Remote Sensing of Subsurface Biomineralization

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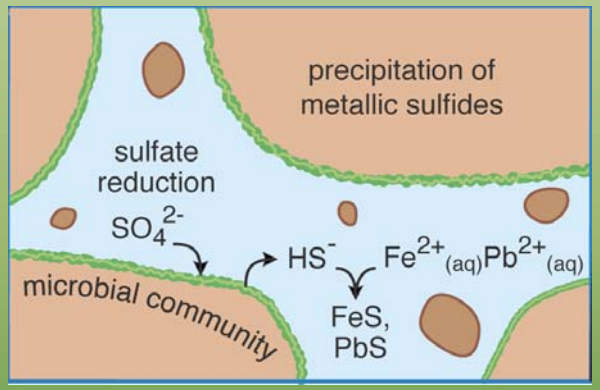
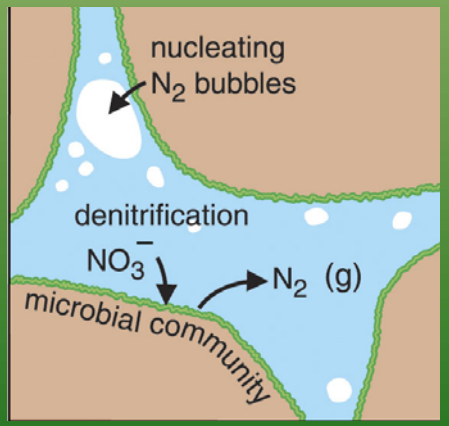
Geophysical Monitoring:

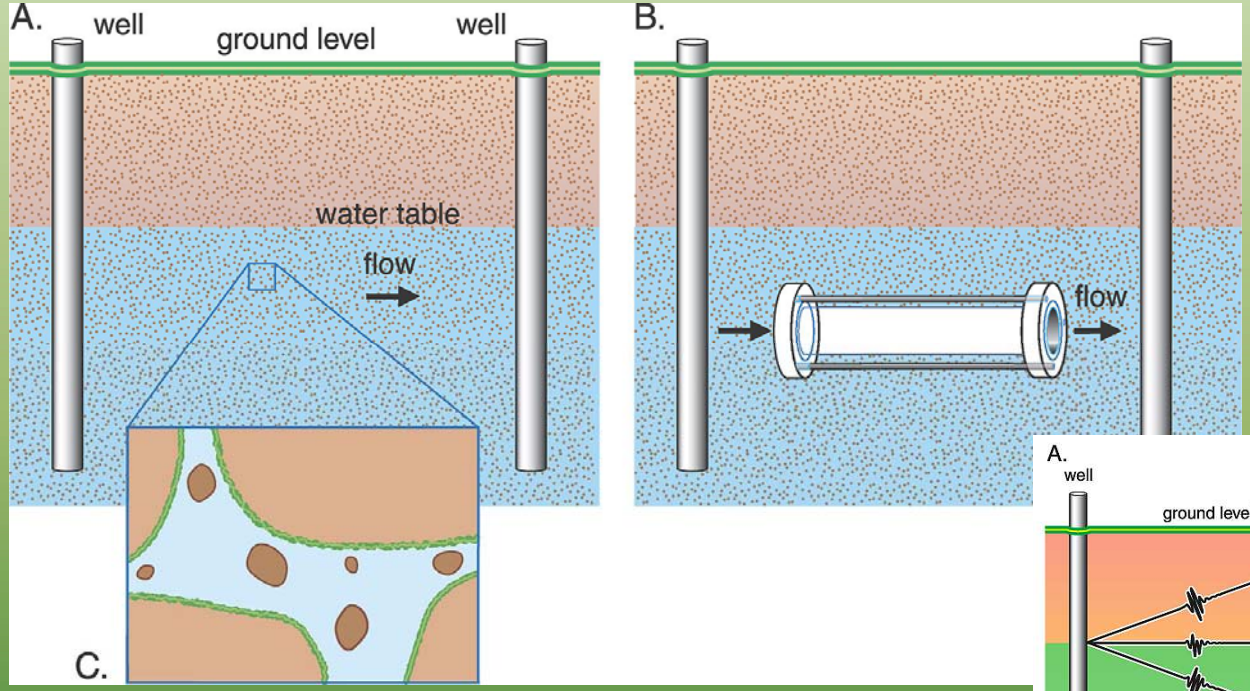
- Governing Hypothesis:
  - Microbial processes in the subsurface create *physical property changes* that can be detected with geophysical methods
- Possible Changes:
  - Evolved gases
    - N_2, CH_4
      - Decreased acoustic wave amplitudes
  - Precipitates
    - Sulfides (ZnS, FeS), Oxides (CrO_3, UO_2):
      - Increased electrode polarization
      - Decreased acoustic wave amplitudes
  - Increasing biomass and biofilm production
    - Pore throat bridging and bulk density changes
      - Increased acoustic wave amplitudes and velocities

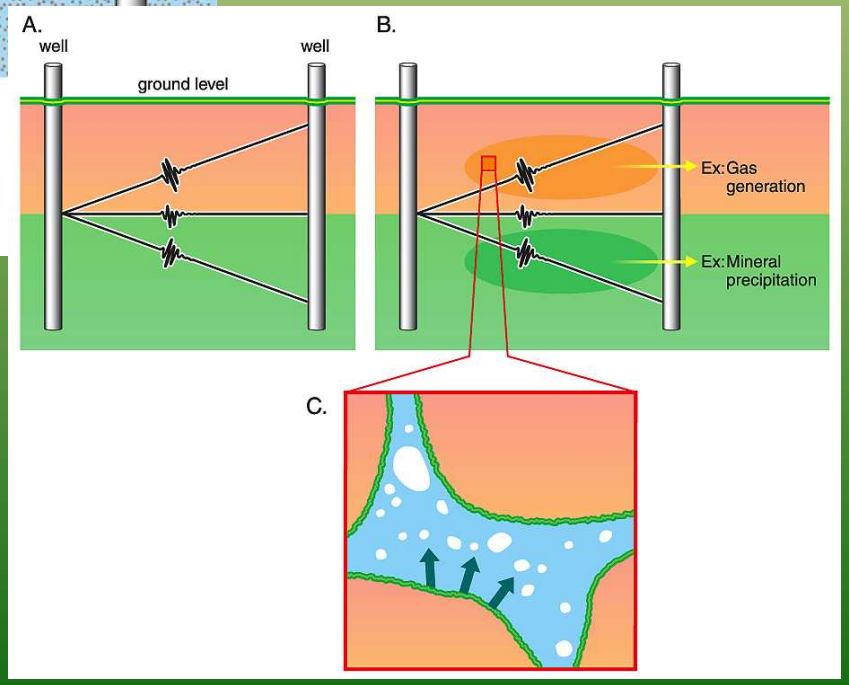




Saturated Column Experiments:

Mimic Subsurface Using *Dynamic Flow* Column Experiments:



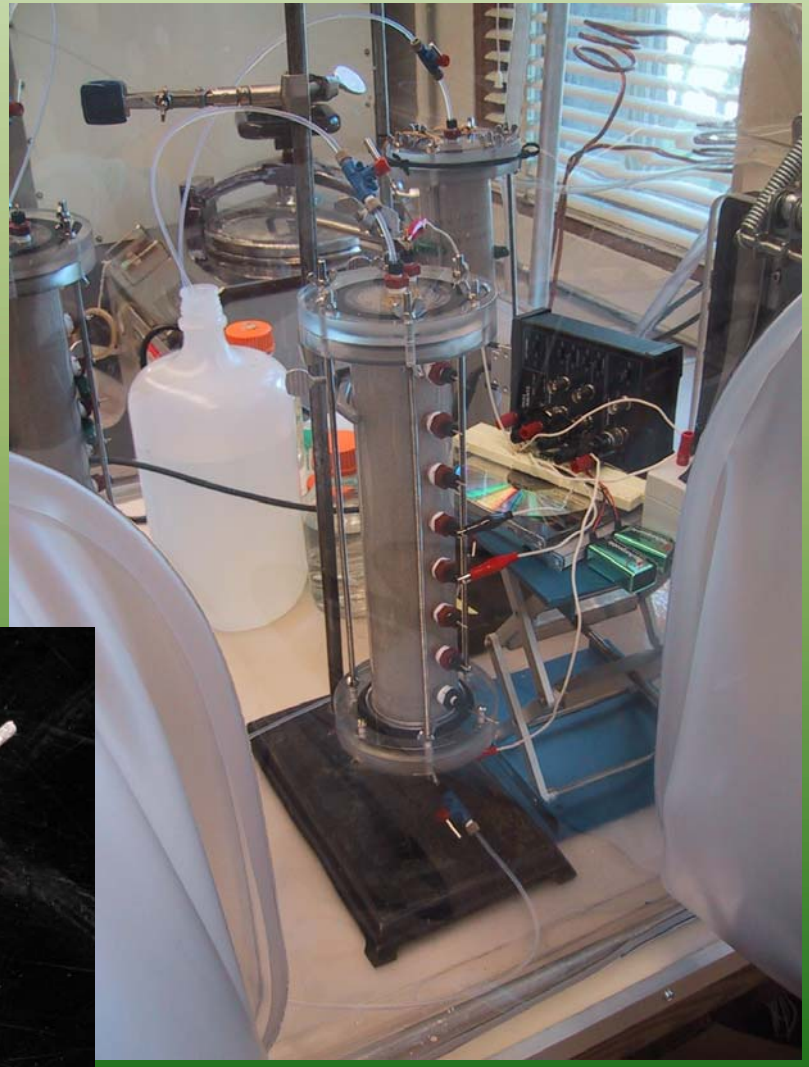


Saturated Column Experiments:

