Biostimulation and Enhancement of Rapid Rhizodegradation of Perchlorate

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Outline

- Introduction:
  - Perchlorate in food chain
  - Bioremediation mechanisms
  - Phytoremediation mechanisms

- Enhancement of Perchlorate Biodegradation in the Root Zone:
  - Hydroponics Studies
  - Soil Studies
Perchlorate:

- ~ 90% is produced as NH₄ClO₄ (Solubility: 200 g/L)

  \[
  \text{NH}_4\text{ClO}_4 (s) \xrightarrow{\text{water}} \text{NH}_4^+ (aq) + \text{ClO}_4^- (aq)
  \]

- Propellant in rocket and missiles (90%), fireworks

- Found in some fertilizers

- ClO₄⁻ acts as a competitive inhibitor of active iodide (I⁻) uptake by the thyroid gland
Perchlorate in the food chain

- ClO$_4^-$ contaminated grass (Kirk et al., 2003)
- Lettuce grown with ClO$_4^-$ contaminated water (Kirk et al., 2005)
- ClO$_4^-$ Phytoremediation (Tan et al., 2004)

Yifru & Nzengung, 2006
Perchlorate Treatment

- Persistent in water

- Physical and chemical treatment
  - Adsorption with activated carbon – Poor ClO$_4^-$ sorption
  - Ion selective resins – Produce excessive waste and expensive
  - Chemical reduction – Relatively slow
  - Air stripping – Perchlorate is non-volatile

- Promising Technologies (effective and economical)
  - Bioremediation
  - Phytoremediation
Bioremediation

- $\text{ClO}_4^- \rightarrow \text{Microorganisms} \rightarrow \text{Cl}^- + \text{O}_2$
  
  Derive energy from oxidation-reduction reactions

- DOC is used as electron donor and $\text{ClO}_4^-$ as a terminal electron acceptor

- $\text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2^- \rightarrow \text{Cl}^- + \text{O}_2 \rightarrow \text{CO}_2, \text{H}_2\text{O}, \text{Biomass}$

- Three preconditions: Microorganisms, anaerobic environment and electron donors
Phytoremediation Mechanisms

Use of plants and their root zone associated microorganisms to either sequester or degrade contaminants or a combination of the two processes.

McCutcheon and Schnoor (2003)
Perchlorate and Plants

Inside plant leaves:
- Storage (Phytoaccumulation)
- Phytodegradation

\[ \text{ClO}_4^- \rightarrow \text{ClO}_3^- \rightarrow \text{ClO}_2 \rightarrow \text{Cl}^- + \text{O}_2 \]

Exudates (e.g., glucose, acetate, ethanol)

Rhizodegradation

\[ \text{ClO}_4^- \rightarrow \text{Cl}^- + \text{O}_2 \]
Perchlorate and Plants

Hydroponics experiment

- **Phase 1**: Uptake and:
  - Slow phytodegradation
  - Phytoaccumulation

- **Phase 2**: Rhizodegradation

Normalized Perchlorate (mg L\(^{-1}\))

Time (days)

- No sand 122 ppm

Nzengung et al., (1999)
Research Objectives

1. Investigate if Dissolved Organic Carbon (DOC) is limiting in the **biostimulation** and **sustainment** of rhizodegradation.

2. Develop an approach to minimize plant uptake of perchlorate and enhance rhizodegradation.

3. Evaluate the use of agricultural waste products as cheap and abundant source of DOC.
Methods

Hydroponics experiment

Soil experiment

Rooting of willow cuttings
Methods

- Perchlorate (Ion Chromatography (IC))
  - 25 mg/L prepared in the lab
  - 40 mg/L from groundwater (LHAAP, Texas)
  - 1.5 ml sample and plant tissue

- 500 mg/L DOC (TOC analyzer)
  - Organic compost tea
  - Chicken litter extract
  - Acetate

- Sterile organic compost and chicken litter extract
  - Autoclaving
  - PCR amplification
Results

Hydroponics Experiment (Control)

![Graph showing the reduction of ClO₄⁻ remaining in solution over time (days)](image)

- **ClO₄⁻ remaining in solution [mg L⁻¹]**
- **Control (No DOC)**
Results

Hydroponics Experiment (Acetate)

![Graph showing the remaining chlorine in solution over time for different treatments including control (No DOC), Acetate - 1, Acetate - 2, and Acetate - 3.]
Results

