Biofilm-Induced Mineralization

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US-Africa Advanced Study Institute, 12/12/11

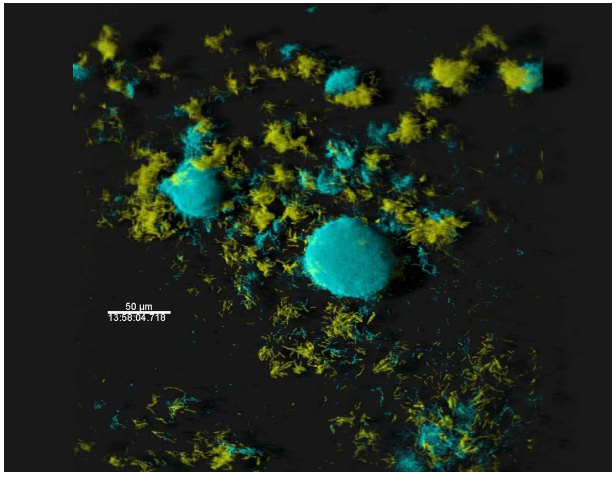
Acknowledgements

- Jack Dockery, Tianyu Zhang (MSU Math)
  Robin Gerlach, James Connolly (MSU CBE)
  Erik Alpkvist (Lund Math)
  Paul Stoodley (Southhampton Engineering)
  Bruce Ayati (Iowa Math)
- Conference organizers

PART I: REVIEW

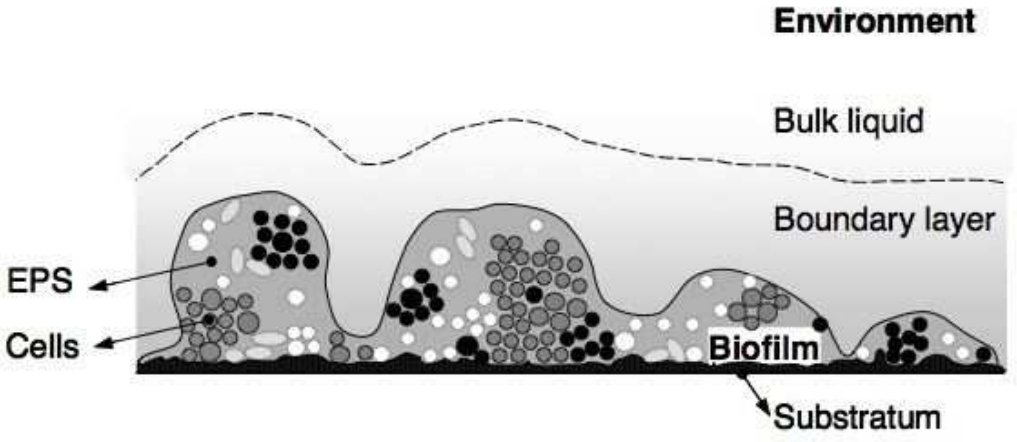
Microbial Biofilms (or Mats)

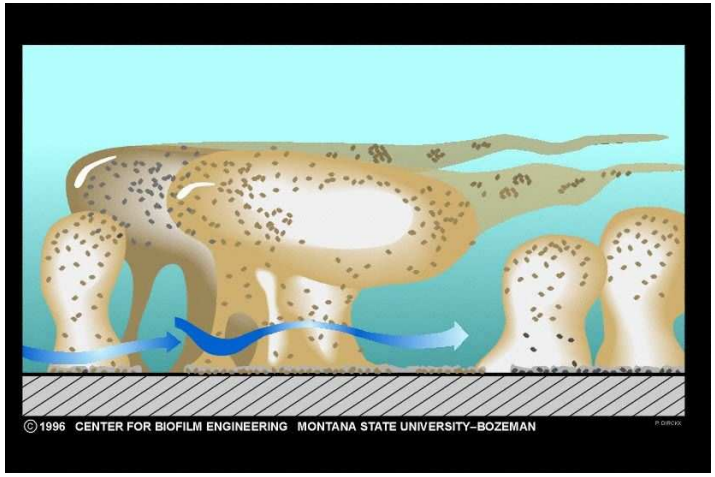
Biofilms are collections of microorganisms (bacteria, archaea, algae, fungi, protozoa) contained in self-secreted matrices of polymers and other substances, a “city of microbes” (Kolter).



Left: algal-bacterial mat, Yellowstone Natl Park (P. Stoodley).
Right: *P. aeruginosa* lab-grown biofilm (B. Klayman).

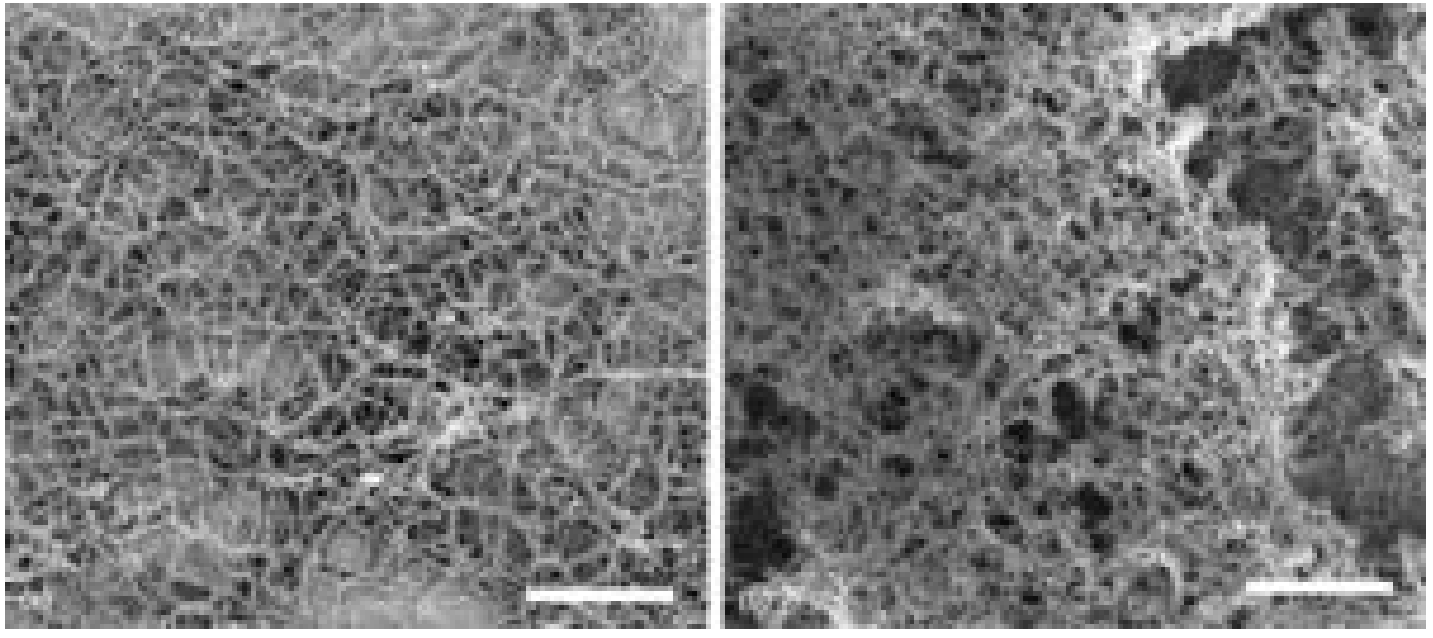
Cartoon Biofilms





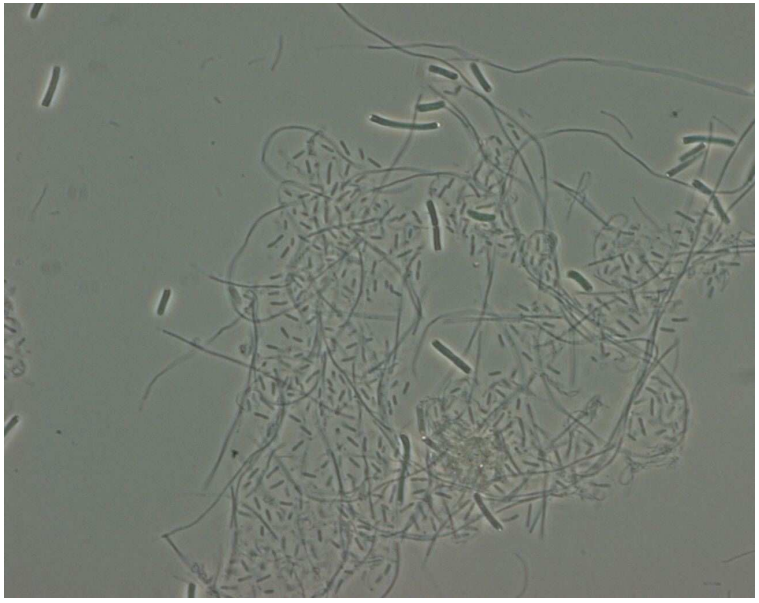
Top: *Mathematical Modeling of Biofilms*, Wanner et al. 2006
Bottom: courtesy of MSU CBE, P. Dirckx.

Extracellular Polymeric Substances (EPS)



Meiothermus biofilm SEM. (BR Johansson)

Spatial Structure

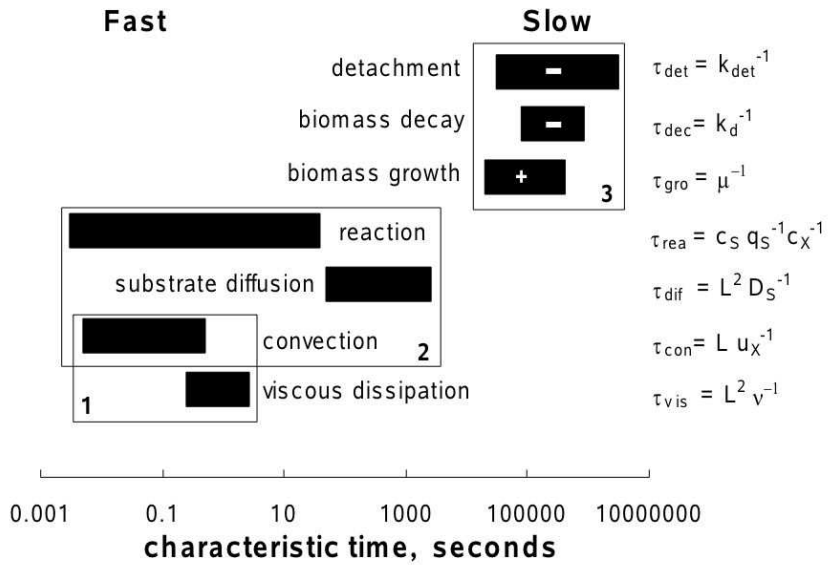


Left: *Synechococcus*, *Chloroflexus*, *Meiothermus* from culture.

Q. What are these cells doing?

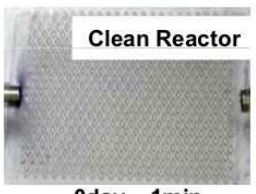
Time Scales

Various time scales for biofilm processes (Picioreanu).

