Modeling Microbial Populations II

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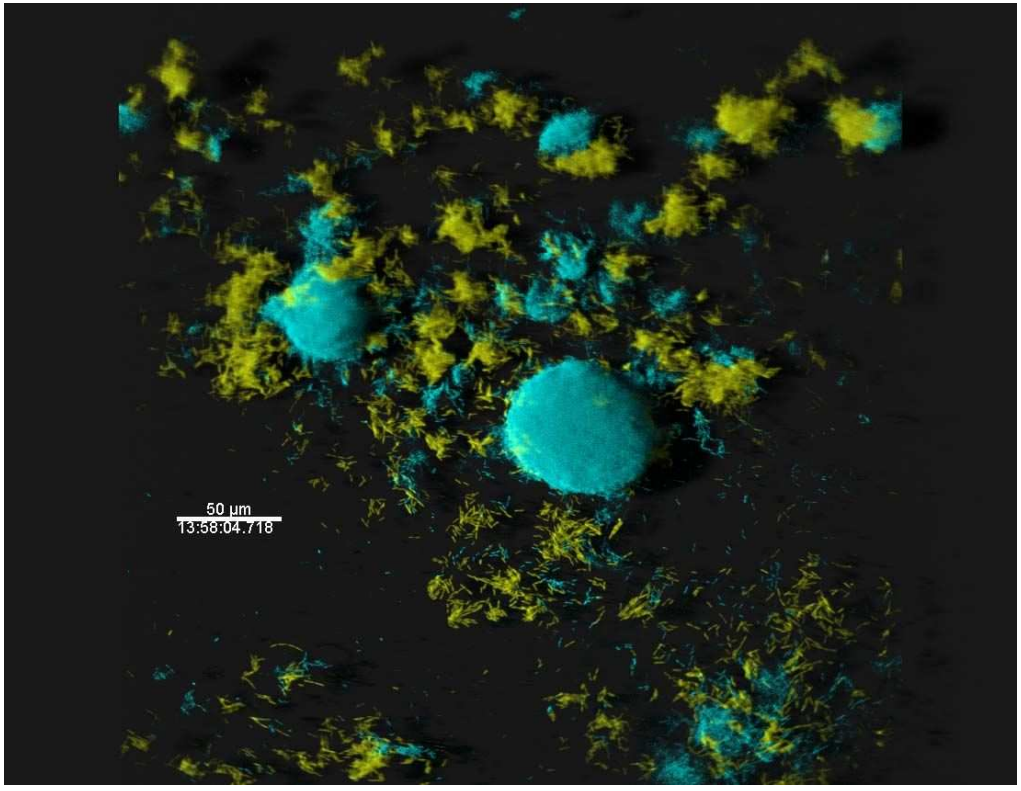
Biofilms

Biofilms are collections of microorganisms contained in self-secreted matrices of polymers and other substances, a “city of microbes” (Kolter) (or “microbe jungle”?).



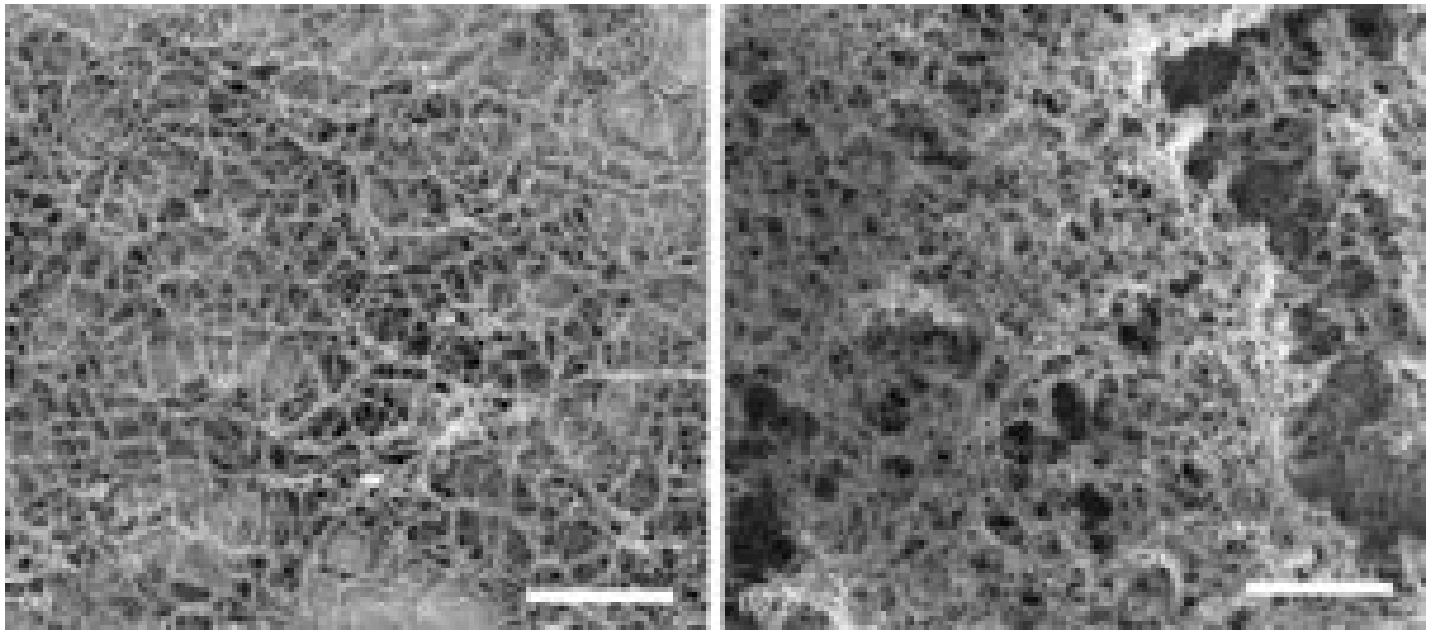
(Picture B. Klayman, *Pseudomonas aeruginosa*)

Some Fundamental Questions

- Community productivity: at what rate does the community convert input products to output products?
- Community ecology: who is there and what are they doing?
- Community tolerance: why (and how) are these communities so tolerant to challenges (in comparison to free-living microbes)?
- Physics and Chemistry: how does the physical environment effect microbial communities and vice-versa?

Extracellular Polymeric Substances (EPS)

Purposes?



