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SYNCHROTRON RADIATION INFRARED SPECTROMICROSCOPY: A NON-INVASIVE CHEMICAL PROBE FOR MONITORING BIOGEOCHEMICAL PROCESSES

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Abstract

A long-standing desire in biogeochemistry is to be able to examine the cycling of elements by microorganisms as the processes are happening on surfaces of earth and environmental materials. Over the past decade, physics, engineering and instrumentation innovations have led to the introduction of synchrotron radiation-based infrared spectromicroscopy. Spatial resolutions of less than ten micrometers and photon energies of less than an electron volt make synchrotron infrared spectromicroscopy non-invasive and useful for following the course of biogeochemical processes on complex heterogeneous surfaces of earth and environmental materials. In this review, we will first briefly describe the technology, and then present several examples demonstrating its application potentials of the technology in probing and imaging biogeochemical processes.

I. Introduction

Microorganisms are important agents in the geochemical cycling of elements. For example, they can change the speciation of metal ions and organic carbons in soils and sediments by releasing complexing agents and by enzymatically catalyzing reactions [1-16]. They also can modify the composition of pore fluid and groundwater through controlled mineral weathering and precipitation [6, 7, 15, 17-32]. Most importantly, they can transform many environmental pollutants to less toxic species [33-48]. With the discovery of diverse microbial communities thriving in every possible environment[49-67], researchers in biogeochemistry are now increasingly focused on expanding their understanding of roles of environmental microorganisms at a more fundamental level. Many important microbial processes happen at the interface between microorganisms and earth or environmental materials. This necessitates a more comprehensive study and analysis of how microorganisms through their wide range of metabolic capabilities interact with their environments, especially at surfaces of earth and environmental materials. This surface biogeochemistry can be highly variable at a microscopic level because of the small-scale (ranging from one micron to hundreds of microns) surface heterogeneity which involves the distributions of clusters of mineral-inhabiting microorganisms and reactive molecules of metal oxides and organic molecules. The methodology commonly employed to study this type of heterogeneous biogeochemical phenomenon is a combination of microscopic imaging and synchrotron radiation-based X-ray spectroscopy techniques. The interested readers can read reviews [68, 69] and other more recent relevant studies [7, 8, 11, 34, 36, 39, 41, 42, 46, 60, 70-94]. SR-based x-

ray spectromicroscopy studies have provided important and unique information about how microorganisms interact with earth and environmental materials. However, the energy range associated with SR-based X-ray spectromicroscopy techniques is between tens to thousands of electron volts (eV), which can adversely affect, harm, or even kill the microorganisms. Consequently it has limited the use of these techniques to measuring the biogeochemical actions only at single time points.

Being able to measure in real-time sequential molecular changes in a biogeochemical system as they are happening on surfaces of earth and environmental surfaces has been a long-standing scientific desire in biogeochemistry. The new availability of SR-based infrared sources to the scientific community in the 1990s provided this opportunity. Our group began developing a SR-based Fourier transform infrared (FTIR) spectromicroscopy approach in 1998 for studying biogeochemical transformation of environmental pollutants, choosing the reduction of hexavalent chromium by living microorganisms on mineral surfaces as the initial application [95]. Prior to the availability of SR-based infrared facilities, this type of *in vivo* and *in situ* measurements was very difficult for two reasons. First, earth materials inherently have low infrared reflectivity surfaces. High quality infrared spectroscopy measurements of earth and environmental materials require a high infrared photon flux on small surface areas. Without a SR-based source, one often needs to co-add thousands to tens of thousands of spectral scans which can be prohibitively time-consuming. Second, the infrared measurements of live microorganisms had been problematic. Investigators were required to feed bacteria with a substantial quantity of deuterated substrates in order to

obtain sufficient signal-to-noise spectra [96]. However, deuterated substrates are known to alter activities and even produce stresses in microorganisms [97, 98].

Presently, there are thirteen synchrotron infrared spectromicroscopy facilities around the world with several more under construction or planned (see, for instance, http://www.lightsource.ca, http://www.diamond.ac.uk,). Within the United States there are four active synchrotron infrared facilities with microscopy capabilities: the National Synchrotron Light Source (NSLS, Brookhaven National Laboratory), the Synchrotron Radiation Center (SRC, University of Wisconsin-Madison), the Center for Advanced Microstructures and Devices (CAMD, Louisiana State University), and the Advanced Light Source (ALS, Lawrence Berkeley National Laboratory); each has similar capabilities and uniqueness. All four are user facilities (i.e., available to qualified scientists). The first SR-based FTIR spectromicroscopy experiments relevant to earth materials were the measurements of composition of clay mineral surfaces [99], followed shortly by measurements of hydrous minerals [100] and of entrapped oil-water inclusions [101]. The first SR-based FTIR spectromicroscopy experiment relevant to cells, although not performed on bacteria, was chemical imaging of single human cells [102], bones [103], and plant tissues [104]. The first SR-based FTIR spectromicroscopy experiments relevant to biogeochemistry in vadose environments were the *in situ* and *in vivo* sequential measurements of reduction of hexavant chromium by a colony of basalt-inhabiting bacteria [95] and of metabolization of pyrene by a colony of soil-inhabiting bacteria from a Superfund site [105]. The first SR-based FTIR spectromicroscopy experiments of

aqueous environments was the characterization of metal-cyanobacteria sorption reactions [106].

The purposes of this chapter are to familiarize readers with SR-based FTIR spectromicroscopy and to realize key issues requiring consideration prior to its application to biogeochemistry. Rather than presenting a comprehensive review of all applications of SR-based FTIR spectromicroscopy, we shall focus on contents that illustrate the requirements and utility of SR-based FTIR spectromicroscopy as a non-invasive molecular probe for tracking molecular changes in a biogeochemical system. The interested readers should read review articles on applications of SR-based FTIR spectromicroscopy to other related areas including ecological and agricultural sciences [107], geochemistry and environmental sciences [108, 109], and biology and biomedicine [110-112]. Readers can also find helpful information in the more recent reports on the applications of SR-based FTIR spectromicroscopy to characterizing chemistry of fossil microorganisms [71], susceptibility of plants to mildew [113, 114], structural-chemical features of feeds and plants [115-119], transport of pollutants in plants [117, 120, 121], carbon in interplanetary dust particles [122], and microbial mineralization and silicification processes [106, 123-129].

II. Synchrotron Radiation-Based FTIR Spectromicroscopy

Synchrotron radiation-based Fourier transform infrared (SR-FTIR) spectromicroscopy takes advantage of three existing technologies: the well-known sensitivity and non-invasive nature of mid-infrared spectroscopy to chemical functional groups in molecules and their conformations, the convenience of a microscope to locate

areas for molecular and composition analysis, and the high signal-to-noise ratio provided by a non-invasive synchrotron radiation-based infrared light source. Mid-infrared spectroscopy also is also a rapid, reagentless, and non-destructive analytical technique which has a wide range of applications in biosciences, be they molecular or organismal. In the following section we shall describe SR-FTIR spectromicroscopy and its issues as a biogeochemical microprobe following the background section.

A. Background

The application of synchrotron radiation-based infrared light as a source of energy to study biogeochemical processes is a recent experimental effort. It is based on the principle of vibrational spectroscopy of molecules in the infrared region. Fourier transform infrared spectroscopy of a sample is the use of a Fourier transform interferometer to study the interaction of incoming infrared light with molecules in the sample. The instrumentation for Fourier transform spectrometry includes a source of infrared light, a means to measure each photon energy, an interface allowing this discreet light to be transmitted or reflected by the sample, a detector, and a data recording and analysis system. The typical measurement recorded is a spectrum of infrared absorbance in the sample as a function of the wavelength of infrared light (typically expressed in units of wavenumber, cm^{-1}). Atoms of a molecule vibrate with characteristic frequencies (normal modes) governed by their chemical bonds and symmetry environment. Incoming infrared light will be absorbed by the molecule if the following two criteria are met: the frequency of the infrared light matches exactly the frequency of the vibrational mode, and the vibration causes an asymmetric change in the charge distribution within the molecule

(dipole moment). The strength of the dipole moment correlates with the strength of the absorption.. Infrared spectroscopy is therefore sensitive to the presence of many chemical functional groups (structural fragments) in molecules in samples, and taken together the set of vibration modes are unique for every molecular configuration. More in-depth readings regarding vibrational spectroscopy of molecules and macromolecules can be found at the following web site: http://infrared.als.lbl.gov/FTIRinfo.html.

Infrared radiation was discovered by William Herschel in 1800 during his investigations of the solar spectrum. However, the potential of using infrared light energy as a source for spectroscopy was not realized until the later part of the 19th Century. William Abney and Festing were the first researchers to successfully use infrared radiation as a light source to obtain infrared spectra of almost fifty organic compounds, and recommended the use of infrared spectroscopy as an analytical tool (Phil. Trans. Roy. Soc. London, 1882, 172, 887-918). In 1905, William Weber Coblentz referenced this empirical evidence and demonstrated in his investigations of infrared spectra that different atomic and molecular groupings absorbed specific and characteristic wavelengths. However, it is the application of Fourier transform spectroscopy in conjunction with a Michelson interferometer in 1911 by Rubens and Woods that laid the foundation for modern Fourier transform infrared spectroscopy. Difficulties associated with computing Fourier transformations manually had hindered the application of the technology. Throughout the first half of the 20th Century, its applications were limited mostly to researchers in physics and astronomy, although it had found its place in WWII as a useful

diagnostic tool in determining the concentration and purity of butadiene in synthetic rubber.

In 1949, Barer [130], Gore [131], and Blout [132] demonstrated the potential importance of joining infrared spectroscopy with microscopy to seeing microscopic structures in a sample, analyzing molecular chemistry and relating composition with the observed microstructures [133-138]. With continued improvement in high quality detectors and spectrometers and the rapid development in microprocessor technology, the first practical infrared micro-spectrometer, which was conceived in 1953 by Coates [139], became available commercially in 1978. Shortly afterwards, the innovative application of the Fast Fourier transform algorithm [140] to FTIR spectroscopy, aided by the availability of low-cost high-speed computers, has led to an explosive growth in mid-infrared spectromicroscopy instrumentation primarily in the 1990s, and making it a popular analytical approach to detecting, identify, and quantify many molecular species mostly in biological samples.

The infrared sources used in these FTIR spectromicroscopy (or microspectroscopy) instruments are thermal emission elements (or thermal globars) that produce a graybody spectrum from a filament heated to between 1000 and 2000 K. These globars can be rod-, coil-, or u-shaped, physically moderate in size (at least several millimeters), and typically radiate in all directions. As shown in Figure 1, the FTIR bench optics collect the light, then collimate and pass it through the scanning interferometer. Next, this modulated light is directed into an infrared microscope. The IR microscope objective and condenser optics are reflective and focus the IR light to a small spot on a

sample. Finally, the light that the sample reflects or transmits is collected, focused onto a detector, and processed by a computer to produce an infrared spectrum. The first FTIR spectromicrosocpy experiments were measurements on coals [141] and polymers [142] during the first half of the 1980s. During the early 1990s, the first set of experiments performed on biological materials were on isolated human cells [143], tissue specimens [144, 145], and plant cells [146]. The FTIR spectromicroscopy measurements of bacteria were first conducted in conjunction with chemometrics to discriminate different bacterial strains [147, 148] almost ten years later. The popularity of FTIR spectromicroscopy in research (as measured in terms of numbers of publications involving the applications of FTIR spectromicroscopy) soared recently (Figure 2).

However, light emitted from thermal globars does not provide sufficient signal-to-noise for the detailed spectral interpretation of microbial assemblies of several to tens of microns. Such measurements were especially difficult to obtain if the microorganisms were on surfaces of earth materials with low infrared reflectivities. High quality infrared spectroscopy measurements of these materials require high infrared photon flux focused to a small spot (brightness). The brightness attainable in infrared spectromicroscopy is governed primarily by how point-like the source is. Thermal emission sources, for example, can be focused with an IR microscope to a spot with a 75 to 100 μm diameter. To measure something smaller, such as a small microbial colony on a mineral surface, one needs to use an aperture to mask away part of the incoming light, or distribute the incoming light among an array of detectors. The use of an aperture can significantly reduces the signal strength.

Our earlier work showed that the brightness (flux per unit area) attainable from a conventional thermal globar infrared source is indeed not sufficient for the use of FTIR spectromicroscopy to study biogeochemical processes on mineral surfaces without a surface treatment [149]. According to the above discussion, it follows that one needs an infrared source that acts like a true point source, i.e., a source that could be focused to a diffraction-limited spot size to maximize for maximum brightness. With the f/1 optics (i.e., the primary focal ratio is f/1), this yields a diffraction-limited spatial resolution of approximately the wavelength of the light without loosing any signal strength. This is the benefit of using a synchrotron as an IR source.

