



# Analysis of Bacterial Random Motility and Chemotaxis in Porous Media using MRI

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# Introduction

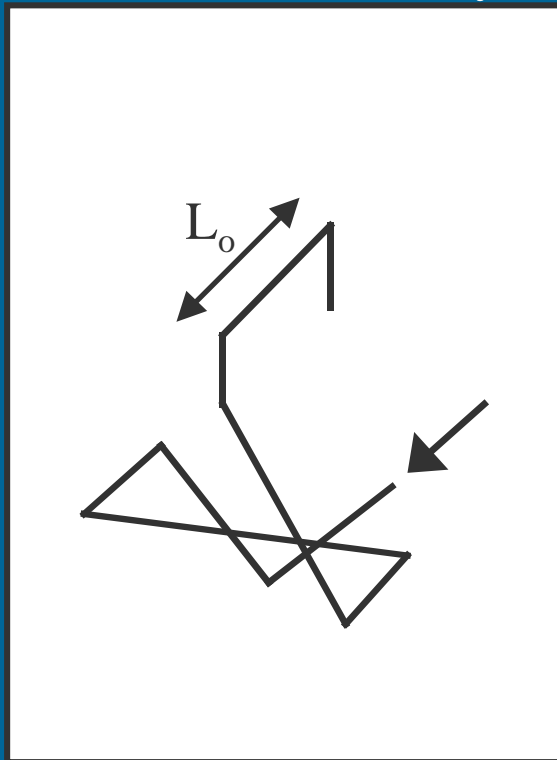
- Conventional pump-and-treat remediation systems often leave contamination in regions of low permeability (aquitards, clay lenses)
- Aquitards can act as long-term reservoirs for contaminants
- Contaminants diffuse into more permeable regions, creating a chemical gradient surrounding the layer of contamination
- *In situ* bioremediation is a common remediation technique, but is limited by bacterial transport



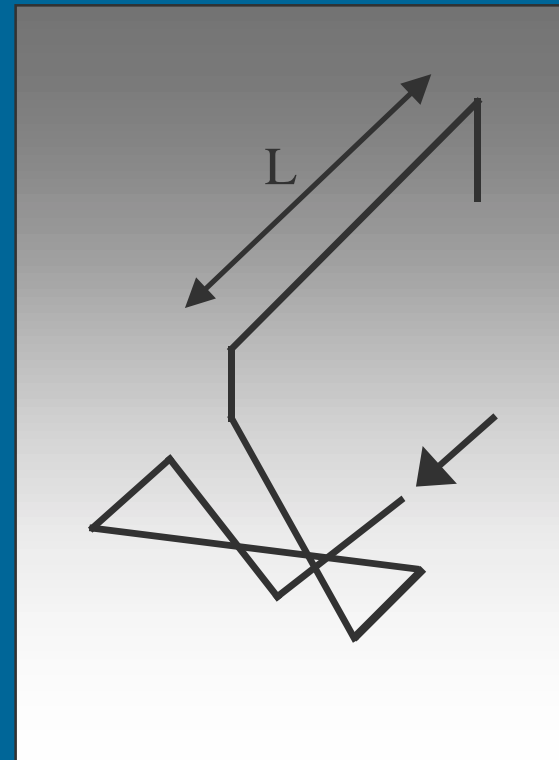
# Bacterial Chemotaxis

Chemotaxis is defined as the ability of bacteria to bias their motion towards regions of high contaminant concentration

## Random Motility



## Chemotaxis



↑  
increasing  
attractant  
concentration

Figure adapted from Macnab, R.M. (1980). Biological Regulation and Development. New York, Plenum



# Objective

- To study how chemotaxis affects the transport of motile bacteria through porous media in the presence of a contaminant gradient
  - Quantify the chemotactic response of *Pseudomonas putida* F1 to TCE
  - Use MRI to image the behavior of magnetically-labeled bacteria in a packed column

