Environmental Biotechnology

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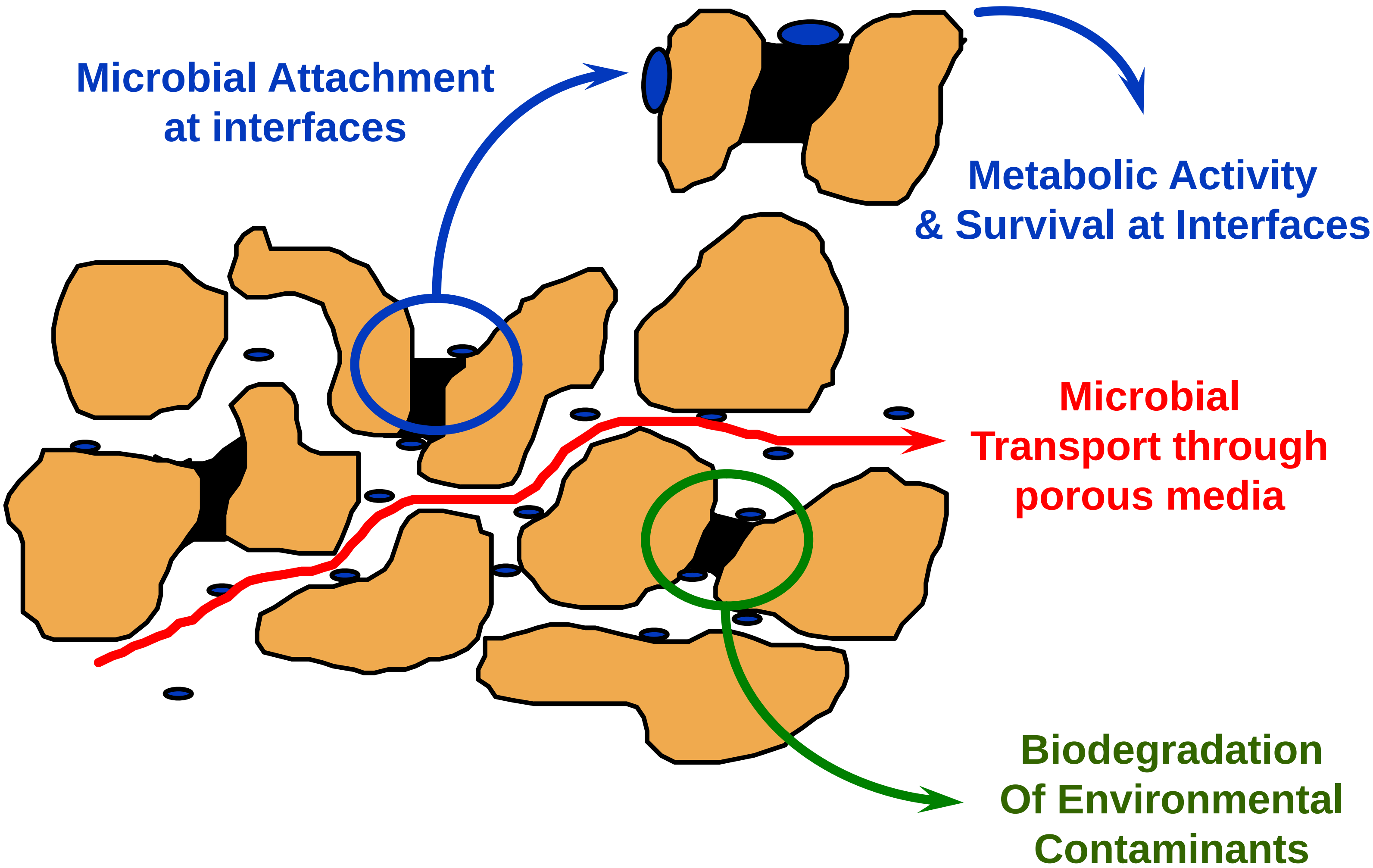
Environmental Biotechnology and Complex Systems



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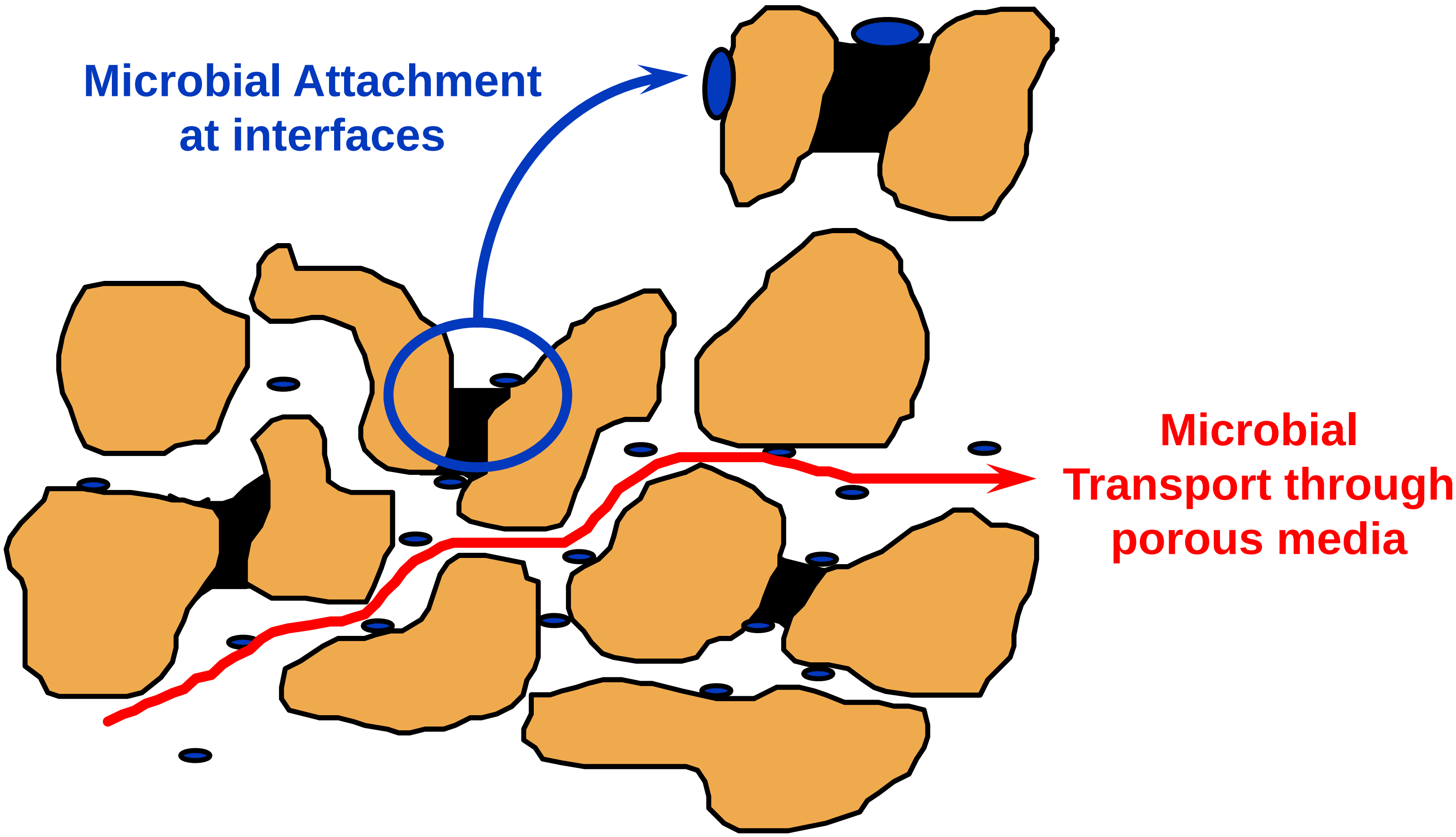
Theme…

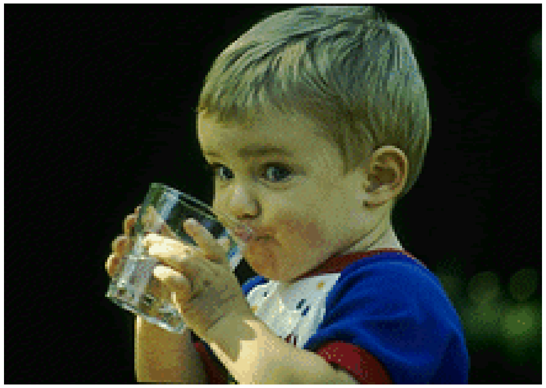
You can learn a lot from simple experiments

Microbial Attachment and Transport



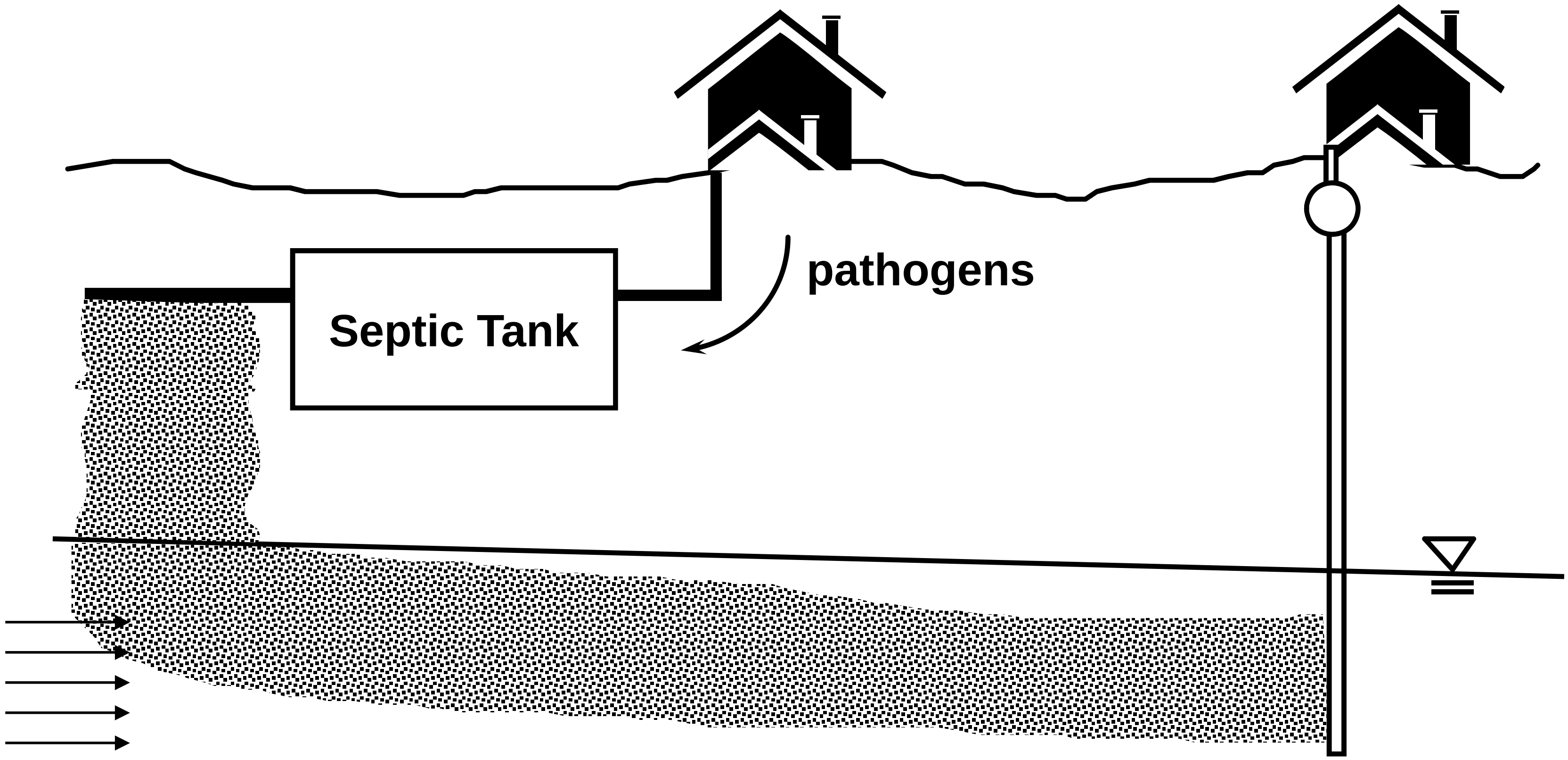
Protection of drinking water supplies

- 77.5 billion gallons of groundwater drawn daily for use in the U.S.
- Groundwater supplies ~50% of the nation's drinking water.



- Typically assumed that groundwater is free from microbial contamination.
- However, a major fraction of drinking water disease outbreaks in the U.S. are due to consumption of untreated groundwater.

