Comparison of *Escherichia coli* and *Bacteriodes fragilis* Transport within Saturated Quartz Sands

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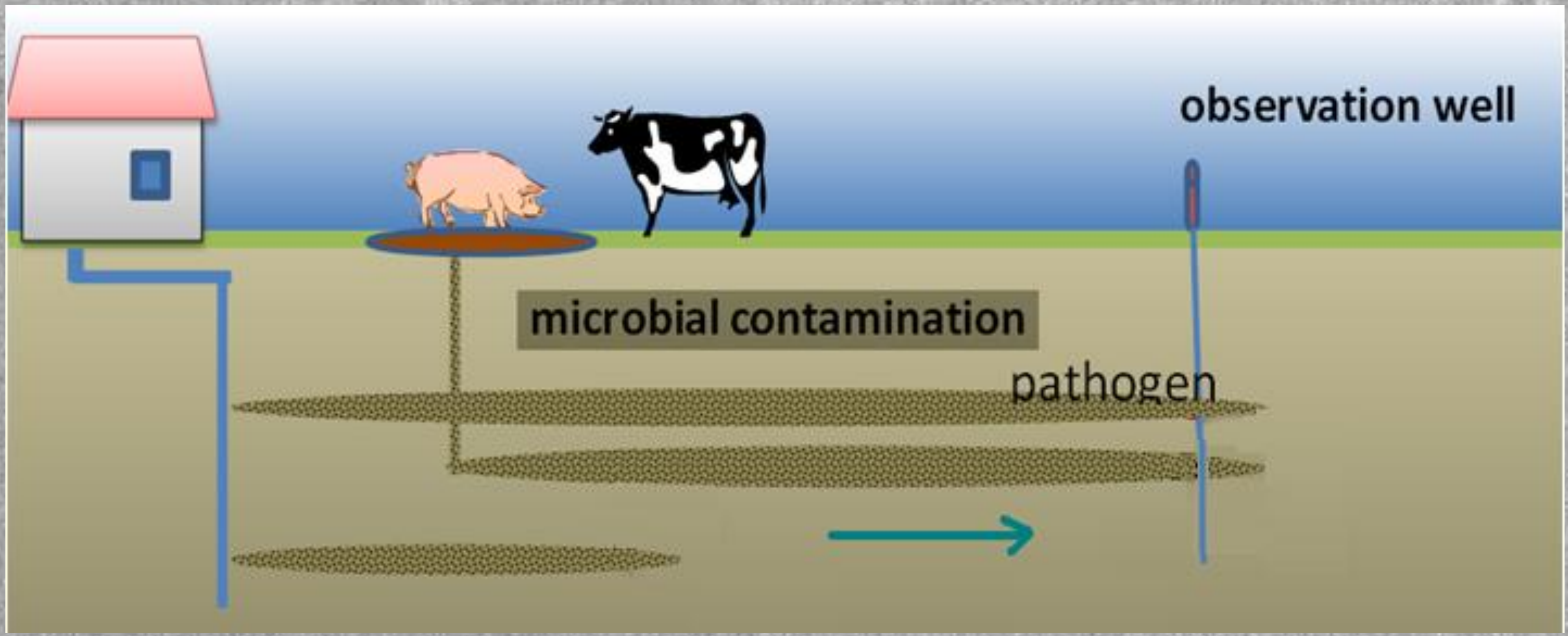
Groundwater Microbial Contamination

- 44% of US population uses GW as primary drinking water source
- GW often untreated
- Pathogenic microbes can cause illness
- Fecal pathogens are the major cause of microbial contamination