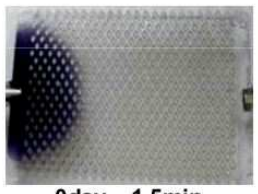


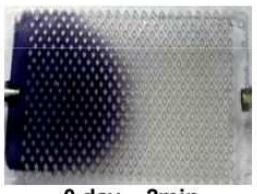
Box 1: bulk fluid (with respect to a biofilm length scale).
Box 2: chemical processes (at biofilm length scales).
Box 3: (some) biological processes

Mechanics

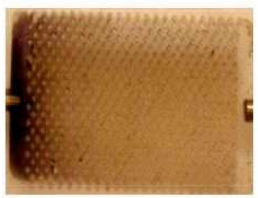


0day – 1min


0day – 1.5min


0 day – 2min

2day – 1min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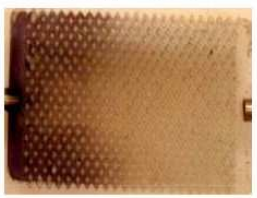
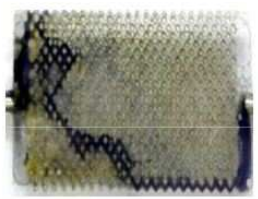
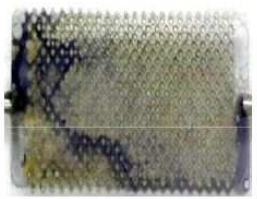
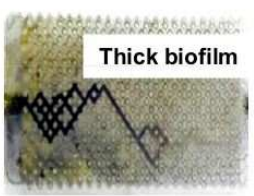

2day – 2min

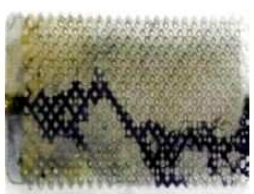
14day – 1min

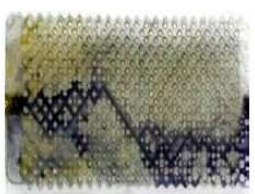

14day – 1.5min


14day – 2min



15day – 1min


15day – 1.5min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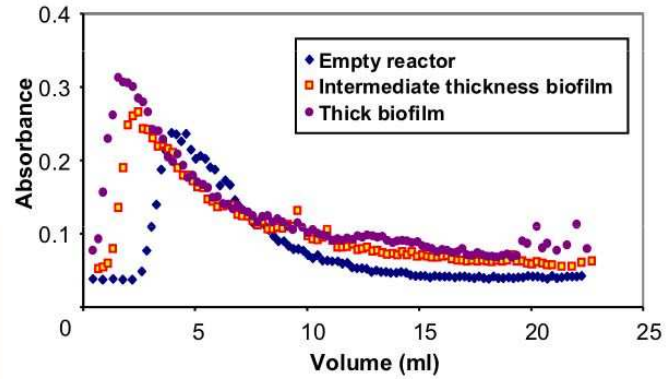
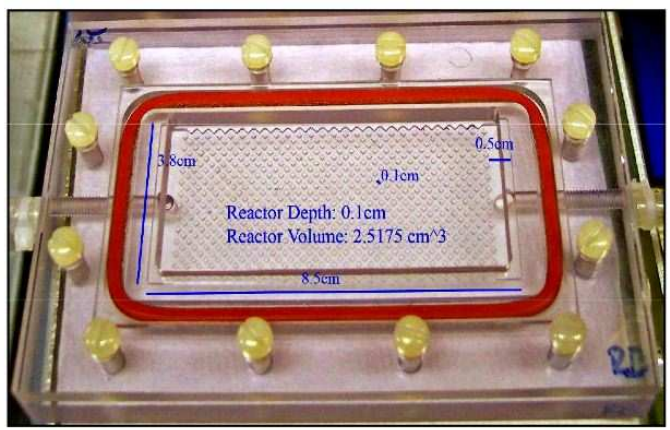
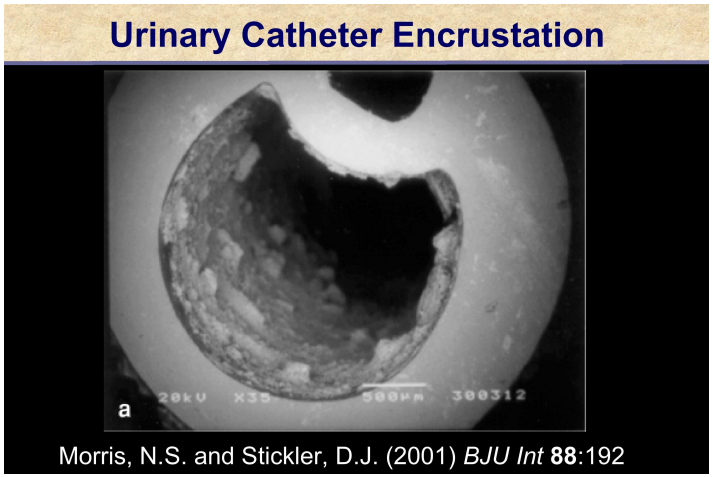
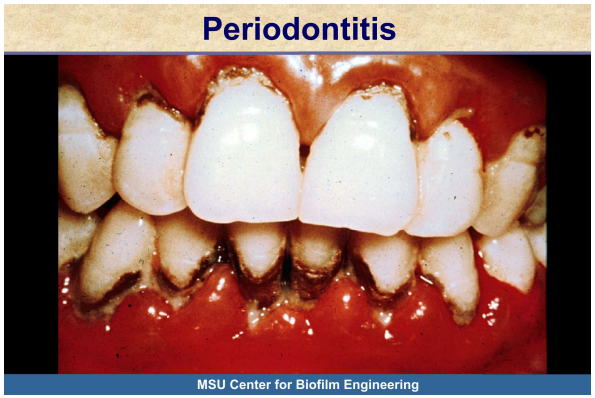




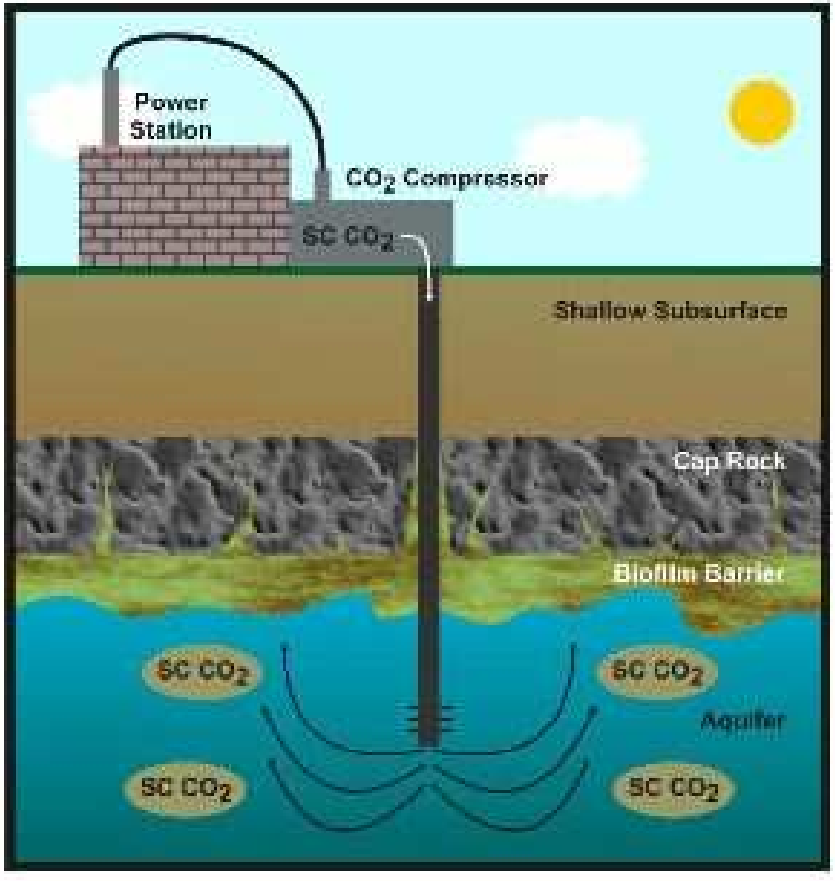
Chemistry and Environmental Impact





Left: struvite formation via of ureolysis (mineralization).
Right: Periodontitus and caries (demineralization).

Application: Biobarrier

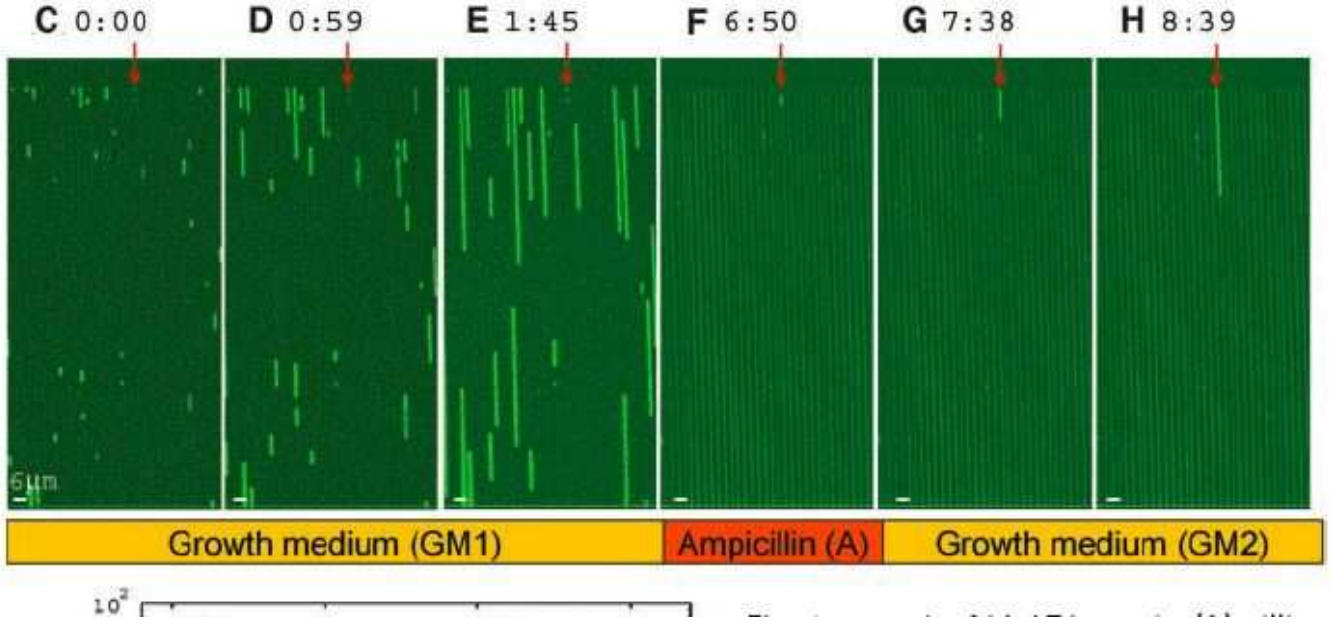


Sequestration of supercritical CO_2

PART II: PERSISTERS

Persisters: What Are They?

