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Strategic Environmental Research and Development Program
Improving Mission Readiness Through Environmental Research

ENVIROGEN
Cost Effective Leadership for a Cleaner Environment

IN SITU BIOREMEDIATION OF PERCHLORATE IN GROUNDWATER

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ENVIROGEN, INC.

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PERSONNEL

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Envirogen, Inc.
Expertise in Environmental Microbiology.

Robert J. Steffan, Ph.D., Co-PI
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Expertise in Molecular Biology and Bioremediation.

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Envirogen, Inc.
Expertise in Hydrogeology, Modeling and In Situ Remediation.

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Indian Head Division, Naval Surface Warfare Center.
Expertise in Engineering and Water Treatment.

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Indian Head Division, Naval Surface Warfare Center.
Expertise in Analytical Chemistry.

Randall Cramer, Ph.D.
Indian Head Division, Naval Surface Warfare Center.
Program Management and Coordination.

TECHNICAL OBJECTIVES

- **Provide a fundamental understanding of perchlorate biodegradation in subsurface environments.**

- **Develop a biological treatment technology for *in situ* remediation of perchlorate in groundwater.**

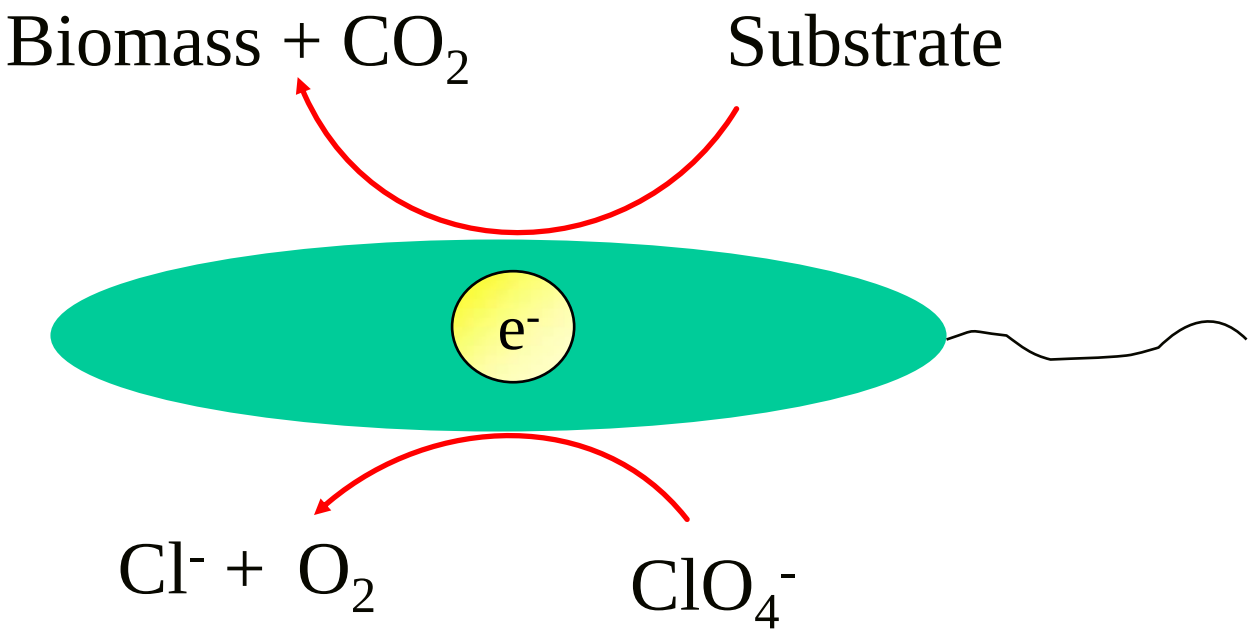
In Situ Perchlorate Bioremediation

Key Question for Technology Development: **Why does perchlorate persist in groundwater?**

Hypotheses:

- **Absence of suitable electron donor (substrate)?**
- **Inhibition by alternate electron acceptors?**
- **Lack of indigenous bacteria capable of perchlorate reduction?**
- **Unfavorable environmental conditions?**

Biological Perchlorate Degradation



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TECHNICAL BACKGROUND

Electron Acceptors

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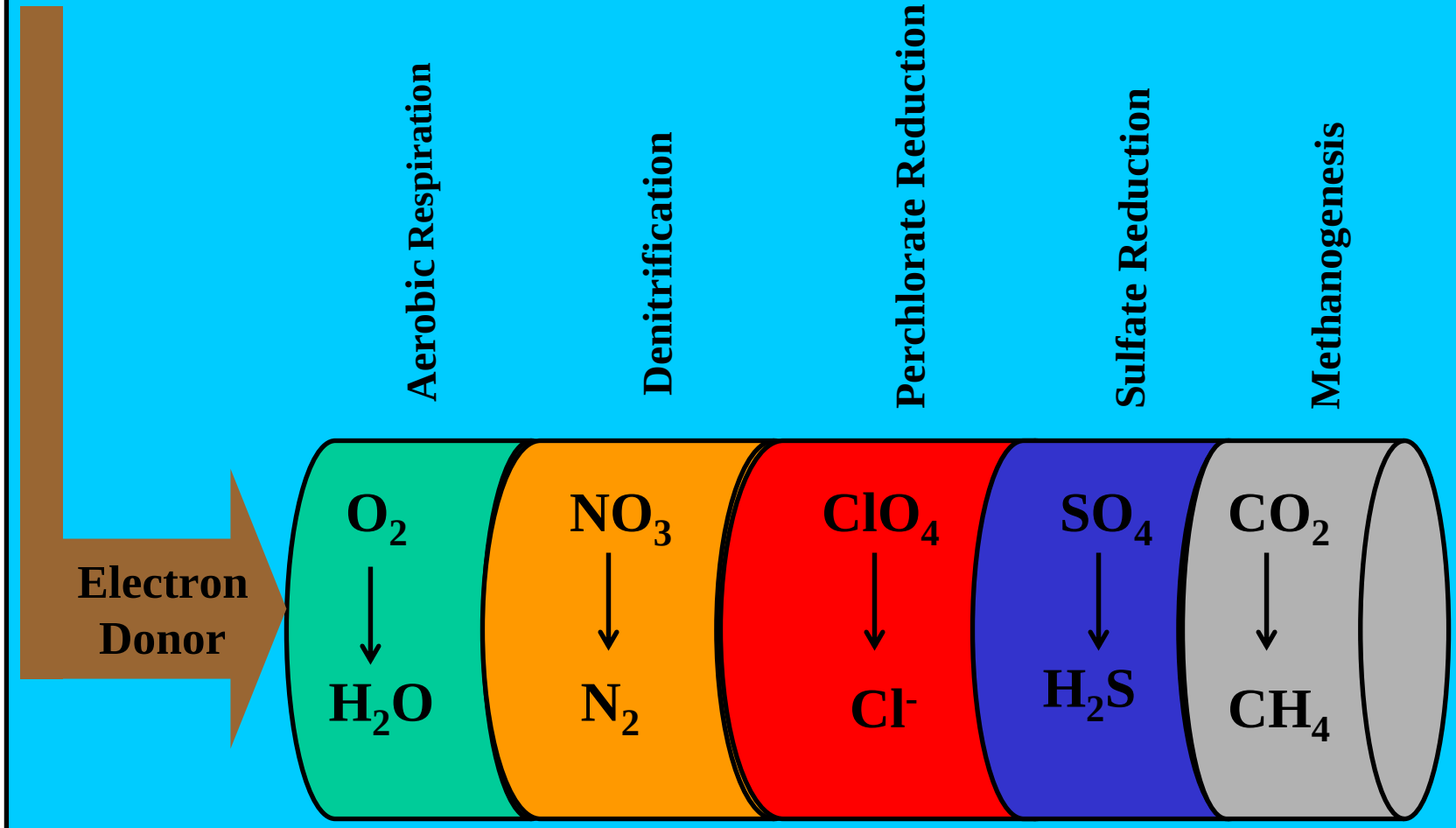