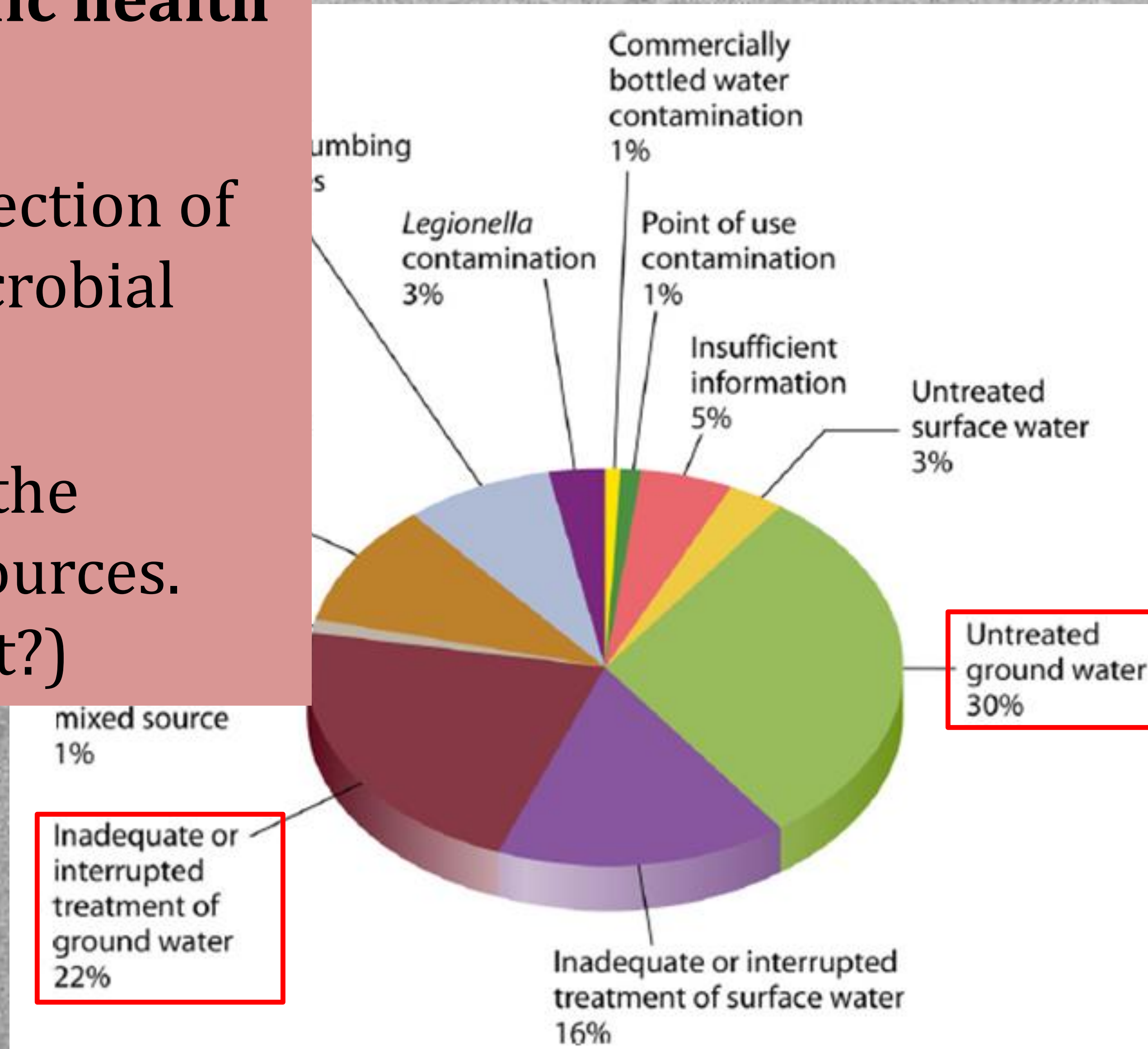
Waikato Regional Council, New Zealand



Outbreaks of Waterborne Diseases
1971-2006

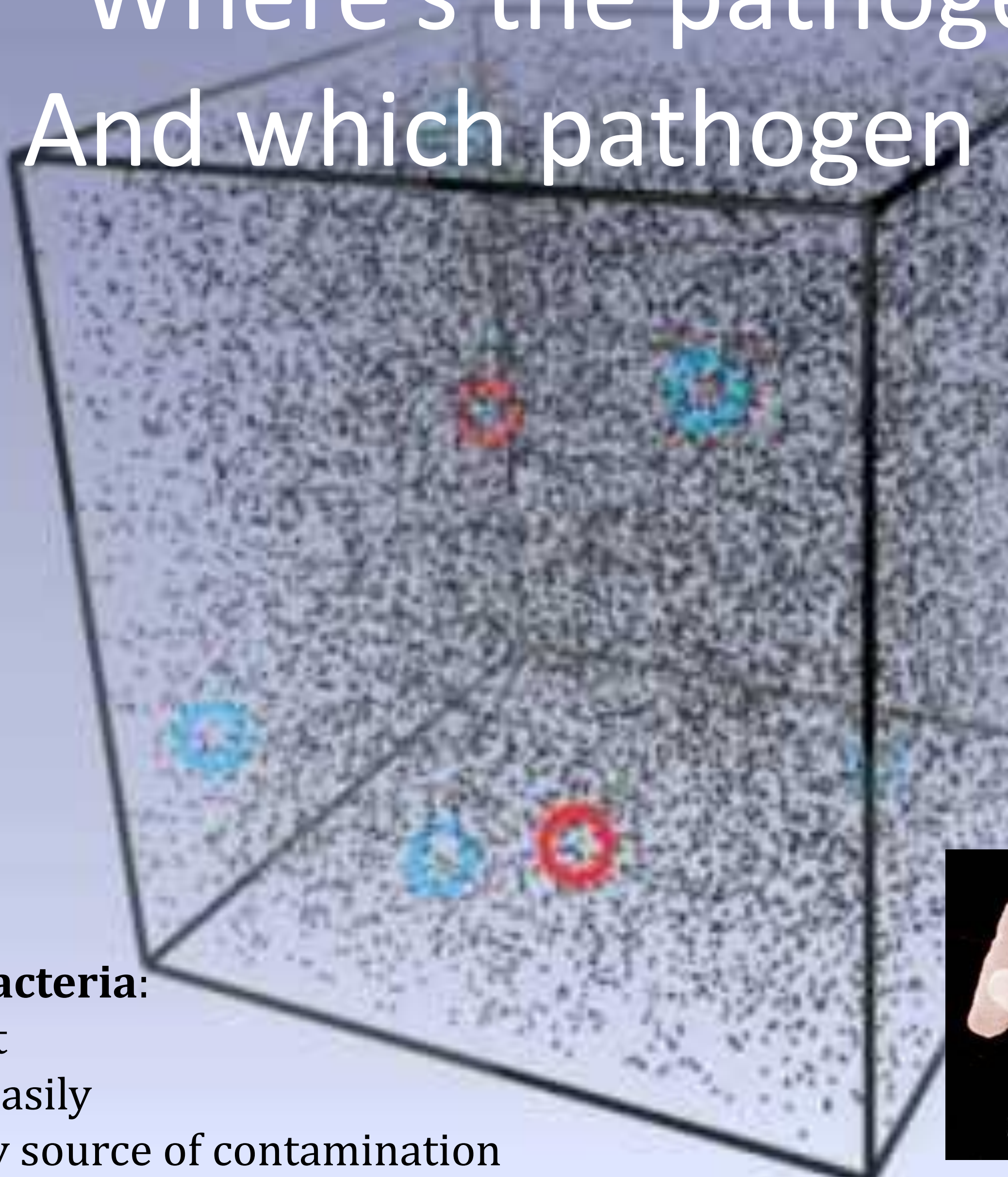
Protection of public health requires:

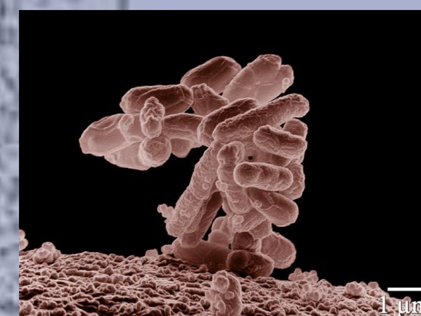
1. Fast, reliable detection of groundwater microbial contamination;
2. Identification of the contamination sources. (Whose poop is it?)



Craun, G. F, et al., 2010.

Where’s the pathogen?
And which pathogen is it?

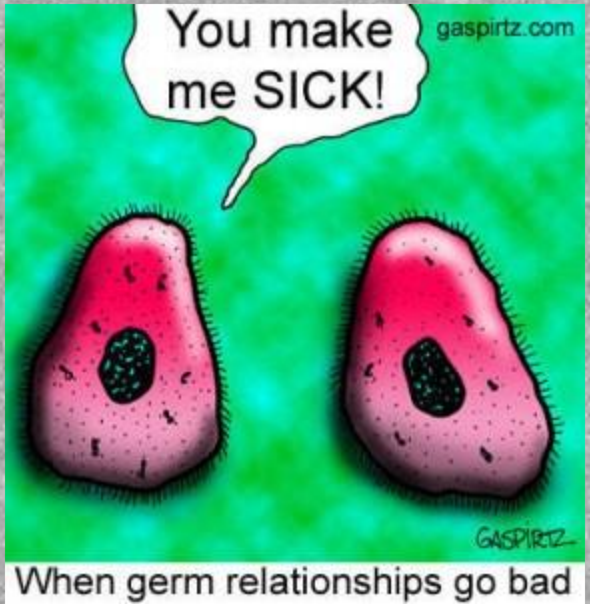




Indicator bacteria:
- Abundant
- Analyze easily
- ….identify source of contamination
- … travel as fast or faster than pathogens

Bacteriodes

- **Anaerobic bacteria 100-1000x more common in gut than aerobic bacteria**
- **4 *Bacteriodes* spp. make up 30% of total gut bacteria**
- **Genetic markers for humans, other species aid in Microbial Source Tracking**



Research Objectives

1. Do both *E. coli* and *B. fragilis* have similar transport properties, or is one type more mobile within quartz sands?

2. If one type of bacteria is more mobile (less attachment to quartz), what are the underlying attachment mechanisms?

Methods

<u>Growth of *E. coli*</u>

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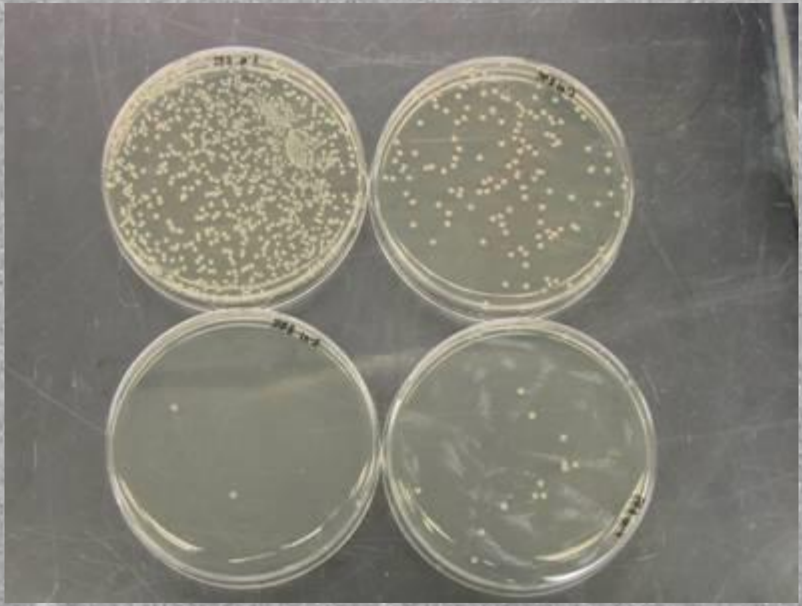


Grow on sterile TS agar,
transfer to sterile TS broth

Methods

Growth of *B. fragilis*.