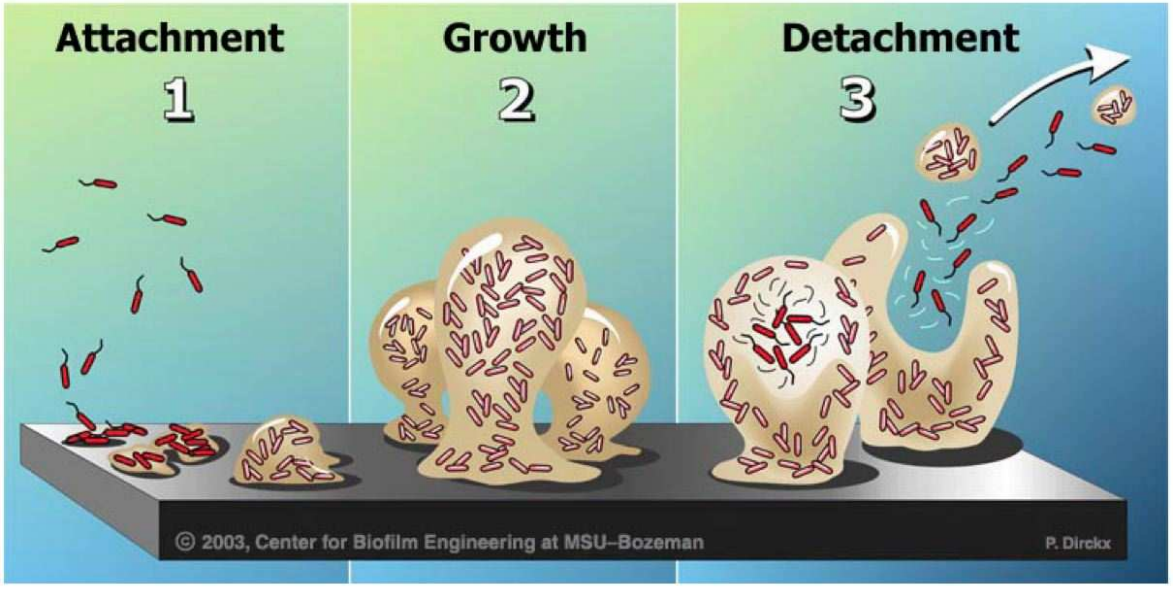
Meiothermus biofilm SEM. (BR Johansson)

Biofilm vs “Plankton”

In biofilms:

- Diffusion (and diffusion-reaction) is dominant transport mechanism for nutrient, electron donor, electron acceptor, signalling, etc. Advective transport is negligible (no turbulent diffusivities) but ...
- competition for space is important for microorganisms (self-generated advection is important).
- close-packing of organisms (well within diffusive lengths) probably has interesting ecological consequences (e.g signalling, consortia building and cheating, ...).
- Physics (due, e.g., to EPS) is more complex but less complicated.

“Life Cycle”


Attachment
1
Growth
2
Detachment
3
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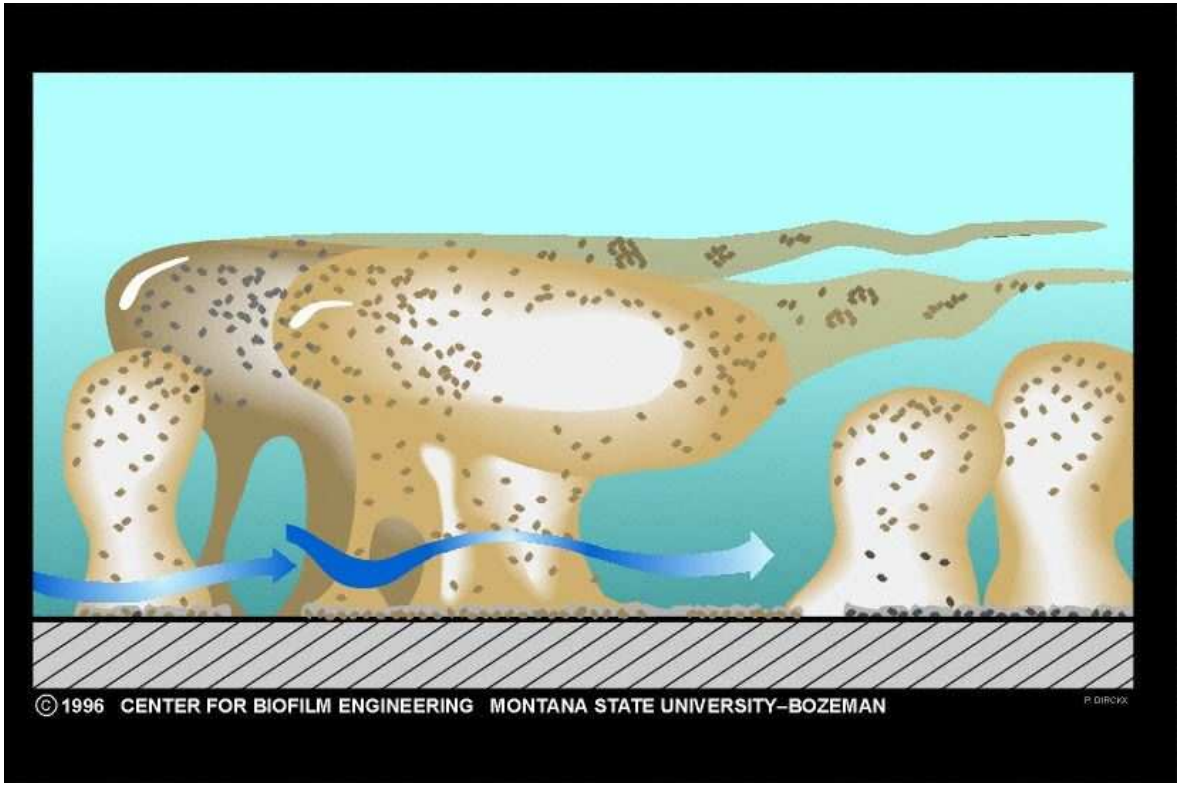
P. Dirckx

