

Impact of *lux* Gene Insertion on Bacterial Surface Properties and Transport

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Potential groundwater contamination with pathogens

Wastewater reclamation for irrigation

- In California, 60% of recycled wastewater used for irrigation



Animal waste applications

- In US, approximately 1.3 billion tons of animal manure generated annually
- Land applications



Objectives

- *lux* genes as genetic marker
- Bacterial surface characterization
- Impact of *lux* gene insertion on bacterial surface property
- Impact of *lux* gene insertion on bacterial transport

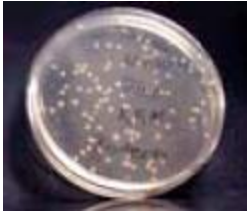


Outline

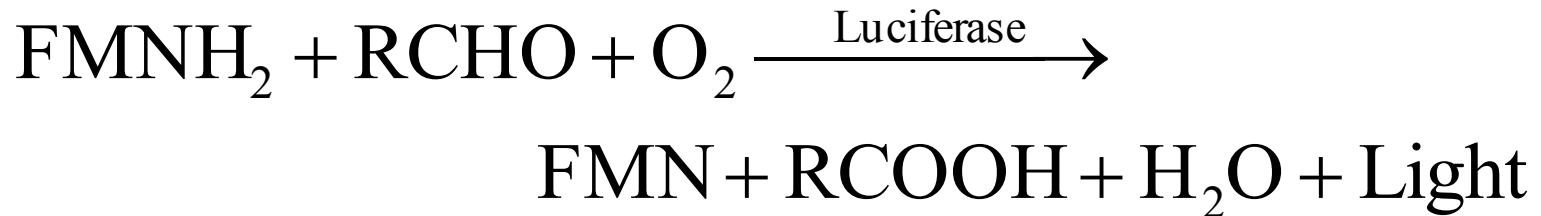
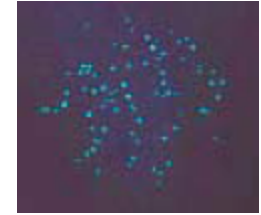
- *lux* genes as bioreporter
- Bacterial surface property quantification
- Bacterium-sediment interaction calculations
 - Lifshitz-van der waals interactions
 - Lewis acid/base interactions
 - Electrostatic interactions
- Bacterial transport and modeling
- Conclusion remarks