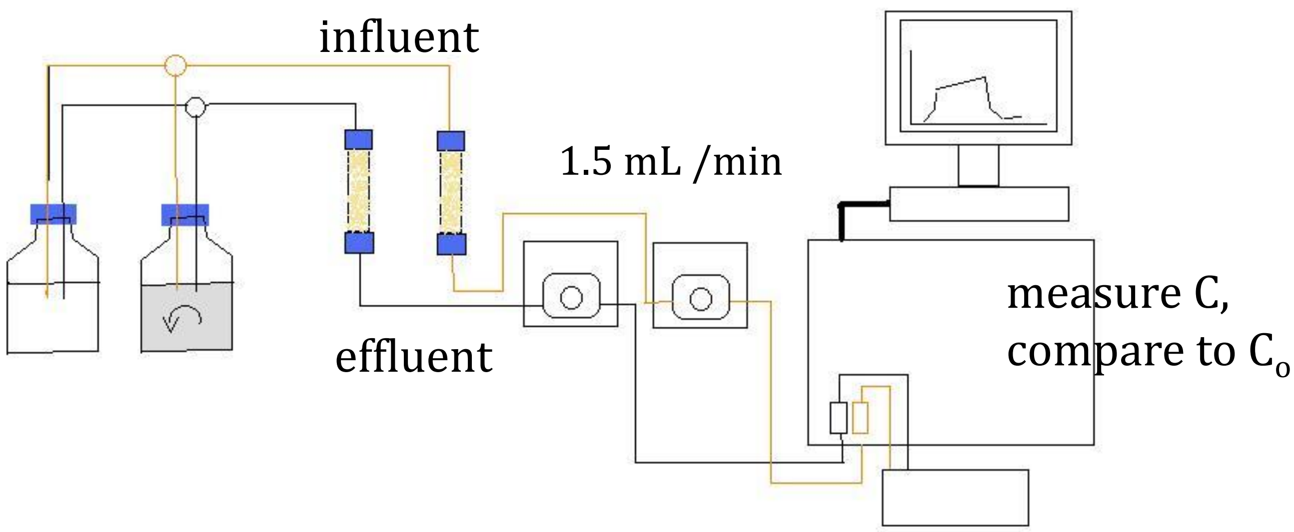
100 mL serum vials,
sealed and crimped

Methods

Assessment of transport behavior

Laboratory column transport experiments performed using quartz sands.

C_o = 4E7 cells/ml



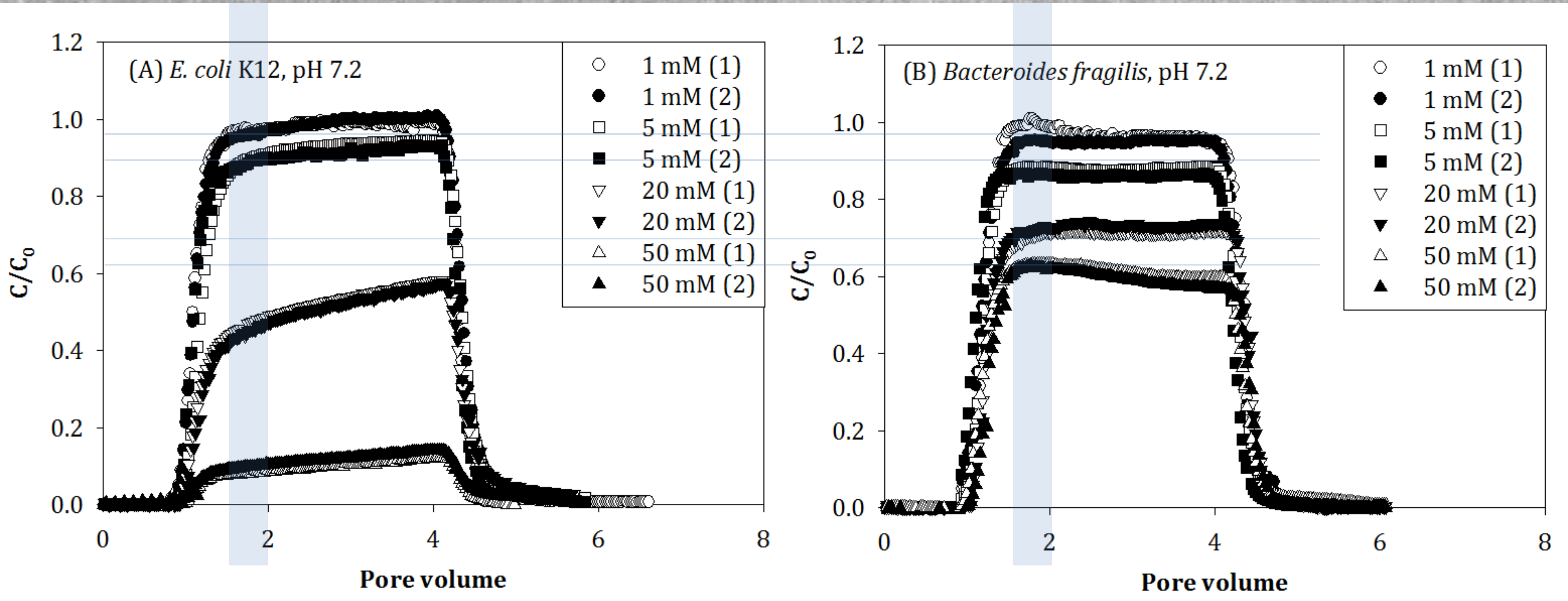
60 min bacterial solution injection (3.5 PV)

1, 5, 20, 50 mM total ionic strength solutions, NaCl buffered with $NaHCO_3$ to pH 7.2