Cartoon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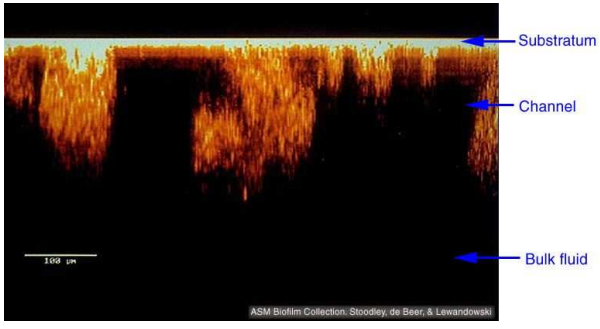
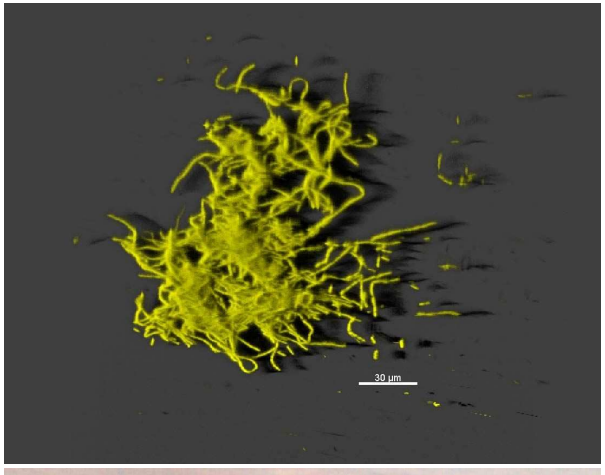
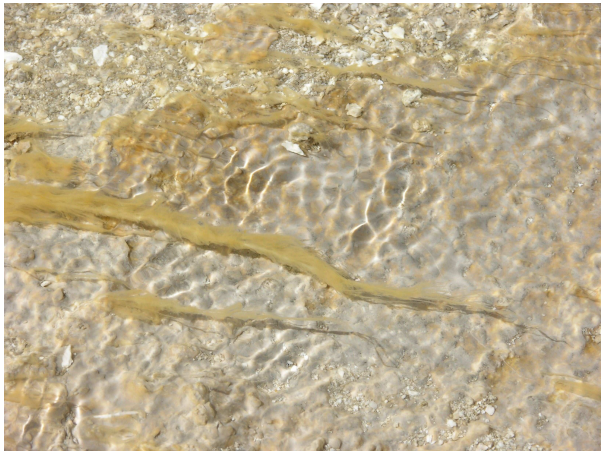
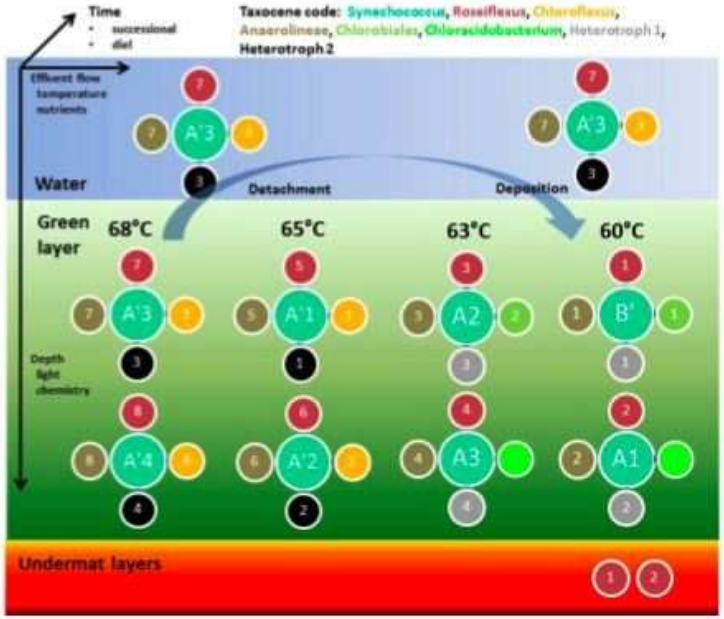
Pictures


Substratum
Channel
Bulk fluid
ASM Biofilm Collection. Stoodley, de Beer, & Lewandowski


30 µm



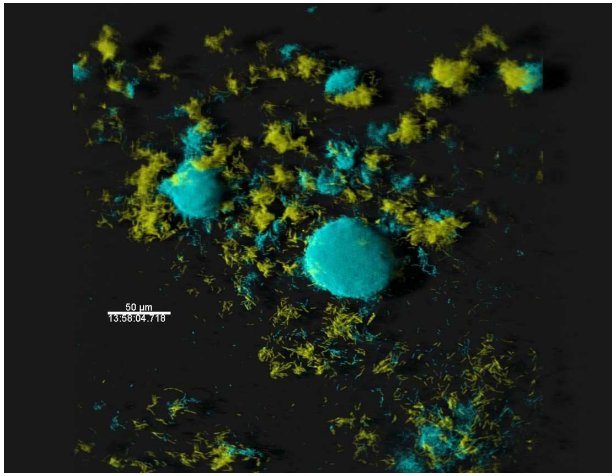
Mat Cartoon

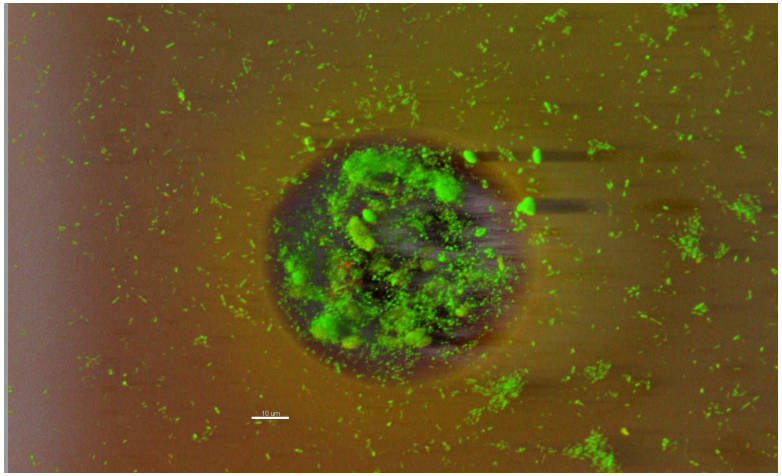


Remark: close neighbors = important interactions (revealed by sequencing data)