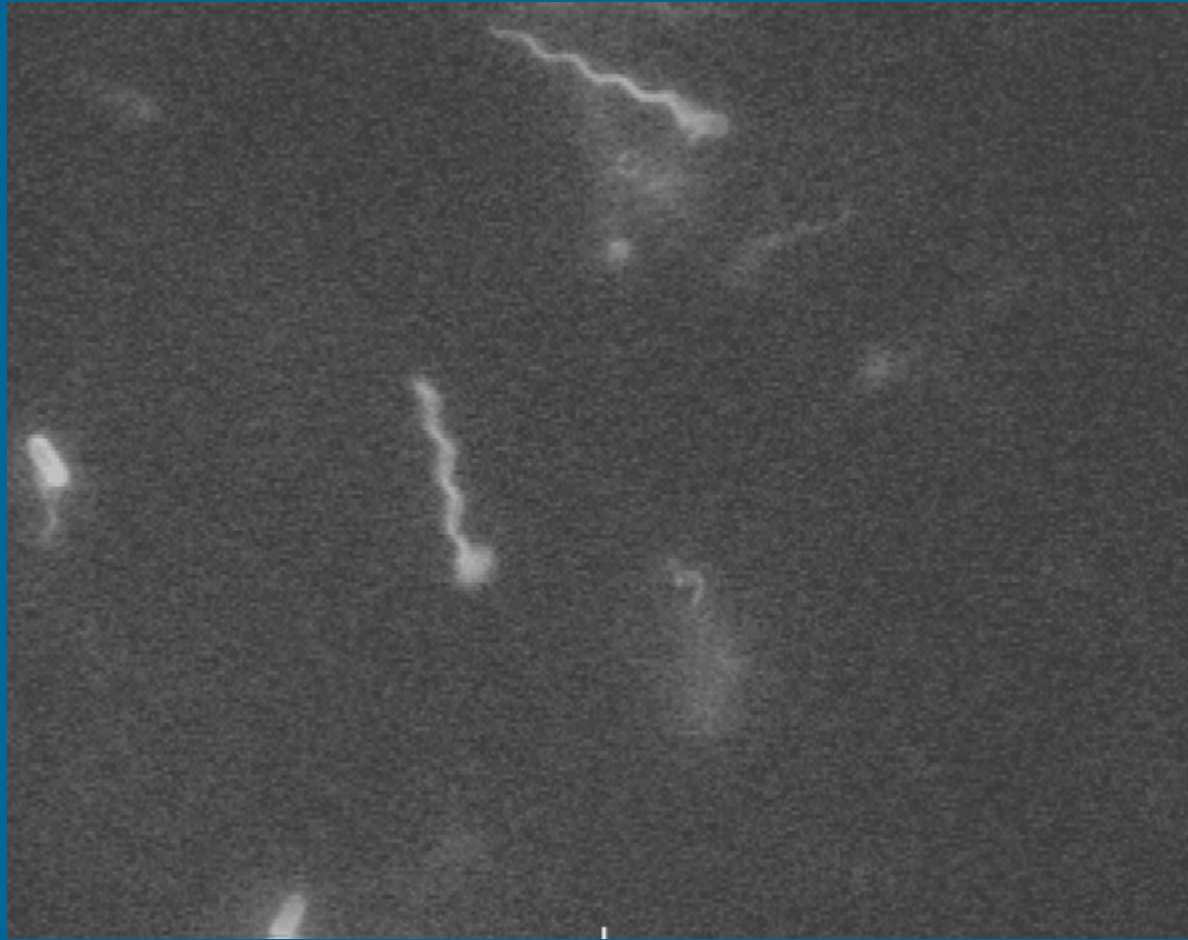


# Experimental System

- *Pseudomonas putida* F1 are a native soil-inhabiting bacterial strain
- Trichloroethylene (TCE) is a common and persistent ground-water contaminant
- *P. putida* exhibit chemotaxis towards TCE, but cannot use it as a growth substrate
- Porous medium is 250-300- $\mu\text{m}$ -diameter glass-coated polystyrene beads