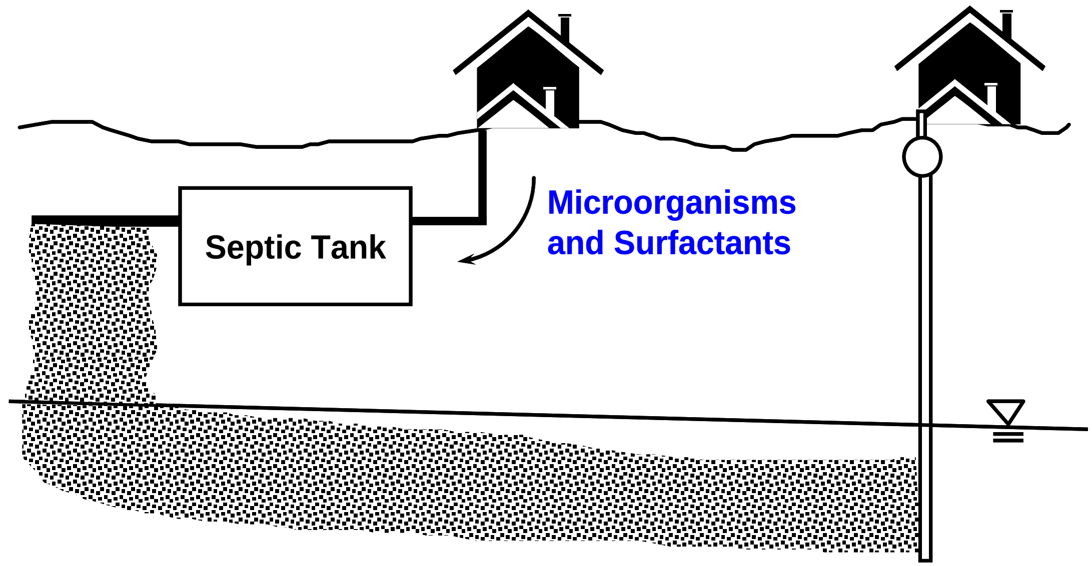
The question is why do pathogens travel farther than expected?



We are investigating bacterial transport through porous media using lab and meso-scale systems

Surfactants and domestic sewage

- There are ~7x10^6 metric tons of surfactants used for laundry detergents in the U.S. every year.
- Surfactants are the major man-made organic constituent in domestic wastewater.



- Grey water contains 150 – 600 mg/L of LAS.
- Total volume of septic waste released into the subsurface in the U.S. is estimated to be one trillion gallons per year.

Examining effects of surfactants on bacterial transport through unsaturated porous media

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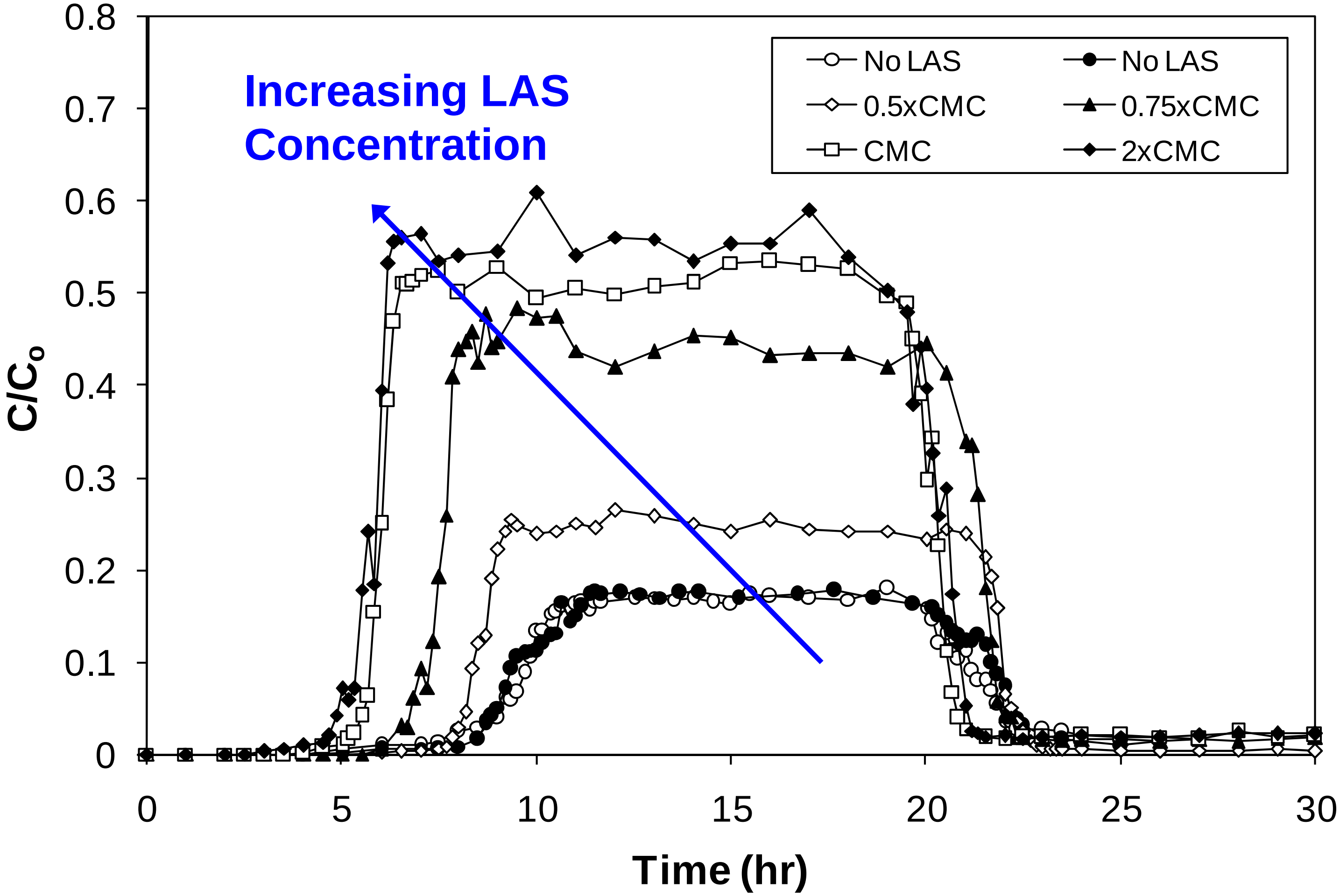
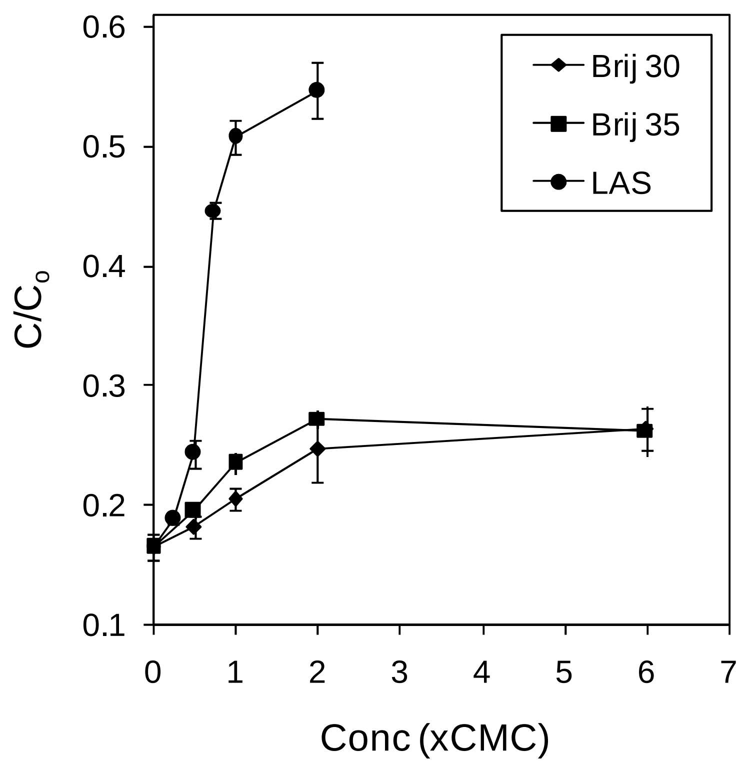
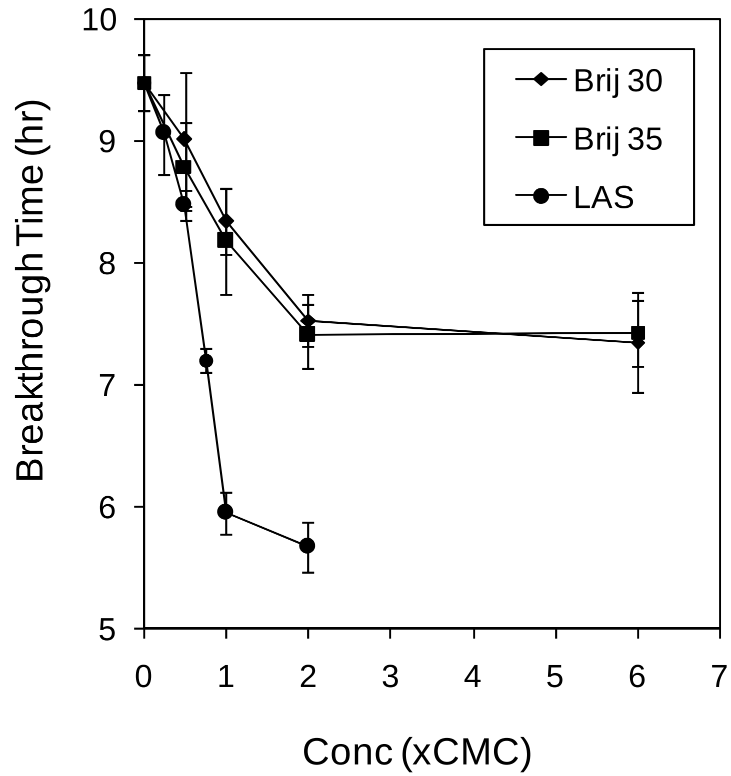
Transport of E. coli through unsaturated porous media in the presence of Linear Alkylbenzene Sulfonate (LAS)



Surfactant affect both increase transport rate and effluent concentration





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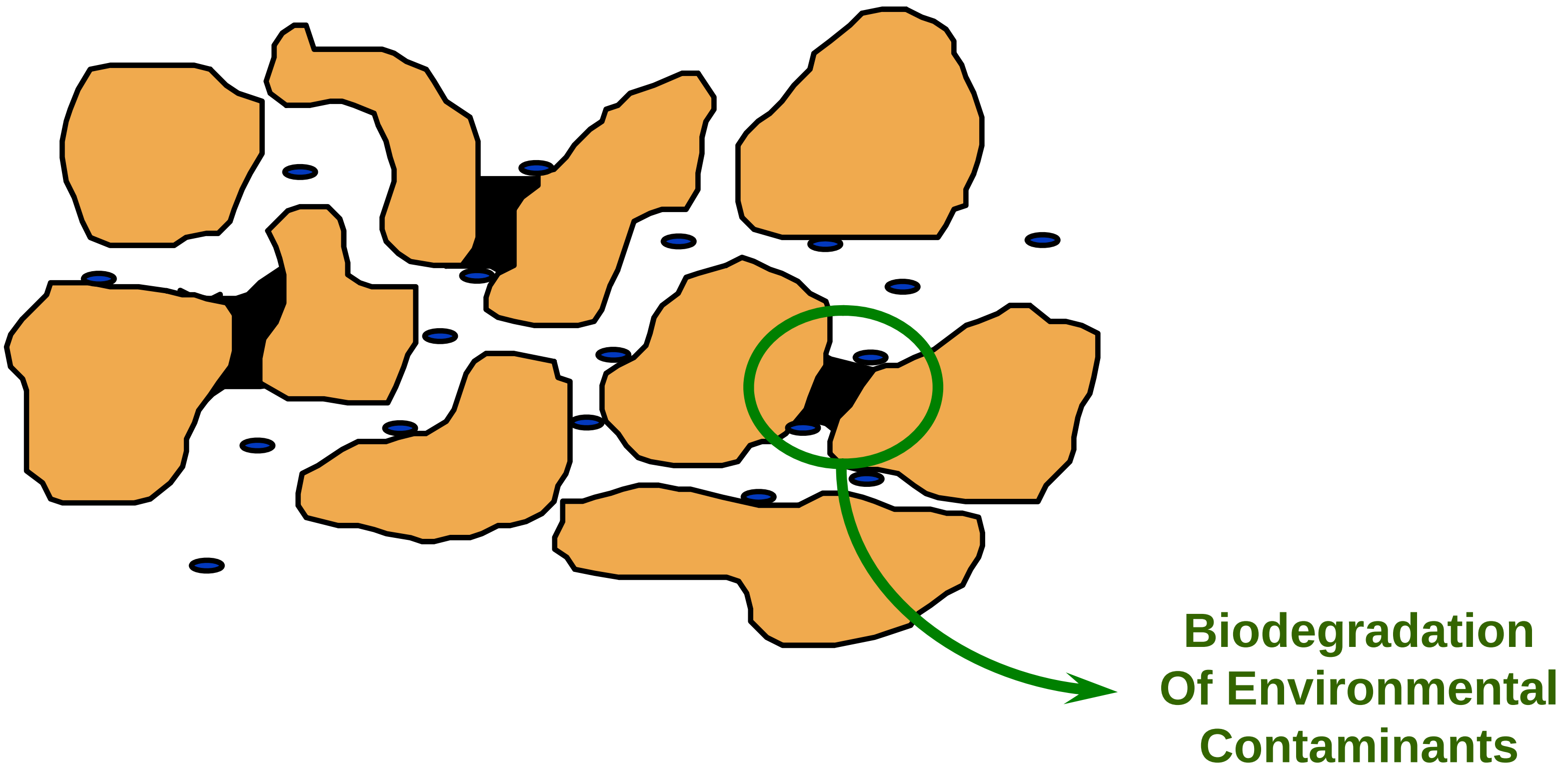
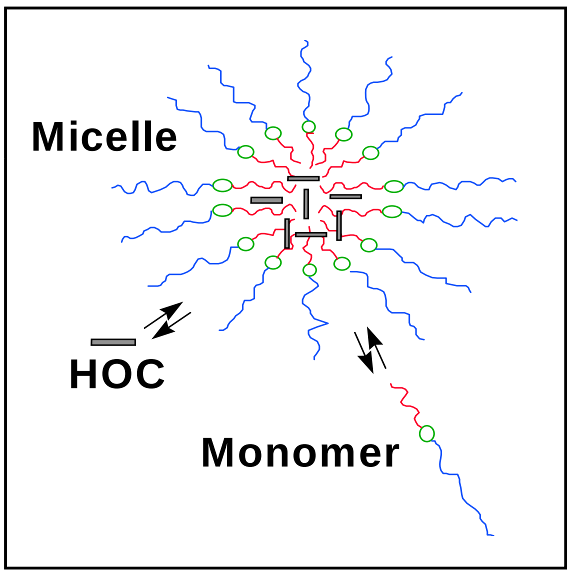
Enhanced biodegradation of environmental contaminants (microbial kinetics)