Bacteria Transport Behavior

breakthrough curves

E. coli B. fragilis

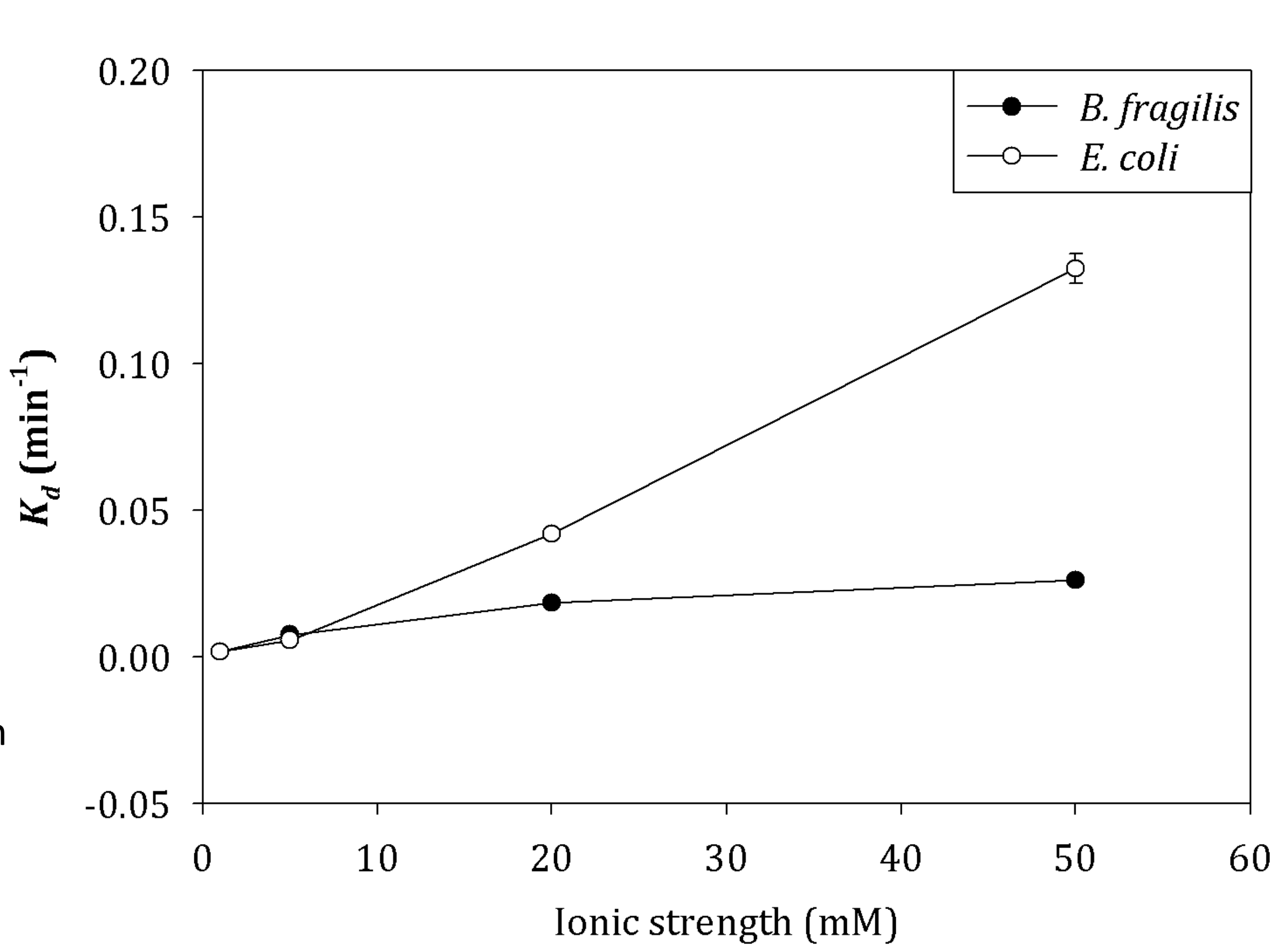


Transport Behavior

k_d First-order deposition rate coefficient

$$k_d = -\frac{v}{\varepsilon L}\ln\left(\frac{C}{C_0}\right)$$

ε = porosity; 0.369
L = column length; 15 cm
v = spec. discharge; 0.31cm/min
C/Co = breakthrough conc.



What explains the attachment difference?

- xDLVO theory: (Extended Dejerguin Landau Verwey Overbeek theory)

Net interaction force is based on 3 factors

$$\Phi^{Total} = \Phi^{LW} + \Phi^{EDL} + \Phi^{AB}$$

Φ^{LW} = Van der Waals attractive forces

Φ^{EDL}= Electrostatic Double Layer

Φ^{AB} = Hydrophobicity

These forces change with separation distance

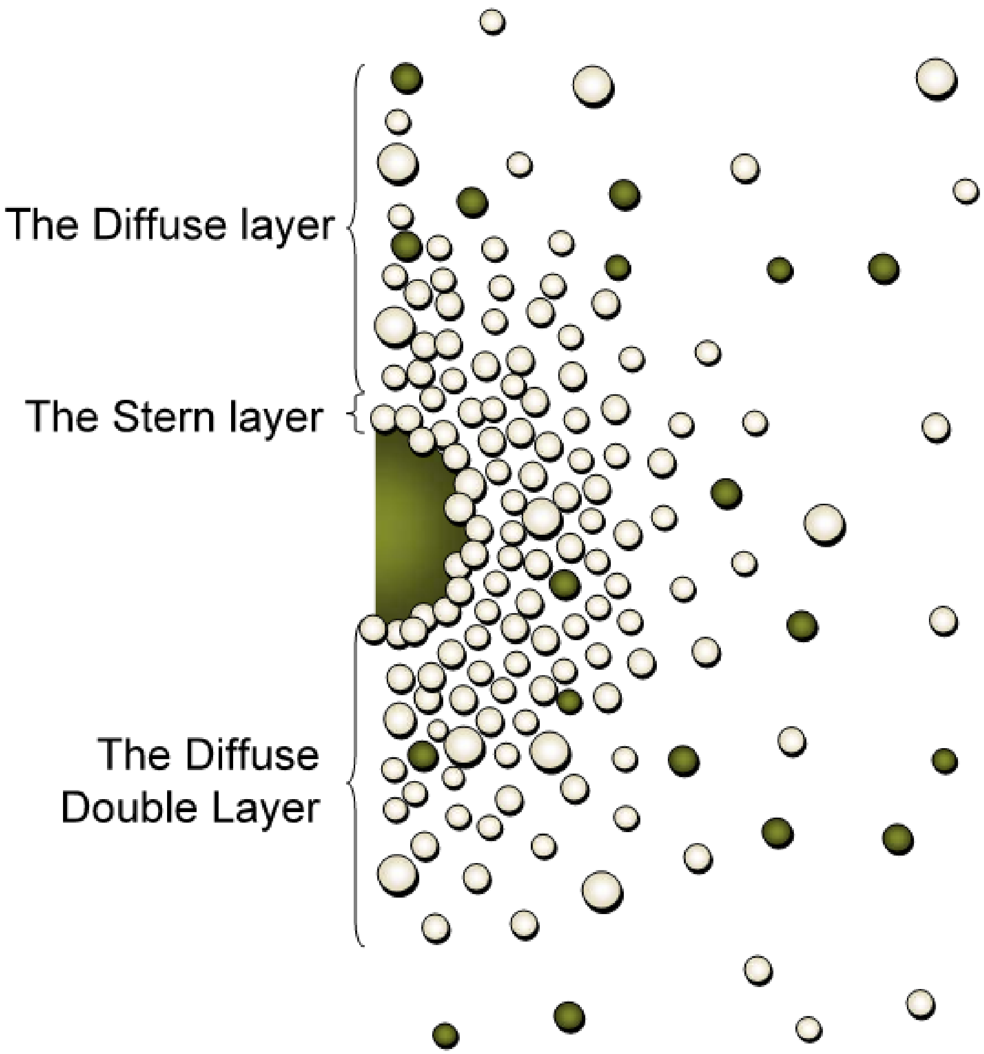
Van der Waals forces (attractive)

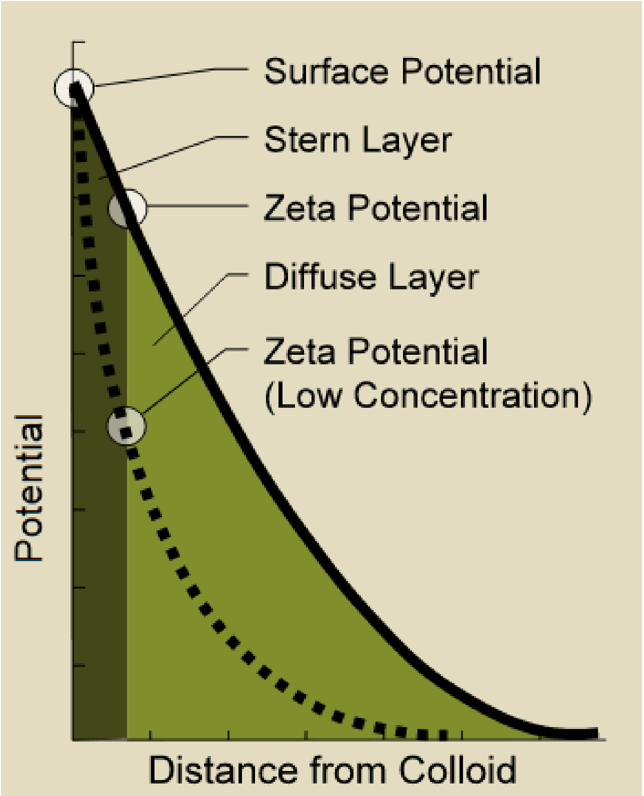
- London dispersion forces (induced attractions)
- Dipole-dipole attractions (permanent attractions)

$$\Phi^{\mathrm{LW}} = \frac{-A a_b}{6h}$$

- *A* = Hamaker constant
  - **f**(interfacial tension parameters, water, sand, *bacteria*) **[contact angle]**
- a_b = ***bacterial radius***
- *h* = bacterium-sand separation **distance (vary to create graph)**

