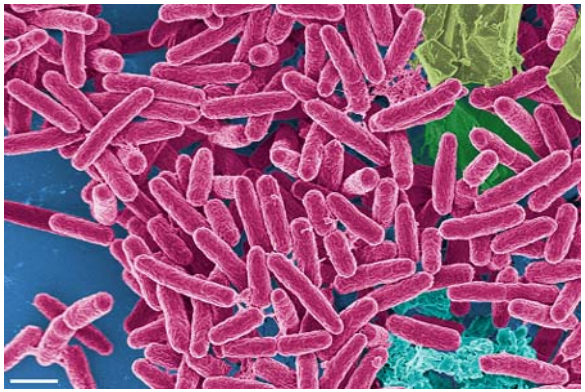
Loughborough University

A free boundary problem in a model for bacterial biolfilms



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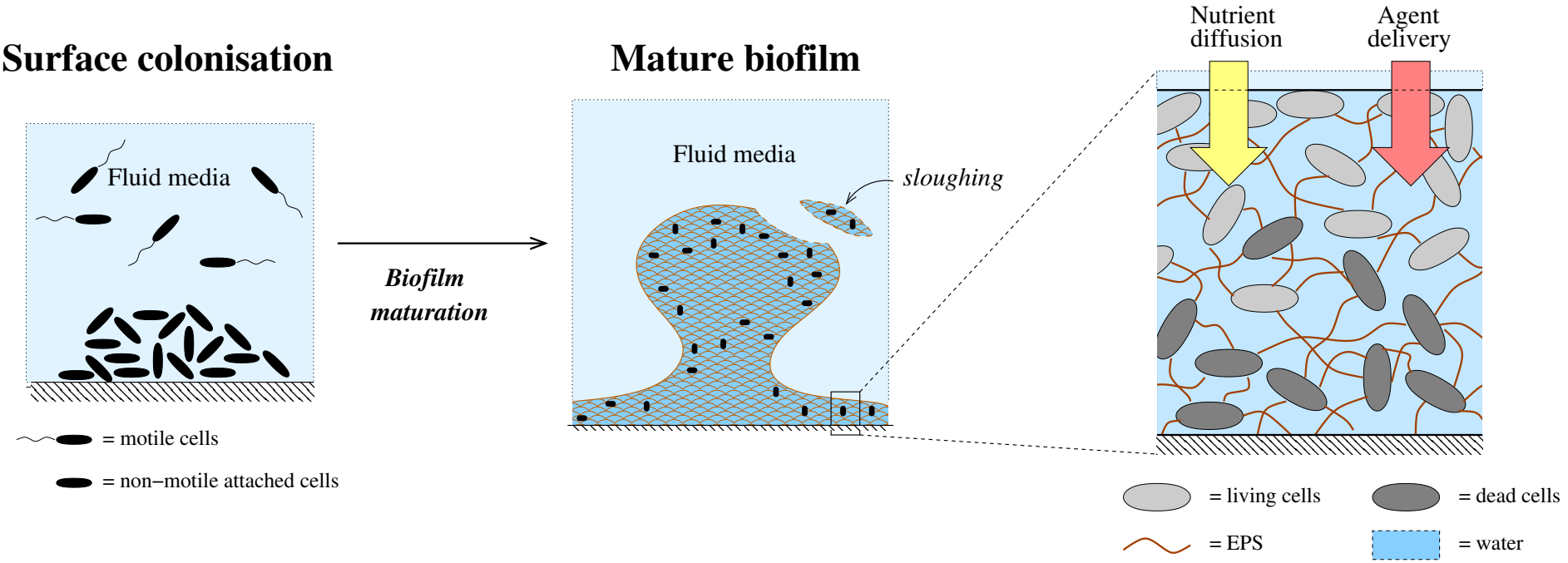
A general overview of biofilms and their development:

- Biofilms are slimy bacterial colonies that grow, usually, on solid-fluid interfaces.
- Growth can be viewed as occurring in distinct phases; a cartoon:

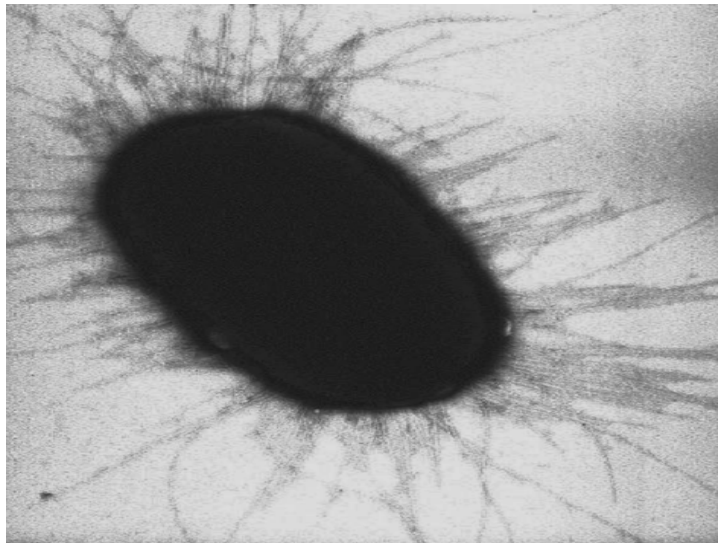


- Bacteria within biofilms typically expend less energy $\Rightarrow$ colonisation of "poor environments".
- **Early** or **undeveloped colonies** $\approx 10\,\mu$m are vulnerable to removal by antimicrobial agents.
- **"Mature" biofilms** can grow up to $\approx O(\text{mm})$ and much less vulnerable to antimicrobial agents.
- Numerous implications in medicine and industry, good and bad.

The role of EPS

- Initial (reversible) attachment to a surface is probably due to electrostatic/van der Waals forces.
- Surface adherence is made permanent by proteinous structures called pili and/or fimbria
  - ... these structures also allow adherence to near by bacteria (range $\approx 2 - 3\mu$m).
- For substantial biofilm expansion, bacteria produce exopolysaccharides
  - ... or alternatively extracellular polymeric substances - either way **EPS**.