Biofilms: Medical

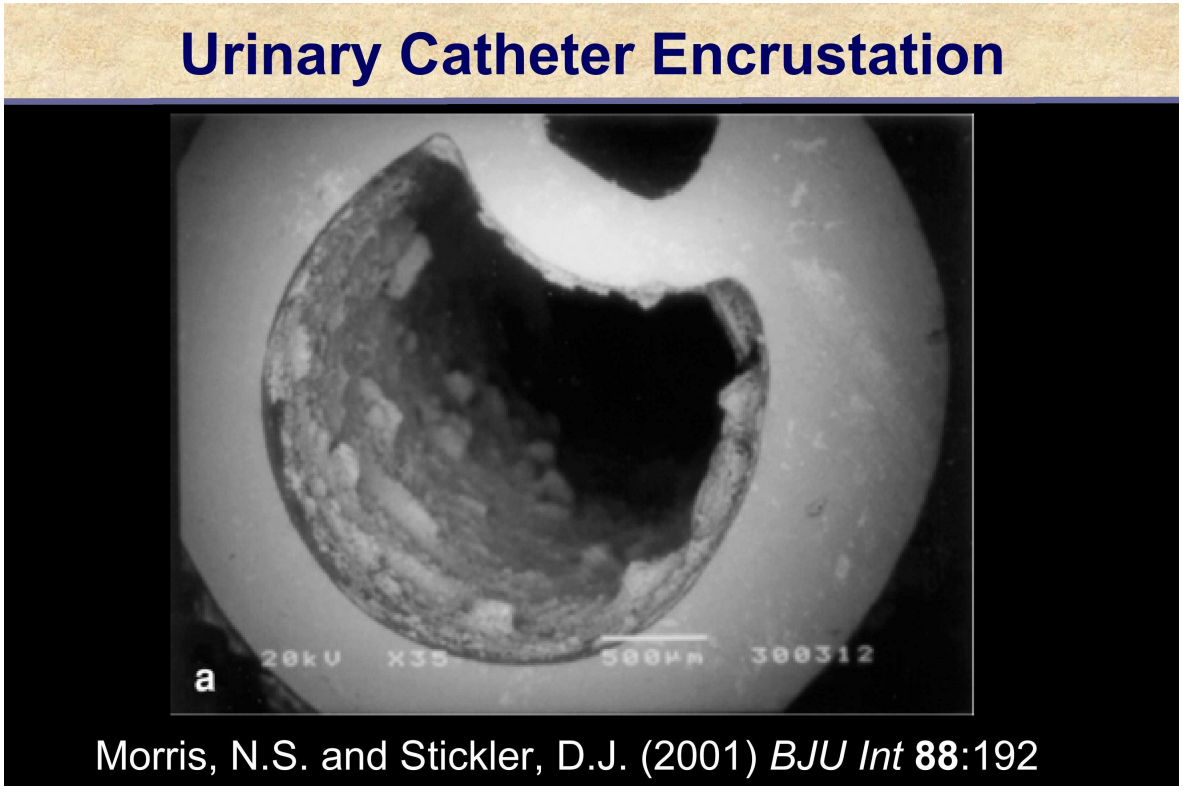
- Every person harbors roughly 10 times more microbial than human cells (mostly in gut biofilms).
- Biofilms are involved in most microbial infections in the body including infections of: gums, ears, eyes, airways/lungs, gastrointestinal tract, all implants (e.g. heart valves, stents, catheters, etc.)





Left: *Pseudomonas aeruginosa* biofilm (Klayman).
Right: biofilm on a contact lens (Pitts).

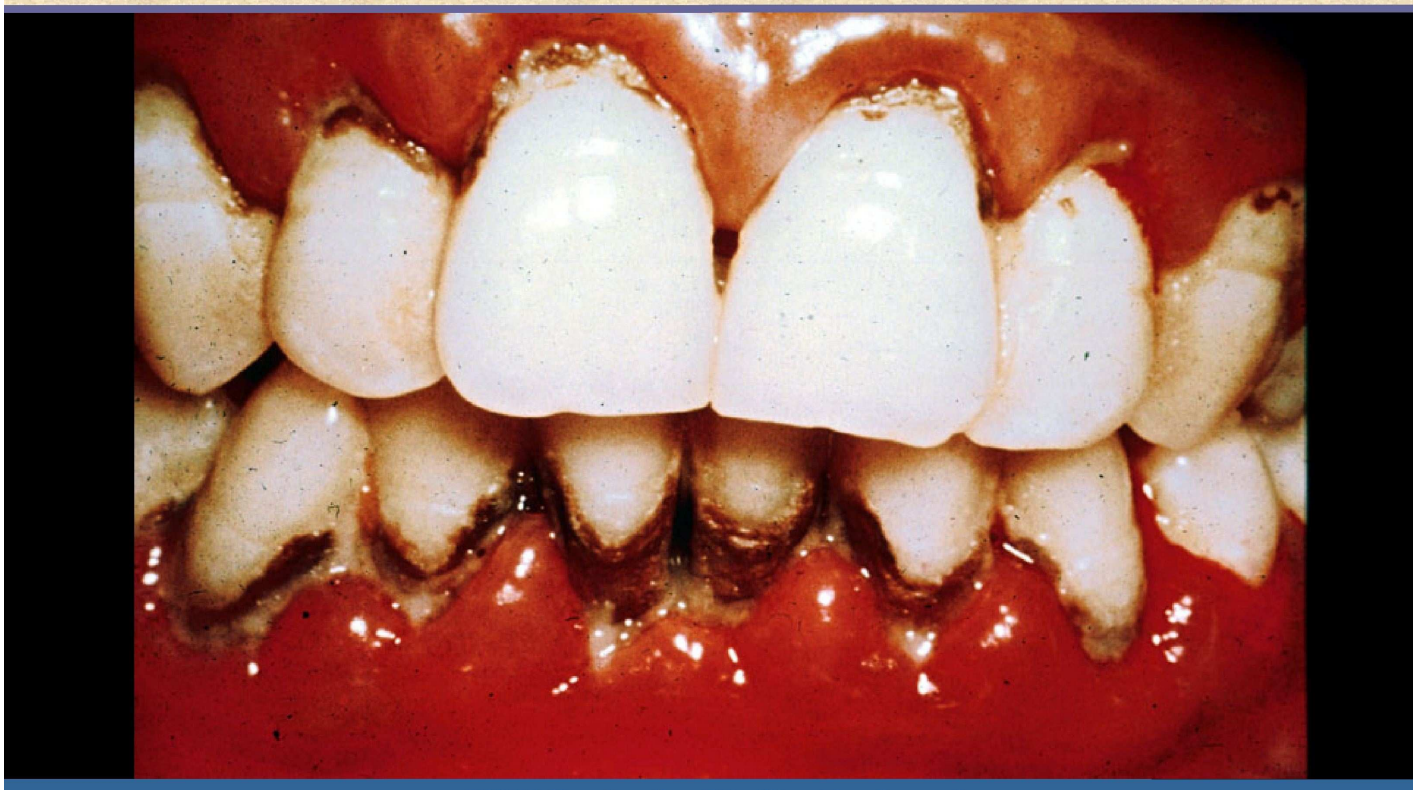
Catheter



Struvite formation as a consequence of ureolysis.