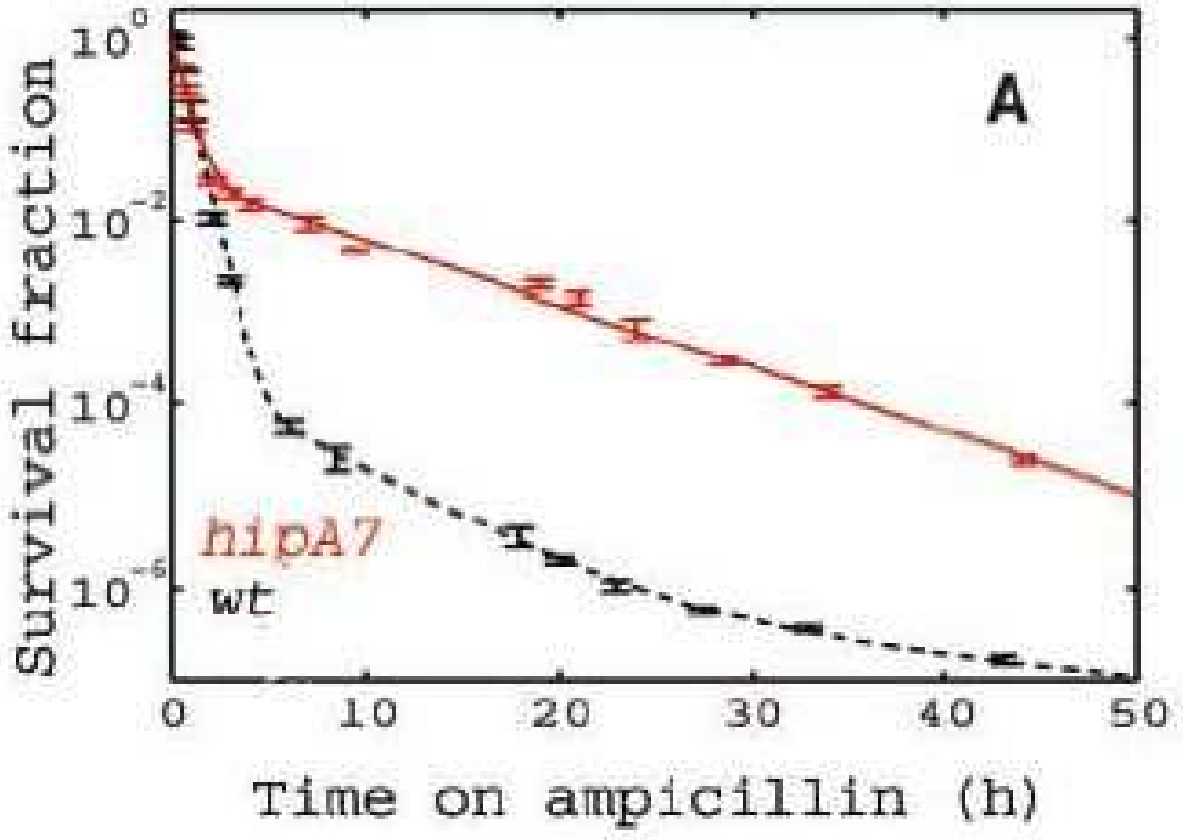
- Many antimicrobials fail to entirely kill bacterial cultures (Lieut.-Colonel Biggers, RAMC, *Lancet* (1944)):



Balaban, Merrin, Chait, Kowalik, Leibler, Science (2004)

Persisters: What Are They?

- More precisely, biophasic killing curves are observed:

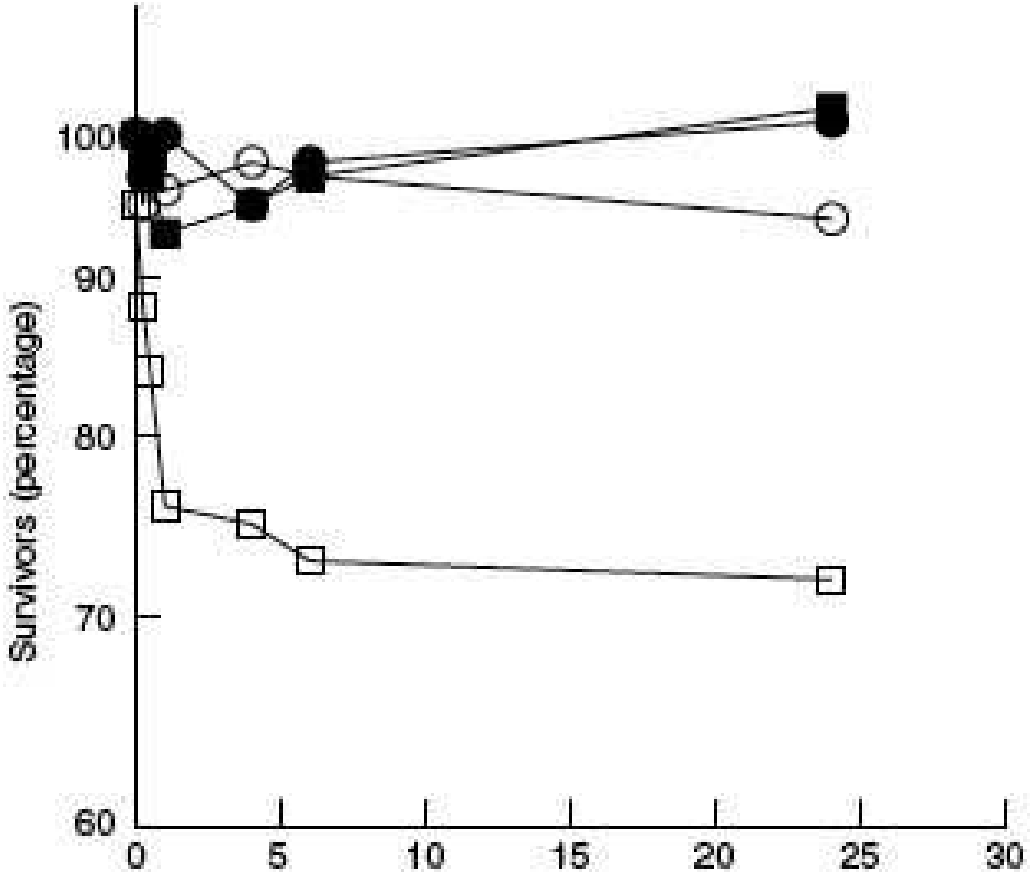


Balaban, Merrin, Chait, Kowalik, Leibler, Science (2004)

Persisters: What Are They?

- Persister cells are cross-resistant:

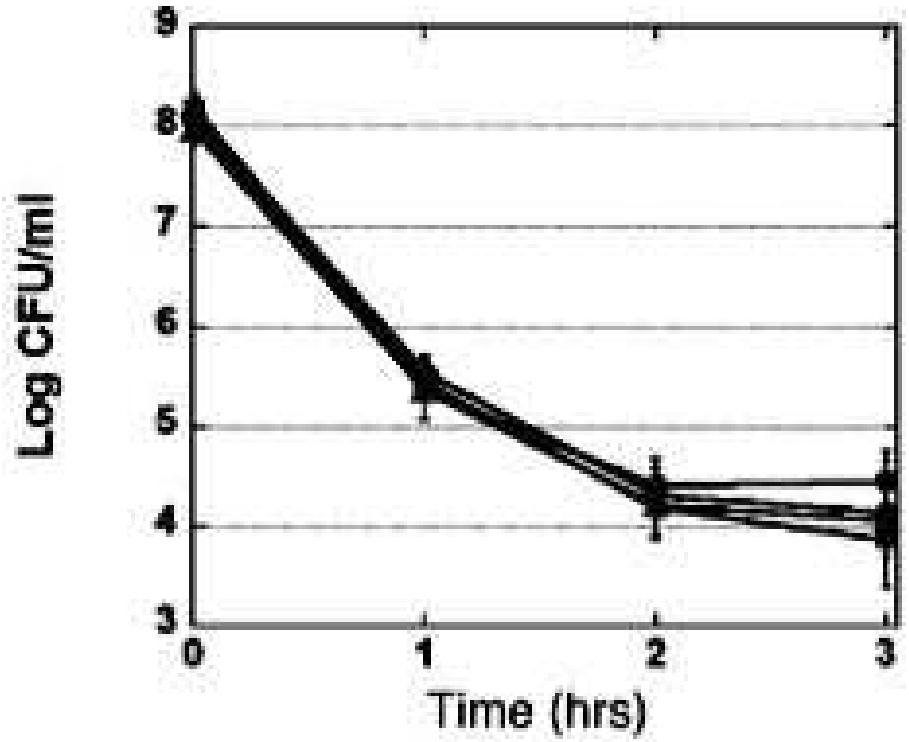
Sufya, Allison & Gilbert, J. Appl. Microbiol. (2003)



Persisters: What Are They?

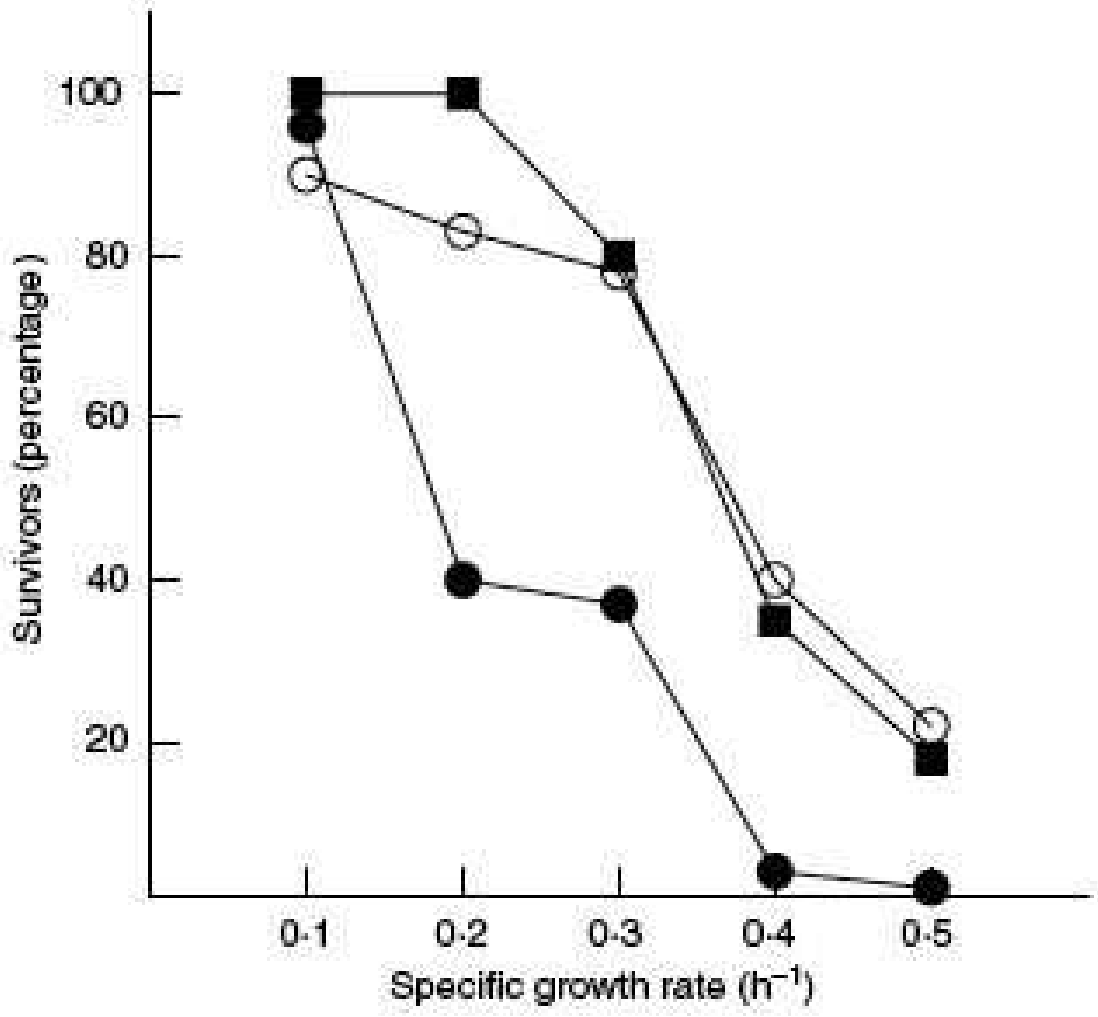
- Persisters are not mutants:

Keren, Kaldalu, Spoering, Wang & Lewis, FEMS Microbiol. (2004)



