Investigating Microbe-Mineral Interactions: Recent Advances in X-Ray and Electron Microscopy and Redox-Sensitive Methods

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Abstract

Microbe-mineral interactions occur in diverse modern environments, from the deep sea and subsurface rocks to soils and surface aquatic environments. They may have played a central role in the geochemical cycling of major (e.g., C, Fe, Ca, Mn, S, P) and trace (e.g., Ni, Mo, As, Cr) elements over Earth's history. Such interactions include electron transfer at the microbe-mineral interface that left traces in the rock record. Geomicrobiology consists in studying interactions at these organic-mineral interfaces in modern samples and looking for traces of past microbe-mineral interactions recorded in ancient rocks. Specific tools are required to probe these interfaces and to understand the mechanisms of interaction between microbes and minerals from the scale of the biofilm to the nanometer scale. In this review, we focus on recent advances in electron microscopy, in particular in cryoelectron microscopy, and on a panel of electrochemical and synchrotron-based methods that have recently provided new understanding and imaging of the microbe-mineral interface, ultimately opening new fields to be explored.

INTRODUCTION AND KEY QUESTIONS ABOUT MICROBE-MINERAL INTERACTIONS

Geomicrobiology is the study of past and present interactions between microorganisms and minerals. Microorganisms have played a major role in the geochemical functioning and shaping of Earth's surface for several billion years (e.g., Ehrlich 1998, Gadd 2009, Cockell 2010, Southam 2012). Microbes can trigger mineral formation, transformation, and dissolution through diverse chemical reactions, including redox transformations. Heavy metal mobility in acid-mine drainages originating from pyrite weathering (e.g., Fortin et al. 1996, Edwards 2000) and pollutant bioavailability governed by the dissolution of Fe-bearing minerals in soils and sediments (e.g., Borch et al. 2010) both illustrate geochemical processes that can be significantly controlled by microbes. Along the same lines, an increasing number of recent investigations have attempted to assess the effective role of microorganisms in the weathering of the oceanic basaltic seafloor (e.g., Templeton et al. 2009, Bach & Fruh-Green 2010, Orcutt et al. 2010, Edwards et al. 2011). Microbes can also mediate mineral precipitation, a process termed biomineralization. It has been proposed that microbes are involved in the formation of several emblematic sedimentary deposits, including stromatolites (e.g., Reid et al. 2000, Baumgartner et al. 2009, Couradeau et al. 2011) and banded iron formations (e.g., Posth et al. 2008).

Such interactions have long been recognized. Kalkowski suggested in 1908 that microorganisms are involved in the formation of stromatolites (Kalkowski 1908). Drew showed in 1911 that bacteria in culture can mediate $CaCO_3$ precipitation (Drew 1911). In 1936, Cayeux suggested that bacteria may be involved in the formation of phosphorites, although he cautioned that convincing images of such relationships were still missing (Cayeux 1936). More recently, the involvement of bacteria in the dissimilatory reduction of Mn and Fe oxides has been widely studied (e.g., Myers & Nealson 1988). Since then, geomicrobiology has followed several research lines:

1. Some studies have revealed new types of microbe-mineral interactions and/or new microbial species involved in such interactions. For example, some bacteria can form intracellular crystals of magnetite or greigite, which they use to orient along the lines of Earth's magnetic field. These crystals can contribute significantly to the magnetization of sediments (e.g., Li et al. 2010, Lefevre et al. 2011, Lin et al. 2011).
2. Other studies have assessed the diversity of modern microbial communities interacting with minerals in order to better understand whether some specific taxa are more important than others in the mediation of geochemical processes and to draw pertinent conclusions about the ecological functioning and the history of past microbe-mineral interactions (e.g., Blank 2009, Uroz et al. 2009, McAllister et al. 2011).
3. Finally, some studies have dissected the molecular mechanisms involved in microbe-mineral interactions through the use of model bacterial systems that can be cultured in the laboratory. Such approaches include the measurement of reaction kinetics, the determination of product speciation, as well as the identification of the genes and proteins involved in these reactions. Examples of studied organisms include Fe(III)-reducing bacteria, for which the various processes of electron transfer have been scrutinized (e.g., Lentini et al. 2012, Richardson et al. 2012, and references therein), and Fe(II)-oxidizing bacteria (e.g., Bose & Newman 2011, Bonnefoy & Holmes 2012).

Many first-order questions remain wide open in geomicrobiology: To what extent has the evolution of microbial species influenced the mechanisms involved in their interactions with minerals (e.g., microbially mediated redox reactions)? How do these interactions benefit microbes? What are their environmental consequences? What parameters control their intensity, and how can we include those parameters in predictive biogeochemical models?

Besides these far-reaching goals, geomicrobiologists usually have more specific and practical concerns when studying a sample: Who is there—i.e., which microbe is associated with an observed mineral? Did microbes contribute to a given mineral's precipitation, transformation, or dissolution? What are the relative contributions of abiotic and biotic processes to mineral formation or transformation? How could environmental conditions (e.g., redox) influence microbially mediated electron transfer reactions? How has the evolution of such microbial electron transfer mechanisms been recorded in mineral assemblages? How can traces of past microbe-mineral interactions (i.e., biosignatures) be identified in ancient rocks?

The specificity of analyzing a microbe-mineral interface relies on the nature of two components: organic matter and mineral. In the past, these components were studied separately, by distinct scientific communities (microbiologists and mineralogists, respectively), sometimes by using different techniques, and sometimes by using the same instruments but following different protocols. For instance, electron microscopy approaches differ markedly depending on whether the sample is a microorganism or a mineral, because these specimens behave differently during sample preparation and when interacting with an electron beam. Along this line, pioneering work in electron microscopy by Beveridge & Murray (1976) triggered methodological developments for imaging and understanding metal–cell surface interactions down to the nanometer scale. Since then, electron microscopy methods and instruments have undergone increasing development, and major recent advances in geomicrobiology have emerged from these new methods—especially cryo-EM approaches, which we discuss more extensively below. In particular, we discuss the potential to retrieve both structural/compositional/mineralogical and genomic/metabolic information.