# Bacterial Swimming Properties

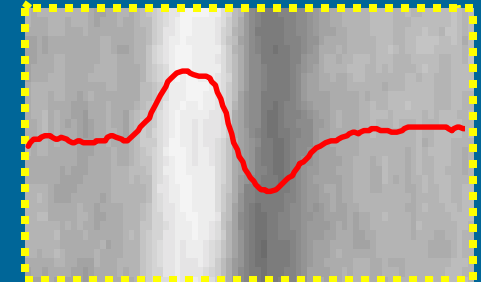
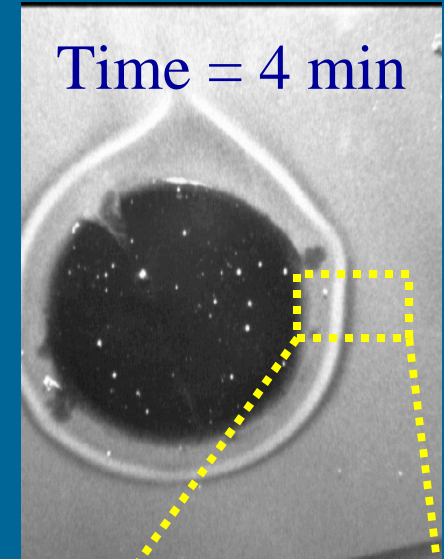
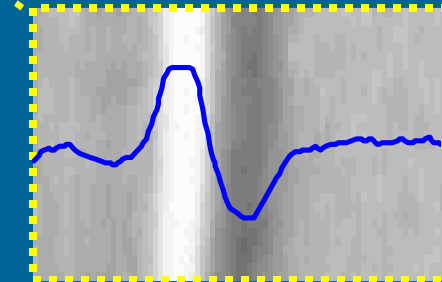
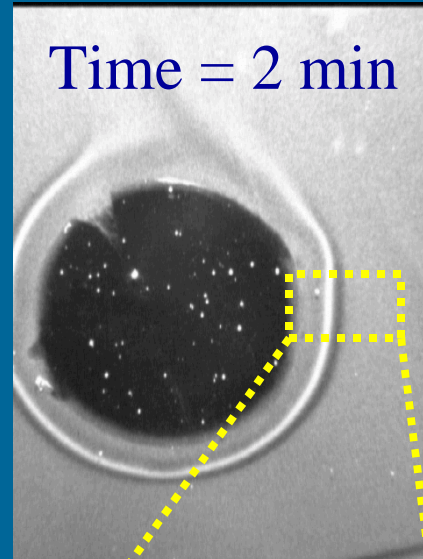
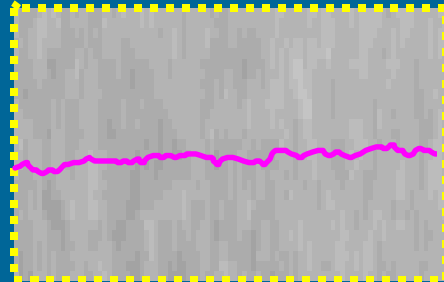
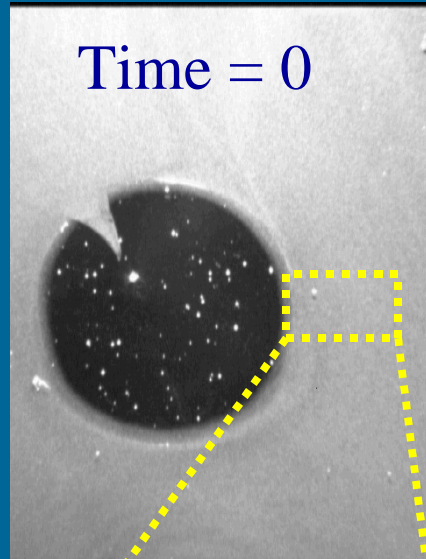


From Howard Berg, Rowland Institute of Science, <ftp://ftp.rowland.org/pub/download/>