Perchlorate - Degrading Bacteria

Few Strains Studied to Date:

Strain CBK (Bruce et al., 1999); Strain Perc1ace (Herman et al., 1999);
Strain GR-1 (Rikken et al., 1996); *Wolinella succinogenes* HAP-1 (Wallace et al., 1996)
Ideonella dechloratans (Malvquist et al., 1994)
Vibrio dechloraticans (Korenkov et al., 1976)

Natural Occurrence Unknown:

Most strains isolated from anaerobic sludge or wastewater.
Occurrence in aquifers unknown?

Differing Respiration:

Most are facultative anaerobes (i.e., can use O_2);
All use ClO_3^-, many use NO_3^-, one Mn (IV), one SO_4^{--}.

Variable Substrate Requirements:

Some use simple carbon (acetate), some require complex carbon (yeast extract), a few use inorganic substrates (H_2, Fe(II)), one has trace mineral requirements.

Environmental Variables

1. Salinity
2. pH
3. Co-Contaminants
   - Chlorinated Solvents (TCE, PCE)
   - BTEX

Research Approach

- Collect Aquifer Solids and Groundwater
- Enumerate and Isolate Perchlorate -Degraders
- Conduct Aquifer Microcosm Studies
- Conduct Studies with Flow-Through Model Aquifers
- Apply Functional Models
- Evaluate Methods for Field Implementation
- Conduct Field Demonstration
- Commercial Application

Collect Aquifer Samples

Current Site List

(1) JET PROPULSION LABS (CA).*

(2) INDIAN HEAD NSWC (MD).*

(3) LONGHORNE ARMY AMMN. DEPOT (TX).

(4) OYSTER VIRGINIA (*Pristine Site*).*

(5) COMMERCIAL SITES.

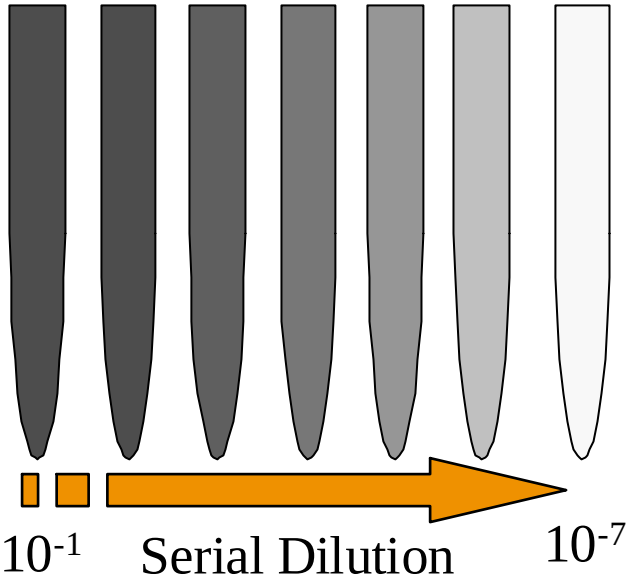
* Studies Underway or Complete

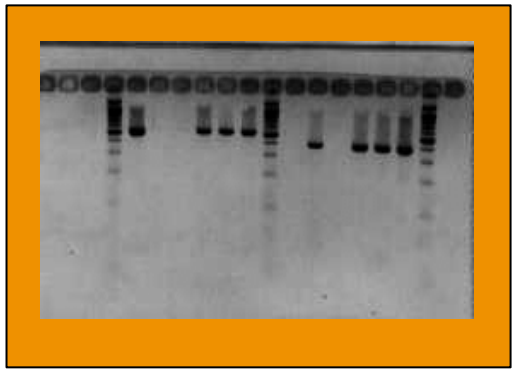
Enumerate and Isolate Perchlorate -Degrading Bacteria

