Influence of Enterococcal Surface Protein (*esp*) on the Transport of *Enterococcus faecium* within Saturated Quartz Sands

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Contaminated GW

- 44% of US population uses GW as primary drinking water source
- Most gw has little to no treatment
- 52% of waterborne disease outbreaks (1971-2006) resulted from GW
- Majority of GW related disease is from microbial contamination
- Fecal matter is the major cause of microbial contamination

Outbreaks of Waterborne Diseases

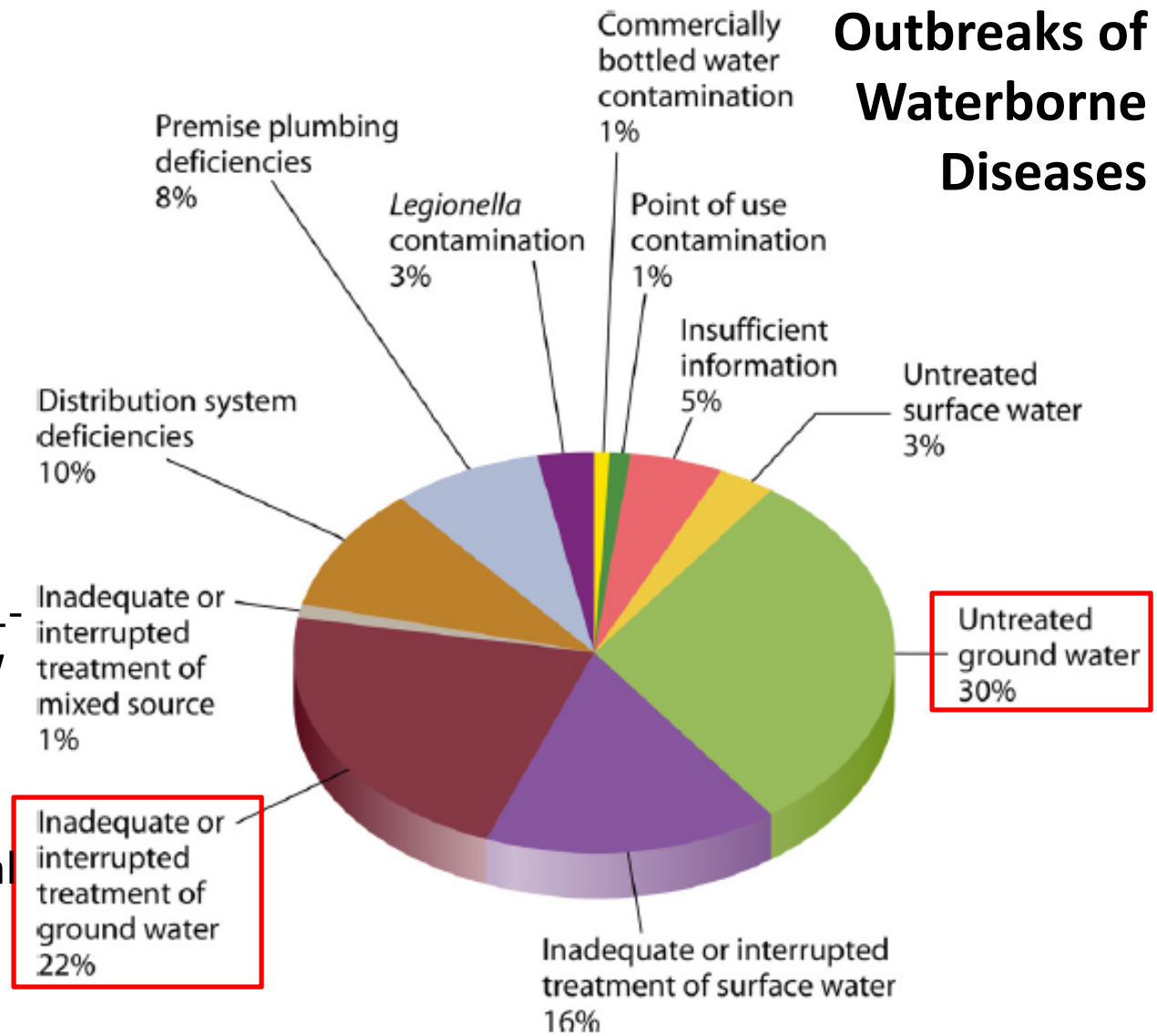
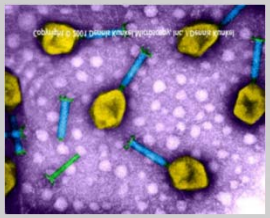


FIG. 5. Percentages of deficiencies (n = 801) in 780 outbreaks associated with drinking water, 1971 to 2006. The untreated groundwater deficiency category includes one outbreak involving treated groundwater where the treatment provided was not expected to remove the chemical contaminants.

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

Common *indicator* microorganisms
for *fecal contamination*

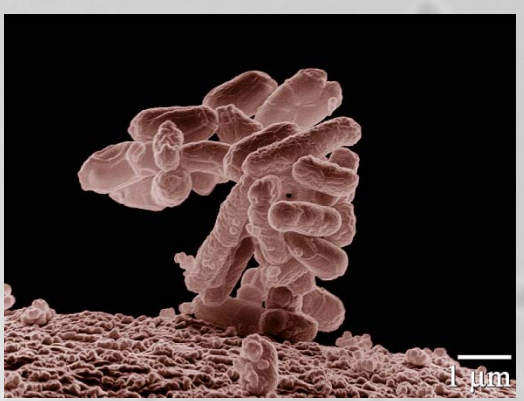
(not necessarily pathogenic, but are plentiful, and detection is cheap, easy, reliable)



-*Escherichia coli*, (E. coli)

coliphage (E. coli-eating virus)

Enterococci (gram positive)



An Aggregation of *E. coli*
Photo from a Public Domain,
Retrieved from Wikimedia Commons

- Not much studied in gw, but sw indicator since 2004
- Possible human pathogen
- Enteroccocal surface protein (esp) may enhance formation of biofilms, facilitating cell attachment to aboitic surfaces

Source Tracking Using *esp*?

