Transport and Survival of Pathogenic Bacteria Associated with Dairy Manure Soil and Groundwater

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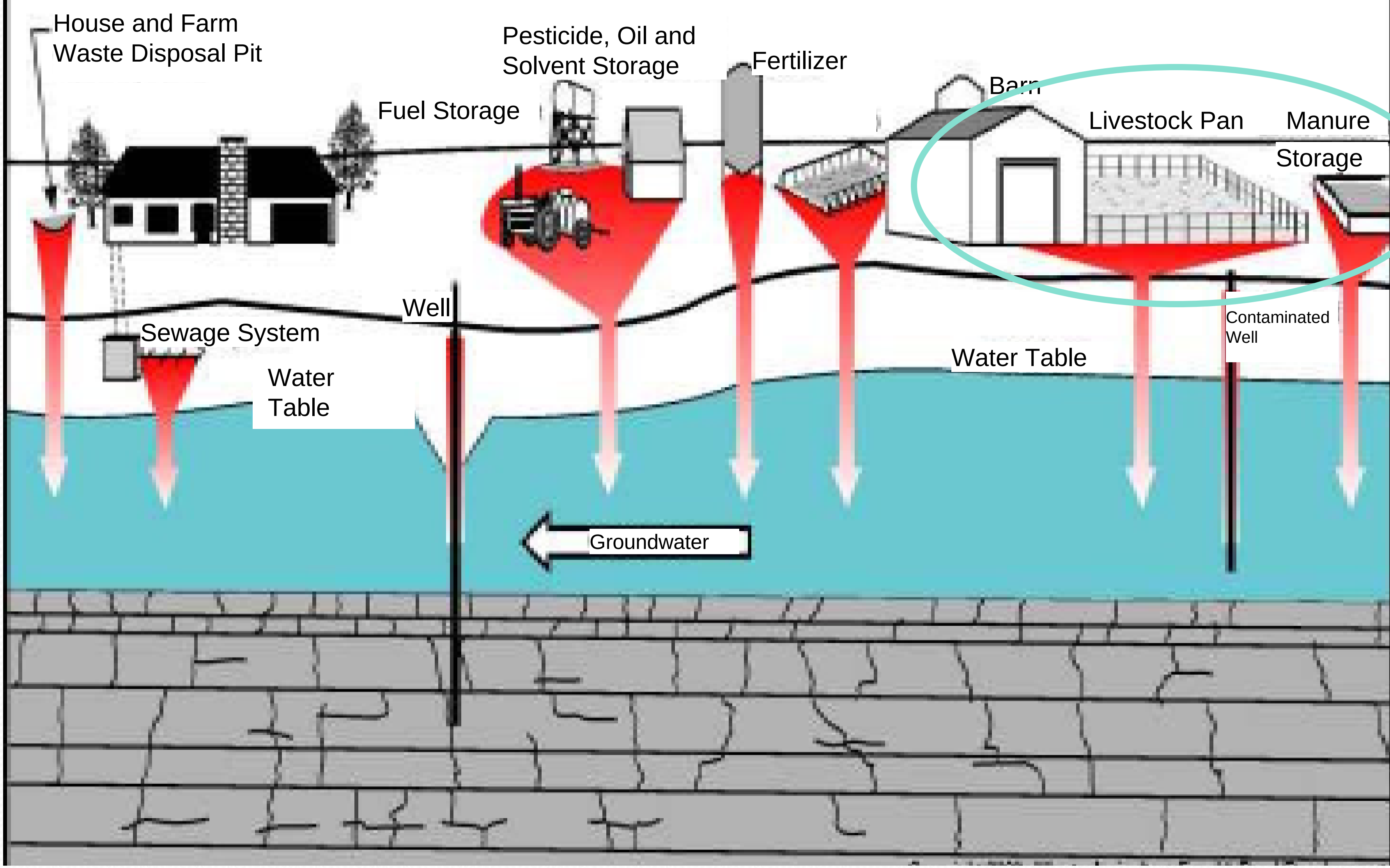
Background

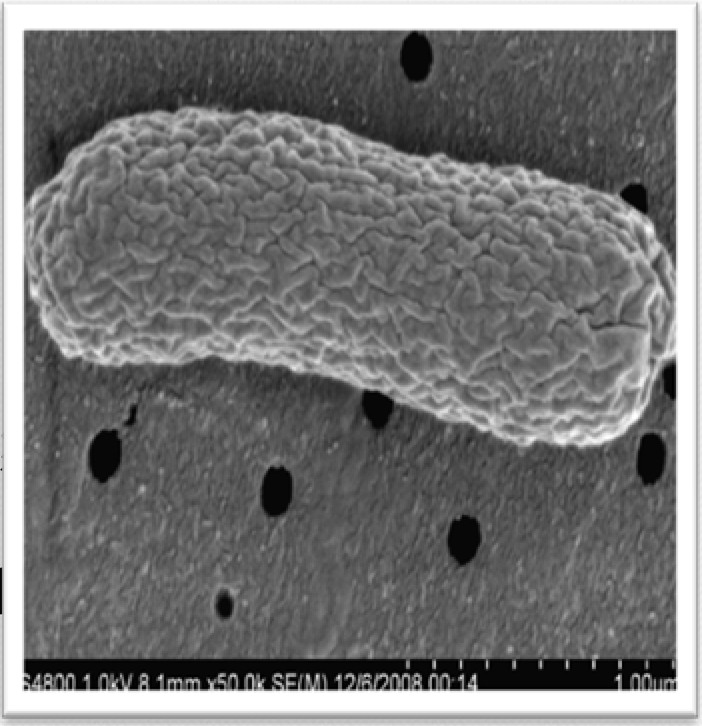
- With over 15,000 dairy farms and 1.2 million cows, the dairy industry is of enormous historical, economical and cultural importance to the State of Wisconsin.
- 48 million tons of manure is produced in Wisconsin annually and nearly two-thirds of all farms spread manure immediately on the surrounding fields because of inadequate storage.
- Concerns were raised over the release of human pathogens into our water and food supply due to the land application of manures for crop fertilization.

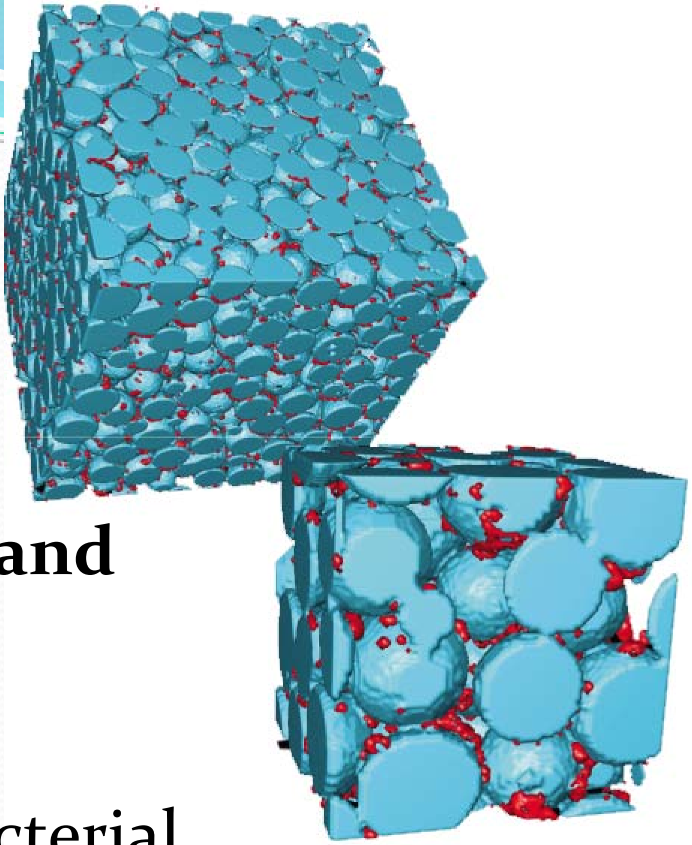
Potential Rural Contamination Sources



Pathogens Associated with Manure

- *E. coli* O157:H7 causes approximately 73,000 illnesses in the United States annually, leading to an estimated 2,168 hospitalizations and 61 deaths;
- Studies have shown that cattle are the principal source of *E. coli* O157 infection with 15% of healthy cattle carrying O157;
- About 1/4 waterborne outbreaks were associated with drinking water many of them were directly or indirectly contaminated with animal manure.