Collaborative with Dr. John Coates, SIU

Enumeration of Perchlorate -Degrading Bacteria

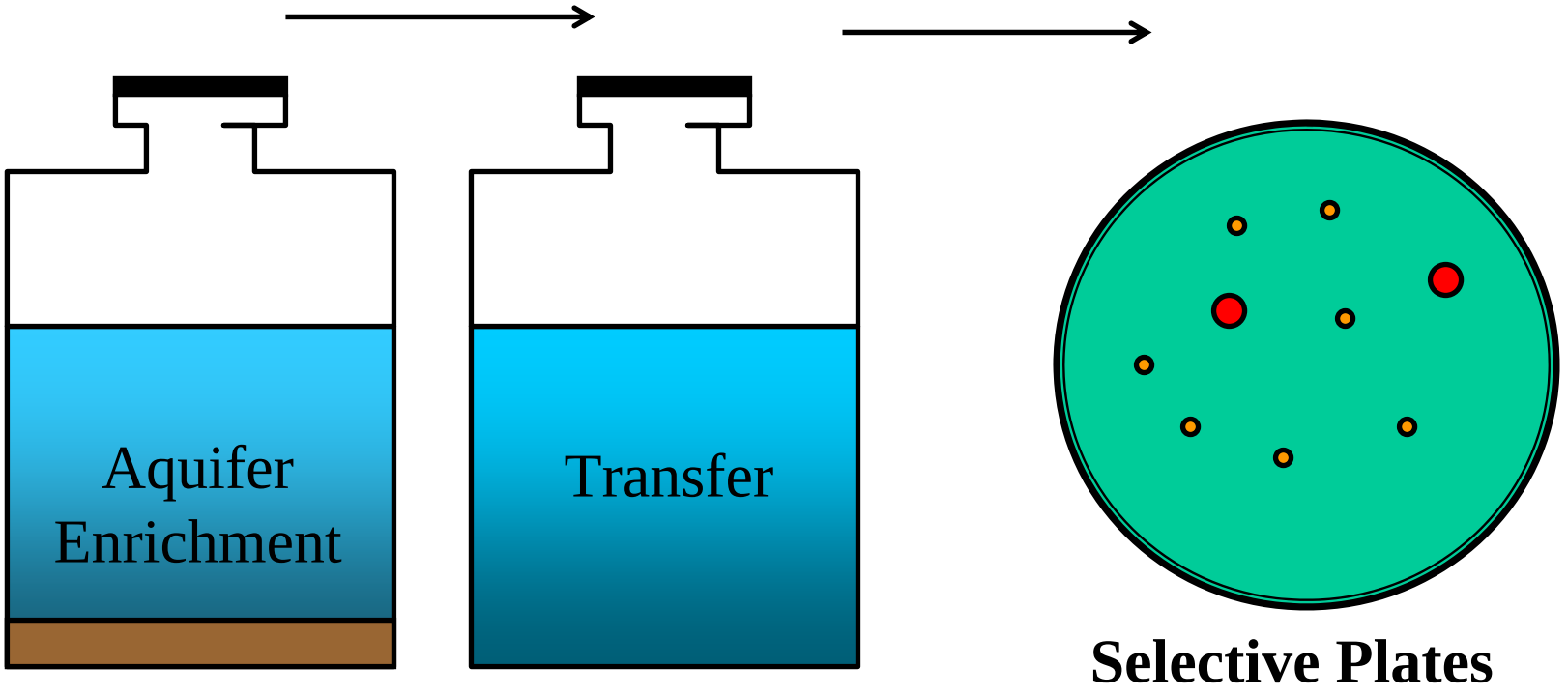
Most Probable Number Counts





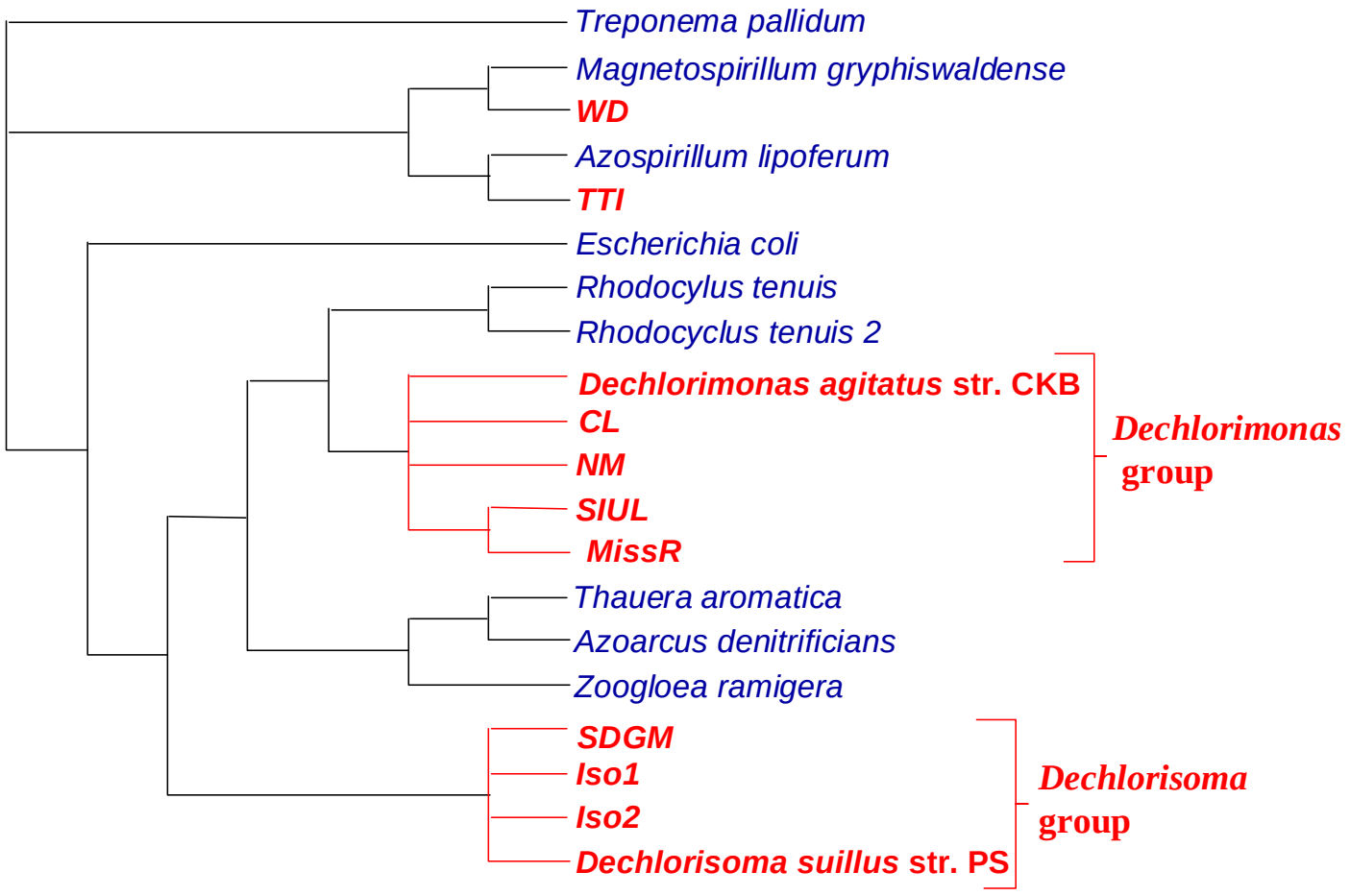
Temperature Gradient
Gel Electrophoresis