Use of surfactants to enhance biodegradation of hydrophobic substrates

$$CH_3 - (CH_2)_{m-1} - (O\text{-}CH_2\text{-}CH_2)_n - OH$$

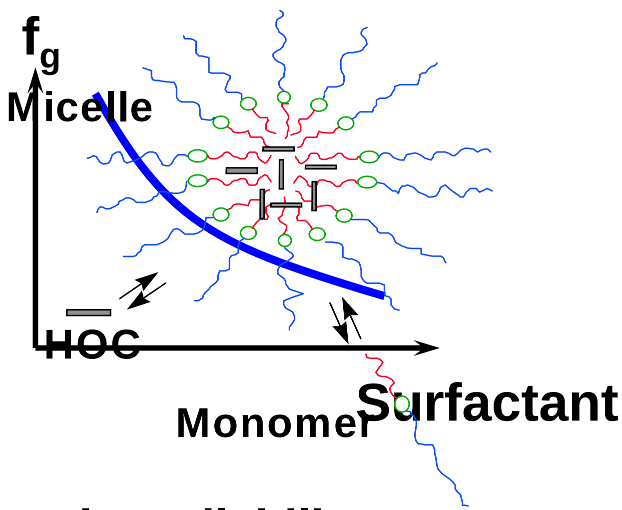
Hydrophobic ⟵|⟶ Hydrophilic



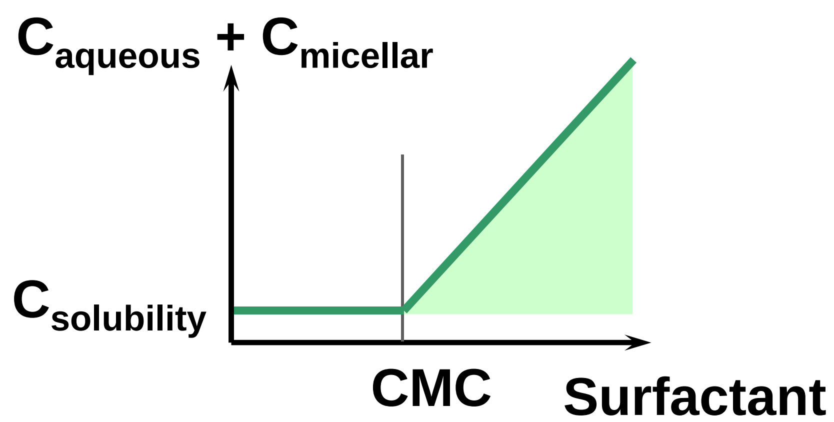
Laundry and Dish Detergents

Enhanced solubility and bioavailability

The bioavailable factor and the enhanced solubilization define the regime of enhanced bioavailability



Bioavailability Factor



Enhanced Solubilization

$$C_{bioavailable} = C_{aqueous} + f_g C_{micellar}$$

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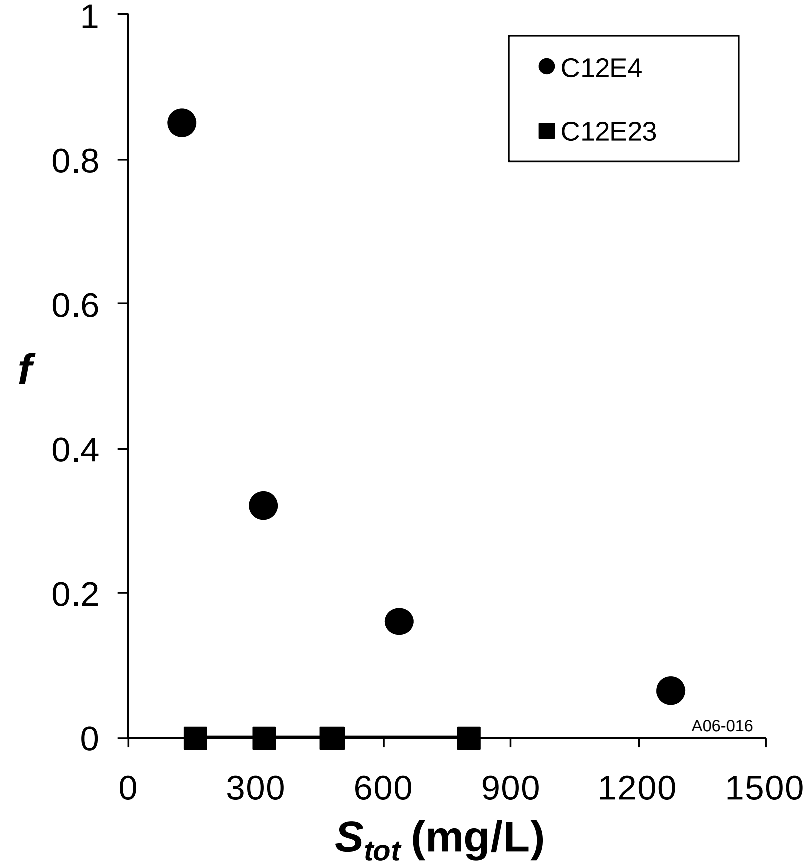
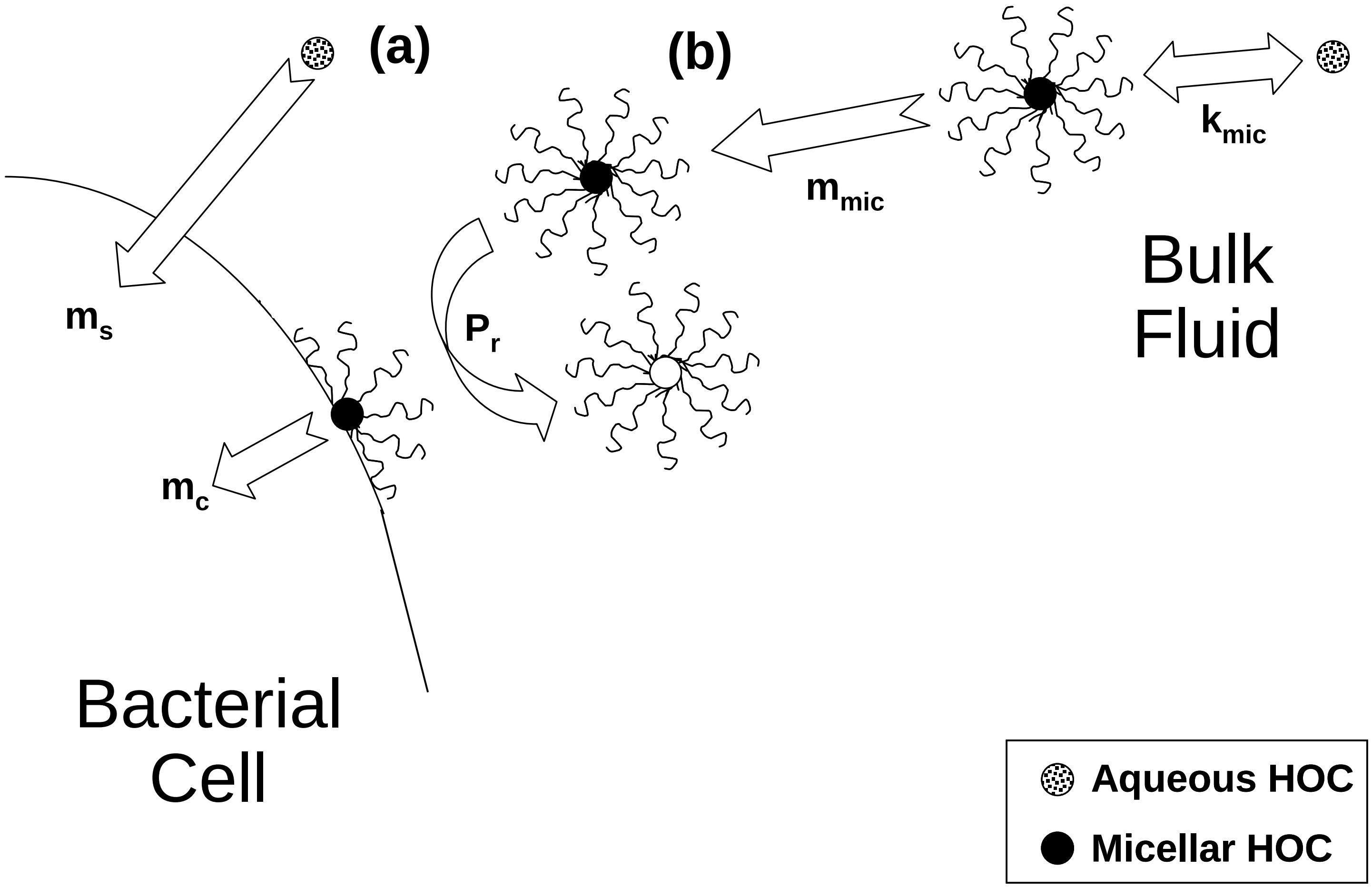
Bioavailability factor experimentally determined for $C_{12}E_y$ surfactants

- **Guha and Jaffe** experimentally determined *f* for $C_{12}E_4$ and $C_{12}E_{23}$ surfactants.
- **Sriwatanapongse** experimentally determined *f* for $C_{12}E_7$, $C_{12}E_9$, and $C_{12}E_{10}$.



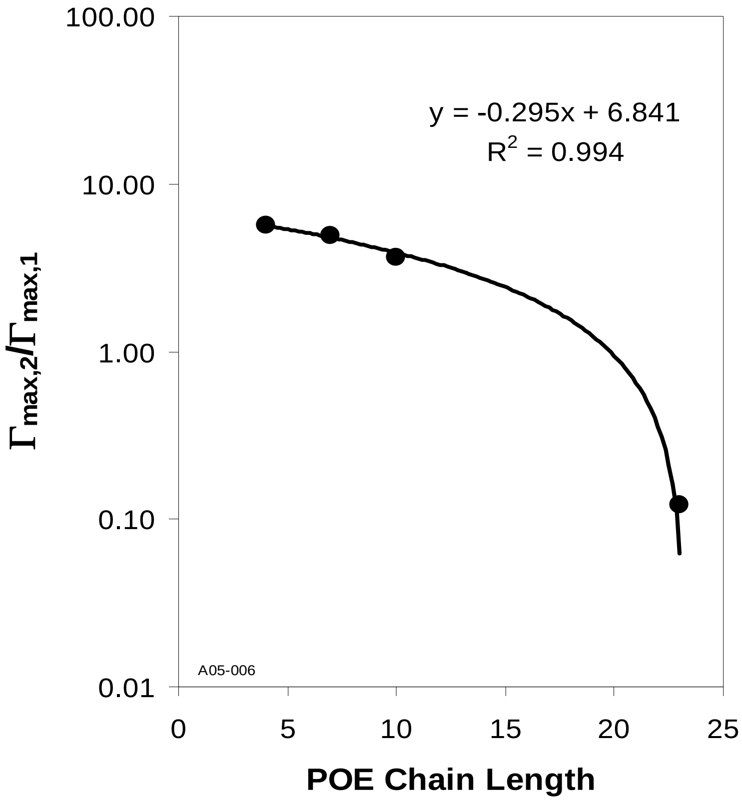
Guha, S. and P.R. Jaffe. 1996. *Environ. Sci. Technol.*, 30(4):1382-1391
Sriwqtanapongse, W., 1997. Princeton University, B.S. Thesis