lux Gene Techniques



Bioluminescence reaction



Luciferase – Enzyme that catalyze bioluminescence reaction

FMNH₂ – Reduced riboflavin mononucleotide

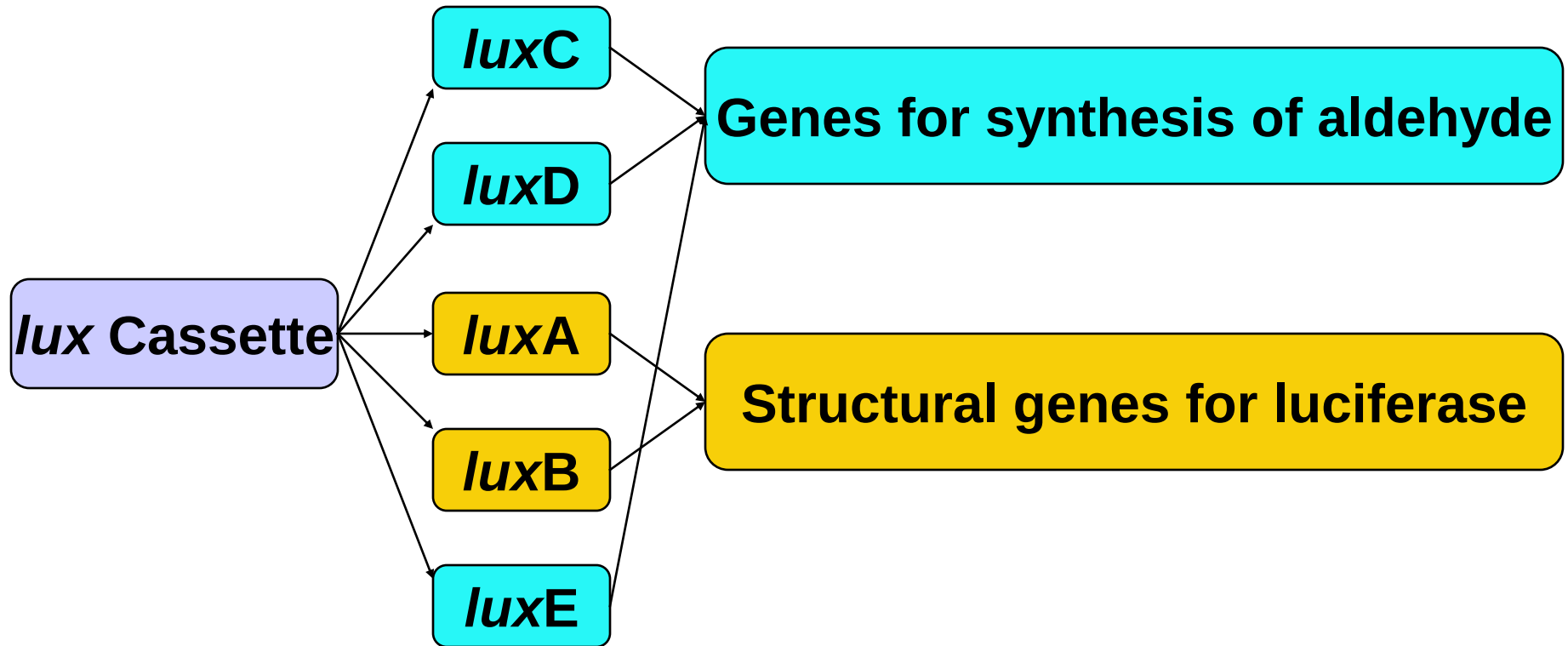
RCHO – Long-chain fatty aldehyde

FMN – Riboflavin mononucleotide

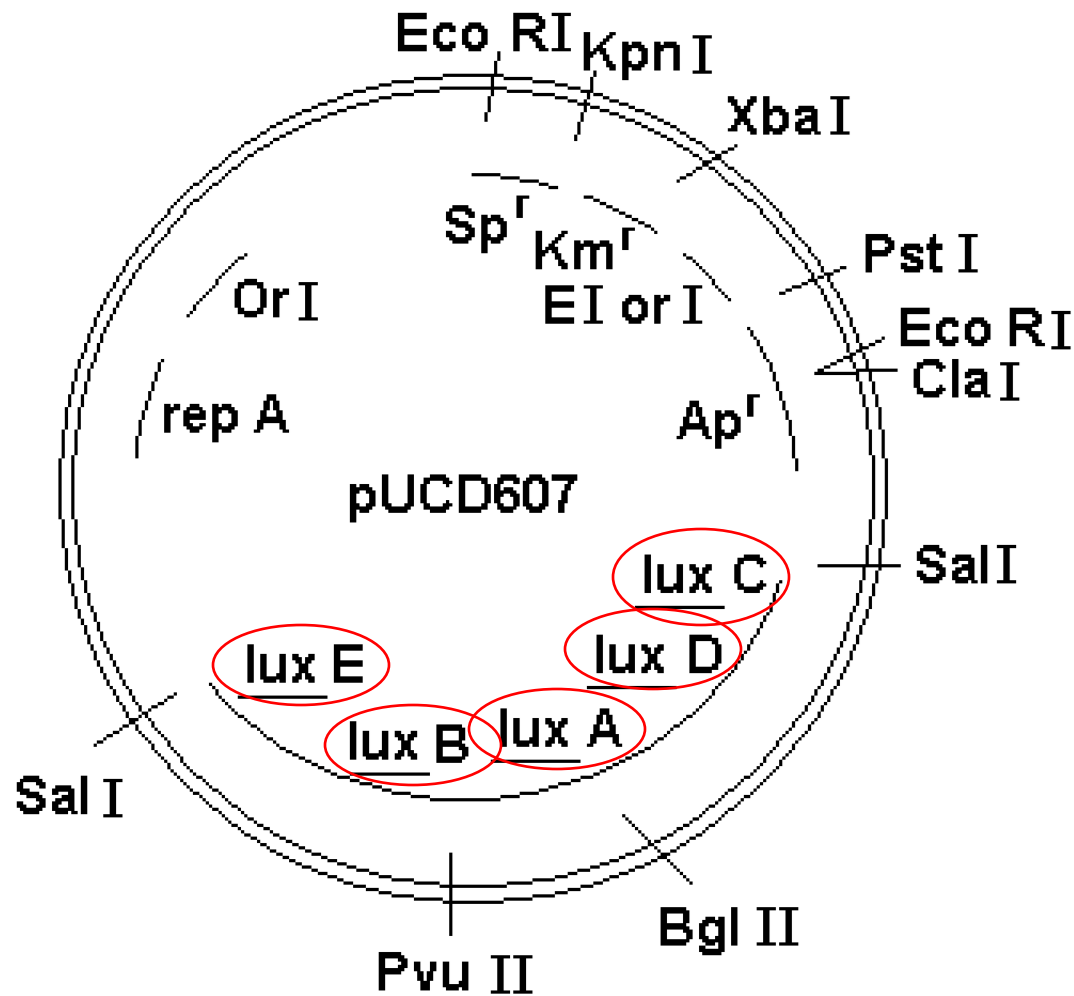
RCOOH – Carboxylic acid



lux Cassette



Plasmid pUCD607



Bacterial Strains



L. casei



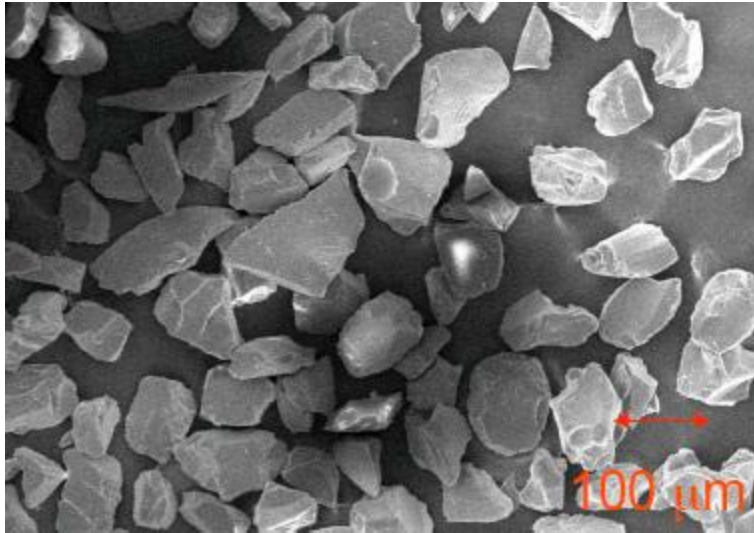
S. mitis



M. luteus

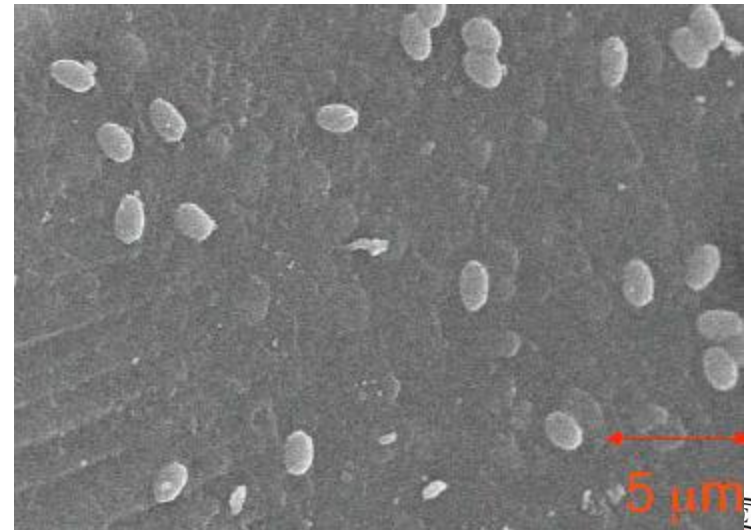


Medium



Silica sand

Silica sand with attached cells



Conjugation of pUCD607 into *P. aeruginosa*

Non-quantitative triparental mating

E. coli HB101/pUCD607

E. coli HB101/pRK2013

P. aeruginosa

Donor Strain

Helper Strain

Recipient

pUCD607

P. aeruginosa/pUCD607



Bacterial Surface Property Quantification

$$\gamma^{LW}$$

$$\gamma^{+}$$

$$\gamma^{-}$$

$$\zeta$$



γ^{LW} – Liftshitz-van der Waals component surface tension (mJ/m²)

γ^{+} – Electron-acceptor parameter of Lewis acid/base component surface tension (mJ/m²)

γ^{-} – Electron-donor parameter of Lewis acid/base component surface tension (mJ/m²)

ζ – Zeta potential (mV)



Surface Property Calculation

van Oss-Chaudhury-Good Equation

$$(1 + \cos \theta) \gamma_L = 2(\sqrt{\gamma_S^{LW} \gamma_L^{LW}} + \sqrt{\gamma_S^+ \gamma_L^-} + \sqrt{\gamma_S^- \gamma_L^+})$$