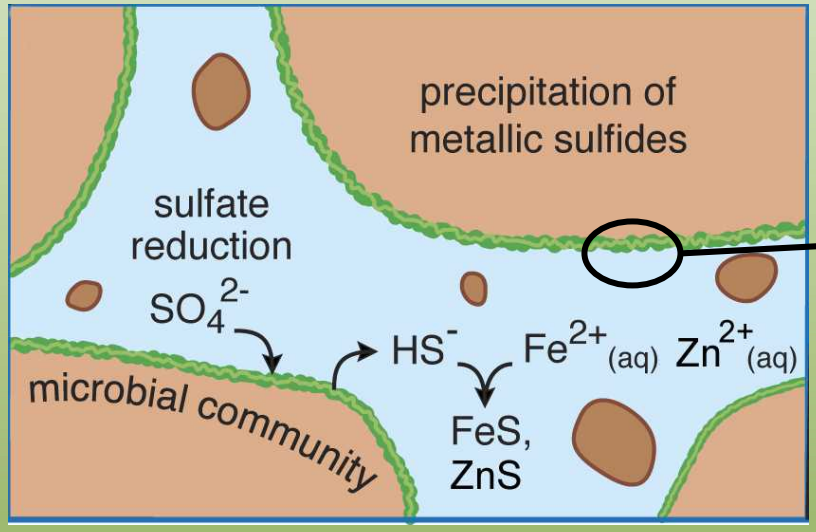
- Acoustic Method:
  - Cross-column acoustic pulses (10^5-10^6 Hz)
  - Velocity and amplitude information
  - Spectral analysis
  - *Non-invasive* measurement

Saturated Column Experiments:

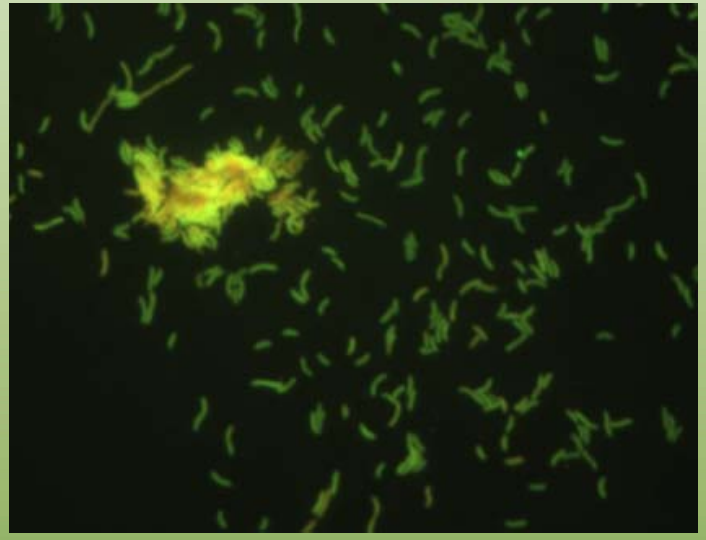
- Electrical Method:
  - Complex Resistivity ($0.1\text{-}10^3$ Hz)
  - Non-polarizing (i.e. low-noise) Ag/AgCl potential electrodes
  - Gold current electrodes
  - *Non-invasive* measurement



Microbe-Induced Sulfide Precipitation in Porous Granular Media

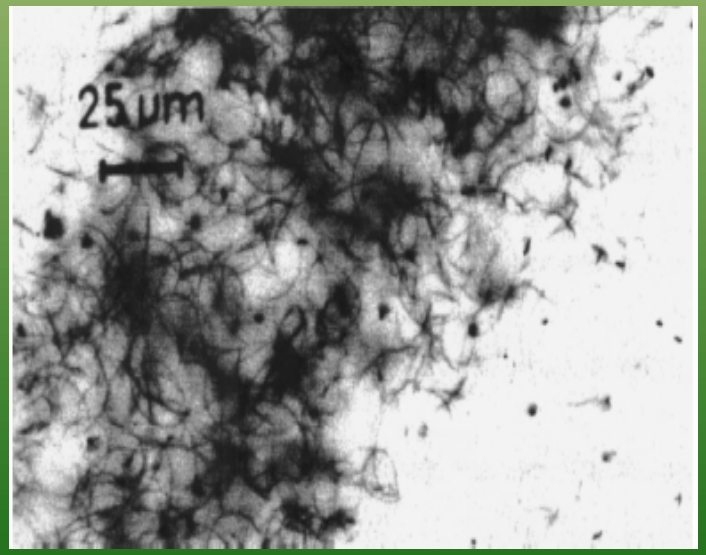


a)



100x Optical Microscope Image

b)



Cypionka, Annu. Rev Microbiol., 54, 2000

- *Desulfovibrio vulgaris (ATCC 29579)*
  - Common soil borne and aquifer SRB
  - Moderately aero-tolerant
  - Motility via single polar flagellum
  - Facultative fermenter; possible hydrogen bursts

Microbe-Induced Sulfide Precipitation in Porous Granular Media

Widdel et al., The Prokaryotes, 1992.

– Experimental Conditions:

- Specific flux = **50cm day^{-1}**
- Incomplete oxidation of lactate = **2.8mM**
- Moderare [SO_4^{2-}] = **3.8mM**
- Low salinity = **~1.5mS/cm**
- Low/Moderate metals concentrations
  - [Fe^{2+}] = **0.36mM = 20ppm**
  - [Zn^{2+}] = **0.30mM = 20ppm**

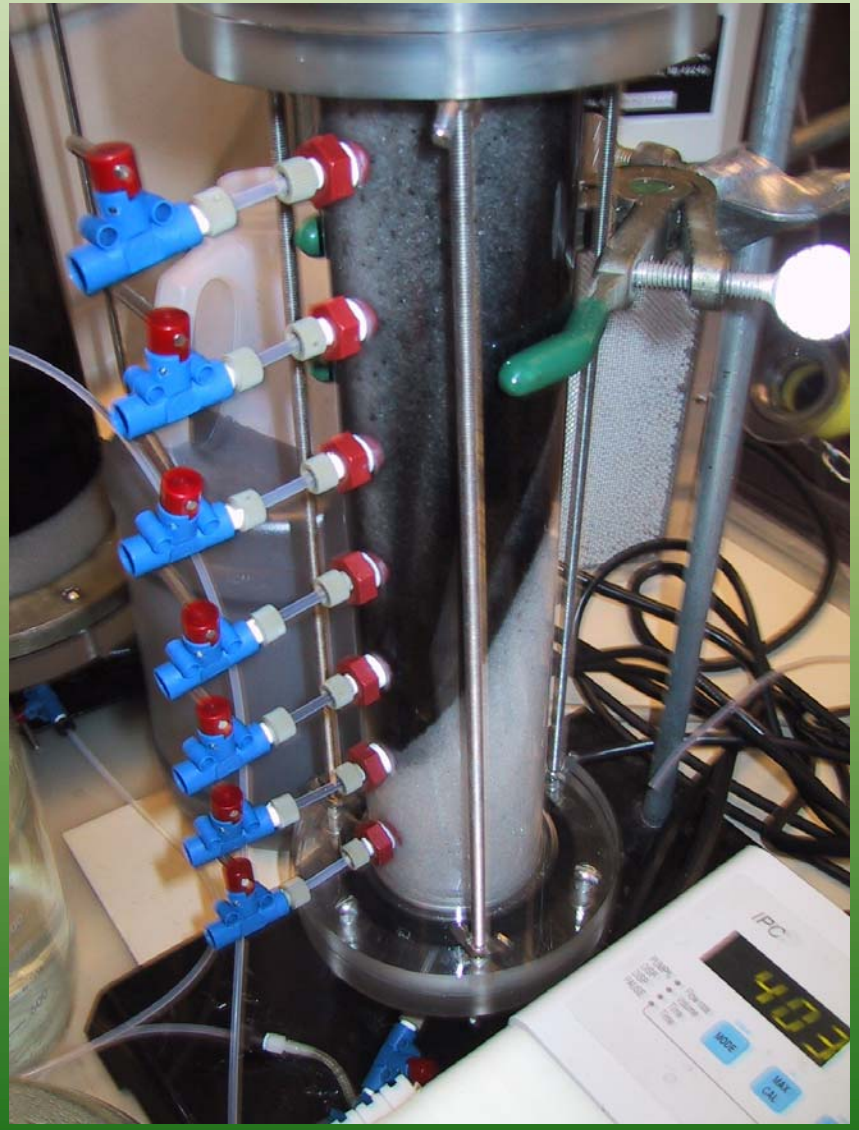
– Predicted Stoichiometric Relations at pH 7:

$$\mathbf{C_3H_5O_3^- + 0.5\ SO_4^{2-} + 0.25\ Fe^{2+}, Zn^{2+} + H^+ \rightarrow}$$
$$\mathbf{C_2H_3O_2^- + 0.25\ FeS, ZnS + 0.25\ H_2S + H_2O + CO_2}$$

Microbe-Induced Sulfide Precipitation in Porous Granular Media

- *General Layout:*
- 4 Columns
  - 3 Inoculated
  - 1 Control
- Syringe injection of 10% pore volume of exp. phase inoculum
- Continuous infiltration of media from ***bottom*** to ***top***
- ***Fixed*** rate of advection
- Measurements and sampling (*except seismic*) made in glovebox

Data and Results: Biogeochemistry



- *Highlights*:
- 7 Sampling Ports:
  - #1 lowest - #7 highest
- Onset of sulfate reduction after short lag phase
- Mobile precipitation front
- Substrate consumption and product accumulation shift toward base
- ***Chemotaxis*** toward higher substrate concentrations (i.e inlet)