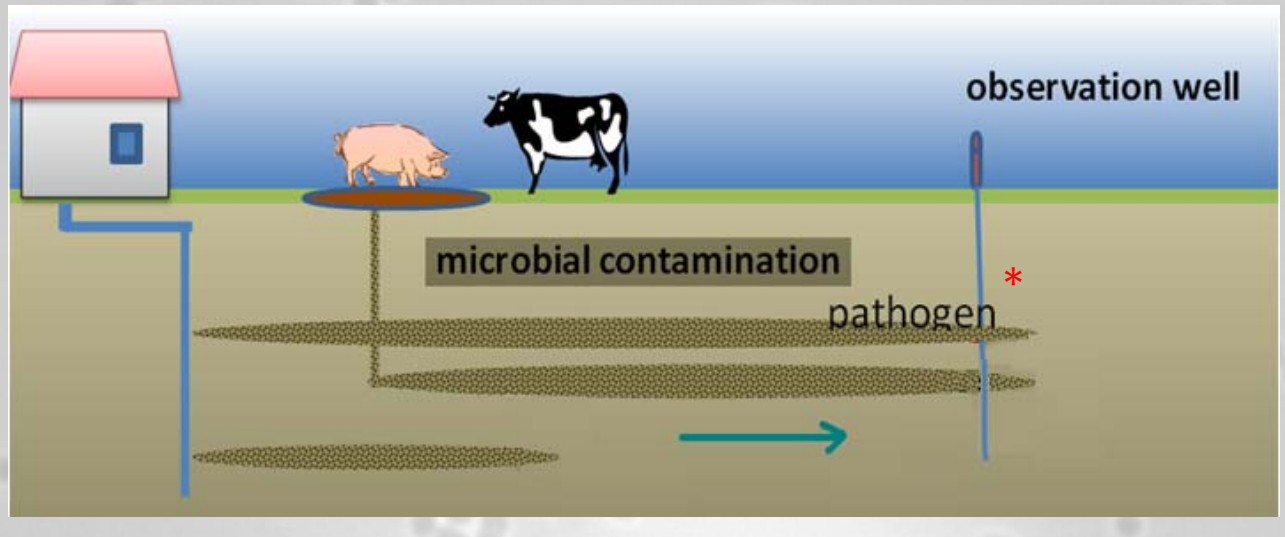
TABLE 1. Detection of the *esp* Gene Sequence in Composite DNA Samples Extracted from Enterococci Isolated from Domestic Sewage, Birds, and Livestock

	host/source	sample type	no. of samples analyzed	PCR results positive	PCR results negative
Human sources	human/sewage	primary influent	40	40	0
	human/sewage	secondary effluent	10	10	0
	human/sewage	filter effluent	5	5	0
	human	septic tank	10	8	2[a]
Animal sources	poultry	feces/lagoon	6	0	6
	swine	feces/lagoon	9	0	9
	dairy cattle	feces/lagoon	28	0	28
	beef cattle	feces	4	0	4
	Canada goose	feces	12	0	12
	seagull	feces	28	0	28
	pelican	feces	7	0	7
	wild birds	feces	8	0	8
	total human		65	63[b]	2
	total animal		102	0[b]	102

[a] Total *Enterococcus* levels were $< 1 \times 10^2$ CFU and showed atypical colony morphology. [b] Statistically significantly different as shown by chi square analysis $p < 0.001$.

Source: Scott et al., 2005

Sources of Groundwater Microbial Contamination (Whose poop is it?)



Protection of public health requires:

1. Fast, reliable detection of groundwater microbial contamination;
2. Identification of the contamination sources.

A poorly managed offal hole can cause groundwater contamination

Waikato Regional Council, New Zealand

Research Objectives

1. Will *esp* affect the transport of *E. faecium* within quartz sands?

- Wild type: *E. faecium* E1162
- *esp*-negative mutant: *E. faecium* E1162Δesp

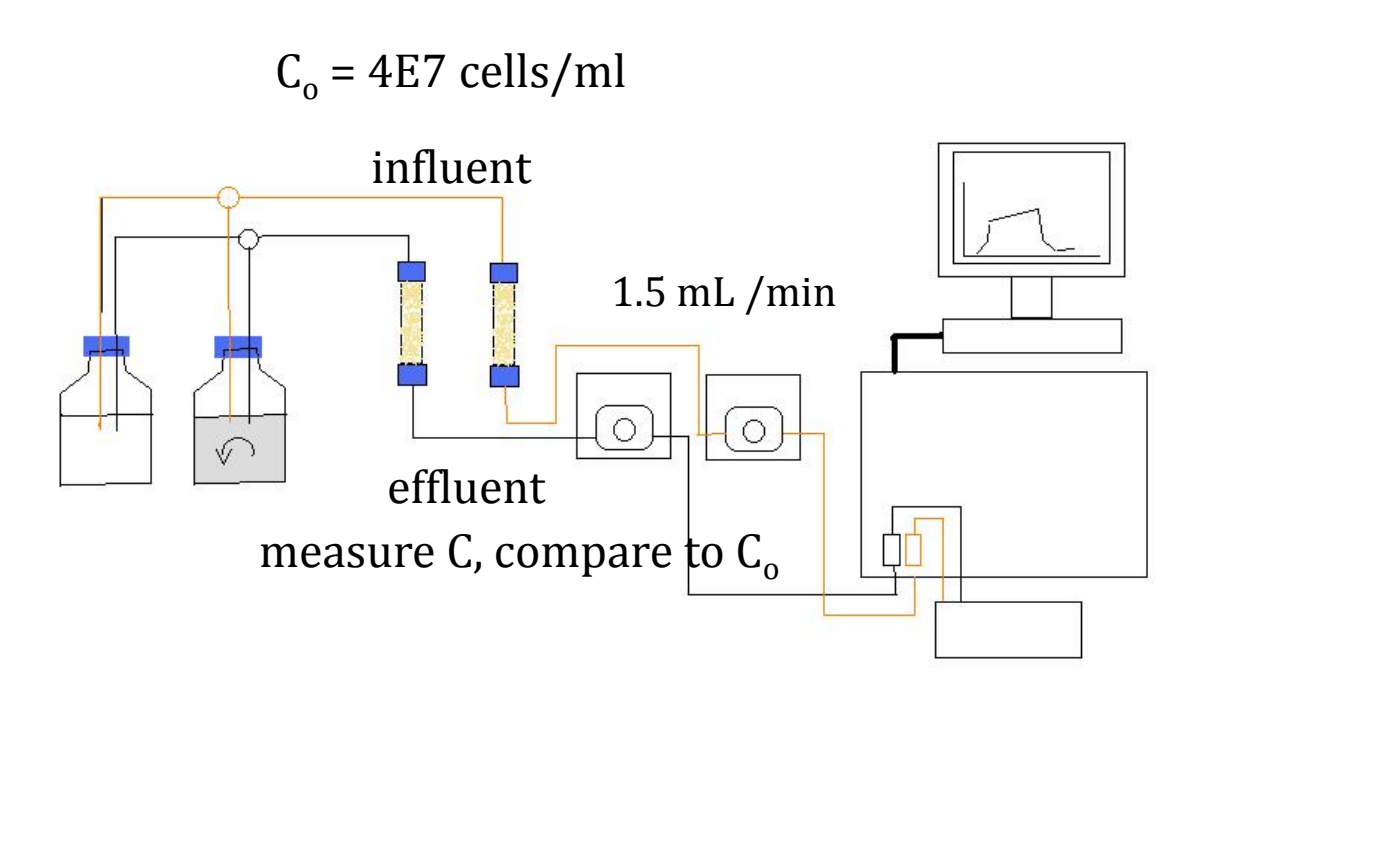
2. If the answer to question (1) is Yes, what are the underlying mechanisms?