The Diffuse Double Layer





Hydrophobicity

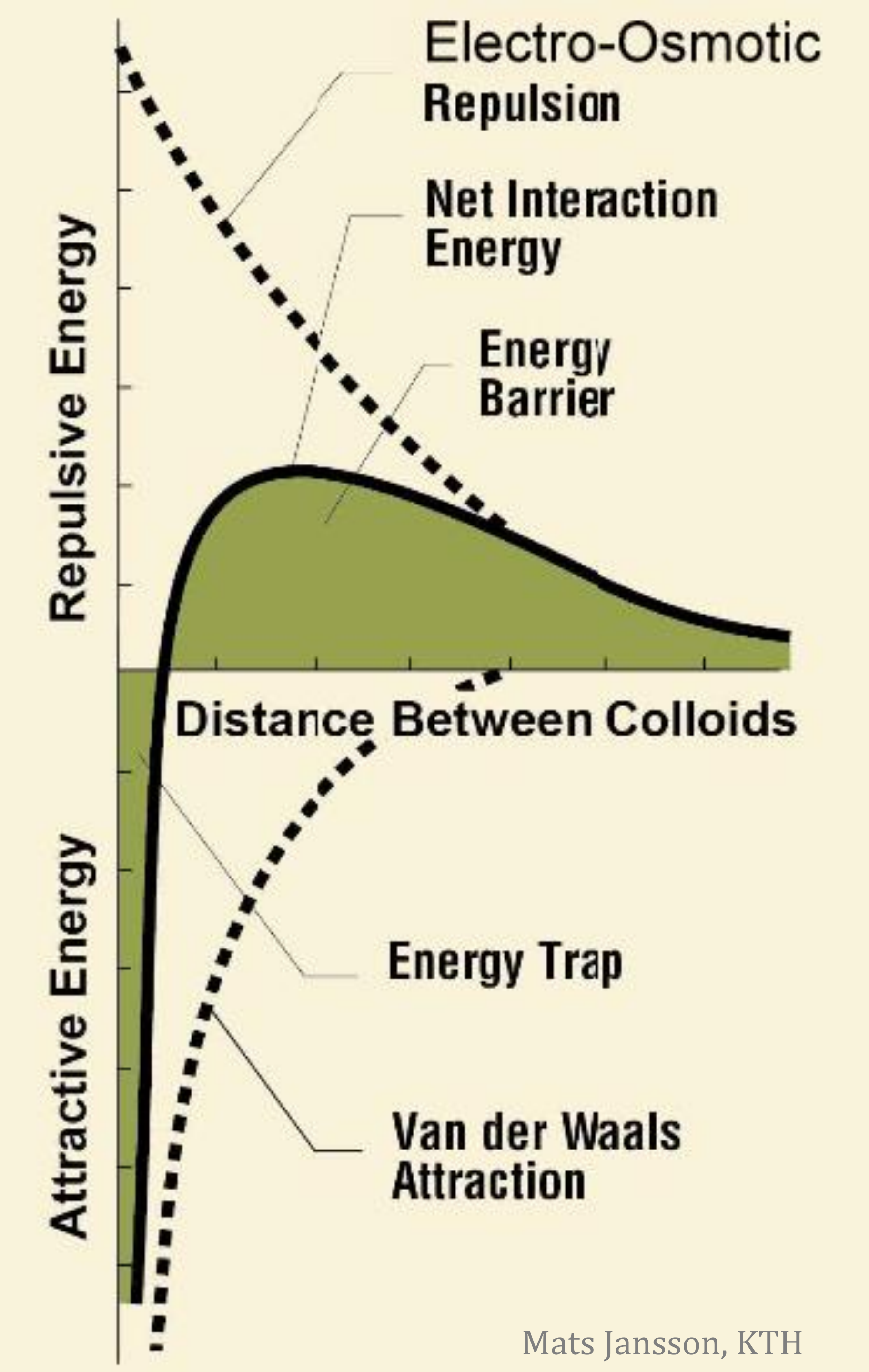
– Lewis acid base parameters;

– the x in xDLVO

- f(radius, hydrophobicity interaction free energy)

$$\Phi^{AB} = 2\pi a_b \lambda_w \Delta G_{h_0}^{AB} \exp\left(\frac{h_0 - h}{\lambda_w}\right)$$

(G measured with interfacial tension parameters from contact angle)



DLVO: EDL and LW forces

- Solve for each force at different distances,
- combine to get overall force
- to calculate forces, characterize cells
  - Radius
  - Zeta potential
  - Contact angle

Cell Characterization

Cell Size: (measured from calibrated photo)

E. Coli about 0.5 μm larger than *B. fragilis*

E. coli : 1.94(±0.25) μm

B. fragilis: 1.44(±0.17) μm

Increases all 3 forces

$$\Phi^{\mathrm{EDL}} = \pi\varepsilon_0\varepsilon_w a_b \left\{ 2\psi_b\psi_s \ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + \left(\psi_b^2+\psi_s^2\right)\ln\left[1-\exp(-2\kappa h)\right] \right\}$$

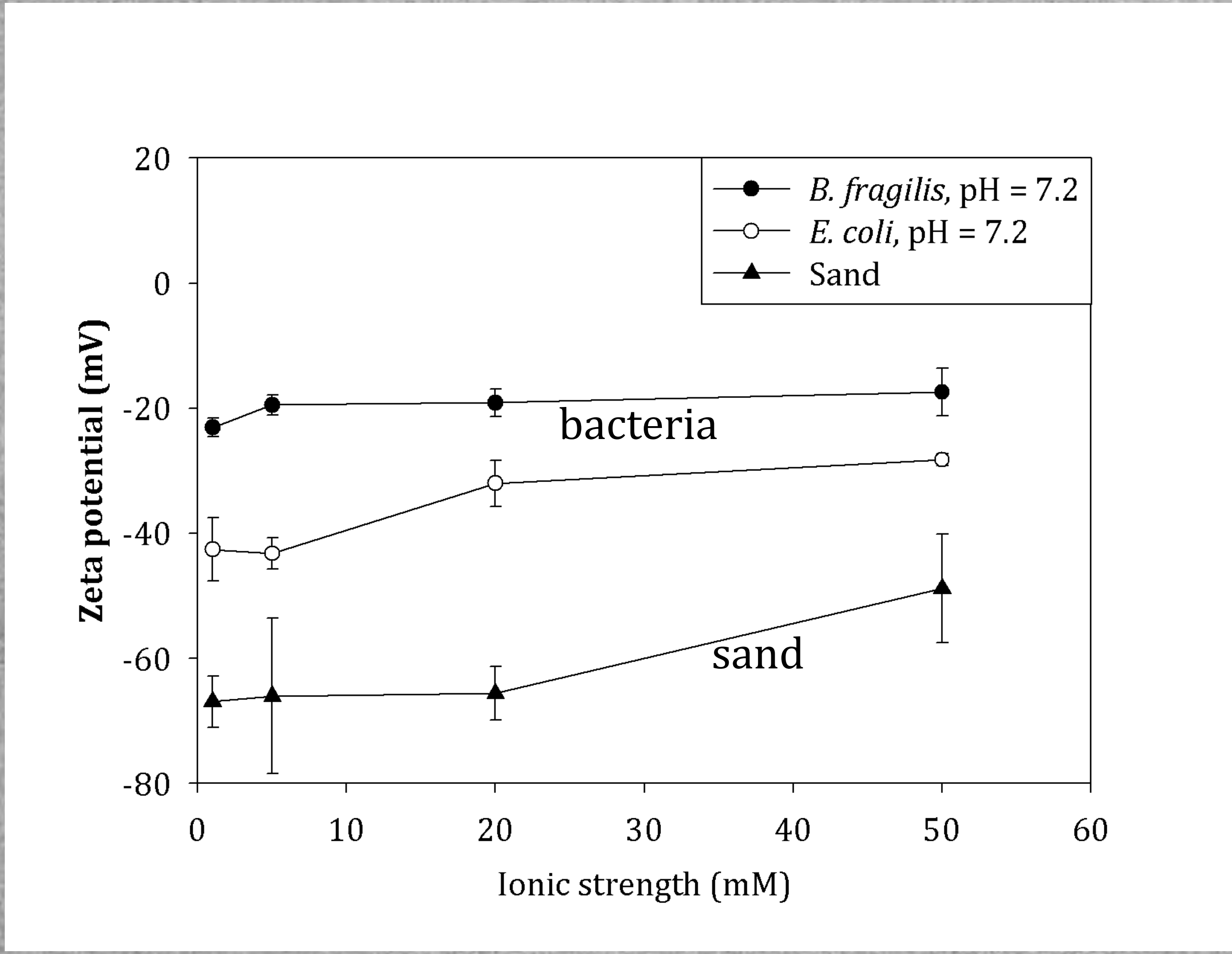
$$\Phi^{\mathrm{LW}} = -\frac{A a_b}{6h}$$

$$\Phi^{\mathrm{AB}} = 2\pi a_b \lambda_w \Delta G_{h_0}^{AB} \exp\left(\frac{h_0-h}{\lambda_w}\right)$$