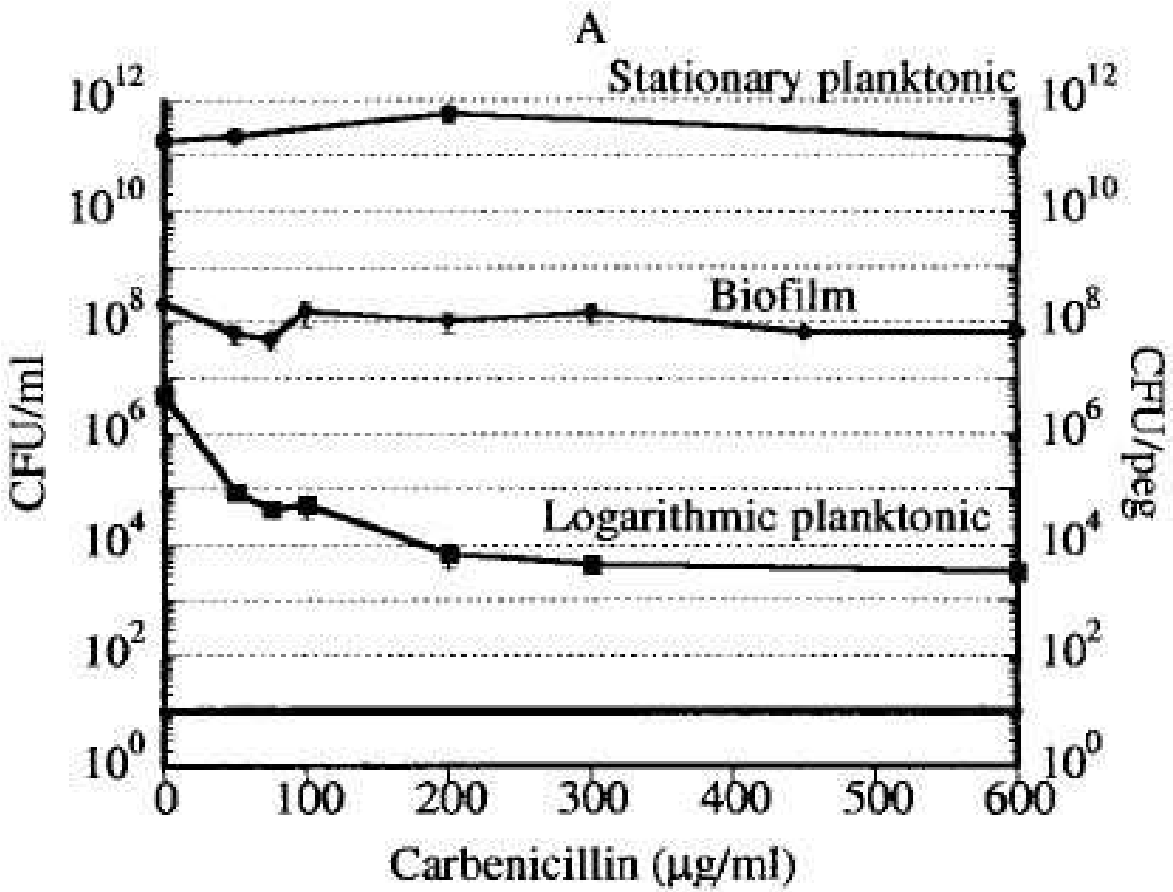
Persisters: What Are They?

- Persister are slow growers:



Persisters: What Are They?

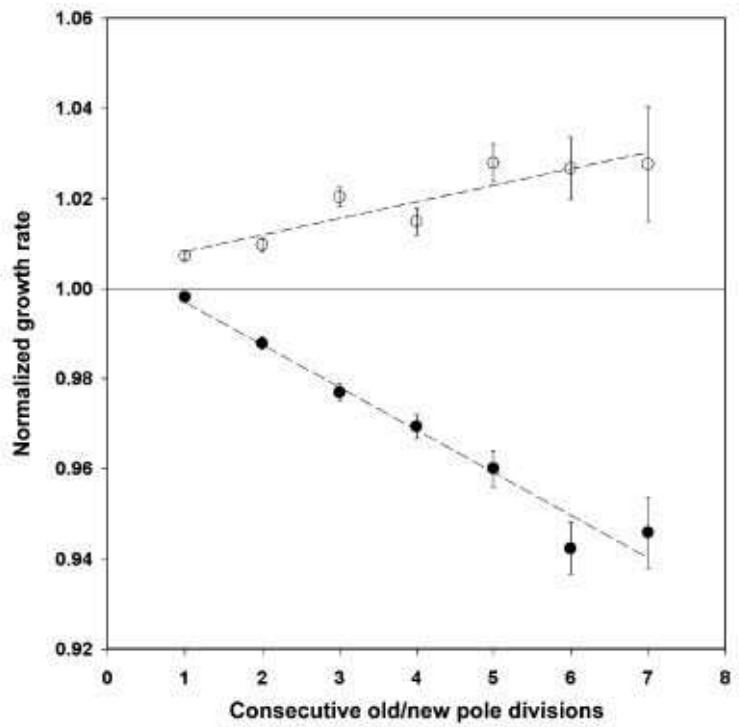
- Persister numbers are phase dependent:



Spoering & Lewis, J. Bacteriol. (2001)

Aging

- Bacteria cells age (and senesce): Upon cell division, one cell is “old“ and the other is “newborn“.
- Remark: senescence had already been observed in many asymmetric dividers.



Senescence May Explain Persistence

Idea:

- Persistent cells are, apparently, relatively inactive cells. This inactivity may explain their antimicrobial tolerance.
- Senescent cells are, apparently, relatively inactive.
- Hence perhaps senescent cells are the elusive persisters.

Quantities of Interest

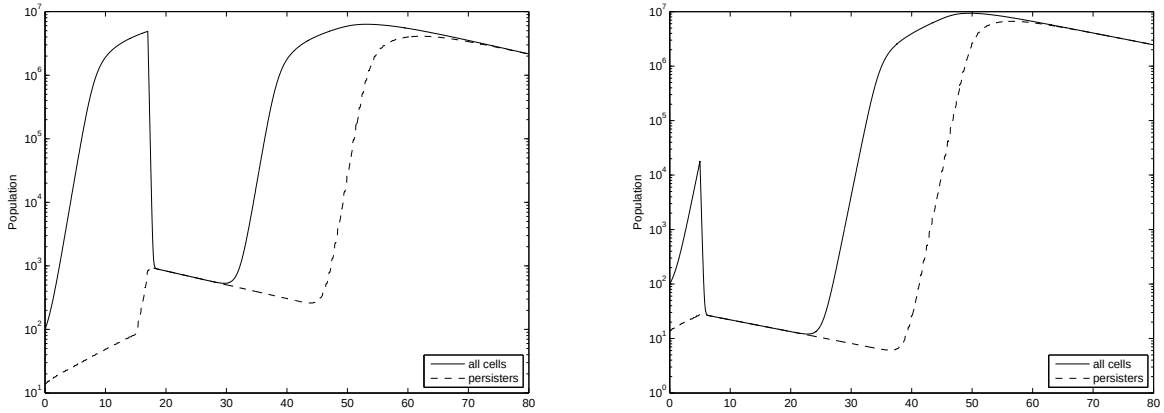
- $b = b(t, a)$ is bacteria/age. ($t =$ time, $a =$ chronological age which we identify with senescence.)
- $c = c(t)$ is limiting substrate concentration.
- $d = d(t)$ is the dosage concentration of applied antimicrobial.
- μ is death rate from natural causes, assumed for simplicity to be independent of a and c.
- $\mu_K = \mu_K(d, a)$ is the killing rate due to application of biocide.

Batch Culture (With Killing)

$$\begin{aligned}
\frac{\partial b}{\partial t} + \frac{\partial b}{\partial a} &= -(\mu + \mu_K(d,a))b, \quad a > 0, \\
b(t,0) &= \alpha\beta c \int_0^\infty s(a)b(t,a)da, \\
\frac{dc}{dt} &= -\alpha c \int_0^\infty s(a)b(t,a)da,
\end{aligned}$$

where $s(a)$ decreases from 1 to 0 monotonically, μ_K is a step function that switches off for a larger than a cutoff parameter λ (the *senescence age*).

Batch Culture (Experiments)

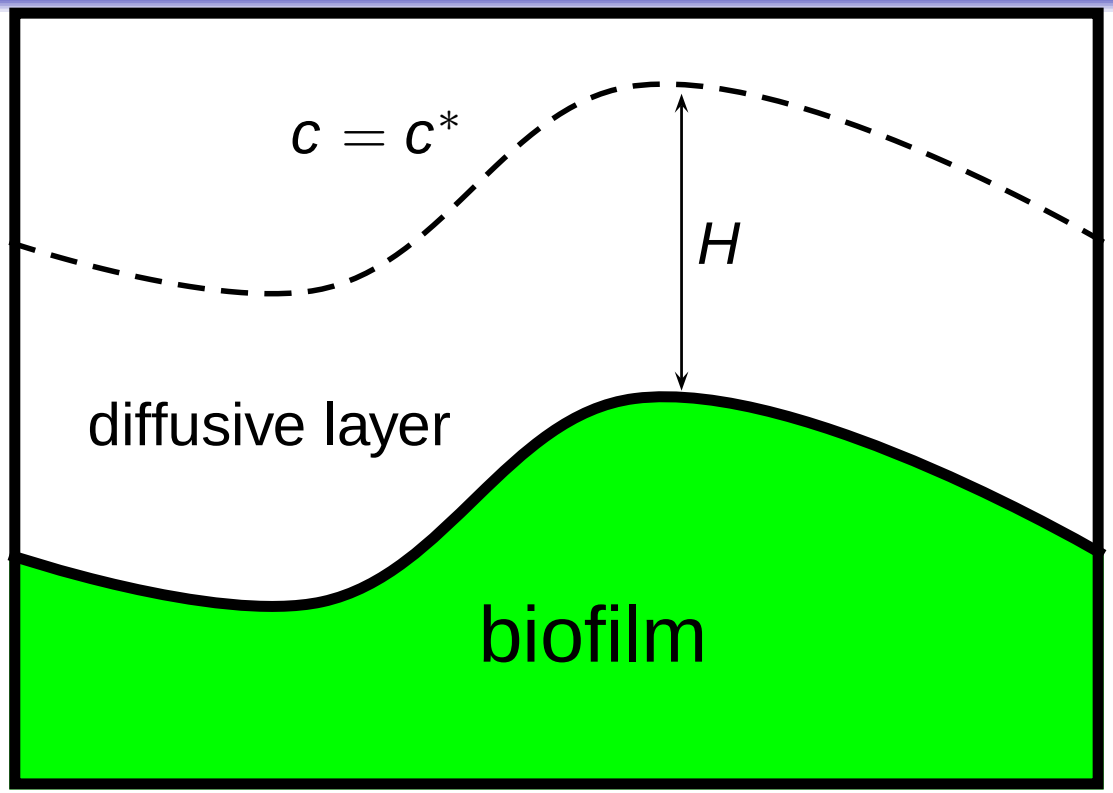


(a) antimicrobial is applied during stationary phase,
(b) antimicrobial is applied during log phase. Recall

- Persister numbers are phase dependent.
- Biphasic killing curves are observed.

PART III: MECHANICS

Growth Induced Mechanics



(1) Substrate diffuses into biofilm through a diffusion layer. (2) Substrate is “eaten” in an active layer (not shown). (3) Growth generates pressure which in turn generates velocity.
(4) Interface moves.

Basic Continuum Model

Growth stress: limiting substrate diffuses into biofilm from "bulk fluid", biofilm eats and expands (homogeneously).

- Substrate reaction/diffusion:

$$\nabla^2 C = G\, r(C)$$

  $C =$ limiting substrate concentration, $G^{-1/2}$= active layer depth, $r(C) = r_0 \chi_b(\mathbf{x})$ is substrate usage rate.