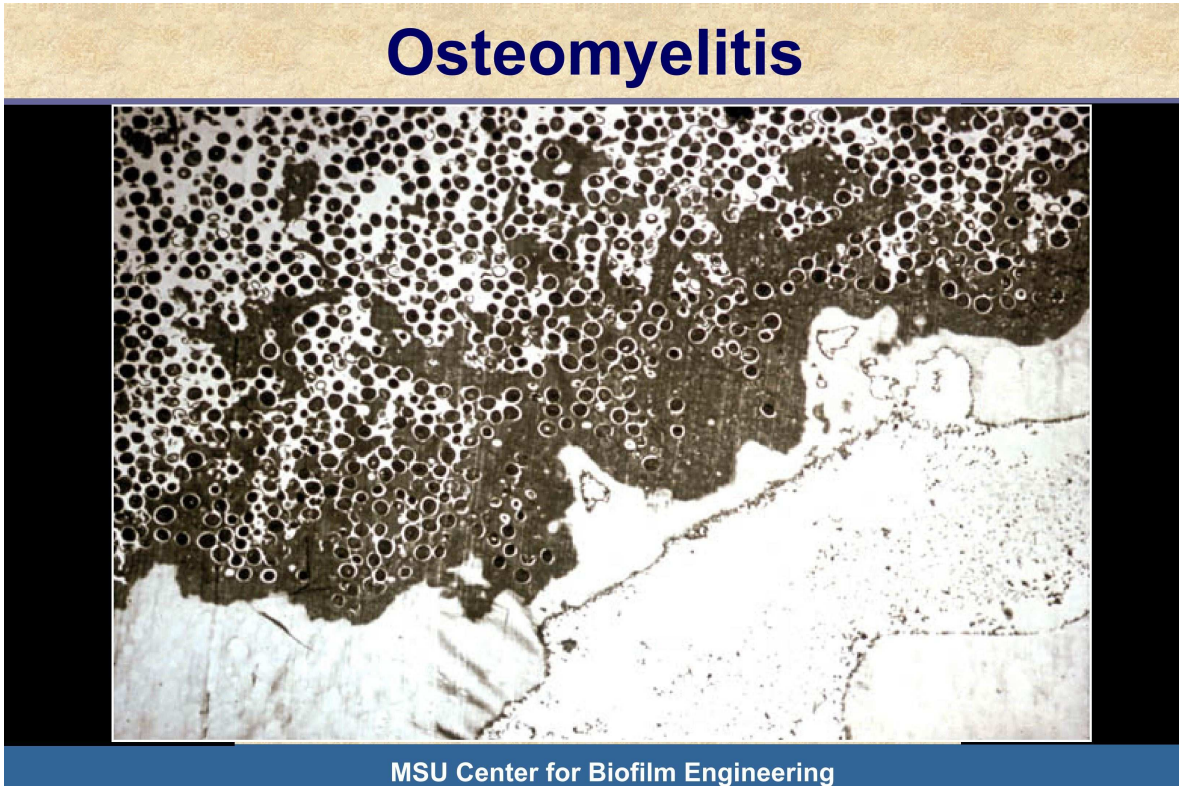
Teeth

Periodontitis



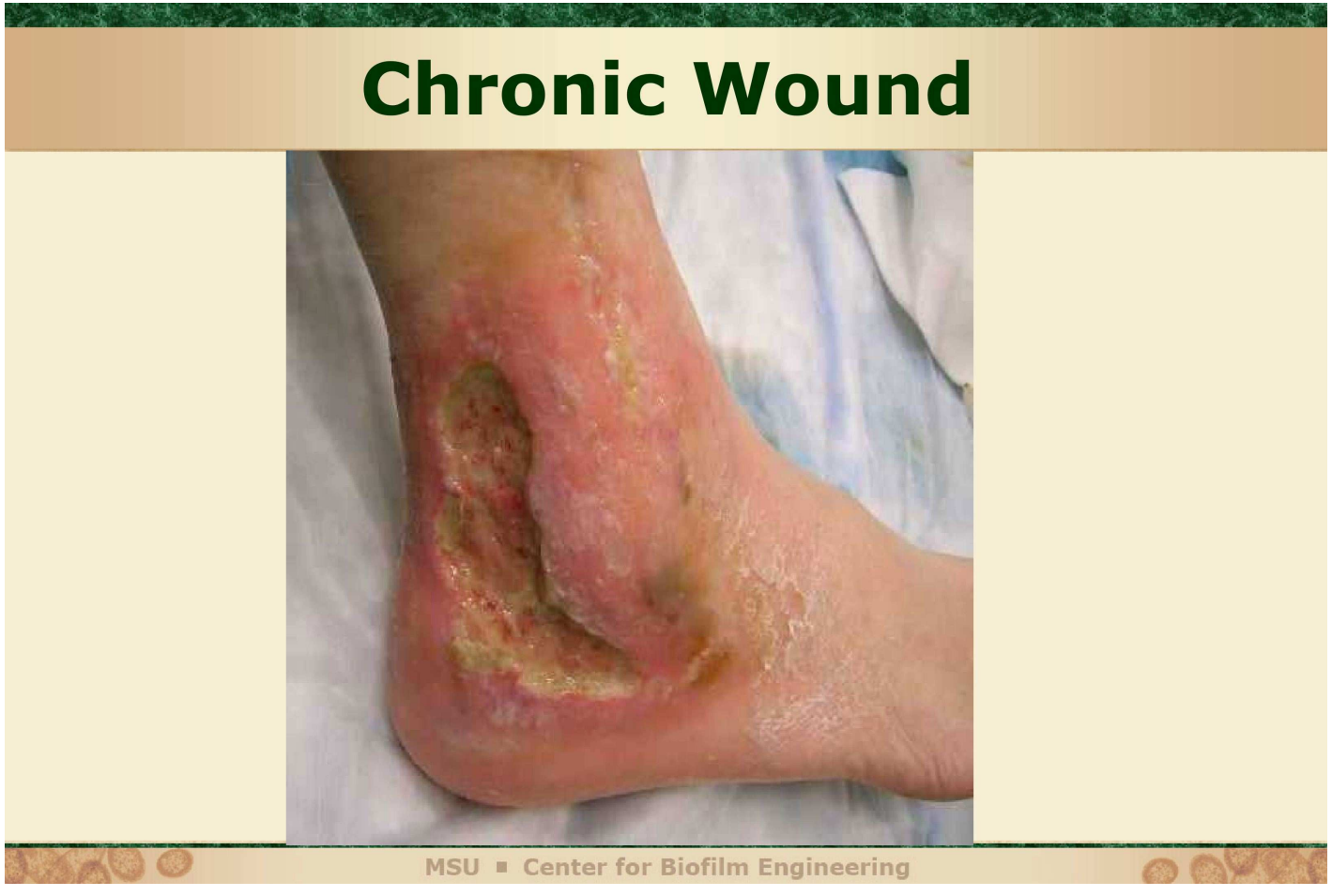
MSU Center for Biofilm Engineering

Osteomyelitis



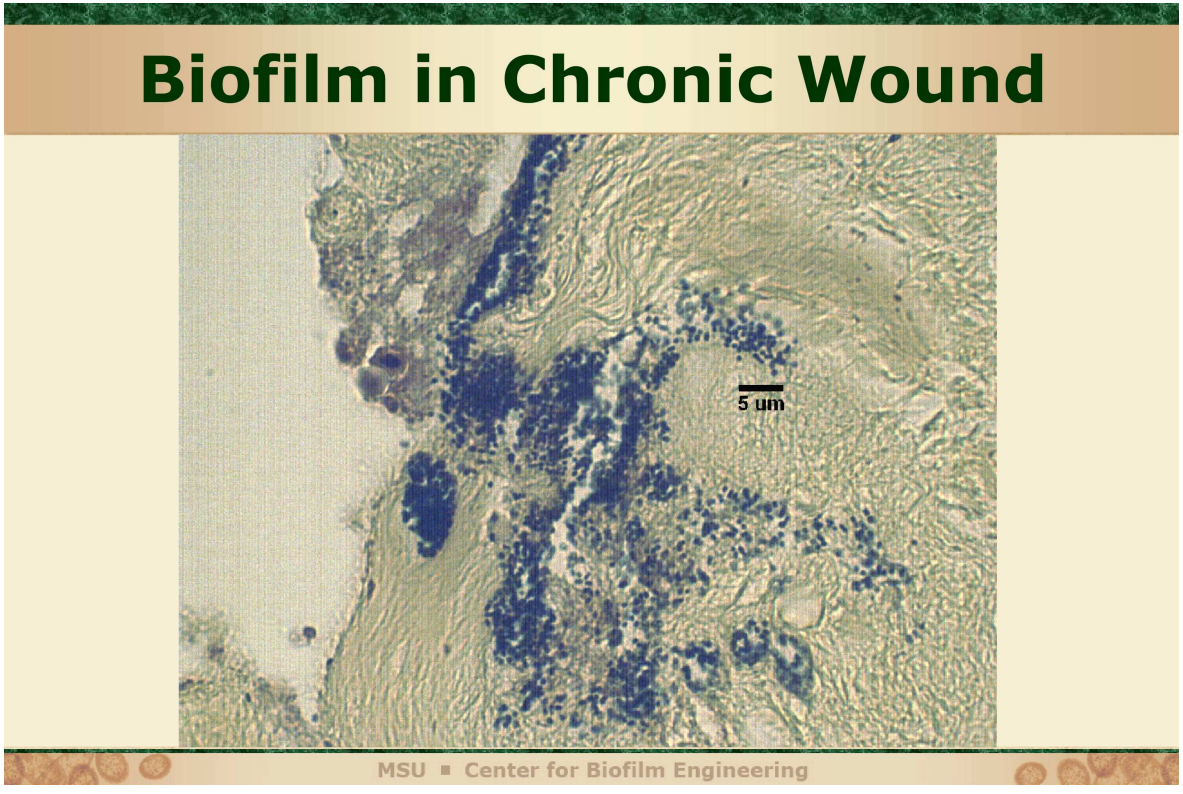
Biofilm that outlives a patient! (From W. Costerton)

Chronic Wounds



Diabetics, bed-ridden patients, etc.

Chronic Wounds



From the lab of P.Stewart, CBE.

Biofilm Model Components

Model Components.

- Microbiology
  - Growth
  - Metabolics
  - Ecology
- Physics
  - Diffusion
  - Mechanics
- Chemistry (and electrochemistry)
  - Microbes as “enzymes”
  - Electrochemistry
  - pH

Biofilms are not well-mixed systems