Data and Results: Biogeochemistry

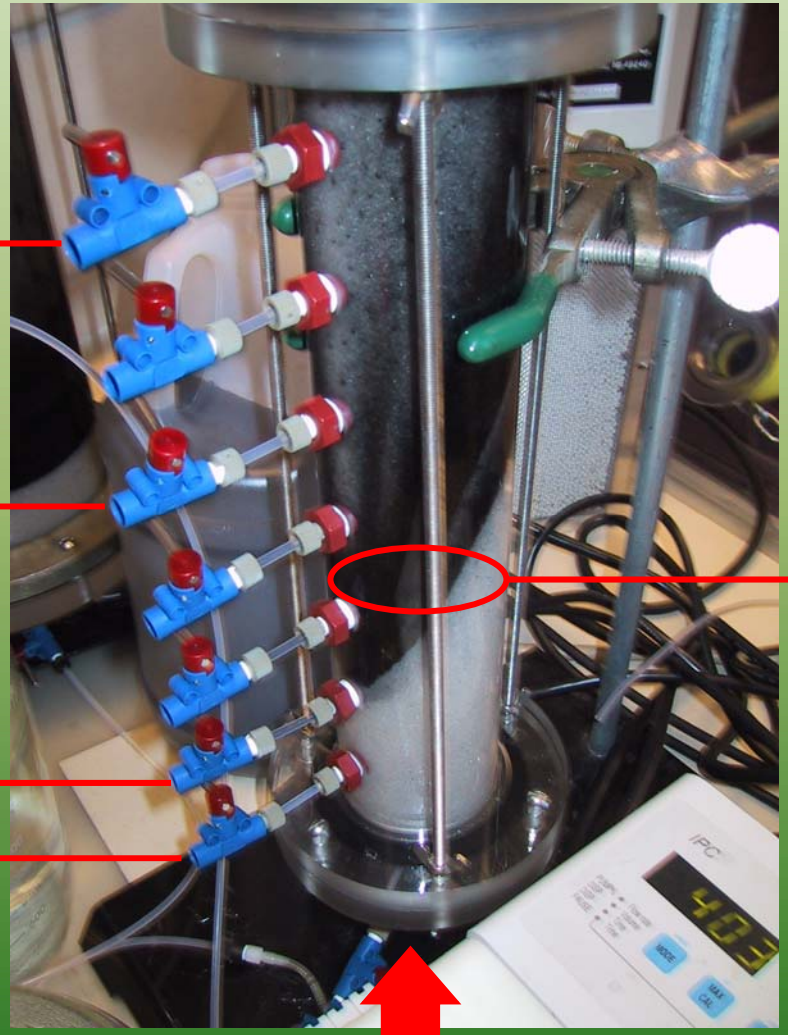
Day 4

Day 6

Day 11

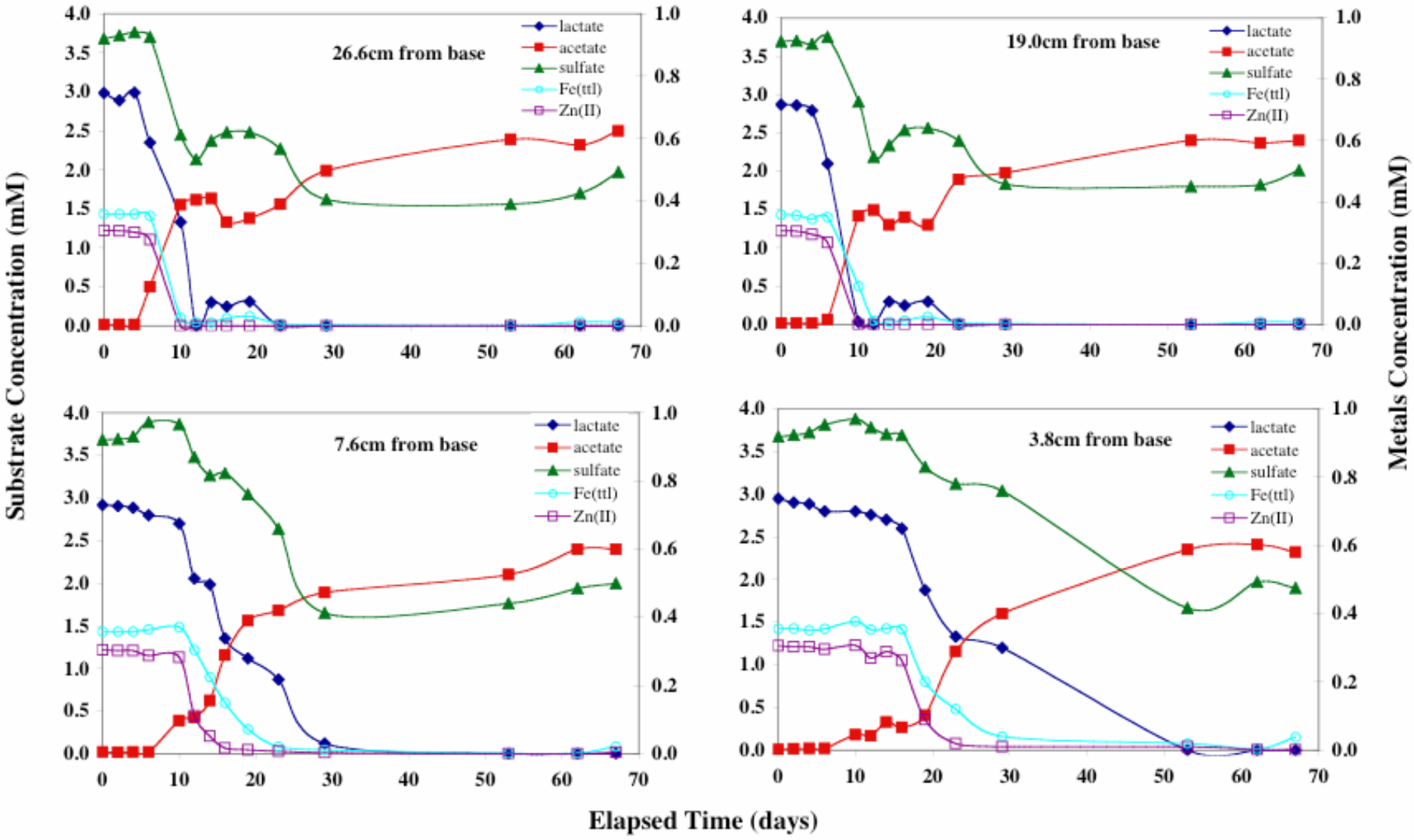
Day 10

Data and Results: Biogeochemistry



Data and Results: Biogeochemistry

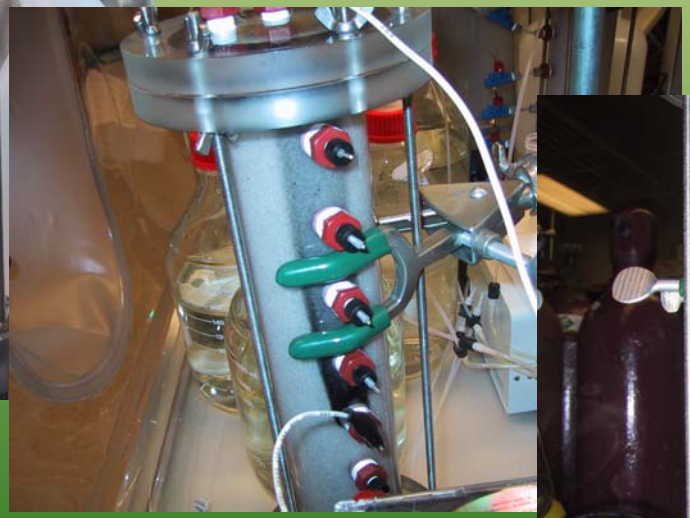
Multi-Port Sampler Data:



Data and Results: Geophysics - Induced Polarization

Day 4

Day 6

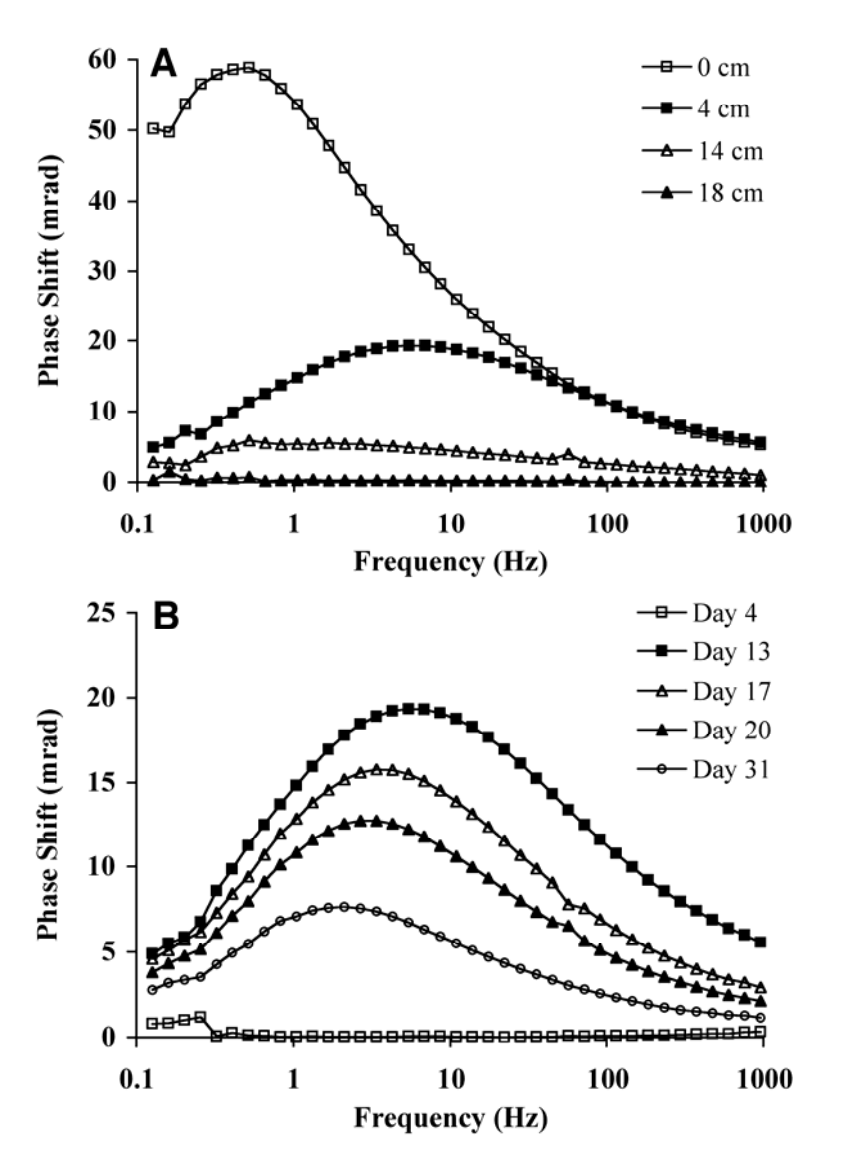


Day 10

Day 31

Data and Results: Geophysics - Induced Polarization

Day 31



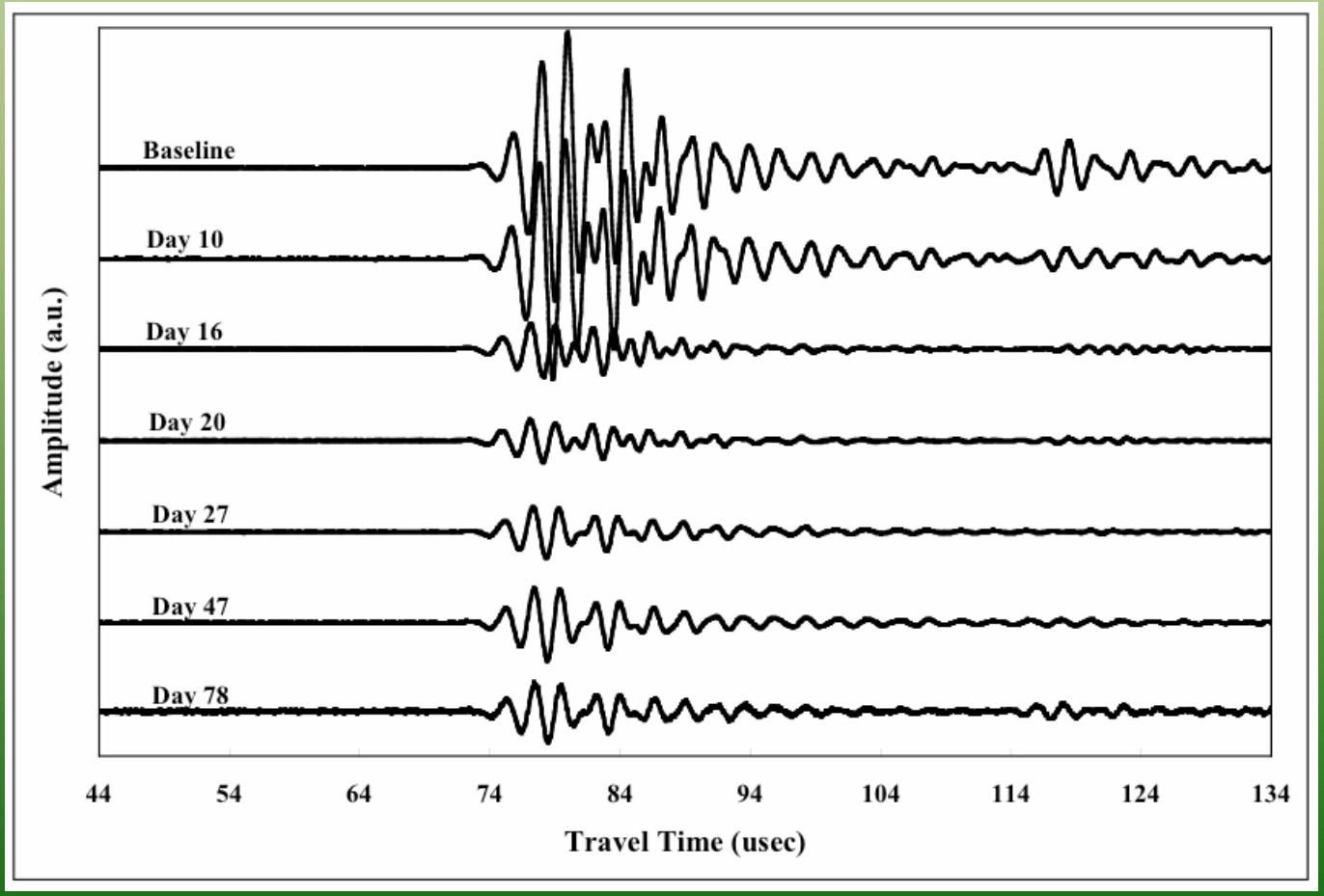
Data and Results: Geophysics - Acoustic Wave

Day 2

Day 27

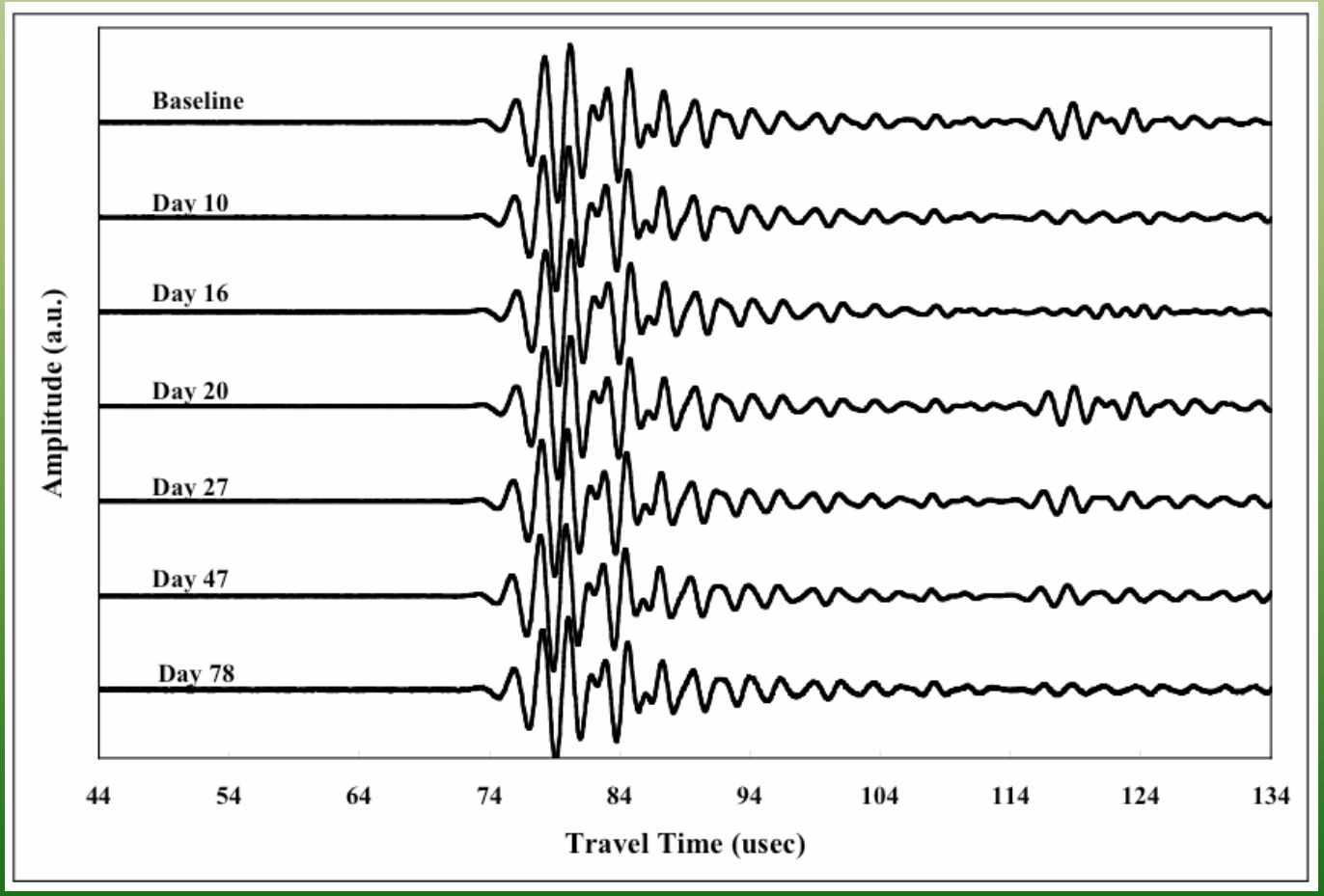
Data and Results: Geophysics - Acoustic Wave