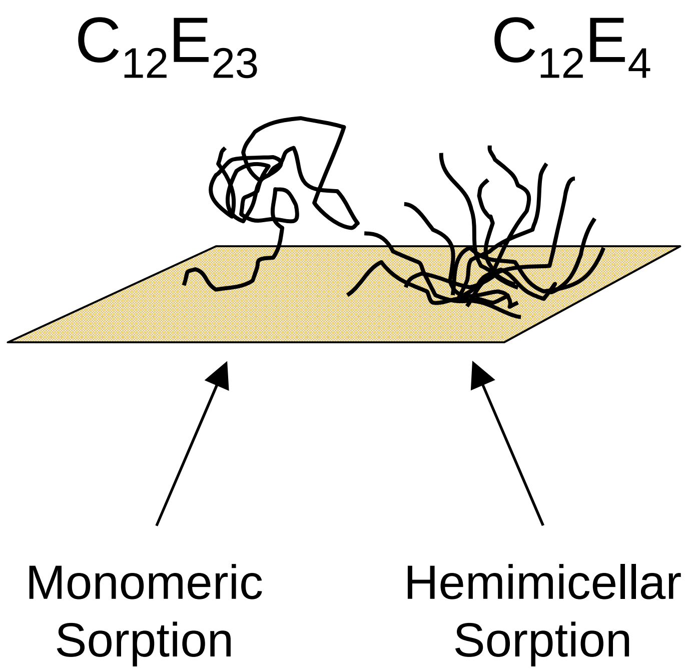
Theory on surfactant-enhanced bioavailability



Guha, S. and P.R. Jaffe. 1996. *Environ. Sci. Technol.*, 30(4):1382-1391

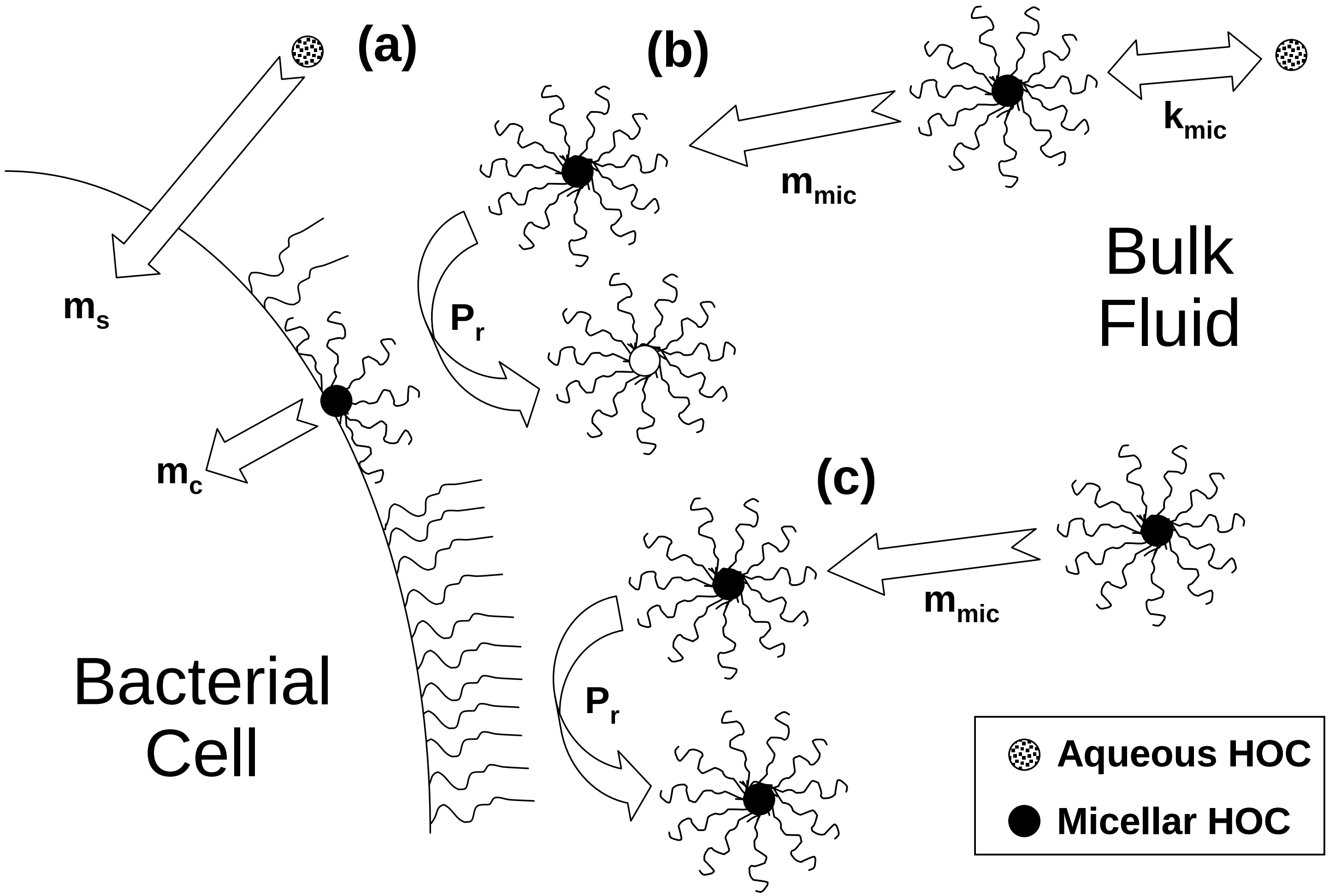
Monolayer and Lateral Interactions Vary as a Function of POE Chain Length





Brown and Al Nuaimi. 2005. *Langmuir* 21(24):11368-11372.

Developed theory on surfactant-enhanced bioavailability



Brown, D.G. 2007. *Environ. Sci. Technol.*, 41(4):1194-1199

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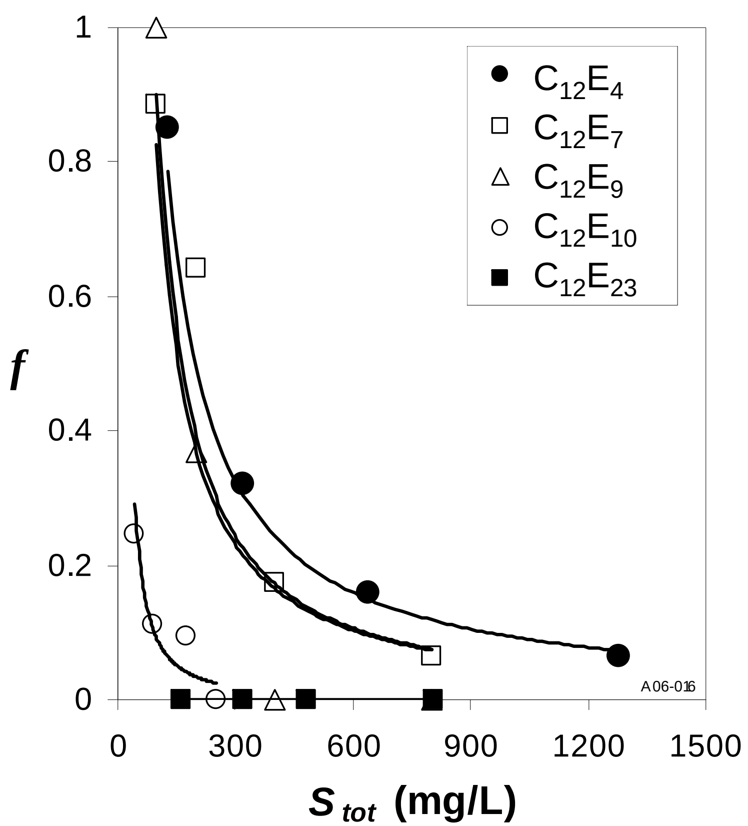
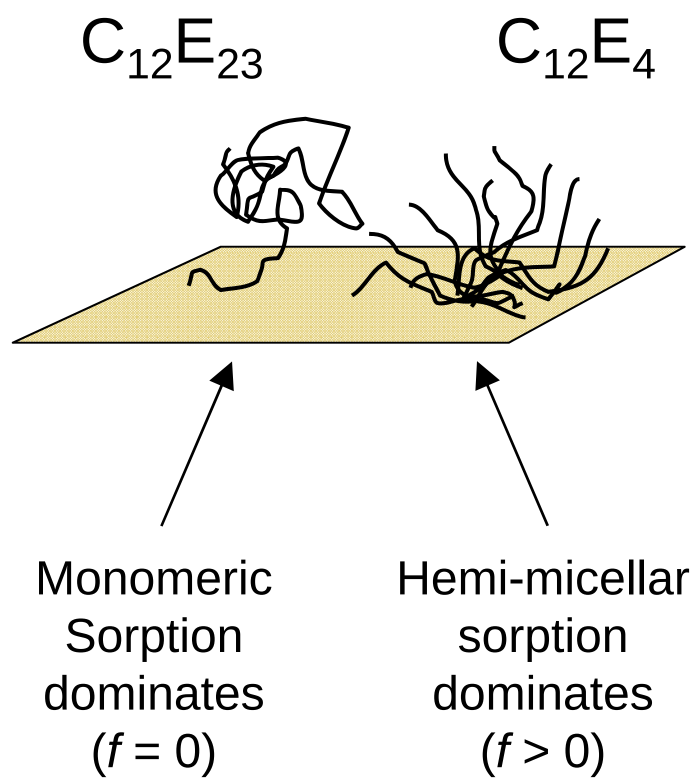
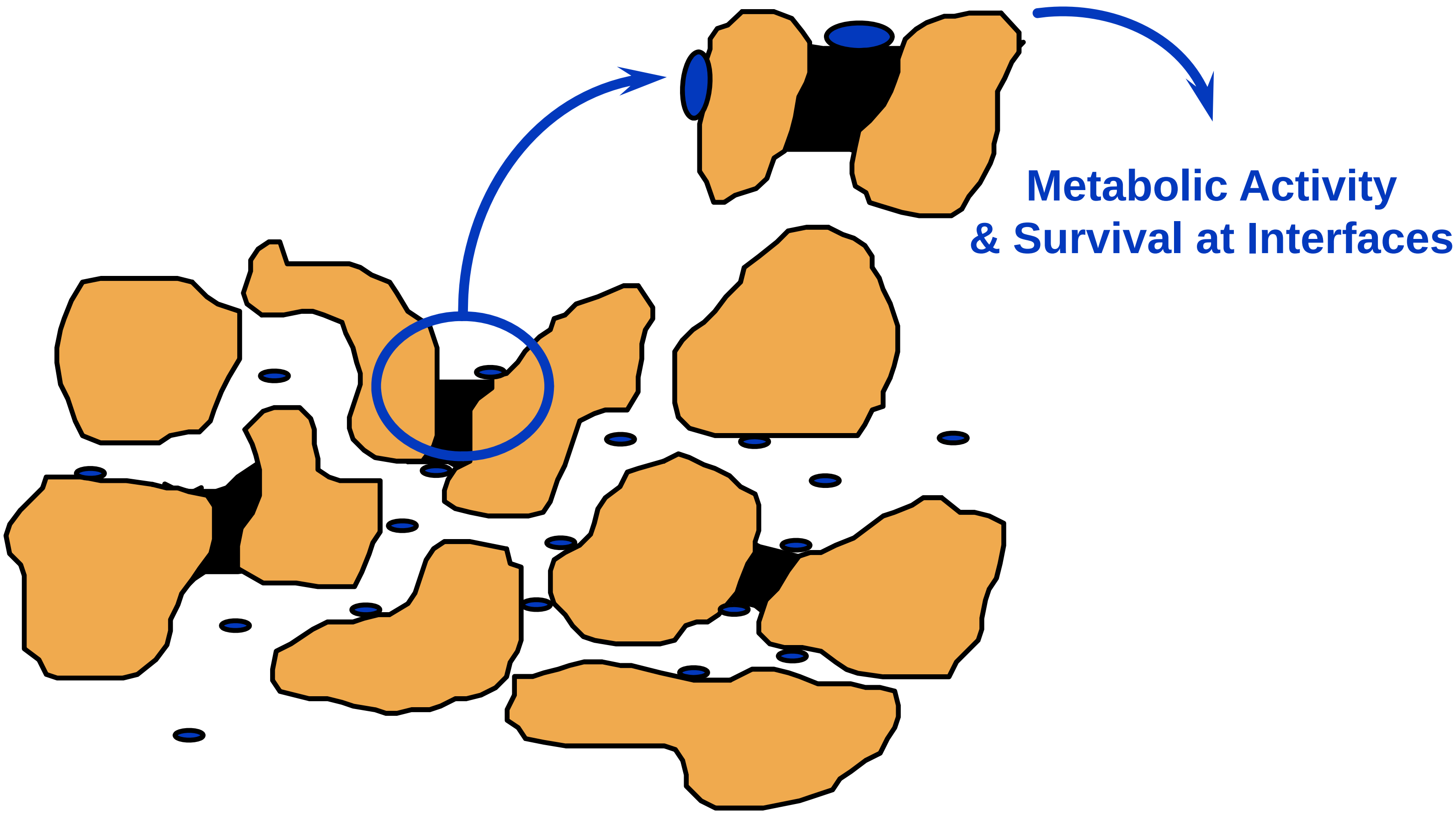
Surfactant-enhanced bioavailability theory is able to replicate multiple experimental data sets



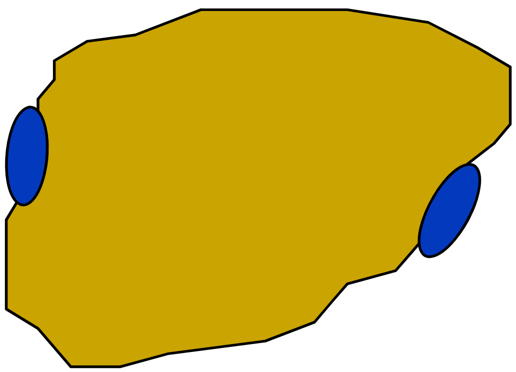


Brown, D.G. 2007. *Environ. Sci. Technol.*, 41(4):1194-1199

Effects of surfaces on metabolic activity and survival



Surfaces have been observed to stimulate bacterial metabolic activity and survival