B. Synchrotron Infrared Light Sources

A synchrotron is a high-energy electron storage ring optimized for the production and collection of the intense light radiated by the electrons upon acceleration. In modern synchrotrons, electrons are first accelerated to near the speed of light and then injected into the storage ring (Figure 3a). Electrons that travel near the speed of light are called relativistic electrons. The storage ring is designed to make the traveling electrons complete a loop via a series of bending magnets and straight sections. When the electrons encounter a magnetic field, they are deflected and they emit electro-magnetic radiation – light. Typical bending magnets have a magnetic field strength of approximately one Tesla. This field strength, coupled with the velocity of the electrons, determines the energies of the emitted photons. This means that higher velocities (higher energy storage rings) and/or higher magnetic fields produce higher energy photons up to hard x-rays. This so-called bending magnet spectrum (Figure 3b) extends from very low energies (far-infrared)

continuously to a critical energy in the soft or hard x-ray, depending on the energy of the synchrotron. Because the radiation pattern from relativistic electrons is such that the opening angle of the emitted radiation is very small, the effective source size of infrared radiation source is dominated by diffraction, and thus can be considered as very close to an ideal point source. Interested readers are directed to an informative overview of synchrotron radiation by Sham and Rivers [150].

As expected, in the mid-IR region — 400 to 4,000 cm^{-1} — the effective source size for a typical synchrotron light source is dominated by diffraction [151-154]. This means that for SR-based FTIR spectromicroscopy, the infrared beam is focused visibly to a spot with a diameter of about 0.7 μm times the wavelength, which for the mid-IR wavelengths of 2.5 to 25 μm, yields a spatial resolution of 1.7 to 17 μm [155] This is smaller than a typical microbial colony, thus providing a spatial resolution smaller than a microbial colony with hundreds to a thousand times the brightness of conventional IR sources.

To demonstrate the advantage of using a synchrotron as an infrared energy source for FTIR spectromicroscopy, we describe here three studies that compare the measured signal-to-noise ratio as a function of aperture size for a conventional thermal globar IR source and the synchrotron. The first two studies were performed using a Thermo Nicolet Nexus 870 FTIR bench and a Thermo SpectraTech Contiuμm infrared microscope at the ALS Beamline 1.4.3. The third study was performed at LURE (Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France).

During the first experiment, we measured 100% reflection lines utilizing a gold-coated glass sample for both sources and for various aperture sizes. We used an MCT-B

detector, co-added 128 scans for background and sample measurements at a spectral resolution of 4 cm^{-1} and a scanning mirror velocity of 1.8988 cm/sec. The signal-to-noise value centered at 2500 cm^{-1} was obtained for both the conventional thermal source and the synchrotron source using different aperture settings. The value was calculated by dividing the measured single beam intensity at this wavenumber by the corresponding root-mean-square (RMS) noise value. The advantage of signal-to-noise improvement is shown in Figure 5. For the thermal globar source, the signal-to-noise level decreases significantly as the aperture diameter decreases. Signals become essentially unusable at aperture sizes below 20 x 20 microns. This is because the size of the thermal globar source when focused to a surface is greater than 70 x 70 microns [151, 152, 154, 156]. By reducing the aperture size, one simply reduces the total IR signal. For the synchrotron source, the signal-to-noise ratio is significantly better for almost all aperture sizes, although the ratio also begins to decrease when the aperture size is smaller than the diffraction-limited spot size. This difference is because the focused spot size of the synchrotron source is diffraction limited (1.7 to 17 microns in diameter) [155, 157]. Consequently, its signal-to-noise ratio is only affected when the aperture size becomes less than the diffraction-limited spot size (starting with the longest wavelengths within the mid-infrared region).

The second experiment compares the signal-to-noise ratio on earth materials. In Figure 6 is a geological example of how the high-brightness (i.e., high signal-to-noise ratio) of the synchrotron infrared source makes very time-consuming and difficult measurements possible. A tiny piece of ocean basalt was mounted in a diamond anvil cell

to achieve extremely high pressures, and the infrared absorbance of the sample was measured at a pressure of 32 GPa. When using a conventional FTIR spectromicroscopy system, a seven-hour signal averaging of over 60,000 scans was required to begin to detect the spectral features. With a synchrotron source, a significantly improved signal-to-noise was achieved after only 2 minutes of averaging 256 scans [158].

The third experiment compared the signal-to-noise ratio on biological materials. In Figure 6 are FTIR spectra from a single living cell using a 6 μm x 6 μm aperture [Figure courtesy of P. Dumas]. In this experiment, the investigators clearly demonstrate that even with significantly longer averaging times, the signal-to-noise of the globar measurement is so poor that the data is not usable whereas the synchrotron-based measurements show all the fine spectral structures required for detailed analysis [159].

C. Synchrotron Infrared Spectromicroscopy of Biogeochemical Systems

The experimental evidence described above reveals that for studying a surface phenomenon with a spatial resolution ranging from 1.7 to 17 microns, the signal-to-noise ratio provided by a synchrotron infrared source is up to 1000 times better than the signal-to-noise ratio provided by a thermal source. Since the SR-based infrared beam does not induce any detectable side-effects in live cells [160] and has negligible sample heating effect [161], SR-based FTIR spectromicroscopy is clearly an ideal microprobe for a noninvasive study of heterogeneous biogeochemical processes *in vivo* and *in situ*, for example, individual microbial colonies or larger biological systems in which local biochemistry may have significant spatial variations.

However, because of the complicated nature of biogeochemical systems, one must consider the following issues carefully before applying this technology to probe the successive biogeochemical processes. Firstly, microorganisms are exceedingly sensitive to their immediate environments. To reliably study molecular changes in a chain of biogeochemical events, SR-FTIR spectromicroscopy measurements must be made in well-controlled experiments that simulate their viability and functionality under *in situ* conditions. This is especially important since microbial cells alter earth and environmental materials mostly via their metabolic activities [15, 16]. Such experimental conditions of biogeochemical processes are best conducted under well-controlled conditions that are similar to the *in situ* conditions. Such similarities can at least be at the appropriate temperature, pH, redox potential (Eh), nutrient, chemistry of bulk water, pore water, relative, humidity, and gas composition. A good example of the importance of controlling the experimental conditions is temperature effects on microbial transformation of redox sensitive elements such as iron and sulfur. An increase in temperature could increase microbial metabolic activity and oxygen removal [162], leading to a decrease in redox potential [163, 164]. These changes could cause shifts in the relative importance of specific terminal electron acceptors used in bacterial respiration [163, 165]. The decrease in redox potential can also affect the chemical and physiochemical state of redox-sensitive elements. In addition to changing chemistry both in bacteria and the elements, these variations may also affect the chemistry of the overlying thin film of water through changes in diffusional fluxes and other processes. To reliably study molecular changes in this chain of biogeochemical events, SR-FTIR spectromicroscopy measurements must be

made in experiments that simulate *in situ* conditions using well-controlled flow-through cells with infrared-transparent windows. Currently there are several research groups developing various types of automated microfluidic incubation platforms to provide a controlled mechanism to rapidly manipulate these experimental conditions. Some of these platforms also control the thickness of the water film to allow for the infrared observation of the biogeochemical processes in aqueous environments. Others have sensors to provide additional measurements of relevant physiological and geochemical parameters.

Secondly, a prior knowledge regarding the type of the pollutants and the pathways of their possible biogeochemical transformation is important for the successful application of SR-based FTIR spectromicroscopy. For heavy metal and metalloid pollutants, they constitute the most difficult environmental problem because they cannot be destroyed once introduced into the environment. A key goal of using SR-based infrared spectromicroscopy is to characterize how intrinsic microorganisms affect the speciation of these heavy metals and metalloids which dictates the overall mobility, bioavailability, toxicity and other health risks in the biosphere. An appropriate SR-based FTIR spectromicroscopy experiment is one that allows investigators to obtain such fundamental knowledge as the stability and mobility of the parent metal compounds, their interactions with the microorganisms, and the altered stability and mobility of the intermediate products under *in situ* and *in vivo* conditions. Our approach to this issue has been both fundamental and applied in nature. We often complement the SR-based FTIR spectromicrosocpy experiments with successive *in vitro* and *in vivo* studies of model

systems of varying complexities to approximate membrane permeability, biotransformation, toxicity, and coupled them with spectroscopic studies. In doing so, we have been able to identify, at least at a functional group level, the targets to be measured and ensure that these targets are likely to be in the biogeochemical system to be investigated.

A good example is the microbial transformation and detoxification of chromium in earth materials. Chromium is a redox-sensitive metal pollutant that enters the environment primarily from industries such as leather tanning, wood preservation, metal plating and alloying. The two important oxidation states of chromium commonly found in environments are trivalent [Cr(III)] and hexavalent [Cr(VI)] states, which have widely contrasting mobility and bioavailability. Most Cr(VI) compounds are highly soluble in water and are readily bioavailable to ecological receptors, while most Cr(III) compounds are less water soluble and less bioavailable. Cr(VI) compounds are amongst the earliest chemicals to be classified as mutagens and human carcinogens [166-168]. Its genotoxic and carcinogenic effects are associated with its ability to enter cells rapidly through non-specific transport. Intracellular biomolecules such as polysaccharides, L-ascorbic acid, glutathione and other reductases readily reduce Cr(VI) species to formed an array of genotoxic Cr(III) complexes and other radicals that can cause single-strand-breaks and plasmid DNA nicking, in addition to a wide variety of DNA lesions and additional oxidative damage [169-175]. Biogeochemical factors that can lead to the reduction of Cr(VI) to insoluble and/or non-genotoxic Cr(III) compounds in environments are very significant for reducing chromium toxicity. Many indigenous bacteria in chromium-

polluted environments possess a multiplicity of survival mechanisms that can potentially transform soluble chromium to less soluble forms. Our experiments show that some Cr-resistant microorganisms immobilize and reduce Cr(VI) to stable Cr(III)-complexes extracellularly via interactions with diverse groups of biomolecules [175-179] and the formation of genotoxic intermediates Cr(V)- and Cr(IV)-complexes [180, 181]. This information, in conjunction with our earlier SR-based FTIR result [95], was applied to the design and execution of an additional in-depth SR-based FTIR spectromicroscopy study of Cr(VI) transformation on mineral surfaces [182]. The speciation of Cr(III) is one of the focal points in the study. Presently there are concerns that Cr(III) (as $Cr(OH)_3$) can be reoxidized to form Cr(VI) compounds [183]. However, our preliminary results indicate that only a small fraction of the Cr(III) compounds is found as $Cr(OH)_3$.

Unlike heavy metals and metalloid pollutants, organic pollutants can be destroyed Once they have entered into the biosphere, they can be degraded, metabolized, and/or mineralized by many intrinsic bacteria via one of the many possible pathways of different complexities and kinetics [184-200]. A large volume of pathway information is available at the University of Minnesota Biocatalysis/Biodegradation Database (http://umbbd.ahc.umn.edu/). However, many of the pathways and toxicity of the intermediates are unknown. The effect of environmental factors on the microbial ability to degrade organic pollutants remains uncertain. Our current approach using SR-based infrared spectromicroscopy to study biodegradation of organic pollutants by intrinsic microorganisms is more applied in nature. Questions to be addressed include whether the microorganisms are capable of decomposing the organic pollutants, and what the

geochemical factors that affect the bioavailability of the organic pollutants to the microorganisms are. For microorganisms that degrade organic pollutants via a known pathway, we would also address if the intermediates are persistent and/or harmful to ecological receptors [105].

Finally, it is important to realize that information derived from SR-based FTIR spectromicroscopy is only the tip of an iceberg of information. Because of the complexity of a biogeochemical system, this information alone is not sufficient for a thorough understanding of how intrinsic microorganisms transform pollutants and what factors could alter the microbial ability to transform the pollutants. It is also not sufficient for making reliable predictions of the potential risks of these pollutants and intermediates to ecological receptors and humans. The use of multiple complementary biochemical, analytical, and imaging techniques is necessary. A good example of the use of complementary techniques is the collaborative study by researchers from the Lawrence Berkeley National Laboratory (USA) and from the Georgian Academy of Science (Republic of Georgia) of chromium reduction by basalt-inhabiting aerobes [180, 181, 201-205]. In addition to the use of SR-based FITR spectromicroscopy to track the sequential reduction of chromium, they also used sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) to identify chromium-induced changes in cell-wall protein composition [204], capillary electrophoresis to determine the effect of cell-wall proteins on the mobility of chromium through cell wall [180], electron spin resonance (ESR) to determine/confirm chromium speciation in bulk cells [181], and μ–XRF and microμ–XAFS imaging of Cr, Fe and Mn distribution. Scanning electron microscopy and

transmission electron microscopy were also employed [201]. Such synergistic use of an array of different analytical and imaging techniques has allowed these researchers to discover the unexpected accumulation and immobilization of stable and toxic chromium intermediates by microorganisms, which will have significant implications in the applications of intrinsic microorganisms to remediate Cr(VI)-polluted earth and environmental materials.

