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

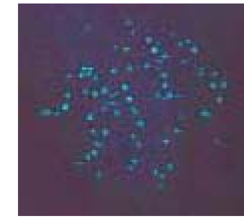
- *lux* genes as genetic marker
- Bacterial surface characterization
- Impact of *lux* gene insertion on bacterial surface property
- Impact of *lux* gene insertion on bacterial transport

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



$$FMNH_2 + RCHO + O_2 \xrightarrow{\text{Luciferase}} FMN + RCOOH + H_2O + \text{Light}$$

Luciferase – Enzyme that catalyze bioluminescence reaction

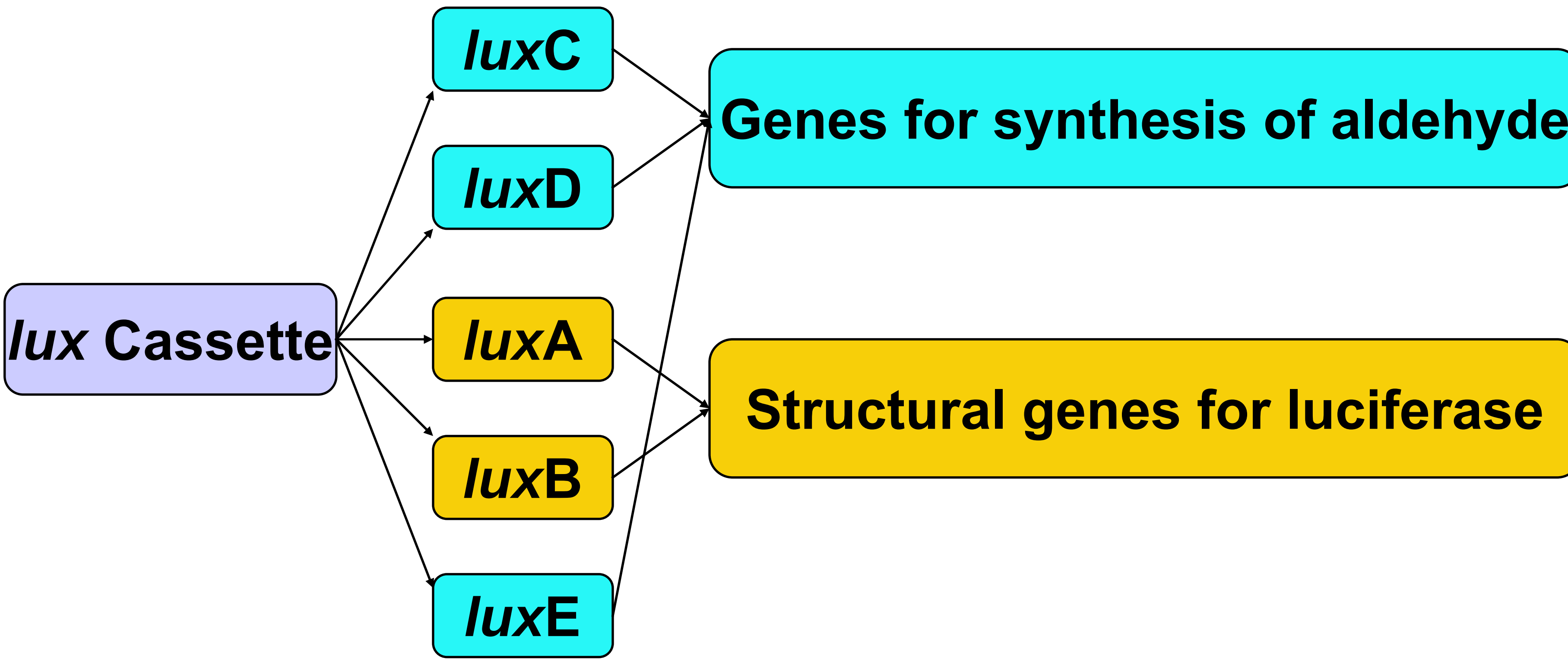
$FMNH_2$ – Reduced riboflavin mononucleotide

