Assessing Microbial Activity in the Laboratory and the Field: Lessons from the USGS Norman Landfill

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Sulfate Reducing Bacteria in the Terrestrial Subsurface: A Duel Edged Sword

Negative Activities

• H₂S Production
• Reservoir Souring
• Corrosion of Metals
• Methylmercury Formation
• Reduction in Hydrocarbon Quality

Positive Activities

• Contaminant Bioremediation
  - Intrinsic
  - Engineered
• Immobilization of Metals and Radionuclides
Approaches for the Control of Sulfate Reduction

• Broad-Spectrum Biocides
• Specific Inhibitors of Sulfate Reduction
• Use of Corrosion Resistant Alloys
• Methods Based on Microbial Ecology
  A) Use of Nitrate/Nitrite
  B) Factors Influencing Metabolic Activity
APPROACHES TO ASSESSING MICROBIAL ACTIVITIES

Field

Assemblages/Enrichments

Pure cultures
whole cells proteins genes

High

Low

ecology

biogeochemistry

biochemistry

Experimental control

Complexity

physiology

molecular biology

High

Property

Low

High

Relevance

Low

So How To Determine in situ Microbial Activities??

Develop Lines of Evidence:
- FIELD: distinguish abiotic/biotic
- LABORATORY: ID controlling factors

multiple convergent independent

Extrapolate Information to Other Locations
For Example

Norman Landfill Research Site

Part of the USGS Toxic Substances Hydrology Program

http://ok.water.usgs.gov/norlan/
SO$_4^-$, Sulfate Reduction, and Iron Sulfide Formation in the Aquifer

1) SO$_4^-$ ~ SR
2) SR ~ Sulfides
3) Impact of Clay
4) S$^{-2}$ oxid. at H$_2$O Table
Apparent Km = 84 µM

In Situ Sulfate ≤ 100 µM
Groundwater flow direction

Norman landfill study site

upgradient well

downgradient well

100m scale

Sewage outfall

North (N)
# Groundwater Chemistry at the Norman Landfill Sites

![Diagram showing landfill, groundwater flow, and distance](image)

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Up-gradient</th>
<th>Down-gradient</th>
<th>Background</th>
</tr>
</thead>
<tbody>
<tr>
<td>dissolved org. C (mM)</td>
<td>~8</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>sp. conductance (µS cm⁻¹)</td>
<td>4990</td>
<td>5940</td>
<td>1570</td>
</tr>
<tr>
<td>sulfate (mM)</td>
<td>0.04</td>
<td>7.1</td>
<td>1.2</td>
</tr>
<tr>
<td>chloride (mM)</td>
<td>9.7</td>
<td>13.6</td>
<td>5.1</td>
</tr>
<tr>
<td>hydrogen (nM)</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>oxygen (mg L⁻¹)</td>
<td>&lt;0.3</td>
<td>&lt;0.3</td>
<td>ND</td>
</tr>
</tbody>
</table>
**Push-pull test procedure**

- Sparge groundwater
- Add reactant/NaBr & inject
- Extract solution and sample for Br\(^{-}\) and reactant vs. time

**Graphs:**
- Tracer breakthrough curve
- Substrate decay curve
- Time vs. normalized concentration (C/Co)

**Graphs:**
- Breakthrough curve for substrate
- Decay curve for [substrate]

**Diagram:**
- Flow direction arrows for push and pull mechanisms
- Vertical section showing groundwater flow

**Legend:**
- Blue arrows: Push mechanism
- Orange arrows: Pull mechanism
Field Sulfate Consumption Rates From Push-Pull Tests at the Upgradient Site at the Norman Landfill

![Graph showing sulfate consumption rates over time.](image-url)

- Formate (+): 14 µM•day\(^{-1}\)
- Formate (-): 3 µM•day\(^{-1}\)
Comparison of Sulfate Reduction Rates Measured in Intact Cores and *in situ* Tests

**SO₄²⁻** reduction rate (µM*d⁻¹)
Comparison of Sulfate Reduction Rates Measured in Intact Cores and \textit{in situ} Tests

SO$_4^{\text{-}}$ reduction rate ($\mu$M*d$^{-1}$)
Sulfate push-pull tests at the downgradient site

push-pull test 1

- sulfate
- bromide

push-pull test 2

- sulfate
- bromide
Sulfate push-pull tests at the downgradient site

No Sulfate Reduction in Lab or Field
Possible hypotheses for the lack of sulfate reduction activity at the downgradient site

- lack of sulfate reducing microorganisms
- presence of an inhibitory compound
- lack of suitable electron donors

To address these issues:

examine microbial sulfate reduction under more controlled conditions

intact cores & aquifer samples
35S-sulfate reduction assay in intact cores

- section core ~ (20 x 5 x 0.5 cm)
- apply 35S-sulfate to core face
- incubate anaerobically
  \[ \text{35SO}_4^- \xrightarrow{\text{SRB}} \text{H}_2\text{35S}^- \] (soluble) (precipitated)
- wash unreacted 35SO_4^- & image 35S=
Sulfate reduction activity in a core segment incubated with $^{35}$S-sulfate, lactate and *Desulfovibrio* preparations

Before treatment (cpm/cm²)

7
5
6
Sulfate reduction activity in a core segment incubated with \(^{35}\)S-sulfate, lactate and *Desulfovibrio* preparations

Before treatment (cpm/cm\(^2\))

<table>
<thead>
<tr>
<th>Lactate</th>
<th>Live</th>
<th>Heat-killed</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
Sulfate reduction activity in a core segment incubated with $^{35}$S-sulfate, lactate and *Desulfovibrio* preparations

Before treatment (cpm/cm$^2$)
- lactate: 7
- live: 5
- heat-killed: 6

Post treatment (cpm/cm$^2$)
- lactate: 66
- live: 44
- heat-killed: 66

17 day incubation
Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater

upgradient sediment/
upgradient water

relative sulfate reduction rate (%)
Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater

- Upgradient sediment/upgradient water
- Upgradient sediment/downgradient water
Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater.

- Upgradient sediment/upgradient water
- Upgradient sediment/downgradient water

Relative sulfate reduction rate (%)

0  20  40  60  80  100

relative sulfate reduction rate (%)
Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater

- Upgradient sediment/upgradient water
- Upgradient sediment/downgradient water
- Downgradient sediment/downgradient water

Relative sulfate reduction rate (%)

0 20 40 60 80 100

relative sulfate reduction rate (%)
Sulfate reduction in aquifer slurries using sediment inocula and sterile groundwater

- upgradient sediment/upgradient water
- upgradient sediment/downgradient water
- downgradient sediment/downgradient water
- downgradient sediment/upgradient water

Relative sulfate reduction rate (%)
What Can We Conclude

• sulfate reduction at the distal site was **not** limited by:
  ✓ sulfate concentration
  ✓ electron donor quantity
  ✓ lack of metabolic potential
  ✓ inhibitory substance

• was limited by electron donor **QUALITY**

• microbial inoculants can be a source of electron donors in bioaugmentation studies