- Biofilm deformation (force balance): $\mathbf{u} = -\lambda \nabla p$
- Growth stress:

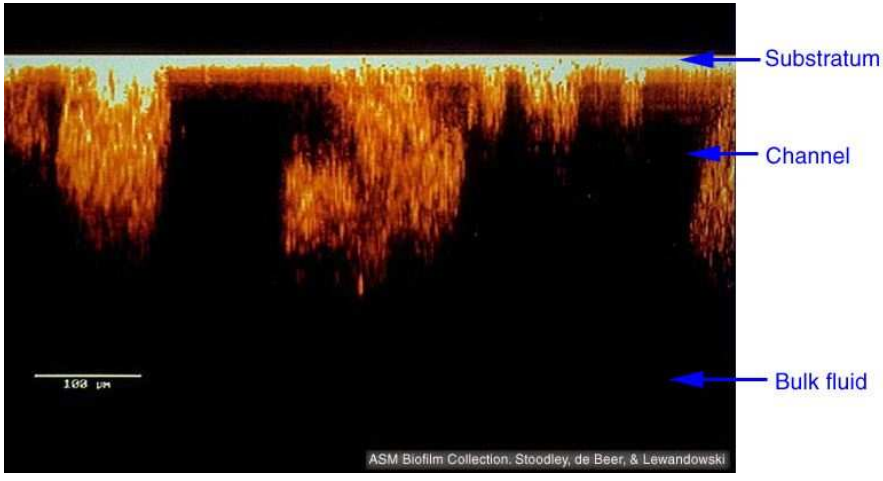
$$\nabla^2 p = -\lambda^{-1} \nabla \cdot \mathbf{u} = -\lambda^{-1} g(r(C))$$

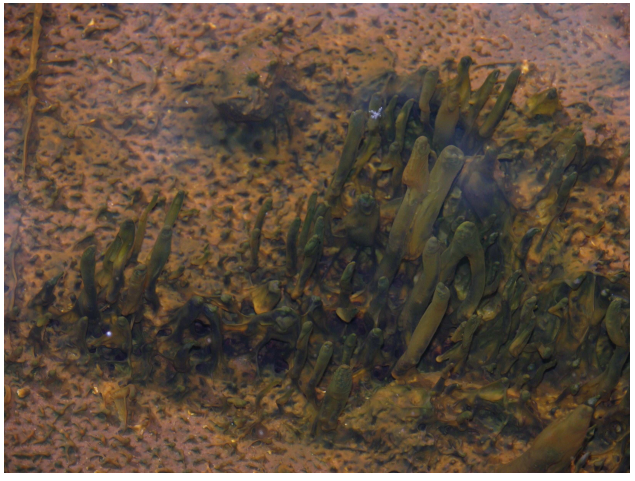
  $g(r(C))$ is a biofilm growth function.

- Interface motion: $\mathbf{u} = -\lambda dp/dn$

Biofilms Are Not Flat (Not Always, Anyway)

Fingering of a sort (P. Stoodley & OS, YNP).





For mechanics, especially fluid-biofilm interaction, as well as for transport, one dimensional representations may not be sufficient.

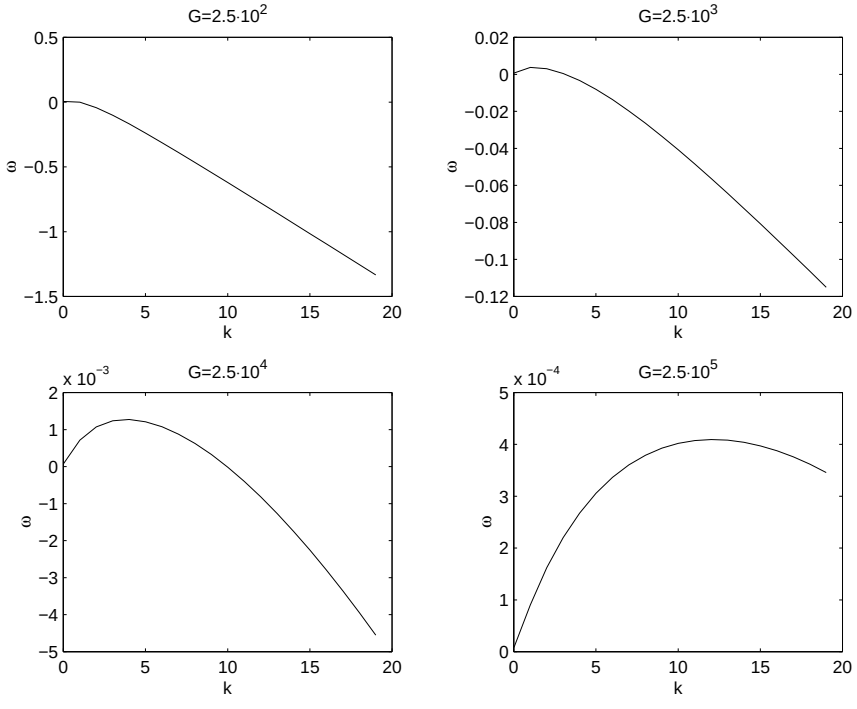
Mullins-Sekerka Instability

Stability: a bump on a flat biofilm has better access to substrate and hence will grow. (Mullins-Sekerka instability)

The most unstable wavelength is $O(G^{-1/2})$, i.e., about the same size as the active layer depth. This system is "self-regularizing". The most unstable wavelength is about the same width as the active layer depth.

Mullins-Sekerka Instability

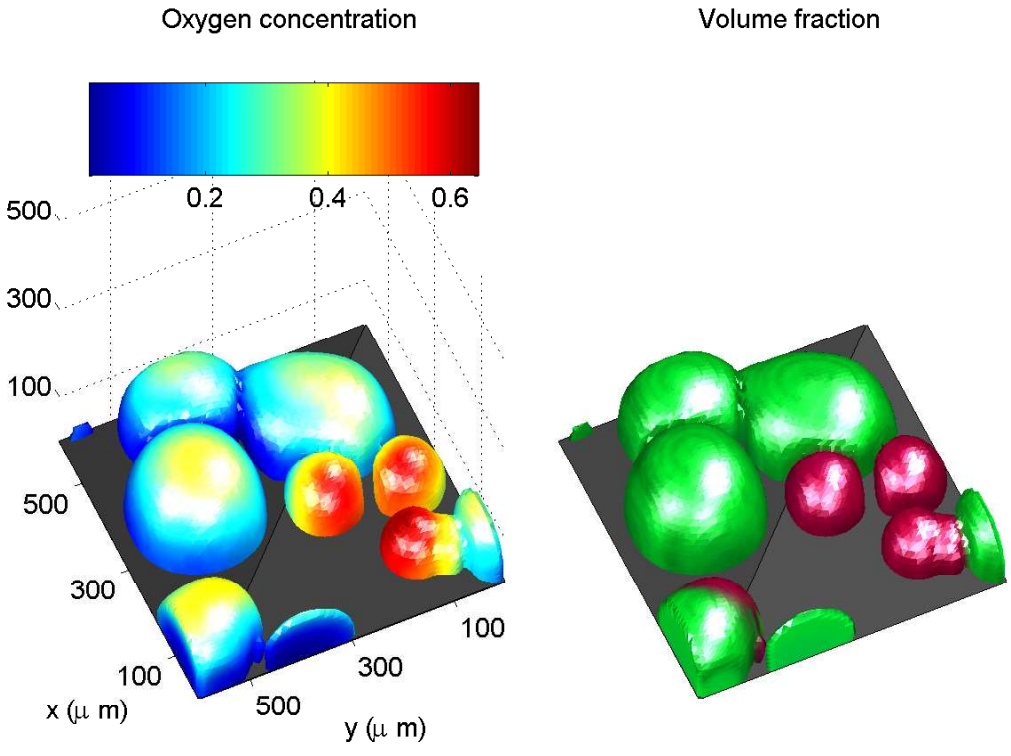
Stability: perturbed 1D solutions are subject to a Mullins-Sekerka instability, even in simple growth models.



The most unstable wavelength is about the same width as the active layer depth. This system is “self-regularizing”.

Growth Mechanics

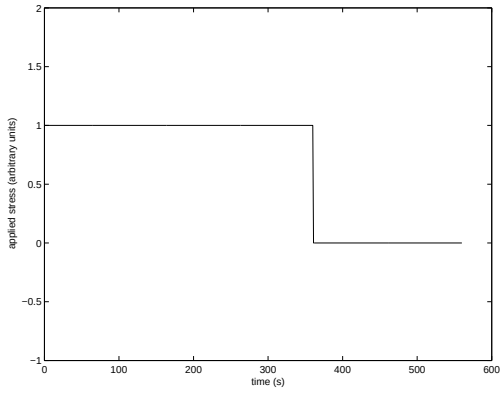
3D Results: Growing mushrooms, multispecies version.
Flat biofilm models exhibit Mullins-Sekerka instability.

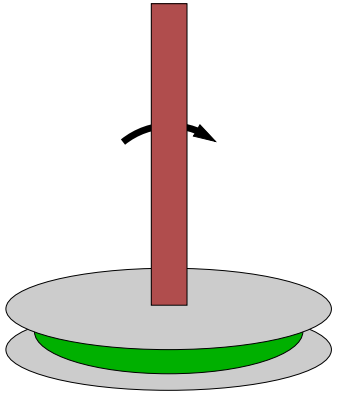


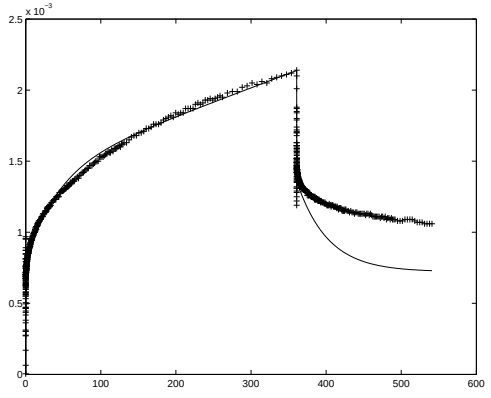
remark: instability reduces overall growth rate.

Biofilm Viscoelasticity

Biofilms are viscoelastic fluids:



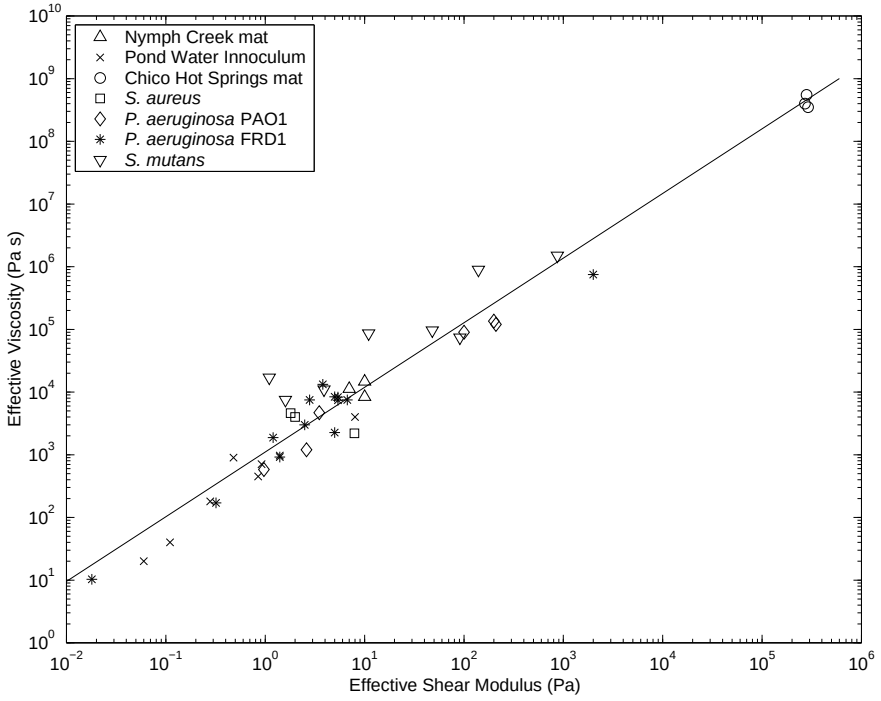




(collected from Nymph creek YNP)

Universality?