Microbial Enrichment and Isolation



Identify and Group Isolates Using rRNA Analysis

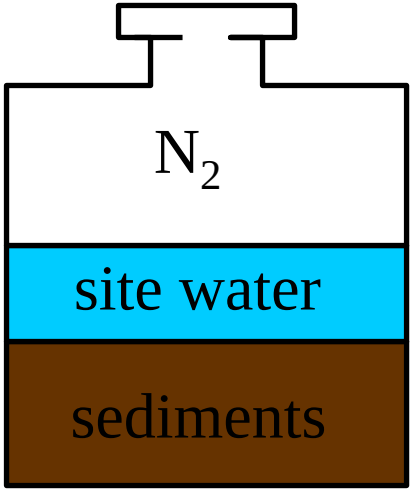
(John Coates, SIU)



Aquifer Microcosms

Serum Bottles:

- Site Sediments
- Site Groundwater
- Perchlorate



Tests:

1. Electron Donors
2. Alternate Electron Acceptors
3. Environmental Variables
4. Biostimulation and Bioaugmentation

JET PROPULSION LABORATORY - MICROCOSM STUDIES

STUDIES

- Electron Donors
- Bioaugmentation
- Alternate Electron Acceptors
- pH
- Salinity

Perchlorate Degradation in JPL Sediment/Groundwater Microcosms Amended with Various Electron Donors or Perchlorate-Degrading Bacteria.

Treatment	Perchlorate Concentration (µg/L)		
Electron Donors	***Day 0***	***Day 10***	***Day 21***
Killed Control	310 ± 0	293 ± 6	320 ± 0
Benzoate	310 ± 0	297 ± 6	150 ± 135
Methanol	310 ± 0	77 ± 57	< 5
Hydrogen	310 ± 0	177 ± 61	< 5
Propane	310 ± 0	283 ± 6	< 5
No Addition	310 ± 0	14 ± 19	< 5
Sucrose	310 ± 0	92 ± 67	< 5
Ethanol	310 ± 0	< 5	NS
Lactate	310 ± 0	< 5	NS
Molasses	310 ± 0	< 5	NS
Yeast Extract/Ethanol	310 ± 0	< 5	NS
Acetate	310 ± 0	< 5	NS
Bacteria Added			
Killed + Inoculum FBR2	310 ± 0	385 ± 7	415 ± 7
Inoculum FBR2+ YE/Etoh	310 ± 0	< 5	NS
Inoculum FBR2+ Acetate	310 ± 0	< 5	NS
Inoculum PC1+ YE/Etoh	310 ± 0	< 5	NS
Inoculum PC1+ Acetate	310 ± 0	< 5	NS

Figure 2. Perchlorate Degradation in Groundwater Microcosms Amended with Various Electron Donors.