<u>Strains (provided by Dr. Rob J. L. Willems and Dr. Toni L. Poole):</u>

Methods

Assessment of transport behavior

Laboratory column transport experiments performed using quartz sands.

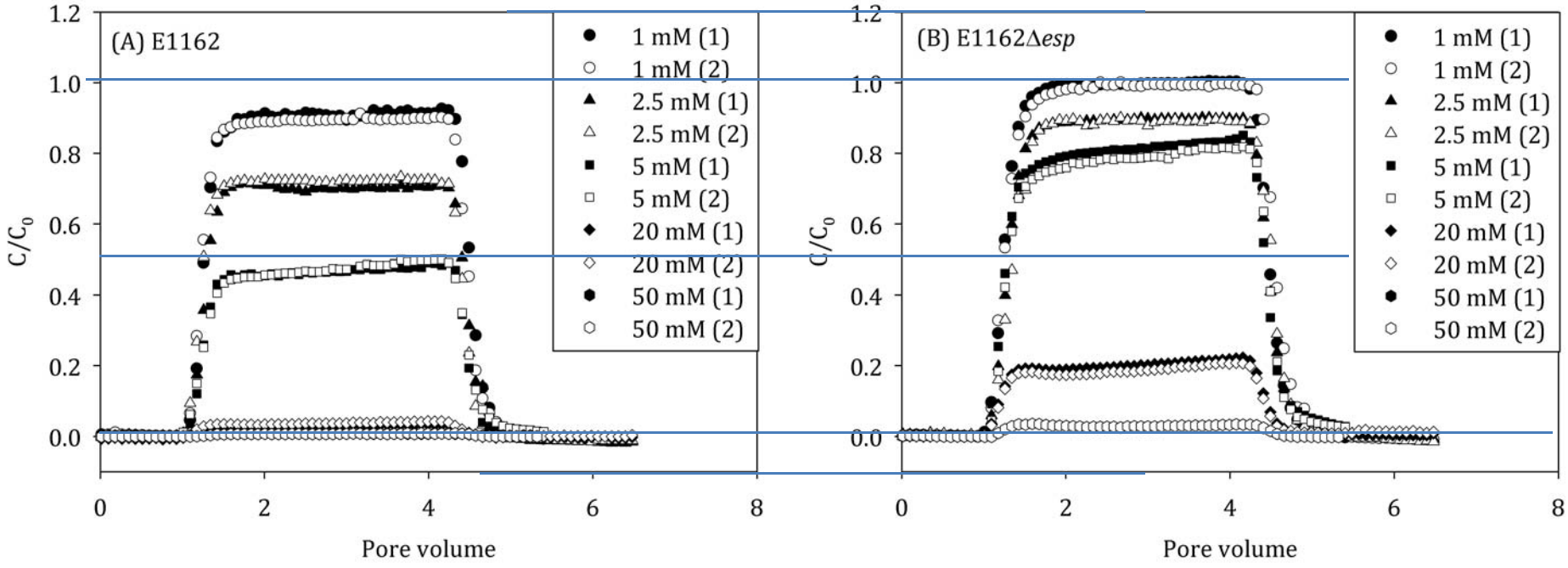


1, 2.5, 5, 20, 50 mM ionic strength solutions, NaCl buffered with $NaHCO_3$ to pH 7.2

Bacteria Transport Behavior

With *esp* (human)

Without *esp* (non-human)



Why is the bacteria with esp more attracted to the sand?

- xDLVO says the interaction is based on 3 factors

(Extended Dejerguin Landau Verwey Overbeek theory)

Φ^{LW} – Van der Waals attractive forces electrostatic

f(Hamaker constant, radius, distance)

