Procedure is based on the biogas profiles produced by a sample when amended with $^{14}$C-acetate in the presence and absence of molybdate – a specific inhibitor of sulfate reduction.
Fe(III) reduction

![Graph showing Fe(III) reduction with and without molybdate over time.](image)

- **10 mM molybdate**
- **No molybdate**

**Axis:**
- **Y-axis:** \(^{14}\text{CO}_2\) (microcuries)
- **X-axis:** Time (hour)
Sulfate reduction

\[ ^{14}\text{CO}_2 \text{ (microcuries)} \]

- No molybdate
- 10 mM molybdate

Time (hour)

\[ 0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \]
Methanogenesis

\[ \text{Methanogenesis} \]

\[ \frac{14\text{CO}_2}{14\text{CH}_4} \text{(microcuries)} \]

- \( \text{CH}_4 \), no molybdate
- \( \text{CH}_4 \), 10 mM molybdate
- \( \text{CO}_2 \), no molybdate
- \( \text{CO}_2 \), 10 mM molybdate

Time (hour)
$^{14}$C-acetate TEAP determination

Problems

- Solid sample collection is required
- Requires supporting geochemical analyses for accuracy
- Requires utilization of radioactive materials
- Requires analyses to be performed on fresh samples
- Requires large amounts of potentially precious samples
Additional factors which control the population

- Relative population sizes
- Other nutritional sources/requirements
- Presence of inhibitors
- Genetic regulation
- Bioavailability of e⁻ acceptor
Bioremediation

Intrinsic remediation

Engineered remediation
Intrinsic remediation

Important Parameters

- Isolation/containment
- Contaminant mobility
- Biodegradation/transformation under intrinsic conditions
- Presence of required microbial community
- Presence of required nutrients

Advantages
1. Ease
2. Low cost

Disadvantages
1. Slow
2. Public perception
3. Long term monitoring required
## Engineered remediation

**Ex-situ**

### Important Parameters

- Accessibility
- Biodegradability
- Rate of Biodegradation
- Is a microbial consortium required
- Will microbial end products cause plugging

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Controlled environment</td>
<td>1. Not applicable to large plumes</td>
</tr>
<tr>
<td>2. Ease of monitoring</td>
<td>2. Not applicable to low level concentration</td>
</tr>
<tr>
<td></td>
<td>3. Cost</td>
</tr>
<tr>
<td></td>
<td>4. Complexity</td>
</tr>
</tbody>
</table>
Engineered remediation  
In-situ

Important Parameters

• Extent of contaminant plume
• Are environmental parameters conducive
• Accessibility of contaminant
• Are the appropriate populations present

Advantages

1. Applicable to large plumes
2. Applicable to low level concentration

Disadvantages

1. Uncontrolled environment
2. Cost
3. Complexity
4. Difficulty of monitoring
Aerobic v’s Anaerobic

**Aerobic**

\[ \text{CH}_3\text{COO}^- + 2 \text{O}_2 \rightarrow 2 \text{HCO}_3^- + \text{H}^+ \quad \Delta G^o = -844 \text{ kJ/mol} \]

**Anaerobic**

\[ 5 \text{CH}_3\text{COO}^- + 8 \text{NO}_3^- + 3 \text{H}^+ \rightarrow 10 \text{HCO}_3^- + 4 \text{H}_2\text{O} + 4 \text{N}_2 \quad \Delta G^o = -792 \text{ kJ/mol} \]

Thermodynamically \( \text{O}_2 \) respiration is much more favorable
Dioxygenase Mediated Destabilization of Benzene Ring

Dioxygenase

O\textsubscript{2} → Benzene Ring → CO\textsubscript{2}

H

OH

OH

H
Monitoring

Geochemical Analyses

Problems:
1. Homogenous Sample collection
2. Not directly indicative of microbial degradation

Microbial Analyses

Molecular v’s physiology

Problems:
1. Homogenous Sample collection
2. Presence not indicative of activity
3. Known biomarkers are required
Monitoring tools

• Stable isotope fractionation
• 16S rDNA Probes
• Immunoprobes
• Functional gene probes
Stable isotope fractionation

Microbial perchlorate reduction
Rayleigh fractionation curve

\[ \frac{R}{R_0} = f(\alpha-1) \]

\( R_0 = \frac{^{37}\text{Cl}}{^{35}\text{Cl}} \text{ of initial } \text{ClO}_4^- \)

\( R = \frac{^{37}\text{Cl}}{^{35}\text{Cl}} \text{ of residual } \text{ClO}_4^- \)

\( f = \text{fraction } \text{ClO}_4^- \text{ reacted} \)

\( \alpha = \text{the isotope fractionation factor associated with reduction.} \)
Culture based methods

Advantages:
- Technically simple
- Robust

Disadvantages:
- Laborious = costly
- Long lead time
-Insensitive
- Biased by media and electron donor
Azospira