$$\left\{ \begin{array}{l} (1 + \cos \theta_1) \gamma_1 = 2(\sqrt{\gamma_S^{LW} \gamma_1^{LW}} + \sqrt{\gamma_S^+ \gamma_1^-} + \sqrt{\gamma_S^- \gamma_1^+}) \\ (1 + \cos \theta_2) \gamma_2 = 2(\sqrt{\gamma_S^{LW} \gamma_2^{LW}} + \sqrt{\gamma_S^+ \gamma_2^-} + \sqrt{\gamma_S^- \gamma_2^+}) \\ (1 + \cos \theta_3) \gamma_3 = 2(\sqrt{\gamma_S^{LW} \gamma_3^{LW}} + \sqrt{\gamma_S^+ \gamma_3^-} + \sqrt{\gamma_S^- \gamma_3^+}) \end{array} \right.$$

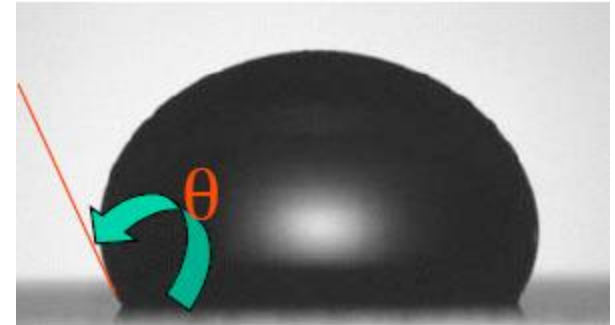
Solve for γ_S^{LW} , γ_S^+ and γ_S^-



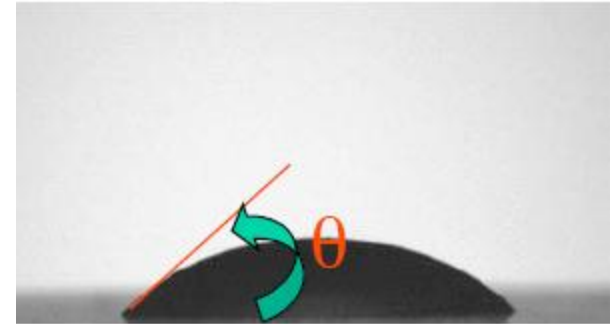
Contact Angle Measurement



- A : Moisture-controlled Chamber
- B : Sample platform
- C : Syringe holder
- D : Camera with imaging lens
- E : Light source



Contact Angle $> 90^\circ$



Contact Angle $< 90^\circ$

Wicking Method



Washburn Equation

$$h^2 = (R_e \cdot t \cdot \gamma_L \cdot \cos\theta) \cdot (2 \cdot \mu)^{-1}$$

h : height of capillary rise (m)

R_e : average interstitial pore size (m)

t : measuring time (sec)

γ_L : Measuring liquid surface tension (mJ/m²)

μ : measuring liquid viscosity (N·s/m²)

Kruss K100

Tensiometer



Bacterial Surface Properties

	θ^{DH} (°)	θ^{F} (°)	θ^{W} (°)	γ^{LW} (mJ/m ²)	γ^+ (mJ/m ²)	γ^- (mJ/m ²)	ζ (mV)
<i>L. casei</i>	22.2 ± 0.3	18.1 ± 0.1	48.6 ± 0.4	47.1	1.18	20.7	- 24.7 ± 0.8
<i>L. casei</i> /pUCD607	34.2 ± 0.4	32.5 ± 0.6	55.1 ± 0.7	42.4	0.99	18.7	- 28.1 ± 0.6
<i>S. mitis</i>	28.8 ± 0.7	28.5 ± 0.6	49.5 ± 0.5	44.7	0.74	23.5	- 27.1 ± 0.4
<i>S. mitis</i> /pUCD607	36.8 ± 0.6	37.2 ± 0.5	55.0 ± 0.3	41.2	0.65	21.2	- 32.8 ± 0.7
<i>M. luteus</i>	37.6 ± 0.3	32.6 ± 0.7	49.5 ± 0.2	40.8	0.89	25.4	- 31.6 ± 0.5
<i>M. luteus</i> /pUCD607	42.0 ± 0.4	40.4 ± 0.5	57.0 ± 0.6	38.6	0.76	20.4	- 38.6 ± 0.7
Silica Sand	70.3 ± 1.2	64.5 ± 0.6	68.5 ± 0.2	22.7	1.57	15.4	- 42.3 ± 0.5