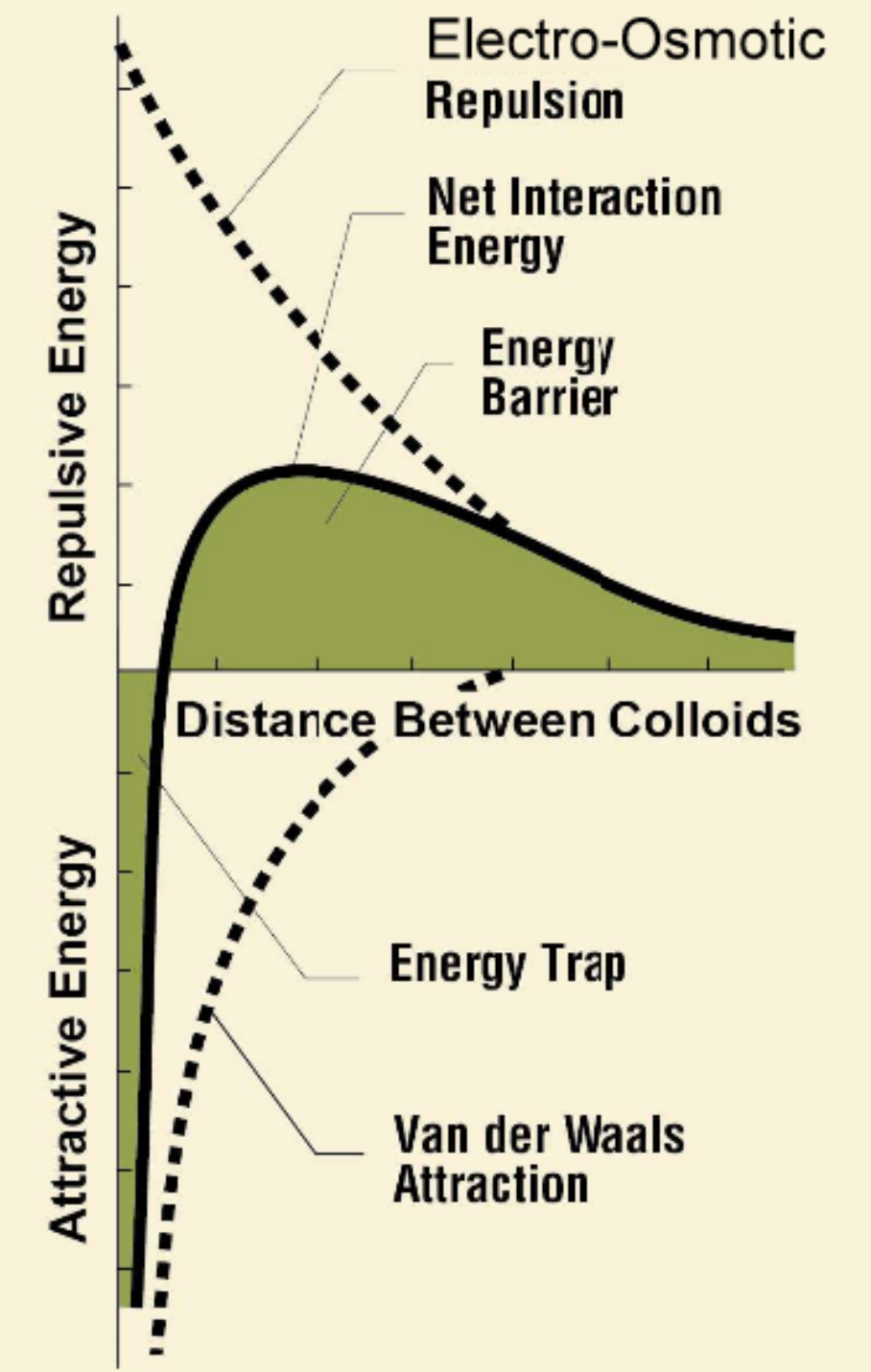
Φ^{EDL} – Electrostatic Double Layer f(dist, radius, surf potential)

Φ^{AB} – Hydrophobicity (Lewis acid base parameters; the x in xDLVO)

f(radius, hydrophobicity interaction free energy)

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

Changes with separation distance



DLVO: electrostatic forces

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

Cell Characterization

Cell Size: (measured from calibrated photo)

<u>E1162 (human)</u>: 1.29(±0.12) μm
<u>E1162Δesp</u>: 1.30(±0.12) μm

No significant difference in size due to esp

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

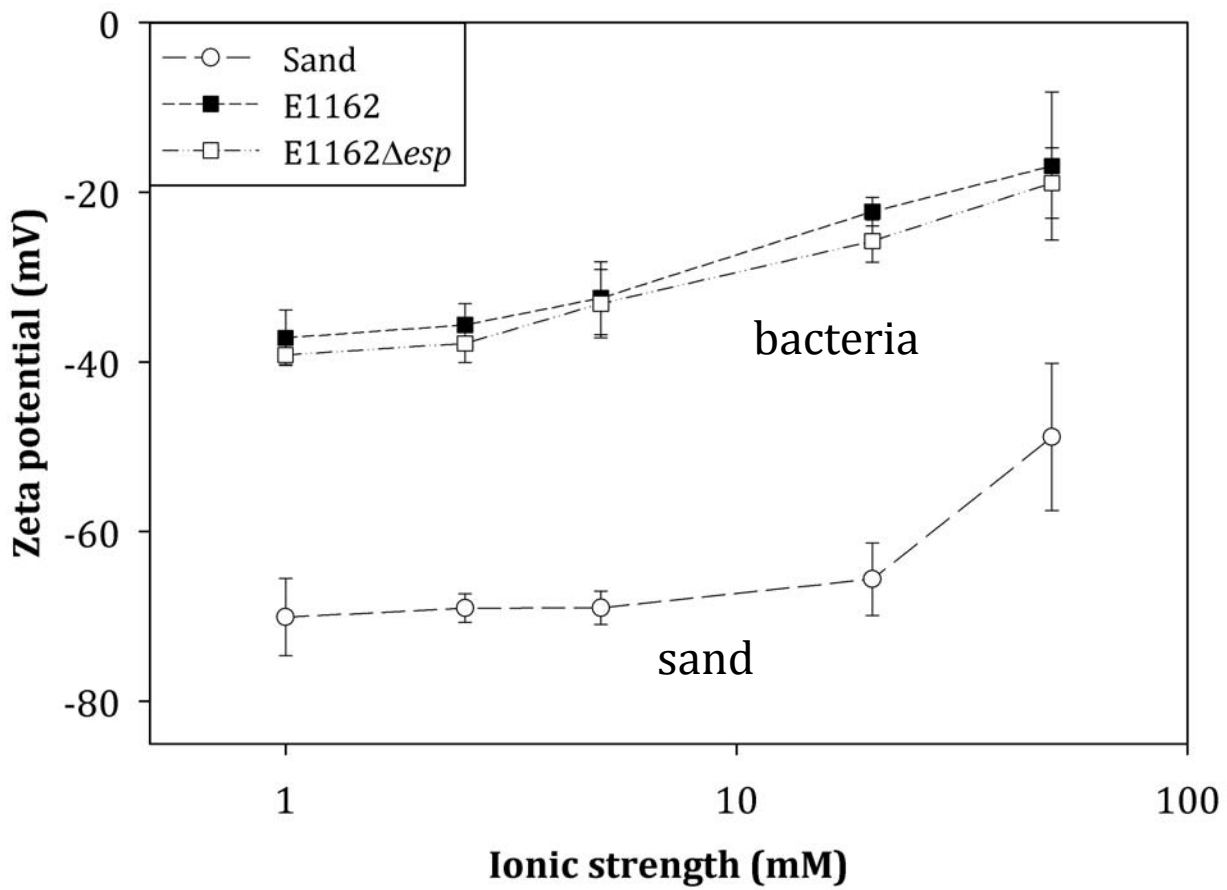
$$\Phi^{\mathrm{LW}} = -\frac{A a_b}{6h}$$