# Chemotaxis - Bulk Response

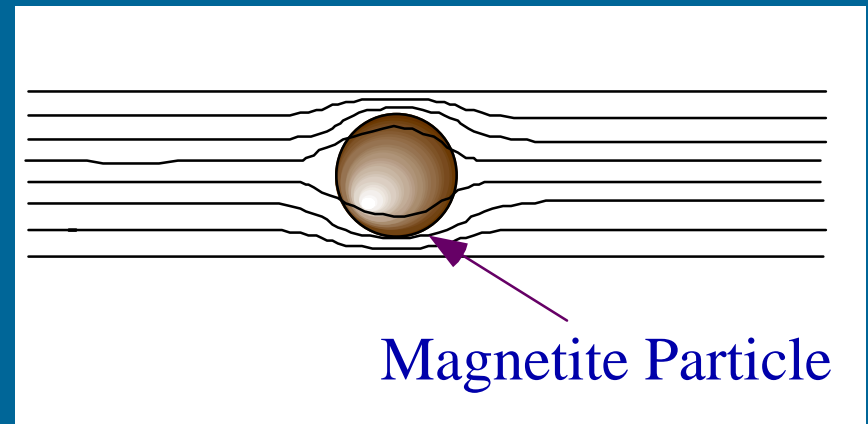
Chemotaxis of *P. putida* to TCE





# Overview of Magnetic Resonance Imaging

- Non-invasive technique for taking measurements in an opaque medium
- Signal is based on H nuclei (protons) in water
- Bacteria can be labeled with contrast medium to make them stand out
- Magnetite causes local magnetic field gradients which cause signal attenuation



