SUBSURFACE MICROBIAL TRANSPORT AT THE CAPE COD SITE

R. Harvey & D. Metge: USGS, Boulder, CO
Factors Affecting Subsurface Bacteria Transport on the Field Scale

(Source: Modified from Harvey, 1991)
Different Scales = Different Answers!!

Field-scale
(USGS Cape Cod, MA array)

Intermediate-scale
(photo courtesy of J. Ryan, CU)
Transport of Waterborne Pathogens

- Chlorine-resistant
- Low infective dose
- Ubiquitous
- Long-lived

Hepatitis Virus

Streptococcus

Cryptosporidium parvum oocysts

Virus 0.01-0.2 µm  ➔ Bacteria 0.2-3.0 µm  ➔ Protozoa 1.5-10 µm
Fluorescent microspheres as surrogates for oocysts?

Considerations:
- Size
- Aspect ratio
- Buoyant density
- Surface charge
- Attachment behavior

Microspheres
- Dia: 1.6 µm
- Type: YG

Dia: 2.9 µm
Type: BB

Dia: 4.9 µm
Type: YG

C. parvum oocysts

Microspheres
C. Parvum oocysts
FLOW CYTOMETER, USGS, Boulder
(Differential quantification of polydisperse microsphere and oocysts)
Injectate

Multi-Level Samplers

Injection Well

Injectate

PRD1 (60 nm)

MS2 (20 nm)

Microspheres (100 nm)

Bromide

Large-scale MLS sampling array

Cape Cod, Massachusetts

Injection sites

Phage Enumeration

Agar Plates Containing Host

Dilution Series
Use of a dual-label to assess phage inactivation

- Infective - Intact
  - $^{32}\text{P}$-labeled capsid
  - $^{35}\text{S}$-labeled DNA

Type 1

- Infective - Non-intact
- Non-infective - Intact
  - $^{32}\text{P}$
  - $^{35}\text{S}$

Type 2

- Non-infective - Disintegrated
  - $^{32}\text{P}$
  - $^{35}\text{S}$

Solid Surface

- Infective
- Non-infective

Iron/Al oxides

Surface-Induced Inactivation
1. *In Situ* Survival Studies: (Ex: Down-well filter-chambers)

- Closures
- Protective Cage
- Membrane
- Plastic ties
- Slotted PVC well screen

Groundwater flow

Contaminant Plume

USGS Well Site 513
Case Study: Groundwater isolate (*Pseudomonas stutzeri*)
(Cape Cod aquifer, 11 m depth, USGS well series F013)

<table>
<thead>
<tr>
<th>Incubation Time (Days)</th>
<th>Cell Concentration (#/mL)</th>
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<tbody>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>16</td>
<td>4</td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>Pre-cessation &quot;Carrying Capacity&quot; (1995)</td>
<td></td>
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<tr>
<td></td>
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<tr>
<td>Current &quot;Carrying Capacity&quot; (8-9-05)</td>
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Design: J. Lisle, USGS
Set of 3 conc’ns bracketing carrying capacity in each well

5 x 10^6/mL 5 x 10^5/mL 5 x 10^4/mL

Bacterium: bfp-labeled P. stuzeri

Acetate injection wells

Down-well Survival/Growth (F513)
Classic “Run & Tumble”
Bacterial Chemotaxis

Water column

“run”

“tumble”

Porous medium

Modified from Ford & Harvey, 2007
Fine-grained sediments (reservoir for attractant)

Coarse-grained sediments (advective transport)

Groundwater flow

Attractant gradient

Chemotactic migration?
Basic Experimental Design
(Proposed to NSF)
% BACTERIAL ATTACHMENT

Uncontaminated Groundwater + LAS (anionic surfactant)- 14 mg/L

Cape Cod site

Uncontaminated Groundwater

pH

0 10 20 30 40 50 60 70 80 90 100

% BACTERIAL ATTACHMENT
Conditioning the Aquifer to Promote Bacterial Transport

1. Initial SDBS Injection

2. Follow-up Injection: NaH$_2$PO$_4$
Preliminary Chemotaxis assessment: Modified down-well filter-chambers

- Saturated Sand
- Attractant
- Pseudomonas stutzeri
- Acetate Gradient
- Bacterial Gradient
- Permeable Membrane
- Impermeable Membrane
- Bacteria

Acetate
Differentiating test and control *Pseudomonas stutzeri*

**Test Bacterium**
Genetically Engineered to produce “BFP”

**Control Bacterium**
Stained with (DAPI + Sybr Gold)

*Cultures Illuminated with ultraviolet light*

- **P. stutzeri** bfp-expressing (chemotactic)
- **P. stutzeri** DAPI/Sybr Gold (Impaired chemotaxis)