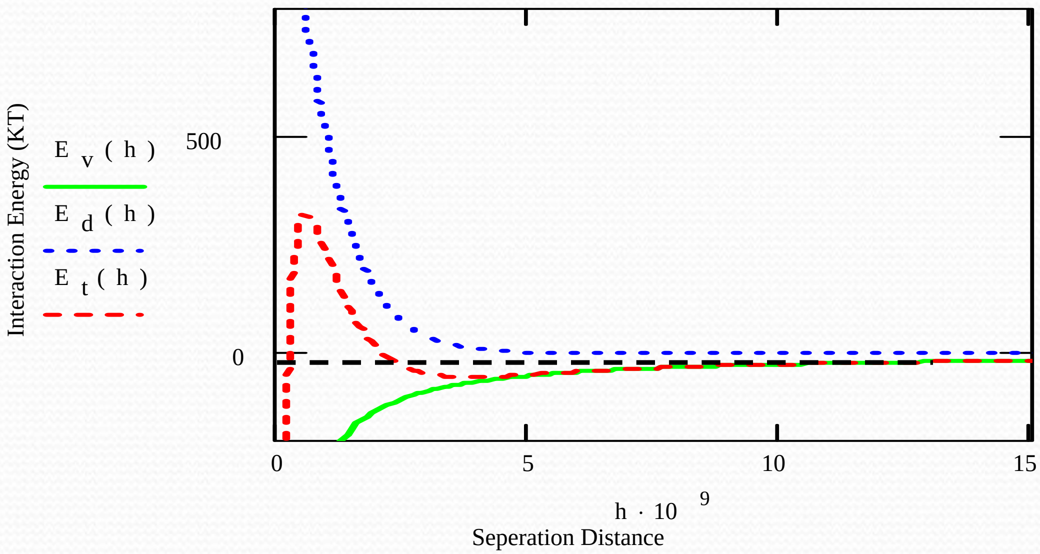


Can we predict bacterial transport and survival?

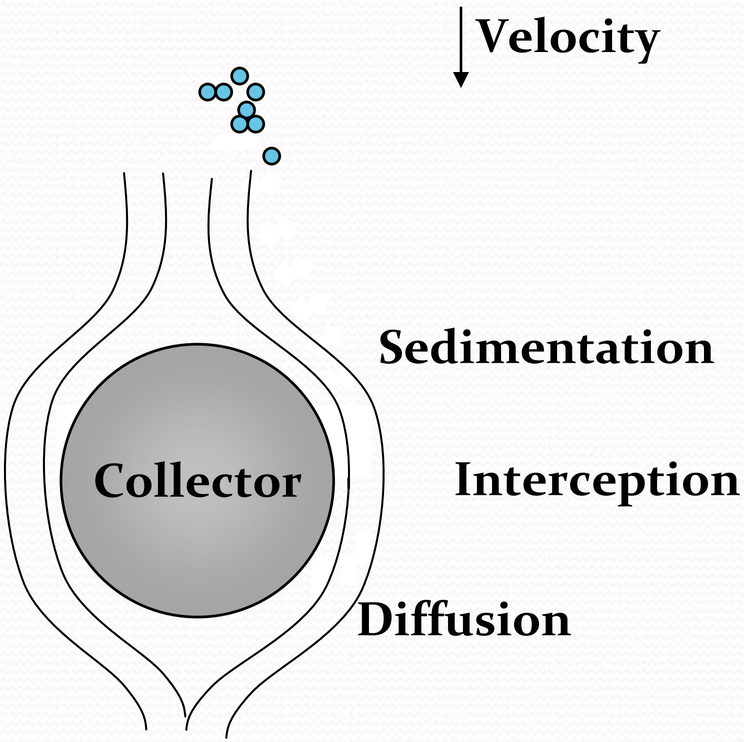
- How far can bacteria transport?
- What are the major factors affect bacterial transport?
- What are the major factors affect bacterial survival?
- Indicator bacteria vs pathogenic bacteria?