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

Cell Characterization

Zeta Potential: used for surface potential

- Both sand and bacteria negatively charged, so EDL repulsive
- Bacterial strains not significantly different
  - **[edl not the factor]**
- At low mM (<10) no significant changes, but the increase (less negative) at high mM is significant

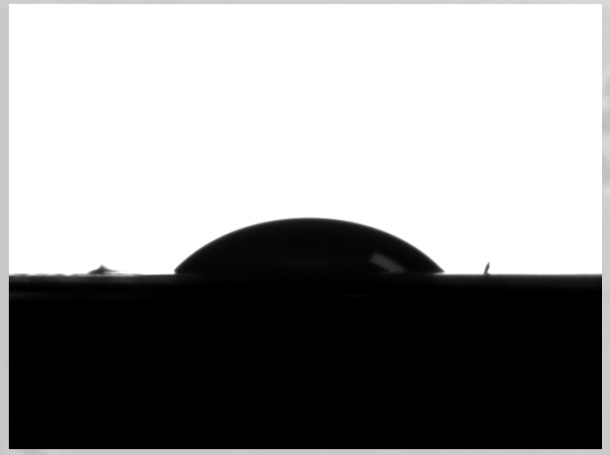


Cell Characterization

Contact angle: Water, Glycerol and diiodomethane

Properties		**E1162**	**E1162Δ*esp***
Contact angle (º) (n≥4)	Water	21.2(±4.1)	16.1(±1.6)
	Glycerol	24.3(±1.6)	32.5(±4.6)
	Diiodomethane	48.2(±2.5)	44.2(±0.2)

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



Diiodomethane on E1162

Solve for the values of γ^{LW} γ^{+} γ^{-}

Used to calculate the values of
Hamaker constant (A),
hydrophobic interaction energy constant, $\Delta G_{h_0}^{AB}$
cell hydrophobicity parameter ΔG_{iwi}

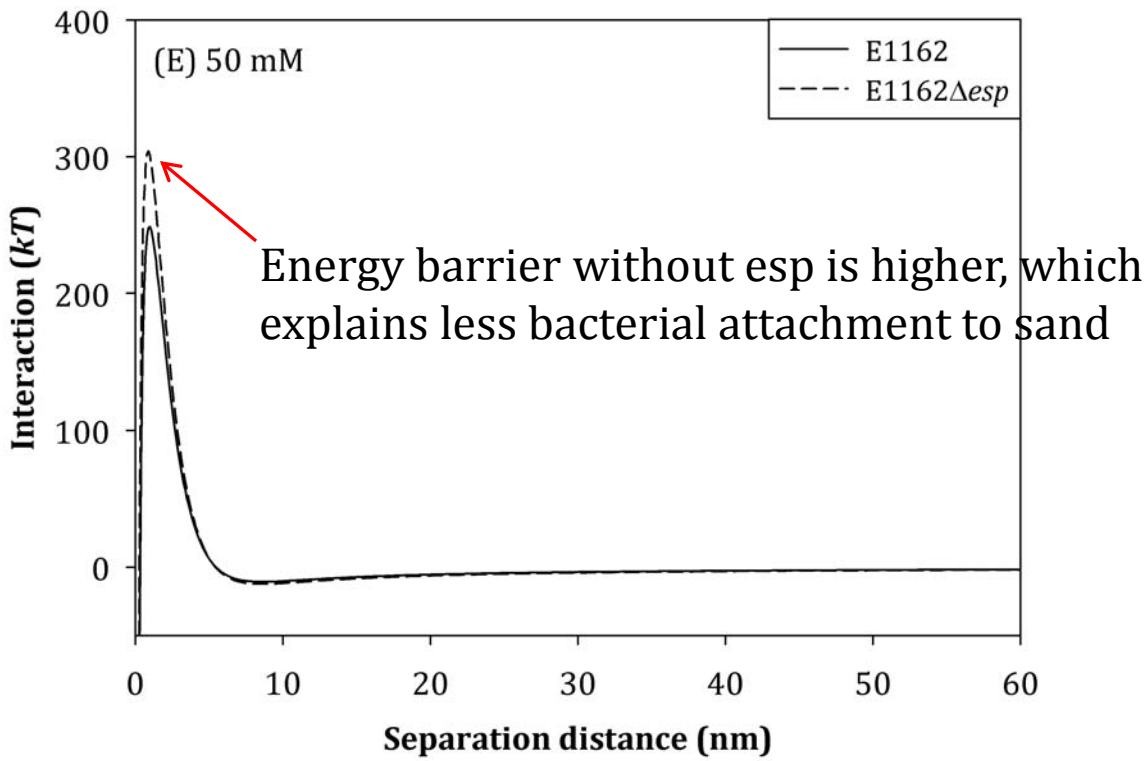
Cell Characterization:
calculated results

Properties		**E1162**	**E1162Δ*esp***
Surface tension components (mJ/m^2)	γ^{LW}	35.3	37.4
	γ^{+} electron accepting	3.1	1.4
	γ^{-}	44.7	53.5
A (10^{-21} J) from vdW Φ^{LW}		4.2	4.8
$\Delta G^{AB}_{h_0}$ (mJ/m^2) Φ^{AB}	**repulsive*	24.1*	31.4**
ΔG_{iwi} (mJ/m^2)	**hydrophylic*	18.3*	31.0**

Note: Microbial Adhesion To Hydrocarbons (MATH) test also indicated hydrophylic results

