Role of Microorganisms in the Speciation and Mobility of Arsenic

Jim A Field, Wenjie Sun, Irail Cortinas and Reyes Sierra

Department of Chemical and Environmental Engineering
The University of Arizona

\[ \text{OH} \quad \text{As} \quad \text{OH} \]
Inorganic Arsenic

Predominant Species of the Biogeochemical Cycle

<table>
<thead>
<tr>
<th>arsenite</th>
<th>arsenate</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(III)</td>
<td>As(V)</td>
</tr>
</tbody>
</table>

- More toxic than As(V)
- Analogue of phosphate
- Reacts with R-SH
- Adsorbed by iron and aluminum oxides
- More mobile
- Less mobile
Organoarsenicals

Methylated Forms are Less Predominant Species in the Biogeochemical Cycle

**Organic Forms**

- Dimethylarsinous Acid: \( \text{DMA}(\text{III}) \)
  - Formula: \( \text{H}_3\text{C}-\text{As}-\text{CH}_3 \)
  - Characteristics: Very Toxic, Metabolite, Unstable

- Methylarsonous Acid: \( \text{MMA}(\text{III}) \)
  - Formula: \( \text{H}_3\text{C}-\text{O}-\text{As}-\text{OH} \)
  - Characteristics: Very Toxic, Metabolite

- Dimethylarsinic Acid: \( \text{DMA}(\text{V}) \)
  - Formula: \( \text{H}_3\text{C}-\text{O}-\text{As}-\text{CH}_3 \)
  - Characteristics: Pesticides, Metabolite

- Methylarsonic Acid: \( \text{MMA}(\text{V}) \)
  - Formula: \( \text{H}_3\text{C}-\text{O}-\text{As}-\text{OH} \)
  - Characteristics: Pesticides
Methanogenesis in Landfill

Phase I: Consumption of $O_2$

Phase II: Formation of organic acids, release $NH_4^+$

Phase III: Conversion of organic acids to $CH_4$ and $HCO_3^-$, continued release $NH_4^+$, mildly alkaline pH
Landfill

![Graph showing gas component percentage by volume in different phases of landfill]

- **Phase I**
  - Carbon Dioxide: 45-60%
  - Methane: 40-60%
- **Phase II**
  - Hydrogen
  - Oxygen
- **Phase III**
  - Nitrogen: 2-5%

**Note:** Phase duration time varies with landfill conditions

**Source:** EPA 1997
Toxicity Arsenic to Methanogens

Dose Response Curves of As(III) on Acetoclastic Methanogenesis

Sierra-Alvarez et al. 2004 AEM 70:5688
## Toxicity Arsenic to Methanogens

50% Inhibitory Concentrations of Arsenicals on Methanogenesis

<table>
<thead>
<tr>
<th>Compound</th>
<th>Substrate</th>
<th>IC50 (µM)</th>
<th>IC50 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As(III)</td>
<td>acetate</td>
<td>15.5</td>
<td>1.16</td>
</tr>
<tr>
<td>As(III)</td>
<td>H₂</td>
<td>27.1</td>
<td>2.03</td>
</tr>
<tr>
<td>As(III)</td>
<td>lactate</td>
<td>4.4</td>
<td>0.33</td>
</tr>
<tr>
<td>As(V)</td>
<td>acetate</td>
<td>&gt;500.0</td>
<td></td>
</tr>
<tr>
<td>As(V)</td>
<td>H₂</td>
<td>&gt;500.0</td>
<td></td>
</tr>
<tr>
<td>MMA(III)</td>
<td>acetate</td>
<td>9.1</td>
<td>0.68</td>
</tr>
<tr>
<td>MMA(V)</td>
<td>acetate</td>
<td>&gt;5000.0</td>
<td></td>
</tr>
<tr>
<td>DMA(V)</td>
<td>acetate</td>
<td>&gt;5000.0</td>
<td></td>
</tr>
</tbody>
</table>

Sierra-Alvarez et al. 2004 AEM 70:5688
Toxicity Arsenic to Methanogens

Sulfhydryl Groups are Central to the Biochemistry of Methanogens

Methyl Reductase: F430 Complex
Reduction of Arsenate

Microorganisms use Two Strategies for Arsenate Reduction

- **Arsenate Reductase (ArsC) for Detoxification**: As(V) is reduced to As(III) to facilitate pumping it from the cell without accidently pumping out phosphate

- **Dissimilatory Arsenate Reductase (ArrA)**: Arsenate is reduced as a terminal electron acceptor during the anoxic respiration coupled to the oxidation of simple substrates
Reduction of Arsenate