DLVO (Derjaguin, Landau, Verwey, Overbeek) Theory

$$E_{total} = E_{van\ der\ Waals} + E_{electrostatic\ forces}$$



Classic Colloid Filtration Theory

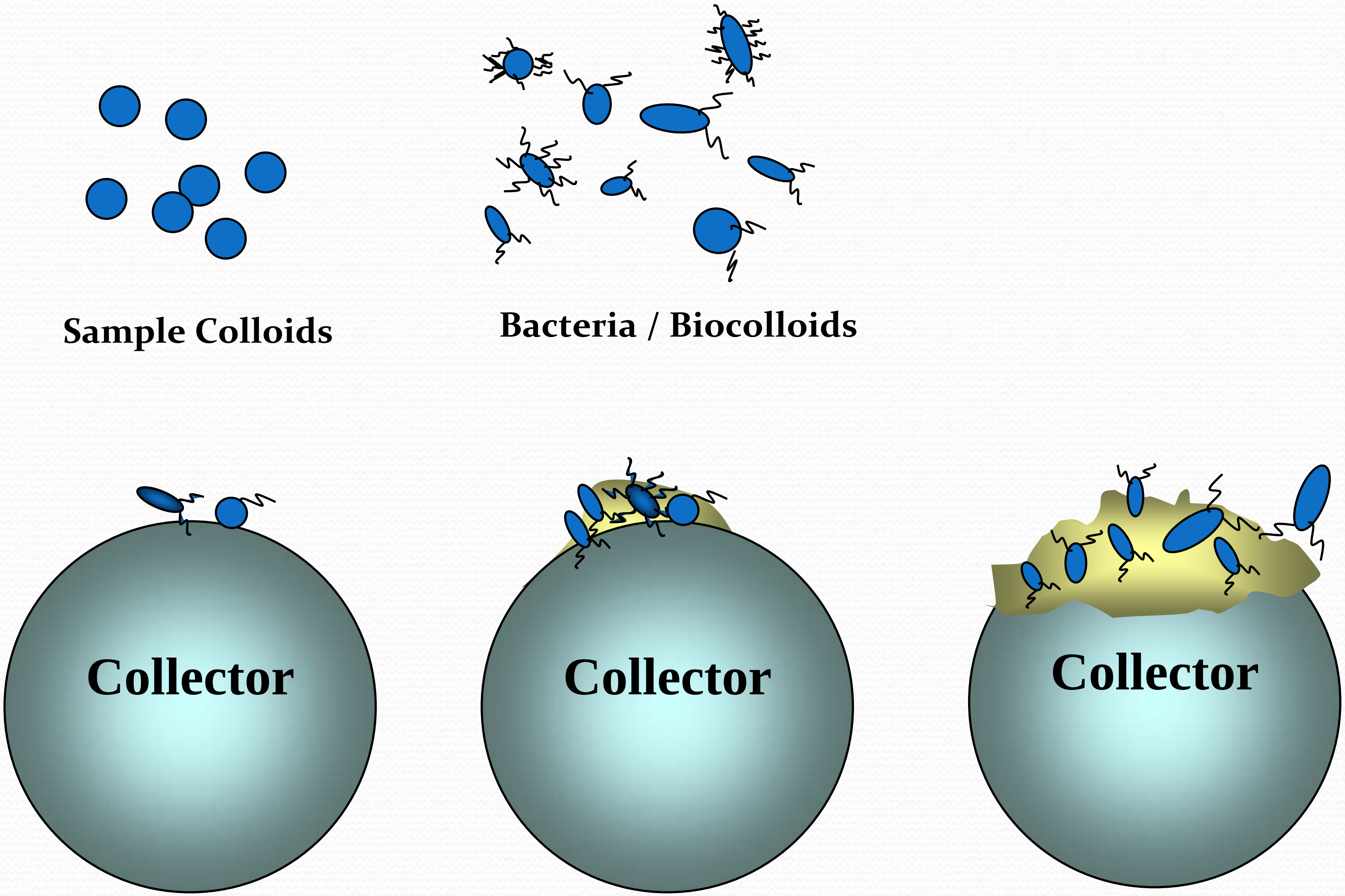


$$k_d = -\frac{U}{\varepsilon \cdot L} \ln\left(\frac{C}{C_0}\right)$$

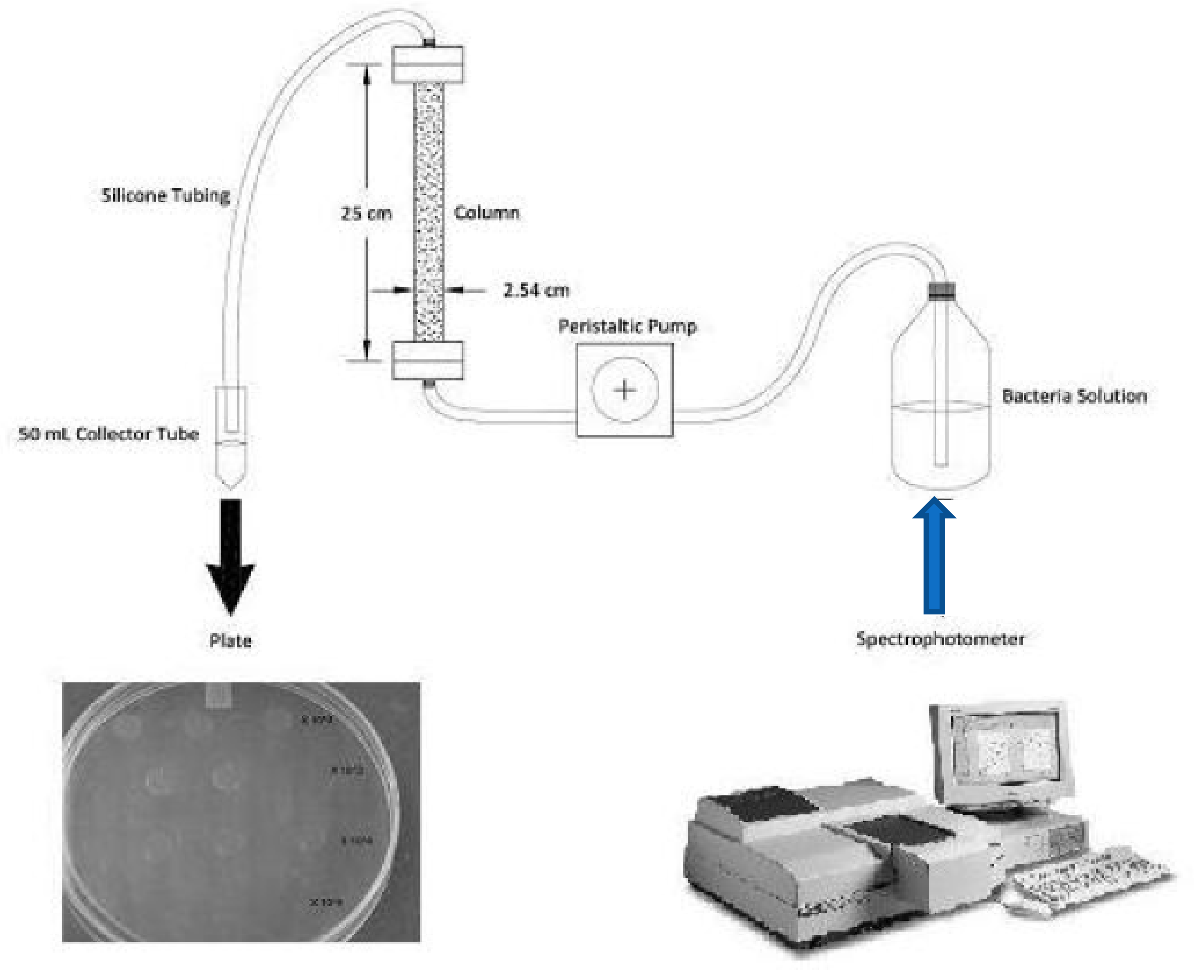
$$S(X) = \frac{t_0 \cdot \varepsilon \cdot k_d \cdot C_0}{\rho_b} \exp\left(-\frac{k_d \cdot X \cdot \varepsilon}{U}\right)$$

ε	bed porosity,
U	approach (superficial) velocity,
L	length of the column,
C/C_o	normalized breakthrough conc.
X	column depth,
ρ_b	porous medium bulk density,
t_o	duration of particle injection.