The tremendous expansion of geomicrobiology since the 1990s is thus due to the advent of revolutionary techniques, not only in microscopy but also in molecular biology combined with mineralogy and isotopic geochemistry. Advances in our understanding of the major changes surrounding the Great Oxidation Event some 2.4 Ga ago provide an emblematic illustration (Anbar et al. 2007; Kump 2008; Rasmussen et al. 2008; Frei et al. 2009; Konhauser et al. 2009, 2011b; Scott et al. 2011; Czaja et al. 2012). These advances have resulted from the combination of complementary approaches to decipher the co-occurrence of geological and microbiological events during this period—a period that totally and uniquely modeled Earth's surface. Recent key studies include (*a*) the mineralogical and isotopic characterization of banded iron formations (e.g., Chi Fru & Ivarsson 2013, Czaja et al. 2013, Koehler et al. 2013), (*b*) strong geomicrobiological arguments suggesting the microbial origin of these formations (Cloud 1965, Hartman 1984, Posth et al. 2013), and (*c*) macroevolutionary data (David & Alm 2010). Electron transfer reactions taking place at the microbe-mineral interface might be sufficiently specific to this interface to leave traces within the geological record. The second part of this article details some major advances in geomicrobiology based upon relatively new analytical methods for probing redox reactions at microbe-mineral interfaces from the bulk to the nanometer scale.

ORGANIC MATTER–MINERAL INTERFACE: HOW TO SCRUTINIZE A SPECIFIC INTERFACE?

Due to their richness in negatively charged carboxyl groups, bacterial cell surfaces and exopolymeric substances are prone to sorption of metal cations (e.g., Beveridge & Murray 1976, Ferris et al. 2000, Phoenix et al. 2002, Lalonde et al. 2008). They are also preferential locations for the nucleation of minerals that might subsequently grow upon crystallization (Banfield et al. 2000). Intracellular accumulations of calcium carbonates in cyanobacteria (Couradeau et al. 2012) and organelles known as magnetosomes that compartmentalize magnetite or greigite in magnetotactic bacteria (e.g., Lefevre et al. 2011) constitute additional mineral–organic matter interfaces.

Electron microscopy has been a dedicated tool to image the nanometer-sized nuclei of these mineral phases as well as their organic templates.

Looking for Organic Matter Remnants in Rocks

Transmission electron microscopy (TEM) is a powerful analytical tool that has provided priceless information in various fields of Earth sciences (e.g., Lee 2010). Modern TEM provides multiple analytical capabilities that are of great interest to Earth scientists, offering the possibility to image samples down to the atomic scale under various conditions (high vacuum, of course, but also cryo- or even wet conditions; e.g., de Jonge & Ross 2011), to map elements with increasing detection limits by energy-dispersive X-ray spectrometry or energy-filtered imaging, to determine the speciation of elements (including redox state) by electron energy loss spectroscopy (e.g., van Aken & Liebscher 2002, Kim & Dong 2011), and to study mineral structures and map crystal orientation on the basis of electron diffraction. However, the study of microbe-mineral assemblages down to the nanometer scale by TEM has long been limited by sample preparation methods. Ultramicrotomy—which has been a pivotal approach for the study of the ultrastructure of bacteria—is, with few exceptions (e.g., Benzerara et al. 2004a), difficult to use on hard or brittle materials and, more notably, does not provide the high spatial precision needed when dealing with geological samples that contain only few and/or micrometer-sized microbe-mineral assemblages. With the advent of focused ion beam (FIB) milling, this limitation disappeared, and it became possible to prepare any interface between microbes and minerals for subsequent TEM analyses (e.g., Benzerara et al. 2005, Obst 2005). Initial studies used mono-beam FIB microscopes, equipped with a gallium ion beam that could ablate or assist deposition of material on the surface of a sample and provided moderate spatial resolution and surface-sensitive ion imaging. Recent FIB microscopes use dual-beam technology, which integrates the ion beam capability into a high-resolution scanning electron microscope. Dual-beam FIB microscopes thus allow easier localization of the area of interest, and their findings can be correlated with those from other microscopy techniques appropriate to the study of microbes, such as confocal laser scanning microscopy (e.g., Gérard et al. 2005, Ehrhardt et al. 2009), making possible additional analyses such as mapping of heavy metals (Hao et al. 2013) and 3D imaging. More details on the general procedures and examples of applications in Earth sciences can be found in de Winter et al. (2009). FIB now has multiple applications in the study of past and modern microbe-mineral interactions (e.g., Benzerara 2005, Obst 2005, Benzerara et al. 2007, Carlut et al. 2010, Fliegel et al. 2011, Lepot et al. 2011). Many of these studies revealed particular nanoenvironments around microbial cells, which stresses the importance of characterizing reaction products at the nanometer scale. Further improvements include a better assessment of artifacts that are produced. Bernard et al. (2009) and Bassim et al. (2012) showed that organic samples that were either FIB milled or ultramicrotomed retained the same C speciation. The development of FIB milling under cryo-conditions (**Figure 1**), as discussed below, is another recent advance.

Cryoelectron Microscopy: Imaging Organic Matter in a Hydrated State

Organic matter (cells, exopolymers, intracellular organelles, or ultrastructures) is very sensitive to dehydration induced by the vacuum in the microscope stage (Schaedler et al. 2008, Dohnalkova et al. 2010). In addition, conventional preparation of thin sections for TEM (embedding in a resin after fixation and dehydration) is also deleterious to organic molecules, in that it can alter their configuration (e.g., Bleck et al. 2010). Although such preparations are informative (e.g., Benzerara et al. 2004a, Miot et al. 2009b, Schaedler et al. 2009), they necessarily induce a loss of information.

