microbial energy metabolism: carbon oxidation coupled to dissimilatory reduction of iron and manganese. *Appl. Environ. Microbiol.* 54, 472–480.
- McLean, J.S., Beveridge, T.J., 2001. Chromate reduction by a Pseudomonad isolated from a site contaminated with chromated copper arsenate. *Appl. Environ. Microbiol.* 67, 1076–1084.
- McLean, J.S., Beveridge, T.J., Phipps, D., 2000. Isolation and characterization of a chromium-reducing bacterium from a chromated copper arsenate-contaminated site. *Environ. Microbiol.* 2, 611–619.
- Meitzner, G., 1998. Experimental aspects of X-ray absorption spectroscopy. *Catal. Today* 39, 281–291.
- Meyer-Ilse, W., 1999. X-ray microscopy and microanalysis. *Encyclopedia of Applied Physics*. VCH Publishers, New York, NY, pp. 324–341.
- Neal, A.L., Techkarnjanaruk, S., Dohnalkova, A., McCready, D., Peyton, B.M., Geesey, G.G., 2001. Iron sulfides and sulfur spe-

- cies produced at hematite surfaces in the presence of sulfate-reducing bacteria. *Geochim. Cosmochim. Acta* 65, 223–235.
- Nelson, Y.M., Waihung, L., Lion, L.W., Shuler, M.L., Ghiorse, W.C., 1995. Lead distribution in a simulated aquatic environment: effects of bacterial biofilms and iron oxide. *Water Res.* 29, 1934–1944.
- Pasteris, J.D., Freeman, J.J., Goffredi, S.K., Buck, K.R., 2001. Raman spectroscopic and laser scanning confocal microscopic analysis of sulfur in living sulfur-precipitating marine bacteria. *Chem. Geol.* 180, 2–18.
- Posfai, M., Buseck, P.R., Bazylinski, D.A., Frankel, R.B., 1998. Iron sulfides from magnetotactic bacteria: structures, composition, and phase transition. *Am. Mineral.* 83, 1469–1481.
- Roden, E.E., Zachara, J.M., 1996. Microbial reduction of crystalline Fe(III) oxides: role of oxide surface area and potential for cell growth. *Environ. Sci. Technol.* 30, 1618–1628.
- Roden, E.E., Urrutia, M.M., Mann, C.J., 2000. Bacterial reductive dissolution of crystalline Fe(III) oxide in continuous-flow column reactors. *Appl. Environ. Microbiol.* 66, 1062–1065.
- Rouxhet, P.G., Genet, M.J., 1991. Chemical composition of the microbial cell surface by X-ray photoelectron spectroscopy. *Microbial Cell Surface Analysis*. VCH, New York, NY, pp. 173–220.
- Singer, P.C., Stumm, W., 1968. Kinetics of the oxidation of ferrous iron. Bituminous Coal Research. Proceedings of the 2nd Symposium on Coal Mine Drainage Research. National Coal Association, Pittsburgh, PA, pp. 12–34.
- Stookey, L.L., 1970. Ferrozine—a new spectrophotometric reagent for iron. *Anal. Chem.* 42, 779–781.
- Storrie-Lombardi, M.C., Hug, W.F., McDonald, G.D., Tsapin, A.I., Nealson, K.H., 2001. Hollow cathode ion lasers for deep ultraviolet Raman spectroscopy and fluorescence imaging. *Rev. Sci. Instrum.* 72, 4452–4459.
- Suci, P.A., Geesey, G.G., Tyler, B.J., 2001. Integration of Raman microscopy, differential interference contrast microscopy, and attenuated total reflection Fourier transform infrared spectroscopy to investigate chlorhexidine spatial and temporal distribution in *Candida albicans* biofilms. *J. Microbiol. Methods* 46, 193–208.
- Suzuki, S., Suzuki, T., Kimura, M., Takagi, Y., Shinoda, K., Tohji, K., Waseda, Y., 2001. EXAFS characterization of ferric oxyhydroxides. *Appl. Surf. Sci.* 169–170, 109–112.
- van der Mei, H.C., de Vries, J., Busscher, H.J., 2000. X-ray photoelectron spectroscopy for the study of microbial cell surfaces. *Surf. Sci. Rep.* 39, 1–24.
- Villinski, J.E., O'Day, P.A., Corley, T.L., Conklin, M.H., 1999. A flow-through cell for in situ, real time X-ray absorption spectroscopy studies of geochemical reactions. In: D.W. Morganwalp, H.T. Buxton (Eds.), U.S. Geological Survey Toxic Substance Hydrology Program, Proceedings of the Technical Meeting, Charleston, SC, March 8–12, 1999. Vol. 1, Contamination from Hard Rock Mining. U.S. Geological Survey Water-Resources Investigations Report 99-4018, pp. 217–225.
- Viollier, E., Inglett, P.W., Hunter, K., Roychoudhury, A.N., van Capellan, P., 2000. The ferrozine method revisited: Fe(II)/Fe(III) determination in natural waters. *Appl. Geochem.* 15, 785–790.
- Warbichler, P., Hofer, F., Hofer, P., Letofsky, E., 1998. On the application of energy-filtering TEM in materials science: III. Precipitates in steel. *Micron* 29 (1), 63–72.
- Watson, J.H.P., Cressey, B.A., Roberts, A.P., Ellwood, D.C., Charnock, J.M., Soper, A.K., 2000. Structural and magnetic studies on heavy-metal-adsorbing iron sulfide nanoparticles produced by sulfate-reducing bacteria. *J. Magn. Magn. Mater.* 214, 13–30.
- Wielinga, B., Mizuba, M.M., Hansel, C.M., Fendorf, S., 2001. Iron promoted reduction of chromate by dissimilatory iron-reducing bacteria. *Environ. Sci. Technol.* 35, 522–527.
- Wu, Q., Hamilton, T., Nelson, W.H., Elliott, S., Sperry, J.F., Wu, M., 2001. UV Raman spectral intensities of *E. coli* and other bacteria excited at 228.9, 244.0, and 248.2 nm. *Anal. Chem.* 73, 3432–3440.