Yao et al (1971), ES&T, 5 (11), 1105-1112


Sample Colloids
Bacteria / Biocolloids
Collector
Collector
Collector

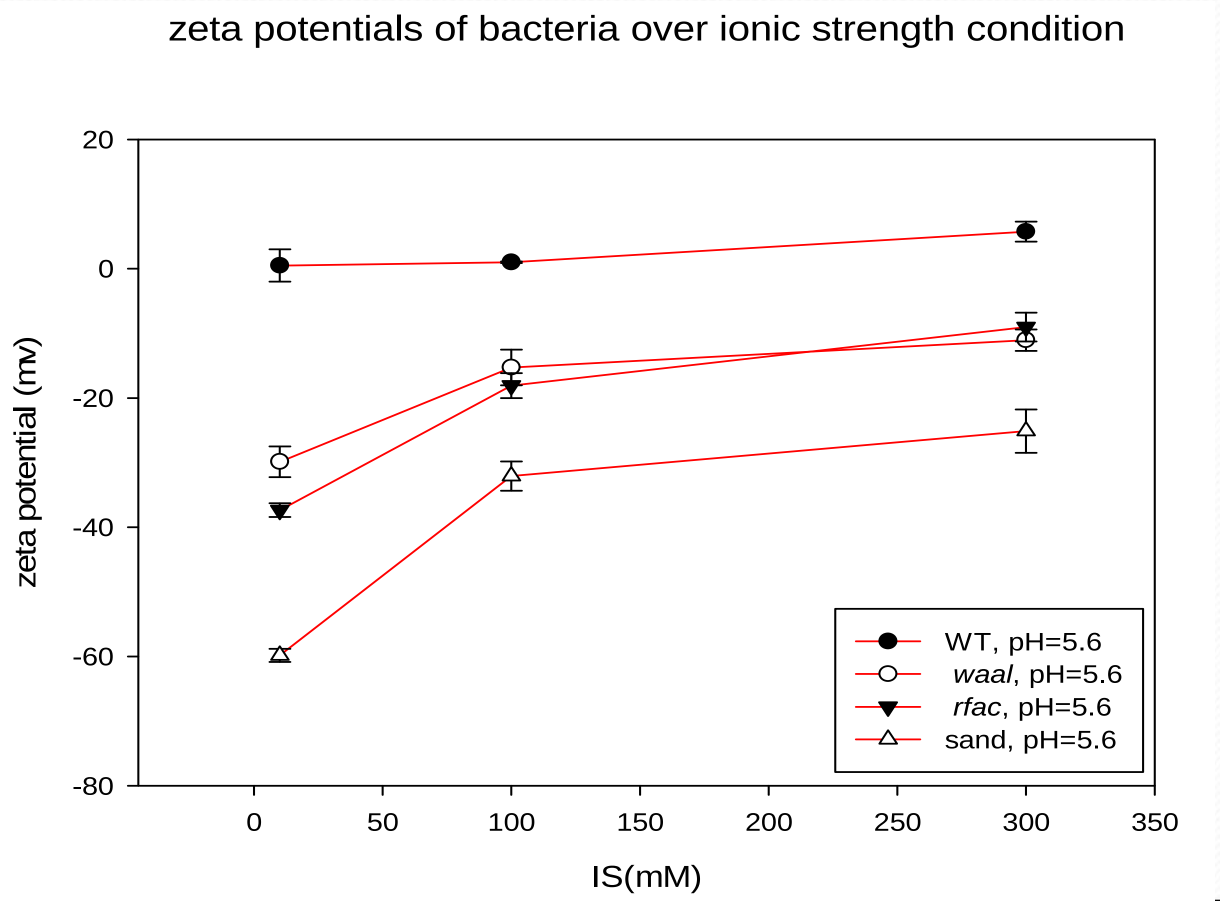
Experimental setup



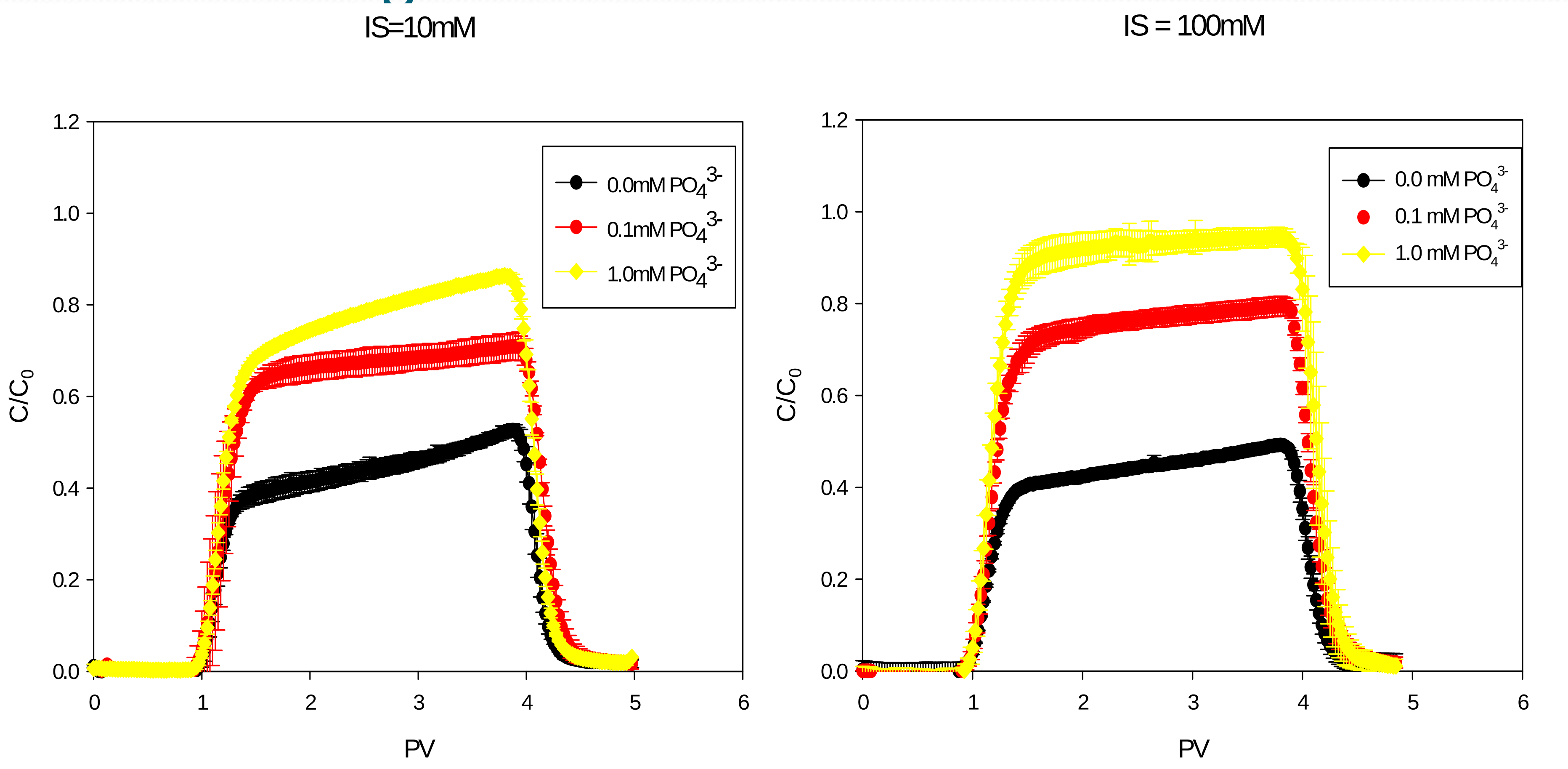
Zeta potential

Solution Chemistry: pH, Ionic Strength, buffers

Bacteria Surface: LPS

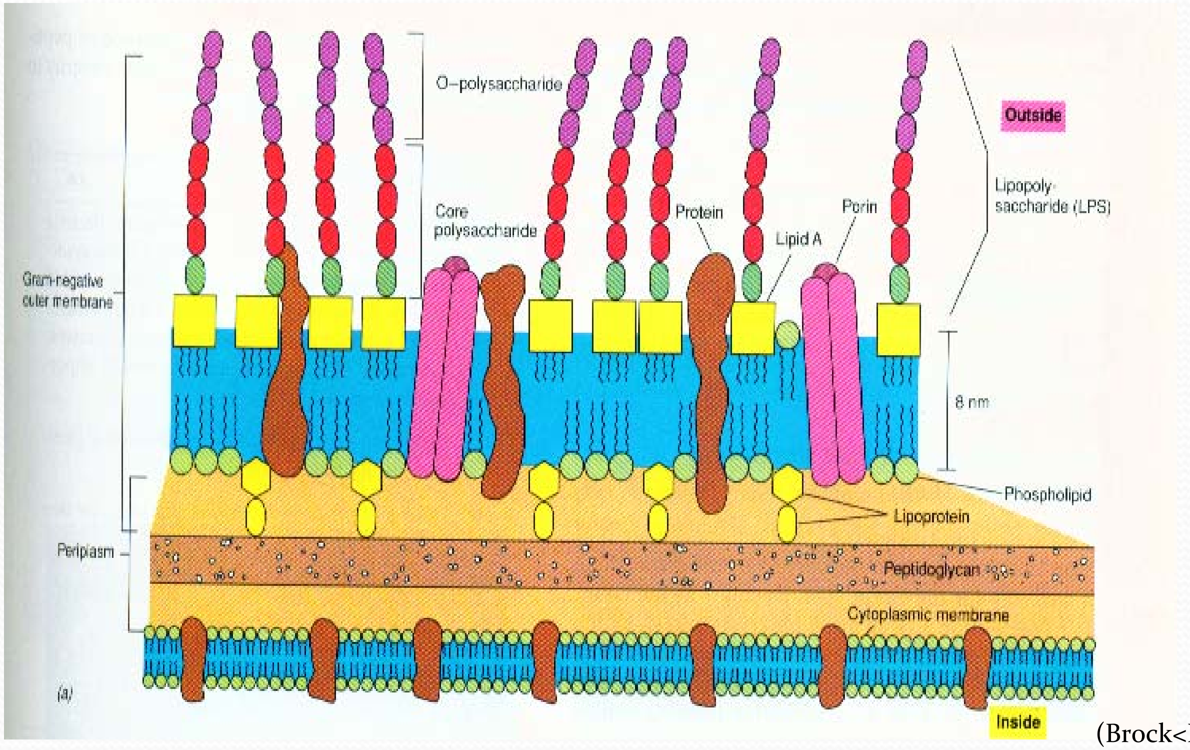


Breakthrough curves of *E.coli* O157:H7



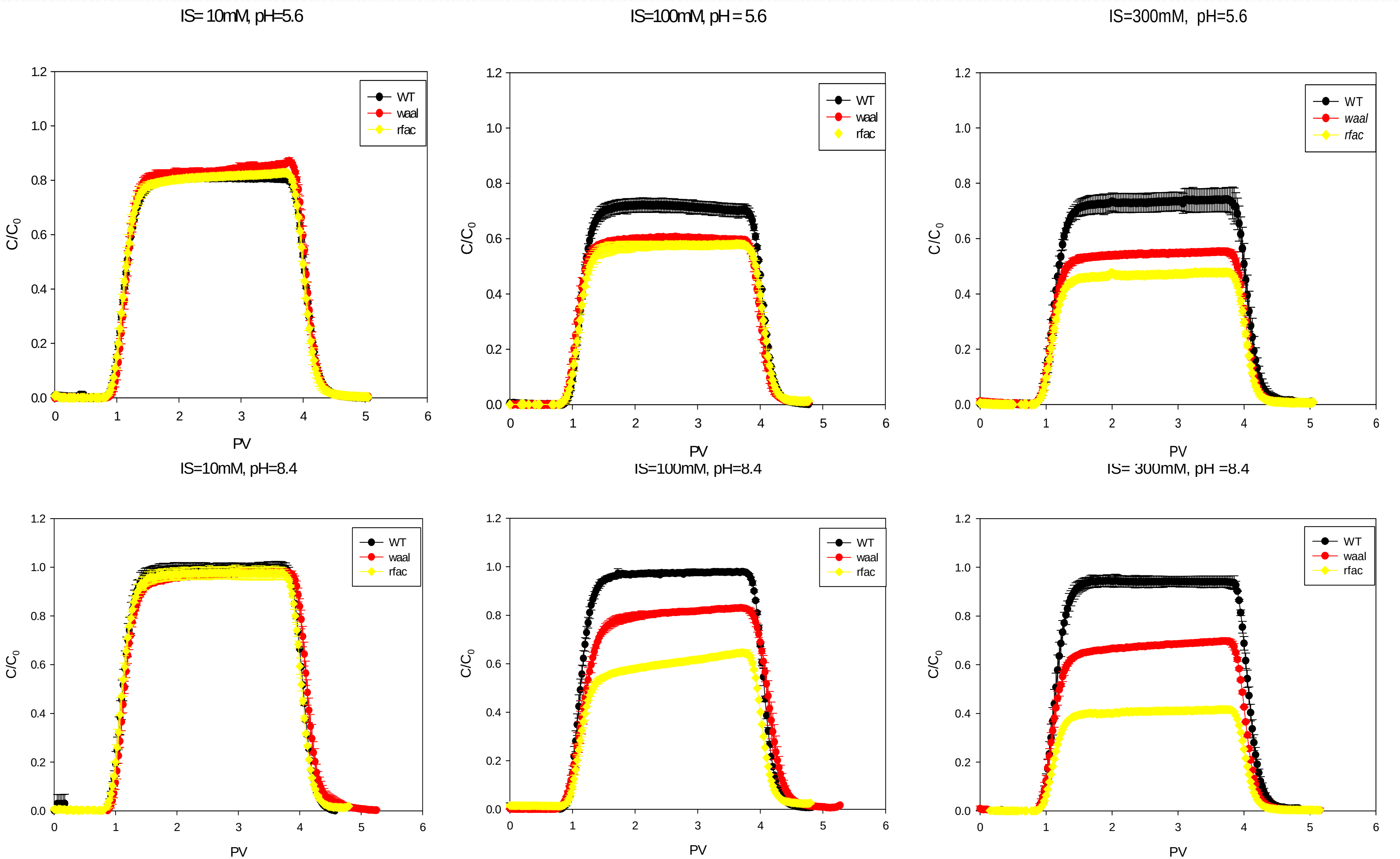
Impact of surface LPS on the transport behavior of *E.coli* O157:H7 in sand columns

- Three strains of *E. coli* O157:H7 EDL933 : wild type, Δ*waa L* (O antigen deficient) , Δ*rfaC* (outercore and O antigen deficient)



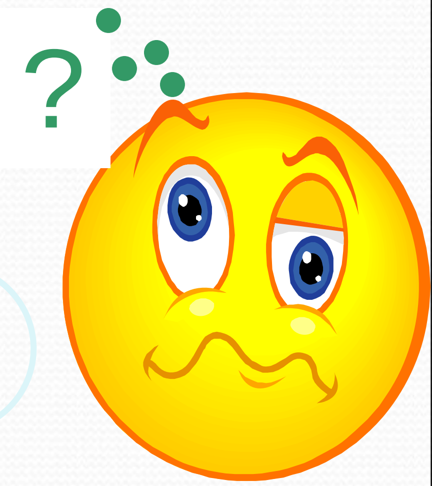
(Brock<Biology of Microorganisms>)