Mathematical Ingredients

- Mass transport (conservation of mass)

$$\text{change in mass} = (\text{transport (in) of mass}) + (\text{"creation" of mass})$$

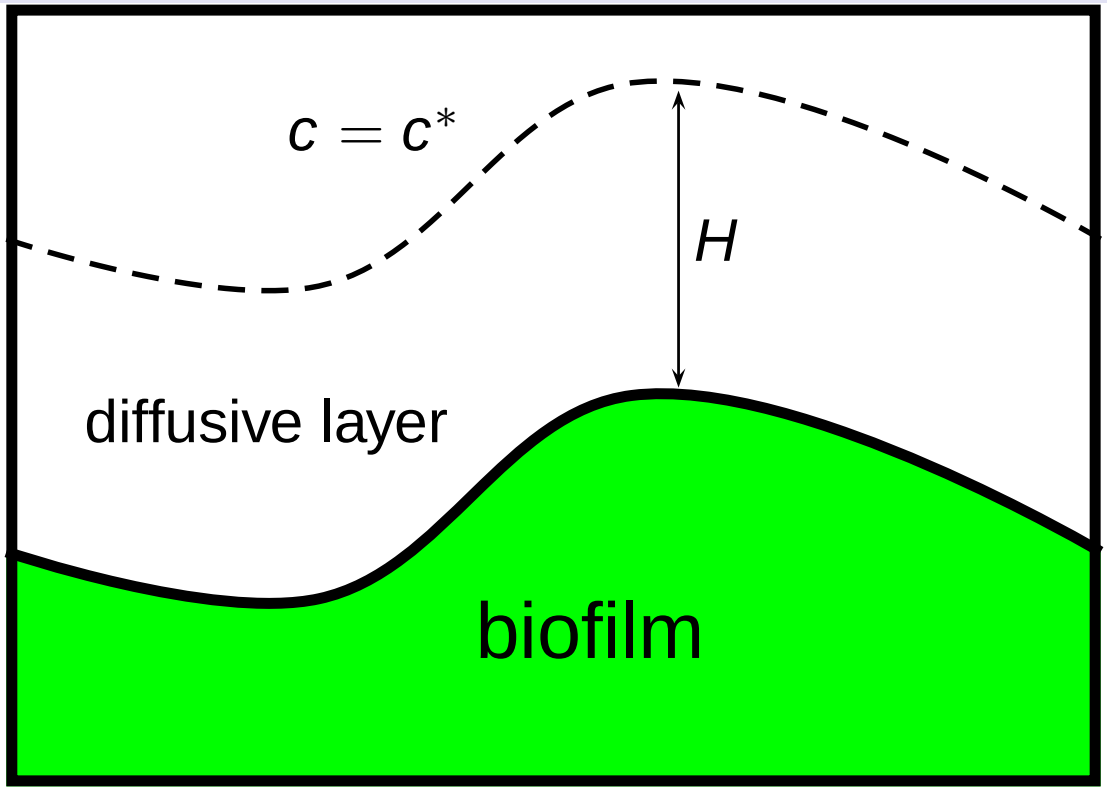
- Force balance (conservation of momentum)

$$\begin{aligned} 0 \quad = \quad & (\text{inertial force}) + (\text{transport of momentum}) + (\text{viscous force}) \\ & + (\text{elastic force}) + (\text{cohesive force}) + \ldots \end{aligned}$$

- Constitutive laws (physics and biology to fill in above terms)
- Chemistry (including pH, electrochemistry)

Can become very complicated! (See also tumor models.)