Bacterial adhesion has been shown to provide:

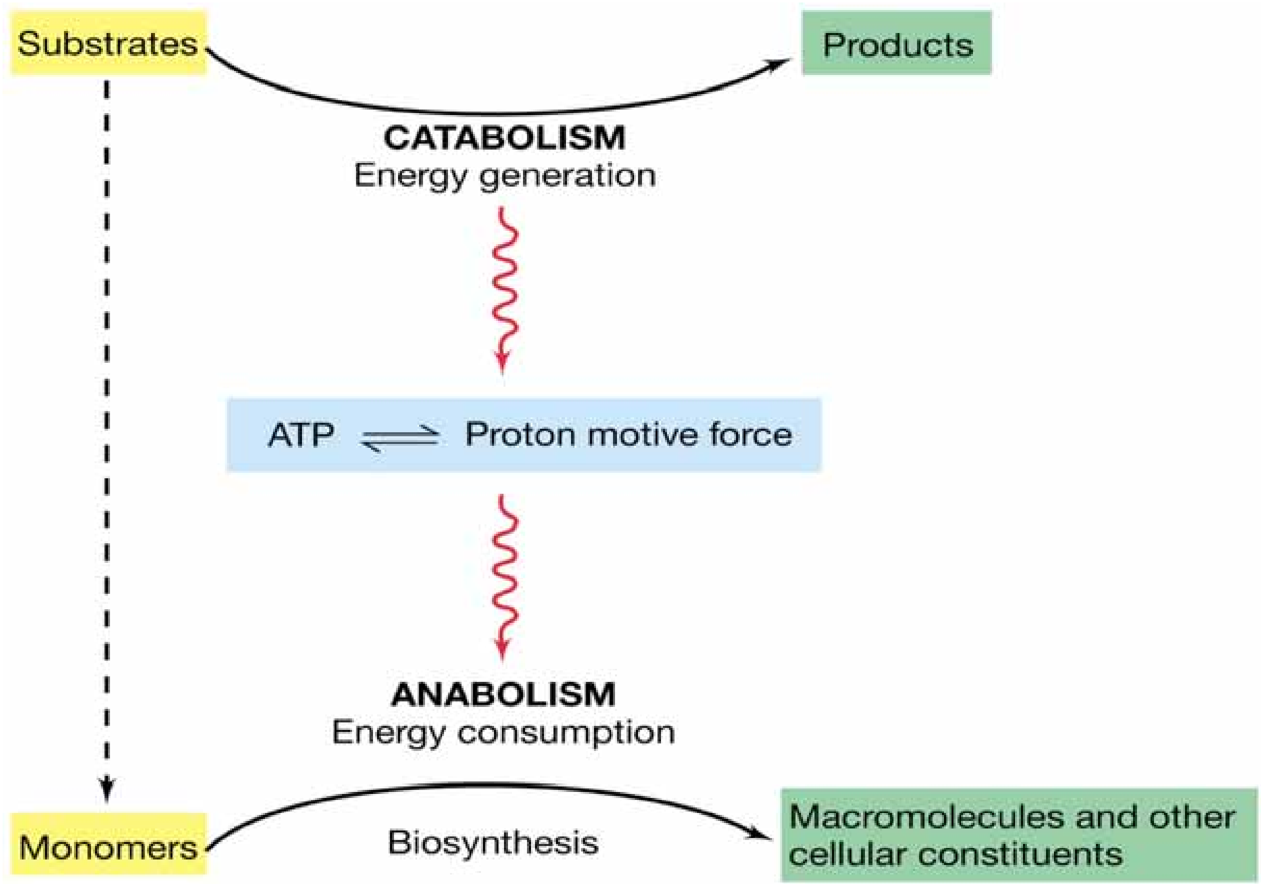
- Enhanced Metabolic Activity
- Enhanced Survivability

↓

Increased ATP levels

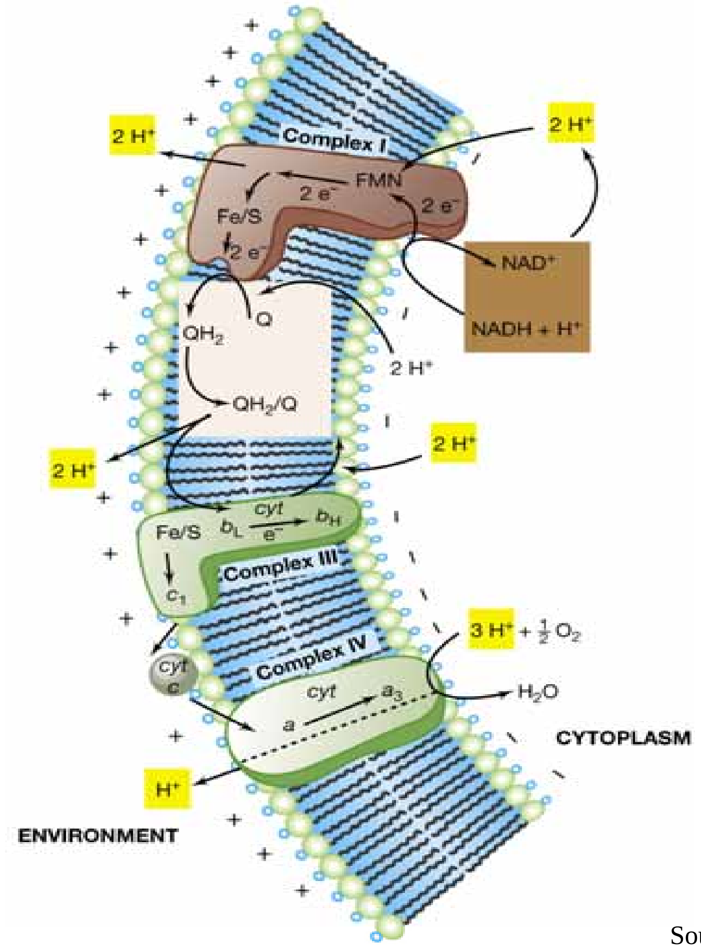
- Not due to nutrients
- Not due to substrate availability

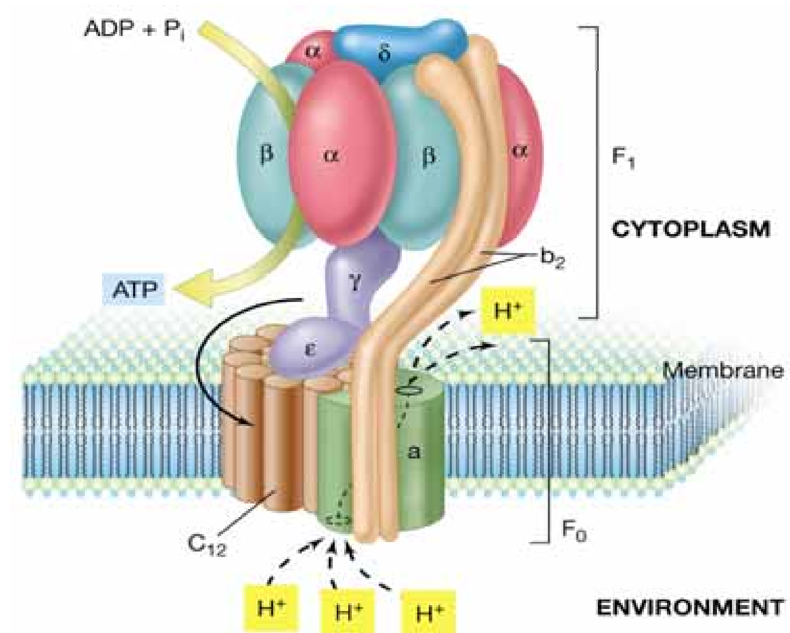
Cellular bioenergetics links catabolism and anabolism



Source: Madigan et al. 2003. Brock Biology of Microorganisms, 10th Edition. Prentice Hall.

Cellular bioenergetics uses a proton gradient across the cell membrane



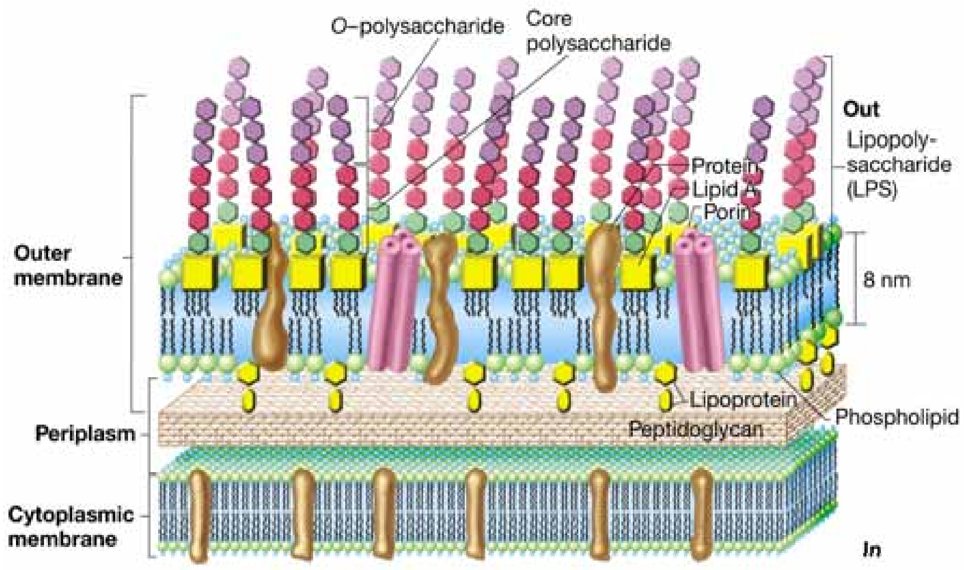


$$\Delta p = \Delta\psi - 59\Delta pH$$

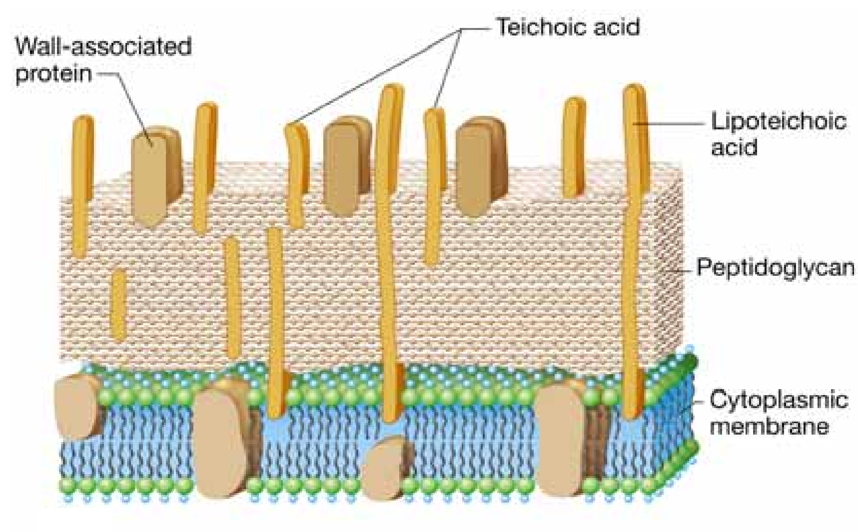
Source: Madigan et al. 2003. Brock Biology of Microorganisms, 10th Edition. Prentice Hall.