RCHO – Long-chain fatty aldehyde

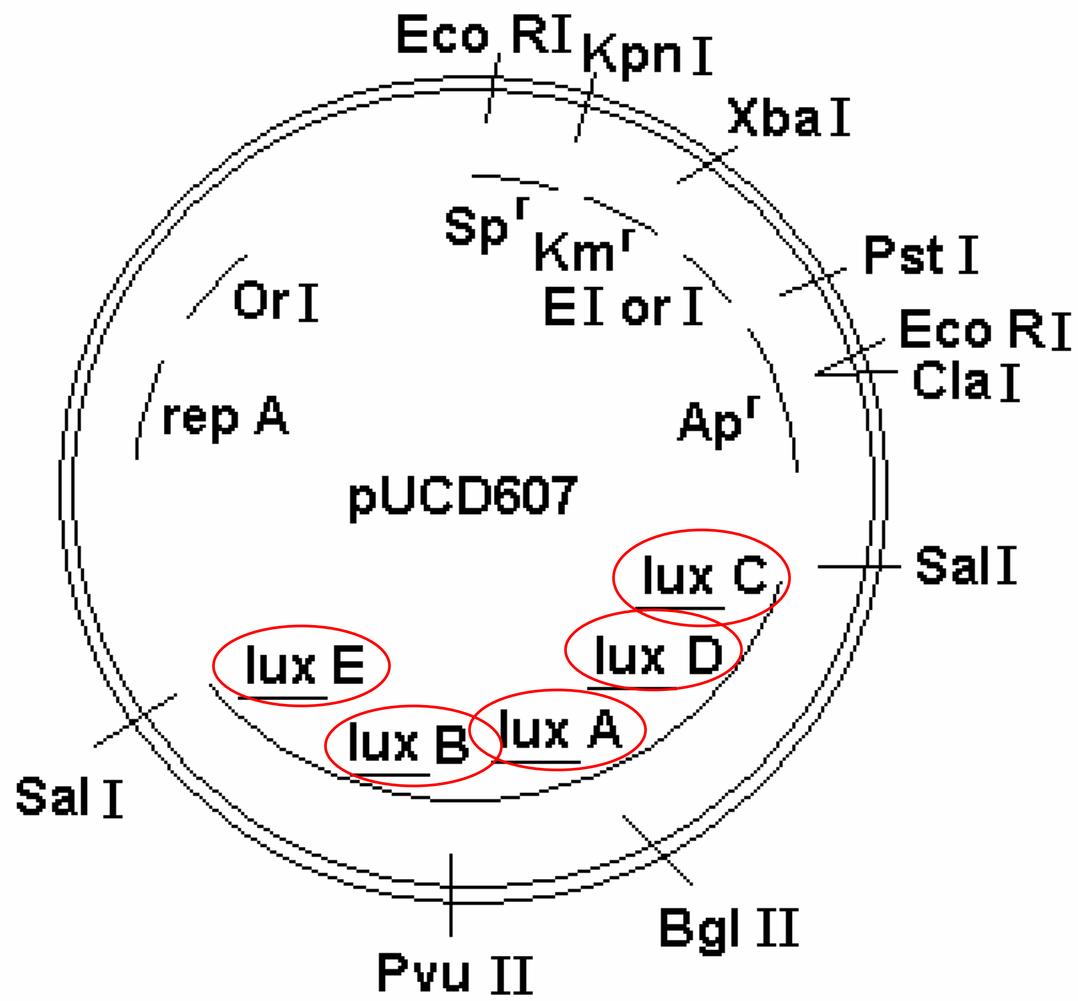
FMN – Riboflavin mononucleotide

RCOOH – Carboxylic acid

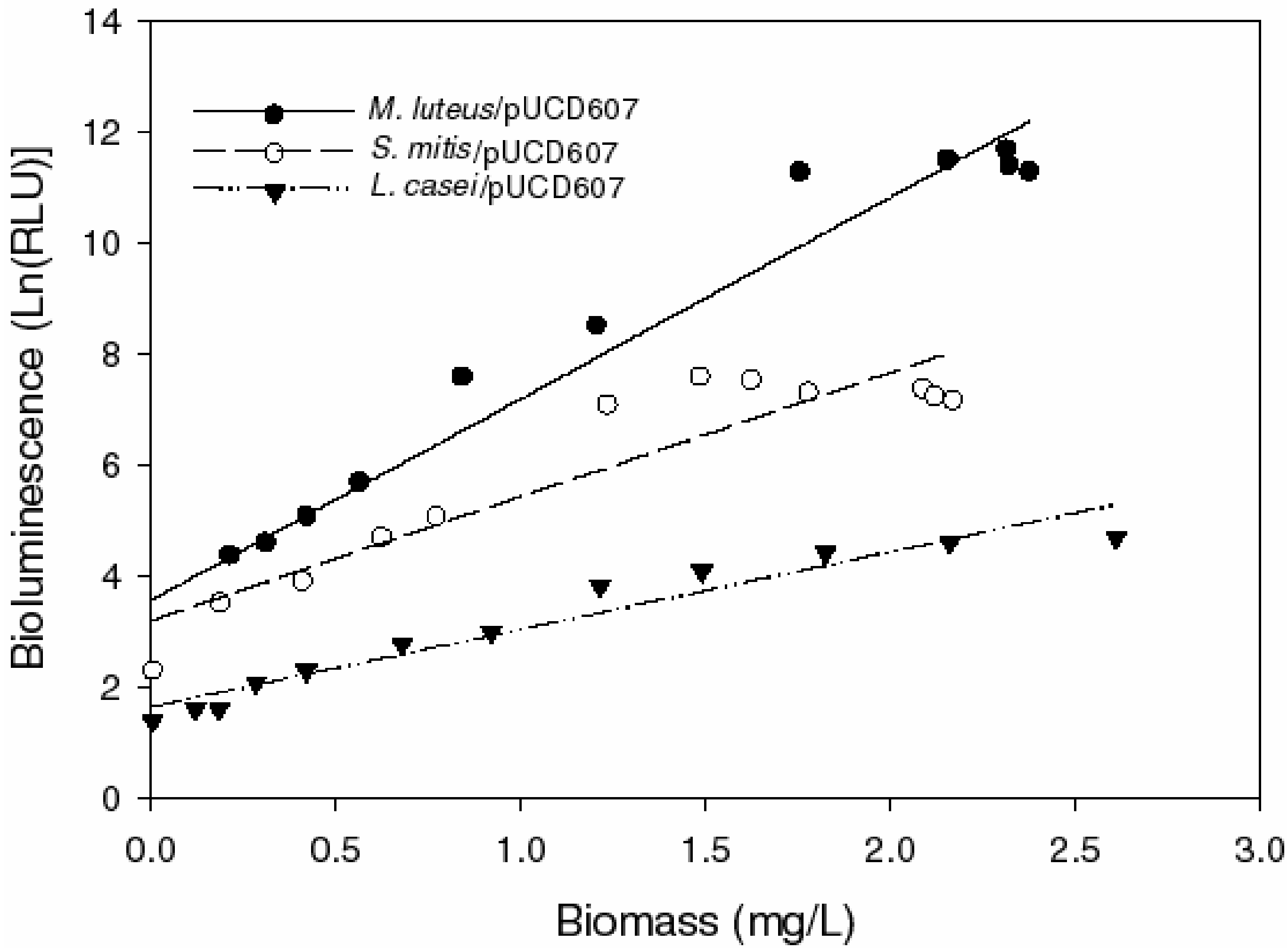
lux Cassette



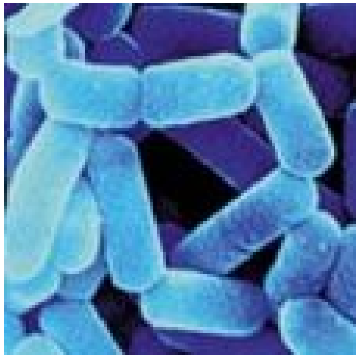
Plasmid pUCD607



Bacterial Concentration Quantification



Bacterial Strains



Lactobacillus casei

Streptococcus mitis

Micrococcus luteus

Bacterial Surface Property Quantification

γ^{LW} γ^{+} γ^{-} ζ

γ^{LW} – Liftshitz-van der Waals component surface tension (mJ/m^2)

γ^{+} – Electron-acceptor parameter of Lewis acid/base component surface tension (mJ/m^2)

γ^{-} – Electron-donor parameter of Lewis acid/base component surface tension (mJ/m^2)

ζ – Zeta potential (mV)

Surface Property Calculation

van Oss-Chaudhury-Good Equation

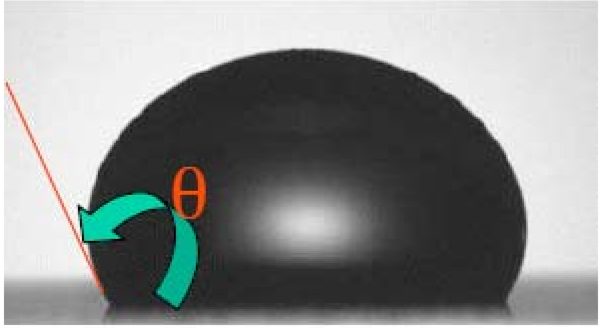
$$(1+\cos\theta)\gamma_L = 2\left(\sqrt{\gamma_S^{LW}\gamma_L^{LW}} + \sqrt{\gamma_S^{+}\gamma_L^{-}} + \sqrt{\gamma_S^{-}\gamma_L^{+}}\right)$$