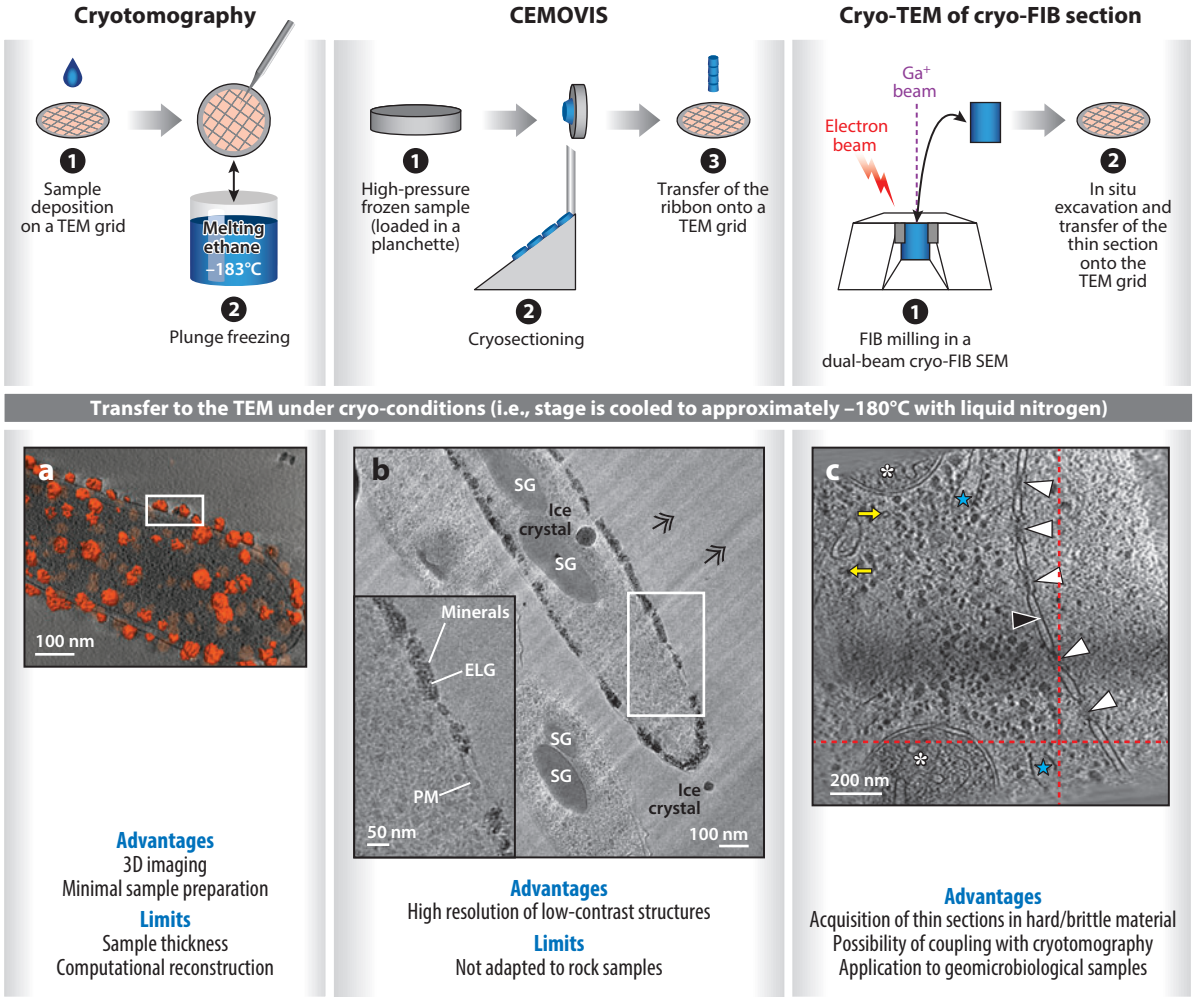


Figure 1

Overview of some cryoelectron microscopy methods adapted to the imaging of microbe-mineral interfaces in their hydrated near-native state. All these methods can suffer from artifacts (e.g., cutting artifacts in CEMOVIS; artifacts related to Ga^+ milling in cryo-FIB) as well as from radiation damage. However, such artifacts can be minimized or even avoided. (*a*) 3D reconstruction of a *Geobacter* sp. cell decorated with nanoaggregates of Fe-rich phases (*red*). Reproduced with permission from Luef et al. 2012. (*b*) CEMOVIS image of *Acidovorax* sp. strain BoFeN1 mineralized by Fe phosphates. Double-headed arrows indicate the direction of knife marks. Reproduced from Miot et al. 2011. (*c*) Slice through the (*x*, *y*) plane of a tomographic reconstruction of a *Dictyostelium discoideum* cell, showing the nuclear envelope (*black arrowhead*) with nuclear pore complexes (*white arrowheads*). Also visible are parts of the rough endoplasmic reticulum (*light blue stars*), tubular mitochondria (*white asterisks*), and microtubules (*yellow arrows*). Reproduced from Rigort et al. 2012. Abbreviations: CEMOVIS, cryoelectron microscopy of vitreous sections; ELG, electron-light globules; FIB, focused ion beam; PM, plasma membrane; SEM, scanning electron microscope; SG, storage granules; TEM, transmission electron microscopy.

Cryo-EM offers a way to bypass these shortcomings by rapidly freezing the sample in vitreous (i.e., amorphous) ice, thereby maintaining the microbe-mineral assemblages in an aqueous, near-native state (**Figure 1**).