Interaction Free Energy Calculation

$$\Delta G_{132}^{\text{TOT}} = \Delta G_{132}^{\text{LW}} + \Delta G_{132}^{\text{AB}} + \Delta G_{132}^{\text{EL}}$$

$$\Delta G(y)_{132}^{\text{LW}} = -4\pi \frac{y_0^2}{y} R [(\sqrt{\gamma_3^{\text{LW}}} - \sqrt{\gamma_2^{\text{LW}}})(\sqrt{\gamma_3^{\text{LW}}} - \sqrt{\gamma_1^{\text{LW}}})]$$

$$\Delta G(y)_{132}^{\text{AB}} = 4\pi R y_0 e^{(y-y_0)/\lambda} [(\sqrt{\gamma_1^+} - \sqrt{\gamma_2^+})(\sqrt{\gamma_1^-} - \sqrt{\gamma_2^-}) - (\sqrt{\gamma_1^+} - \sqrt{\gamma_3^+})(\sqrt{\gamma_1^-} - \sqrt{\gamma_3^-}) - (\sqrt{\gamma_2^+} - \sqrt{\gamma_3^+})(\sqrt{\gamma_2^-} - \sqrt{\gamma_3^-})]$$

$$\Delta G(y)_{132}^{\text{EL}} = \pi \epsilon \epsilon_0 R [2\psi_1 \psi_2 \text{Ln}(\frac{1+e^{-\kappa y}}{1-e^{-\kappa y}}) + (\psi_1^2 + \psi_2^2) \text{Ln}(1-e^{-2\kappa y})]$$



Interaction Free Energies

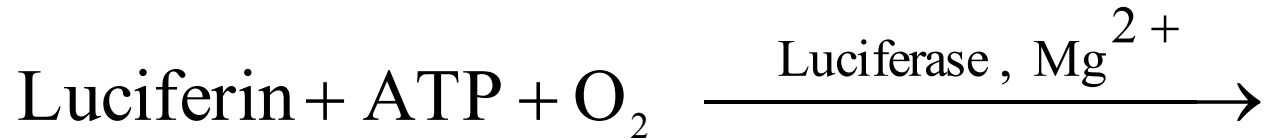
	ΔG_{132}^{LW} (kT)	ΔG_{132}^{AB} (kT)	ΔG_{132}^{EL} (kT)	ΔG_{132}^{TOT} (kT)
<i>L. casei</i>	- 50.2	- 1525.5	236.2	- 1339.5
<i>L. casei</i> /pUCD607	- 42.2	- 1755.5	312.7	- 1485.0
<i>S. mitis</i>	- 46.2	- 1315.2	290.9	- 1070.5
<i>S. mitis</i> /pUCD607	-40.0	- 1551.4	407.4	- 1184.0
<i>M. luteus</i>	- 39.2	- 1117.7	384.4	- 772.5
<i>M. luteus</i> /pUCD607	- 35.3	- 1613.6	506.4	- 1142.5