Breakthrough curves

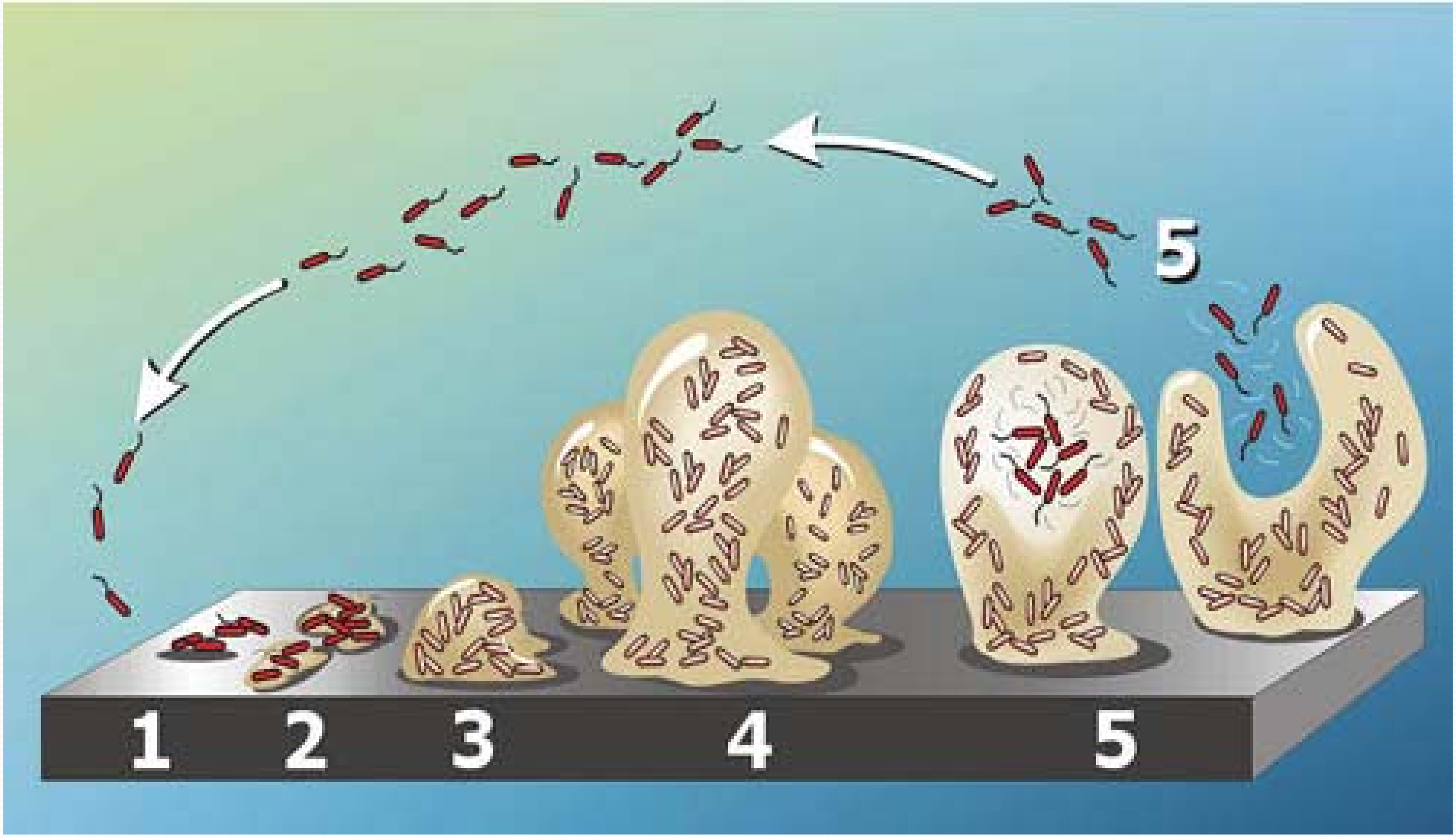


Fate of Bacteria in Biofilm Mediated Porous Media

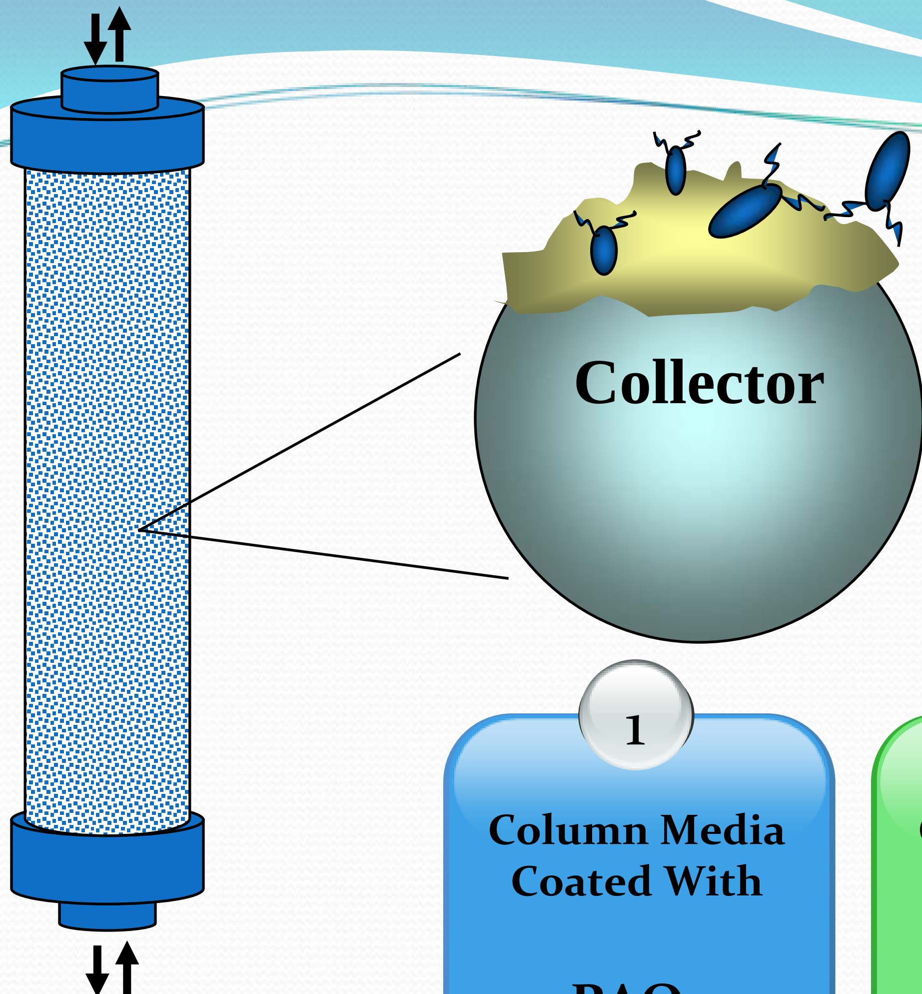
- Successful adhesion and colonization
- Compete with the indigenous organisms on porous media for nutrients
- Survive and grow in the biofilm
- Detach from/together with the biofilm