Extended DLVO calculation

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

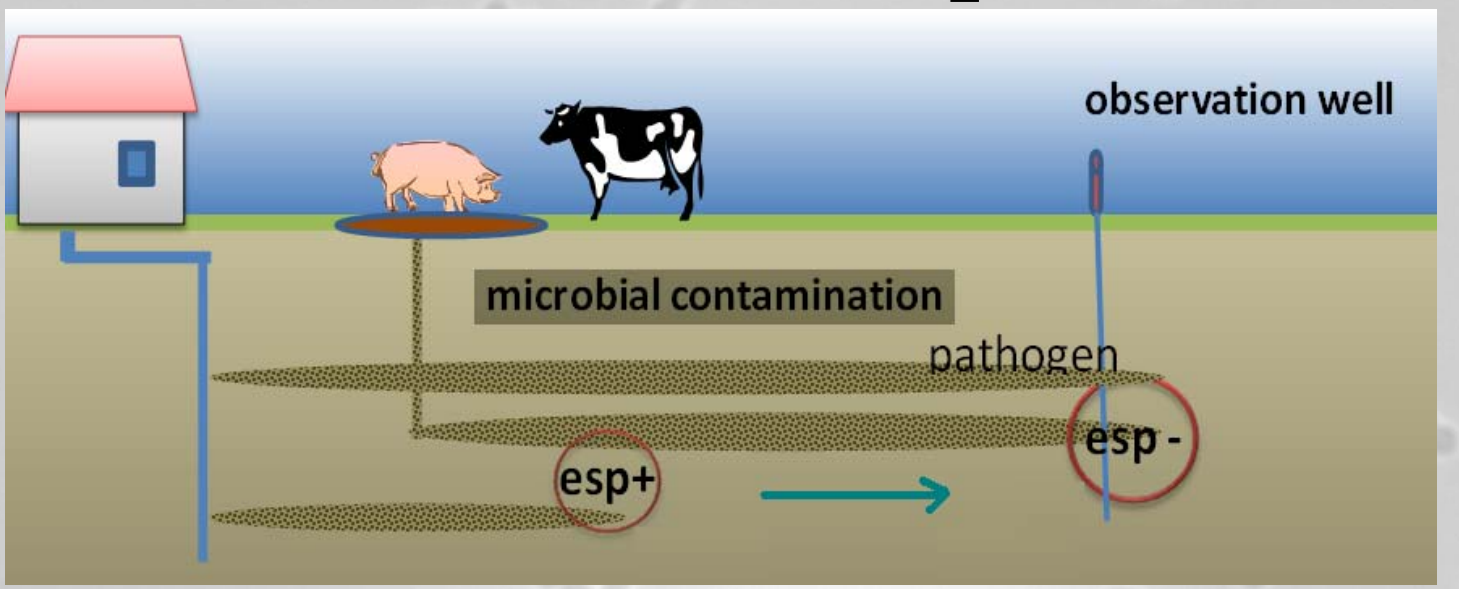


Energy barrier without esp is higher, which explains less bacterial attachment to sand

Conclusions

1. *esp* enhances the attachment of *E. faecium* to quartz sands.
2. *esp* led to alterations in cell surface properties and the effects of *esp* on the transport behavior of *E. faecium* can be explained by the XDLVO theory.

Environmental Implications



1. Sewage-derived *E. faecium* may have lower mobility within sand and gravel aquifers.

2. The effects of *esp* on the transport of *E. faecium* complicates its effectiveness as a groundwater microbial source tracking tool.

Acknowledgement

Dr. Rob J. L. Willems of University Medical Center Utrecht, Utrecht, The Netherlands.

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Lixia Wang and Dr. Jin Li of UWM.

American Water Resources Association - Wisconsin Section

Van der Waals attractive forces

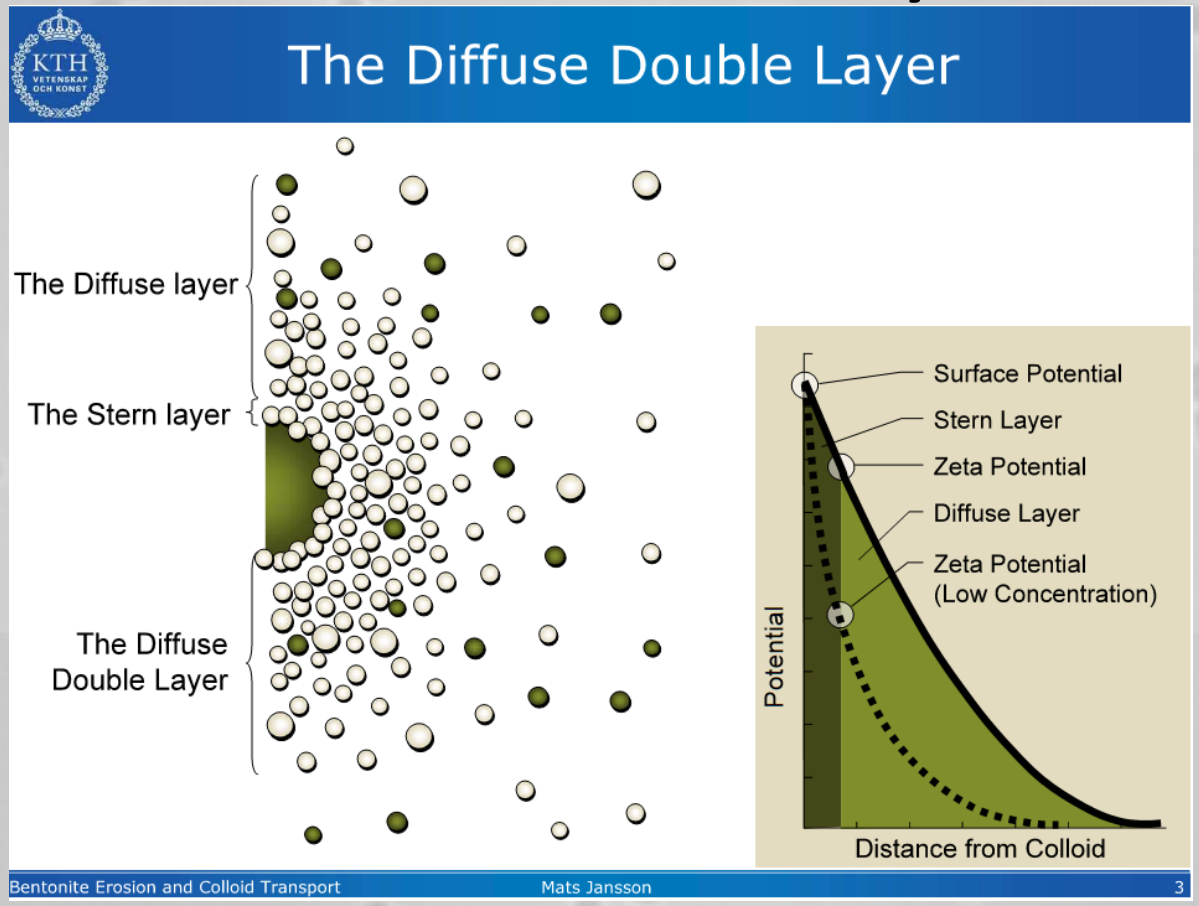
Act at close distances

- 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, *bacteria*, water, sand) [contact angle]
- a_b = *bacterial radius*
- *h* = separation distance bacterium to sand (varies to create graph)