# Bacterial Labeling Procedure

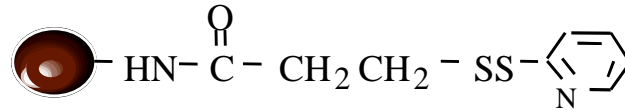
Magnetite Particle  
w/BSA Coating



+ SPDP



Magnetite w/Derivatized Surface

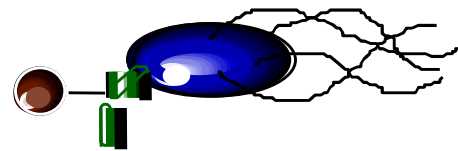


+ DTT

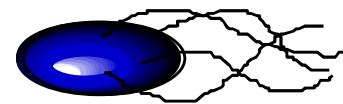


Reduced Antibody

Anti- *P. putida*  
Antibody

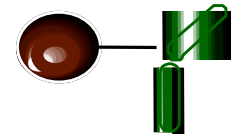


*P. putida* with  
Conjugated Magnetite



*P. putida* Bacterium

+



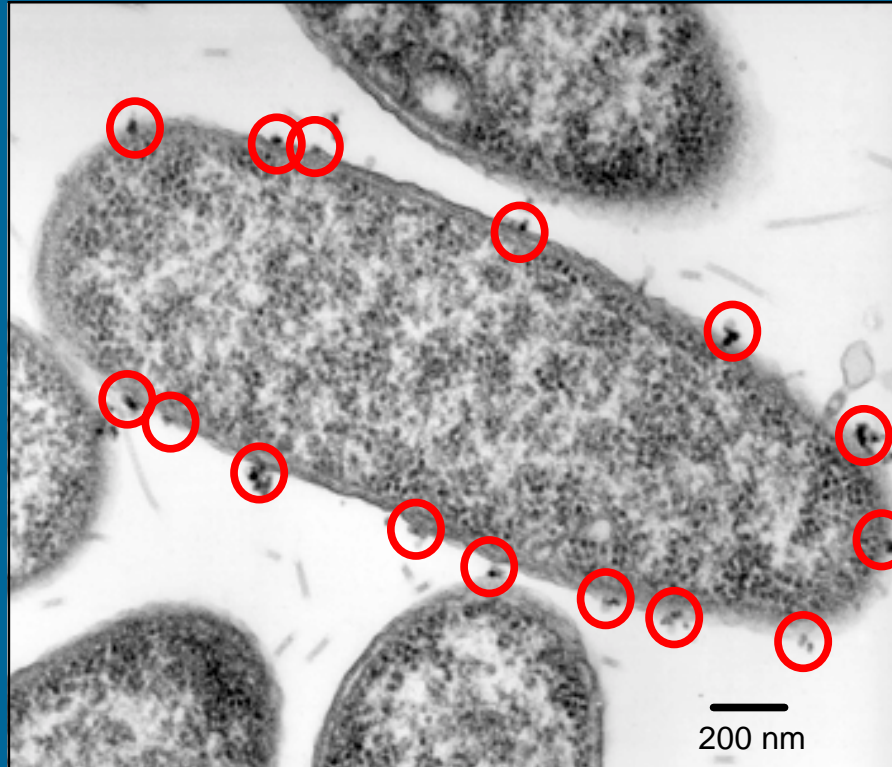
Magnetite w/  
Conjugated  
Antibody

Labeling technique adapted from Nakamura et al., 1993

Antibody to *P. putida* supplied by M.I. Ramos-Gonzalez (CSIC)



# Attached Magnetite



*E. Coli* Bacterium with attached magnetite



# Outline of Experiments

- One-dimensional bacterial transport with no flow:

$$\frac{\partial b}{\partial t} = \frac{1}{\tau} \mu_{bulk} \frac{\partial^2 b}{\partial x^2} - \frac{\partial(v_c b)}{\partial x}$$

$b$  = bacterial concentration

$\tau$  = tortuosity

$\mu_{bulk}$  = motility coefficient

$v_c$  = chemotactic velocity

$$v_c = \frac{2}{3\tau} v \cdot \tanh\left(\frac{\chi_o}{2v} \frac{K_d}{(K_d + a)^2} \frac{\partial a}{\partial x}\right)$$

$v$  = bacterial swimming speed

$\chi_o$  = chemotactic sensitivity

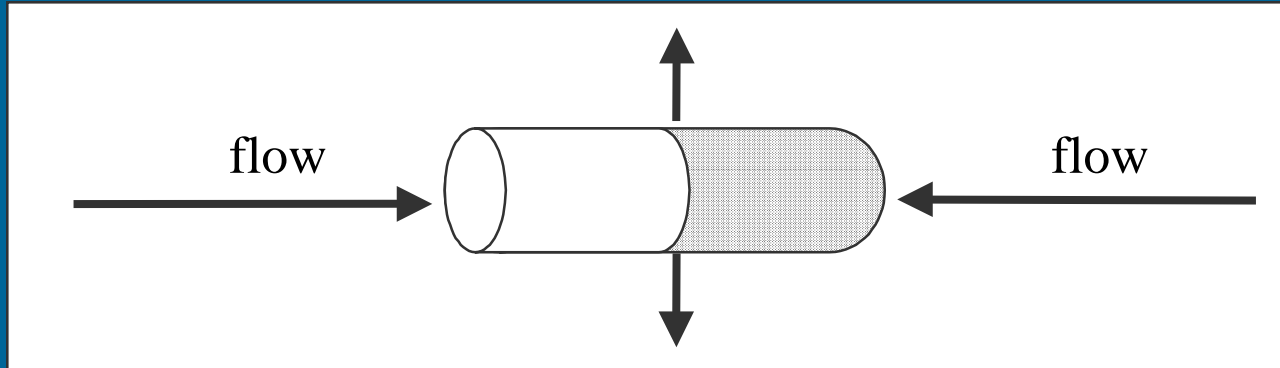
$K_d$  = effective dissociation constant

$a$  = TCE concentration

- » 1.  $\text{MnCl}_2$  Experiments to Determine Tortuosity,  $\tau$
- » 2. Bacterial Motility Experiments to Determine Random Motility Coefficient,  $\mu_{bulk}$
- » 3. Bacterial Chemotaxis Experiments to Determine Chemotactic Sensitivity Coefficient,  $\chi_o$