Cell wall structure of Gram-negative and Gram-positive bacteria

Gram-negative

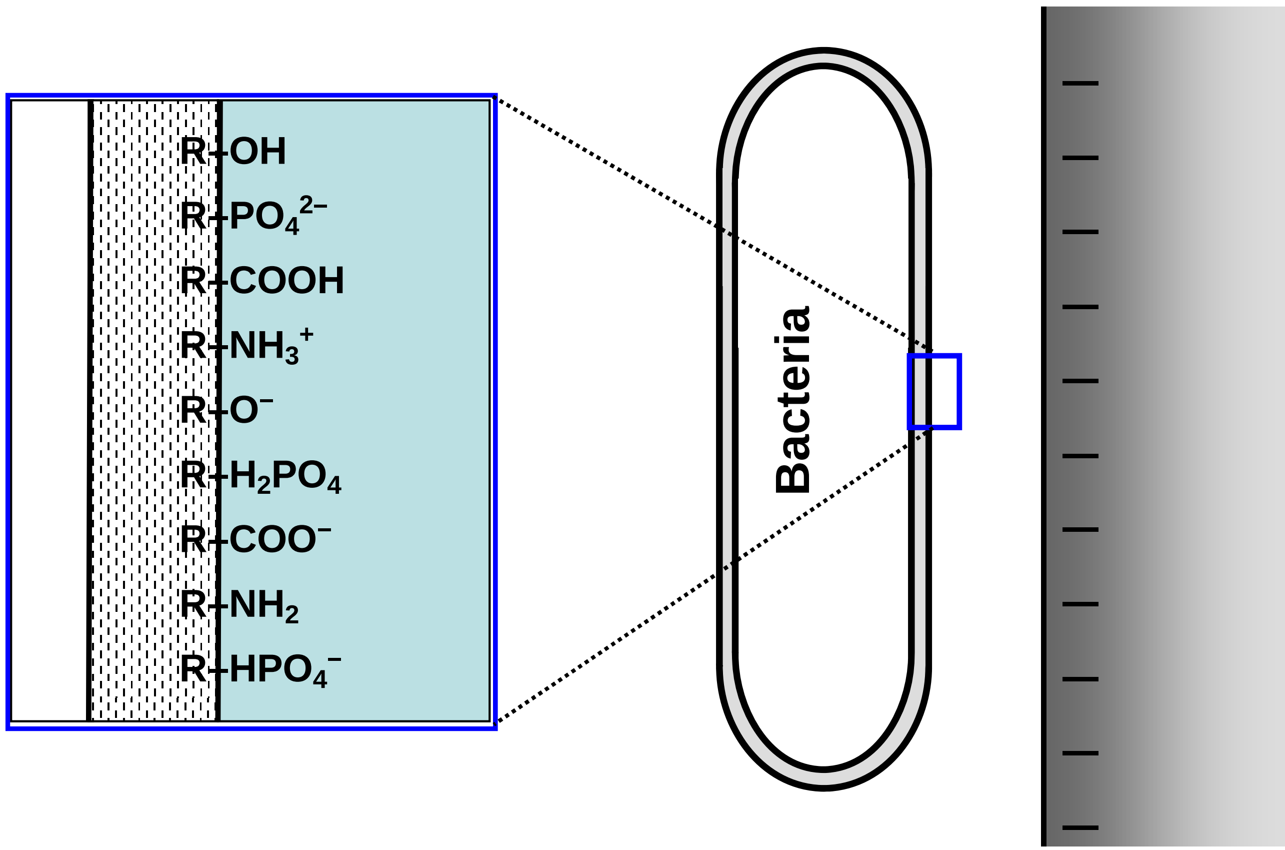


Gram-positive

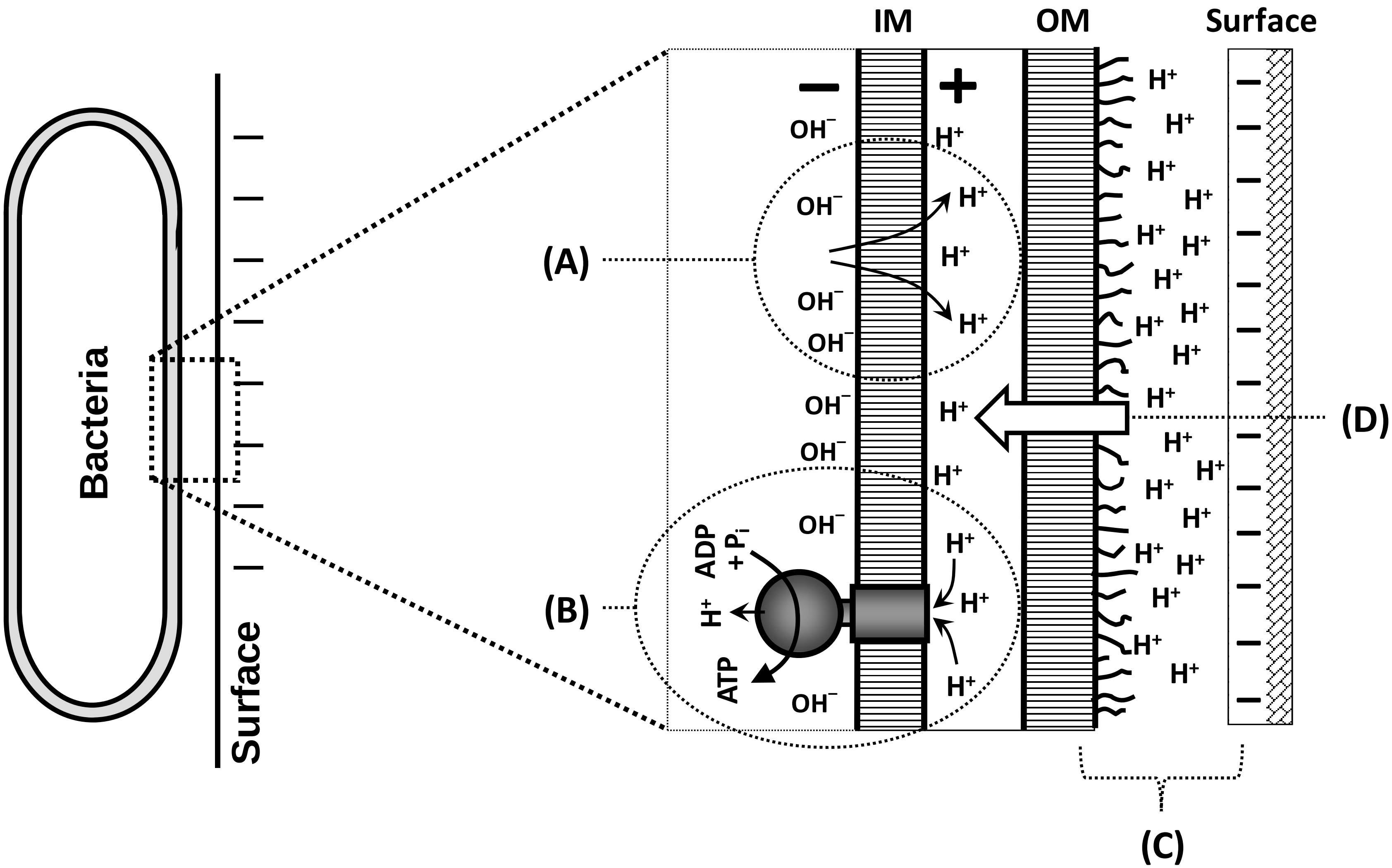


Source: Madigan et al. 2003. Brock Biology of Microorganisms, 10th Edition. Prentice Hall.

Charge-regulation effect can result in surface pH changes upon adhesion



Hypothesis: Charge regulation process alters surface pH, which alters the proton motive force



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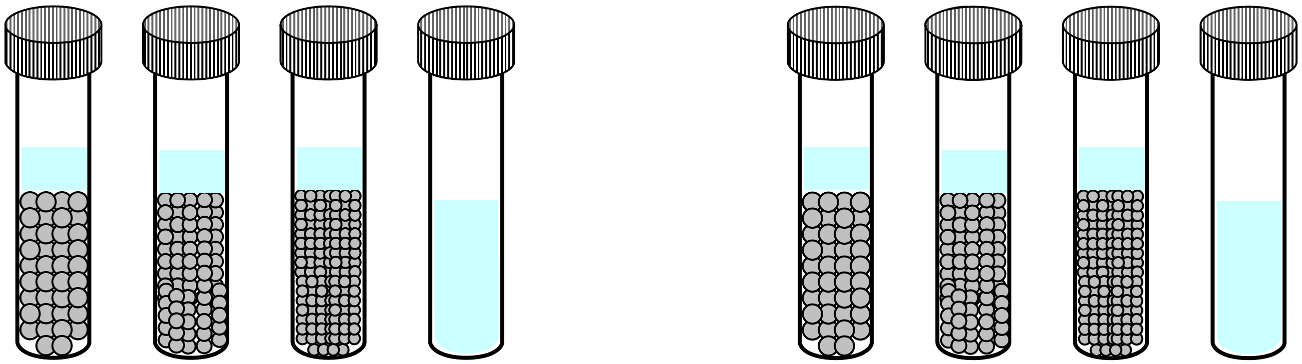
Experimental Approach

Bacteria | **Bacteria + Surfactant**

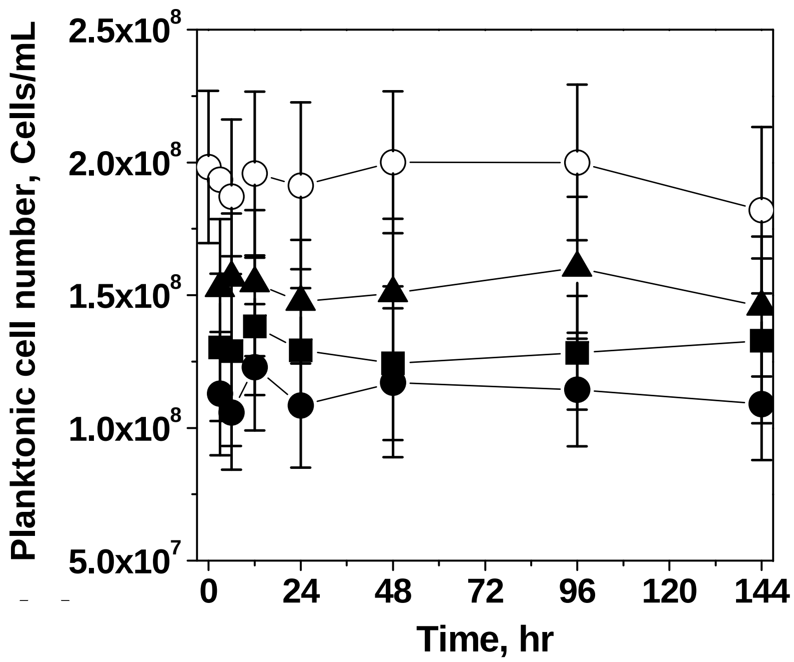


Diameter	Surface Area
2.5 mm	76.8 cm^2
1.0 mm	192 cm^2
0.5 mm	384 cm^2

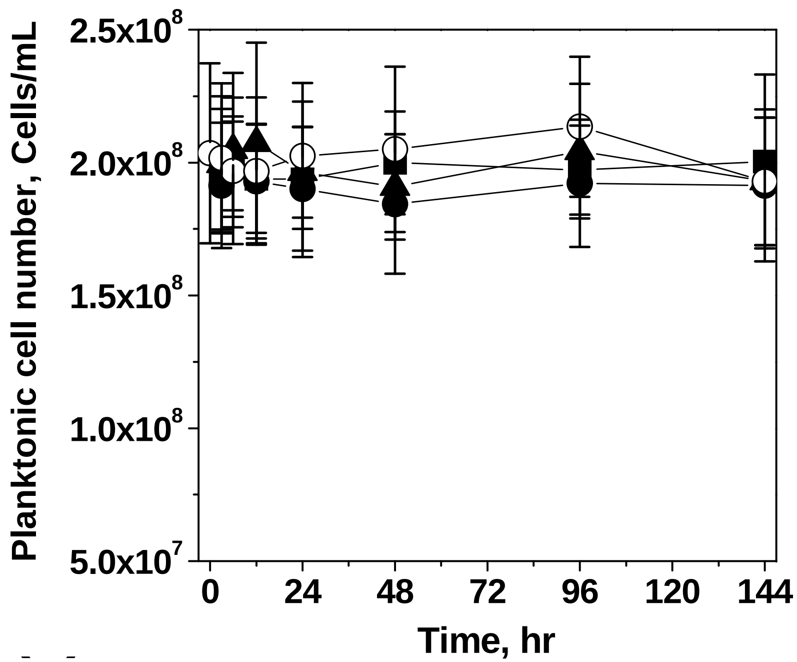
- Orbitron Shaker
- Samples taken over time
  - Adhesion
  - ATP

Adhesion as a function of surface area

E. coli in 0.01 M Phosphate buffer



E. coli in 0.01 M Phosphate buffer + Tween 80



● 0.5 mm ■ 1.0 mm ▲ 2.0 mm ○ No beads

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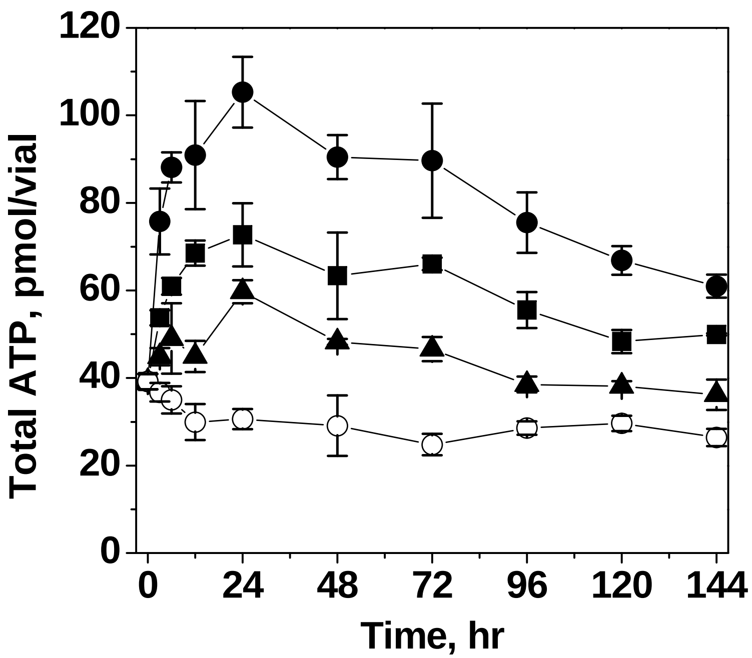
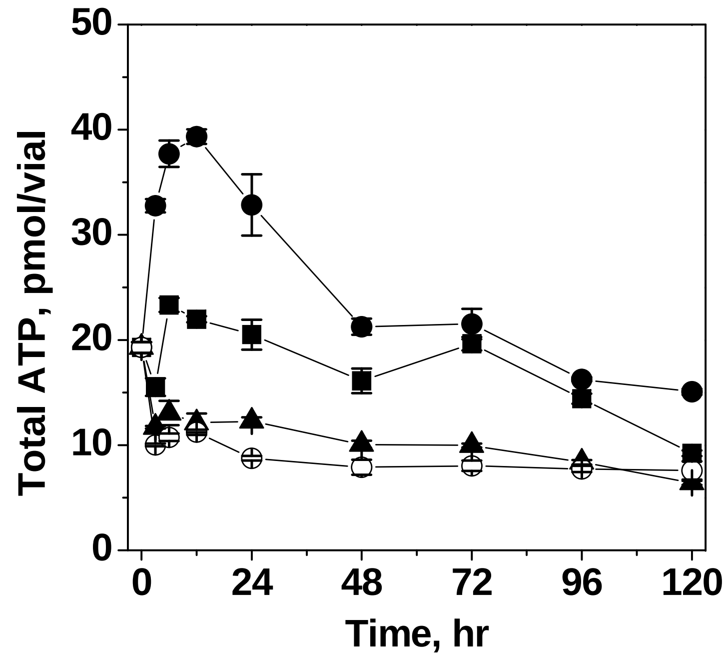
Total ATP as a function of adhesion