First developed by molecular biologists mainly in the 1980s (Adrian et al. 1984, Al-Amoudi et al. 2004, Dubochet 2012), this technique offered unprecedented visualization of biological

ultrastructures (Beveridge 2006, Leforestier et al. 2012) and of the organization of key macromolecules (e.g., Beck et al. 2004, Eltsov et al. 2008, Fischer et al. 2010, Celler et al. 2013). Observations in the TEM are performed at low temperature (approximately −170°C), and low exposure conditions are required to limit radiation damage (usually approximately 10 electrons/Å^2). The low contrast of images obtained by cryo-EM results from the density difference between the organic molecules and the cryoprotectant (e.g., dextran). However, in geomicrobiological samples, metals can act as in situ contrasting agents, and very fine details thus can become visible provided that the samples are mineralized or bound to metals (Miot et al. 2011).

Cryoelectron tomography: imaging of a vitrified sample at multiple angles so that a 3D image of the specimen can be reconstructed

In a cryo-EM analysis, a drop of the sample is deposited onto a conventional carbon-coated TEM grid, rapidly frozen in liquid ethane (or an ethane/propane mixture) cooled by liquid nitrogen, and then transferred to a TEM (**Figure 1**). Whole frozen cells can be visualized by cryoelectron tomography (see Milne & Subramaniam 2009 for technical details). Optimal transmission of the electron beam through the specimen (i.e., with minimal inelastic scattering) is achieved with objects less than 0.5 μm thick (0.5 μm at 0°, i.e., approximately 1 μm at 60°). The ability to prepare sufficiently thin samples is thus crucial (Bouchet-Marquis & Hoenger 2011). Thin sections can be produced by cryo-ultramicrotomy of frozen hydrated samples (i.e., cryoelectron microscopy of vitreous sections, or CEMOVIS) [see Adrian et al. (1984), Matias et al. (2003); see also Miot et al. (2011) for a geomicrobiological application (**Figure 1**)]. A limit of this method, however, is its inadequacy for preparing thin sections of brittle or hard samples (e.g., rocks). Because cryo-FIB can achieve this preparation (Rigort et al. 2010, 2012), it is a promising approach to image microbe-mineral interfaces in ancient rocks. To address a need for complementary biomolecular information, many molecular biologists (e.g., Tokuyasu 1973) combine cryo-EM with immunolabeling of specific molecules on the same sample (see the review by Ripper et al. 2008). As an alternative, correlative methods are now being implemented (Briegel et al. 2010) and will likely provide breakthroughs in future geomicrobiological studies. A promising approach would involve the correlative combination of cryo-EM techniques with light microscopy, in particular fluorescent light microscopy, enabling analyses of microbe-mineral interactions at the population level. Recently, much progress has been made in confocal laser scanning microscopy, which allows the retrieval of phylogenetic and metabolic information. Combining this method with cryo-EM will enable us not only to combine structural observations with label-specific information (on either the phylogeny of the microorganisms or the functional nature of the molecules/proteins) (Celler et al. 2013), but also to determine more rapidly and efficiently the areas to be imaged by cryo-EM.

Cryoelectron Microscopy Study of Microbe-Mineral Interfaces: Main Results and Future Prospects

The widest use of cryo-EM tools in geomicrobiology has been in studies of magnetotactic bacteria (MTB). Cryotomography revealed the subcellular arrangement of the magnetosome membranes that consist of invaginations of the inner membrane in *Magnetospirillum magneticum* strain AMB-1 (Komeili et al. 2006, Murat et al. 2012). Cryo-EM also revealed the association of these organelles with a cytoskeletal network that shares homology with previously described bacterial actin-like proteins (Komeili et al. 2006). Recently, the high-resolution structure of the bacterial actin (MamK) filaments involved in the organization of magnetosomes in AMB-1 was elucidated using cryo-EM (Ozyamak et al. 2012), extending our knowledge of the functional and structural diversity of proteins involved in magnetite biomineralization. Cryo-EM methods have also highlighted substantial interspecies differences in magnetosome membrane genesis (Scheffel et al. 2006, Byrne et al. 2010, Katzmann et al. 2010). However, membranes are difficult to

discriminate when too closely associated with the mineral phase (Jogler et al. 2010). These studies highlight the necessity of correlating results from complementary approaches (e.g., biomolecular, genetic, and microscopic methods) to gain insights into the mechanisms of microbe-mineral interactions.

Apart from studies of magnetotactic bacteria, cryo-EM studies of geomicrobiological samples have been scarce. Notably, they all concern Fe biomineralization (e.g., Miot et al. 2009a, Luef et al. 2012). Cryo-EM has revealed organic structures and molecules that play roles in Fe biomineralization, as nucleation sites and potentially as drivers of Fe oxidation. These include stalks and surface structures in *Gallionella* sp. and *Mariprofundus* sp. (Comolli et al. 2011) and the peptidoglycan and proteins located within the 40-nm-thick mineral layer in *Acidovorax* sp. strain BoFeN1 (Miot et al. 2011) (**Figure 1**).

Generalizing the use of cryo-EM approaches in geomicrobiology will undoubtedly be a pivotal strategy to retrieve information about the mechanisms of biomineralization and microbe-induced weathering processes, as well as to reveal biosignatures in geological samples. The broader use of this technique has been limited mainly by the demands for specialized instrumentation and technical expertise (Beveridge 2006), but these barriers are progressively lifting in an increasing number of laboratories. Beyond Fe biomineralization, there are a multitude of geomicrobiological systems that will benefit from cryo-EM approaches.

One powerful aspect of cryo-EM, described in the final section of this review, is the possibility of combining this method with other analyses such as genetic tools, proteomics, high-resolution secondary ion mass spectrometry (NanoSIMS), Raman spectromicroscopy, and various synchrotron-based techniques (e.g., scanning transmission X-ray microscopy, micro-X-ray fluorescence, micro-X-ray absorption spectroscopy).