2cm from bottom

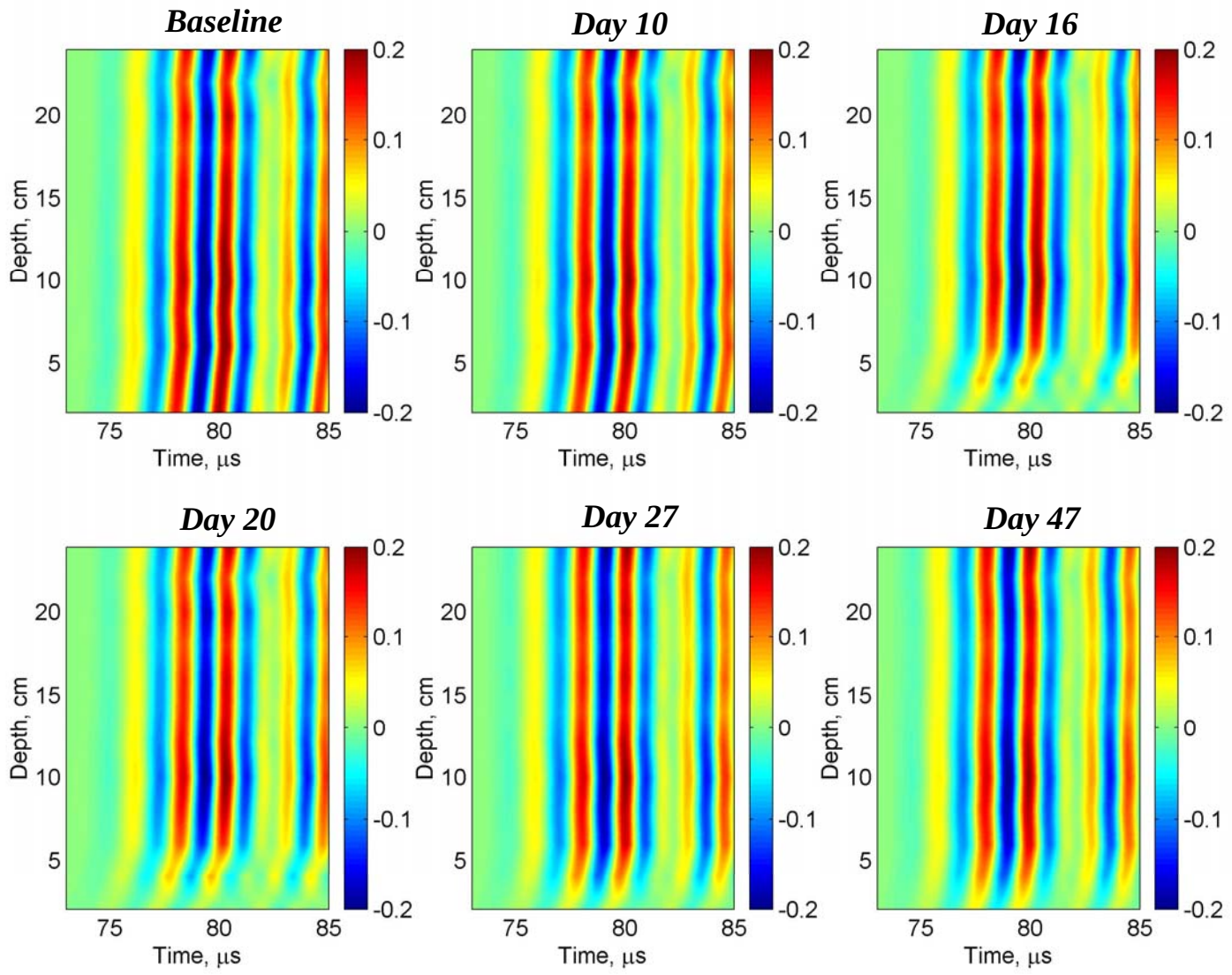


Data and Results: Geophysics - Acoustic Wave

8cm from bottom

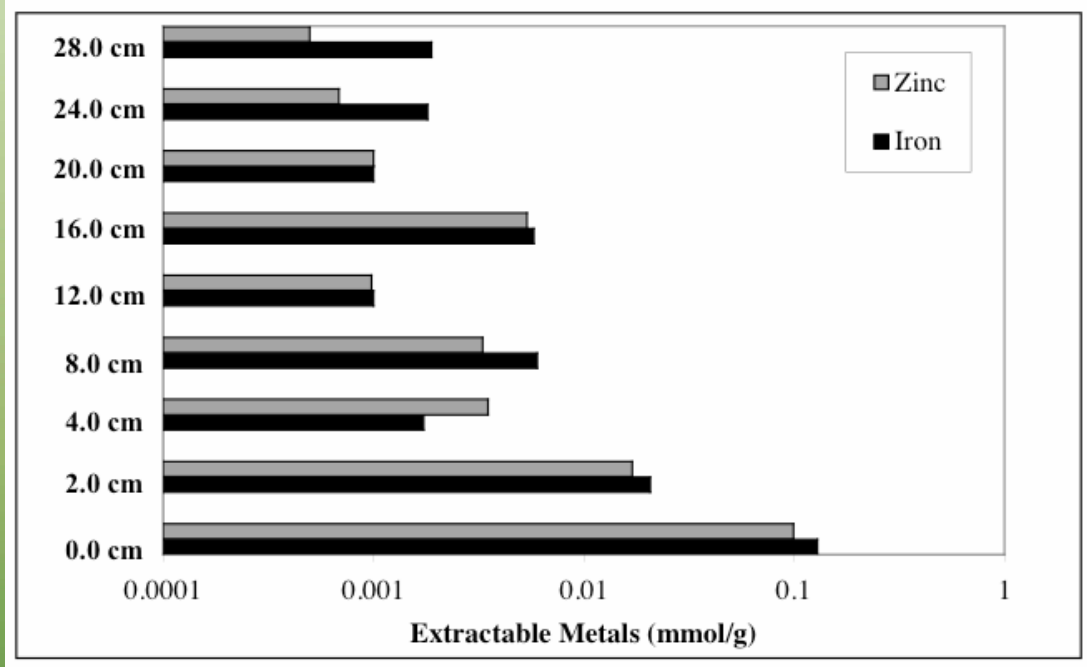


Data and Results: Geophysics - Acoustic Wave

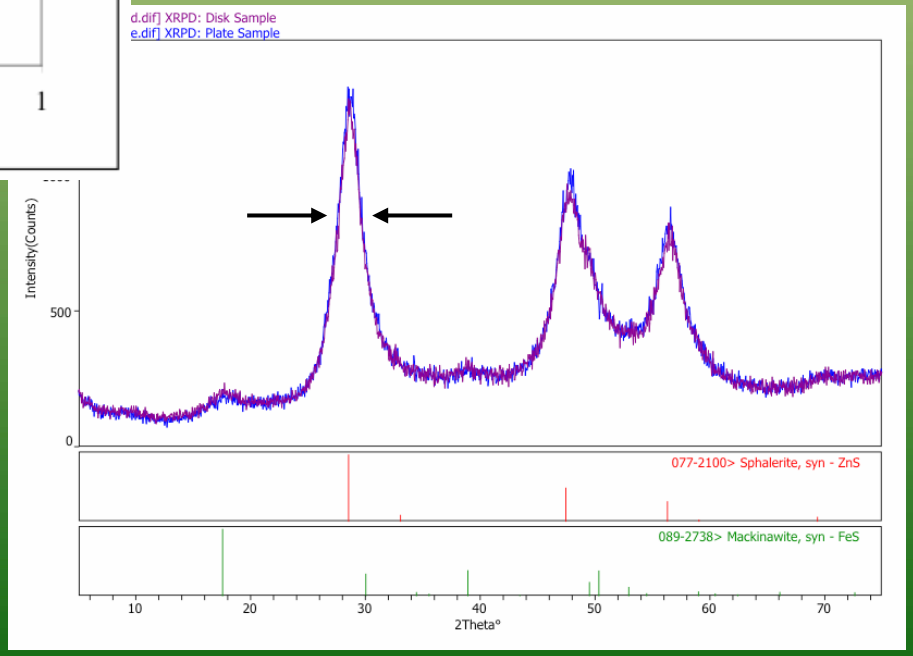


Data and Results: Destructive Evaluation

Extractable Metals as Function of Distance



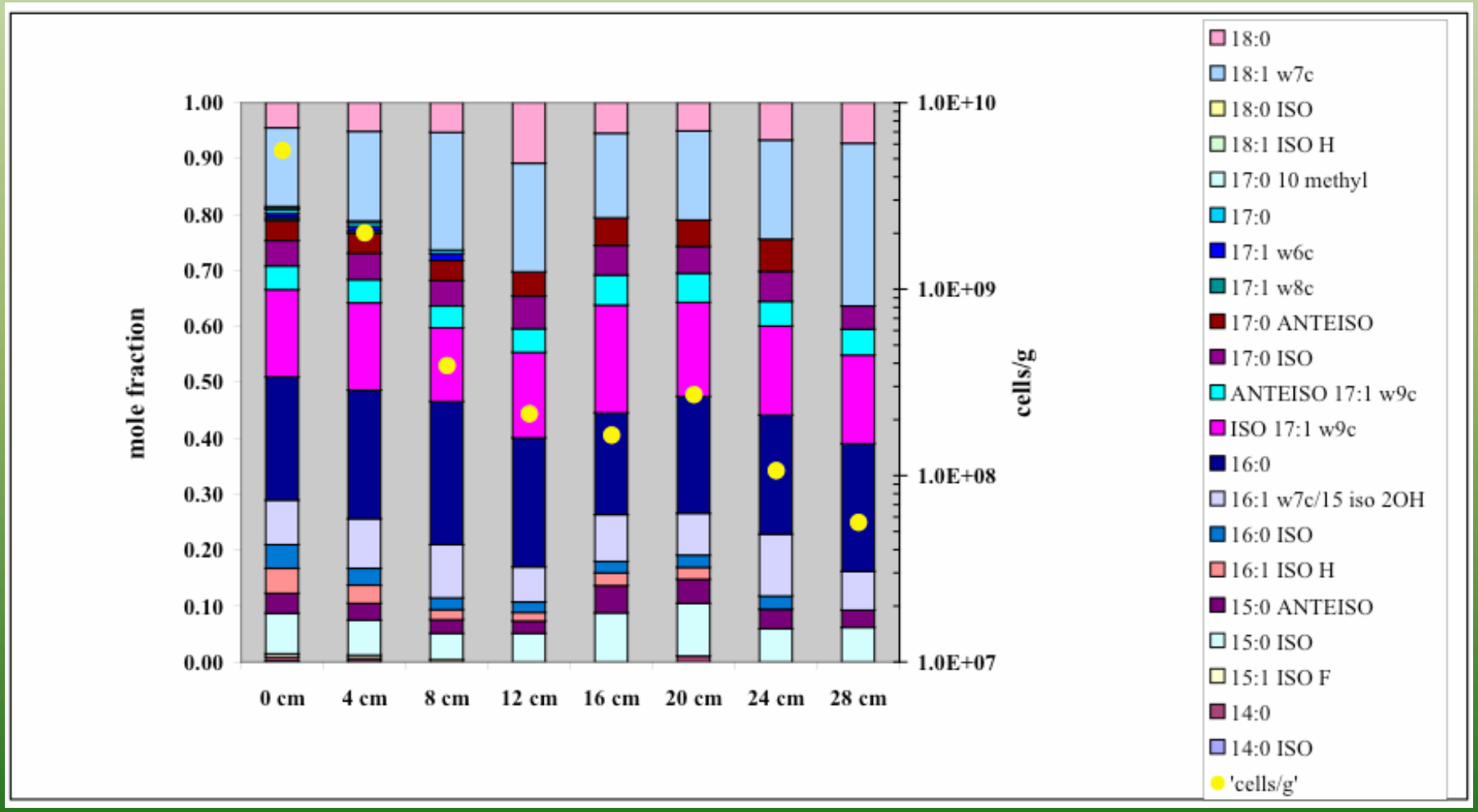
XRD Analysis of Biogenic Sulfides



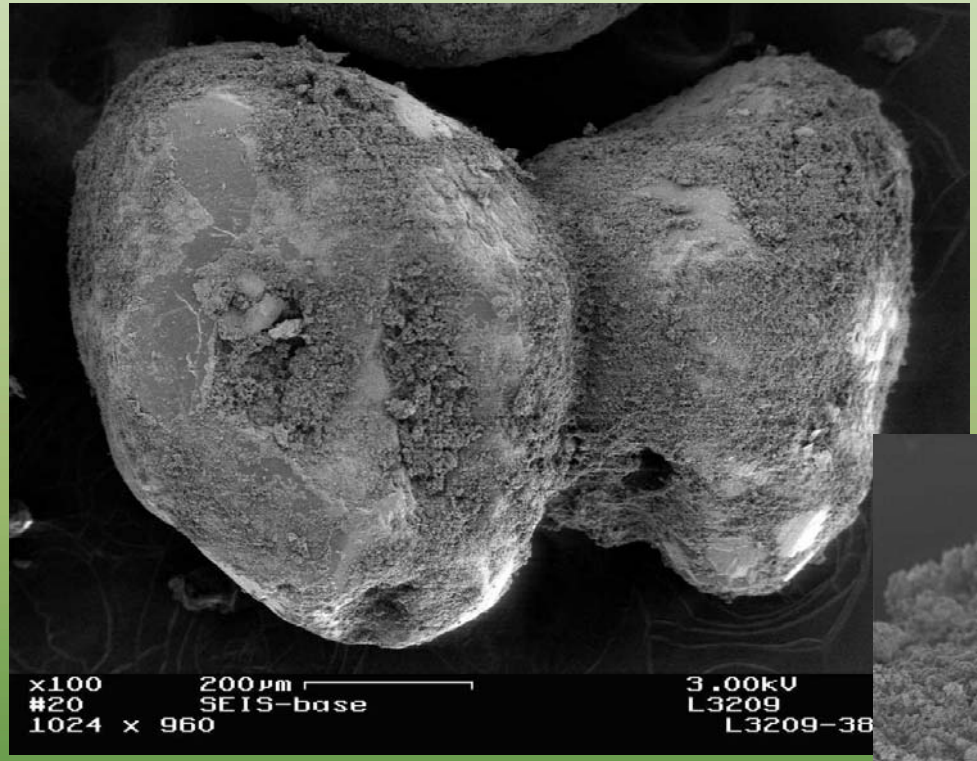
- *XRD Results:*
  - ~ 85/15 weight mixture of ***Sphalerite/Mackinawite***