Hydroponics Experiment (Organic compost)

[Diagram showing the remaining ClO$_4^-$ in solution over days for different compost conditions, including Sterilized Mushroom Compost 1, Sterilized Mushroom Compost 2, Unsterilized Mushroom Compost, and Control (NO DOC).]
Results

Hydroponics Experiment (Chicken litter extract)

![Graph showing the remaining ClO₄⁻ in solution over days for different treatments: Sterilized Chicken Litter 1 (dotted), Sterilized Chicken Litter 2 (purple circles), Unsterilized Chicken Litter (blue line), and Control (No DOC) (red line). Each line shows a decrease in ClO₄⁻ with increasing days.](image-url)
Results

Hydroponics Experiment (Groundwater from LHAAP, TX)

![Graph showing ClO₄⁻ remaining in solution over days for different treatments: Sterilized Chicken Litter 1, Sterilized Chicken Litter 2, 500 mg L⁻¹ DOC from acetate, and Control (no DOC).]
Results

- Distribution of perchlorate fraction taken-up by plants

- Perchlorate phytoaccumulated in leaves

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ClO$_4^-$ [mg kg$^{-1}$ fresh weight]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetate</td>
<td>16.53 ± 0.09</td>
</tr>
<tr>
<td>Chicken Litter</td>
<td>30.34 ± 0.00</td>
</tr>
<tr>
<td>Organic Compost</td>
<td>32.52 ± 0.20</td>
</tr>
<tr>
<td>Control</td>
<td>424.19 ± 1.4</td>
</tr>
</tbody>
</table>
Soil Experiments

- Bioreactors irrigated with perchlorate-contaminated groundwater 4 times
- DOC added during the first and fourth perchlorate doses
- 1.5 ml water samples – daily
- Willow leaves analyzed for perchlorate
Results

Soil Experiment (PCR amplification)

4 Organic compost
6 Control
5 Chicken litter extract
Results

Soil Experiment (Dissolved organic carbon)

Dose 1: 35 mg/L Perchlorate
300 mg/L DOC
11.8 mg/kg Perchlorate in leaves

Acetate
Chicken litter extract
Results

Soil Experiment (Dissolved organic carbon)

Dose 2: 30 mg/L Perchlorate
281 mg/L DOC
39 mg/kg Perchlorate in leaves
Results

Soil Experiment (Dissolved organic carbon)

Dose 3: 40 mg/L Perchlorate
33 mg/L DOC
95 mg/kg Perchlorate in leaves
Results

Soil Experiment (Dissolved organic carbon)

Dose 4: 44 mg/L Perchlorate
300 mg/L DOC
120 mg/kg Perchlorate in leaves
Results

Soil Experiment (Controls)

Dose 1: 33 mg/L Perchlorate
55 mg/L DOC
104 mg/kg Perchlorate in leaves
Results

Soil Experiment (Controls)

![Graph showing perchlorate concentration remaining over days with control treatment.]

Dose 2: 17 mg/L Perchlorate
32 mg/L DOC
186 mg/kg Perchlorate in leaves
Results

Soil Experiment (Controls)

Dose 3: 39 mg/L Perchlorate
18.3 mg/L DOC
211 mg/kg Perchlorate in leaves
Results

Soil Experiment (Controls)

Dose 4: 43 mg/L Perchlorate
387 mg/kg Perchlorate in leaves
< 18 mg/L DOC
## Results

<table>
<thead>
<tr>
<th>Bioreactor</th>
<th>Dose</th>
<th>ClO$_4^-$ (mg L$^{-1}$)</th>
<th>DOC (mg L$^{-1}$)</th>
<th>ClO$_4^-$ in leaves (mg kg$^{-1}$)</th>
<th>K$_{Zero}$ (mg L$^{-1}$ day$^{-1}$)</th>
<th>K$_{First}$ day$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOC</td>
<td>1</td>
<td>30.3 ± 4.2</td>
<td>309.1 ± 22.5</td>
<td>11.8 ± 0.1</td>
<td>4.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>32.8 ± 0.5</td>
<td>54.6 ± 5.3</td>
<td>103.7 ± 16.8</td>
<td>0.36 ± 0</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>2</td>
<td>26.3 ± 6.3</td>
<td>280.7 ± 48.6</td>
<td>39 ± 7.3</td>
<td>8.7 ± 3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>17.2 ± 0.1</td>
<td>32 ± 12</td>
<td>185.6 ± 88.5</td>
<td>0.35 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>3</td>
<td>40.7 ± 2.8</td>
<td>32.8 ± 5.2</td>
<td>95.1 ± 36.3</td>
<td>0.2 ± 0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>38.7 ± 5.4</td>
<td>18.3 ± 5.6</td>
<td>210.6 ± 97.8</td>
<td>0.33 ± 0</td>
<td></td>
</tr>
<tr>
<td>DOC</td>
<td>4</td>
<td>43.7 ± 9.5</td>
<td>300 ± 0</td>
<td>120.6 ± 22.4</td>
<td>21.1 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>42.8 ± 2.7</td>
<td>386.6 ± 27</td>
<td>0.17 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Summary