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

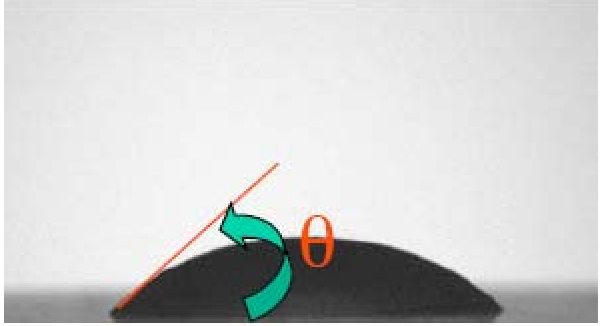
Solve for γ_S^{LW}, γ_S^{+} and γ_S^{-}

Contact Angle Measurement

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

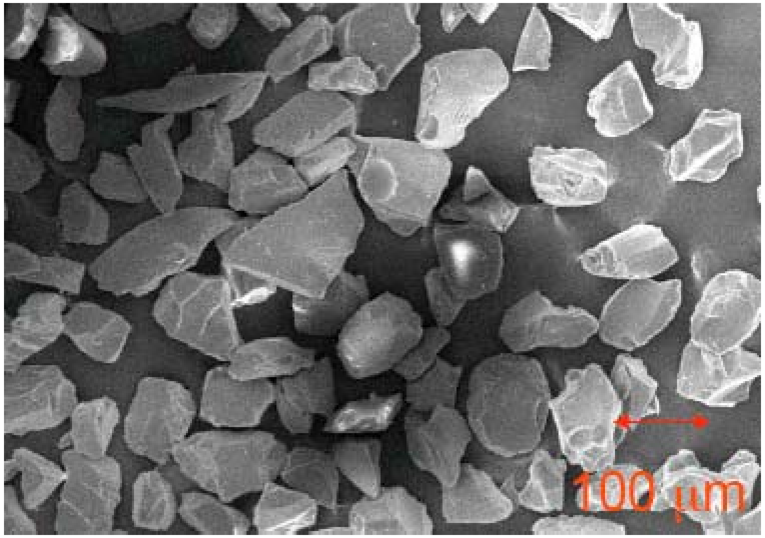


Contact Angle > 90°



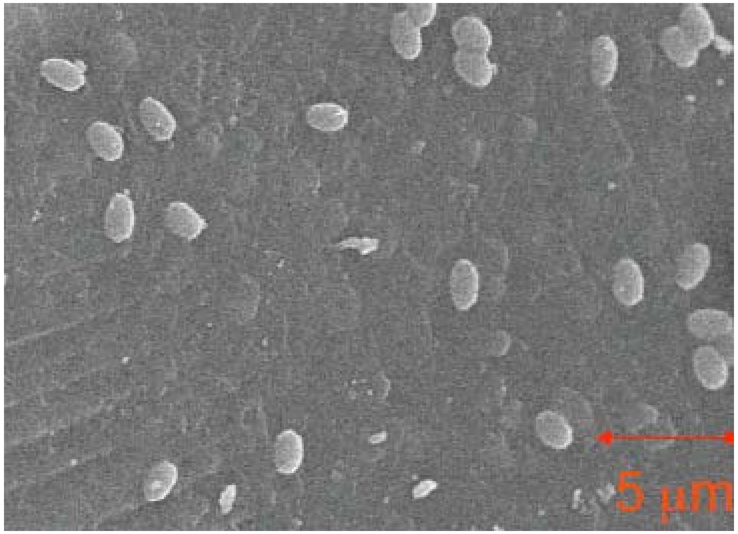
Contact Angle < 90°

Medium



Silica sand

Silica sand with attached cells



5 µm

Wicking Method

Kruss K100 Tensiometer

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/m^2)

μ : measuring liquid viscosity ($N \cdot s/m^2$)

Bacterial Surface Properties

	θ^{DII} (°)	θ^{F} (°)	θ^{W} (°)	γ^{LW} (mJ/m^2)	γ^{+} (mJ/m^2)	γ^{-} (mJ/m^2)	ζ (mV)
L. casei	22.2 ± 0.3	18.1 ± 0.1	48.6 ± 0.4	47.1	1.18	20.7	- 24.7 ± 0.8
L. casei/pUCD607	34.2 ± 0.4	32.5 ± 0.6	55.1 ± 0.7	42.4	0.99	18.7	- 28.1 ± 0.6
S. mitis	28.8 ± 0.7	28.5 ± 0.6	49.5 ± 0.5	44.7	0.74	23.5	- 27.1 ± 0.4
S. mitis/pUCD607	36.8 ± 0.6	37.2 ± 0.5	55.0 ± 0.3	41.2	0.65	21.2	- 32.8 ± 0.7
M. luteus	37.6 ± 0.3	32.6 ± 0.7	49.5 ± 0.2	40.8	0.89	25.4	- 31.6 ± 0.5
M. luteus/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

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

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

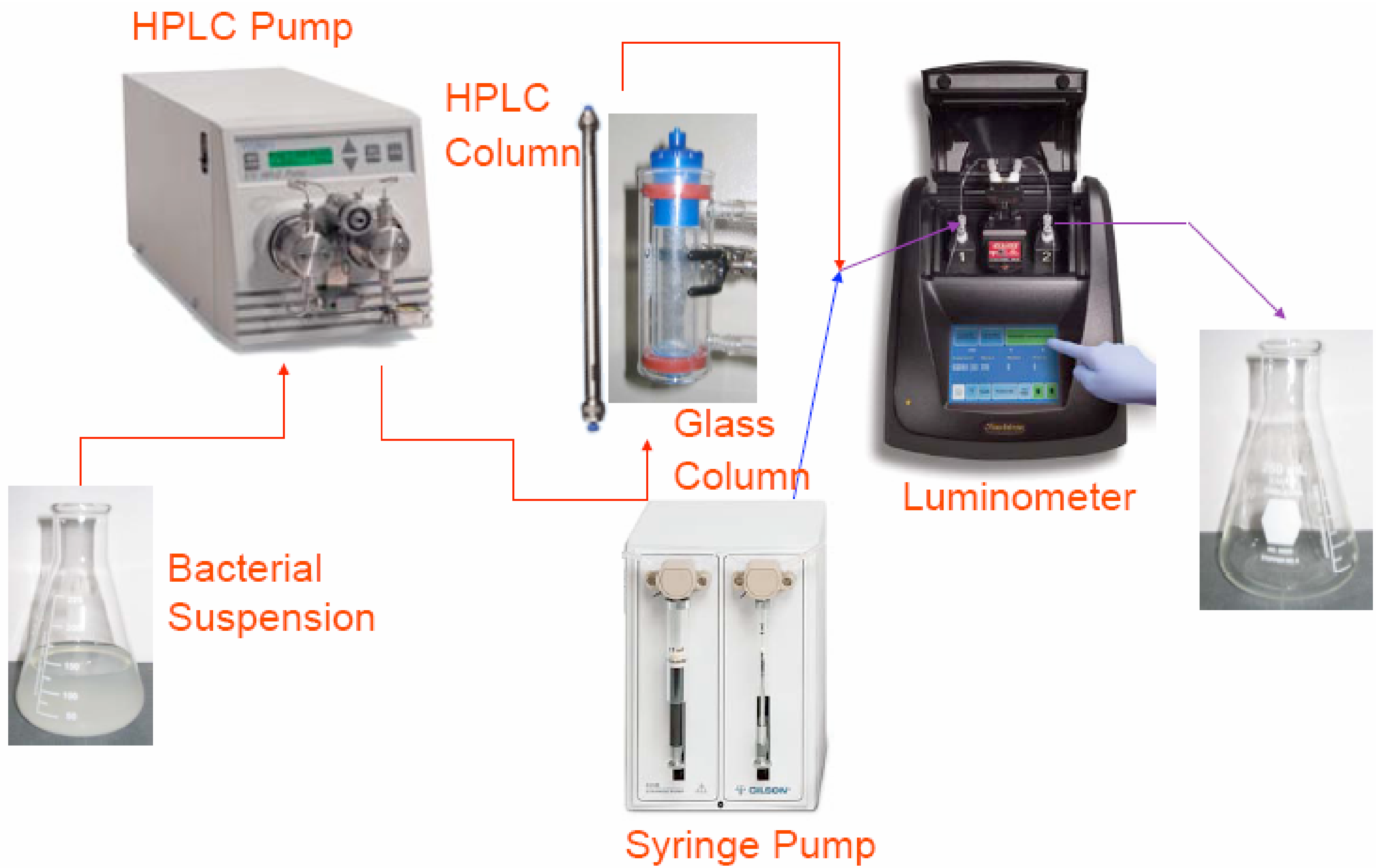
$$\Delta G(y)_{132}^{AB} = 4\pi R y_0 e^{(y-y_0)/\lambda} [(\sqrt{\gamma_1^+} - \sqrt{\gamma_2^+})(\sqrt{\gamma_1^-} - \sqrt{\gamma_2^-}) - (\sqrt{\gamma_1^+} - \sqrt{\gamma_3^+})(\sqrt{\gamma_1^-} - \sqrt{\gamma_3^-}) - (\sqrt{\gamma_2^+} - \sqrt{\gamma_3^+})(\sqrt{\gamma_2^-} - \sqrt{\gamma_3^-})]$$