III. Biogeochemical Processes Measured by SR-Based FTIR Spectromicroscopy

The measurement and imaging of biogeochemical processes by means of SR-based FTIR spectromicroscopy involves the use visible light and reflecting optics to view a magnified image of the sample and to select a microscopic surface area on the sample for infrared reflection-absorption spectroscopic analysis. In this section, three biogeochemical studies conducted at the ALS are highlighted, following the description of instrumentation and spectral analysis. Interested readers are directed to read applications in other related areas [71, 106, 113-129, 206-208].

A. Instrumentation

The instrumentation at Beamline 1.4.3 at the ALS is similar to FTIR spectromicroscopy systems that are commercially available, except that the thermal source is replaced by an infrared beam from the synchrotron (Figure 7). Additionally, the beam is also passed through a beam position locking system [209, 210] to minimize the effect of the beam motion. Without this system, the beam tends to move on the sample during data acquisition for a variety of reasons. Such movements can be as large as several microns and cause artifacts and/or noise in the data. The addition of the beam-

locking system is exceedingly helpful when studying biogeochemical materials that often have fine and highly heterogeneous surface features.

The samples are maintained inside a mini-incubator which is mounted on the microscope stage. The sample is positioned using a computer-controlled x-y-z stage with 0.1-μm precision, allowing mapping measurements of FTIR spectra (through the incubator's ZnSe window) as a function of x- and y-position on the sample. The selection of the area is relatively subjective and relies on the geometry, color, crystallographic properties, and other material-specific features of the sample surface. Once the sample area is selected, the spectroscopic information of the selected surface area can be recorded *in situ* in a reflection mode.

B. Spectral Analysis

Because of the complexity of a biogeochemical system, one of our key efforts has been to carefully determine infrared spectral features that are truly the molecular markers of the biogeochemical phenomena to be investigated. Infrared spectra of biomolecules in microbes [211-226], of many relevant minerals [227-244] and common environmental pollutants [245-250] [251-273] are already well-known with specific peaks and groups of peaks that can be related to specific biochemical and chemical groups of single molecules in an ideal system. The traditional approach of spectral analysis, which is intended to identify particular compounds, involves a band-shape analysis followed by direct assignment of characteristic absorption bands in the infrared spectrum. However, in a complicated and often transient biogeochemical system under *in situ* and *in vivo* conditions, these specific peaks and bands of peaks may shift, and the overall pattern may

even change and deviate from the well established features. To date, our general approach has focused on a small number of important spectral features that could be derived from a series of simplified model systems prior to the SR-based FTIR spectromicroscopy experiment. For time-course experiments, we also would combine the traditional direct assignments and the difference spectroscopy approach to guide the interpretation of the absorption bands as a function of exposure time. We evaluated the intensity of each absorption band by means of the method of the most probable baseline [274]. It is important to note that as the beam current of the synchrotron decreases with time between electron refills, the beam intensity decreases proportionally, which needs to be taken into account if one wants to accurately measure absorption band intensity. We have found that rescaling the intensity of the absorption bands by means of an internal-standard-equivalent approach works reliably.

C. Application Examples

With the completion of sequencing of genomes of many organisms and the continuous success in identifying gene products (proteins) and metabolic pathways, one of the central interests in biogeochemical and environmental research is to apply this wealth of information to understand and to design appropriate strategies to utilize metabolic capabilities in living microorganisms to remediate pollutants in earth and environmental materials. The success of these directions will ultimately be determined by how well one can measure without disturbing the relevant dynamic processes in a biogeochemical system, for example, the redox transformations of heavy metals by metal-reducing bacteria, or degradation of carcinogenic organic pollutants. These

examples will illustrate how SR-Based FTIR spectromicroscopy can be a useful tool that allows one to get a step closer to achieve this important goal.

Reduction of Hexavalent Chromium by Basalt-Inhabiting Aerobes [95]

"Compounds containing chromium atoms can be potentially hazardous contaminants in the environment. The degree of the hazard depends on the chemical state of the chromium in the compounds in which it occurs. Chromium at its hexavalent state (Cr(VI)) is usually highly soluble in water and therefore mobile in the environment, so the contamination spreads, and it is toxic and suspected to be carcinogenic. However, chromium at its trivalent state (Cr(III)) is relatively insoluble in water and significantly less harmful. Geochemical and biogeochemical processes that convert chromium from the hexavalent to the trivalent state are potentially useful for environmental remediation. We demonstrated the use of SR-FTIR spectromicroscopy to illustrate that certain bacteria found naturally in basalt are effective agents in the "biogeochemical" transformation of chromium from the undesirable hexavalent state to the less harmful trivalent state, thereby resolving an on-going controversy about the nature of the conversion."

This is the first time that biogeochemical transformation of Cr(VI) by microorganisms on a mineral surface has been nondestructively monitored and studied where it occurs. Distinct and relevant infrared absorption bands (Table 1) were used as chemical markers to detect the presence of microorganisms and to identify different chromium species, on specimen surfaces. In addition, the brightness of the infrared

radiation from the synchrotron infrared beamline makes spatially resolved spectroscopy (spectromicroscopy) possible for imaging biogeochemical systems.

Two reduction mechanisms in polluted geological materials have previously been postulated for the reduction of Cr(VI) compounds. The biological mechanism requires the presence of microorganisms to aerobically reduce the Cr(VI). The chemical mechanism relies on metal oxides, such as Fe(II) compounds, to catalyze the Cr(VI)-reduction reaction. We conducted synchrotron FTIR spectromicroscopy experiments to distinguish the relative significance of these two mechanisms. In addition, we evaluated the effects of common organic co-contaminants, such as toluene vapor, on the biotic reduction process (Figure 10).

For magnetite surfaces of mixed iron oxides that contain no living microorganisms, a five-day exposure to Cr(VI) compounds resulted in statistically insignificant changes in the infrared chemical markers, indicating that little catalysis of Cr(VI) reduction was occurring. On samples with living microorganisms, however, some Cr(VI) reduction was detected (Figure 8). Moreover, when the samples with living microorganisms were incubated in dilute toluene vapor, statistically significant changes in both infrared-absorption intensity and characteristic band shapes were observed for Cr(VI), as were new bands signaling the existence of intermediate Cr(V). FTIR spectromicroscopy showed that the changes in the infrared absorption bands occurred at the sites of bacterial concentration. Measured images of the surface at characteristic absorption bands showed a strong correlation between peak depletion of Cr(VI) and depletion of toluene and peak concentration of biological molecules (Figure 9).

In a study to determine if this microbial reduction process could occur in real geological samples, composite mineral surfaces of basalt rock chips containing resident communities of microbes were exposed to solutions of Cr(VI) and toluene vapor. At the end of four months, FTIR spectromicroscopy showed that Cr(VI)-tolerant and Cr(VI)-reducing natural microorganisms were thriving in association with Cr(III) (Figure 10). The reduced Cr(III) state was confirmed by x-ray absorption fine structure (XAFS) spectroscopy at ALS Beamline 10.3.2 (Figure 11). The nondestructive infrared spectromicroscopy studies, combined with XAFS spectroscopy and microbiological techniques, show that highly mobile and toxic Cr(VI) contaminants can be biologically reduced into less soluble, less toxic Cr(III) compounds. The FTIR method can now be expanded to examine other infrared-amenable microbial/chemical contaminant systems.

Mycobacterial Metabolization of Pyrene in Humic Acid [204]

"Contaminants in the environment come in many forms, one of which is the relatively recalcitrant toxic organic (carbon-based) family of chemicals known as polycyclic aromatic hydrocarbons (PAHs). These include more than 100 different chemicals resulting from incomplete burning of coal, oil and gas, garbage, or other organic substances like tobacco or grilled meat. Converting PAHs into non-toxic chemicals removes the hazard, but learning how to do this in an efficient and cost-effective way remains to be accomplished. Here we made use of synchrotron infrared spectromicroscopy to show that the speed of biodegradation can be dramatically increased (by almost a hundred fold) by adding a certain soil-derived organic (humic) acid along with the bacteria to a PAH spot on a mineral surface."

The role of humic acid (HA) in the biodegradation of toxic polycyclic aromatic hydrocarbons (PAHs) has been the subject of controversy, particularly in unsaturated environments. By utilizing an infrared Fourier transform spectromicroscope and a very bright, nondestructive synchrotron photon source (SR-FTIR spectromicroscopy), we monitored *in situ* and over time the influence of HA on the degradation of pyrene (a model PAH) by a bacterial colony on a magnetite surface. Our results indicate that HA dramatically shortens the onset time for PAH biodegradation from 168 to 2 hours. These results will have significant implications for the bioremediation of contaminated soils.

The pyrene-degrading bacterium used for this study is *Mycobacterium sp*. JLS (Figure 12), a gram-positive, rod-shaped bacterium recently isolated from PAH-contaminated soil at the Libby Groundwater Superfund Site in Libby, Montana, USA. Abiotic (no bacteria present) results (inserts in Figures 13a and 13b) show that almost all of the pyrene remains on the mineral surface for the duration of the study, owing to slow removal mechanisms. After introduction of *M. sp*. JLS in the absence of HA, it took the bacteria about 168 hours to produce sufficient glycolipids to solubilize pyrene. At this point, biodegradation could proceed, resulting in a rapid decrease of pyrene and a rapid increase of biomass within the next 35 hours. After the pyrene was depleted, the biomass signal significantly decreased, presumably as the *M. sp*. JLS bacteria transformed themselves into ultramicrocells, a starvation-survival strategy commonly observed among bacteria in oligotrophic environments.

In the presence of HA, pyrene biodegradation began within an hour, and the pyrene was depleted by the end of the fourth hour, with a concurrent increase of biomass

(Figure 13b). Both the degradation of pyrene and the increase of biomass corroborate the effectiveness of Elliott Soil Humic Acid (ESHA) in radically accelerating biodegradation of pyrene. It is likely that the water-insoluble pyrene is solubilized into the cores of ESHA pseudo-micelles and therefore becomes directly available for bacterial uptake and consumption.

Over longer times, the remaining infrared absorption bands of pyrene on magnetite surfaces first showed a slight increase and subsequently a decrease. The increase is probably due to diffusion of pyrene trapped in micropores (< 0.5 μm in diameter) of the magnetite and/or neighboring surfaces of higher pyrene concentration after the first wave of rapid depletion of pyrene by *M. sp*. JLS set up a diffusion gradient from the pyrene-containing micropores toward the bacterial colony. For the surface containing HA, the biomass remained almost constant over a period of more than 200 hours, indicating that the flux of pyrene from the micropores was sufficient to maintain the bacterial colony. For the surface free of HA, there is little evidence of the presence of a quasi-steady state biomass (Figure 14).

At the end of the time-resolved experiment (about 460 hours), spatial distributions of pyrene, *M. sp*. JLS, and ESHA were measured by acquiring infrared spectra at 5-micron intervals across the center of the bacterial colony with HA. Figure 15 shows contour maps of the spatial distribution of measured infrared absorbance corresponding to *M. sp*. JLS, HA, and pyrene. The central region of the maps has a high population density of *M. sp*. JLS and a high concentration of HA, but the pyrene in this region was

completely biodegraded. Where pyrene is present without *M. sp.* JLS, there is no significant degradation.

We conclude that SR-FTIR spectromicroscopy can assess real-time interactions between multiple constituents in contaminated soils. Combined with conventional mineralization measurements, which monitor respiration through carbon dioxide production, SR-FTIR spectromicroscopy is thus a powerful tool for evaluating bioremediation options and designing bioremediation strategies for contaminated vadose zone environments.

Rapid Screening for Remediation Capability of a Microbial Community

"Can infrared light from a synchrotron be used to screen for metabolic activities in a living microbial community that can degrade organic pollutants? If so, it would open up possibilities for the eventual use of synchrotron infrared light in environmental diagnostics or environmental health research. The experiment summarized here is an infrared imaging of transformation of toluene by a microbial community on vesicular basalt surfaces. Our preliminary results suggest that some day it may be routine to study a tiny microbial colony, by using synchrotron infrared spectroscopy, and to screen for microbes and conditions that are most effective in detoxifying environmental pollutants."

The possibility of utilizing the capability of intrinsic microorganisms to decompose and even mineralize organic pollutants has stimulated intensive interests in exploring if these biotransformation reactions actually take place on surfaces of geologic

materials. Recent conceptual and technological improvements in environmental microbiology have advanced our ability to partly address these issues. For example, the use of the DNA probes for specific enzymatic activities enables researchers to determine if certain genes are present in the bulk microorganisms that can initiate and sustain the desirable transformation of pollutants [275]. Detection of unique intermediate metabolites in site-derived samples provides evidence for the occurrence of *in situ* contaminant biotransformation. Together with microcosm experiments they globally address the questions of whether or not the bacteria interact with contaminants. However, these efforts are labor intensive and time-consuming. We are currently conducting feasibility study to evaluate if SR-based FTIR spectromicroscopy can be an ideal screening tool to rapidly identify microbial remedial capability on mineral surfaces.