- Perchlorate degraders are ubiquitous and DOC is a limiting factor in soils during rhizodegradation.
- Rhizodegradation of perchlorate can be enhanced by DOC.
- Plant perchlorate uptake and phytoaccumulation could be significantly reduced by adding DOC to the rhizosphere.
- Cheap agricultural wastes can be used as abundant sources of DOC to enhance rhizosphere degradation of perchlorate.
Thank You
Uptake of N-Nitrosodimethylamine (NDMA) by Phreatophytes from Water in the Absence and Presence of Perchlorate as a Co-contaminant
$^{14}$C-NDMA (Experimental Setup)

1: 1N NaOH
2: Ambersorb® 572
3: GAC
4: GAC
$^{14}$C-NDMA
Results

Fate of NDMA in plants

- Uptake and
  - Phytovolatilization
  - Phytoaccumulation
  - Phytodegradation

No Rhizodegradation
Results

- Summer: 98.3 ± 1.7% NDMA taken up in 80 days
- Winter: 81.4 ± 10.3% NDMA taken up in 102 days
- Unplanted controls: 3.5 ± 0.9% NDMA was removed in 86 days (Winter)
Results

Relationship between transpiration and mass NDMA removed

![Graph showing the relationship between transpiration and mass NDMA removed.](image)
Results

$^{14}$C-activity Distribution in Plants and Traps

- Leaves: 18.8 ± 2.3
- Stems: 15.7 ± 8.9
- GAC: 1.3 ± 1
- Branches: 7.7 ± 3.5
- CO$_2$: 0.6 ± 0.1
- Ambersorb® 572: 3.7 ± 0.6
- Phytovolatilized: 42.5 ± 1.6
- Removed by sampling: 0.4 ± 0.1

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NDMA and Perchlorate

The graph shows the remaining perchlorate in solution over 80 days. The x-axis represents the number of days, ranging from 0 to 80, and the y-axis represents the perchlorate remaining in solution in mg L⁻¹, ranging from 0 to 30.

There are two lines on the graph:
- The blue line represents perchlorate only.
- The red line represents perchlorate with NDMA as a co-contaminant.

The data points for both lines are marked with error bars, indicating variability in the measurements.
N-Nitrosodimethylamine (NDMA)

- Produced by the oxidation of unsymmetrical dimethylhydrazine (UDMH) containing rocket fuel
- Produced during disinfection of wastewater
- Highly soluble in water with log $K_{ow}$ of -0.57
Treatment of NDMA

- Henry’s law constant ($K_H$) for NDMA is low at $2.6 \times 10^{-7}$ atm-m$^3$ M$^{-1}$, volatilization and air stripping is not effective.

- NDMA is hydrophilic ($\log K_{ow} = -0.57$) and sorbs poorly to GAC, soil and other sorbents.

- Commonly used treatment method is photolysis by UV radiation (not cost-effective):

$$\begin{align*}
\text{H}_3\text{C} & \quad \text{N} \quad \text{N} \quad \text{O} \\
\text{H}_3\text{C} & \quad \text{UV} \quad \text{H}_3\text{C} \\
\text{H}_3\text{C} & \quad \text{N} \quad \text{O} 
\end{align*}$$

- Bioremediation: Recent studies showed biodegradation of NDMA under aerobic conditions.

- The potential for phytoremediation of NDMA is currently unknown.
Research objectives

- Investigate the potential of green plants to remove NDMA from contaminated water
- Identify the mechanisms of NDMA decontamination
- Verify if there is competitive removal of NDMA and perchlorate, when present as co-contaminants
Thank You