Arsenate Reductase (ArsC): Detoxification

Silver & Phung 2005. AEM 71:599
Reduction of Arsenate

Dissimilatory Arsenate Reductase (ArrA): Respiration

Silver & Phung 2005. AEM 71:599
Reduction of Arsenate

Dissimilatory Arsenate Reducing Bacteria

Reduction of Arsenate

Dissimilatory Arsenate Reducing Bacteria

**Example of Reactions**

**Complete oxidation organic matter**

\[
\text{CH}_3\text{COOH} + 4 \text{AsO(OH)}_3 \rightarrow 2 \text{CO}_2 + 4 \text{As(OH)}_3 + 2 \text{H}_2\text{O}
\]

- acetate
- As(V)
- CO₂
- As(III)

**Partial oxidation organic matter**

\[
\text{CH}_3\text{CHOHCOOH} + 2 \text{AsO(OH)}_3 \rightarrow \text{CO}_2 + \text{CH}_3\text{COOH} + 2 \text{As(OH)}_3 + \text{H}_2\text{O}
\]

- lactate
- As(V)
- CO₂
- acetate
- As(III)
# Reduction of Arsenate

## Dissimilatory Arsenate Reducing Bacteria

Electron Donors Known to Support Dissimilatory Arsenate Reducing Microorganisms

<table>
<thead>
<tr>
<th>Organic Acids</th>
<th>Alcohol, Sugar</th>
<th>Aromatic</th>
<th>Inorganic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>Ethanol</td>
<td>Phenol</td>
<td>H₂</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>Glycerol</td>
<td>Benzoate</td>
<td>H₂S</td>
</tr>
<tr>
<td>Fumarate</td>
<td>Glucose</td>
<td>Syringate</td>
<td></td>
</tr>
<tr>
<td>Malate</td>
<td></td>
<td>Ferulate</td>
<td></td>
</tr>
<tr>
<td>Succinate</td>
<td></td>
<td>Toluene</td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Niggemeyer et al 2001. AEM 67:5568
Hoeft et al 2004. AEM 70:2741
Does Arsenate Reduction Occur in a Methanogenic Consortium?
Arsenate Reduction In a Methanogenic Consortium with Sulfate Added

Methylation of Arsenic

Both Eukaryotes and Prokaryotes Methylate Arsenic

- Challenger Mechanism
- Enzymes
  - Cyt19 in fungi, mammals
  - ArsM in bacteria, archaea
- Methyl Donors
  - S-adenosyl methionine
  - Methyl-Vitamin B12

Bentley & Chasteen 2002. MMBR 66:250
Oxidation of Arsenite

There are two physiologically distinct classes of Arsenite Oxidizers:

- **Chemoorganoheterotrophic Arsenite Oxidizers:** Detoxification Mechanism
- **Chemolithoautotrophic Arsenite Oxidizers:** Arsenite is used as an energy source (electron-donor); CO₂ is fixed

**Oxic**

**Anoxic**

\[
\begin{align*}
\text{Oxic} & : \quad \text{NO}_3^- + 2 \text{H}^+ + 5 \text{As(OH)}_3 & \rightarrow & \text{N}_2 + 5 \text{AsO(OH)}_3 + \text{H}_2\text{O} \\
\text{Anoxic} & : \quad \text{O}_2 + 2 \text{As(OH)}_3 & \rightarrow & 2 \text{AsO(OH)}_3
\end{align*}
\]
Reduction of Arsenate

Respiratory Arsenite Oxidase

Silver & Phung 2005. AEM 71:599
Oxidation of Arsenite

Arsenite Oxidizing Bacteria (▲ ▼)

Anoxic Arsenite Oxidation by Municipal Anaerobic Digester Sludge

Formation of As(V) from 0.5 mM As(III)
### Anoxic Oxidation of Arsenite is Ubiquitous

<table>
<thead>
<tr>
<th>Sample</th>
<th>As(V) formation</th>
<th>Time† (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>anaer. bioreactor sludge, distillery</td>
<td>+</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>anaer. bioreactor sludge, paper</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>municipal anaer. digester sludge</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>thiosulfate-denitrification enrichment</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>municipal aerobic active sludge</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>duck pond sediments</td>
<td>+</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Winogradsky column sediment</td>
<td>+</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Pinal Creek sediments (high Mn)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>groundwater</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

† time to oxidize 0.5 mM As(III) to As(V) linked to denitrification
Bioreactor Test for Continuous Anoxic Oxidation of Arsenite
Bioreactor Results