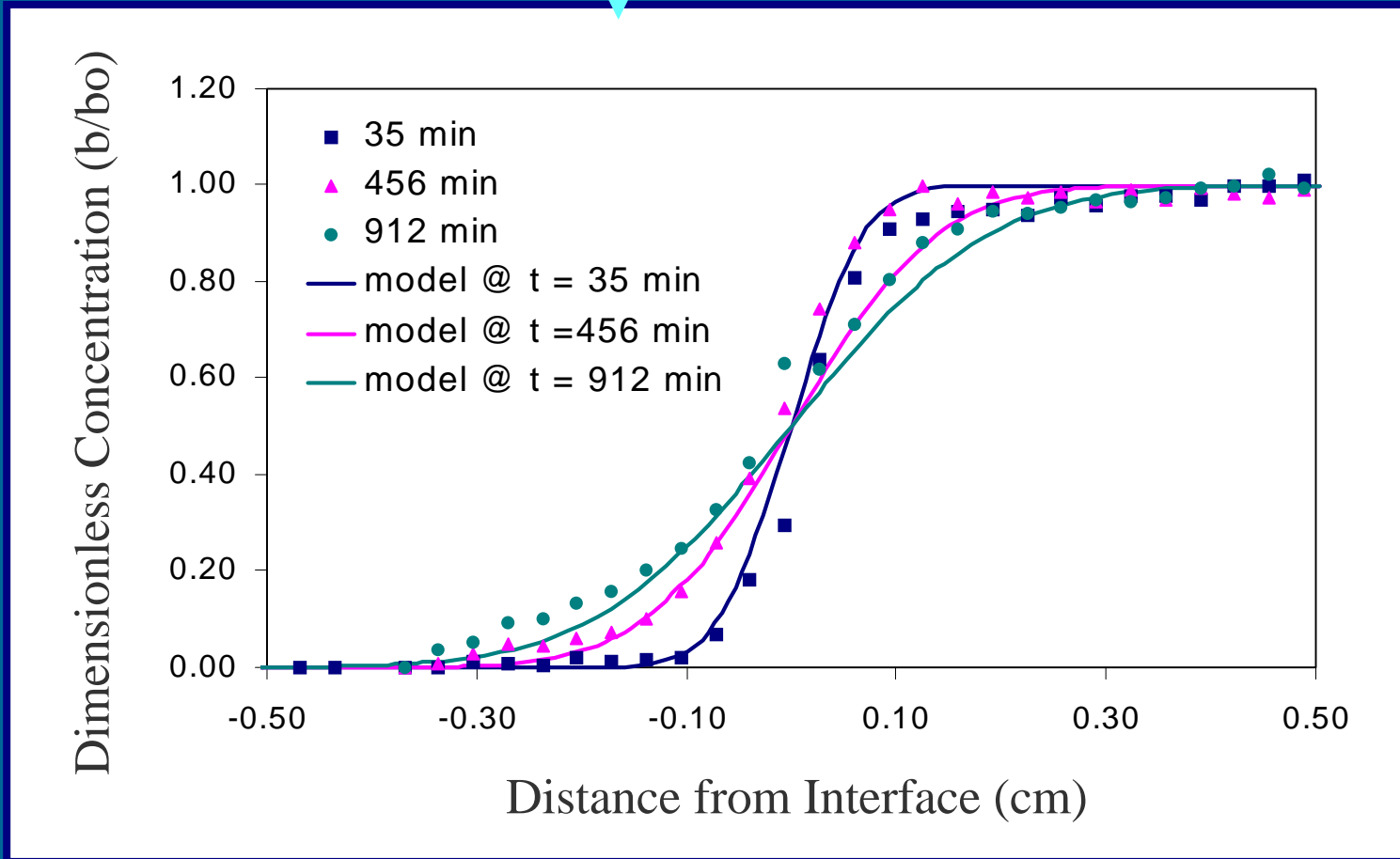
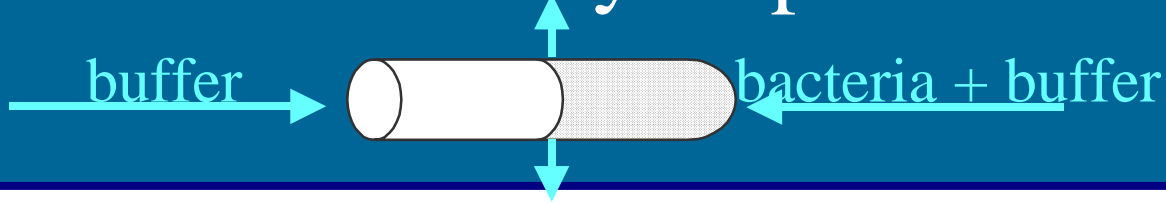
# Experimental Design