Biofilm

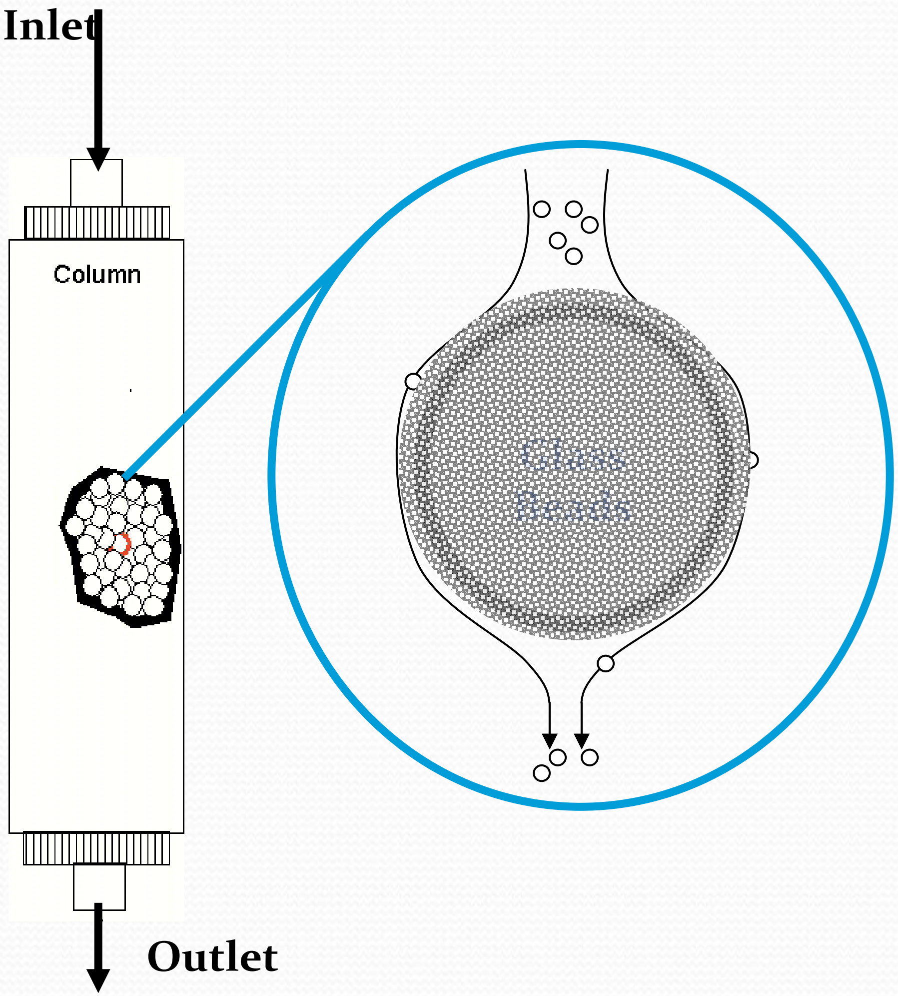


Experimental Design



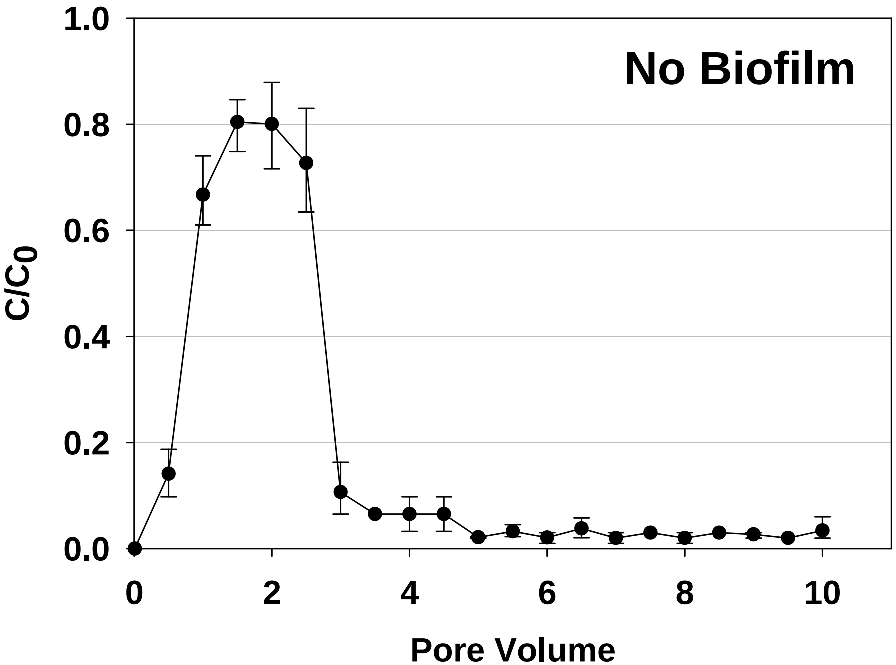
1. Column Media Coated With **PAO1 Biofilm**
2. Column Media Coated With **PDO300 Biofilm**
3. Column Media With **No Biofilm**

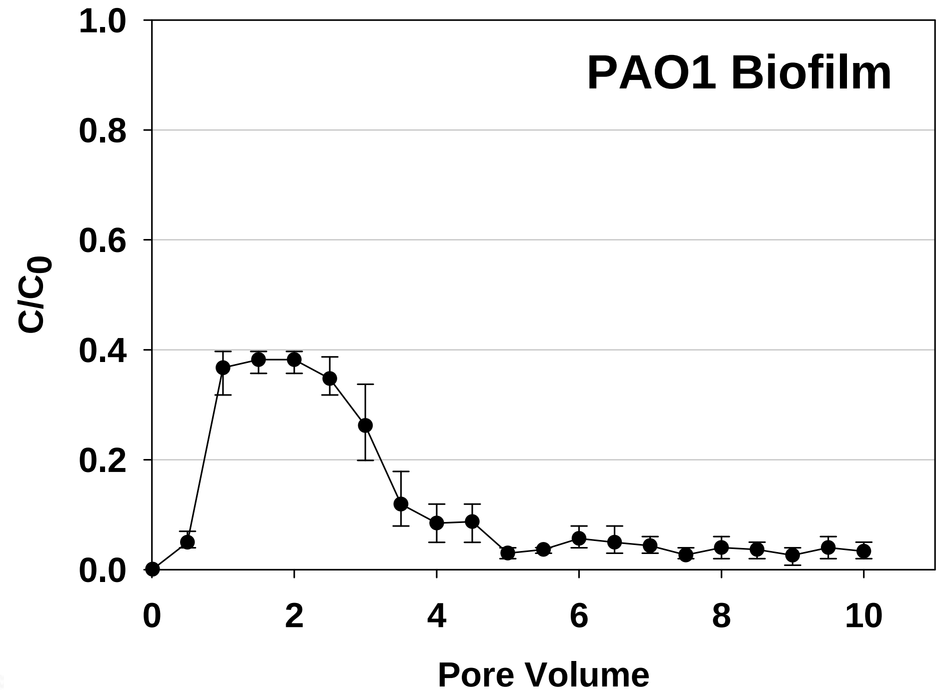
Transport Experiment



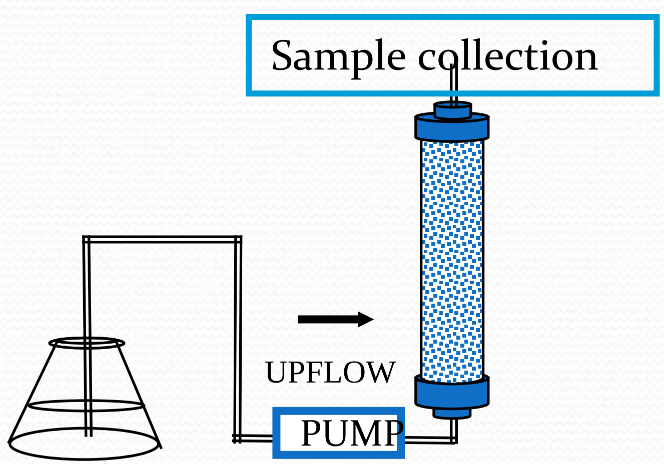
1. Examine how biofilm thickness affect introduced bacterial adhesion
2. Compare the impact of biofilm EPS composition on bacterial adhesion

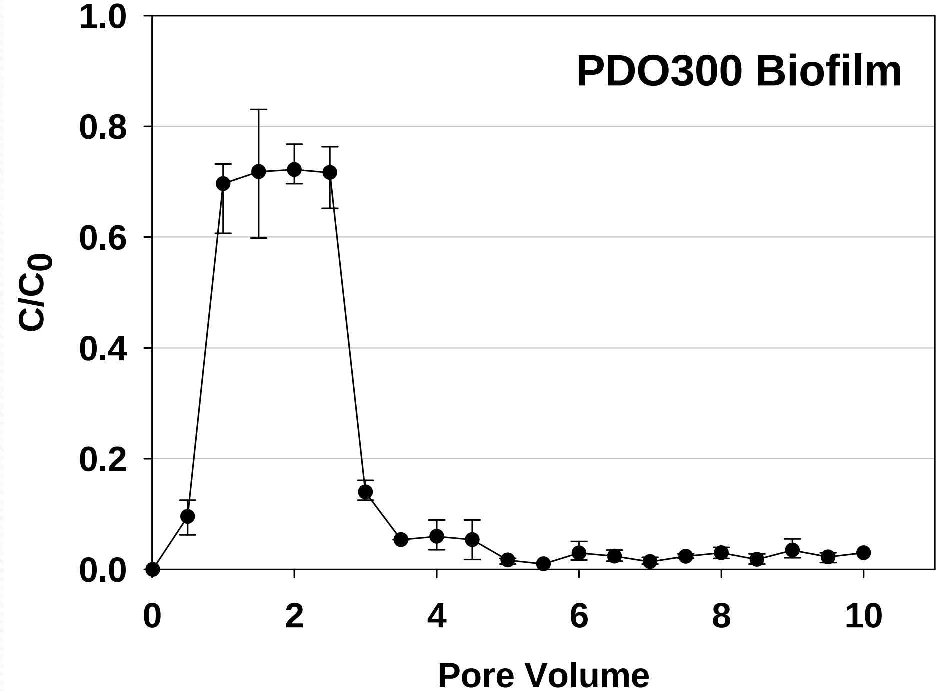
Main mechanism controlling bacterial transport in biofilm mediated porous media?