$$\Delta G(y)_{132}^{EL} = \pi\varepsilon\varepsilon_0 R[2\psi_1\psi_2 Ln(\frac{1+e^{-\kappa y}}{1-e^{-\kappa y}}) + (\psi_1^2 + \psi_2^2) Ln(1-e^{-2\kappa y})]$$

Interaction Free Energies

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

Column Experiment



Column Experiment

Bacterial Transport Modeling

Two Site Model

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

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

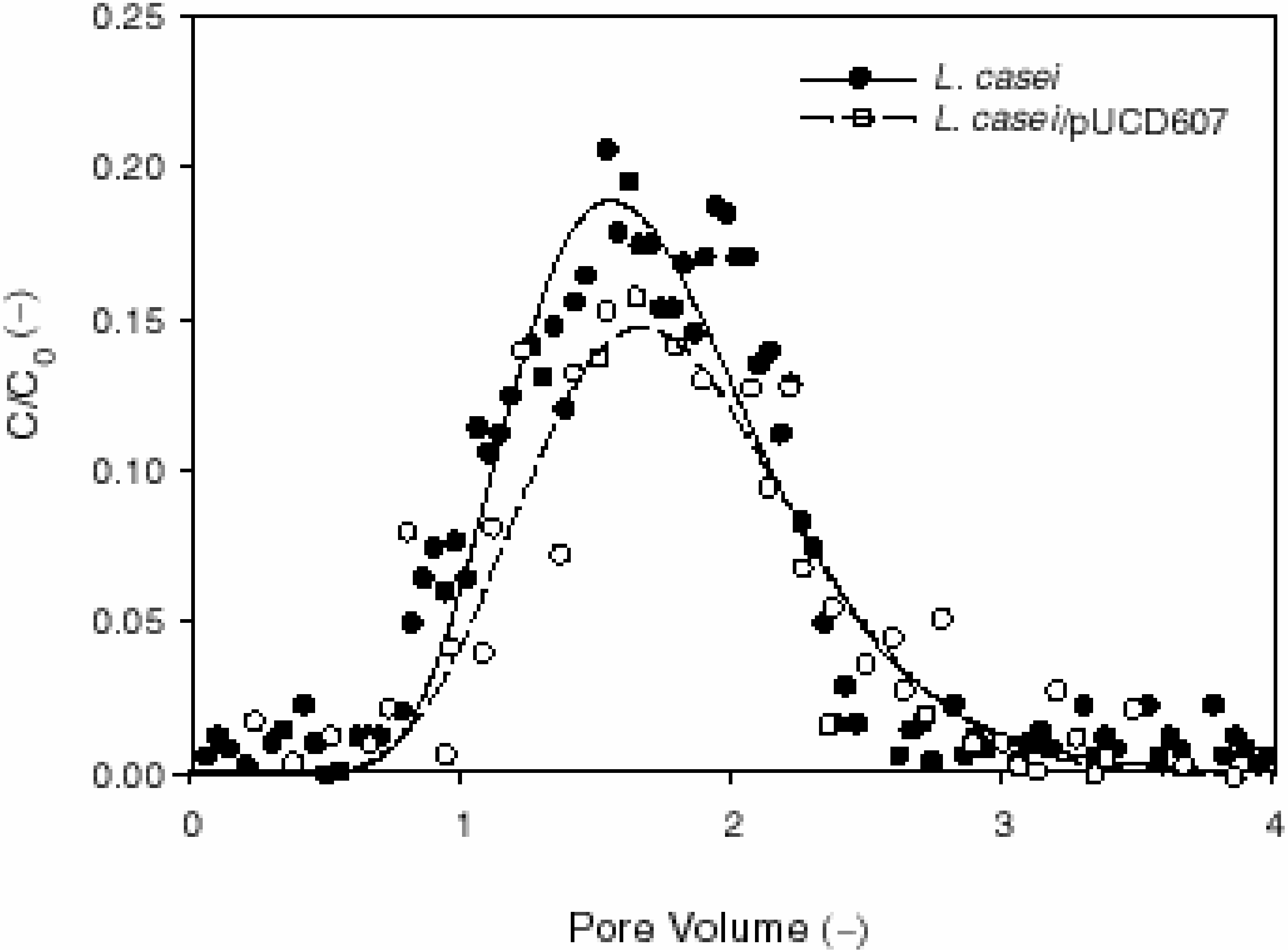
f – Fraction of equilibrium sorption sites