The geological sample used was a fragment of a vesicular basalt rock from a site formerly polluted with volatile organic compounds (VOCs). The sample was exposed to 100-ppm of toluene vapor at 100% relative humidity for five days. Distinct and relevant infrared absorption bands of toluene and its metabolites from a common degradation pathway (Figure 16) are used to mark the progression and capability of toluene degradation (Table 2). At the end of fifth day, chemical images from synchrotron-radiation-based Fourier-transform-infrared (SR-FTIR) spectromicroscopy showed that the native microorganisms were thriving in association with various capabilities of toluene degradation (Figure 17). This demonstrates that the excellent spatial resolution of SR-FTIR spectromicroscopy provides a means for determining the degree to which the toxic toluene was metabolized by the microorganisms.

VI. Future Possibilities and Requirements

Although synchrotron radiation-based FTIR spectromicroscopy is an emerging analytical and imaging technology for studying biogeochemical processes *in vivo* and *in situ*, considerable experience has already been obtained in its use in evaluating microbial interactions with environmental pollutants. It seems as though only a small part of this non-invasive technology has been explored so far. For example, the quantitative capability of infrared spectroscopy for accurately quantifying the transformation of metal ions or organic substrates, for defining the inter-relationship between such transformation and metabolic activities, and even for measuring the chemical or activity gradient and thus the chemical fluxes across a microbial colony have not been fully utilized. Such utilities can be enhanced by a number of emerging or hoped-for advances in other relevant technologies. Improved software for the automated and accurate analysis of the spectra will make accurate quantitation easier.

Improved experimental systems are also essential. To date the major experimental obstacles lie not in the synchrotron infrared instruments themselves. Instead, they lie in two difficulties: in rapidly controlling the optimum conditions for experiments before products of microbial functions are measured, and in optimizing immediate data processing and interpretation. The existing techniques are relatively time consuming and labor intensive. Their fragility frequently results in major losses of sample and experimental time, and they require many steps that can take days to achieve the optimum experimental conditions.

Additionally, the future utility of the technique will also be enhanced by combining SR-FTIR spectromicroscopy with other techniques of higher specificity. For example, the most popular approach that is beneficial to this combination appears to be the visible/infrared imaging along with fluorescence microscopy. Whilst visible imaging provides information on the physical features of the biogeochemical system and infrared imaging yields global chemical information of the system, fluorescence microscopy allows one to observe localized environments (e.g., redox conditions, molecular cluster dimensions) [276-278] and key dynamical processes that govern the function and structure of cells [279-283]. The fluorescent probes can be endogenous molecules such as NAD(P)H [284-286], genetically-encoded specific fluorescent proteins [287, 288], or passive markers of specific fluorescent molecules/dyes [289]. By establishing an associative analysis that links the genetically-encoded molecules and marked cellular events from fluorescence microscopy to the global chemical information derived from SR-based FTIR spectromicrosocpy, one can truly expand the existing understanding of biogeochemical capabilities in living microbes and developing biotechnologies for utilizing such capabilities for the benefit of environmental management.

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Table 1. Spectral regions and distinct absorption bands within each region for microorganisms (including bacteria), Cr(VI)-, Cr(V)-, and Cr(III)-compounds, toluene, and catechols in our mineral/microorganisms/Cr/toluene system [95].

Compounds	Spectral regions (cm^{-1})	Absorption bands (cm^{-1})
Microorganisms (protein)	1800 - 1500	~1650; ~1550
Cr(VI)-compounds	900 - 800	~ 846; ~ 890
Cr(V)-compounds	900 - 700	~ 830; ~ 764
Cr(III)-compounds	850 - 750	~ 810; ~ 798
Toluene	800 - 650	~ 728; ~ 695
Catechols	800 - 700	~ 770; ~ 742

Table 2. Spectral regions and distinct absorption bands within each region for microorganisms (including bacteria), toluene, benzoic acid, and catechols in our basalt/microorganism/toluene system.

Compounds	Spectral regions (cm^{-1})	Absorption bands (cm^{-1})
Microorganisms (protein)	1800 - 1500	~1658; ~1548
Toluene	1250 - 650	~1029; ~ 728; ~ 695
Benzyl alcohol	1250 - 650	~1200; ~ 1022
Benzoic acid	1250 - 650	~930;
Catechols	1250 - 700	~1096; ~ 770; ~ 742

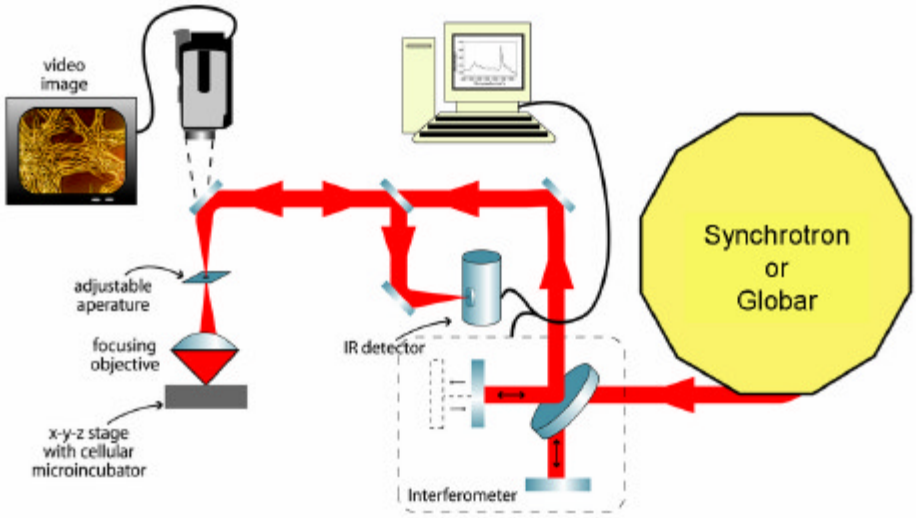

Figure 1. Schematic diagram of Fourier transform infrared (FTIR) spectromicroscopy experimental setup. Mid-infrared radiation from either a synchrotron or a globar is transported to a FTIR interferometer bench. After modulation by the interferometer, an infrared microscope focuses the beam onto the sample with all-reflecting optics. Microbial or biogeochemical samples can be placed inside an on-stage mini-incubator with environmental controls. The stage is computer controlled and rasters the sample in the x-y-z plane to ± 0.1mm precision to obtain spectral maps across the sample. The light reflected from the sample is collected by the same microscope optics and sent to an IR detector. A computer performs a Fourier transform on the measured interferogram to obtain an infrared spectrum.

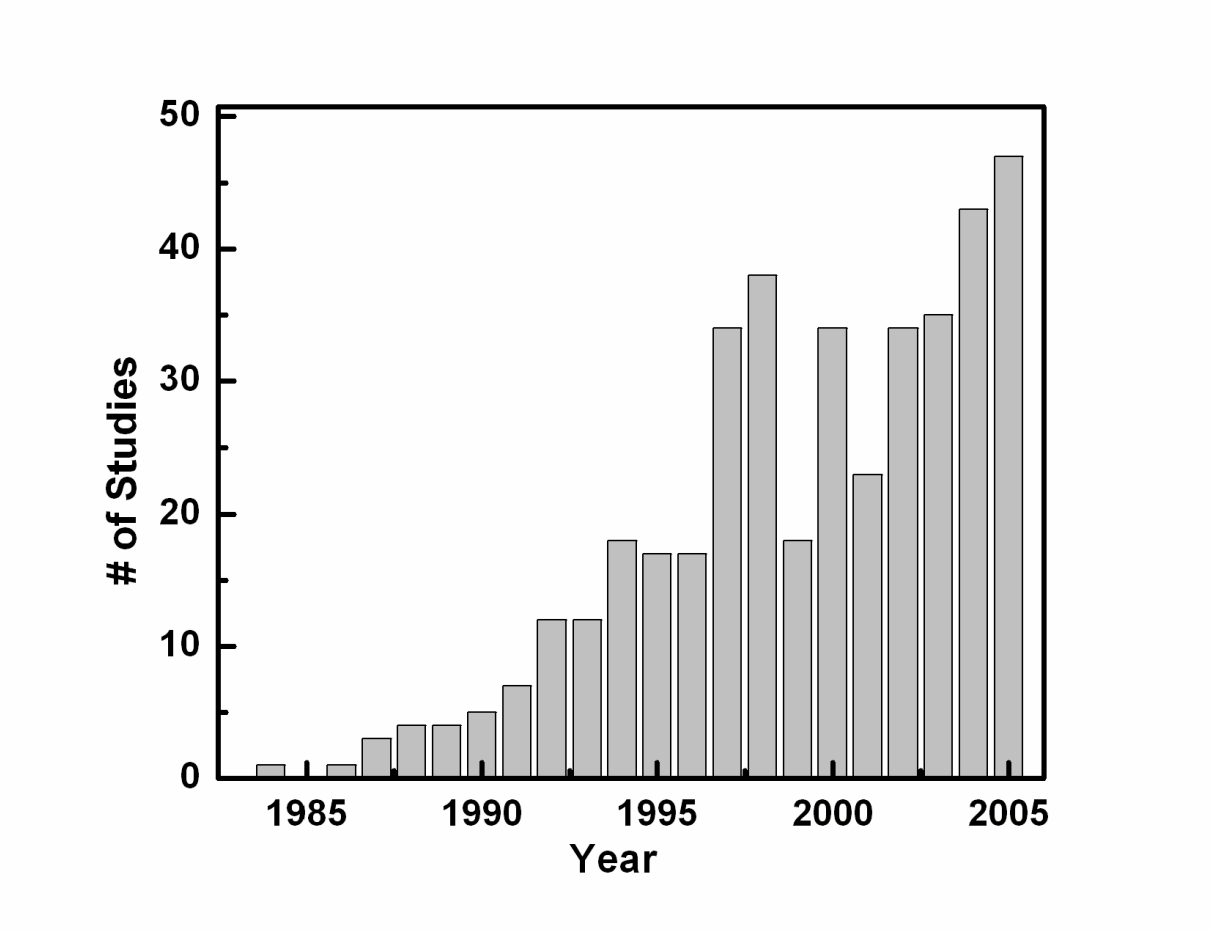

Figure 2. An almost five-fold increase in the popularity of FTIR spectromicroscopy (or microspectroscopy) as indicated by the number of published paper that present FTIR spectromicroscopy measurements.

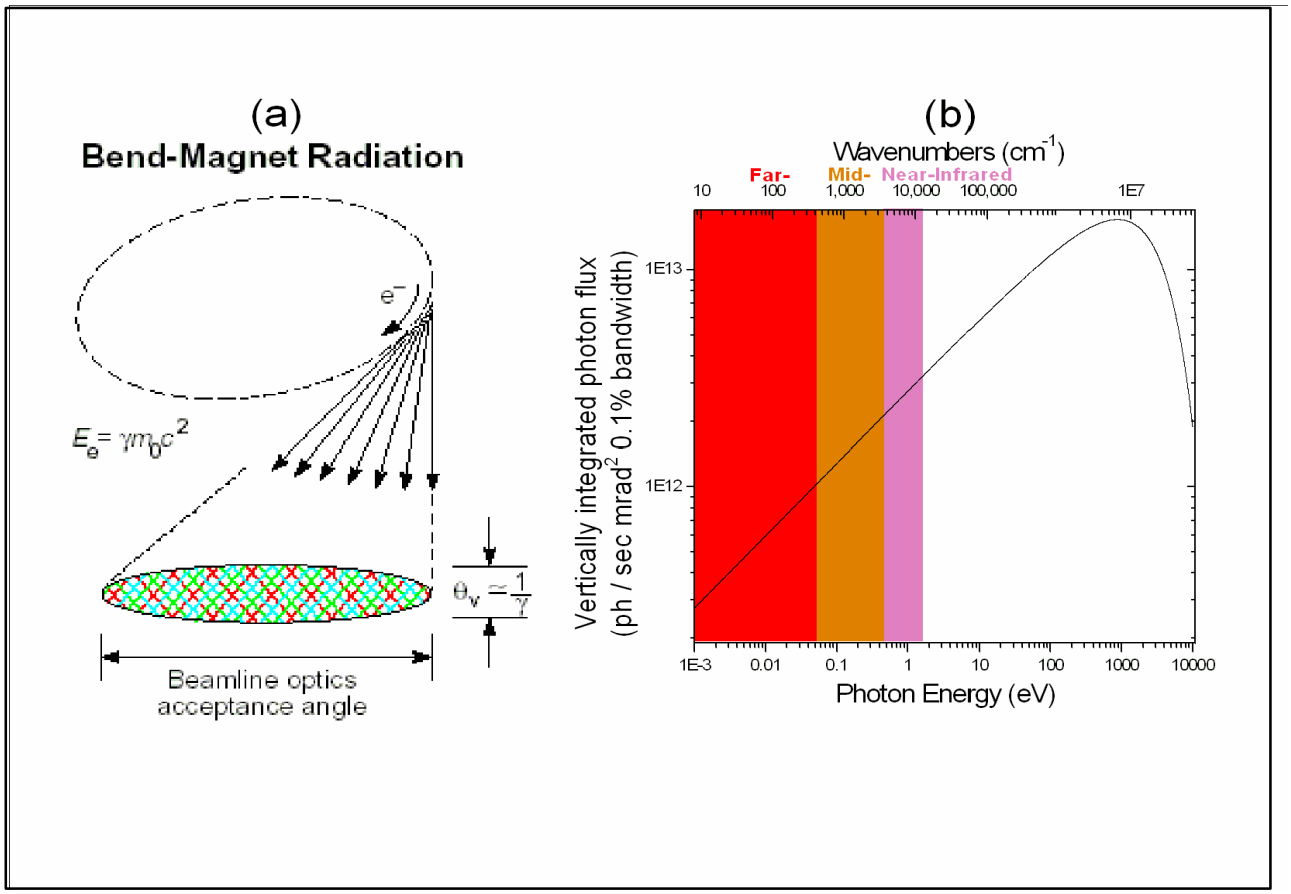

Figure 3. An overview of synchrotron radiation. (3a) Guided by a series of bending magnets and straight sections, relativistic electrons inside a storage ring complete a loop. When the relativistic electrons encounter a magnetic field, they are deflected and they emit electro-magnetic radiation with energy photons up to hard x-rays. (3b) This so-called bending magnet spectrum extends from very low energies (far-infrared) continuously to a critical energy in the soft or hard x-ray, depending on the energy of the synchrotron. The radiation pattern from relativistic electrons is such that its effective source size can be considered as very close to an ideal point source.