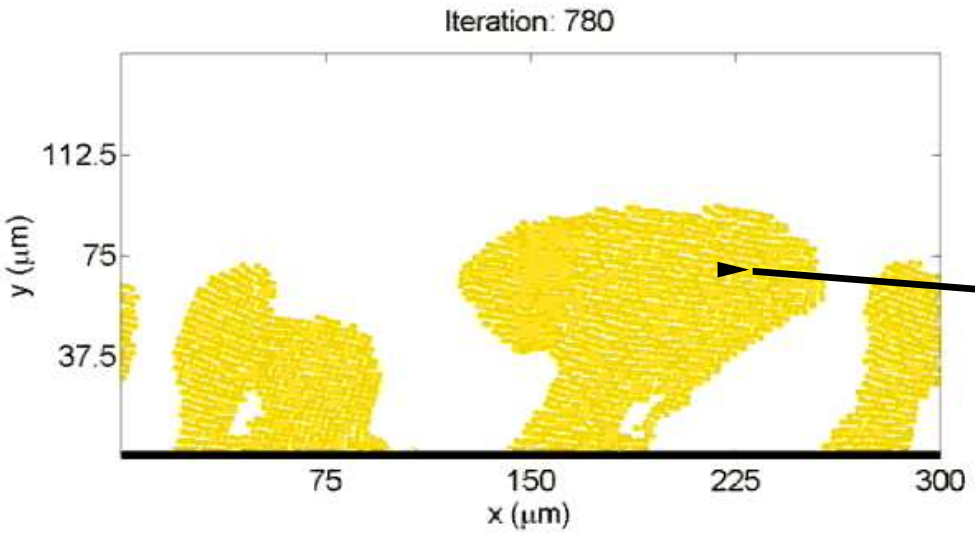
Elastic Relaxation Time: Approximately 20 min.

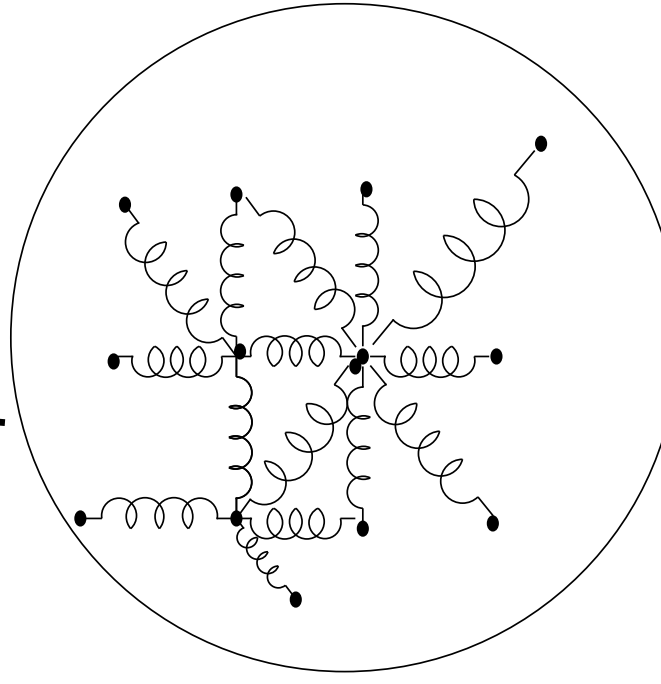


Viscosity η vs. shear modulus D for varied biofilms. Note large viscosities.

Immersed Boundary-Based Method: Biofilm

Use the method to model mechanical properties of the Biofilm: Rheometry and Detachment (E. Alpkvist).

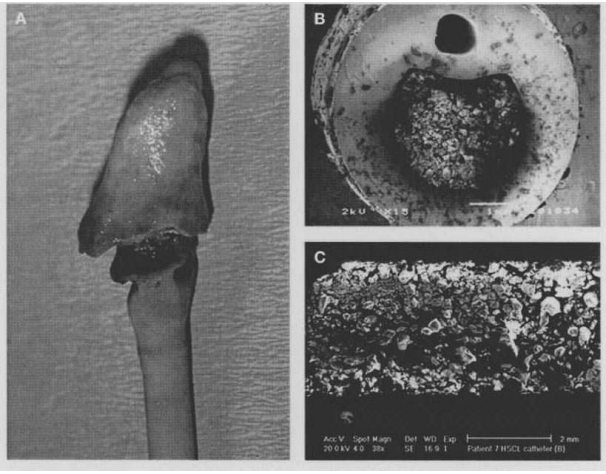


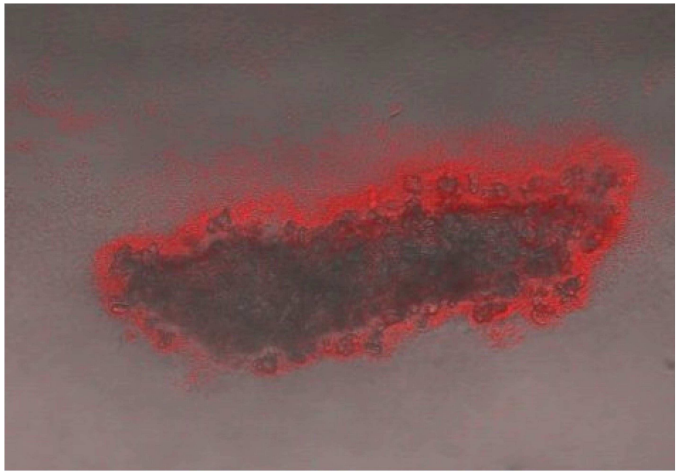


PART IV: SOME CHEMISTRY

Chemical Environment: pH and Biomineralization

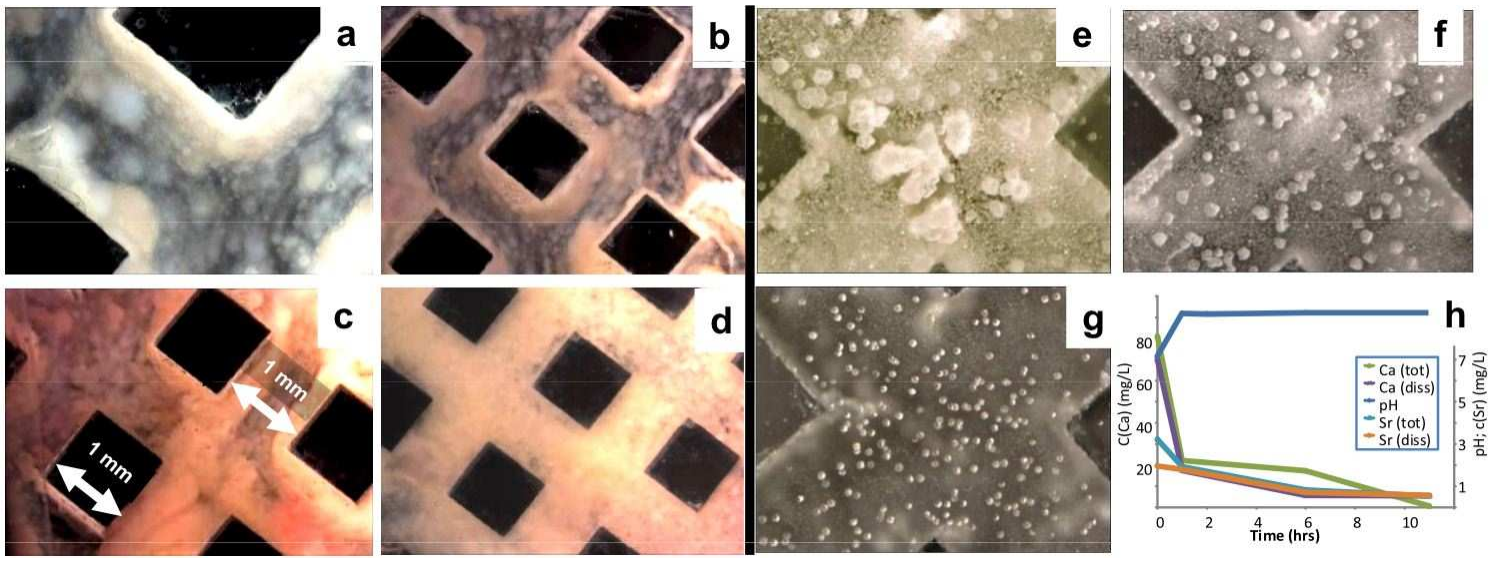
Biofilms can produce microenvironments where mineral formation or loss can occur, e.g. biobarriers, urinary catheter mineralization, tooth decay.





Left: Stickler, *Nature Clinical Practice. Urology* **5**, 598-608 2008
Right: Mallette & Stewart 2009

Experimental System (Gerlach lab)



(a)-(d) biofilm developing in a porous media reactor.
(e)-(g) calcite formation by *Sporosarcina pasteurii* biofilm.
(h) effluent measurements of pH, Ca, Sr show an increase in pH within hours and decreases in Ca and Sr.

Mixture Model

Three phases: solvent and two non-soluble phases.

$$\begin{aligned} \phi_s &= \text{Solvent Phase Volume Fraction} \\ \phi_b &= \text{Biomaterial Phase Volume Fraction} \\ \phi_c &= \text{Calcite Phase Volume Fraction} \end{aligned}$$

Remarks:

- $\phi_s + \phi_b + \phi_c = 1$ with $0 \leq \phi_\alpha \leq 1$, $\alpha = s, b, c$.
- Mixture idea borrowed from polymer physics (N. Cogan).

Free Energy

Chemical Free Energy of Mixing: $F = \int e(\phi_s, \phi_b, \phi_c)\, dV$ with Flory-Huggins form

$$\begin{aligned} e(\phi_c, \phi_b, \phi_s) &= \chi_b \phi_b \phi_s + \chi_c \phi_c (1 - \phi_c) \\ &\quad + \frac{\Gamma_c}{2} \|\nabla \phi_c\|^2 + \frac{\Gamma_b}{2} \|\nabla \phi_b\|^2 + \frac{\Gamma_s}{2} \|\nabla \phi_s\|^2 \\ &\quad + kT \left[\frac{1}{N} \phi_b \ln \phi_b + \phi_s \ln \phi_s + \phi_c \ln \phi_c \right] \end{aligned}$$

Remarks:

- Top terms are mixing energies: $\chi_b < 0$ and $\chi_c > 0$.
- Middle terms are transitional energy density terms.
- Bottom terms are mixing entropies.

Chemistry

$$CO(NH_2)_2 + 2H_2O \rightarrow 2NH_3 + H_2CO_3 \quad \text{(Urea hydrolysis)}$$

$$2NH_3 + 2H_2O \leftarrow\rightarrow 2NH_4^+ + 2OH^- \quad \text{(pH increase)}$$