As(V) in Reactor Fed NO₃⁻
Bioreactor Results

As(V) in Control Reactor not Fed NO₃⁻
As(III) Oxidation Linked to Complete Denitrification (3.5 mM AsIII)
Microbial Mobilization of Arsenate

Experimental: Continuous Columns
Microbial Mobilization of Arsenate

Experimental: Continuous Columns

- C3: Abiotic Control
  - NH₃ + NaHCO₃

- C2: Sludge Control
  - sludge
  - NH₃ + NaHCO₃

- C1: Biological
  - volatile fatty acids
  - sludge
  - NH₃ + NaHCO₃
Microbial Mobilization of Arsenate

Experimental: Continuous Columns

Activated Aluminum (AA) operated for 257d:


**Biologically Active Column** = 17.4% of arsenic released as freely soluble identified arsenic species; loss of 37.2% from AA

The predominate species released was As(III), 85% of soluble species

**Abiotic Column** = 3.4% of arsenic released as freely soluble identified arsenic species; loss of 7.6% from AA

Granular Ferrihydrite (GFH) operated for 387d:

Results reported in next slides (Cortinas et al. 2006. In preparation)
Microbial Mobilization of Arsenate

Granular Ferrihydrite

Vol = 270 ml

Effluent

Gas (CH₄)

Anaerobic Sludge
(10 g VSS/L)

GFH
(100.5 g)
(6.01 mg As(V)/g GFH)

Simulated leachate
(inorg. nutr. + VFA)

Cortinas et al 2006 in prep.
Microbial Mobilization of Arsenate

Experimental: Continuous Columns
Microbial Mobilization of Arsenate

Experimental: Continuous Columns

Granular Ferrihydrite

Freely Soluble Arsenic

% of As Species

Period

DMA(V)
As(III)
As(V)
Microbial Mobilization of Arsenate

Experimental: Continuous Columns

Granular Ferrihydrite

Acidified Arsenic

% of As Species

Period

1 2 3 4

DMA(V) As(III) As(V)
Microbial Mobilization of Arsenate

Experimental: Continuous Columns

Granular Ferrihydrite

Recovery of Mass after 387 days

Start of experiment

Day 387

% mass recovered

Column

C1 t0  C2 t0  C3 t0  C1end  C2end  C3end

mix  GFH  sludge
Microbial Mobilization of Arsenate

Schematic Overview GFH Columns

As(V) Fe(OH)₃ As(V)

Fe²⁺ + e⁻ → As(III)

Arsenite methylation

CH₃-R

DMA(V)

Iron reducers

As(V) Fe(OH)₃ As(V)

Fe²⁺ + e⁻ → As(III)

Arsenite methylation

CH₃-R

DMA(V)

Iron reducers

As(V) Fe(OH)₃ As(V)

Fe²⁺ + e⁻ → As(III)

Arsenite methylation

CH₃-R

DMA(V)

Iron reducers

As(V) Fe(OH)₃ As(V)

Fe²⁺ + e⁻ → As(III)

Arsenite methylation

CH₃-R

DMA(V)

Iron reducers

As(V) Fe(OH)₃ As(V)

Fe²⁺ + e⁻ → As(III)

Arsenite methylation

CH₃-R

DMA(V)

Iron reducers
Conclusion 1

Arsenic in Matrix with Fe/Al Oxide Minerals

Arsenite oxidation

O₂ NO₃⁻

As(V) As(V)

Fe(II) Oxidation

Fe(II) + As(V)

arsenite, As(III)

arsenate, As(V)

As cycle

organic matter

arsenate reduction

Fe(III) reduction

Aerobic Conditions: Arsenic immobile

Anaerobic Conditions: Arsenic mobile
**Conclusion 2**

**Arsenic in Matrix with Sulfide Minerals**

- **S cycle**: 
  - As(III) + SO$_4^{2-}$ (a aqueous) → As(III)$_2$S$_3$ (solid) → arsenite oxidation → As(III) + SO$_4^{2-}$ (a aqueous) via organic matter → sulfate reduction
  - As(III)$_2$S$_3$ (solid) → arsenite oxidation → As(III) + SO$_4^{2-}$ (a aqueous) via organic matter → sulfate reduction

- **As cycle**: 
  - HO$_2$AsOH (arsenate) → arsenite reduction → HO$_2$AsOH (arsenate) via organic matter → arsenate reduction

- **Aerobic Conditions**: Arsenic mobile
- **Anaerobic Conditions**: Arsenic immobile
Graduate Students

Wenjie Sun

Irail Cortinas