Cell Characterization

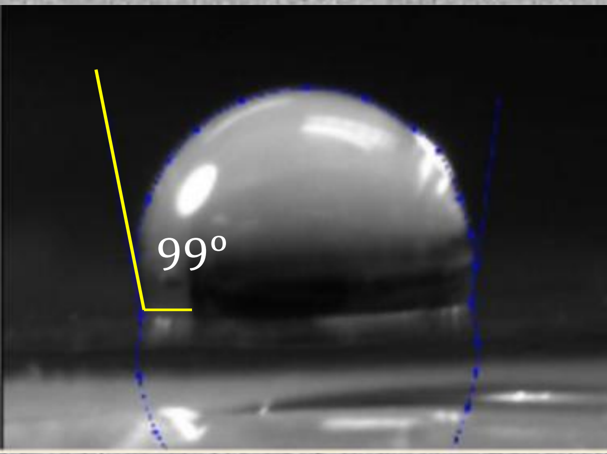
Zeta Potential: used for surface potential

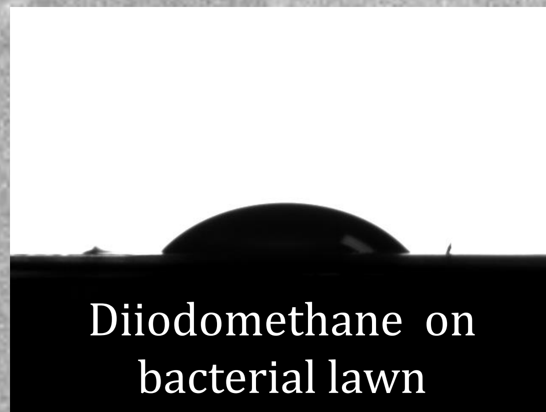
- Both sand and bacteria negatively charged, so EDL repulsive
- *B. fragilis* slightly less negative than *E. coli*
- At low mM (<10) no significant changes,
- Both sand and *E. coli* are less negative (lower repulsion) at higher ionic concentration

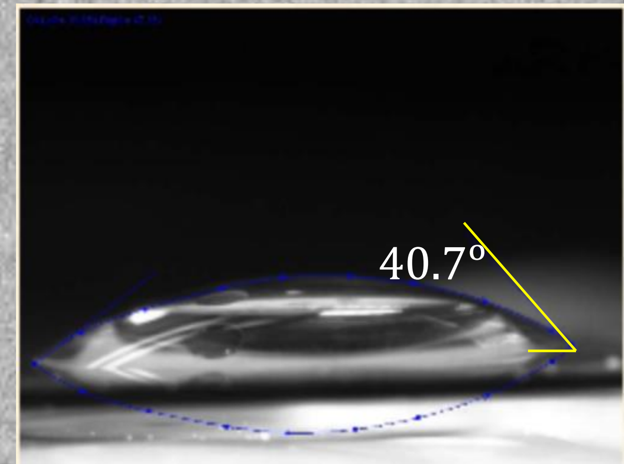


Cell Characterization

Contact angle







Properties		***E. coli K12***	***B. fragilis***
Contact angle (º) (n≥4)	Water	16.0(±3.9)	27.6(±4.1)
	Glycerol	19.4(±0.3)	34.2(±7.8)
	Diiodomethane	54.7(±5.2)	59.5(±3.8)

$$\gamma_i^L(1+\cos\theta) = 2\sqrt{\gamma_i^{LW}\gamma^{LW}} + 2\sqrt{\gamma_i^+\gamma^-} + 2\sqrt{\gamma_i^-\gamma^+}$$

Solve for the values of γ^{LW} γ^+ γ^- (electron accepting, donating)

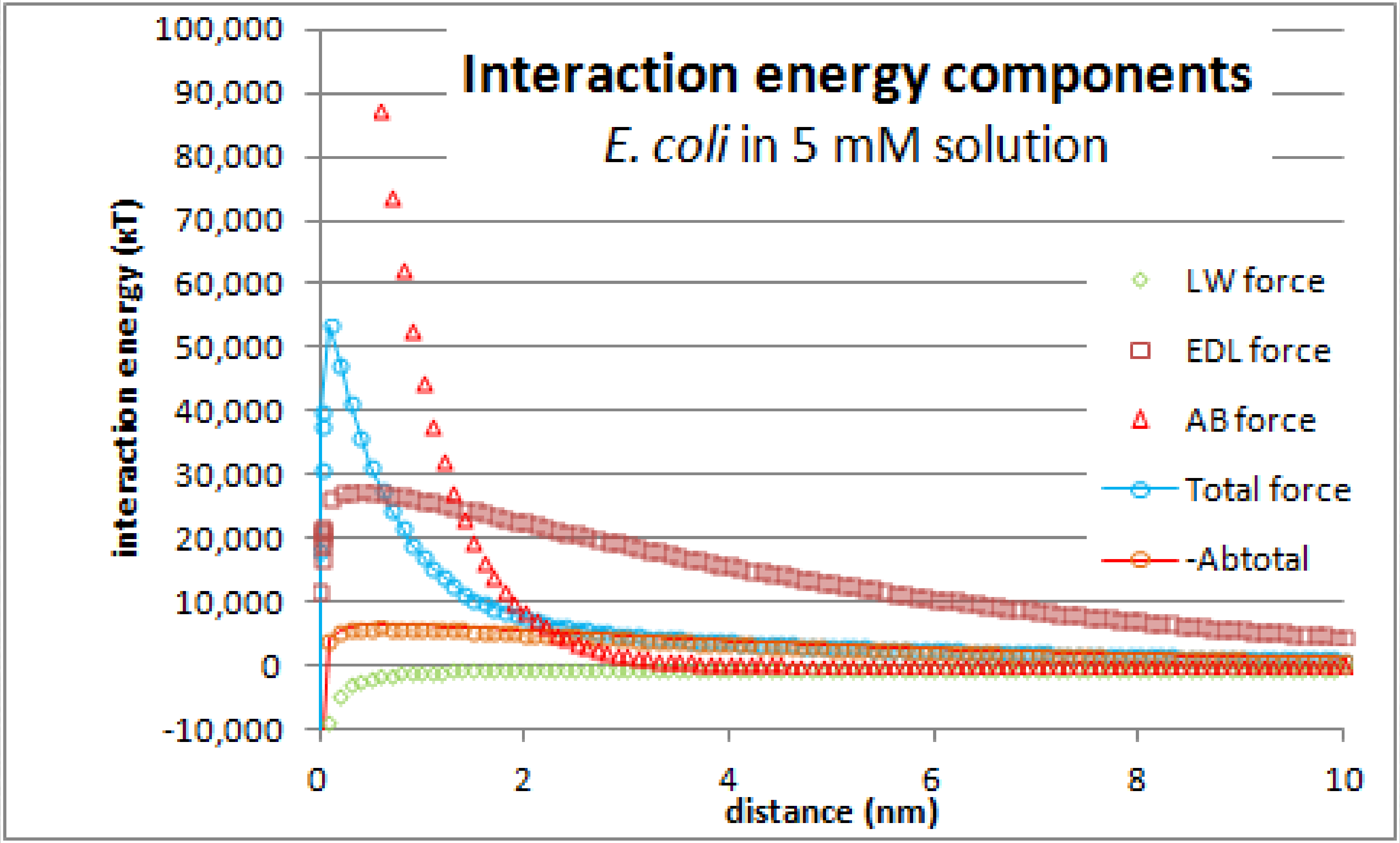
Cell Characterization:
calculated results