  - Peak widths indicate average crystal size of ~3.0nm

Data and Results: Destructive Evaluation

Extractable Lipids as Function of Distance



Data and Results: Electron Microscopy (SEM)

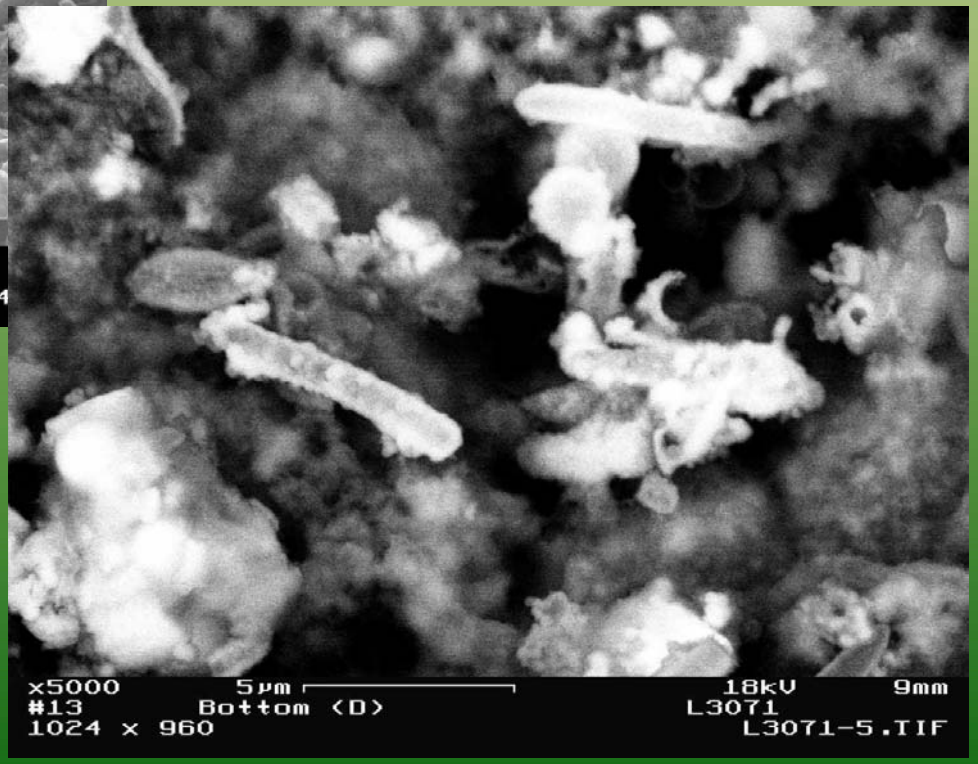


Zn/Fe-sulfide crusts on quartz grains

Data and Results: Electron Microscopy (SEM)

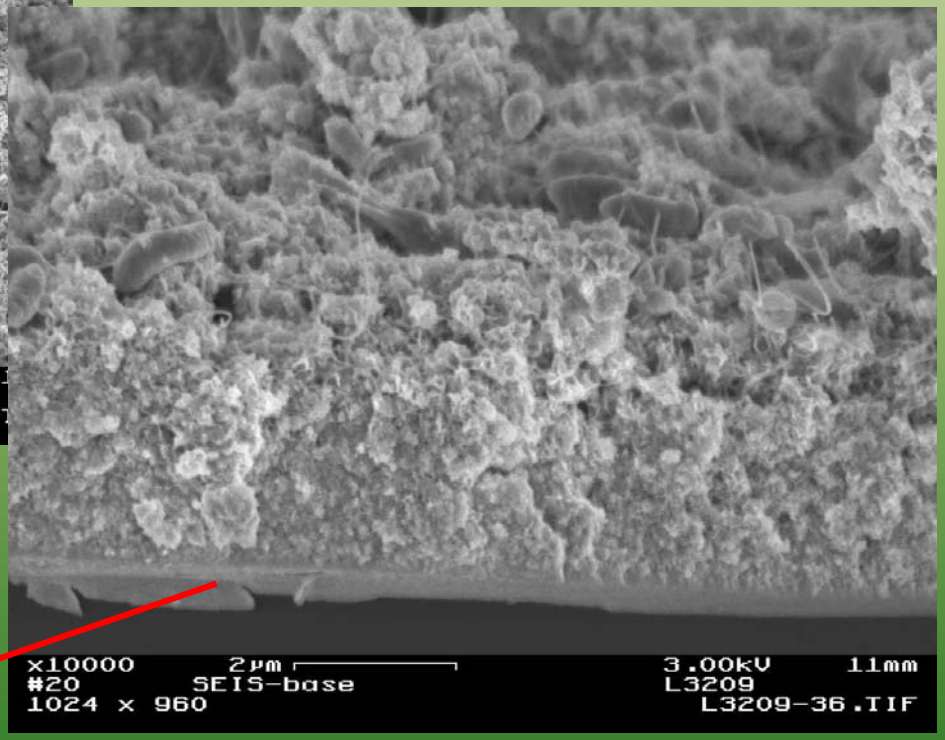
Magnified image of encrusted cells of D. vulgaris

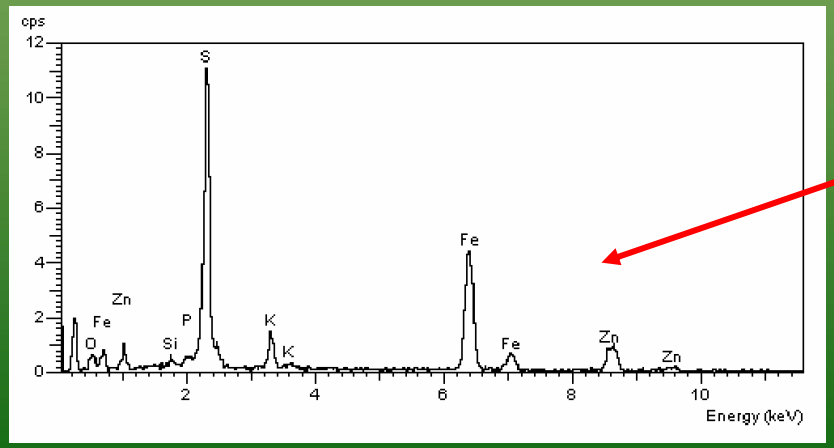
Secondary electron image of same



Data and Results: Electron Microscopy (SEM)

Biofilms and exopolymer acting as screens (?)





