To foster interest and excellence in ground water science and technology, the Henry Darcy Distinguished Lecture Series in Ground Water Science was established in 1986. The series—which has reached more than 72,000 ground water students, faculty members, and professionals—honors Henry Darcy of France for his scientific discoveries of 1856.
Beyond the Black Box: Integrating Advanced Characterization of Microbial Processes with Subsurface Reactive Transport Models

Quantifying Flow and Reactive Transport in the Heterogeneous Subsurface Environment: From Pores to Porous Media and Facies to Aquifers

http://subsurface.pnl.gov/resources/darcy/schedule.shtml
A challenge to quantitative hydrogeology as a discipline to improve the predictive capability of our simulation tools...

**PART Measure:** The Subsurface Biogeochemical Research Program supports a long-term measure to "provide sufficient scientific understanding such that DOE sites would be able to incorporate physical, chemical and biological processes into decision making for environmental remediation and long-term stewardship."

“The current focus of the activity is to predict the impact of biogeochemical processes on the fate and transport of contaminants in the subsurface…”
1940’s: Establishment of fundamental theory; flow to single wells.
50’s and 60’s, regional aquifer flow.
Late 70’s: Love Canal: contaminant transport
1980: CERCLA
1983: MODFLOW
80’s/90’s Failures of P&T
Current: Focus on in situ remediation, biogeochemistry

http://www.isws.illinois.edu/hilites/achieve/gwmodpic.asp?p=06
A Grand Challenge:

The scales at which critical processes and properties are best defined and understood are orders of magnitude smaller than the scales at which practical problems are defined (observations and predictions).
Some specific examples to illustrate these pressing issues

Areas of recent and ongoing research that offer promise
- Characterization
- Simulation
- Integration Across Scales

Putting it all together: A framework for predictive simulation
Beyond the Black Box: Integrating Advanced Characterization of Microbial Processes with Subsurface Reactive Transport Models

Timothy D. Scheibe, Ph.D.
Pacific Northwest National Laboratory
To understand me, you need to know something about the world I live in...

**Planet Earth:**
- 70% surface covered by water; 30% land
- Solid iron inner core, molten outer core, plastic mantle, brittle crust
- Gaseous atmosphere about 100 km thick, composed of about 78% nitrogen, 21% oxygen, and less than 1% of other gases
- Supports carbon-based life forms
To understand me, you need to know something about the world I live in...

North America / United States:
- US population approx. 300 million
- Northern hemisphere
- Bordered by Pacific and Atlantic Oceans, Gulf of Mexico, nations of Mexico and Canada
- Primary language: English


To understand me, you need to know something about the world I live in...

Washington State:
- Pacific Northwest, known for temperate climate with rain, green forests, mountains, and salmon
- Major population center: Seattle/Tacoma
The World We Experience

To understand me, you need to know something about the world I live in...

Richland:

- Small city on the banks of the Columbia River
- In the “rain shadow” of the Cascade mountains; approximately 17 cm annual precipitation\(^1\)
- Shrub-steppe desert

\(^1\) Gee et al., PNNL-14143, 2002, Table 3.1
The World We Experience

What’s the point?

- Our world contains incredibly diverse environments with a large range of temporal dynamics.
- My experiences can vary dramatically depending on my specific location in space and time.
- My behavior is influenced by my local environment.
The same is true for microorganisms

- Their world contains incredibly diverse environments with a large range of temporal dynamics
- Their environment can vary dramatically depending on their specific location in space and time
- Their behavior is influenced by their local environment
The rest of the presentation:

- A glimpse into the fascinating world of subsurface microbes
- Some potential beneficial effects of subsurface microbial activity
- A new quantitative approach to predicting microbial behavior that accounts for local environmental dynamics
Until as little as 25 years ago it was widely believed that life existed in the subsurface environment only within the upper few meters of the earth's crust.

In 1985, the U. S. Department of Energy (DOE) initiated a research program called "Microbiology of the Deep Subsurface."


"Information about deep subsurface microbiology is likely to increase understanding of the transport and fate of groundwater contaminants, and it may offer new opportunities for in situ bioremediation…"
• Deep Microbes

  • **Bacillus infernus**: A “thermophilic” bacterium that lives in the deep terrestrial subsurface. It was found at a depth of circa 2700 m below ground surface in Virginia and thrives at 50 °C (122 °F).¹

  • **Strain 121**: Found to grow at temperatures up to 121 °C (250 °F).³

“The microbes brought to the surface are sometimes unique, including the first bacillus

"It's a very hot topic," said Dr. Henry L. Ehrlich, editor of The Geomicrobiology Journal. ²

---

The Fascinating World of Subsurface Microbes

- Rock-Eating Microbes

“...A bug discovered deep in a goldmine and nicknamed ‘the bold traveller’ has got astrobiologists buzzing with excitement. Its unique ability to live in complete isolation of any other living species suggests it could be the key to life on other planets.”