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:

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

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

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

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

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

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

Derivation

GO TO BOARD

1D Growth: Exact Solutions

Given a flat biofilm with "top" at $z = h(t)$:

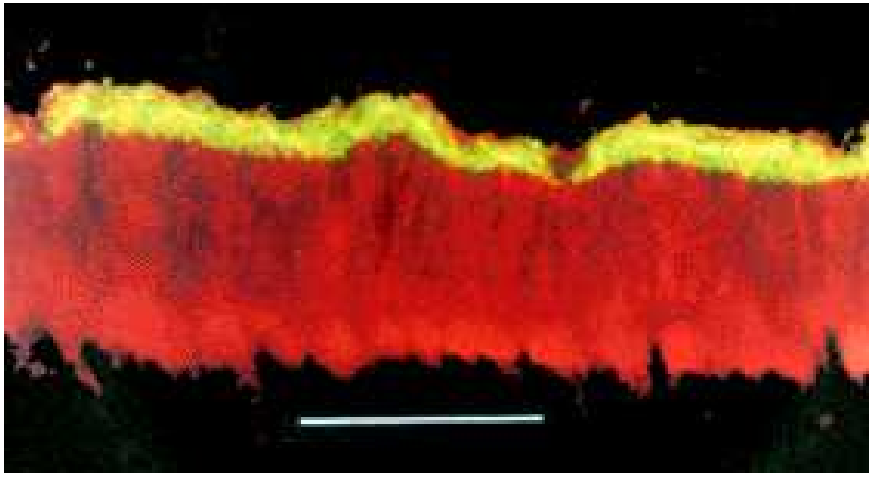
- Nutrient concentration S has a limited penetration depth below which S decays exponentially. Most growth takes place in this layer, the so-called *active layer*.
- For small h, biofilm grows exponentially.
- For large h, biofilm grows linearly.

The active layer has depth $\sim \sqrt{\text{diffusivity}/\text{reaction rate}}$.

REMARK: balance of diffusive transport with reactive sources/sinks can explain a lot!

1D: Active Layers

- Substrate is “eaten” by an active layer at the top of the biofilm.



Pseudomonas aeruginosa from the lab of P.Stewart.

- **Penetration Barrier**: “reaction layer” protection
- **Adaptive Response**: sublethal exposure below
- **Altered Microenvironment**: inactivity below active layer
- **Dormancy**: protected environment

1D: Substrates

In one dimension:

$$\frac{\partial}{\partial z}\left(D_j \frac{\partial}{\partial z} S_j\right) = r_j$$

with diffusivity D_j assumed piecewise constant with jump across the biofilm-bilk fluid interface. Note $r_j = 0$ for $z > L(t)$.

For simplicity, suppose a single, *limiting* substrate (e.g. oxygen) with concentration c, as well as a single microbial species.

1D: Single Substrate

Then

$$\frac{d^2S}{dz^2} = \begin{cases} 0 & z > L \\ D_{\text{bi}}^{-1} r(S) & z \leq L \end{cases}$$

Boundary/interface conditions.

- Interface $z = L(t)$ continuity conditions:

$$S|_{L^+} = S|_{L^-}, \quad -D_{\text{aq}}(dS/dz)|_{L^+} = -D_{\text{bi}}(dS/dz)|_{L^-}$$

- Wall $z = 0$ no-flux condition: $(dS/dz)|_0 = 0$.
- Well-mixed boundary $z = L(t) + H$: $S = S_0$.

Use (for simplicity) linear kinetics $r(S) = \gamma S$.

1D: Single Substrate Solution

Solve to obtain

$$S(z,t) = \frac{S_0}{1 + (D_{\text{bi}}/D_{\text{aq}})H\sqrt{\gamma D_{\text{bi}}^{-1}}\tanh\left(\sqrt{\gamma D_{\text{bi}}^{-1}}L\right)} \frac{\cosh\left(\sqrt{\gamma D_{\text{bi}}^{-1}}z\right)}{\cosh\left(\sqrt{\gamma D_{\text{bi}}^{-1}}L\right)}$$

for $0 \leq z \leq L(t)$.

1D: Single Substrate Solution, large L

For L large ($\sqrt{\gamma D_{\text{bi}}^{-1}} L \gg 1$),

$$S(z,t) \approx \frac{S_0}{1 + (D_{\text{bi}}/D_{\text{aq}}) H \sqrt{\gamma D_{\text{bi}}^{-1}}} e^{\sqrt{\gamma D_{\text{bi}}^{-1}}(z-L)},$$

up to exponentially small corrections, for $0 \leq z \leq L(t)$.

- Large L limit = a *thick* biofilm, substrate does not penetrate in significant quantity to the bottom.
- S decays quickly below a layer of depth roughly $1/\sqrt{\gamma D_{\text{bi}}^{-1}}$ (the active layer) so that, below this layer, activity is limited by low substrate concentration.
- If S = concentration of a reactive antimicrobial, the same analysis predicts that antimicrobial will be largely depleted within a reactive layer of depth roughly $1/\sqrt{\gamma D_{\text{bi}}^{-1}}$.

1D: Microbes

Microbe volume fraction equations

$$\frac{\partial}{\partial t}(\rho_j X_j) + \underbrace{\nabla \cdot (\mathbf{u}_j \rho_j X_j)}_{\text{advection}} = \underbrace{\nabla \cdot (\kappa_j \nabla(\rho_j X_j))}_{\text{diffusion}} + \underbrace{\rho_j g_j}_{\textit{growth}}$$

reduce, for a single microbial species, to $du/dz = g(S)$ so that $u(z) = \int_0^z g(S(z'))dz'$.

Thus

$$\frac{dL}{dt} = u(L(t)) = \int_0^L g(S(z'))dz' \approx \int_{L-h}^L g(S(z'))dz',$$

where $h = 1/\sqrt{\gamma D_{\text{bi}}^{-1}}$.