Electrostatic Double Layer forces



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

f(radius, surface potential, Debye length [T, ionic strength, e charge])

Hydrophobicity

– Lewis acid base parameters;

– the x in xDLVO

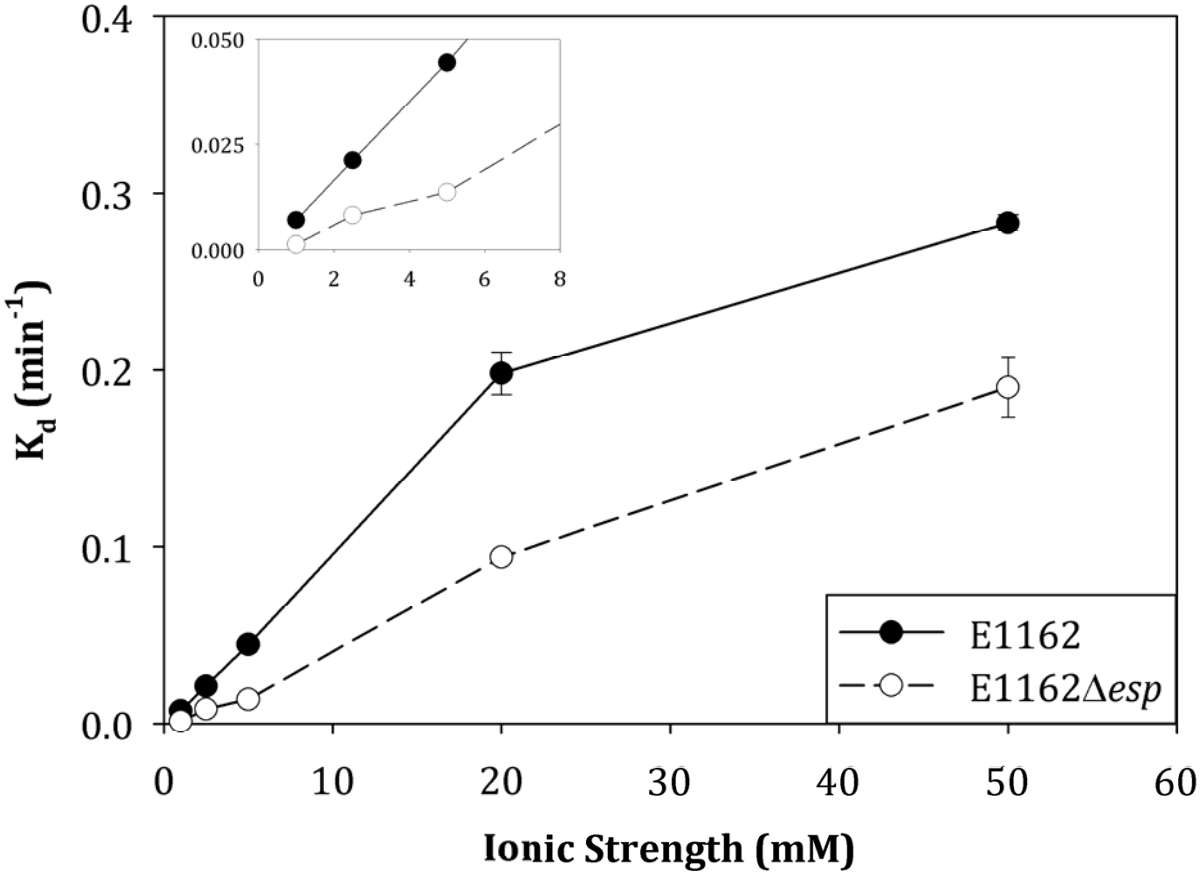
- f(radius, hydrophobicity interaction free energy)