1 Chivian et al., Science, 322: 275-278, 2008
2 http://abcnews.go.com/Technology/story?id=5998582&page=1
Super-Tough Microbes

- **Deinococcus radiodurans**: This organism was first discovered in meat that had been “sterilized” by radiation. It can tolerate unusually high levels of ionizing radiation, as well as cold temperatures, dehydration, vacuum, and high acidity.
- “Conan the Bacterium” is in the Guinness Book of World Records as the “world’s toughest bacterium.”
Metal-Breathing Microbes:

- The organism shown here (*Geobacter metallireducens*) was first isolated in 1987 from the Potomac River.
- This was the first organism shown to oxidize organic compounds to carbon dioxide using iron oxides as the electron acceptor.
- *Geobacter metallireducens* gains energy by using metal (specifically iron oxide or “rust”) in the same way that humans use oxygen.

http://www.geobacter.org
The Beneficial Effects of Subsurface Microbes

- The U. S. Geological Survey also started a research program in the early 1980’s called the “Toxic Substances Hydrology Program” in which they studied bioremediation at several sites:
  - Bemidji, MN: Crude oil spill
  - Cape Cod, MA: Sewage effluent
  - Picatinny Arsenal, NJ: Chlorinated solvents

“One of the principal findings of this program was that microorganisms in shallow aquifers affect the fate and transport of virtually all kinds of toxic substances.” (Dr. Frank Chapelle, USGS)


Early bioremediation efforts focused on hydrocarbons and other organic substances impacted by aerobic organisms. These can be broken down to carbon dioxide and water in the presence of oxygen.

\[ \text{CH}_3\text{CH}_2\text{OH} + 3\text{O}_2 \rightarrow 2\text{HCO}_3^- + \text{H}_2\text{O} + 2\text{H}^+ \]
The Beneficial Effects of Subsurface Microbes

- How can we use unique bacterial abilities (such as metal-reducing)?
  - Metals can’t be “broken down” – they are elemental
  - However, their chemical state can be changed (in particular, their speciation and valence state or redox condition)

\[
\text{CH}_3\text{CH}_2\text{OH} + 4\text{FeOOH} + 7\text{H}^+ \rightarrow \text{CH}_3\text{COO}^- + 4\text{Fe}^{2+} + 7\text{H}_2\text{O}
\]

(Fe(III) $\rightarrow$ Fe(II))
The Beneficial Effects of Subsurface Microbes

Microbial reduction of metals/radionuclides

Microbially Significant Half-Reaction
Reduction Potentials

<table>
<thead>
<tr>
<th>Transformation</th>
<th>Reaction</th>
<th>Eh, Volts (@ pH 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₂ depletion</td>
<td>0.5O₂ + 2H⁺ = H₂O</td>
<td>0.82</td>
</tr>
<tr>
<td>Denitrification</td>
<td>NO₃⁻ + 6H⁺ + 5e⁻ = 0.5N₂ + 3H₂O</td>
<td>0.71</td>
</tr>
<tr>
<td>Mn reduction, Mn(IV) to Mn(II)</td>
<td>MnO₂ + 4H⁺ + 2e⁻ = Mn²⁺ + 2H₂O</td>
<td>0.54</td>
</tr>
<tr>
<td>Sulfate reduction, S(VI) to S(II)</td>
<td>SO₄²⁻ + 10H⁺ + 8e⁻ = H₂S + 4H₂O</td>
<td>-0.22</td>
</tr>
<tr>
<td>Methane generation, C(IV) to C(−IV)</td>
<td>HCO₃⁻ + 9H⁺ + 8e⁻ = CH₄ + 3H₂O</td>
<td>-0.26</td>
</tr>
<tr>
<td>H₂ generation, H(I) to H(0)</td>
<td>H⁺ + e⁻ = 0.5H₂</td>
<td>-0.41</td>
</tr>
</tbody>
</table>
Three examples:
1. Oak Ridge FRC – Area 2
The Beneficial Effects of Subsurface Microbes

Three examples:
1. Oak Ridge FRC – Area 2
2. Hanford 100D/H chromium bioremediation

Petersen, S. W., presentation to Hanford S&T Workshop
June 10, 2009, HNF-41489; CHPRC0905-05
The Beneficial Effects of Subsurface Microbes

Three examples:
1. Oak Ridge FRC – Area 2
2. Hanford chromium bioremediation
3. Rifle IFRC

http://ifcrifle.pnl.gov/
Question: Can we understand and even quantitatively predict the behavior of subsurface microorganisms?