- Pre-treat column with unlabeled bacteria
- Set up column with initial step change of bacteria (for random motility experiments) and TCE (for chemotaxis experiments)
- Image column for 17 hours to obtain spatial bacterial concentration profiles over time
- Bacterial concentrations are correlated to reduction in signal caused by contrasting agent (magnetite)



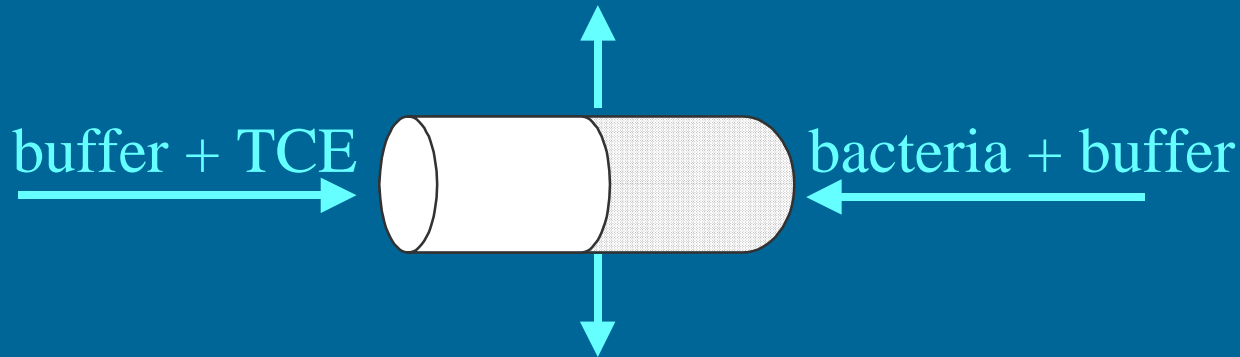
# Bacterial Motility Experiments



Random Motility of *P. putida* F1,  $\mu = 1.14 \times 10^{-6}$  cm<sup>2</sup>/sec, assuming a tortuosity of  $\tau = 6.71$