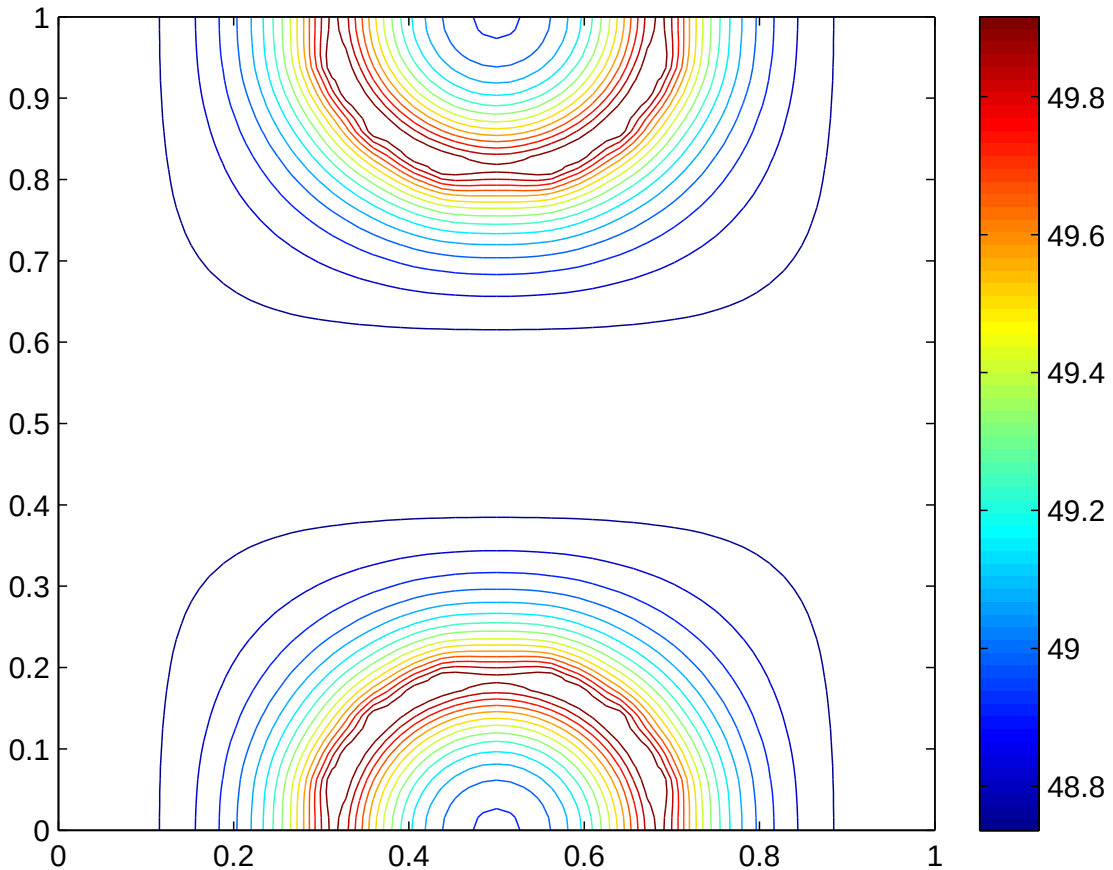
$$H_2CO_3 + 2OH^- \leftarrow\rightarrow HCO_3^- + H_2O + OH^- \leftarrow\rightarrow CO_3^{2-} + 2H_2O$$

$$CO_3^{2-} + Ca^{2+} \leftarrow\rightarrow CaCO_3 \quad \text{(carbonate precip.)}$$

Remarks:

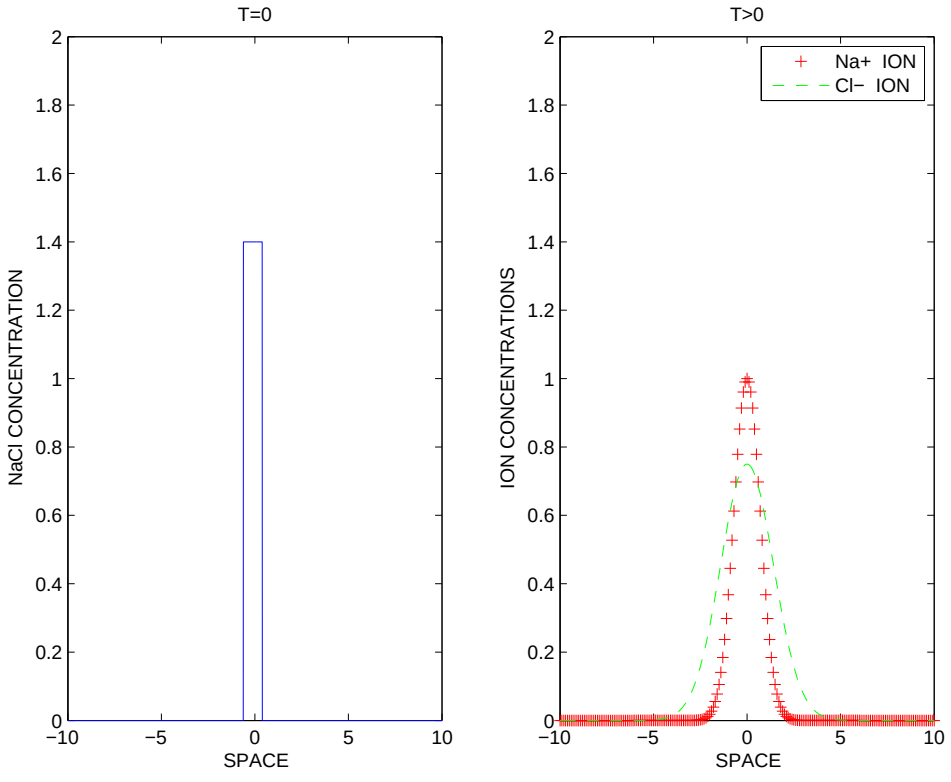
- Urea hydrolysis carried out by ureolytic organisms, *S. pasteurii*, (this is the rate-limiting step), results in pH increase and production of carbonic acid (H_2CO_3).
- H_2CO_3 buffers pH, producing carbonate (CO_3^{-2}).
- Carbonate precipitates into calcite (in presence of Ca^{2+}).

Precipitation: Saturation Layer



Precipitation occurs in a thin layer at the biofilm-fluid interface.

Generation of Electric Field by Diffusion



Left: $t = 0$, initial (charge neutral) seeding of NaCl in water. Right: $t > 0$, in the absence of electric field, ions diffuse at different rates ($D_{\mathrm{Na}^+} = 1.33$, $D_{\mathrm{Cl}^-} = 2.03$ in units of $10^{-5}\mathrm{cm}^2/\mathrm{sec}$) (not charge neutral!)

Solution

FIND THE ELECTRIC FIELD WHICH
PREVENTS CHARGE SEPARATION.

Remarks.

- Charge neutrality is preserved, but electrical currents may be induced.
- This idea is similar to use of a pressure to enforce fluid incompressibility.

Q. HOW IS THIS ELECTRIC FIELD COMPUTED?

Nernst-Planck Equations

Consider N ionic species with concentations S_i, $1 \leq i \leq N$.

$$\frac{\partial S_i}{\partial t} + \nabla \cdot \mathbf{J}_i = R_i, \quad 1 \leq i \leq N,$$

where $\mathbf{J}_i$, the flux of S_i, is given by

$$\mathbf{J}_i = S_i \mathbf{v} - \alpha_i S_i \nabla \mu_i.$$

Here R_i is the reaction term for species i, α_i is the mobility of species i, and μ_i is the electrochemical potential of species i, with

$$\nabla \mu_i = \frac{kT}{S_i} \nabla S_i + \hat{a} z_i \nabla \psi$$

where ψ is the electric potential (discussed below), z_i is the valence, and $\hat{a}$ is a units-related constant.

Electrodiffusion-Advection-Reaction Equations

Using the Einstein relation $D_i = \alpha_i kT$ for the diffusion coefficient D_i of species i, we obtain

$$\frac{\partial S_i}{\partial t} + \nabla \cdot (S_i \mathbf{v}) = \nabla \cdot (D_i \nabla S_i + a z_i D_i S_i \nabla \psi) + R_i, \quad 1 \leq i \leq N,$$

where $a = \hat{a}/kT$ with

$$\begin{aligned} z_i &= \text{charge valency of species } i \\ \psi &= \text{electrostatic potential} \\ R_i &= \text{reaction rate of species } i \end{aligned}$$

Remark. In the multiphase system, replace S_i by $\phi_s S_i$.

Electric Potential: Computation

Equation for species concentration S_i (with reaction source R_i):

$$\frac{\partial S_i}{\partial t} + \nabla \cdot (S_i \mathbf{v} - D_i \nabla S_i - a S_i z_i \nabla \psi) = R_i \tag{1}$$

Assume

- $\sum z_i S_i = 0$ (Charge neutrality)
- $\sum z_i R_i = 0$ (Charge conservation).

Multiply (1) by z_i and sum over i to obtain

$$\nabla \cdot \left[\left(\sum S_i z_i^2 \right) \nabla \psi \right] = -\frac{1}{a} \nabla \cdot \left(\sum z_i D_i \nabla S_i \right),$$

a (non-constant coefficient) Poisson equation for the potential ψ which enforces charge neutrality. (Note (1) is independent of a.)

Electric Potential: 1D Example

Consider two species with $z_1 = -z_2 = 1$ and $S_1 = S_2 = S$.

The Poisson equation for ψ reduces to

$$\nabla\psi = -\frac{1}{a}\frac{D_1 - D_2}{D_1 + D_2}\frac{\nabla S}{S}$$

The electrodiffusive flux for both species 1 and 2 becomes

$$D_i \nabla S_i + a z_i D_i S_i \nabla\psi \equiv D_{eff} \nabla S_i = \frac{2}{D_1^{-1} + D_2^{-1}} \nabla S_i,$$

(1) D_{eff} is the harmonic average of D_1 and D_2.
(2) Note enhanced diffusion of less diffusive species.
(3) Given $D_1 \leq D_2$, then $D_1 \leq D_{eff} < 2D_1$.

Punchline

1. Chemistry in non-mixed systems leads to spatial variation.
2. Spatial variation leads to diffusive transport.
3. Variation in ion diffusivities leads to charge separation.
4. Charge separation leads to electric fields.
5. Electric field leads to enhanced reaction rates.

Electro-Enhanced Chemistry

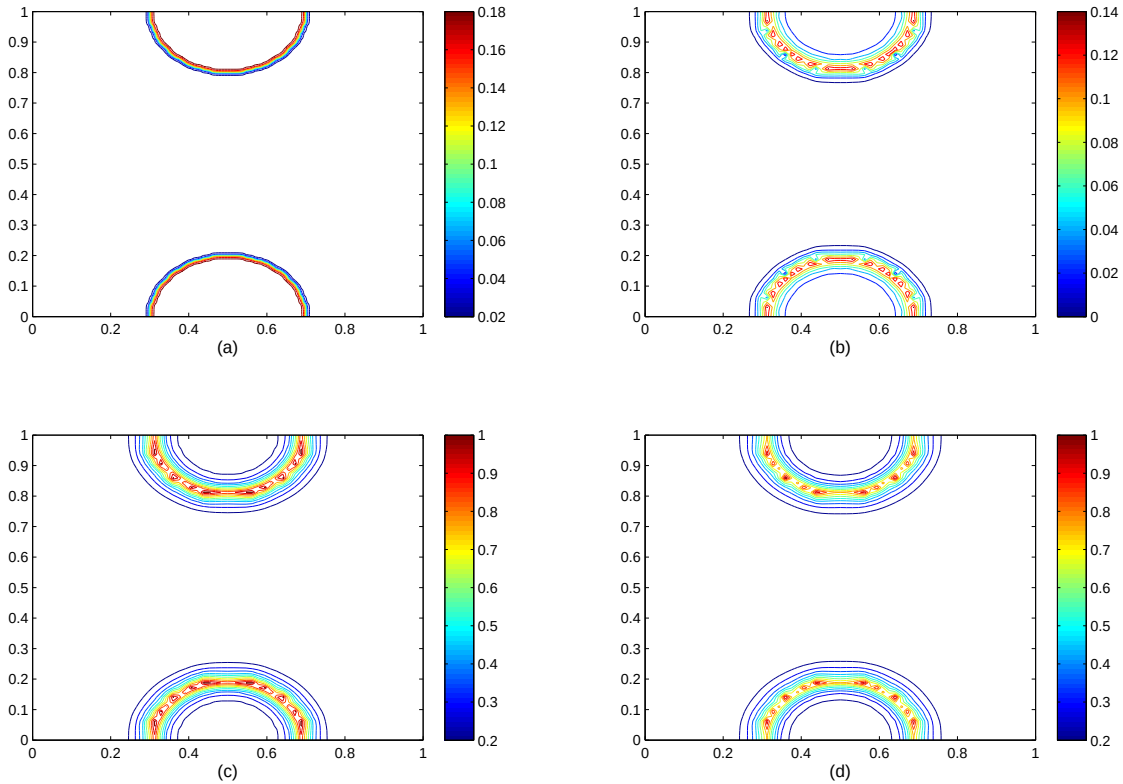
Electro-enhancement.

- Reactions locally deplete reactants
- Slowest diffusing reactant is limiting.
- Electric field generally increases diffusivity of the slowest diffusing chemical species.

$\Rightarrow$ electrodiffusion generally enhances reaction.