- Design of effective bioremediation strategies
- Scientifically defensible decisions for environmental management (bioremediation and/or monitored natural attenuation)
Modeling uranium bioreduction at the Rifle, CO mill tailings site – 2002 experiment:

- Injection of acetate and bromide for 109 days
- 1-D steady flow
- Multicomponent reactive transport with iron, sulfate and uranium TEAPs
- Multi-step calibration process to define parameters

State-of-the-art numerical simulators represent microbial action in terms of biogeochemical reaction models, e.g.:

\[ R = V_{\text{max}} \frac{[BM]}{K_{BM} + [BM]} \frac{[Ac]}{K_{Ac} + [Ac]} [Fe(III)_{\text{site}}] f(\Delta G_{\text{rxn}}) \]

Monod-Type Kinetics
Understanding Behavior of Subsurface Microbes

- Model results:
State-of-the-art numerical simulators represent microbial action in terms of biogeochemical reaction models, e.g.:

\[
R = V_{\text{max}} \frac{[BM]}{K_{BM} + [BM]} + \frac{[Ac]}{K_{Ac} + [Ac]}[Fe(III)_{\text{site}}]f(\Delta G_{\text{rxn}})
\]

- Three “fitting” parameters (constants)

In reality, many different reaction pathways exist within the cellular mechanisms; which are active under specific environmental conditions? Can we predict rates from fundamental understanding?
Beyond the “Black Box” – Integrating Microbiological Understanding

Levels of Increasing Complexity

1 Graphics from R. Mahadevan, Univ. of Toronto
Beyond the “Black Box” – Integrating Microbiological Understanding

- Constraint-based *in silico* modeling:
  - Genetic characterization of reaction pathways
  - Laboratory characterization of flux constraints
  - Optimization under specific conditions

In *silico* Cellular Models

- Understand Metabolism
- Predict Growth Physiology
- Analyze high-throughput data

Graphics from R. Mahadevan, Univ. of Toronto
Application to Bioremediation: *Geobacter sulfurreducens*

- Steady-state representation of fluxes through the metabolic system under given environmental conditions
- Flux constraints in the form of maximum uptake rates as a function of external concentration
- Optimization requires specification of an “objective” – e.g., maximize biomass growth

Mahadevan et al., *Appl. and Envir. Microbiology*, 2006.
Uptake experiments conducted at Univ. of Mass.
Predicted biomass yield significantly varies under a reasonable range of environmental conditions.
Integrating with Reactive Transport Models:

- Reactive transport model (with geochemical speciation) provides local chemistry (defines microbial uptake flux constraints)
- *In silico* model with given flux constraints provides reaction fluxes (rates) that replace Monod kinetic rate formulations
Comparison of coupled (dashed) and original (solid) models with observations (colored symbols)

Both models gave very similar results and trends matched observations reasonably well.

*In silico* coupled model requires fewer calibration parameters:
- Initial biomass density (could be constrained by measurement)
- Uptake rates (flux constraints) taken from literature were reduced by x10 to represent effects of local diffusional gradients (scale effect)

\[ C_{Ac}(bulk) > C_{Ac}(surface) \]
- Pore-scale simulation of iron reduction at grain surfaces
- Initial distribution of attached microbes shown in red

Tartakovsky et al., AGU Fall Meeting, 2009
- Add acetate at inflow boundary (right edge)
- Microbes are allowed to grow and partition to aqueous phase (transport with flow)
- Concentration of acetate at grain surfaces is less than the average bulk concentration.
Increasing pressure on water supplies and natural ecosystems give hydrogeology a prominent role into the future.

Research opportunities are diverse, ranging from field studies to lab experiments to high-performance computing.

The field of hydrogeology is becoming increasingly multidisciplinary and collaborative.
The fascinating world of subsurface microorganisms offers an amazing opportunity for new research and development activities that will have significant impact on our future.

The intersection of hydrogeology and subsurface microbiology is an exciting and fruitful area of research.
Acknowledgments

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- Derek Lovley, U. Mass., Microbiology
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My family…
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January 4-8
UC Santa Barbara
Florida State Univ.
Florida Intl. Univ.

January 11-15
Michigan Tech. Univ.
Univ. of Georgia
UC Davis

January 18-22
Stanford Univ.
UC Berkeley

January 25-29
Univ. of Arizona
Northern Arizona Univ.

February 1-5
Univ of Colorado - Denver
Colorado State Univ.
Colorado School of Mines
Univ. of Colorado - Boulder

February 8-12
Penn State Univ.
Michigan State Univ.

February 15-19
Wright State Univ.
Univ. of North Carolina

February 22-26
Virginia Tech
Univ. of Virginia

March 1-5
Los Alamos Natl Lab
Univ. of New Mexico
USGS Tacoma
New Mexico Tech.

March 8-12
Univ. of British Columbia
Univ. of Washington

March 17
WA Dept. of Ecology

March 22
Univ. of Nevada