# Chemotaxis Experiments



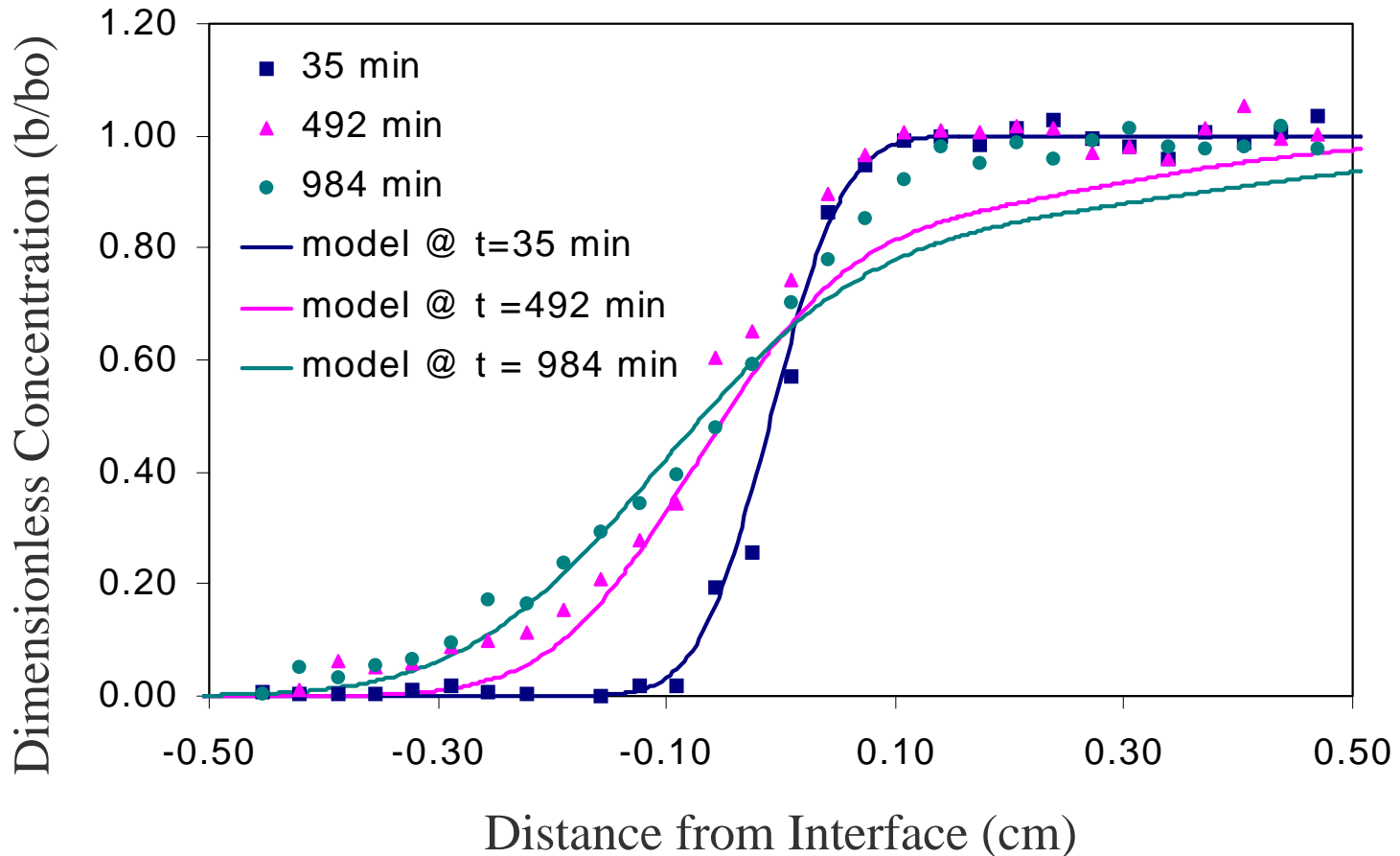
$$\frac{\partial b}{\partial t} = \frac{\mu_{bulk}}{\tau} \frac{\partial^2 b}{\partial x^2} - \frac{\partial(v_c b)}{\partial x} \quad v_c = \frac{2}{3\tau} v \cdot \tanh\left(\frac{\chi_o}{2v} \frac{K_d}{(K_d + a)^2} \frac{\partial a}{\partial x}\right)$$

$v_c$  = chemotactic velocity     $\chi_o$  = chemotactic sensitivity coefficient  
 $K_d$  = equilibrium dissociation constant     $a$  = TCE concentration

- Given the column tortuosity and the bacterial random motility coefficient, we solve the bacterial transport equation for the chemotactic sensitivity coefficient,  $\chi_o$



# Chemotaxis Experiments



Chemotactic Sensitivity of *P. putida* F1 to TCE,  $\chi_0 = 2.9 \times 10^{-4}$  cm<sup>2</sup>/sec, assuming a random motility coefficient of  $\mu = 1.14 \times 10^{-6}$  cm<sup>2</sup>/sec and a tortuosity of  $\tau = 6.71$



# Conclusions

- A noninvasive imaging technique was used to quantify bacterial transport through porous media
- Preliminary data suggests that the increased accumulation of bacteria in regions of favorable contaminant concentration is due to chemotaxis
- Numerical simulations predict the following parameters:
  - Column tortuosity,  $\tau = 6.71 \pm 0.25$
  - Random Motility of *P. putida* F1,  $\mu = 1.14 \times 10^{-6}$  cm<sup>2</sup>/sec
  - Chemotactic Sensitivity of *P. putida* F1 to TCE,  $\chi_o = 2.9 \times 10^{-4}$  cm<sup>2</sup>/sec