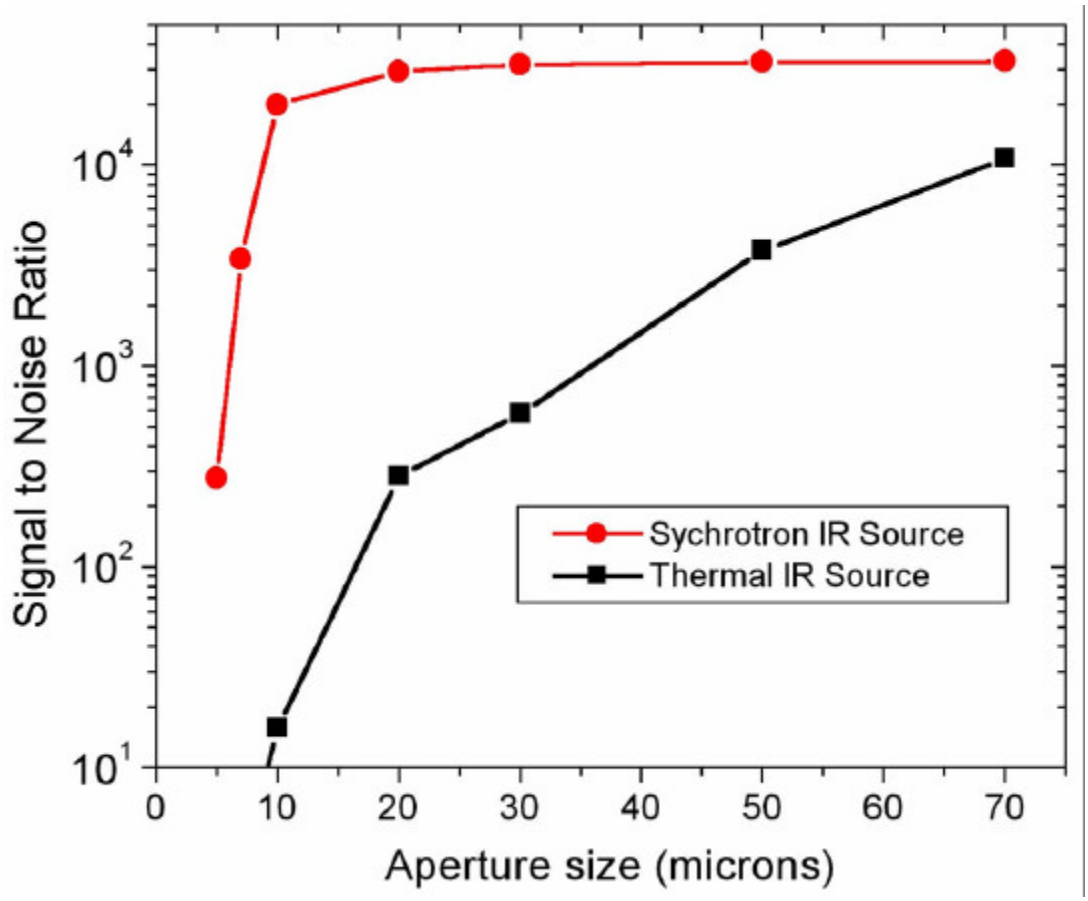

Figure 4. Comparison of measured noise around 100% reflectance for the thermal and synchrotron IR sources with different aperture size expressed in terms of signal to noise ratio on a log scale as a function of aperture size for the synchrotron and thermal IR sources. The synchrotron source extends FTIR spectromicroscopy to below 20 micron spatial resolution with a signal-to-noise advantage over conventional IR sources of at least 100.

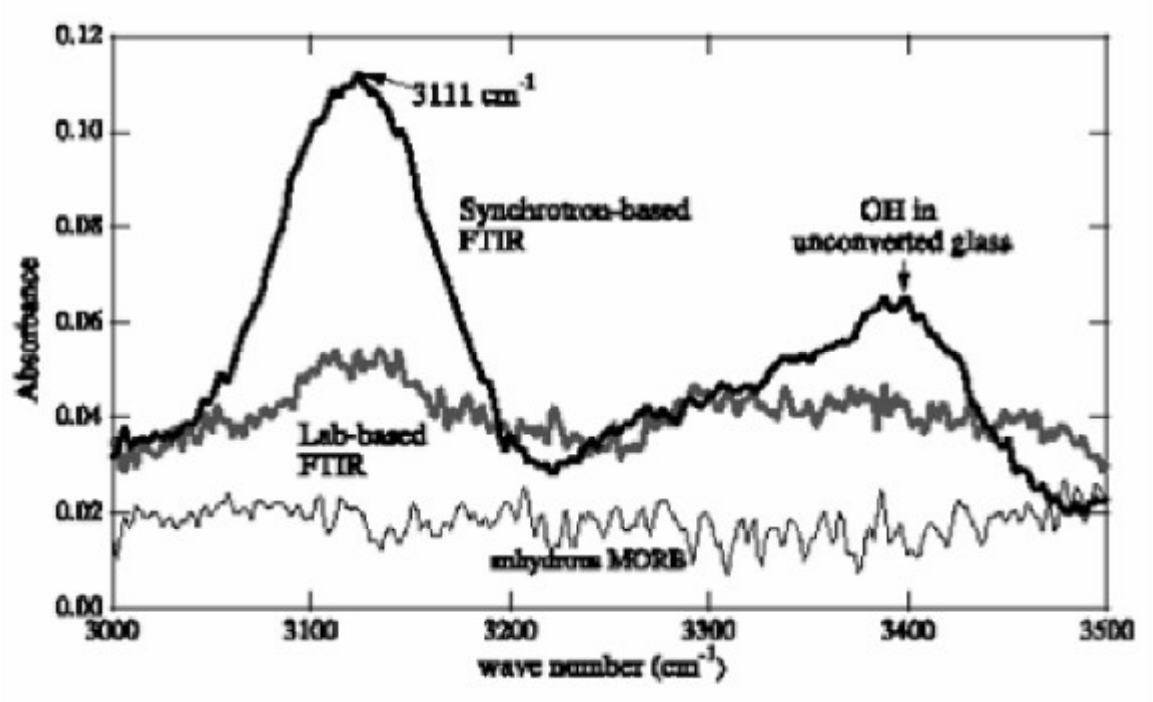

Figure 5. Spectra of a sample synthesized at 32 (±2) GPa and 2850 (±150) K, comparing results from a synchrotron-based system (black, Advanced Light Source beamline 1.4.3; Nicolet Magna 760 with KBr beamsplitter and an MCT detector) to the spectrum from a lab-based system (gray, Bruker IFS-66v using a CaF2 beamsplitter and an InSb detector). The collection time for the synchrotron-based system was about 2 min (256 scans, top), compared to about 7 hours (60,000 scans, bottom) for the lab-based system. While both show a distinct peak at 3111 cm^{-1} corresponding to OH vibrations in stishovite, the synchrotron-based spectrum has a better signal-to-noise ratio, as well as better spatial resolution. There is no detectable absorption at 3450 cm^{-1}, where OH in Mg-perovskite is expected to absorb. A control experiment was performed on a dry, synthetic basalt glass starting material (sample 1114b_6); synthesis conditions were 33 (±1) GPa and 2130 (±150) K. No absorption features were found in the 3000–3500 cm^{-1} region for this sample (thin line, bottom), again collected by synchrotron FTIR [158].

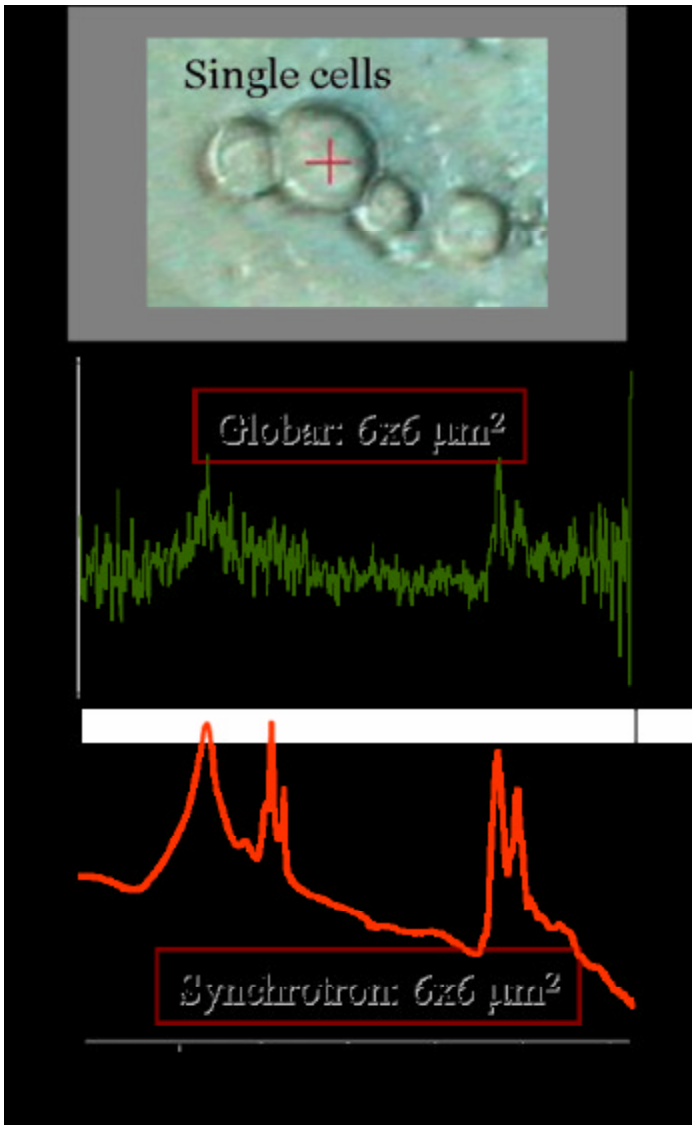

Figure 6. FTIR spectra of a single cell using a 6 μm x 6 μm aperture, comparing results from a synchrotron-based system (red, at Laboratoire pour l'Utilisation du Rayonnement Electromagnétique, Orsay, France) to the spectrum from a lab-based system (green). The collection time for the synchrotron-based system was about 16 seconds (32 scans, bottom), compared to about 500 seconds (1,000 scans, middle) for the lab-based system. These investigators clearly demonstrate that even with significantly longer averaging times, the signal-to-noise of the globar measurement is so poor that the data is not usable whereas the synchrotron-based measurements show all the fine spectral structures required for detailed analysis. [Figure courtesy of P. Dumas].