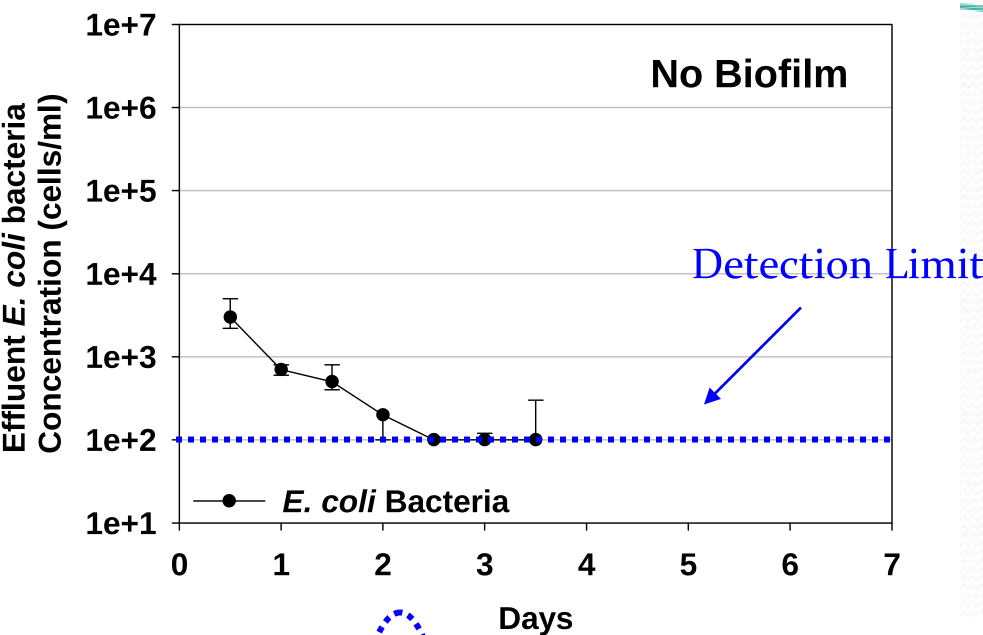


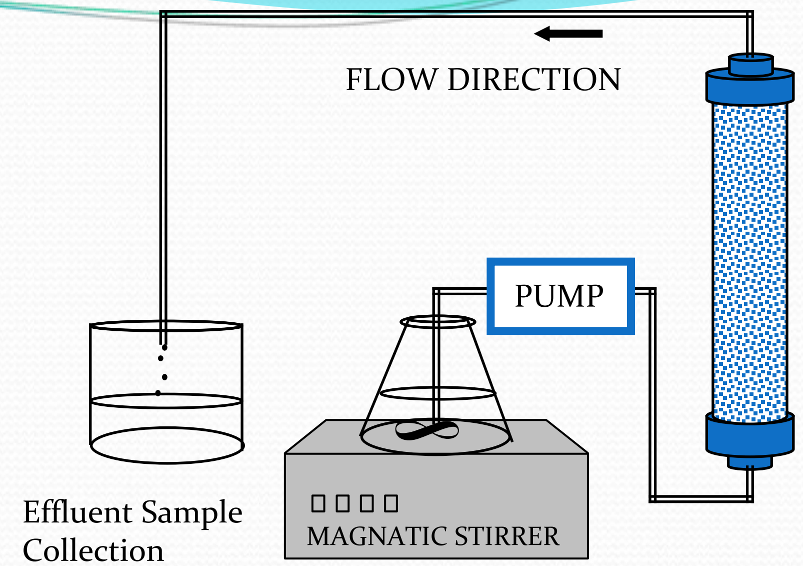


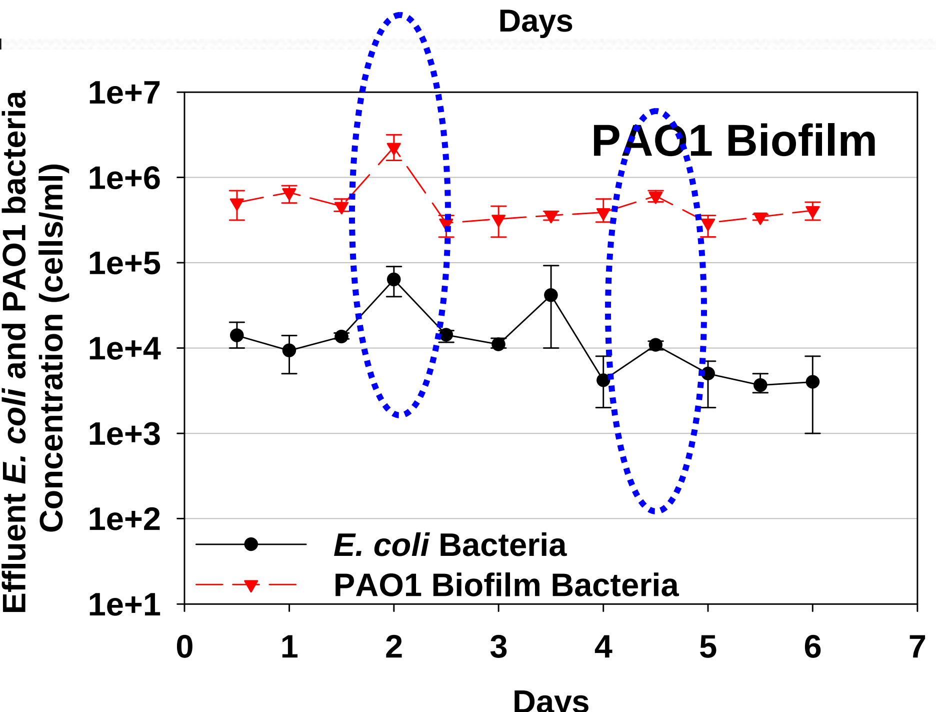
Transport Experiment
Effluent Breakthrough Curves