E. coli **in 0.01 M Phosphate buffer + Tween 80**



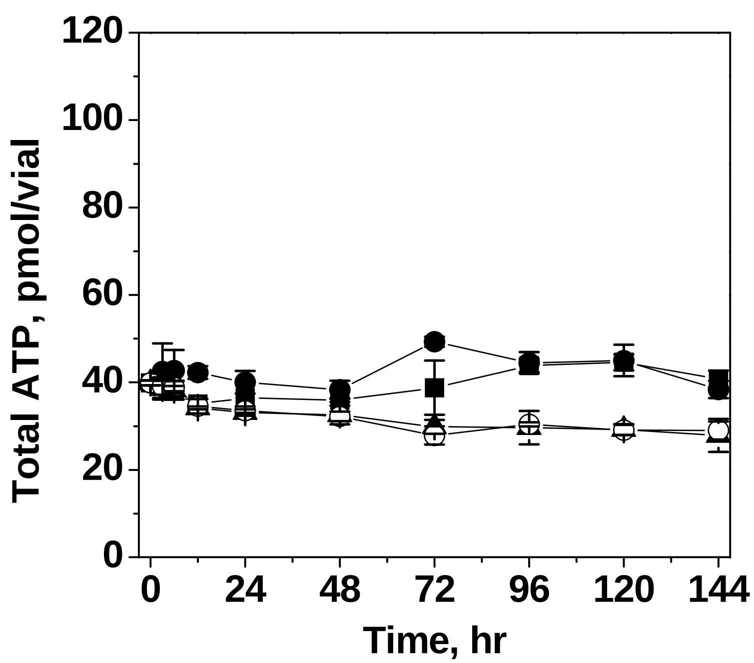
B. brevis **in 0.01 M Phosphate buffer + Tween 80**



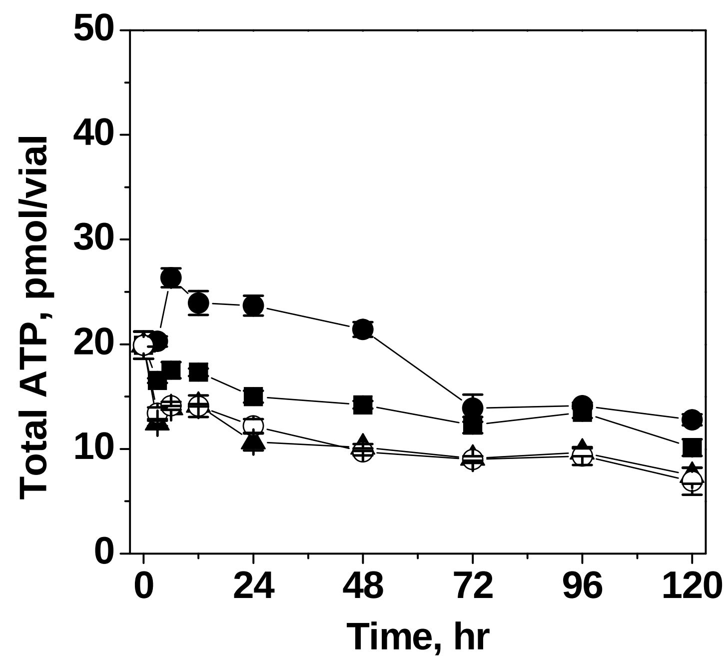
● **0.5 mm** ■ **1.0 mm** ▲ **2.0 mm** ○ **No beads**

Total ATP as a function of adhesion

E. coli in 0.01 M Phosphate buffer + Tween 80

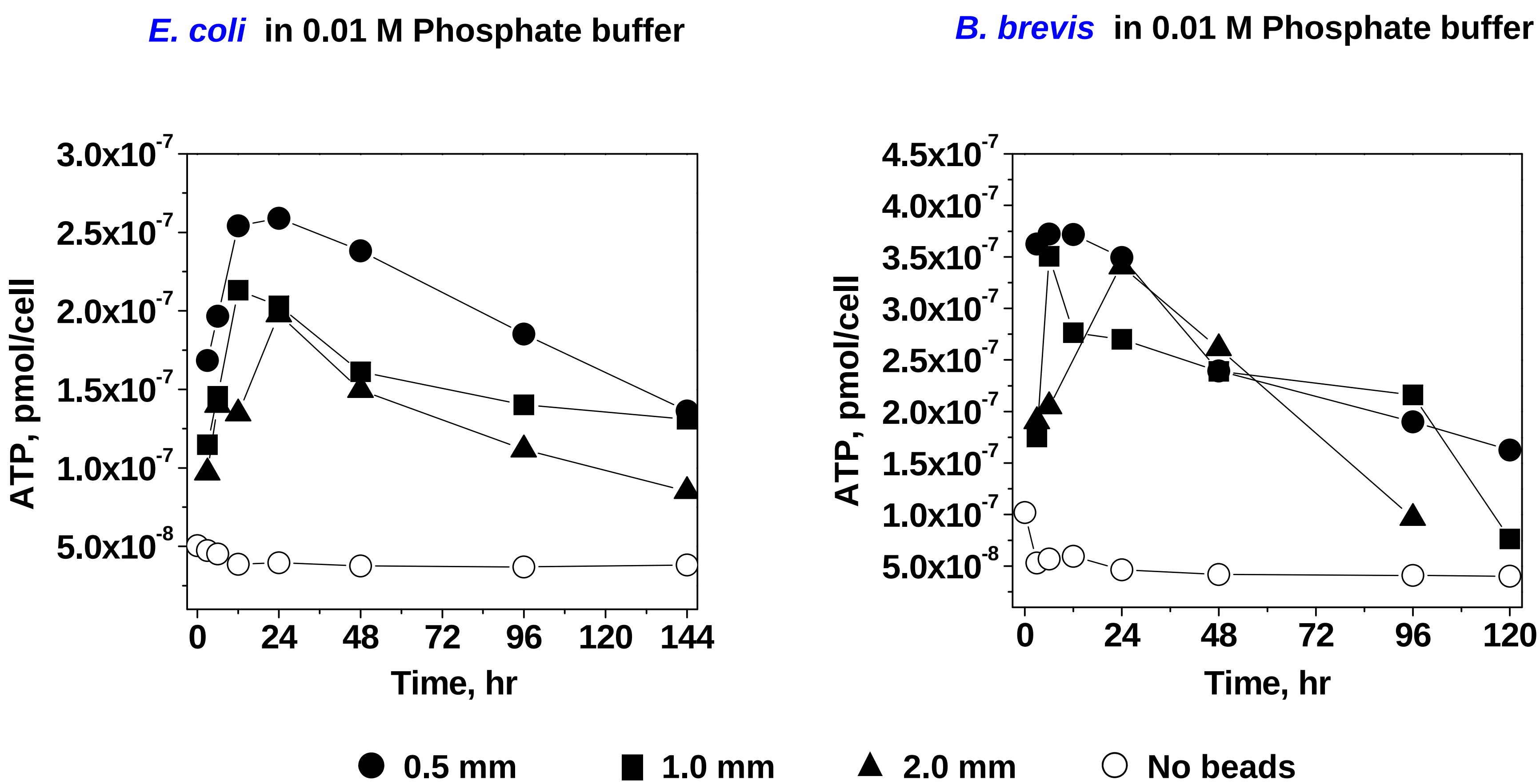


B. brevis in 0.01 M Phosphate buffer + Tween 80

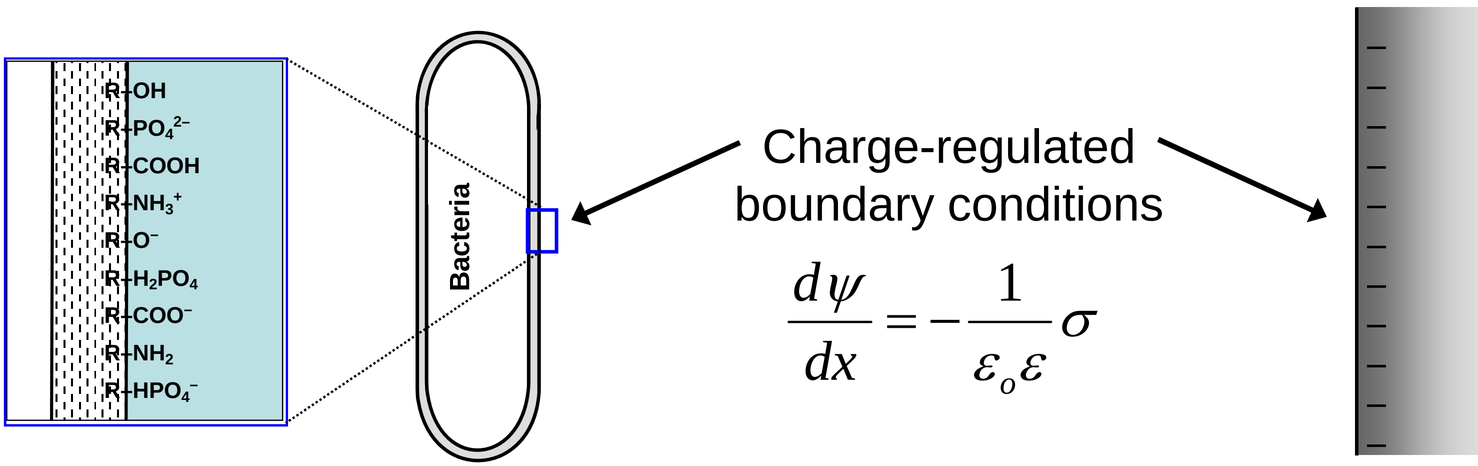


● 0.5 mm ■ 1.0 mm ▲ 2.0 mm ○ No beads

ATP per cell for attached and planktonic bacteria



Charge-regulation model accounts for acid/base properties of the glass and bacterial surfaces



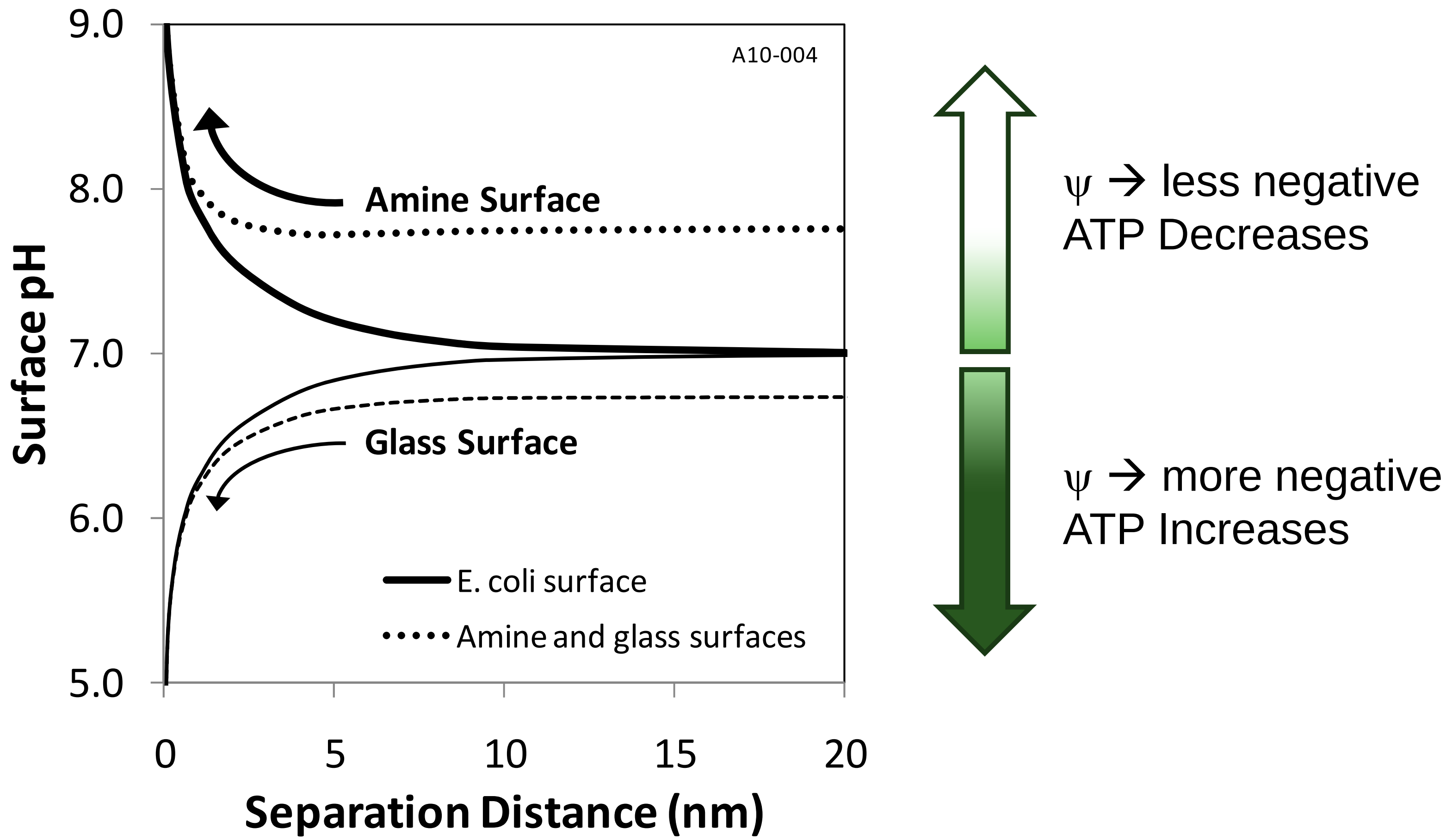
$$\frac{d\psi}{dx} = -\frac{1}{\varepsilon_o \varepsilon}\sigma$$

$$\frac{d^2\psi}{dx^2} = -\frac{1}{\varepsilon_o \varepsilon}\sum_{i=1}^{I} n_{ri} z_i e \exp\left(\frac{-z_i e \psi}{kT}\right)$$