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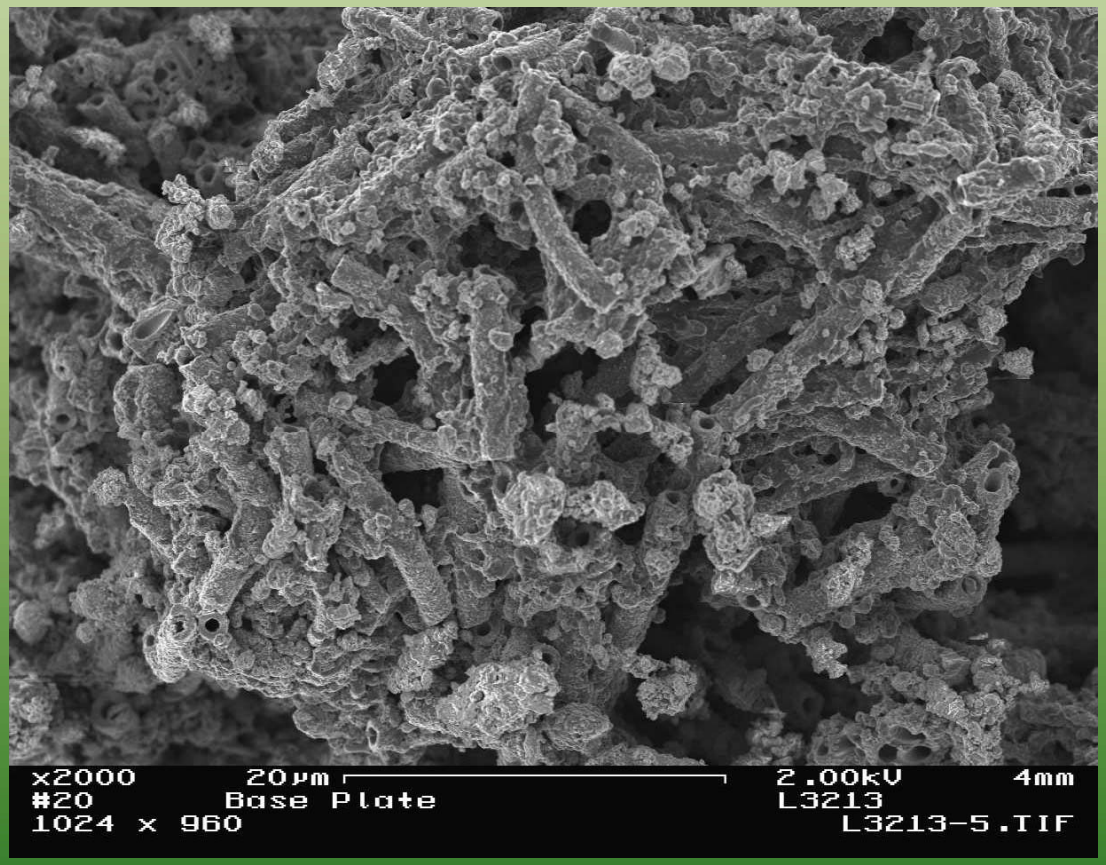
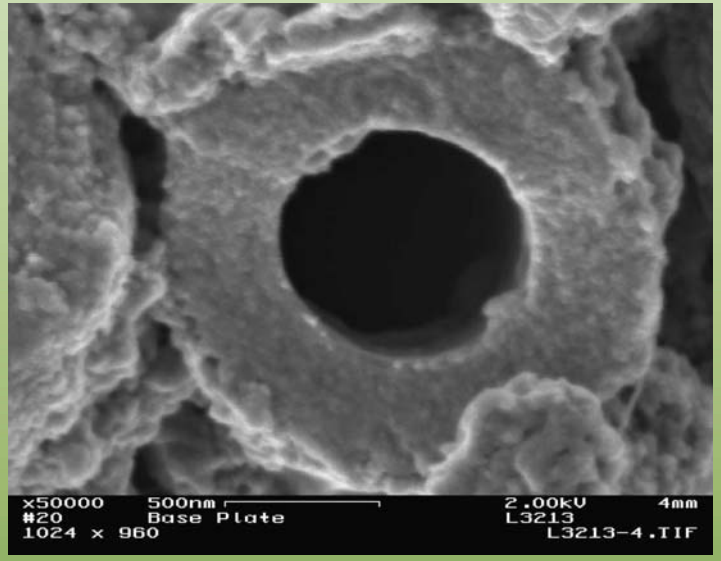
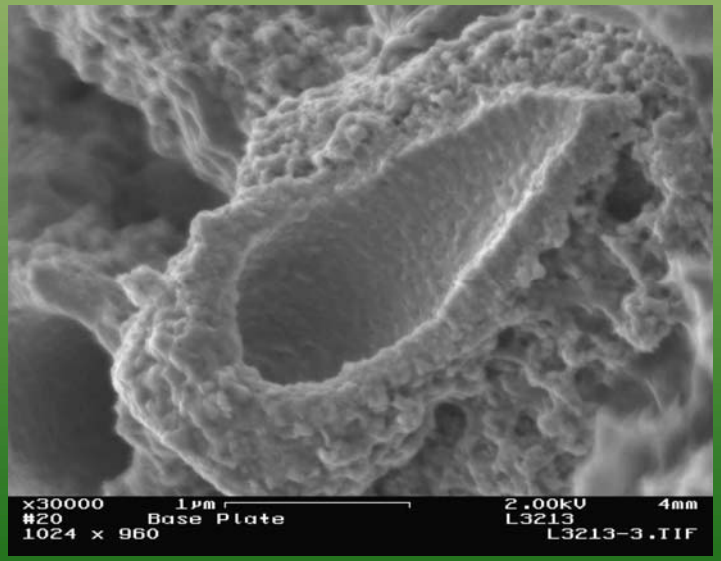
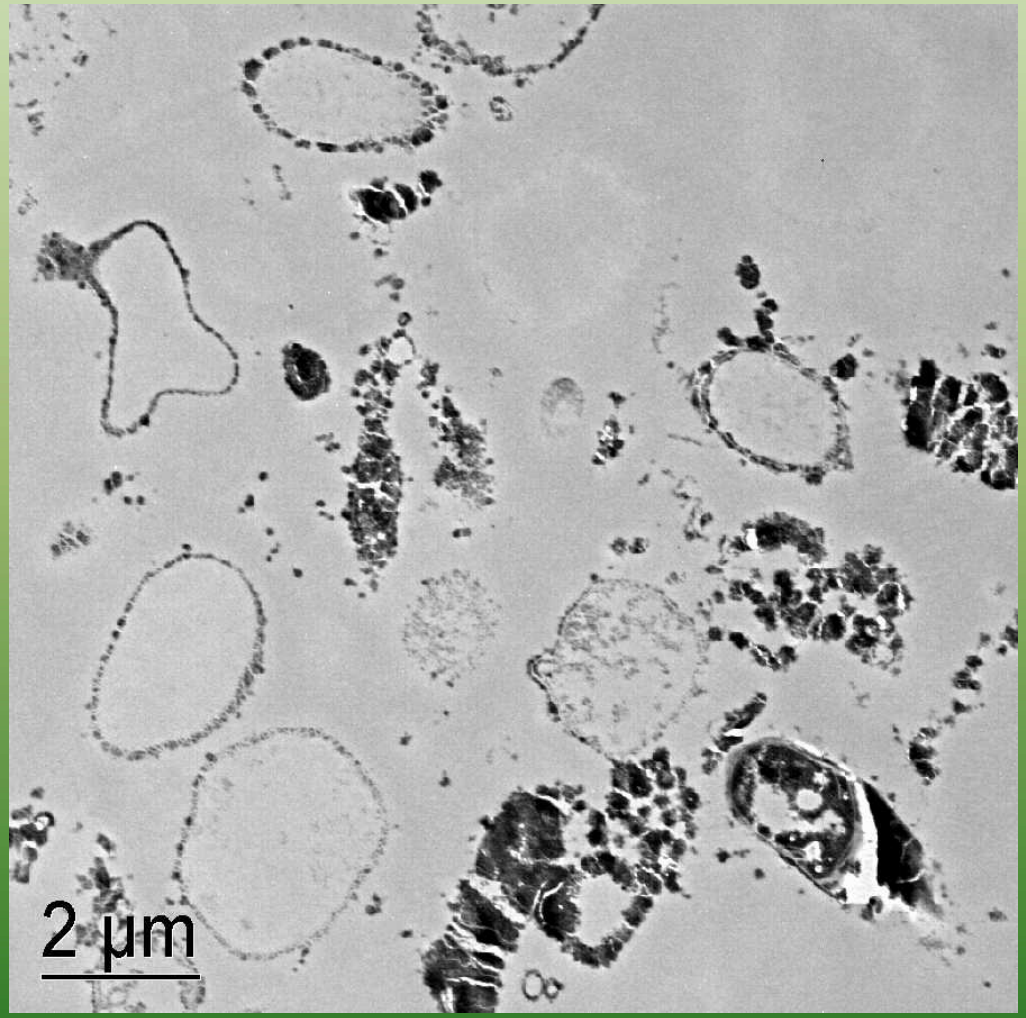


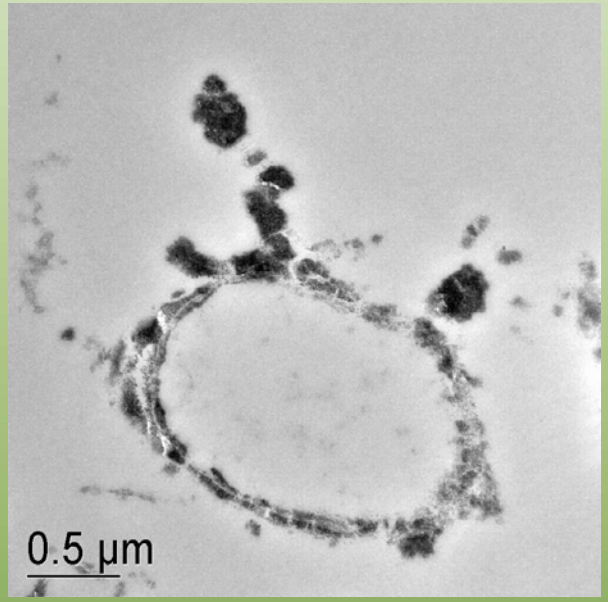


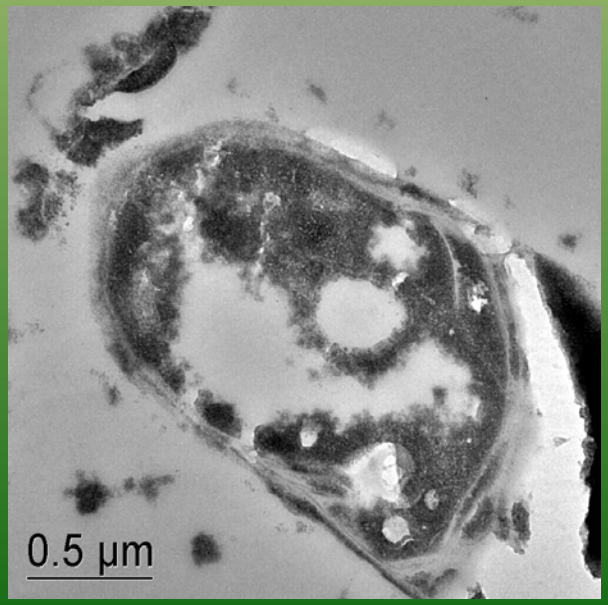


Non glutaraldehyde-fixed; note morphologic preservation!