1D: Active (Reactive) Layer

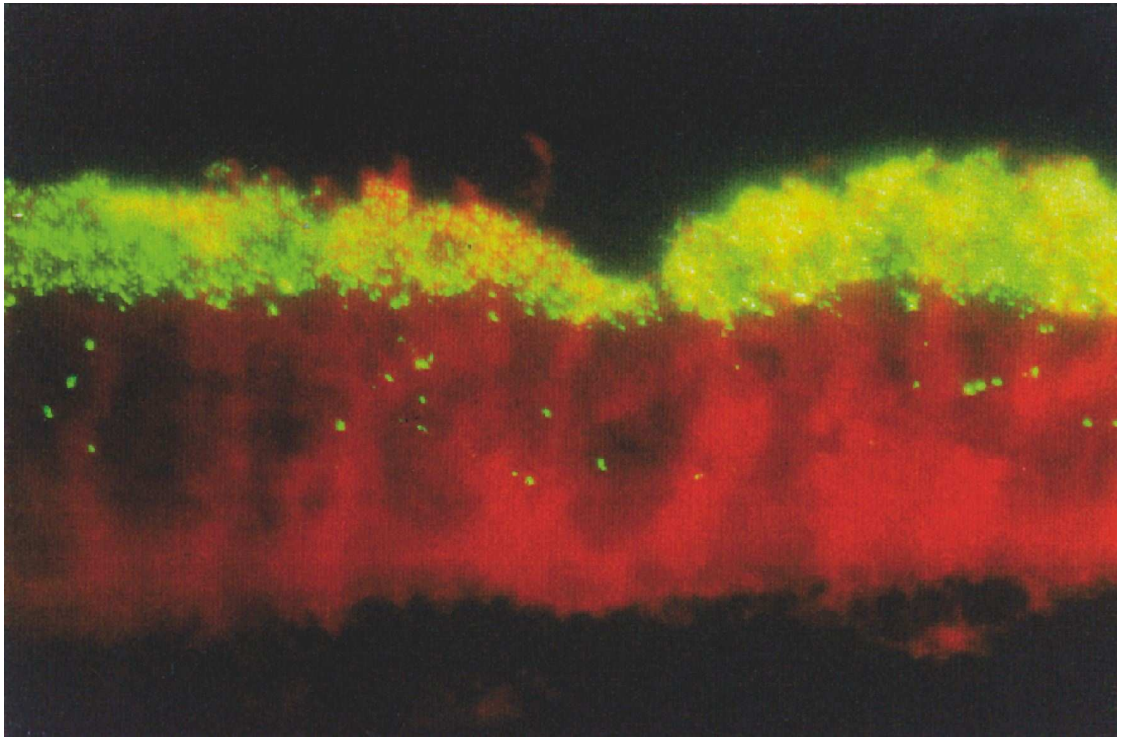
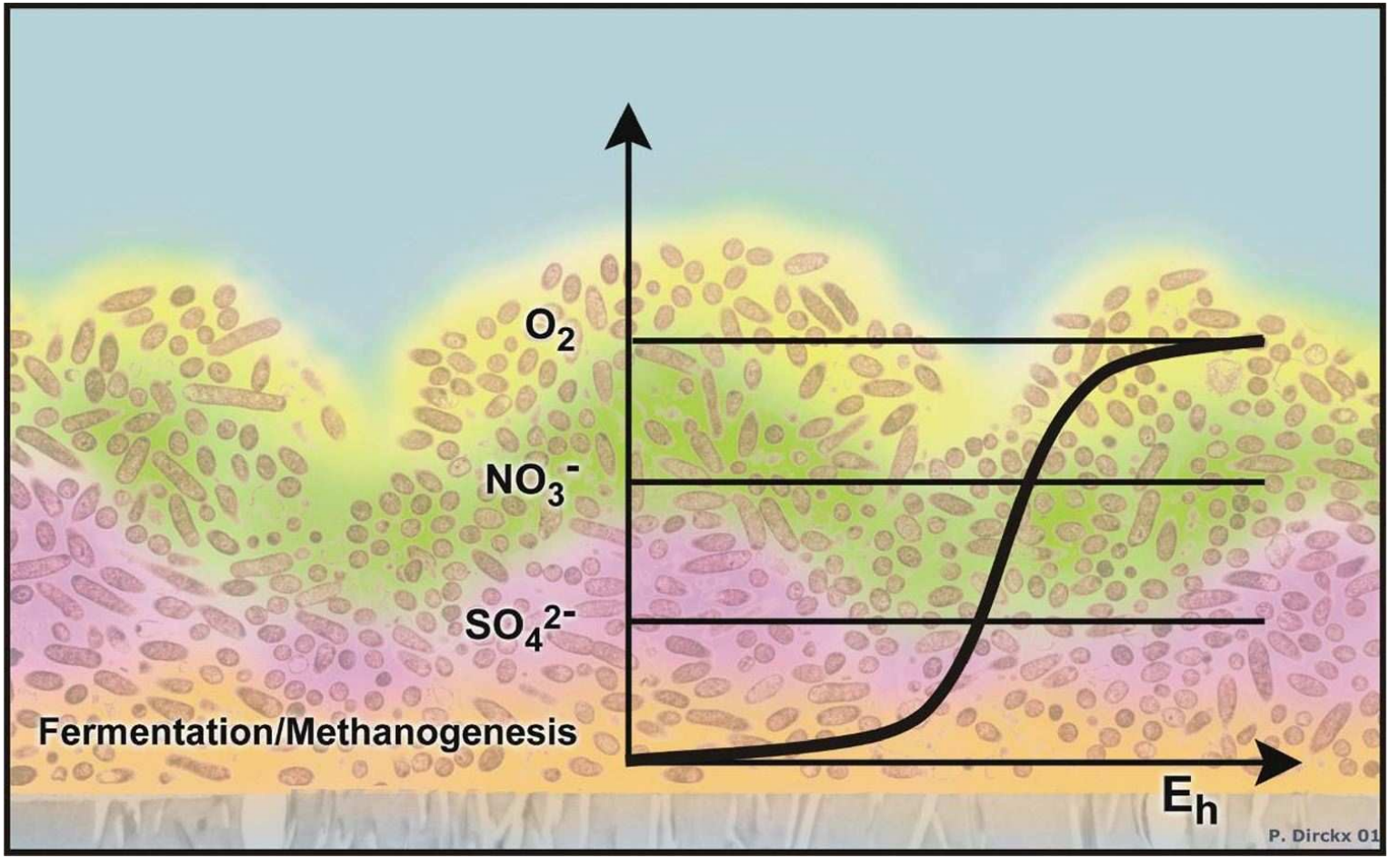


Figure: Microscopic cross-section of a *Pseudomonas aeruginosa* biofilm stained for protein-synthetic activity (green) and counterstained for biomass independent of activity (red). Image courtesy of Karen Xu and Phil Stewart, Center for Biofilm Engineering, Montana State University.

Multiple Active Layers



Reaction-diffusion is dominant. (E_h = redox potential.)

Final Remark

1. Microbial ecology needs more theory
2. Theorists need to know microbial ecology