K_b – Partitioning coefficient of bacteria to equilibrium sorption sites (m^3/g)

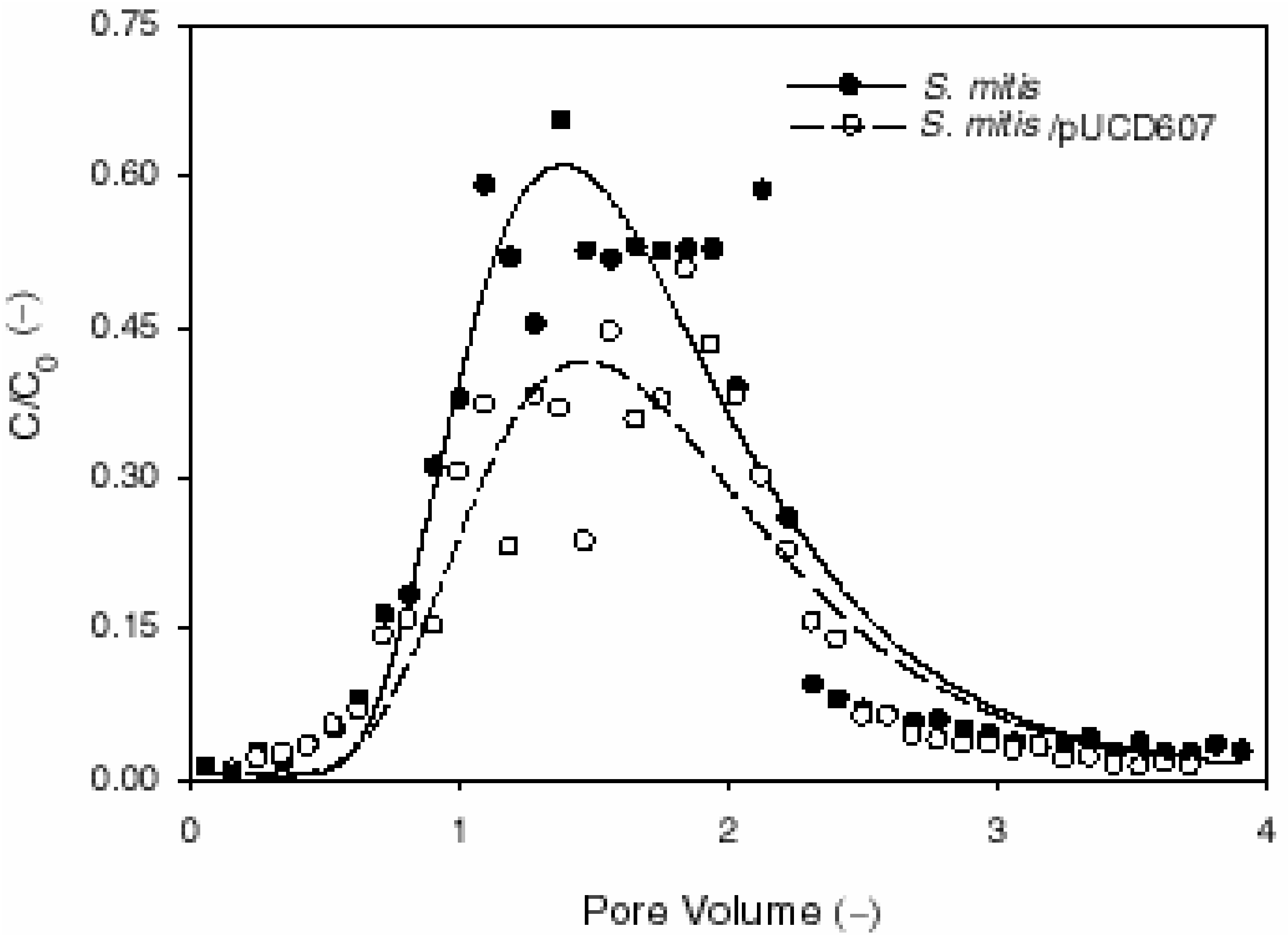
α – Mass transfer coefficient (min^{-1})

$\mu_{s,k}$ – Bacterial deposition coefficient on kinetic sorption sites (min^{-1})