Synchrotron-based X-ray absorption spectroscopy methods: based on the interaction of an X-ray beam with the sample at a given energy

X-ray fluorescence (XRF): results from the emission of fluorescence following X-ray absorption and is indicative of the chemical composition of the area scanned

ELECTRON TRANSFER AT THE MICROBE-MINERAL INTERFACE: FROM THE POPULATION SCALE TO THE NANOMETER SCALE

Minerals can serve either as electron acceptors or as sources of energy and electrons for microbes (Lovley et al. 1996, Fredrickson & Zachara 2008). Diverse microbial strategies have been evidenced that support the existence of electron transfers either between microbes and minerals (**Figure 2**) or between different microbial species (Konhauser et al. 2011a, Lovley 2011). For instance, geomicrobiological iron cycling (Emerson et al. 2010, Roden 2012) proceeds through the activity of (*a*) Fe(II)-oxidizing bacteria that convert (dissolved or solid) Fe(II) to (usually solid) Fe(III), thus producing Fe-bearing minerals [(oxyhydr)oxides or phosphates], and (*b*) Fe(III)-reducing bacteria that transfer electrons toward a solid Fe(III) phase, leading to its reduction and even dissolution. As such redox reactions might have also played a key role on the early Earth, especially around the time of the Great Oxidation Event (Konhauser et al. 2005, 2009), it will be important to decipher these electron transfer mechanisms in laboratory (model), modern, and ancient specimens from the sediment scale to the nanometer scale (Wessel & Hmelo 2013).

Mechanisms of Electron Transfer Between Microbes and Electrodes or Minerals

Electrical currents have been measured in marine sediments, connecting organic matter and sulfide oxidation in the anoxic zone (deep in the sediment) to aerobic respiration (in the overlying surface oxic zone) (Risgaard-Petersen et al. 2012); these currents were recently attributed to the activity of filamentous bacteria (*Desulfobulbaceae*) functioning as living electrical cables (Pfeffer et al. 2012). Microelectrode measurements of key parameters such as oxygen fugacity and pH provided evidence of electron transport at the sediment scale. Electrochemical devices are increasingly used

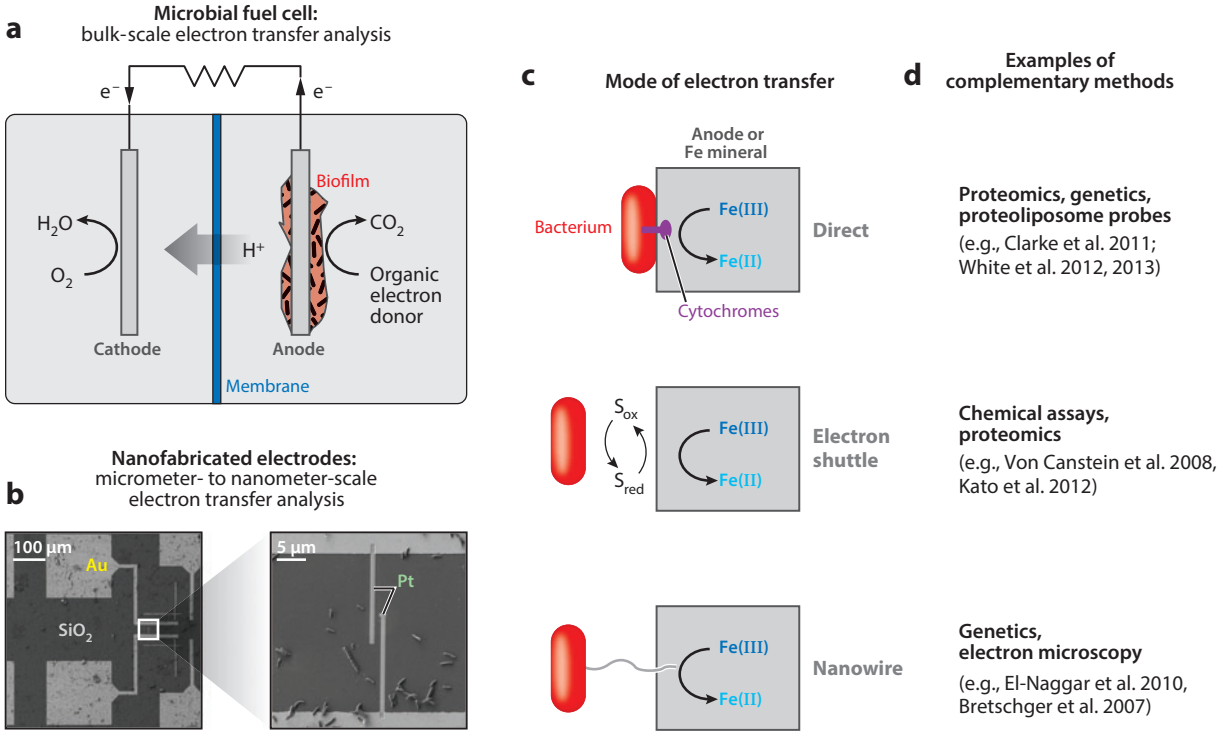


Figure 2

Electrochemical (*a*,*b*) and complementary (*d*) methods for investigation of electron transfer at the microbe-mineral interface. (*a*) A microbial fuel cell evaluates electron transfer at the bulk scale. Biofilm (*pink*) that developed at the anode surface transfers electrons derived from oxidation of organic compounds to the electrode. (*b*) Nanofabricated electrodes provide a direct measurement of electron transfer along nanowires at the micrometer to nanometer scale. Reproduced from El-Naggar et al. 2010. (*c*) Diagram of the different modes of electron transfer observed for Fe-reducing bacteria. (*d*) Examples of complementary nonelectrochemical, non-synchrotron-based methods that provided information on the mechanisms of electron transfer for each of the three modes described in panel *c*. Abbreviations: S_{ox}, oxidized electron shuttle; S_{red}, reduced electron shuttle.

not only to retrieve energy from microbial reactions (e.g., Lovley 2008, Miot et al. 2014) but also as tools to investigate the mechanisms of electron transfer between microbes and electrodes or minerals (Reimers et al. 2001, Lovley 2008). Such an approach was augmented by the advent of microbial fuel cells (MFCs) (e.g., Lovley 2008) (**Figure 2**), which led to the identification of a diversity of microorganisms (Bond 2002, Logan 2009). Predominant among these are *Desulfuromonas* and *Geobacter* species, which can completely oxidize organic matter and use the electrode as the sole electron acceptor (Bond & Lovley 2003) in reactions involving proteins implicated in extracellular electron transfer to solid Fe- or Mn-bearing phases (Nevin et al. 2009, Tremblay et al. 2011).

