The Dynamics of Growing Biofilm

J. P. Keener

Department of Mathematics
University of Utah
Biofilms

Biofilm fouling of filter fibers

Plaque on teeth
Some Interesting Questions

How do gels grow?

- *P. aeruginosa* (on catheters, IV tubes, etc.)
- Mucus secretion (bronchial tubes, stomach lining)
- Colloidal suspensions, cancer cells
- Gel morphology (the shape of sponges)

Why are gels important?

- Protective capability
- Friction reduction
- High viscosity (low washout rate) for drugs
- Acid protection
Biofilm Formation in *P. Aeruginosa*

Wild Type  Biofilm Mutant  Mutant with autoinducer
Dynamics of Growing Biogels

I: Quorum sensing:
- What is it?
- How does it work?

II: Heterogeneous structures
- How do cells use polymer gel for locomotion?
- What are the mechanisms of pattern formation?
**I: Quorum Sensing in *P. aeruginosa***

Quorum sensing: The ability of a bacterial colony to sense its size and regulate its activity in response.

Examples: *Vibrio fisheri, P. aeruginosa*

*P. Aeruginosa*:

- Major cause of hospital infection in the US.
- Major cause of death in intubated Cystic Fibrosis patients
- In planktonic form, they are non-toxic, but in biofilm they are highly toxic and well-protected by the polymer gel in which they reside. However, they do not become toxic until the colony is of sufficient size, i.e., quorum sensing.
Stages of Growth

Planktonic
Stages of Growth

Small Dense Colony
Stages of Growth

Biofilm Colony
Biochemistry of Quorum Sensing

$\textit{lasR}$

$\textit{lasI}$
Biochemistry of Quorum Sensing

\[ \text{LasR} \rightarrow 3\text{–}\text{oxo–C}12\text{–HSL} \rightarrow \text{LasI} \]

\[ \text{lasR} \]

\[ \text{lasI} \]

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Biochemistry of Quorum Sensing

lasR

LasR

3-oxo-C12-HSL

LasI

lasI
Biochemistry of Quorum Sensing
Biochemistry of Quorum Sensing

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Biochemistry of Quorum Sensing

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Modeling Biochemical Reactions

Bimolecular reaction $A + R \leftrightarrow P$

$$\frac{dP}{dt} = k_+ AR - k_- P$$
Bimolecular reaction $A + R \rightleftharpoons P$

$$\frac{dP}{dt} = k_+ AR - k_- P$$

Production of mRNA

$$\frac{dl}{dt} = \frac{V_{max} P}{K_l + P} - k_- l$$
Bimolecular reaction $A + R \rightleftharpoons P$

\[
\frac{dP}{dt} = k_+ AR - k_- P
\]

Production of mRNA

\[
\frac{dl}{dt} = \frac{V_{max} P}{K_l + P} - k_- l
\]

Enzyme production $l \rightarrow L$

\[
\frac{dL}{dt} = k_l l - K_L L
\]
Full system of ODE’s

\[
\begin{align*}
\frac{dP}{dt} &= k_{RA}RA - k_PP \\
\frac{dR}{dt} &= -k_{RA}RA + k_PP - k_RR + k_1r, \\
\frac{dA}{dt} &= -k_{RA}RA + k_PP + k_2L - k_AA, \\
\frac{dL}{dt} &= k_3l - k_lL, \\
\frac{dS}{dt} &= k_4s - k_SS, \\
\frac{ds}{dt} &= V_s \frac{P}{K_S+P} - k_ss, \\
\frac{dr}{dt} &= V_r \frac{P}{K_r+P} - k_r r + r_0, \\
\frac{dl}{dt} &= V_l \frac{P}{K_l+P} \frac{1}{K_S+S} - k_ll + l_0
\end{align*}
\]
\[
\frac{dA}{dt} = F(A, R, P) + \delta(E - A)
\]
\[
\frac{dE}{dt} = -k_E E + \delta(A - E)
\]
The dynamics of growing biofilm involve the following equations for the rate of change of two variables, $A$ and $E$:

\[
\frac{dA}{dt} = F(A, R, P) + \delta(E - A)
\]

\[
\frac{dE}{dt} = -k_E E + \delta(A - E)
\]

These equations describe the evolution of the biofilm's properties over time, reflecting the impact of environmental factors and internal dynamics.
\[ \frac{dA}{dt} = F(A, R, P) + \delta(E - A) \]

\[ \frac{dE}{dt} = -k_E E + \delta(A - E) \]

rate of change, production or degradation rate,
The rate of change, production or degradation rate, diffusive exchange.