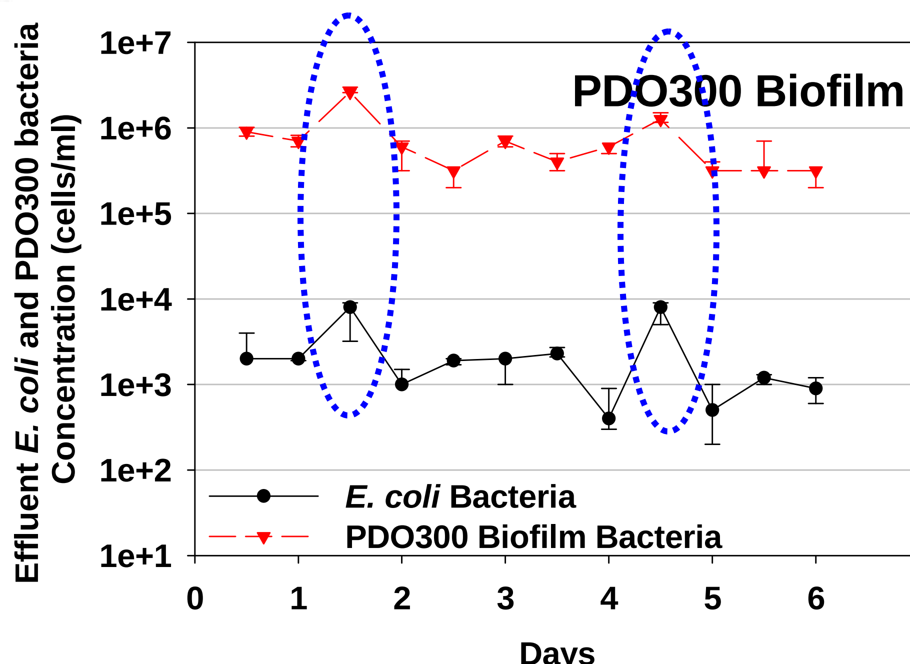


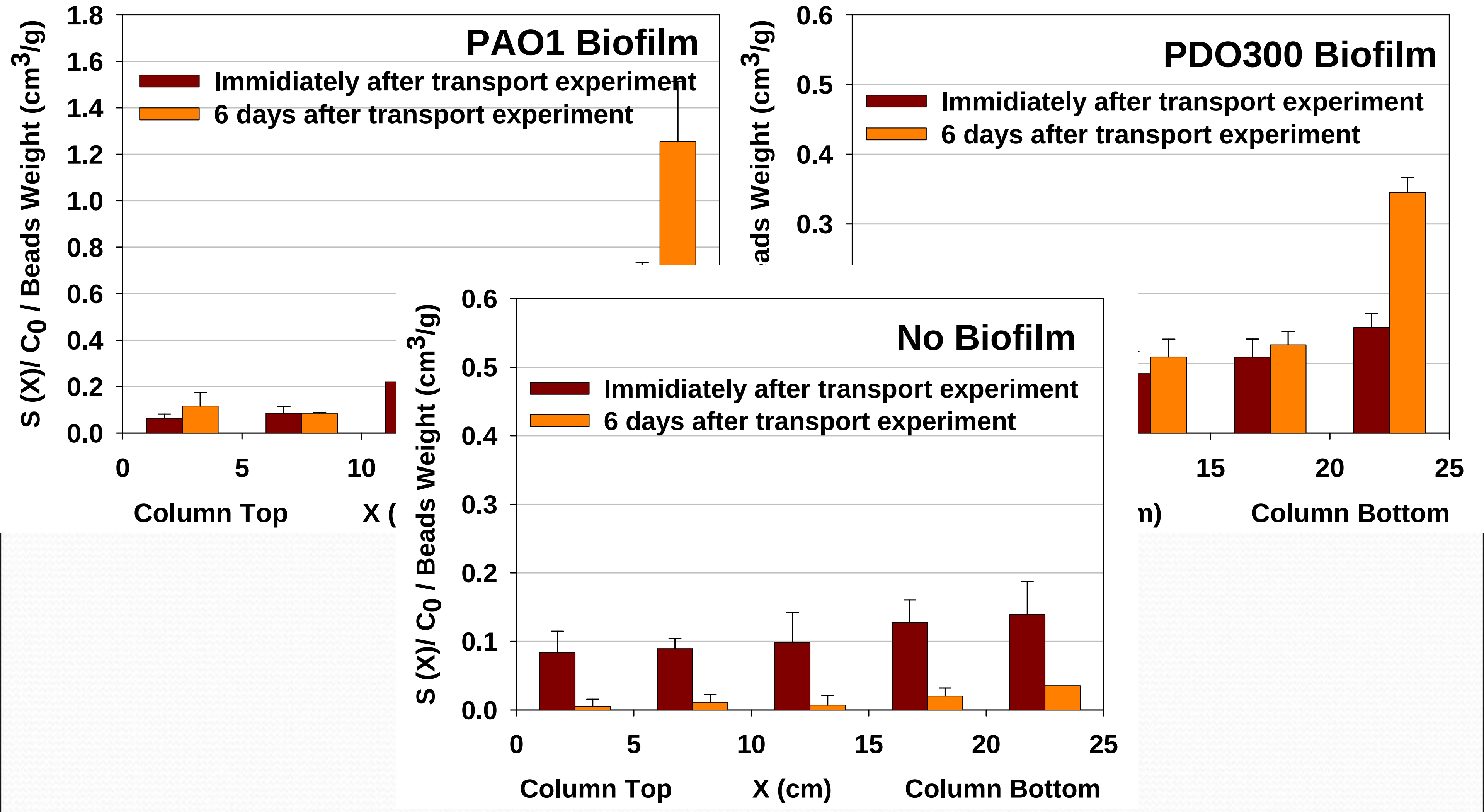



No Biofilm
Detection Limit
Effluent E. coli bacteria Concentration (cells/ml)
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
0
1
2
3
4
5
6
7
Days
E. coli Bacteria


FLOW DIRECTION
PUMP
Effluent Sample Collection
MAGNATIC STIRRER

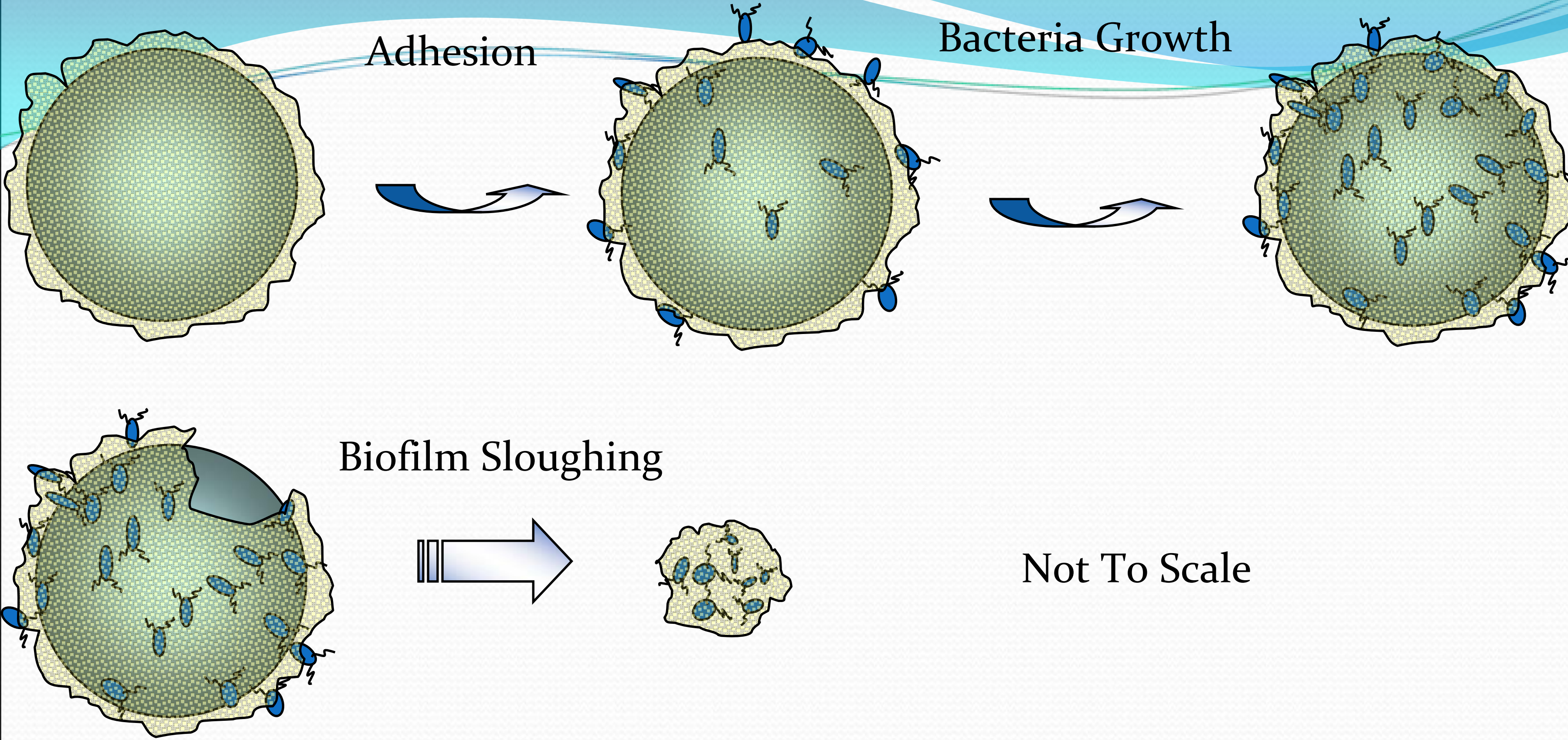

PAO1 Biofilm
Effluent E. coli and PAO1 bacteria Concentration (cells/ml)
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
0
1
2
3
4
5
6
7
Days
E. coli Bacteria
PAO1 Biofilm Bacteria


PDO300 Biofilm
Effluent E. coli and PDO300 bacteria Concentration (cells/ml)
1e+7
1e+6
1e+5
1e+4
1e+3
1e+2
1e+1
0
1
2
3
4
5
6
7
Days
E. coli Bacteria
PDO300 Biofilm Bacteria



Liu et al. (2008) *Environmental Science & Technology, 42*. 443-449

		No Biofilm	PAO1 Biofilm	PDO300 Biofilm
Transport experiment	Column effluent[(a)]	80.40%	53.56%	81.40%
	Column media[(b)]	20.10%	42.67%	15.20%
	Recovery rate[(a+b)]	100.5%	96.23%	96.6%
Survival experiment	Column effluent[(c)]	1.00%	76.68%	12.70%
	Column media[(d)]	3.00%	81.85%	31.25%
	Recovery rate[(a+c+d)]	84.40%	212.09%	125.35%
E. coli growth rate (day^{-1})		-0.32	0.11	0.12



- After initial attachment, *E. coli* bacteria were able to survive and grow in the biofilm matrix with a relatively low nutrient supply.
- *E. coli* cells became an integral part of the biofilm and biofilm sloughing was the major mechanism that introduces the *E. coli* bacteria to the bulk fluid long after the contamination event.

Conclusions

- The composition of manure can impact the deposition and survival of microorganisms, especially during events of heavy precipitation, through physical, chemical and microbiological mechanisms, e.g., changes of pH, salinity of pore fluid, blocking of the favorable deposition site by organic matters, aggregations between manure suspensions and bacterial cells, and formation of biofilms.
- An important phenomenon that we observed in this study is that the transport and adhesion behavior of *E. coli* O157 differ significantly from model prediction and from the behavior of other *E. coli* strains. Therefore the use of indicator bacteria to predict the risk of pathogen contamination in groundwater needs to be evaluated in detail in future study.

Related Publications:

- Liu, Y., Li, J. 2008, "Role of *Pseudomonas aeruginosa* biofilm in the initial adhesion, growth and detachment of *Escherichia coli* in porous media", *Environmental Science and Technology*, 42(2), 443–449.
- Marissa Jablonski, 2009, "Comparison of the Role of Ionic Strength and Surface Charge Heterogeneity on the Initial Adhesion, Distribution and Detachment of *E. coli* O157 and JM109 in Glass Beads-Packed Column Experiments", Master Thesis
- Yamazaki, A., Li, J., Hutchins, W. C., Wang, L., Ma, J., Ibekwe, A.M., Yang, CH, 2011, "Commensal effect of pectate lyases secreted from Dickeya dadantii on the poliferation of Escherichia coli O157:H7 EDL933 on lettuce leaves", *Applied and Environmental Microbiology*, 77(1):156-162.

Thank you!