Miniaturization of electrodes down to the nanometer scale (**Figure 2**) enabled the direct and local measurement of electron transport along electrically conductive appendages known as nanowires in the Fe(III)-reducing bacterium *Shewanella oneidensis* (Gorby et al. 2006, El-Naggar et al. 2010). The nanofabrication of Pt electrodes and the subsequent electric measurements were all performed in a FIB. Similar results were also obtained using conducting probe–atomic force microscopy (Reguera et al. 2005; El-Naggar et al. 2008, 2010). Complementary techniques, such as

proteomic and electrochemical analyses, are critical for understanding the mechanisms of electron transfer along these appendages and the conductive properties of the pili themselves, as well as for detecting these structures in natural environments (Bretschger et al. 2007, El-Naggar et al. 2010, Boesen & Nielsen 2013, Vargas et al. 2013); this undertaking deserves increasing attention in the coming years.

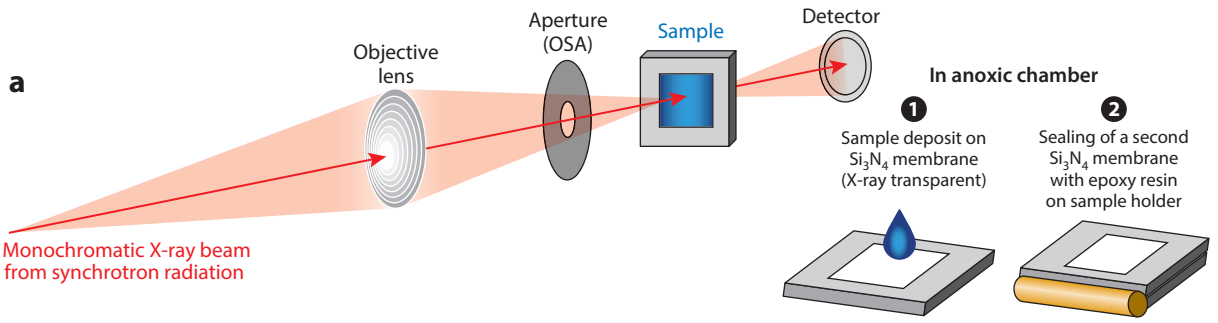
Much like electron shuttles released by bacteria (e.g., Torres et al. 2010), naturally abundant redox-active organic molecules (namely humic acids) were shown to act as electron acceptors for microbial respiration (Lovley et al. 1996, Wolf et al. 2009). In the same way, Fe(III) oxides were also shown, by using a combination of electrochemical and chemical methods, to play a role in electron transfer between bacterial species (Kato et al. 2012). The rates of direct electron transfer from c-type cytochromes to extracellular solid Fe(III) compounds were also recently revisited (Clarke et al. 2011, White et al. 2013). Using proteoliposomes—including the enzymatic MtrCAB complex from *S. oneidensis* and a mixture of dithionite and methyl viologen playing the role of both electron reservoir and redox indicator—as well as a combination of cryo-EM and immunolabeling, White et al. (2012, 2013) showed that this molecular wire mechanism (Fredrickson & Zachara 2008) might support the rates of electron transfer needed for anaerobic solid-phase Fe(III) respiration.

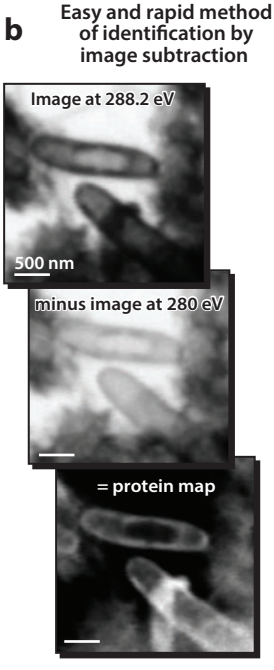
Future challenges for improving bioelectrochemical devices and bettering our understanding of electron transfer mechanisms between bacteria and minerals include optimizing the viability of cells within the biofilms that may develop at the electrode contact (e.g., Nassif et al. 2002, Gautier et al. 2006). We must also more extensively explore the diversity of electron transfer mechanisms in the tree of life (to date, studied species are almost exclusively model microorganisms such as *Geobacter*, *Shewanella*, and *Sideroxydans*). Not only are these questions central to optimizing technological devices such as MFCs, but they also connect to fundamental issues for geomicrobiologists: How did environmental (in particular redox) conditions influence the evolution of life (in particular electron transfer mechanisms)? How did these electron transfer systems evolve during Earth's history? How has this evolution shaped the mineral record? Undoubtedly, combined biochemical, geochemical, and phylogenetic approaches will provide priceless clues.