Properties		*E. coli*	*B. fragilis*
Surface tension components (mJ/m^2)	γ^{LW}	31.6	28.9
	γ^{+} electron accepting	4.37	3.24
	γ^{-}	46.9	46.6
A (10^{-21} J) from vdW Φ^{LW}		2.83	2.09
$\Delta G_{h_0}^{AB}$(mJ/m^2) Φ^{AB} **repulsive*		24.9*	25.8**
ΔG_{iwi} (mJ/m^2) **hydrophilic*		19.5*	22.1**

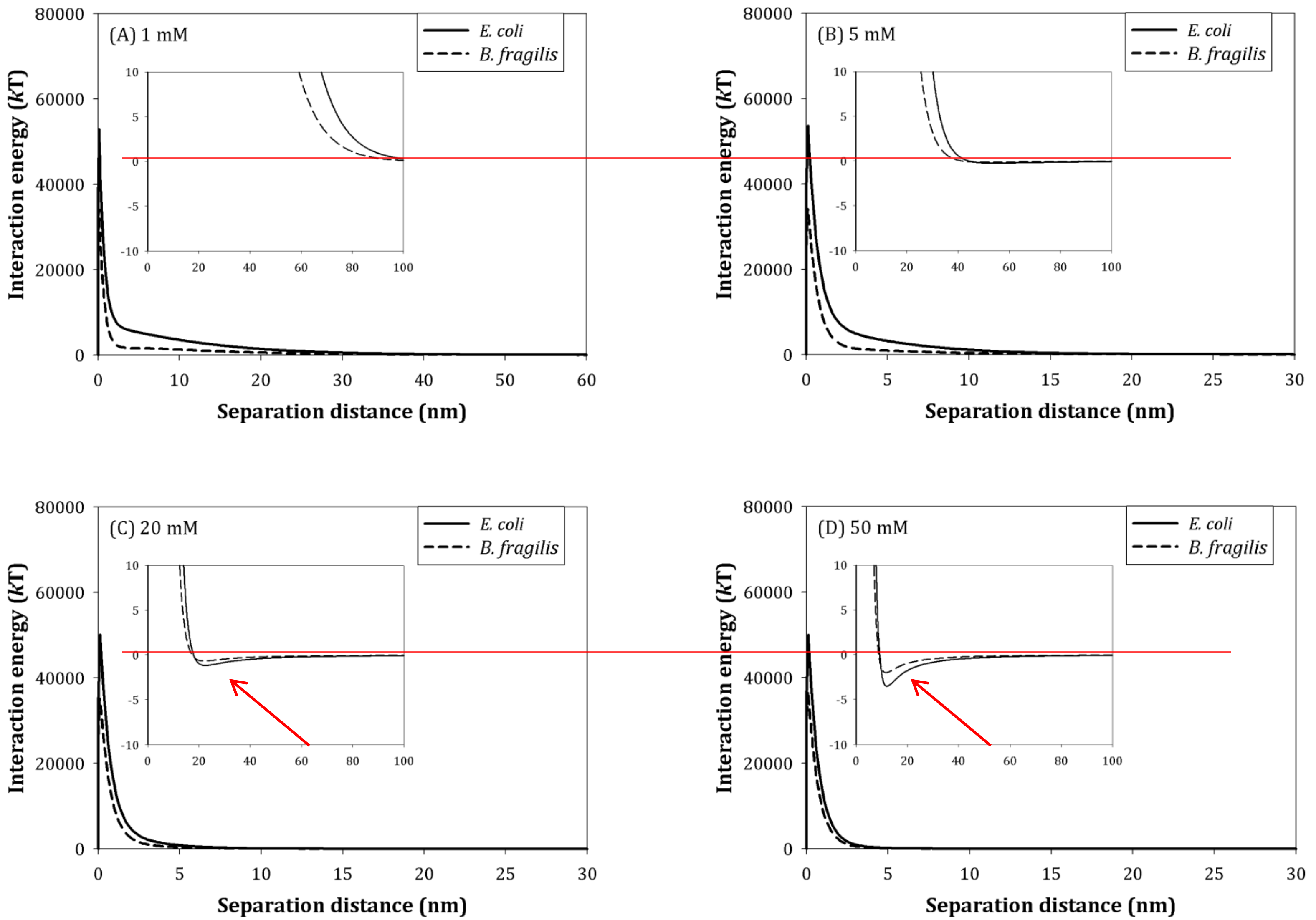
$$\Phi^{Total} = \Phi^{LW} + \Phi^{EDL} + \Phi^{AB}$$

Extended DLVO calculation

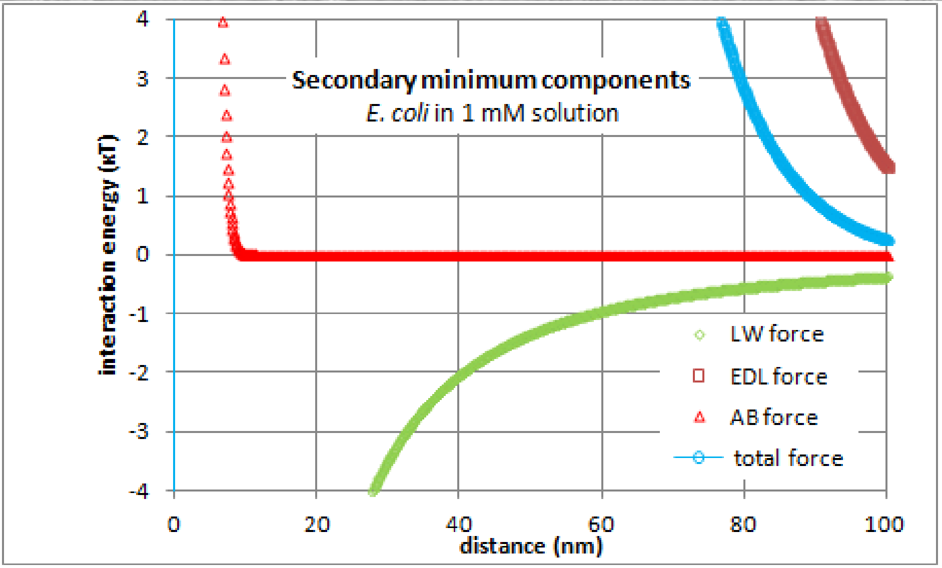
$$\Phi^{Total} = \Phi^{LW} + \Phi^{EDL} + \Phi^{AB}$$