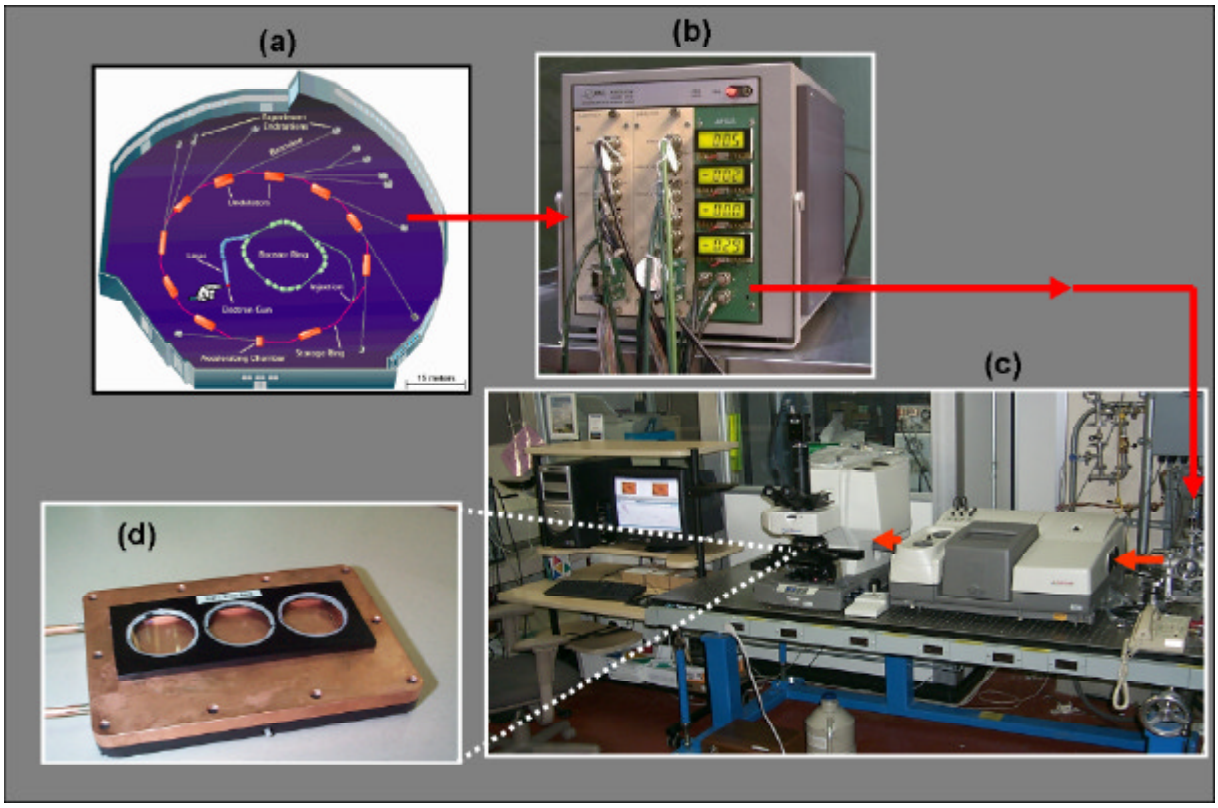

Figure 7. The beam position locking system instrumentation at Beamline 1.4.3 at the ALS. It is specifically made for probing biogeochemical processes *in situ* and *in vivo*. The beam from the synchrotron (a) is passed through the beam position locking system (b), then enters the commercially available FTIR spectromicroscopy system (c). During the experiment, samples are kept inside a stage mini-incubator (d). The addition of the beam-locking system is exceedingly helpful when studying biogeochemical materials that often have fine and highly heterogeneous surface features.

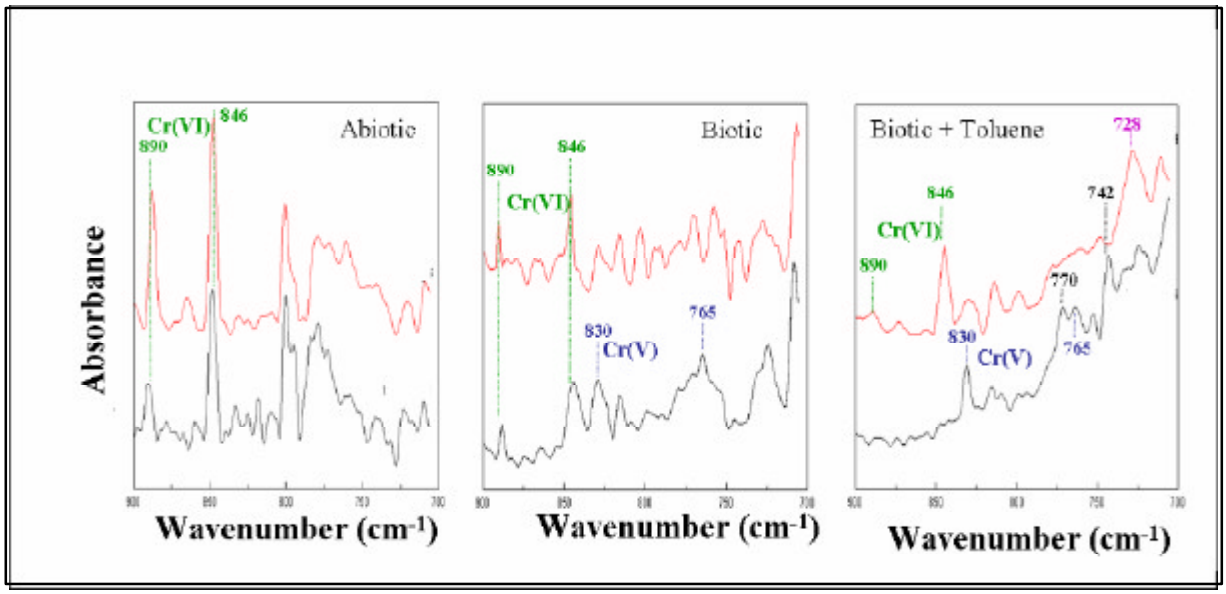

Figure 8. SR-based FTIR spectra of chromate on magnetite surfaces during the 5-day experiment of (left) abiotic reduction, (middle) biotic reduction in the absence of other organic compounds, and (right) biotic reduction in the presence of toluene vapor (as a model volatile organic compound). (—) t < 1 day, shifted vertically for visual clarity. (—)t = 5 days. Although the total chromate concentration for each of the three experiments were the same, microbial-mineral surface roughness and redistribution during evaporation results in heterogeneous spatial distributions of Cr(VI) concentrations. The most relevant vibrational frequencies identified are marked: 890 and 846cm^{-1} correspond to Cr(VI), 830 and 765cm^{-1} correspond to Cr(V), 770 and 742 are catechols, and 728 is toluene. We observe that microbial reduction of Cr(VI) is the dominant mechanism in our experimental system. The microbial chromium reduction is further enhanced during the microbial degradation of the organic compound toluene [95].

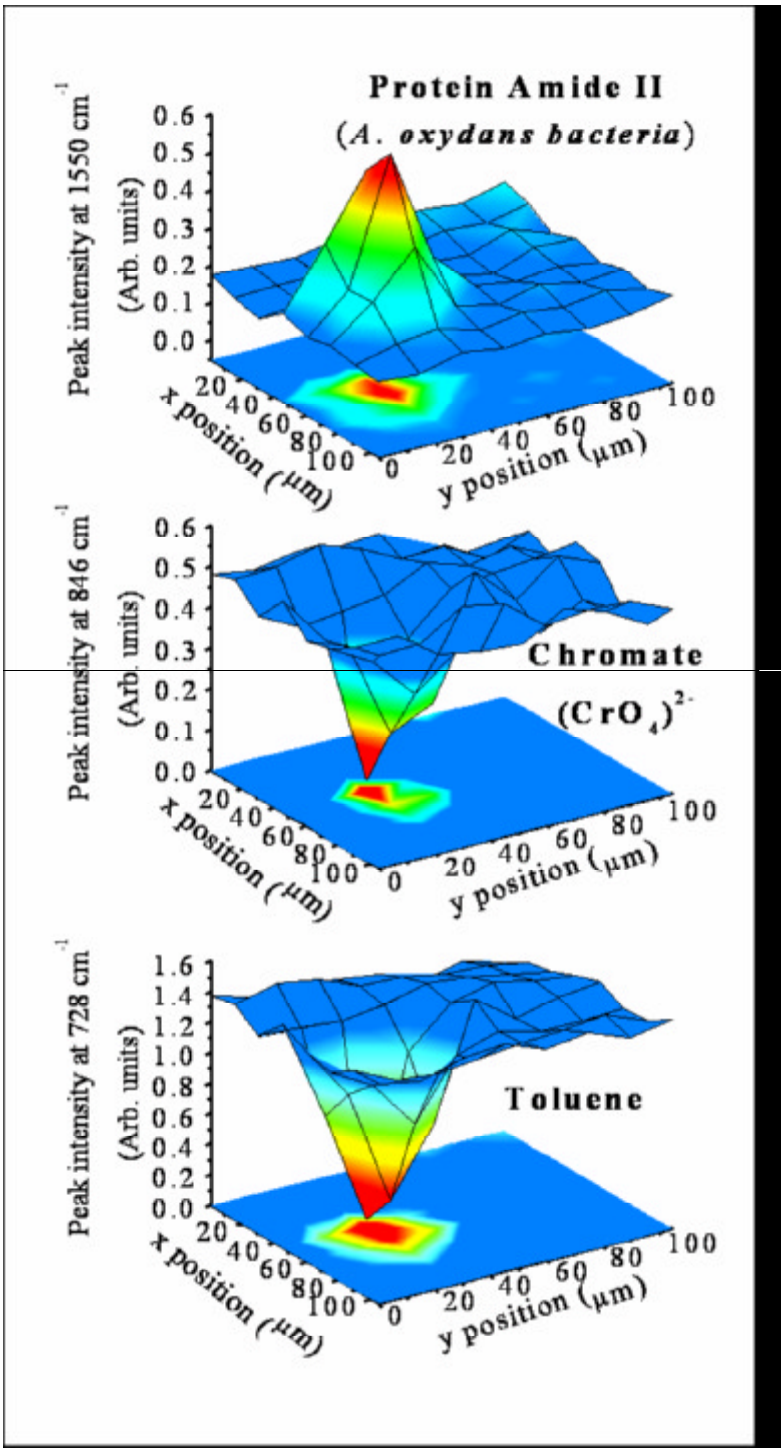

Figure 9. During the 5-day study period, *Arthrobacter oxydans* bacteria (isolated from the basalt core sample) attached themselves to magnetite surfaces. They reduced Cr(VI) while degrading toluene. SR-based FTIR spectromicroscopy measurements at the end of the

experiment show the spatial distribution of (top) *A. oxydans*, (middle) chromate, and (bottom) toluene, as measured by their spectral signatures [95].

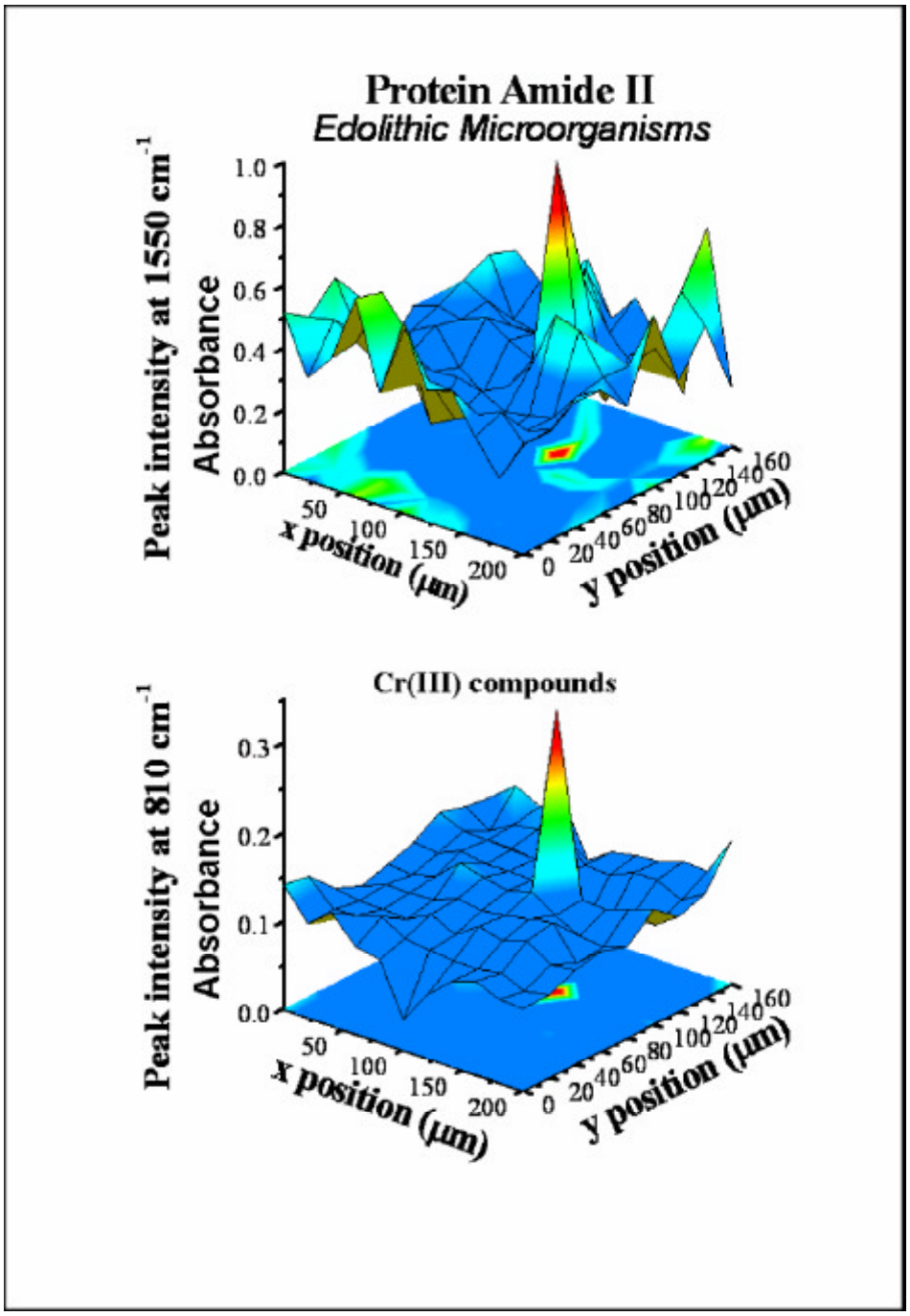

Figure 10. Distribution of indigenous endolithic microorganisms (top) and the Cr(III) compounds (bottom) asmeasured by SR-based FTIR spectromicroscopy at the end of the 4-month Cr(VI)–microbe–basalt experiment. Only chromium-tolerant and chromium-reducing microorganisms proliferated during the study period [95].