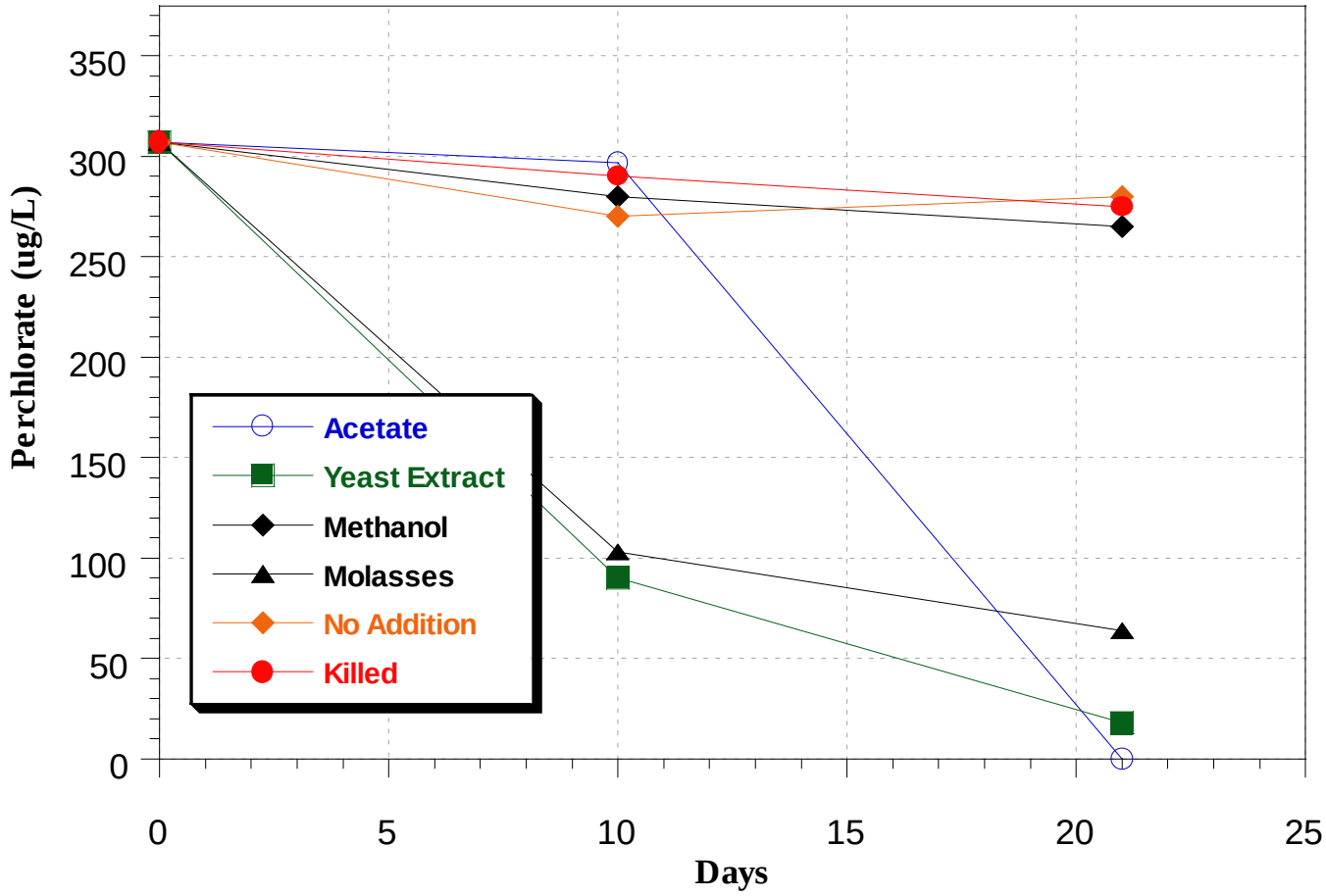


Figure 3. Degradation of Perchlorate (100 mg/L) and Nitrate (100 mg/L) in Aquifer Microcosms from JPL with Ethanol as a Substrate

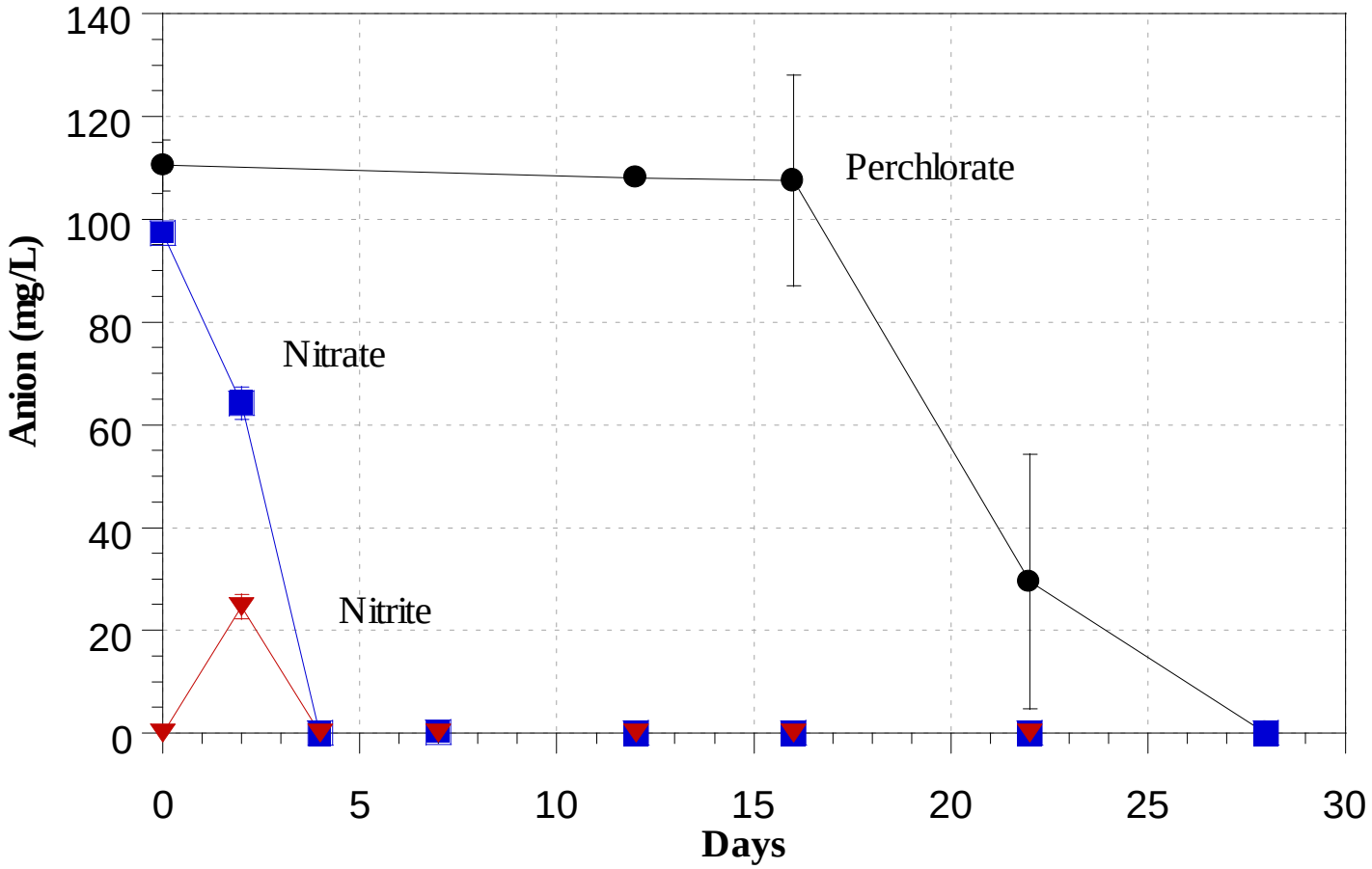


Figure 4. Influence of Nitrate (100 mg/L) on Perchlorate Biodegradation in Aquifer Microcosms from JPL