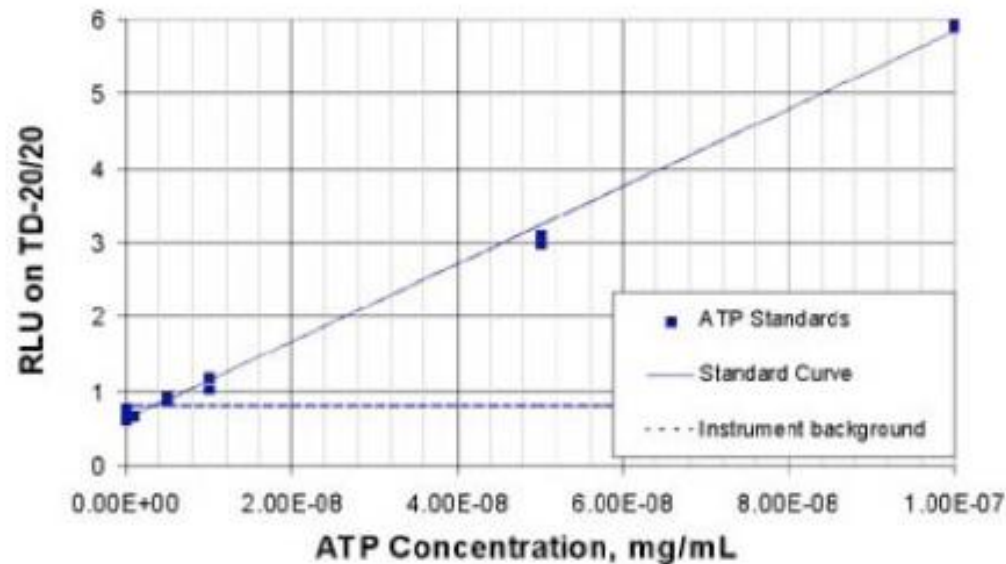
Column Experiment



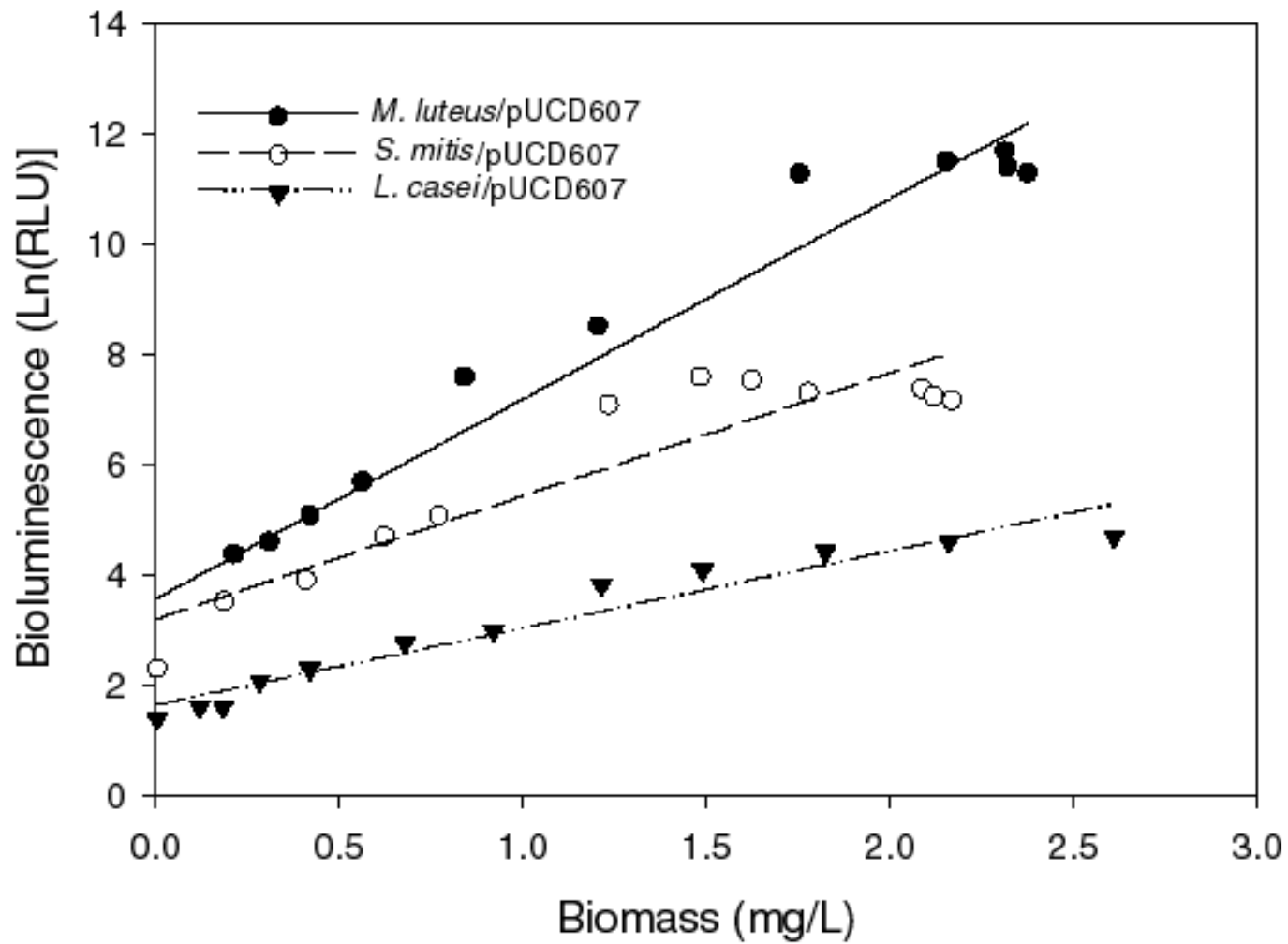
ATP Measurement



Oxyluciferin + AMP + CO₂ + PP_i + Light (560 nm)