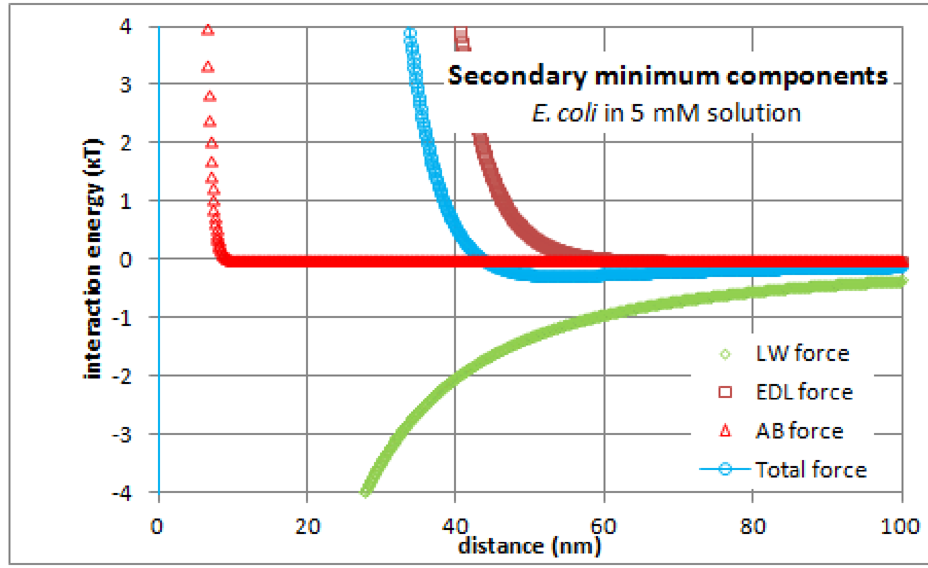


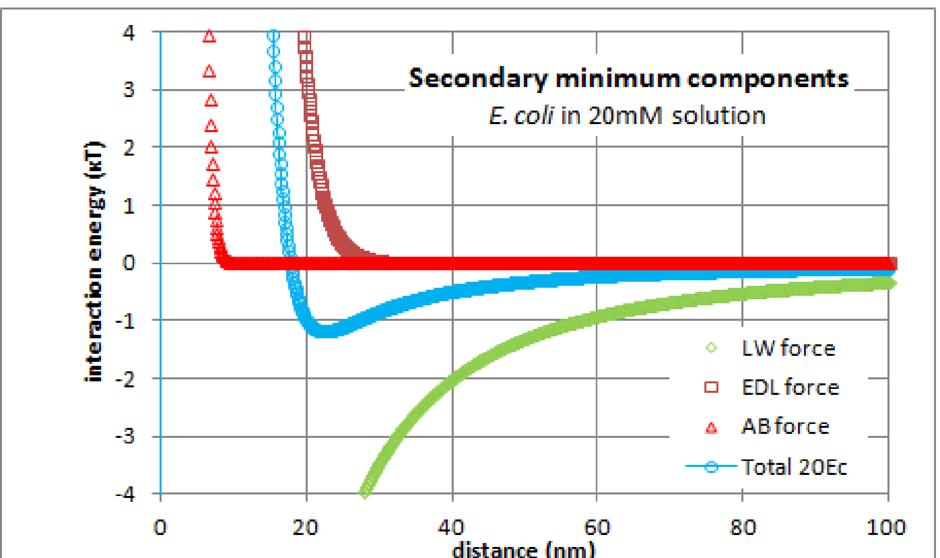
Extended DLVO calculation

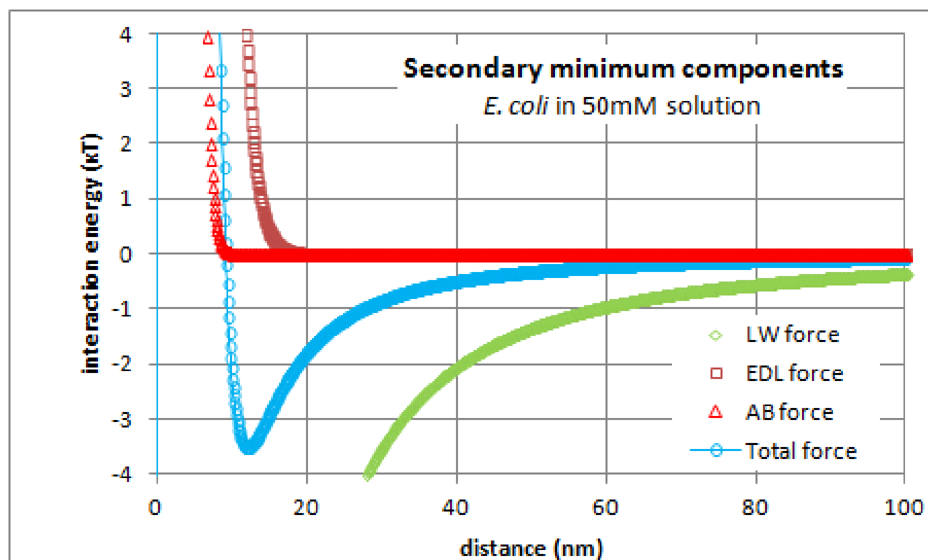


Extended DLVO secondary minimum





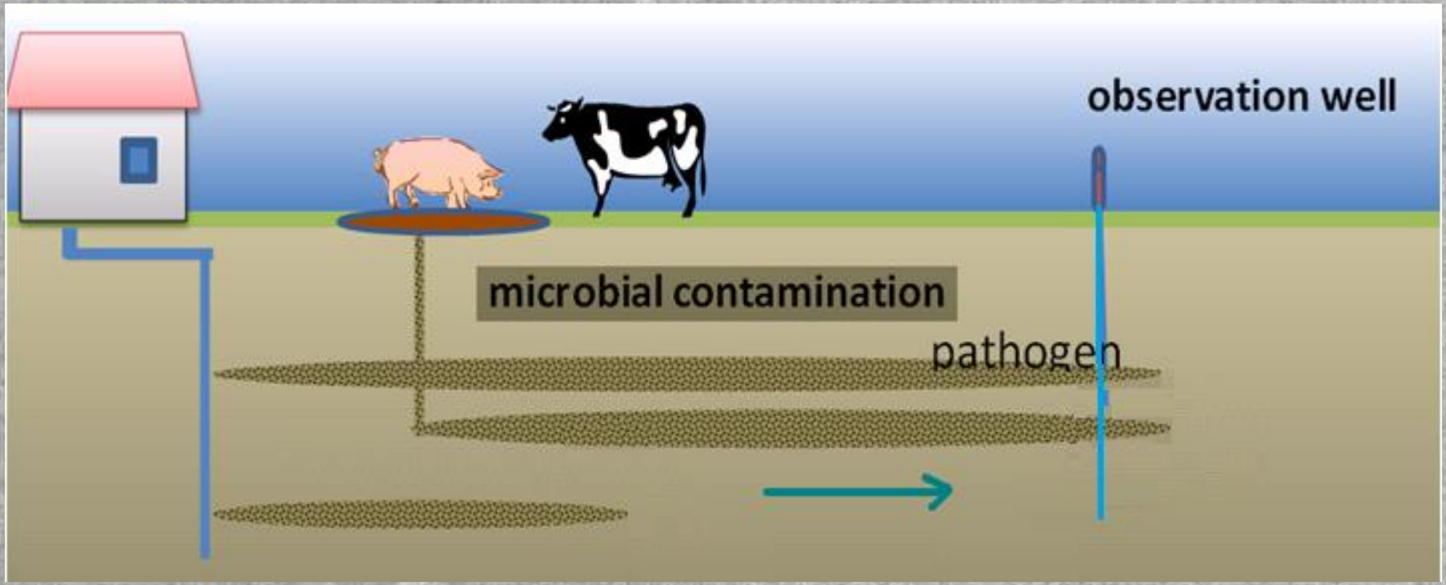




Conclusions

1. *E. coli K12* attaches to quartz sands at a higher rate than *B. fragilis*, therefore *B. fragilis* has greater mobility, but only in higher ionic strength solutions
2. Differences in attachment is explained by differences in the depth of the secondary energy minimum using the XDLVO theory.

Environmental Implications



1. In groundwater near-source (higher ionic strength) *E. coli* may have lower mobility within sand and gravel aquifers.

2. The greater mobility of *B. fragilis* in high ionic strength, combined with the potential for microbial source tracking increases its effectiveness as a groundwater indicator.

Acknowledgements

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American Water Resources Association - Wisconsin Section