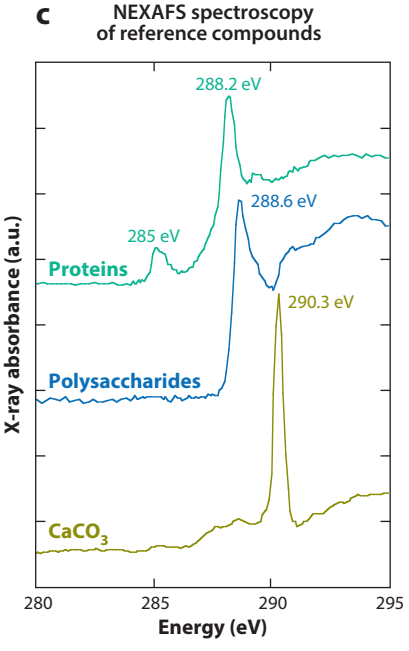
Traces of Microbe-Induced Electron Transfer Recorded in Minerals

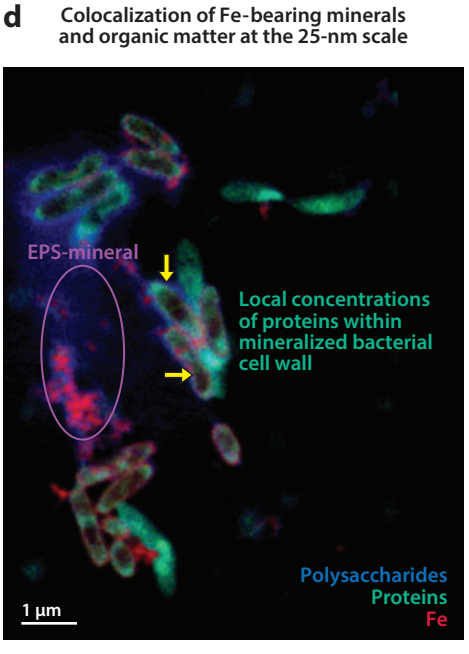
Biofilms are central in the process of electron transfer at mineral interfaces, for instance at the surface of basaltic glass in the oceanic crust. At the scale of the biofilm, microenvironments may exhibit rather specific and locally constant physicochemical properties, whereas at the scale of microorganisms (micrometer to nanometer scale), strong redox heterogeneities may result from their metabolic activity (Wessel & Hmelo 2013). Such redox heterogeneities, sometimes associated with persistent organic compounds, may constitute biosignatures to be sought in ancient samples. It is thus crucial to deploy a panel of methods to determine the speciation and redox state of major elements such as Fe and to localize organic molecules from the bulk scale, through the micrometer (biofilm) scale, to the nanometer scale.

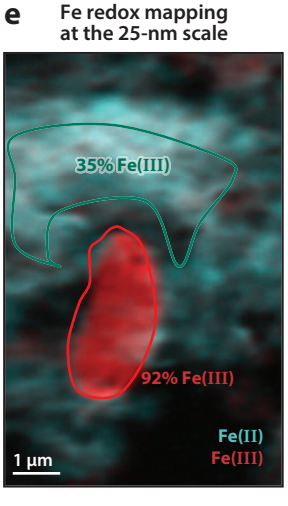
Some synchrotron-based tools are specifically suited to the study of microbe-mineral interactions in laboratory samples as well as in modern and ancient geological specimens, from the micrometer to the nanometer scale. A thorough review of the methods dedicated for geomicrobiology applications can be found in Templeton & Knowles (2009). Applications of synchrotron-based X-ray techniques for the investigation of redox heterogeneities at microbe-mineral interfaces are well illustrated by studies of oceanic crust weathering and iron biomineralization (e.g., Benzerara et al. 2007, Templeton et al. 2009, Carlut et al. 2010, Fliegel et al. 2011) (**Figure 3**).

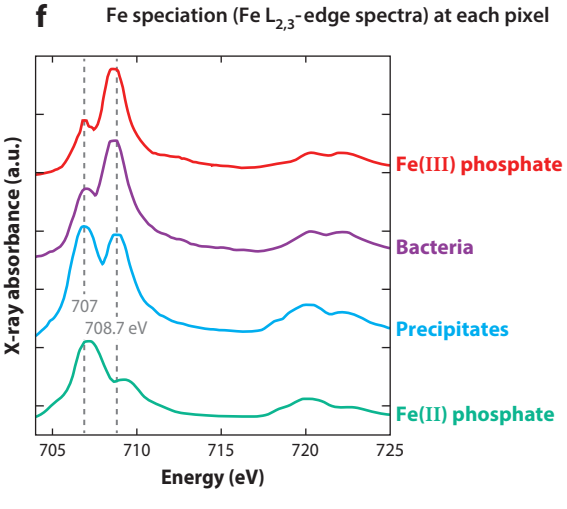


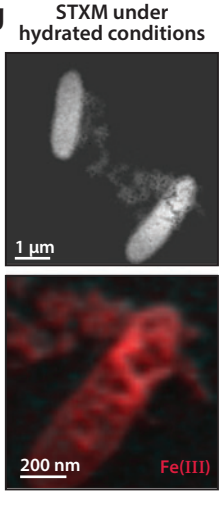












Complementary to bulk-scale methods [e.g., X-ray absorption near-edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS)] that allow determination of the mean metal speciation in amorphous and crystalline phases (e.g., Petit et al. 2001, Miot et al. 2009b, Hohmann et al. 2011, Pantke et al. 2012), X-ray absorption spectroscopy (XAS) methods that provide micrometer-scale resolution are of great interest for geomicrobiological issues. This is indeed the adequate scale to map the microenvironments produced by biofilms interacting with solid matrices. Micro-X-ray fluorescence (micro-XRF) provides elemental maps at this scale. For example, it gave strong evidence (combined with XANES data at the Mn K edge) that Fe-Mn crusts at the surface of basaltic glass are derived from dissolved Fe and Mn species in seawater (Templeton et al. 2009).