$$\frac{dA}{dt} = F(A, R, P) + \delta(E - A)$$

$$\frac{dE}{dt} = -k_E E + \delta(A - E)$$
\[
\frac{dA}{dt} = F(A,R,P) + \delta(E - A)
\]

\[
(1 - \rho)(\frac{dE}{dt} + KE') = \rho \delta(A - E')
\]

rate of change, production or degradation rate, diffusive exchange, density dependence.
Two (possible) ways to proceed:

- Numerical simulation (but few of the 22 kinetic parameters are known),
- Qualitative analysis (QSS reduction)
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- Numerical simulation (but few of the 22 kinetic parameters are known),
- Qualitative analysis (QSS reduction)

\[
\frac{dA}{dt} = F(A) + \delta(E - A), \quad (1 - \rho)(\frac{dE}{dt} + kE) = \rho\delta(A - E)
\]
Two Variable Phase Portrait
Suppose cells are immobile, so internal variables do not diffuse, but extracellular autoinducer $E$ diffuses

$$\begin{align*}
\frac{\partial A}{\partial t} &= F(A, U) + \delta(E - A), \\
\frac{\partial U}{\partial t} &= G(A, U), U \in \mathbb{R}^7, \\
\frac{\partial E}{\partial t} &= \nabla \cdot (D_E \nabla E) - k_E E + \frac{\rho}{1 - \rho} \delta(A - E)
\end{align*}$$

in $\Omega$ with Robin boundary conditions

$$n \cdot D_e \nabla E + \alpha E = 0$$

on $\partial \Omega$. 

\[\text{A PDE Model}\]
Autoinducer as function of cell density
A Hydrogel Primer

- What is a hydrogel?
  A tangled polymer network in solvent.

- Examples of biological hydrogels
  Micellar gels
  Jello (a collagen gel $\approx 97\%$ water)
  Extracellular matrix
  Blood clots
  Mucin - lining the stomach, bronchial tubes, intestines
  Glycocalyx
  Sinus secretions
A Hydrogel Primer - II

Functions of a biological hydrogel

- Decreased permeability to large molecules
- Structural strength (for cell walls)
- Capture and clearance of foreign substances
- Decreased resistance to sliding/gliding
- High internal viscosity (low washout)

Important features of gels

- Usually comprised of highly polyionic polymers
- Can undergo volumetric phase transitions in response to ionic concentrations, temperature, etc.
- Volume is determined by combination of forces (entropic, electrostatic, hydrophobic, cross-linking, etc.)
How gels grow

- Polymerization/deposition
  - Monomers → Polymers → Network

- Secretion
  - Condensed → Secretion → Swollen
A two phase material with polymer network volume fraction $\theta$

\[
\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n \\
\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0 \\
\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b \\
\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u)) = g_u
\]

Network Phase (EPS)
Solute Phase
Bacterial concentration
Resource Concentration

where $\theta + \theta_s = 1$, 
Modelling Biofilm Growth

A two phase material with polymer network volume fraction $\theta$

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$  

Network Phase (EPS)

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$  

Solute Phase

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$  

Bacterial concentration

$$\frac{\partial \theta_s u}{\partial t} + \nabla \cdot \left( \theta_s (V_s u - D_u \nabla u) \right) = g_u$$  

Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction,
A two phase material with polymer network volume fraction $\theta$

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$  \hspace{1cm} \text{Network Phase (EPS)}

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$  \hspace{1cm} \text{Solute Phase}

$$\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b$$  \hspace{1cm} \text{Bacterial concentration}

$$\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u)) = g_u$$  \hspace{1cm} \text{Resource Concentration}

where $\theta + \theta_s = 1$, solute volume fraction, network velocity,
A two phase material with polymer network volume fraction $\theta$

$$\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n$$  

Network Phase (EPS)

$$\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0$$  

Solute Phase

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Bacterial concentration

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Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction, network velocity, solute velocity,
A two phase material with polymer network volume fraction $\theta$

\[
\frac{\partial \theta}{\partial t} + \nabla \cdot (V_n \theta) = g_n + \epsilon \nabla^2 \theta
\]

Network Phase (EPS)

\[
\frac{\partial \theta_s}{\partial t} + \nabla \cdot (V_s \theta_s) = 0
\]

Solute Phase

\[
\frac{\partial b}{\partial t} + \nabla \cdot (V_n b) = g_b
\]

Bacterial concentration

\[
\frac{\partial \theta_s u}{\partial t} + \nabla \cdot (\theta_s (V_s u - D_u \nabla u)) = g_u
\]

Resource Concentration

where $\theta + \theta_s = 1$, solute volume fraction, network velocity, solute velocity, artificial network diffusion.
Force Balance