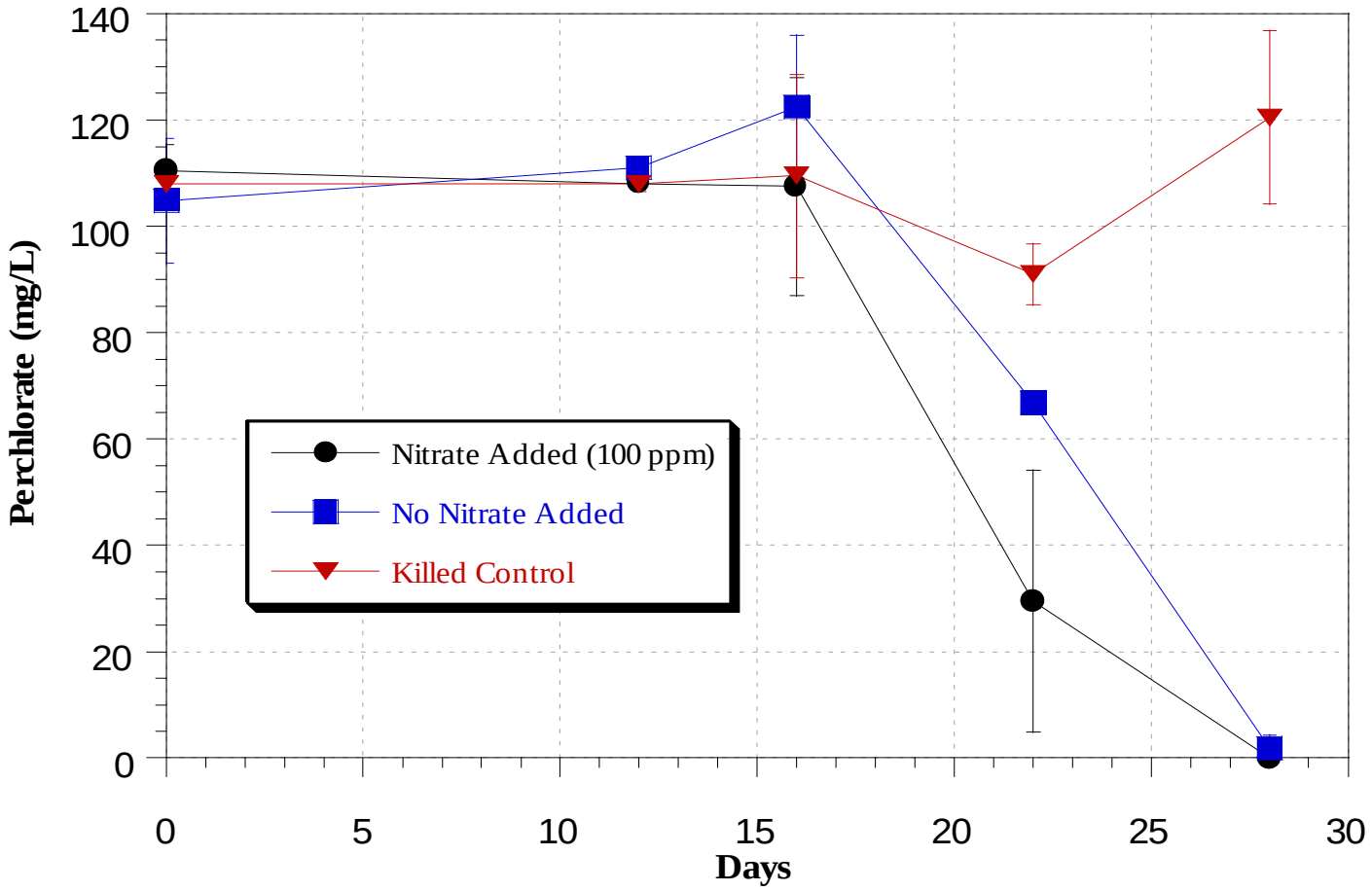


Figure 5. Biodegradation of Perchlorate (100 mg/L) and Nitrate (100 mg/L) in JPL Microcosms with No Substrate Added.