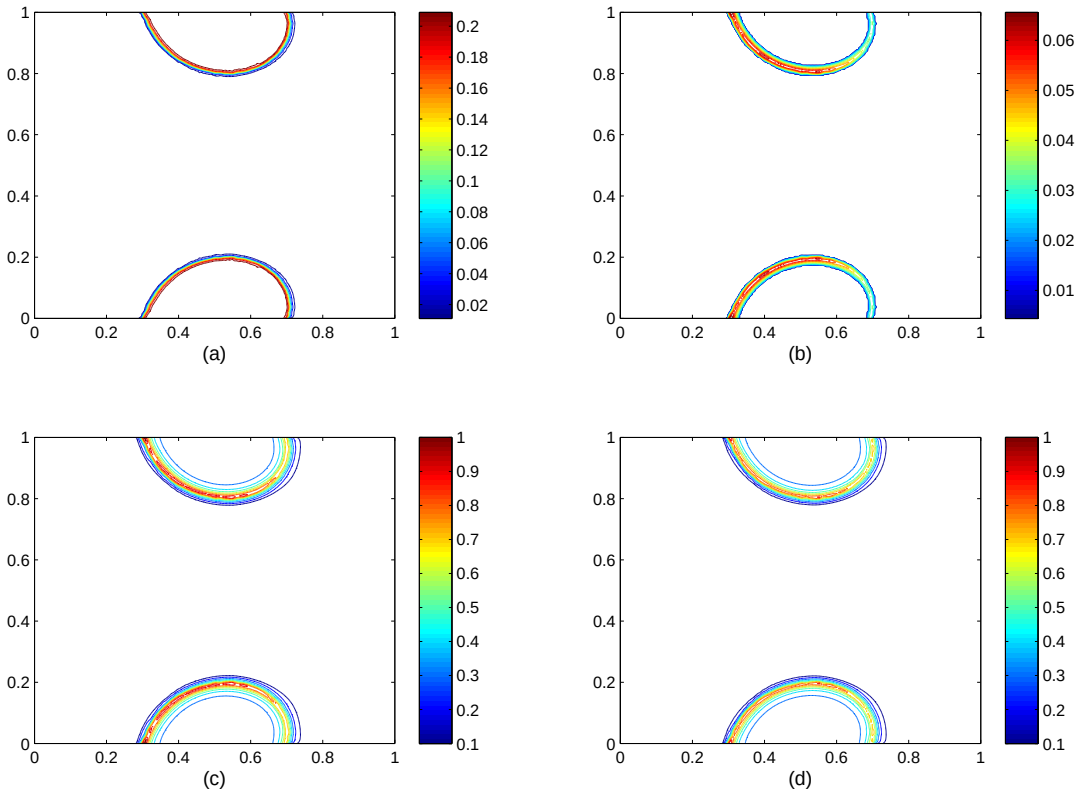
Example. Electrodiffusion of Ca^{2+} enhances carbonate precipitation $\mathrm{CO}_3^{2-} + \mathrm{Ca}^{2+} \leftarrow \rightarrow \mathrm{CaCO}_3$.

Biomaterial and Calcite: No Flow



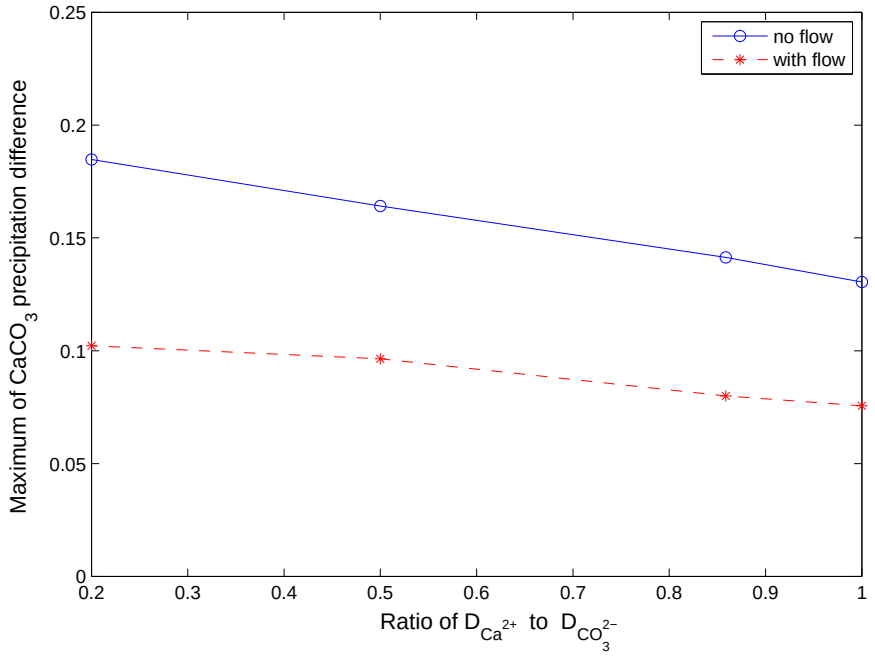
Contours of (a) ϕ_B at $t = 0$, (b) difference at $t = 1$ day of ϕ_c with and without electric field, (c) ϕ_c (with electric field) at $t = 1$ day, (d) ϕ_c (without electric field) at $t = 1$ day.

Biomaterial and Calcite: With Flow



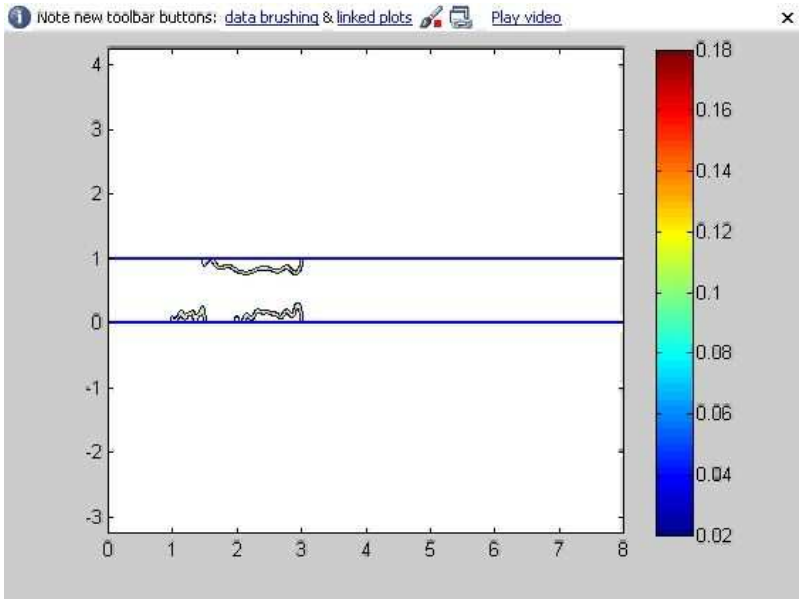
Contours of (a) ϕ_B at $t = 0$, (b) difference at $t = 1$ day of ϕ_c with and without electric field, (c) ϕ_c (with electric field) at $t = 1$ day, (d) ϕ_c (without electric field) at $t = 1$ day.

Electrodiffusive Enhancement Effect

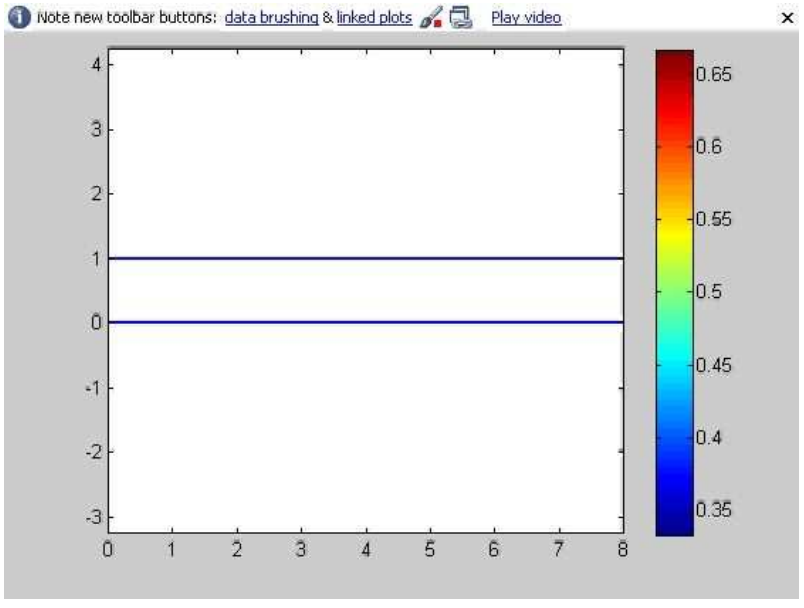


Largest difference between ϕ_c calculated with and without the electric field, plotted against the ratio of calcium and carbonate ion diffusivities.

Example Computation



Biomaterial



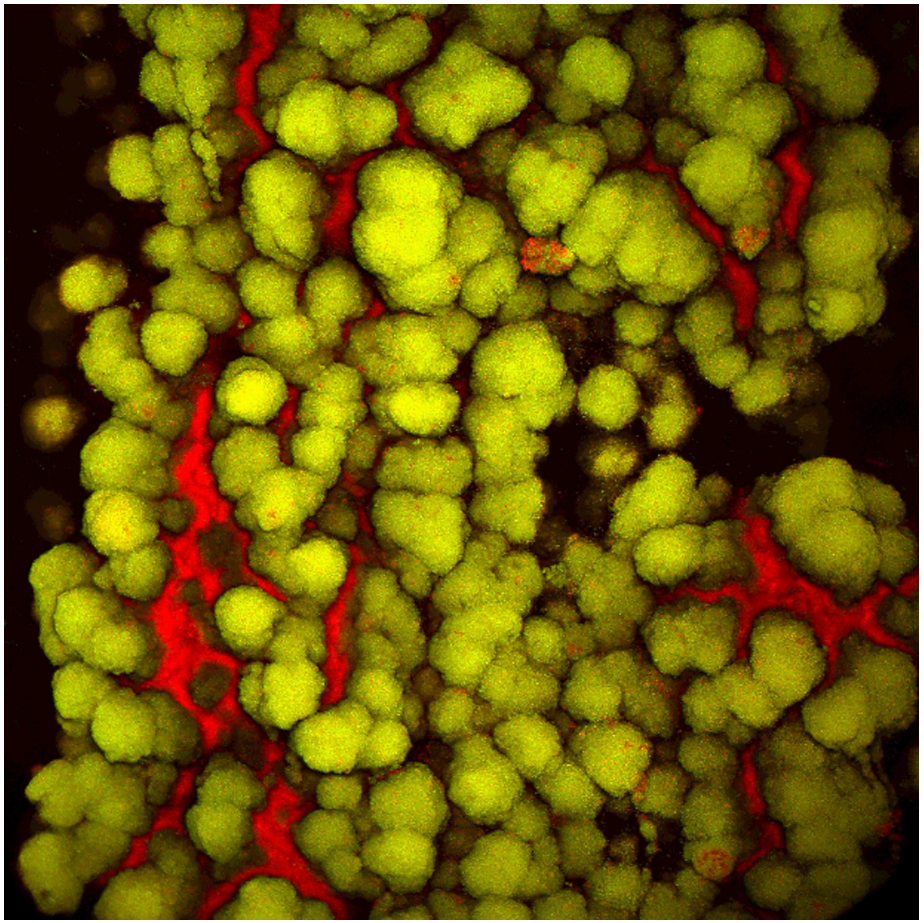
Calcite

Conclusions

1. Electrodiffusion is at least moderately important.
2. Electrodiffusion is at least moderately inconvenient.

Where Next?

Thank You for Listening!



S. epidermidis biofilm, courtesy of Betsy Pitts, Center for Biofilm Engineering, MSU

Ending

THINK SNOW!!

(*Bacillus* biofilms, Alessandra Agostinho)