Scanning transmission X-ray microscopy (STXM) is a choice method to characterize the microbe-mineral interface at the microorganism scale (Cosmidis & Benzerara 2014) (**Figure 3**). This synchrotron-based transmission microscopy method provides images with spatial resolution of approximately 25–50 nm. Using soft X-rays (<4 keV), it also gives information about the speciation of elements from carbon (C K edge) to arsenic (As L edge), i.e., of light elements entering the composition of the organic (microbe) portion and of heavier elements from the mineral portion. This semiquantitative method allows estimation of the Fe redox state (Bourdelle et al. 2013) as well as the C speciation (e.g., Bernard et al. 2007, Solomon et al. 2012) at the 50-nm scale via calibration with reference spectra. STXM has increasingly been used in recent years to characterize bacteria-mineral interfaces and to reveal redox heterogeneities at the cellular scale (i.e., <1 μm) that are associated with organic compounds (e.g., Benzerara et al. 2004b, Chan et al. 2009, Miot et al. 2009b, Carlut et al. 2010, Cosmidis et al. 2013) and might constitute valuable biosignatures to be sought in the rock record. For instance, STXM analyses (Benzerara et al. 2007) have contributed evidence to the ongoing debate about the potential biogenicity of tubular microchannels commonly observed in weathered oceanic basaltic glass (Banerjee & Muehlenbachs 2003, Furnes et al. 2005). Indeed, these microtubes were shown to be filled with palagonite (a secondary weathering product of basalt) exhibiting a slightly higher Fe(III)/[Fe(II)+Fe(III)] ratio than that of the surrounding basaltic glass and to be associated with organic carbon and carbonate (Benzerara et al. 2007). Such features were interpreted as potential biosignatures of past Fe biooxidation. Similarly, redox heterogeneities and gradients of Fe(II) oxidation state over a micrometer range were demonstrated to be produced through anaerobic microbial Fe(II) oxidation in laboratory experiments on the nitrate-reducing Fe(II)-oxidizing BoFeN1 strain and the phototrophic Fe(II)-oxidizing strain SW2, respectively (Miot et al. 2009b,c). STXM is also well suited for the identification of organic templates produced by bacteria, e.g., proteins, polysaccharides, and lipopolysaccharides (Chan et al. 2009; Miot et al. 2009b,c; Solomon et al. 2012).

X-ray absorption near-edge spectroscopy (XANES): provides information about the speciation of an element when it absorbs X-rays at or above a characteristic energy

Extended X-ray absorption fine structure (EXAFS): provides information about the nature of the atoms surrounding an element absorbing X-rays and about their distance from the absorber

Figure 3

Methods for scanning transmission X-ray microscopy (STXM) study of microbe-mineral interfaces. (*a*) Diagram of an STXM. (*Inset*) Method for the preparation of air-sensitive samples (e.g., for Fe redox study). (*b*) Method of image subtraction for a rapid determination of C speciation within a sample (e.g., for localization of bacteria within a geological sample). (*c*) Near-edge X-ray absorption fine structure (NEXAFS) spectra at the C K edge recorded on reference compounds used for the mapping of proteins and polysaccharides in panel *d*. (*d*) STXM composite map of Fe, proteins, and polysaccharides derived from Fe $L_{2,3}$ edges and C K-edge stacks as described in Miot et al. (2009a). Fe is associated with proteins accumulated within the cell wall and with exopolymeric substances in these cultures of the iron-oxidizing bacterium *Acidovorax* sp. strain BoFeN1. (*e*) Fe redox map, showing Fe(III)-rich precipitates at the cell contact and extracellular Fe(II)-rich phases. (*f*) Corresponding Fe $L_{2,3}$-edge spectra compared with reference Fe(II) and Fe(III) phosphate spectra. (*g*) STXM image and Fe(III) map recorded on a hydrated sample. Beam radiation damage may lead to an overestimation of the Fe(III)/Fe_{total} ratio. Abbreviation: EPS, exopolymeric substance; OSA, order-sorting aperture.

Combining STXM analyses with other methods—such as NanoSIMS (Behrens et al. 2012, Remusat et al. 2012), angle-scan tomography (Schmid & Obst 2014, Schmid et al. 2014), X-ray magnetic circular dichroism (Coker et al. 2012), and low-energy X-ray fluorescence in the STXM (Hitchcock et al. 2012)—can provide complementary information. STXM analyses of fully hydrated samples (**Figure 3**) may also gain some technical advances and provide a better understanding of microbe-mineral interactions in the future. Such an approach has been used in a few studies (e.g., Toner et al. 2005, Leung et al. 2009, Schmid et al. 2014). However, radiation damage induced by the copresence of redox-sensitive phases and organic matter under the X-ray beam is nonnegligible and must be quantified for each system. Although cryo-TXM has recently been developed, some technical challenges remain for the advent of cryo-STXM (Schneider et al. 2012).

SUMMARY POINTS

1. Detailed imaging of the microbe-mineral interface can be obtained from a combination of electron microscopy tools adapted to the study of laboratory or modern environmental samples as well as of ancient rocks, for example, through the FIB milling preparation of thin sections for the TEM.
2. Substantial progress in the characterization of microbe-mineral assemblages is just beginning with the application of cryo-EM methods to the study of geomicrobiological samples.
3. Electrochemical approaches provide quantitative information about the mechanisms of electron transfer at the microbe-mineral interface.
4. Synchrotron-based X-ray absorption techniques can be used to probe the traces left by recent or ancient microbial redox reactions at the microbe-mineral interface, from the bulk scale (XANES, EXAFS), through the biofilm scale (micro-XRF), to the nanometer scale (STXM).
5. Future breakthroughs in the understanding of microbe-mineral interactions will likely originate from the correlative combination of cryo-EM methods with light (fluorescence) microscopy as well as from the concomitant use of complementary methods, such as synchrotron-based analyses, electrochemistry, and isotopic, genetic, phylogenetic, and proteomic analyses.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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