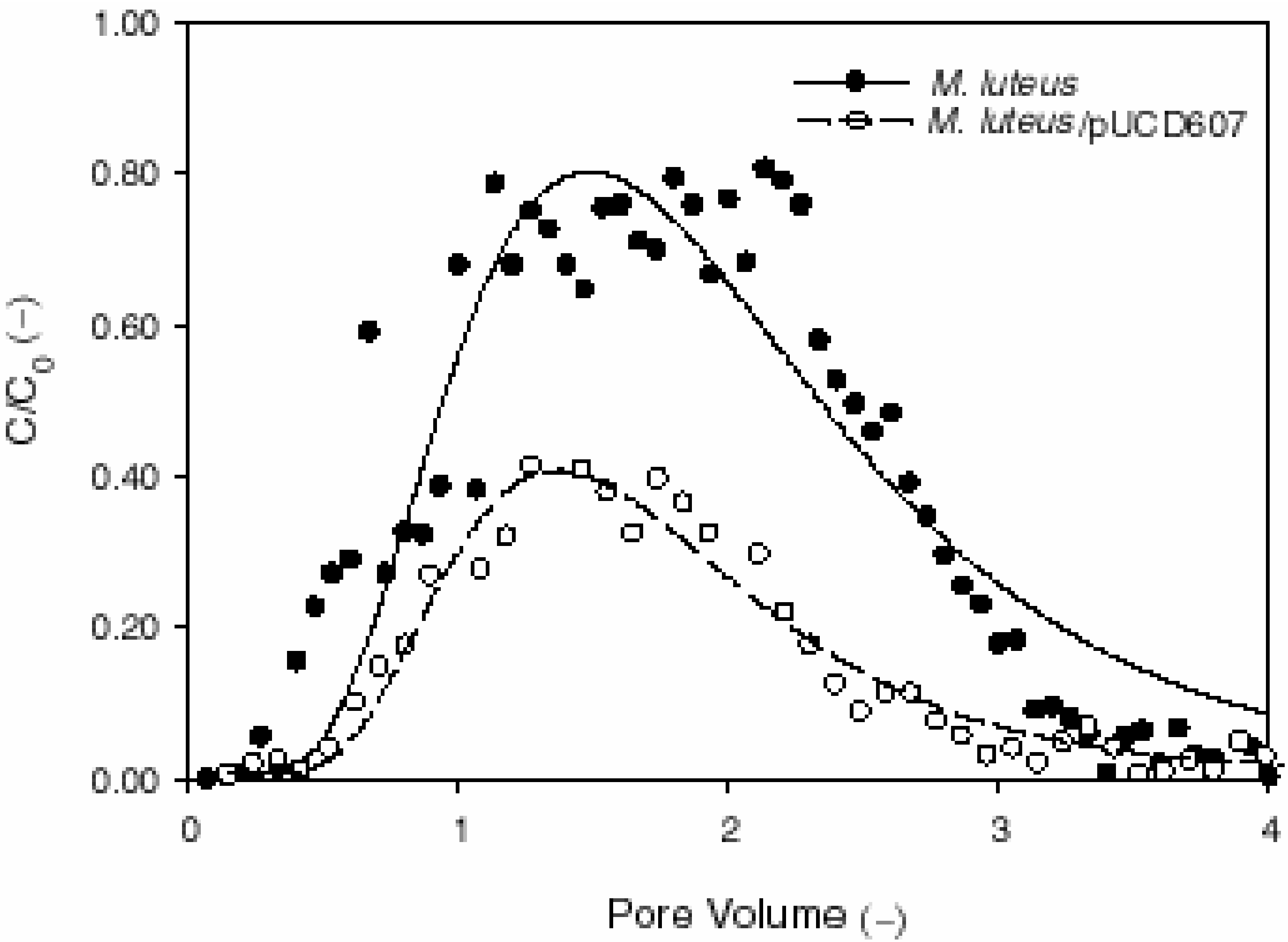
L. casei Breakthrough Curve



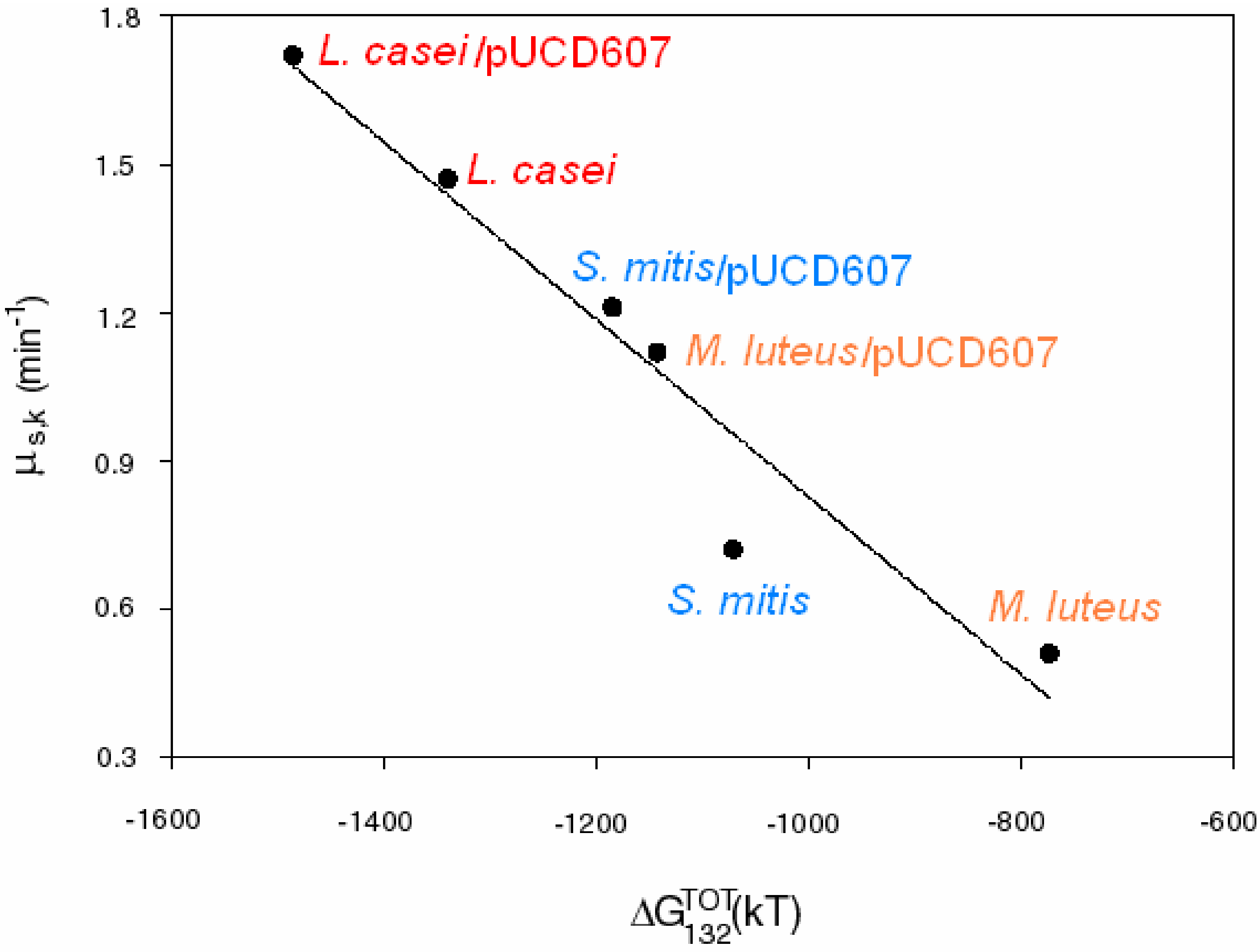
S. mitis Breakthrough Curve



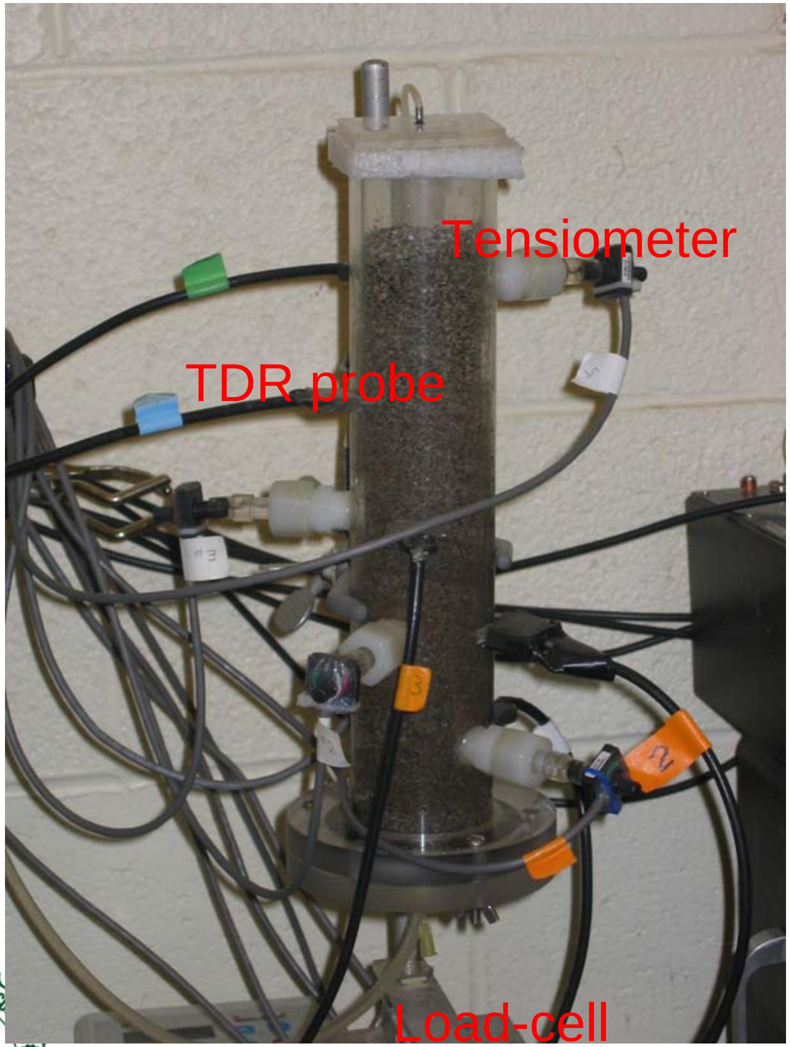
M. luteus Breakthrough Curves



Deposition Coefficient



Future Work – Column Assembly



- 5 TDR probes
- 5 Tensiometers
- Mounted on a load-cell
- -10 cm-H_2O maintained using a hanging water column

Conclusion Remarks

- *lux* genes as bioreporter
- *lux* gene insertion impacts bacterial surface property and interactions
- *lux* gene insertion impacts bacterial transport
- Bacterial transport to be linked with their interactions

Acknowledgements

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Questions?