Poisson-Boltzmann Equation

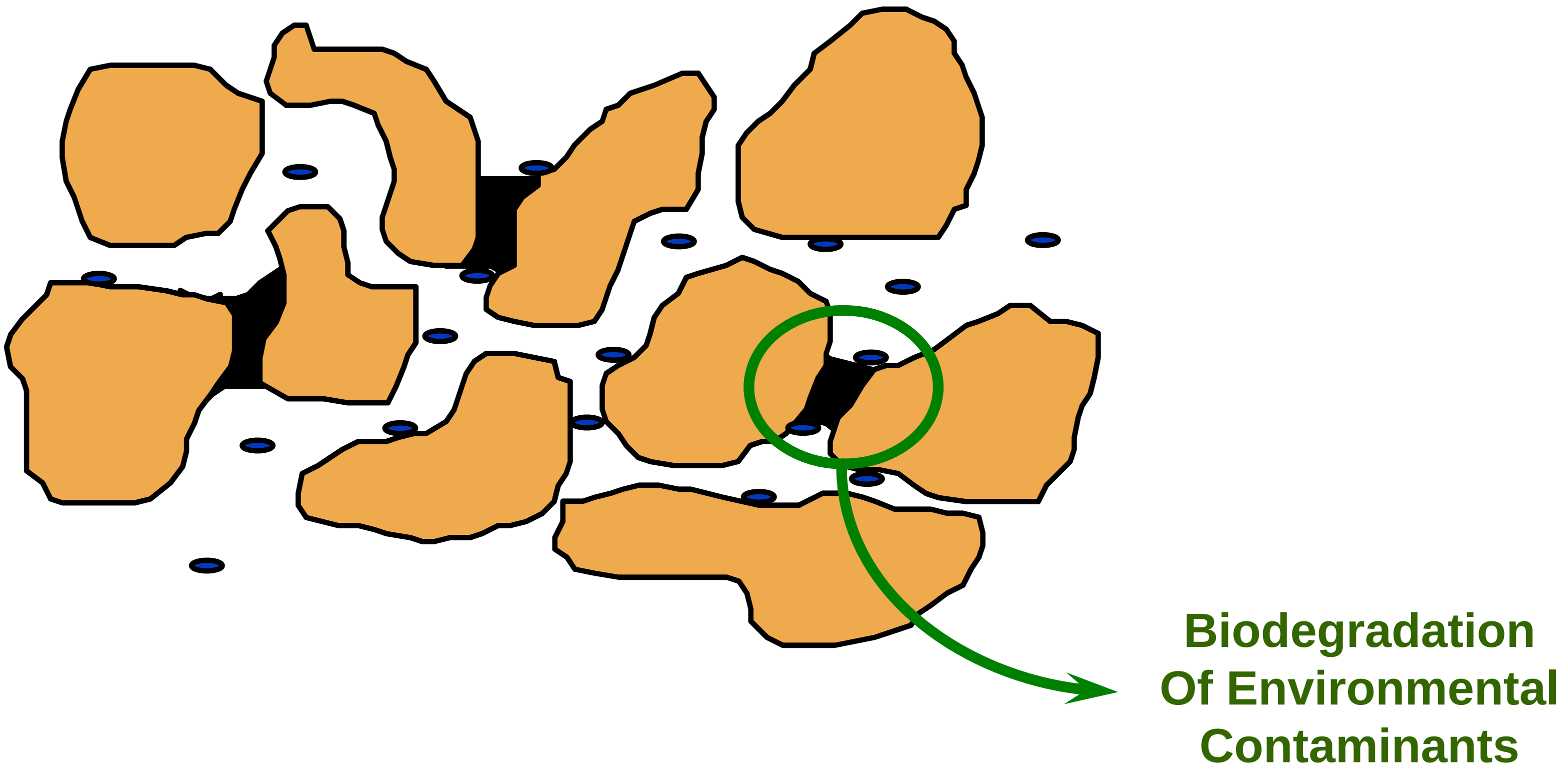
Hong and Brown. 2006. *Colloids and Surfaces B: Biointerfaces*. 50(2):112-119.
Hong and Brown. 2008. *Langmuir*. 24(9):5003-5009.

How surface charge affects surface pH and cellular ATP



Hong and Brown. 2010. *Biotechnology and Bioengineering.* 105(5):965-972.

Impacts of microbial viruses (bacteriophages) on the design and operation of engineered systems



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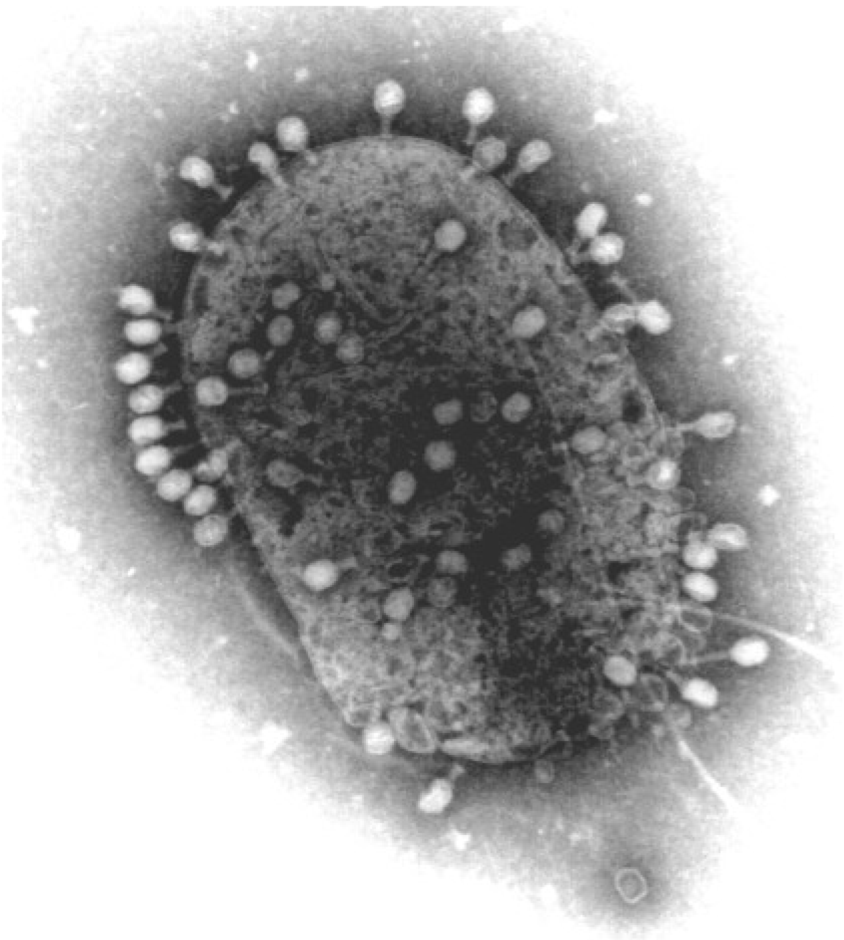
Wastewater treatment plants are big bioreactors!

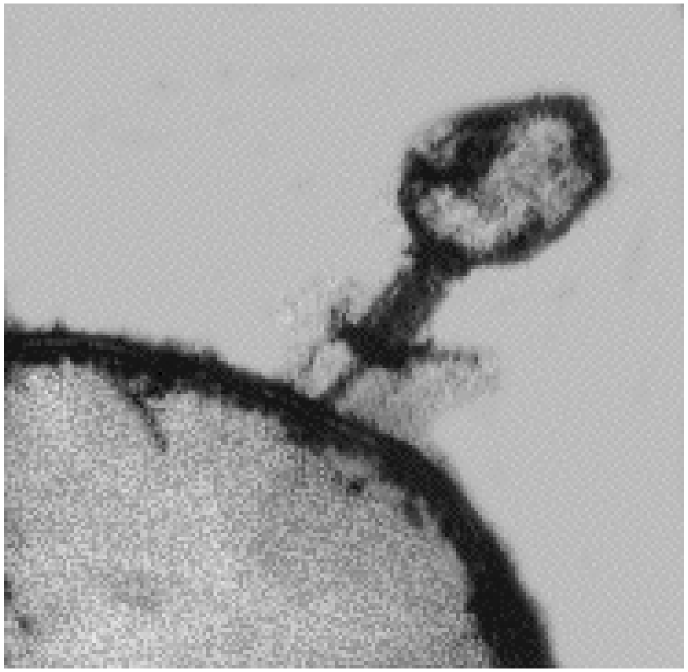
We design systems like these considering biological kinetics

Bacteria: $$\frac{dX}{dt} = \mu_{max} \frac{SX}{K_s + S} - k_d X$$

Growth Substrate: $$\frac{dS}{dt} = -\frac{\mu_{max}}{Y} \frac{SX}{K_s + S}$$

This approach does not consider the impacts of bacteriophage on the system dynamics





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Add a few more equations and we can model the systems by considering the impact of bacteriophages

Susceptible Bacteria: $$\frac{dX}{dt} = \mu_{max}^{X} \frac{SX}{K_S^X + S} - k_d^X X - \delta PX\left(1 - e^{-\lambda}\right)$$

Infected Bacteria: $$\frac{dI}{dt} = \delta PX\left(1 - e^{-\lambda}\right) - \frac{d\hat{I}}{dt} - k_d I$$

Resistant Bacteria: $$\frac{dR}{dt} = \mu_{max}^{R} \frac{SR}{K_S^R + S} - k_d^R R$$

Bacteriophages: $$\frac{dP}{dt} = B\frac{d\hat{I}}{dt} - k_d^P P - \delta P\left(X + I + R\right)$$

Growth Substrate: $$\frac{dS}{dt} = -\frac{\mu_{max}^{X}}{Y^X} \frac{SX}{K_S^X + S} - \frac{\mu_{max}^{R}}{Y^R} \frac{SR}{K_S^R + S} - q_{max}^{I} \frac{SI}{K_S^I + S}$$

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All the model parameters can be determined from independent experiments

$$\frac{dS}{dt} = -\frac{\mu^x_{max}}{Y_x}\frac{SX}{K^x_S + S} - \frac{\mu^R_{max}}{Y_R}\frac{SR}{K^R_S + S} - q_{max}\frac{SI}{K^I_S + S}$$

$$\frac{dX}{dt} = \mu^x_{max}\frac{SX}{K^x_S + S} - k^x_d X - \delta FX\left(1 - e^{-\lambda}\right)$$

$$\frac{dR}{dt} = \mu^R_{max}\frac{SR}{K^R_S + S} - k^R_d R$$

$$\frac{dI}{dt} = \delta FX\left(1 - e^{-\lambda}\right) - \frac{dI}{dt} - k_d I$$

$$\frac{dP}{dt} = B\frac{dI}{dt} - k^P_d P - \delta P(X + I + R)$$

Substrate utilization parameters for infected bacteria determined from batch experiments with an MOI > 3.

Bacterial kinetic parameters determined from batch and chemostat experiments with no phage present.

Adsorption rate (δ) is determined from batch experiments.

Lysis rate is calculated via t_{burst}, which is obtained from single-step growth experiments.

Phage decay rate determined from batch experiments with phage only.

Burst size determined from single-step growth experiments.

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Undergraduates are involved in all this research

- **Metabolic activity** – Long-term survival of attached and planktonic bacteria
- **Metabolic activity** – Development of method for determination of surface charge properties of macroscopic materials

- **Bacteriophage kinetics** – Effects of bacterial growth rate on phage burst size and time to burst.
- **Bacterophage kinetics** – Factors that affect bacteriophage adhesion rate.

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Theme…

You can learn a lot from simple experiments

Environmental Biotechnology