Medium



Bacterial Transport Modeling

Two Region Model

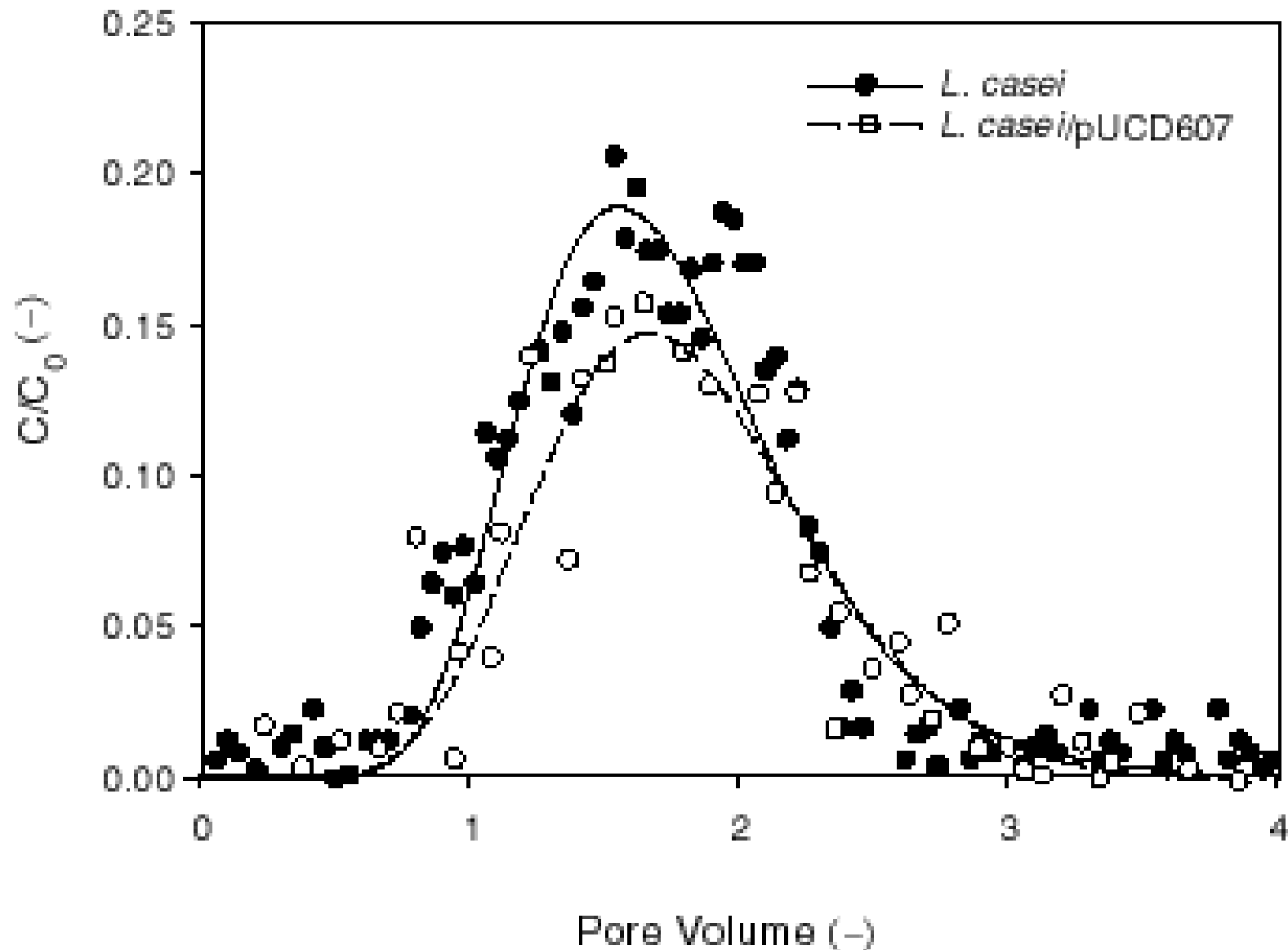
$$\left[1 + \frac{f\rho_b K_b}{\eta}\right] \frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} - v \frac{\partial C}{\partial x} - \alpha \frac{\rho_b}{\eta} [(1-f)K_b C - S_k]$$

$$\frac{\partial S_k}{\partial t} = \alpha [(1-f)K_b C - S_k] - \mu_{s,k} S_k$$

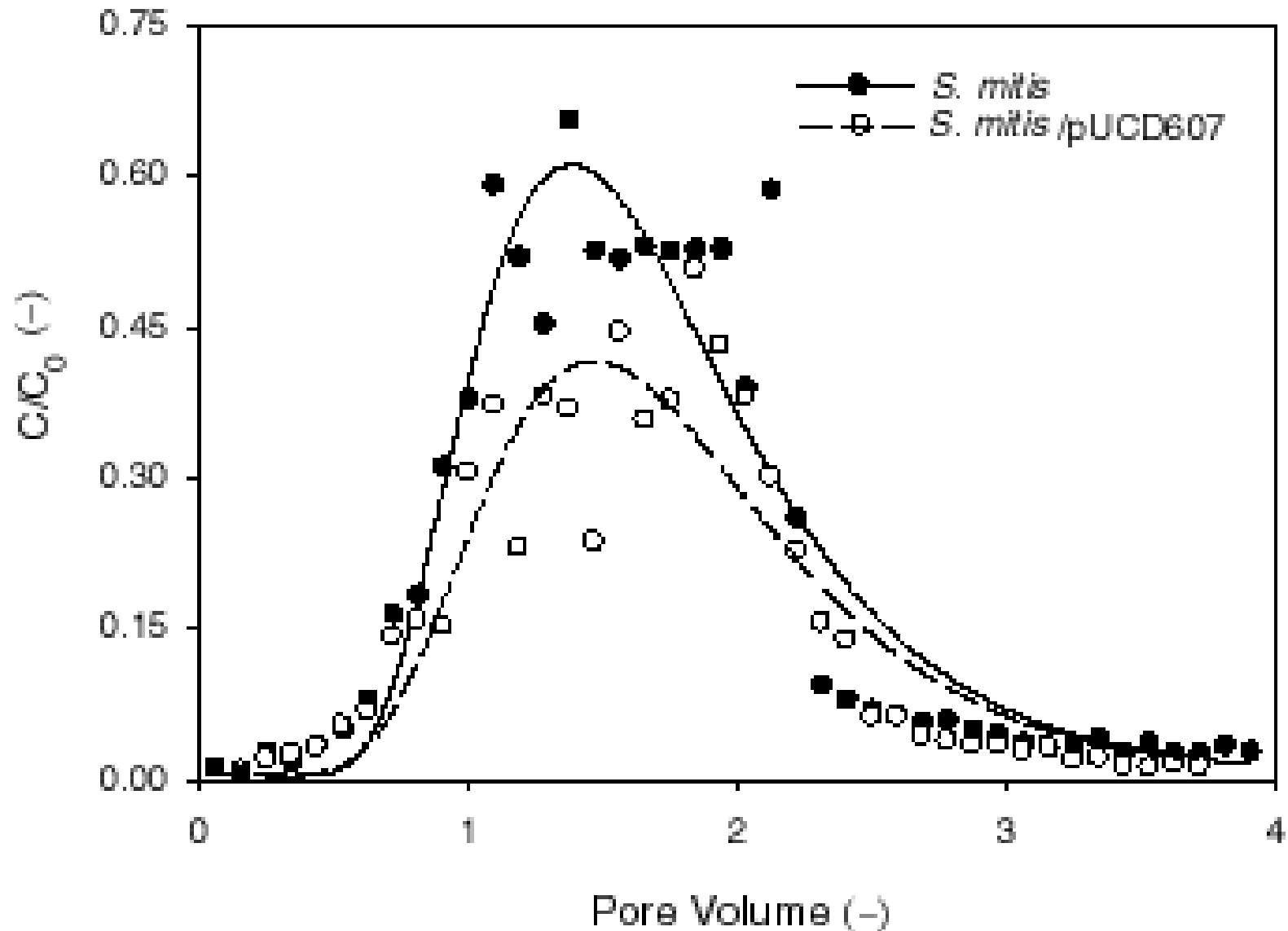
- f – Fraction of equilibrium sorption sites
- K_b – Partitioning coefficient of bacteria to equilibrium sorption sites (m^3/g)
- α – Mass transfer coefficient (min^{-1})
- $\mu_{s,k}$ – Bacterial deposition coefficient on kinetic sorption sites (min^{-1})