Solute Phase (an inviscid fluid)

\[ h_f \theta_s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction
Solute Phase (an inviscid fluid)

\[ h_f \theta s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction \hspace{1cm} \text{pressure}
**Force Balance**

**Solute Phase** (an inviscid fluid)

\[ h_f \theta \theta_s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction pressure

**Network Phase** (a viscoelastic material)

\[ \eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) - h_f \theta \theta_s (V_n - V_s) - \nabla \psi (\theta) - \theta \nabla p = 0 \]

network viscosity
**Force Balance**

**Solute Phase** (an inviscid fluid)

\[ h_f \theta \theta_s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction  \( \theta \)  \( \nabla \)  pressure

**Network Phase** (a viscoelastic material)

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network viscosity  solute-network friction
**Force Balance**

**Solute Phase** (an inviscid fluid)

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network viscosity solute-network viscosity osmosis
Force Balance

Solute Phase (an inviscid fluid)

\[ h_f \theta_\theta s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction \hspace{1cm} \text{pressure}

Network Phase (a viscoelastic material)

\[ \eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) - h_f \theta_\theta s (V_n - V_s) - \nabla \psi (\theta) - [\theta \nabla p] = 0 \]

network viscosity \hspace{1cm} \text{solute-network viscosity} \hspace{1cm} \text{osmosis} \hspace{1cm} \text{pressure}
**Force Balance**

**Solute Phase** (an inviscid fluid)

\[ h_f \theta \theta_s (V_n - V_s) - \theta_s \nabla p = 0, \]

solute-network friction pressure

**Network Phase** (a viscoelastic material)

\[ \eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) - h_f \theta \theta_s (V_n - V_s) - \nabla \psi(\theta) - \theta \nabla p = 0 \]

network viscosity solute-network viscosity osmosis pressure

**Imcompressibility**

\[ \nabla \cdot (\theta V_n + \theta_s V_s) = g_n \]
Osmotic Pressure

What is the meaning of the term $-\nabla \psi(\theta)$?

$\psi'(\theta) > 0$ gives expansion (swelling)
$\psi'(\theta) < 0$ gives contraction (deswelling)
Osmotic Pressure

What is the meaning of the term $-\nabla \psi(\theta)$?

$\psi'(\theta) > 0$ gives expansion (swelling)
$\psi'(\theta) < 0$ gives contraction (deswelling)

To maintain an edge, $\psi(\theta)$ must be of the form $\psi(\theta) = \theta^2 F(\theta)$
Movement by Swelling

WT Biofilm Growth

Mutant Cell Growth
"Nutrient Poor" Fingering Instability
Modified Network Model: Include elastic strains, \( \sigma_n = \gamma \epsilon \)

\[
\eta \nabla (\theta (\nabla V_n + \nabla V_n^T)) + \nabla \cdot (\gamma \theta \epsilon) - h_f \theta \theta_s (V_n - V_s) - \nabla \psi (\theta) - \theta \nabla p = 0
\]

and displacements \( D \)

\[
\frac{\partial D}{\partial t} + \nabla \cdot (V_n D) = V_n
\]

where \( \epsilon \) is the Cauchy-Green strain tensor.
Remark: The existence of this channeling "Moses Bifurcation" can be established using singular perturbation arguments.
Summary

- Quorum sensing is via a hysteretic switch involving diffusible autoinducer
- Fingering and mushrooming may be driven by a substrate deficiency-fingerprinting instability.
- Channeling may be driven by a gel-osmosis "Moses Bifurcation".
Acknowledgments

Collaborators
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- Nick Cogan, Tulane University

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- This talk can be viewed at http://www.math.utah.edu/~keener/lectures/biofilmmdynamics
- No Microsoft products were used or harmed during the production of this talk.

The End
Structure of the "Moses Bifurcation"

The steady state equation is

\[
\epsilon \theta \frac{d}{dy} \left( \frac{d\theta}{\theta} \right) + \frac{1}{\theta} H(\theta, y) = k
\]

subject to

\[
\int_{0}^{1} \theta dy = \hat{\theta}
\]

where

\[
H(y, \theta) = G^2(y - \frac{1}{2})^2 - \theta \Psi(\theta) + \hat{\theta}^2 - \theta^2, \quad \Psi(\theta) = \kappa \theta^2 (\theta - \theta_{ref})
\]
This is a singular perturbation problem.

For $\epsilon = 0$ (the "outer solution"), we must solve an algebraic equation for $\theta$ as a function of $y$. However, the equation $H(\theta, y) = k\theta$ has (possibly) multiple solutions.
The governing equation is a "bistable equation", so transition layers can be inserted at certain locations.

It is possible that boundary layer solutions coexist with non-boundary solutions, as is seen in the bifurcation diagram.