Data and Results: Electron Microscopy (TEM)

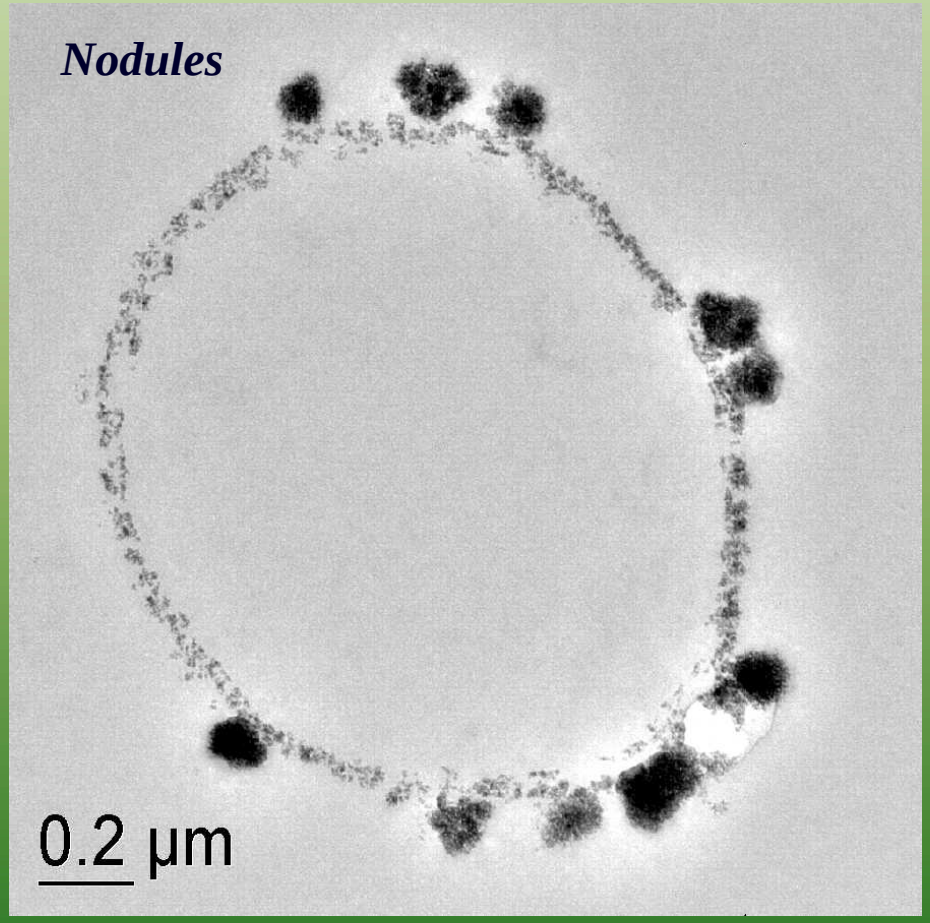


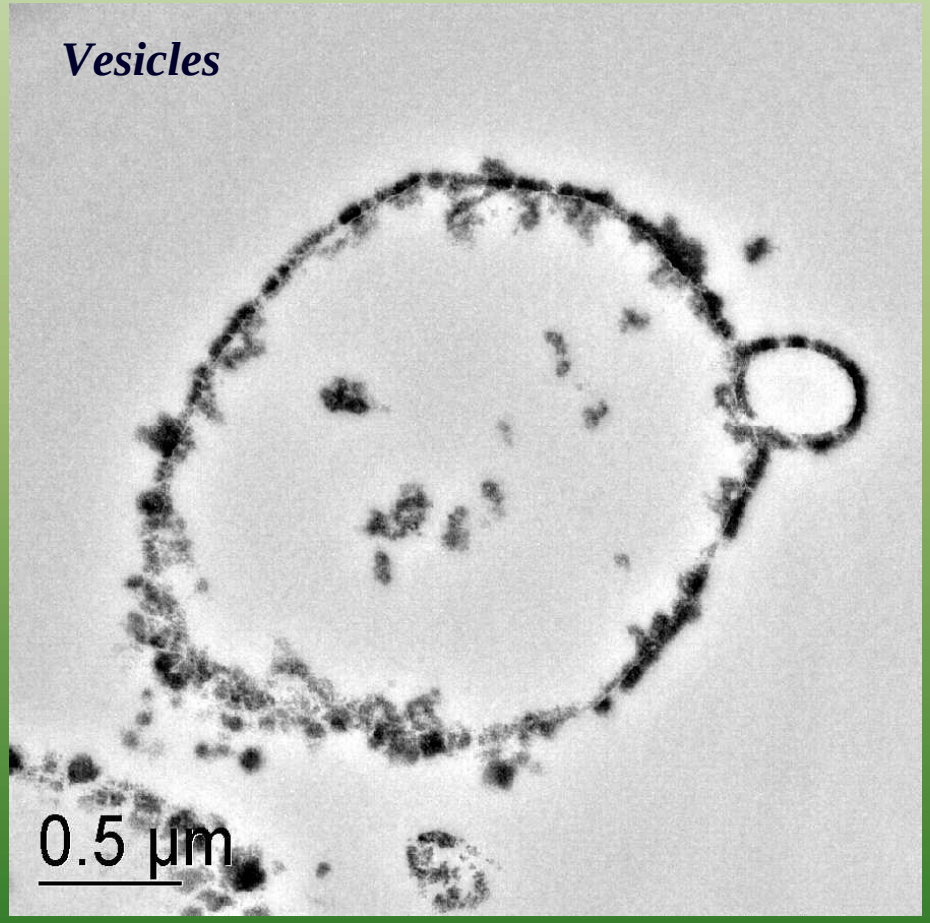




Wide range of microbe-sulfide associations

Data and Results: Electron Microscopy (TEM)





Data and Results: Electron Microscopy (TEM)

Predominantly Zn-rich sulfides

QuickTime™ and a TIFF (Uncompressed) decompressor are needed to see this picture.

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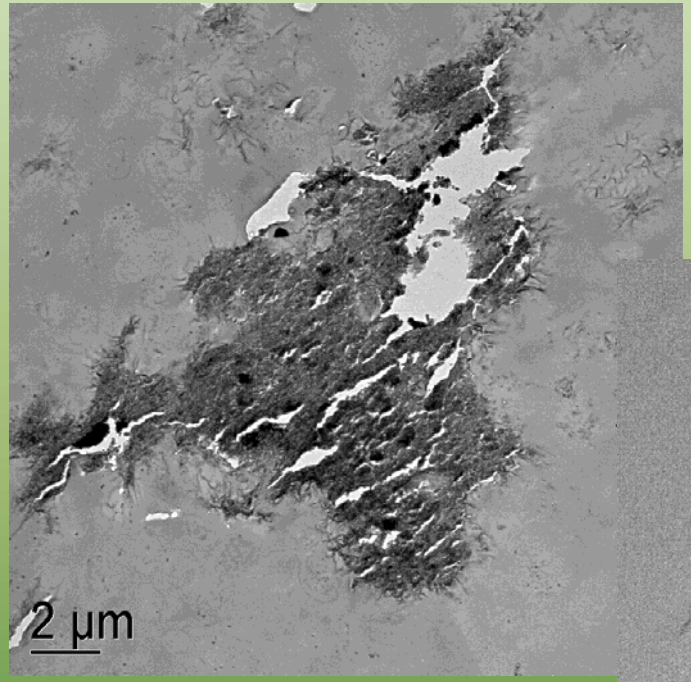
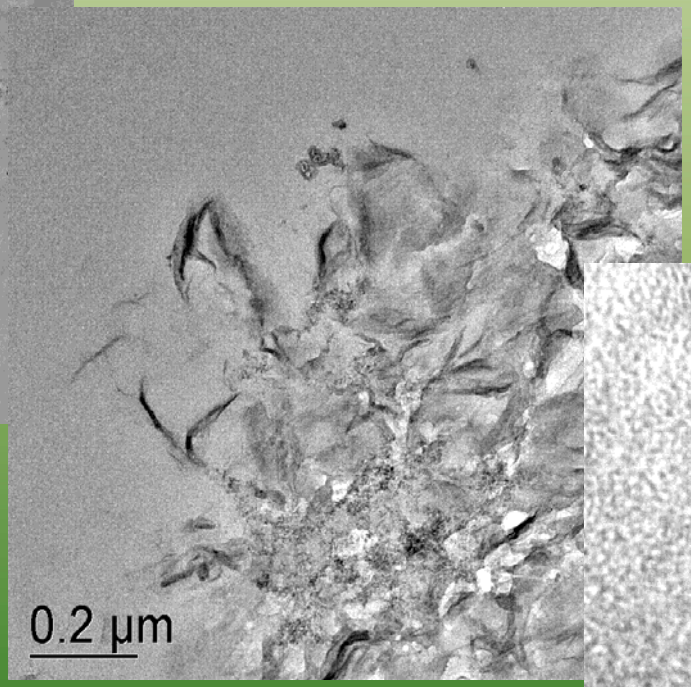
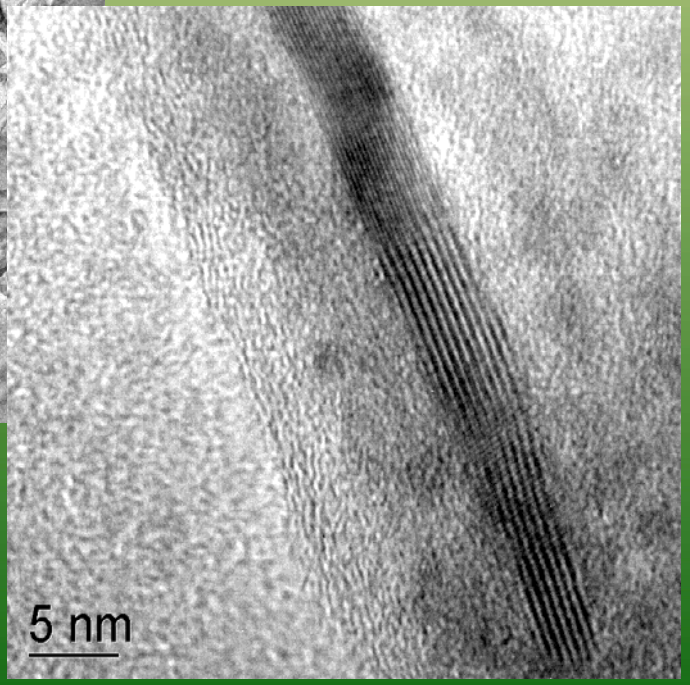
Data and Results: Electron Microscopy (TEM)

Predominantly Fe-rich sulfides







Primary Project Results To-Date:

Improved understanding of…

- Coupled processes that *influence* contaminant metals remediation using stimulated microbial sulfate-reduction
- Potential of using *geophysical methods* as a minimally invasive and potentially *field-scale* approach for investigating and monitoring such processes

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    - **Special thanks to Alice Dohnalkova and Jim Young (PNNL) for invaluable assistance with the electron microscopy**
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