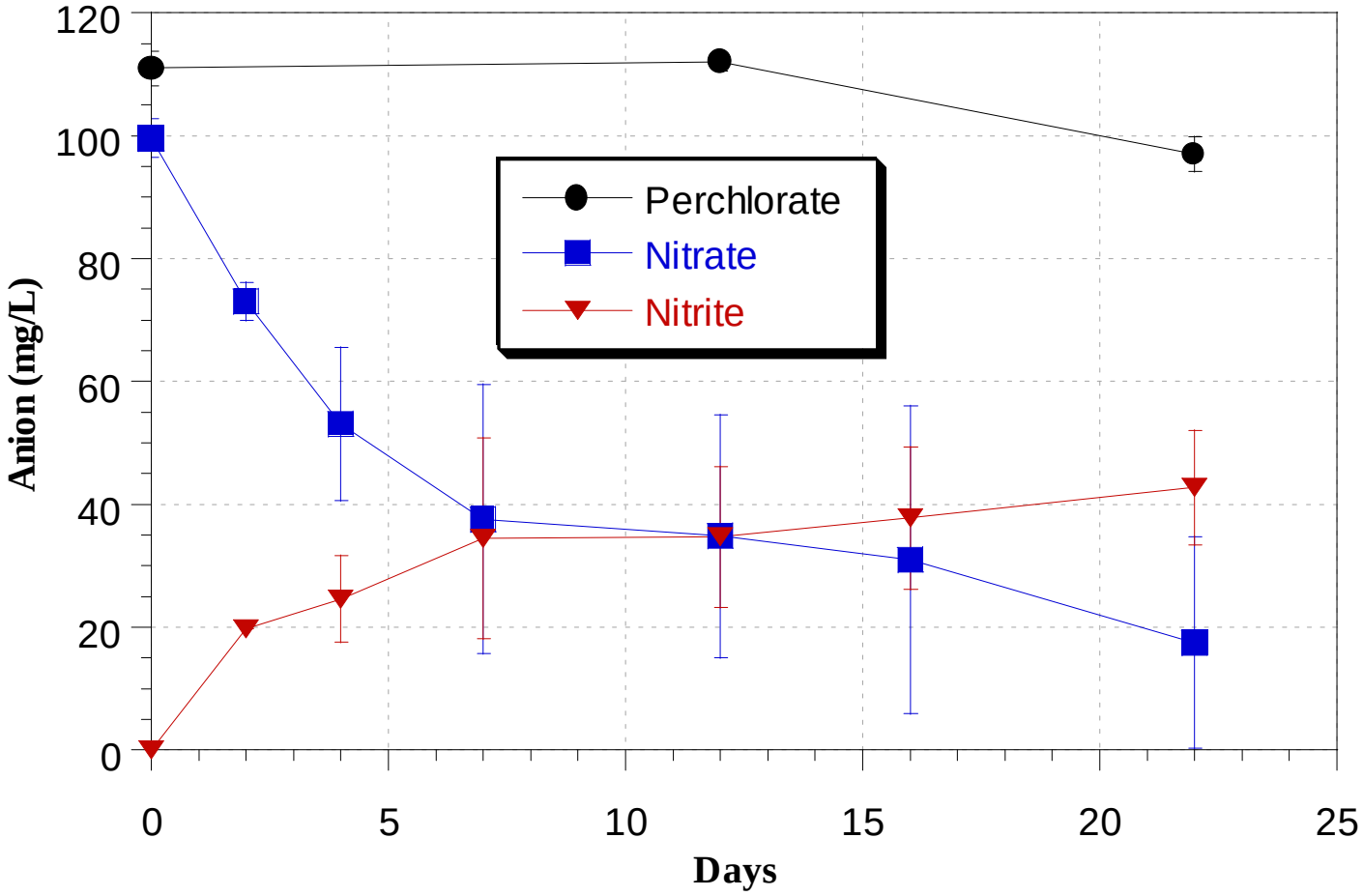
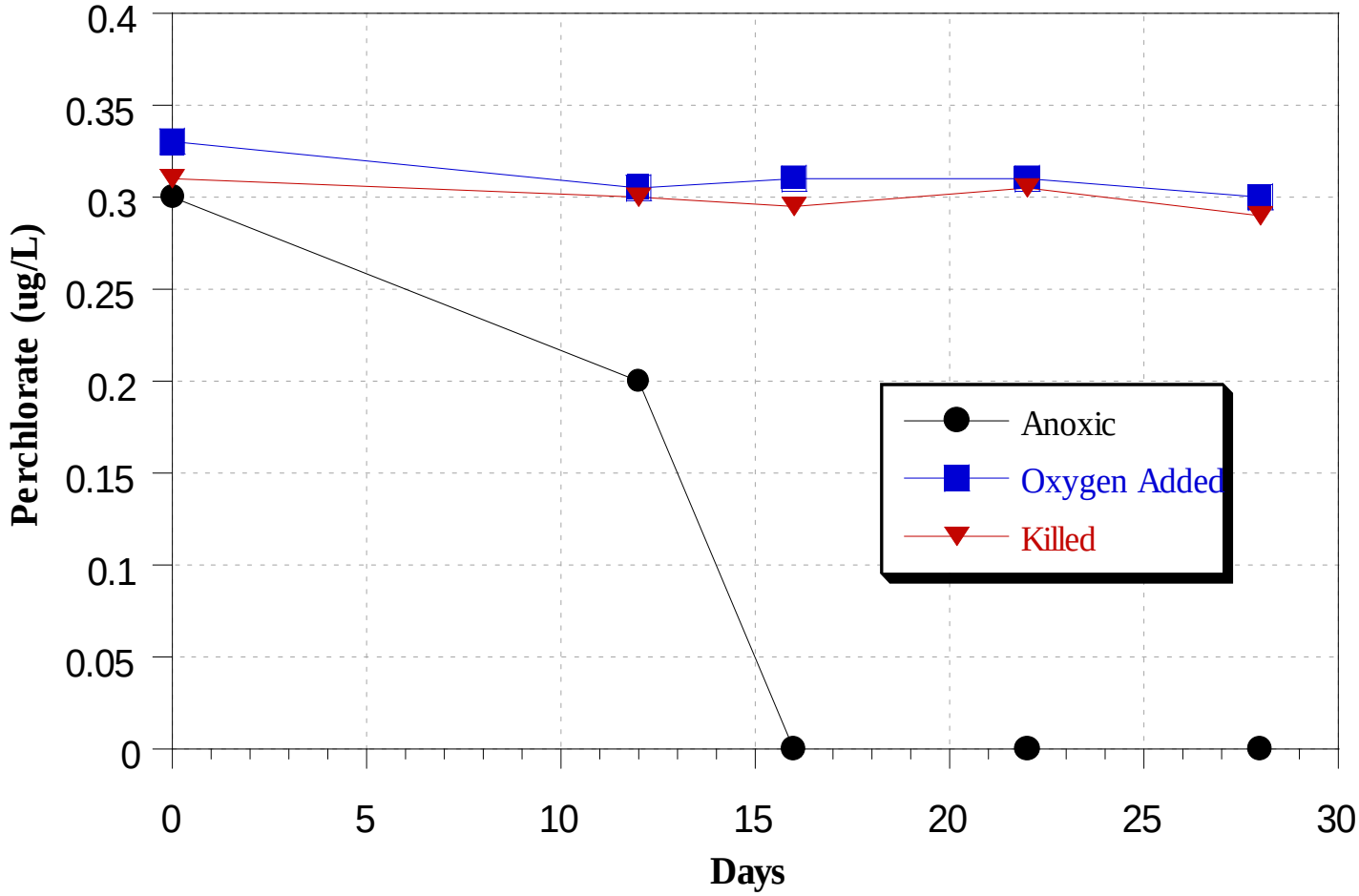


Figure 6. Influence of Oxygen on Perchlorate Degradation in Aquifer Microcosms from JPL