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

» Radius, contact angle measurement

Transport Behavior

k_d First-order deposition rate coefficient



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

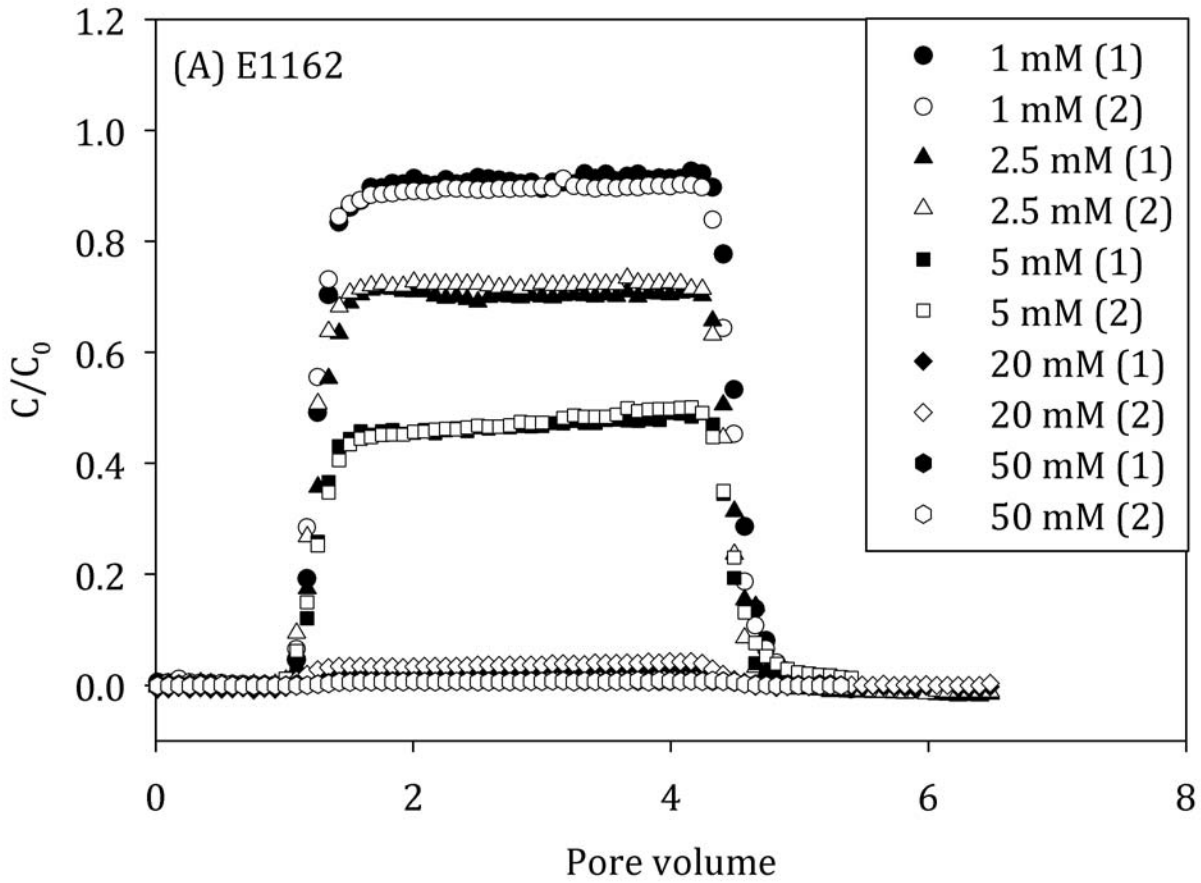
$\epsilon = porosity = 0.37$

$L = column\ length = 15\ cm$

$v = specific\ discharge = 0.31\ \frac{cm}{min}$

$\left(\frac{C}{C_o}\right) = normalized\ breakthrough\ concentration$

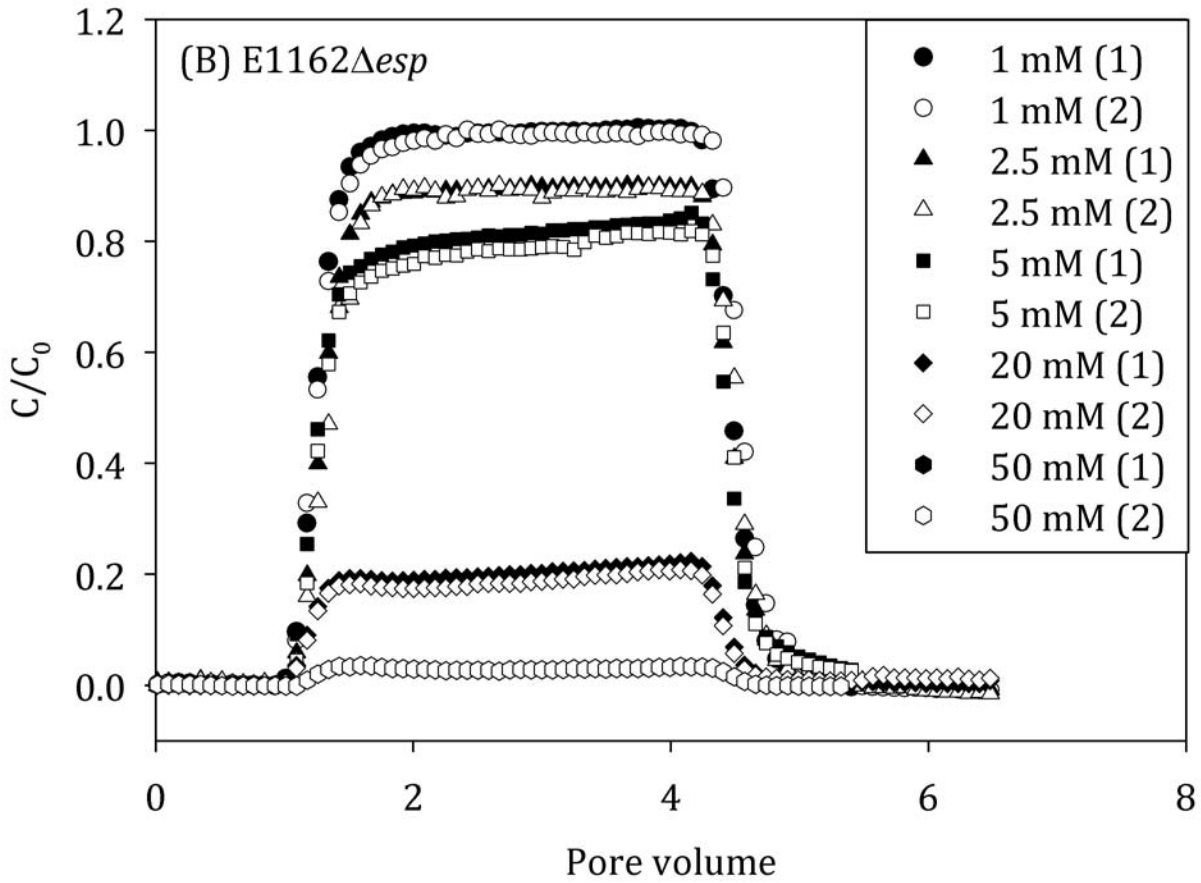
Transport Behavior with esp (human)



RECOVERY

- I mM ~ 90+%
- 2.5 mM ~ 75%
- 5 mM ~50%
- 20 and 50 mM <5%

Transport Behavior with no esp (non-human)



RECOVERY

- I mM ~ 100+%
- 2.5 mM ~ 90%
- 5 mM ~75%
- 20 mM ~20%
- 50 mM ~5%

Source Tracking Using *esp*?

source	number of samples	number of positives[b]
***esp*$_{fm}$ Gene**		
sewage influent	29	27
pit toilets	15	0
septic trucks	20	6
cats	34	0
deer	4	0
dogs	43	9
geese	18	0
gulls	34	2
mice	22	0
raccoons	23	0
songbirds	55	0

Source: Whitman et al., 2007