PS-type

Dechloromonas

CKB-type

RCB-type

Treponema pallidum

Magnetospirillum magnetotacticum

Strain SN1

Dechloromonas strain WD

Azospirillum brasiliense

Strain DB

Strain TTI

Comamonas testosteroni

Ideonella dechloratans

Strain FL2

Strain FL8

Strain FL9

Dechloromonas agitata

Strain CL

Strain NM

Strain MLC33

Strain JM

Strain HZ

Strain CL24plus

Strain CL24

Strain CCO

Dechloromonas aromatica

Strain SIUL

Strain MissR

Rhodococcus tenuis

Strain AH

Azospira suillum

Strain Iso1

Strain Iso2

Strain SDGM

Strain PDX

Strain KJ

Propionivibrio limicola

Strain LT-1

Gill symbiont of Thyasira flexuosa

Dechloromonas strain NSS

Escherichia coli

Pseudomonas stutzeri

Pseudomonas strain PK

Strain CFPBD

Pseudomonas chloritidismutans

Strain PDA

Strain PDB

Wolinella succinogenes strain HAP-1

Helicobacter pylori

10 changes

α proteobacteria

β proteobacteria

γ proteobacteria

ε proteobacteria
## San Nick Island

<table>
<thead>
<tr>
<th>Sample</th>
<th>MPN</th>
<th>CKB type</th>
<th>RCB type</th>
<th>PS type</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drainage channel from EOD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H-3’</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
<td>8.0</td>
</tr>
<tr>
<td>H-6’</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td>H-8’</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
<td>8.4</td>
</tr>
<tr>
<td><strong>Explosive Ordinance Disposal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-3’*</td>
<td>7.49 + 3.35 x 10^4</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>8.6</td>
</tr>
<tr>
<td>M-6’</td>
<td>7.49 + 3.35 x 10^4</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>8.4</td>
</tr>
<tr>
<td>M-9’</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
<td>8.7</td>
</tr>
<tr>
<td><strong>1000 Springs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P-3’</td>
<td>No growth</td>
<td></td>
<td></td>
<td></td>
<td>8.9</td>
</tr>
<tr>
<td>P-5.5’</td>
<td>2.40 + 1.74 x 10^5</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>8.8</td>
</tr>
<tr>
<td>P-8’*</td>
<td>2.40 + 1.74 x 10^5</td>
<td>negative</td>
<td>negative</td>
<td>negative</td>
<td>9.0</td>
</tr>
</tbody>
</table>
### MPN's for Longhorn, Texas

<table>
<thead>
<tr>
<th>Site</th>
<th>MPN</th>
<th>pH</th>
<th>CKB type</th>
<th>RCB type</th>
<th>PS type</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 Sediment</td>
<td>$2.40 \pm 1.74 \times 10^6$</td>
<td>6.6</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>16 Ground Water*</td>
<td>$2.15 \pm 0.81 \times 10^6$</td>
<td>6.4</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>25 Surface Soil</td>
<td>$9.33 \pm 4.17 \times 10^5$</td>
<td>4.2</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>25 Sediment</td>
<td>$2.40 \pm 1.74 \times 10^5$</td>
<td>5.6</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>25 Ground Water</td>
<td>$1.12 \pm 0.64 \times 10^3$</td>
<td>6.5</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
The biochemistry offers some targets

Acetate

ClO_4^-

Perchlorate reductase

ClO_2^-

Chlorite dismutase

O_2

H_2O

CO_2

2H^+ + 2e^-

QH_2

Perchlorate reductase complex

Quinone Pool

2H^+ + 2e^-

O_2

6H^+

Cytochrome oxidase

Cyt o

4H^+

ATPase

ADP + Pi

ATP

Cytoplasm

Periplasm

ClO_4^-/ClO_3^-

ClO_2^-

Cl^-
Antibody to the Chlorite Dismutase Enzyme

<table>
<thead>
<tr>
<th>Organism</th>
<th>Whole cells</th>
<th>Cell lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dechloromonas strain CKB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dechloromonas strain RCB</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azospira strain PS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. dechloratans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dechlorospirillum strain WD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dechloromarinus strain NSS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas strain PK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. coli*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. stutzeri*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*These organisms are incapable of reductive (per)chlorate respiration.
Many uses of the CD Specific Antibody

1. **Fluorescent tagging.**
   CD antibody can be conjugated to a fluorescent label and visualized using immunofluorescence micrographs.

2. **Functional detection.**
   Antibody only detects cells actively reducing perchlorate.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Whole cells</th>
<th>Cell lysate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain CKB perchlorate grown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strain CKB aerobic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3. **Immuno-Based Assay for the Rapid Screening of Environmental Samples**

Analysis of two environmental samples for the presence of the CD enzyme using the ELISA test. The left sample is positive for CD, while the right sample is negative.
4. Rapid ELISA assay for Perchlorate-reducing Bacteria

- Environmental groundwater sample containing perchlorate-reducing bacteria
- Positive for perchlorate-reducing bacteria

- Secondary Goat-anti-rabbit peroxidase IgG
- Primary rabbit-anti-CD IgG
- Expressed chlorite dismutase (CD)
- Perchlorate-reducing organism

Graph showing absorbance (450 nm) vs. D. agitata cell number (x10⁶.ml⁻¹), with R² = 0.9912
Immunoprobe summary

Advantages:
- No culture biases
- Rapid
- Simple
- Cheap
- Sensitive
- Functional probe

Disadvantages:
- Not applicable to soil or sediments
- Potential false positives
Genetic probes targeting the chlorite dismutase gene

First amplification gives 484 bp cld gene fragment plus spurious products.

Second amplification gives clean 408 bp cld gene fragment.
Quantification of Perchlorate-reducing bacteria with MPN-PCR

1) Extract DNA from an environmental sample
2) Sequentially dilute DNA in triplicate
3) PCR amplify the chlorite dismutase gene from the dilutions
4) Score each dilution for the presence or absence of the product
5) Quantify the perchlorate-reducing population using the MPN equation

Most Probable Number Counts

Serial Dilution
10^{-1} - 10^{-7}

DNA amplification by polymerase chain reaction (PCR) and analysis by gel electrophoresis
Genetic probe summary

Advantages:
- Rapid
- Sensitive
- Will work with soil samples
- Can be adapted to a functional probe
- Combined with PR probe can remove false positives

Disadvantages:
- More expensive than immunoprobe
- Technically involved requires specialized equipment