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TECHNICAL APPROACH

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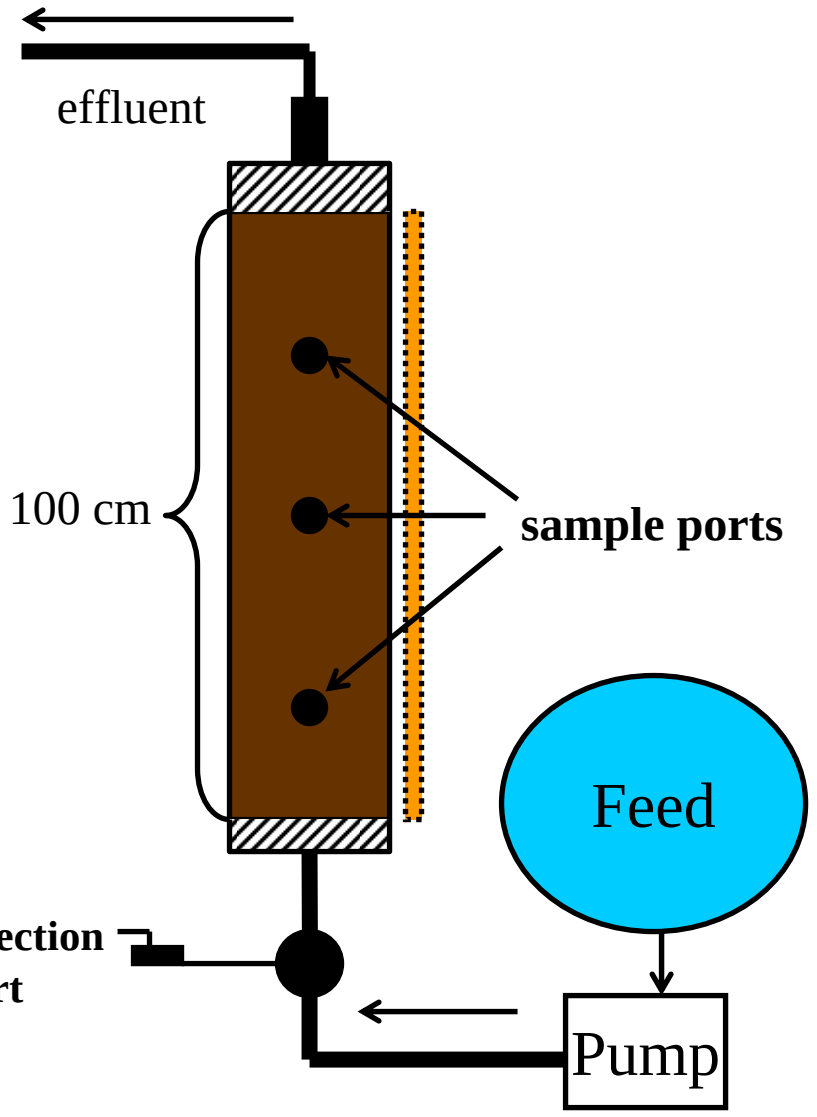
Flow-Through Aquifers

Tests: Based on Microcosms:

1. **Biostimulation**
2. **Bioaugmentation**
3. **Environmental Variables**

Results:

Degradation Rates and Extents
Substrate Requirements
Inoculum Activity/ Transport
Modeling Parameters



Modeling

Apply existing functional flow and reactive transport models

- **"MODFLOW-MT3D-RT3D"**
- **"hydrobiogeochem"**

Modeling results

- **validate laboratory studies**
- **evaluate field implementation strategies**
- **identify hydrogeological settings where the technology is applicable**

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TECHNICAL APPROACH

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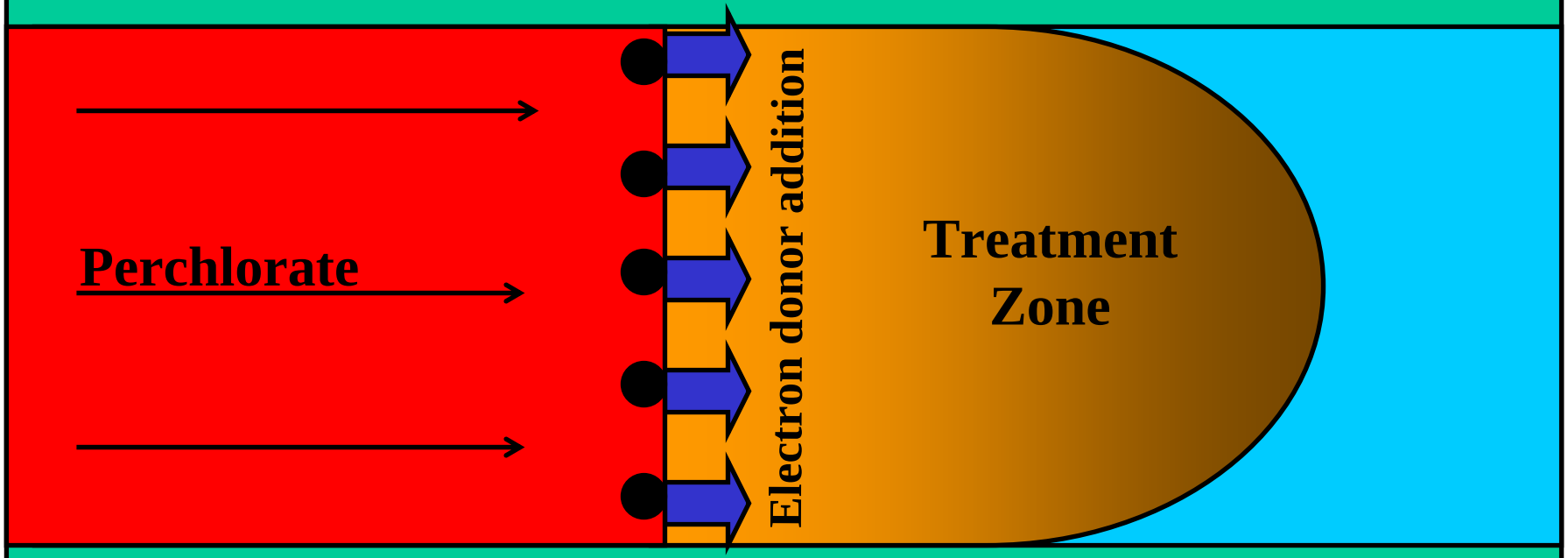
Field Demonstration

Reactive Barrier Technology



REACTIVE BARRIER TECHNOLOGY - DOVER LANDFILL

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In Situ vs *Ex Situ* Treatment Both !

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Factors

- Depth to Groundwater
- Plume Characteristics
- Hydraulic Control
- Economics
- Waste Generation
- Water Use and Reuse
- Aquifer Geochemistry
- Co-Contaminants
- Political Considerations
- Social Acceptance
- Regulatory Issues