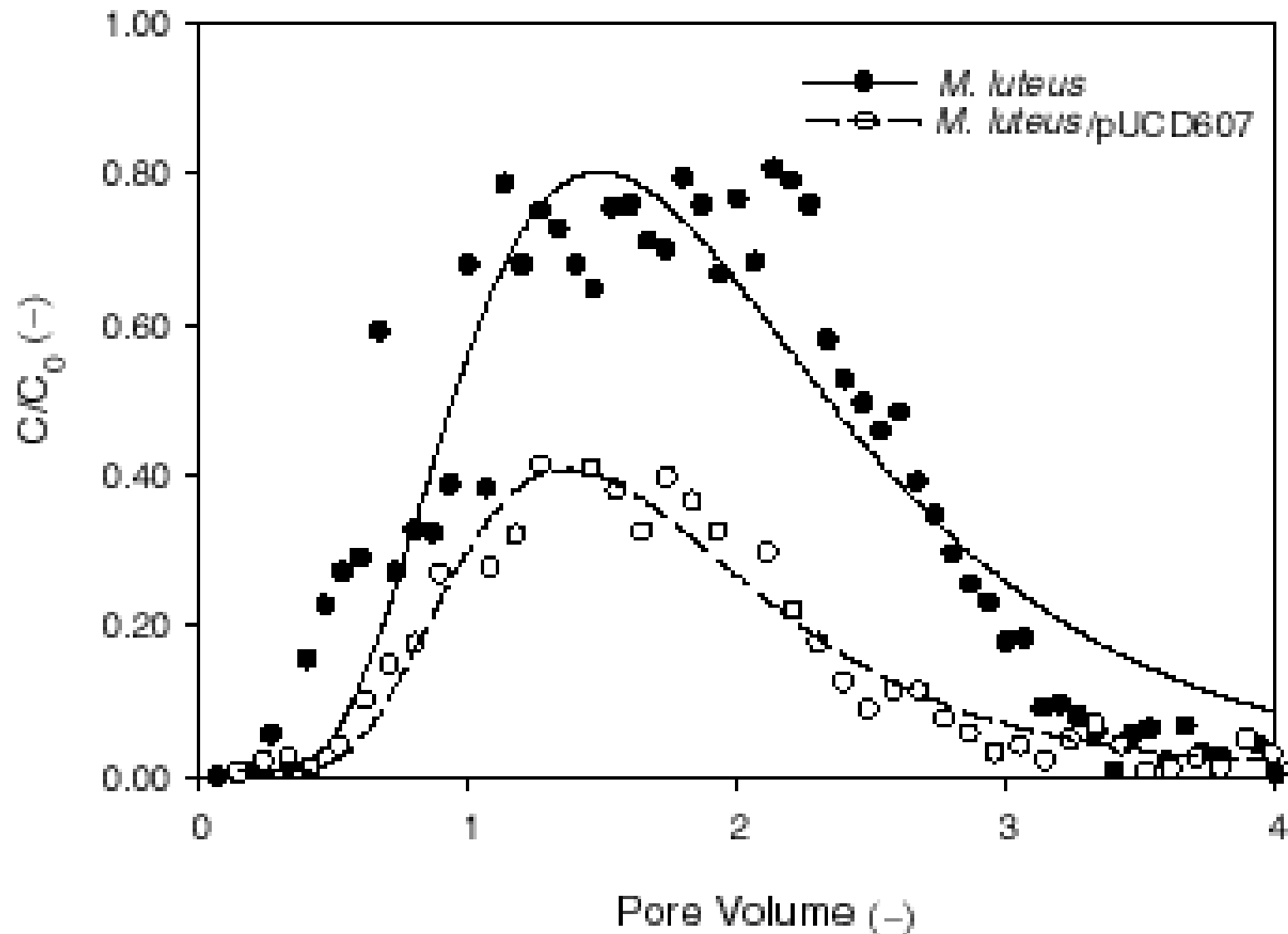
E. coli Breakthrough Curve



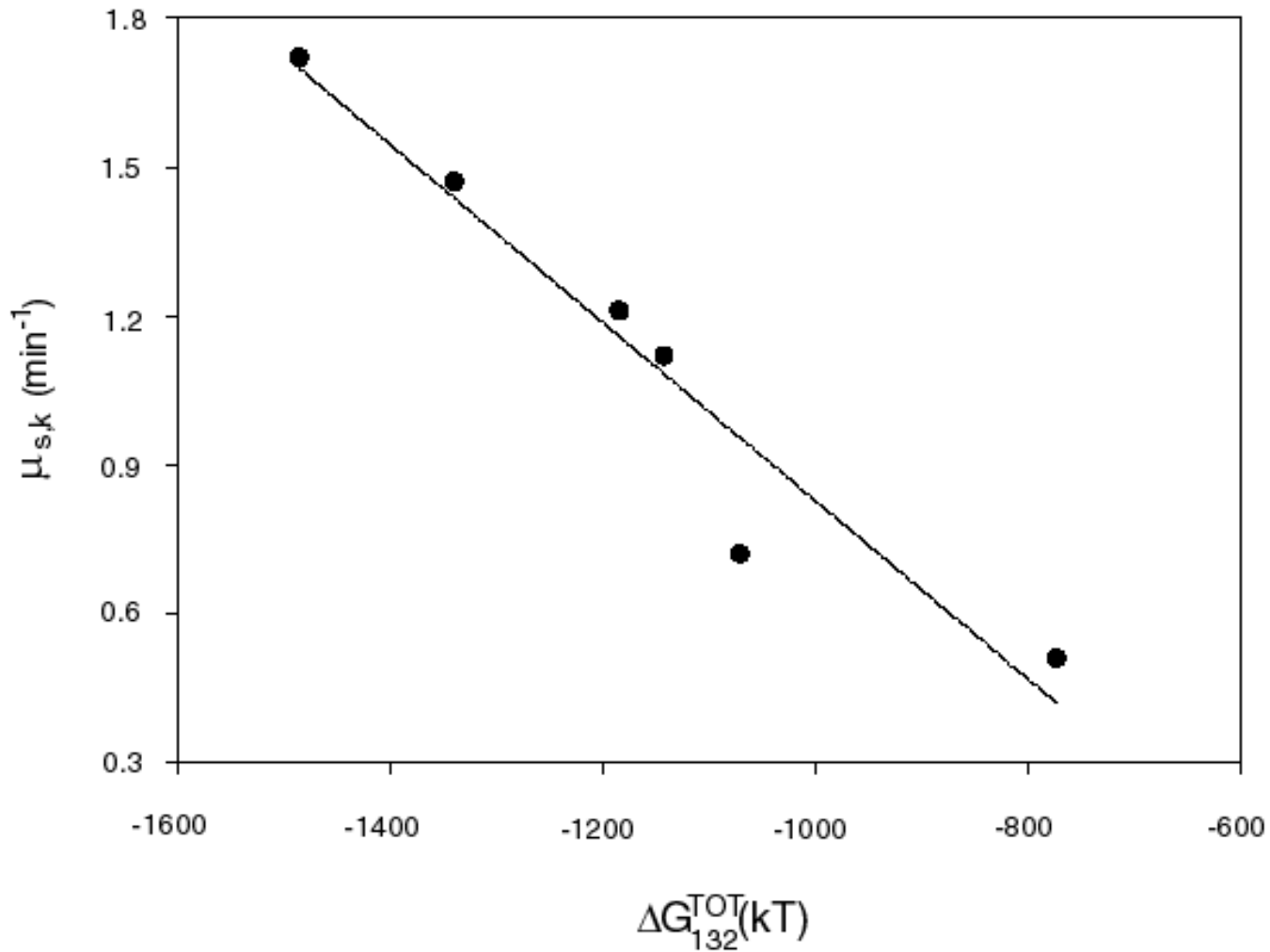
E. coli Breakthrough Curve



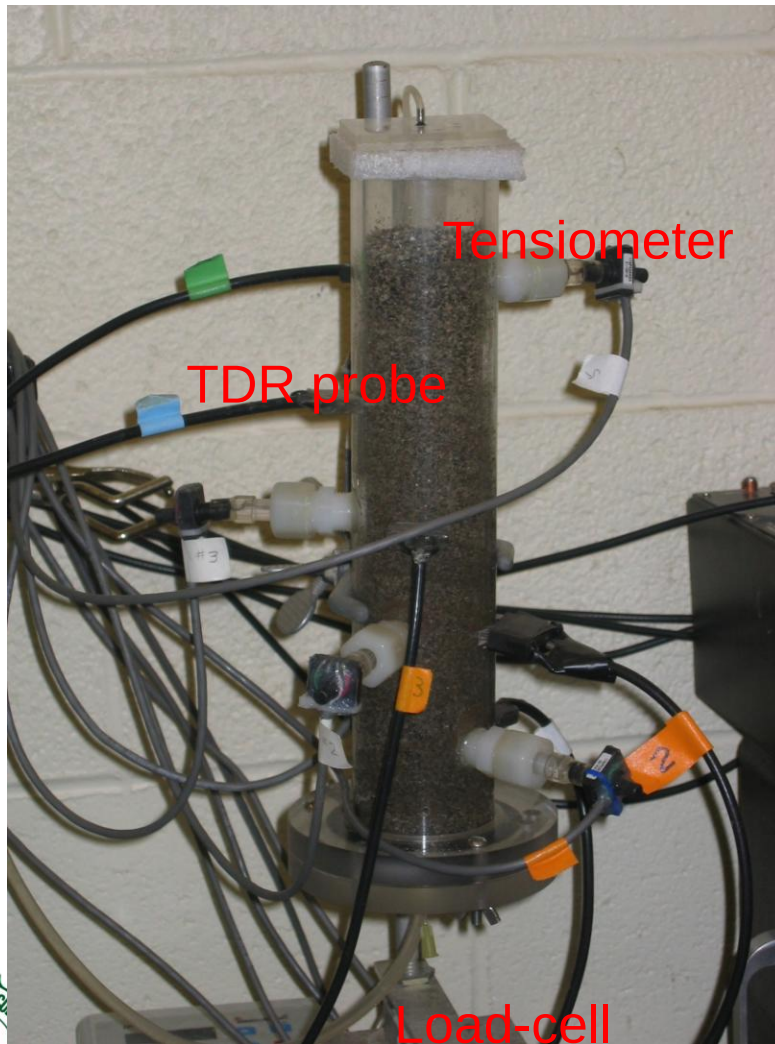
Deposition Coefficient



Deposition Coefficient



Column Assembly



- 5 TDR probes
- 5 Tensiometers
- Mounted on a load-cell
- -10 cm-H₂O maintained using a hanging water column

Conclusion Remarks



Questions?