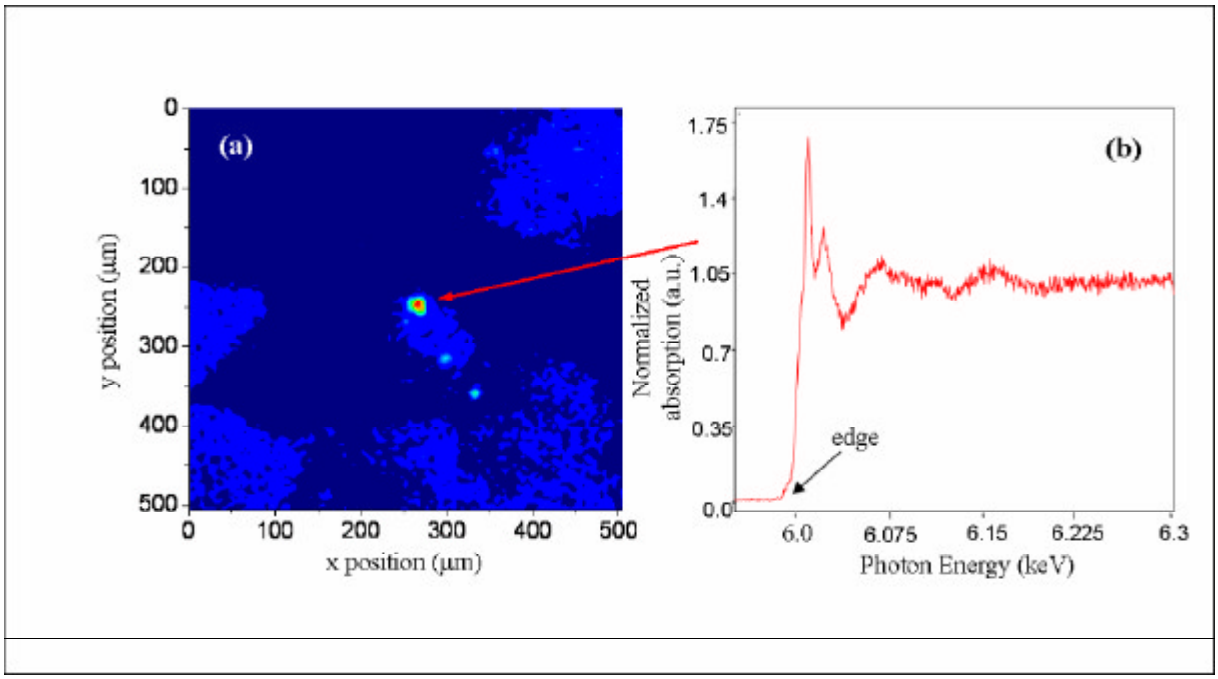

Figure 11. Confirmation of chromium (III) oxidation state by micro-X-ray analysis on the similar area of the identical sample studied by SR FTIR (see Figure 5). **(a)** Chromium elemental mapping by micro-X-ray fluorescence analysis (μ-XRF) . The colors go from black (chromium concentration below detection limit) to red (high chromium concentration). **(b)** Average of 9 micro-X-ray absorption fine structure (μ-XAFS) scans taken at the highest concentration spot shows no Cr(VI) pre-edge peak and is consistent with Cr(III) compounds. Each data point represents 20 seconds counting time. The energy increments are 0.5 eV [95].

Figure 12. A TEM image of the newly isolated Gram-positive cocci Mycobacterium sp. JLS (GenBank accession no. AF387804). It appears that *M. sp. JLS* degrades polycyclic aromatic hydrocarbons such as pyrene via a novel pathway. However, it gained biomass rapidly while degrading the compounds [105]. Time-resolved analysis of spectra from SR-based FTIR spectromicroscopy did not reveal fingerprints of known metabolites. This is further confirmed by follow-up mass spectrometry analysis of the sample.

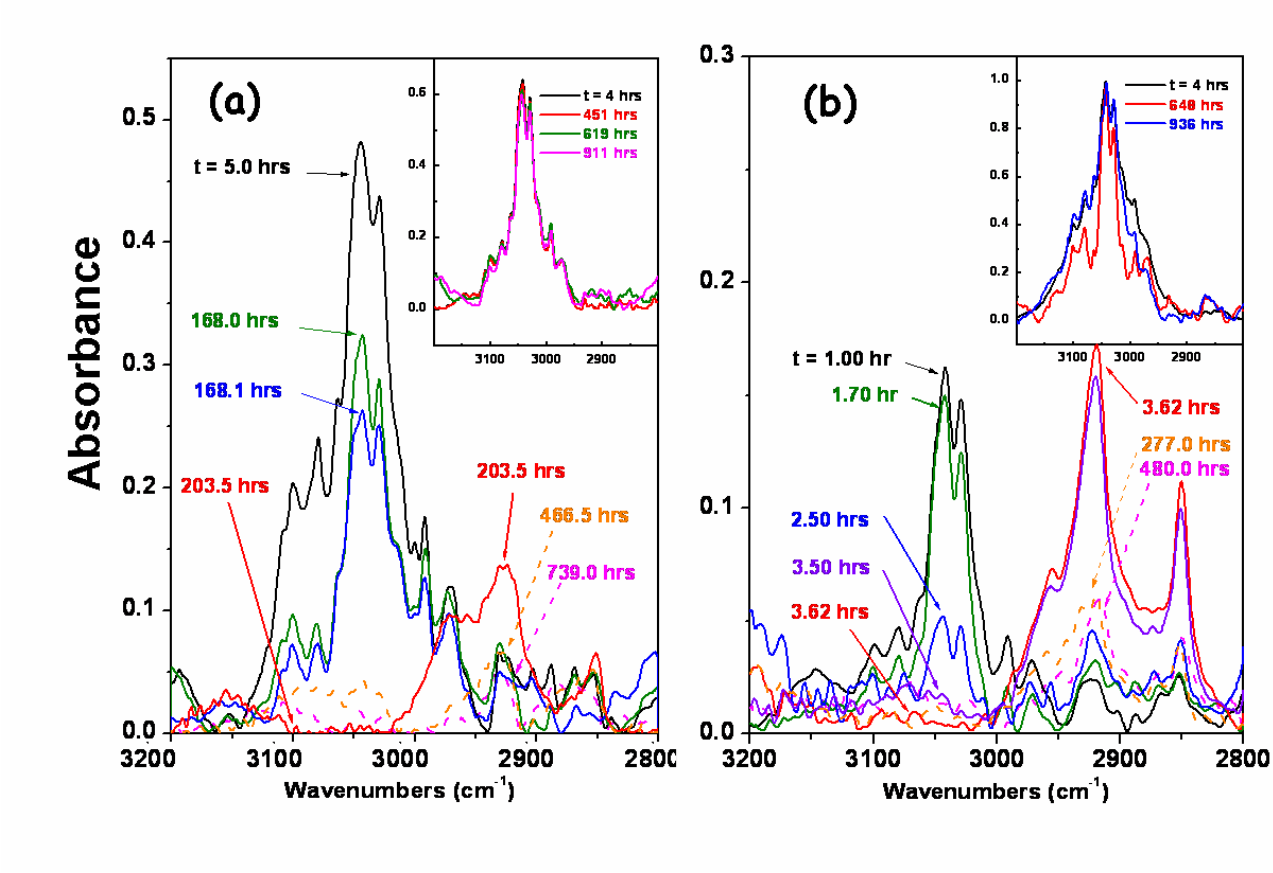

Figure 13. Time series of SR-based FTIR absorption bands corresponding to pyrene and biomass formation following the degradation of pyrene by *Mycobacterium sp*. JLS on magnetite surfaces. Panels a and b are from a sample free of and with ESHA. The time at which each spectrum as acquired is labeled. They show the transient behavior of pyrene doublet at 3044 and 3027 cm^{-1} as well as and biomass IR absorption bands at 2921 and 2850 cm^{-1}. Similar behavior was observed for pyrene absorption band centered at 1185 cm^{-1}. Inserts are time series from abiotic control experiments [105].

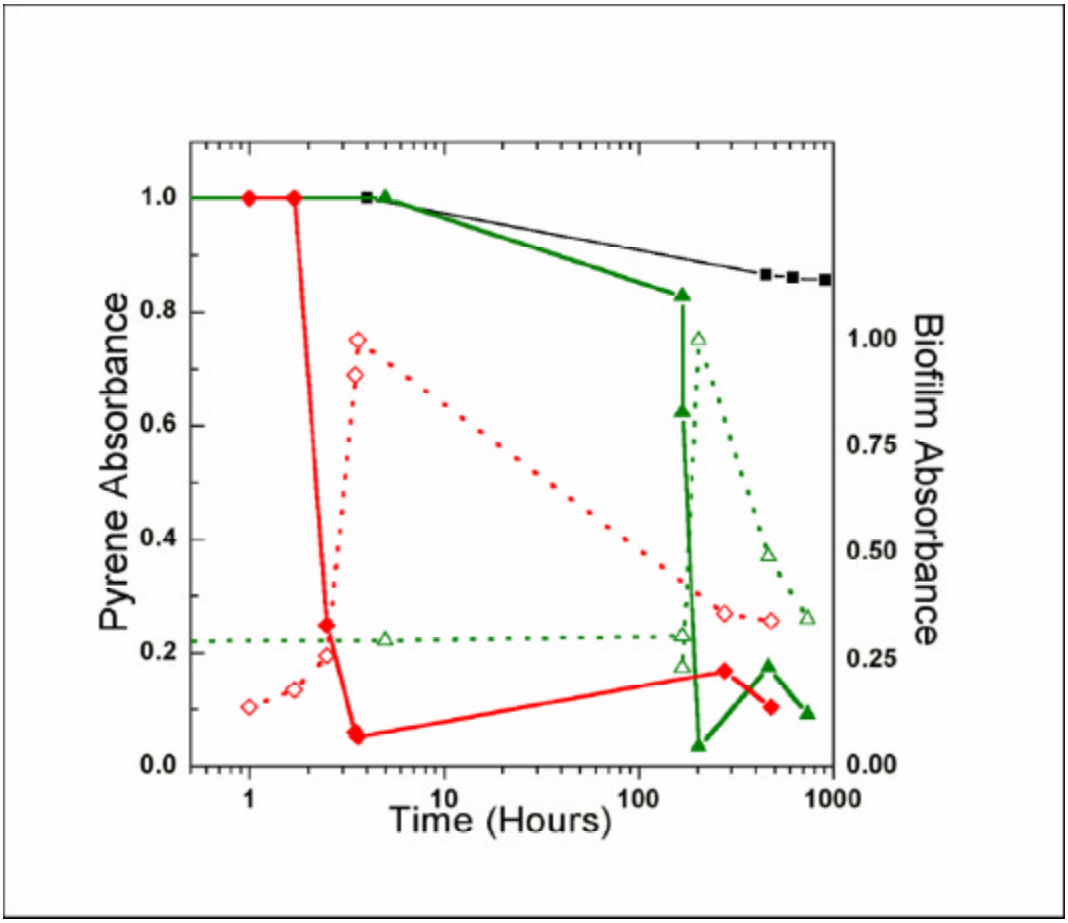

Figure 14 Summary of the SR-based FTIR results showing that pyrene degradation occurs much faster when ESHA is present (note the log scale on the time axis). They pyrene absorbance was measured at 1185 cm^{-1} and biomass IR absorption band at 2921 cm^{-1}. The color scheme is black for abiotic, green for biotic without ESHA, and red for biotic with ESHA. The solid lines show the pyrene amount as a function of time for each experiment. The dotted lines show a subsequent increase in *Mycobacterium sp*. JLS biomass after pyrene degradation [105].

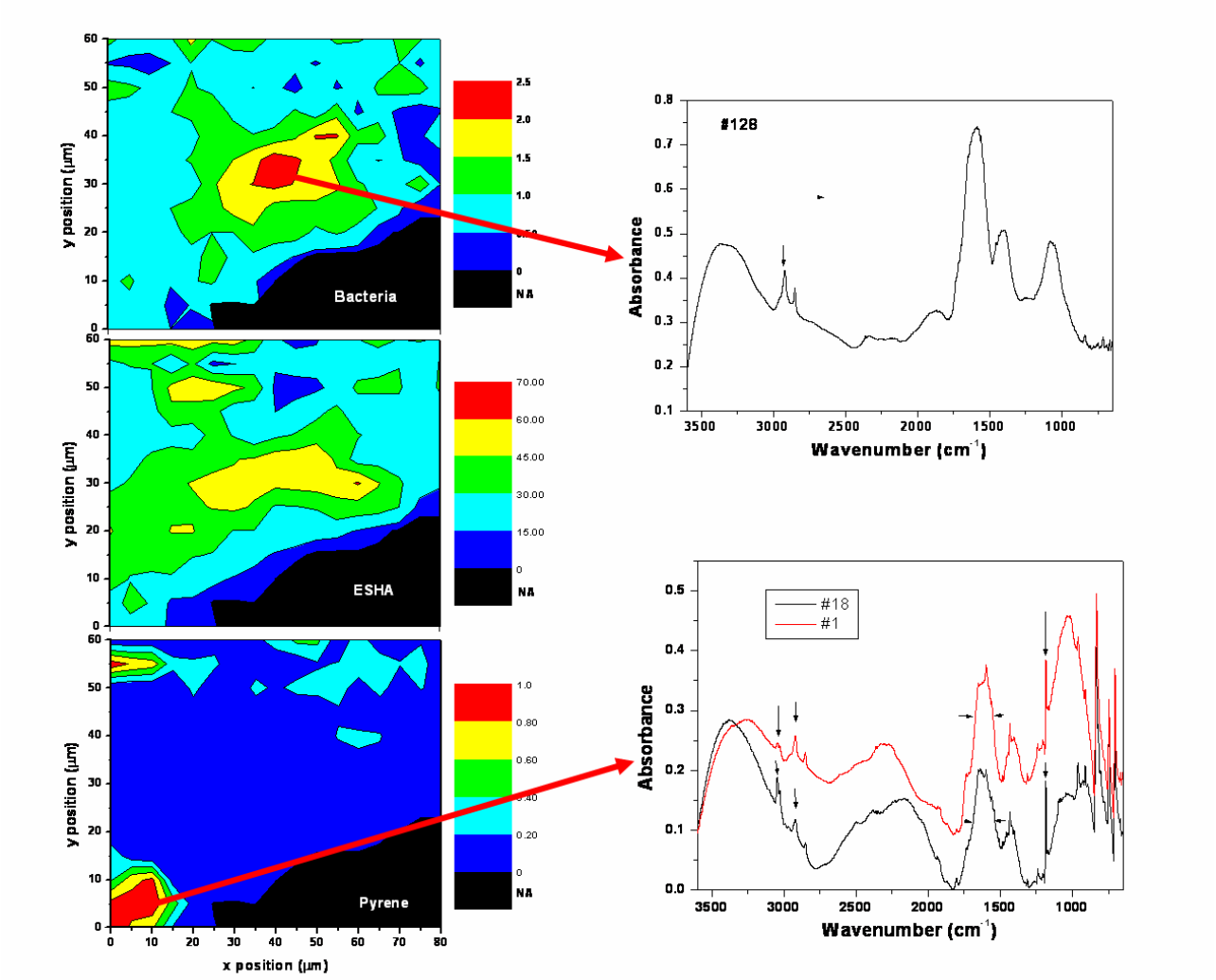

Figure 15. Contour diagrams from infrared mapping obtained at the end of the experiment showing the spatial distribution of the infrared absorption peaks corresponding to (top) *Mycobacterium sp*. JLS bacteria, (middle) ESHA, and (bottom) pyrene. Appropriate spectral regions were integrated for each point on the maps. The color scales for each contour plot are red for high integrated IR peak area (high concentration of the corresponding component) and blue for low peak area (low concentration); black is an out-of-focus region of the sample. The center of the map shows a region with high density of bacteria and high concentration of ESHA where pyrene has been completely degraded [105]. Note that the quality of the spectra is excellent even on such a complicated surfaces of earth materials. (Arrows are pointing at some of marker peaks employed in this study).

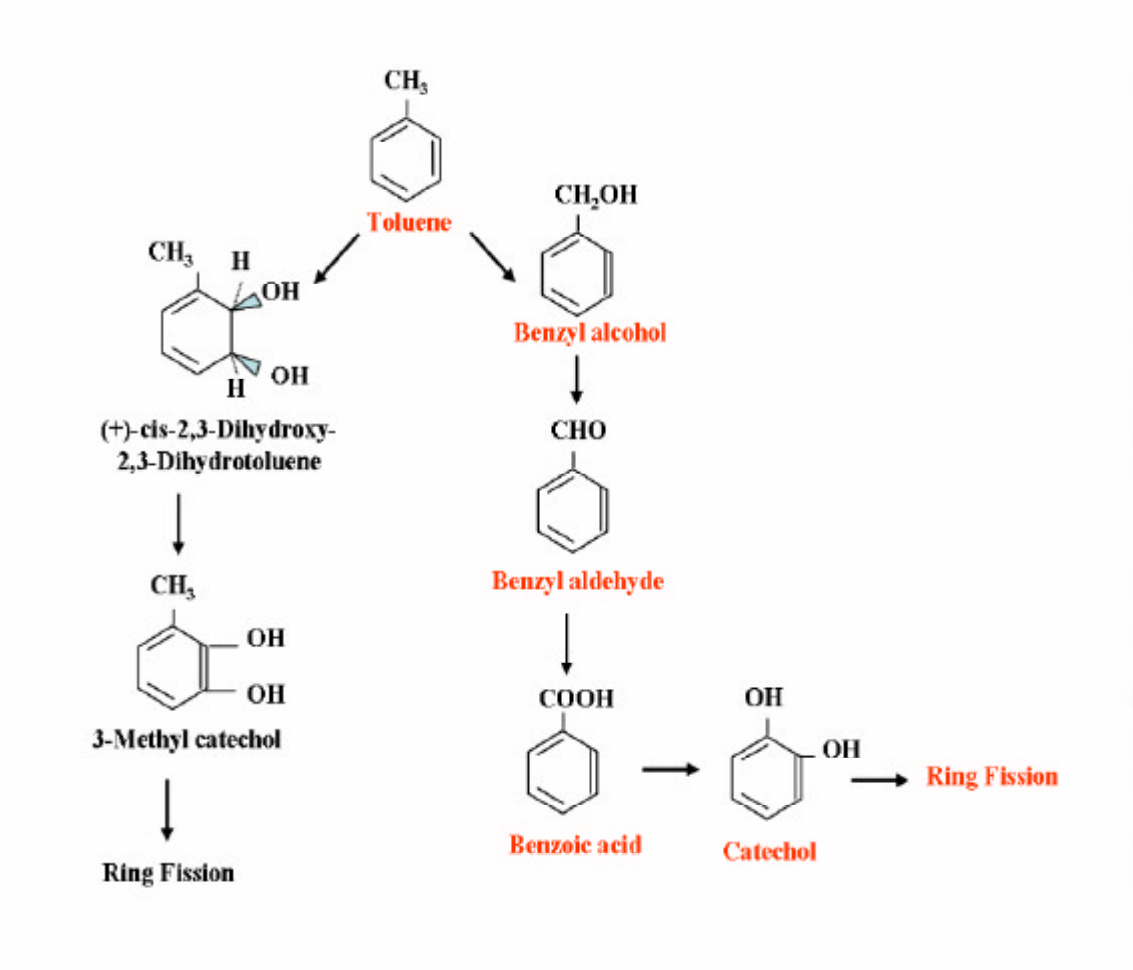

Figure 16. The possible pathway for the metabolic degradation of toluene by the intrinsic microbial communities in the earth materials. Due to interference, we only tracked the marker peaks for toluene, benzyl alcohol, benzyl alcohol, benzoic acid, and catechol in this study (see Table 2).

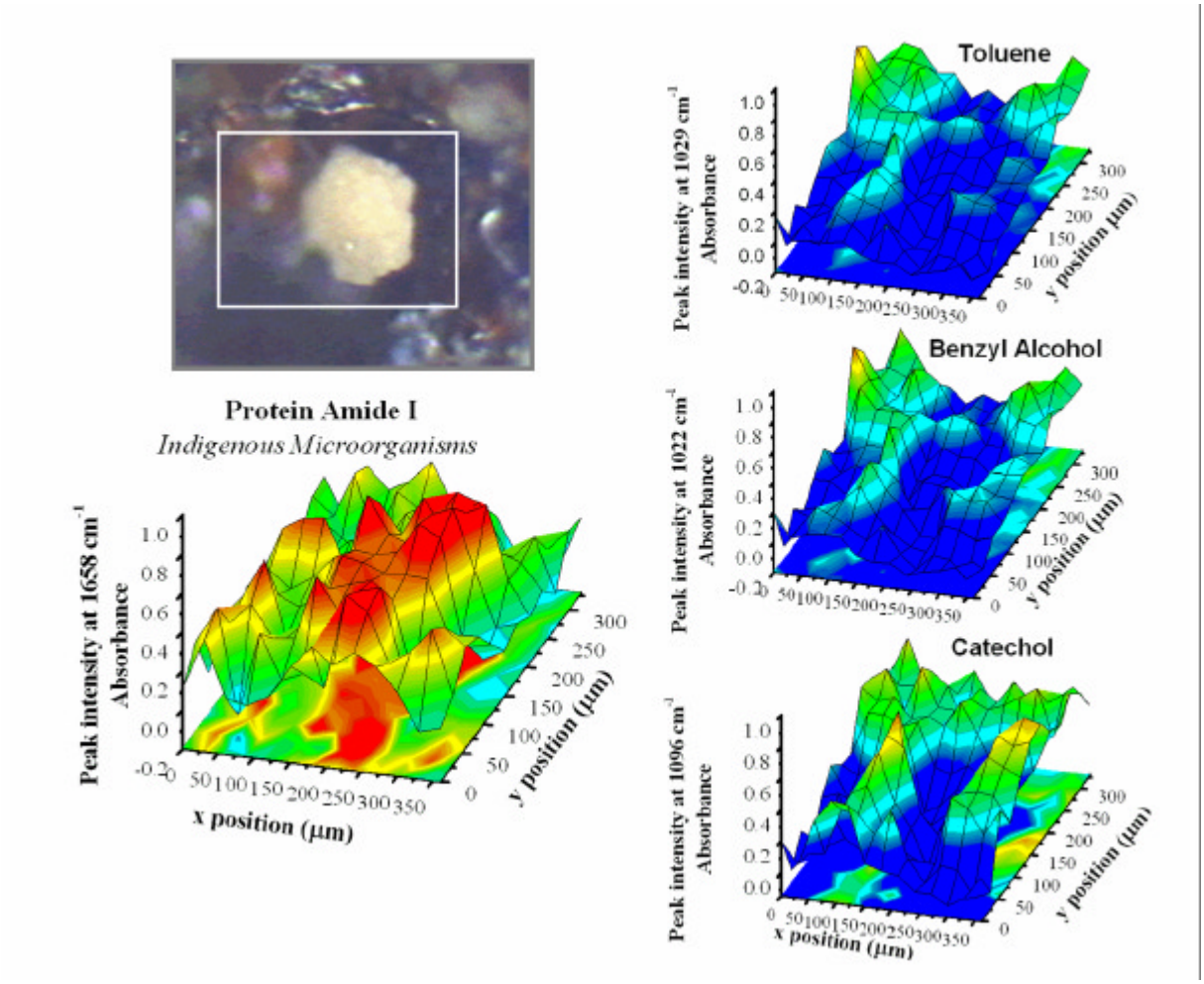

Figure 17. Chemical images from synchrotron-radiation-based Fourier-transform-infrared (SR-FTIR) spectromicroscopy showed that the native microorganisms were thriving during toluene degradation. (Left top) A bright-field micrograph of microbial colonies formed on the basalt surfaces after exposure to 100-ppm toluene vapor for five days. The spatial distribution of infrared absorption peaks corresponding to (left bottom) indigenous microorganisms, (right top) toluene and the metabolites (right middle and bottom). It appears that many native microbes metabolized nearly all the toluene immediately, with some accumulation of the nontoxic metabolites benzyl alcohol and catechol. No accumulation of benzoic acid was detected. This implies that intrinsic microbial communities at the former polluted site remained efficient in detoxifying toluene.