fimbria



EPS

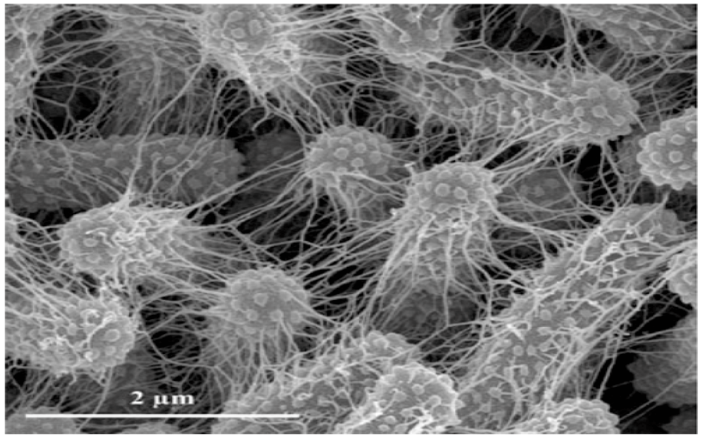
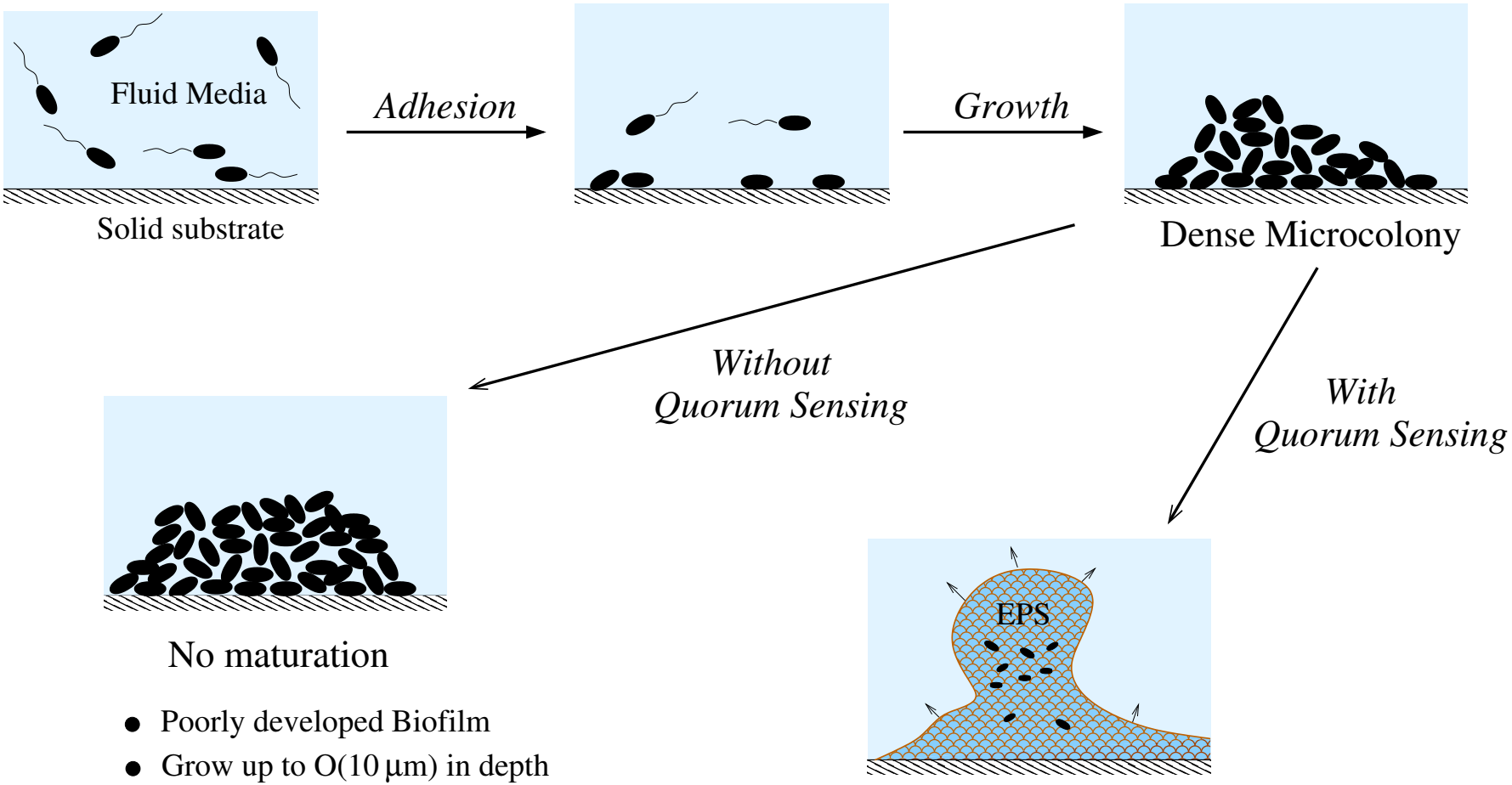


Fig. 6. Thin threads emerged from bacterial body surface. Scanning electron microscopy of sessile *K. pneumoniae* LM21 performed in mature biofilm formed on Thermanox® slides in microfermentor system after 48 h of development at X20,000 magnification.

- **KEY POINT**:
  - **Biofilms with low EPS** are slow growing and vulnerable, e.g. to anti-microbial agents.
  - **Biofilms with high EPS** are fast growing and are much more resistant to outside effects.

Role of quorum sensing in EPS production (*Pseudomonas aeruginosa*)



No maturation

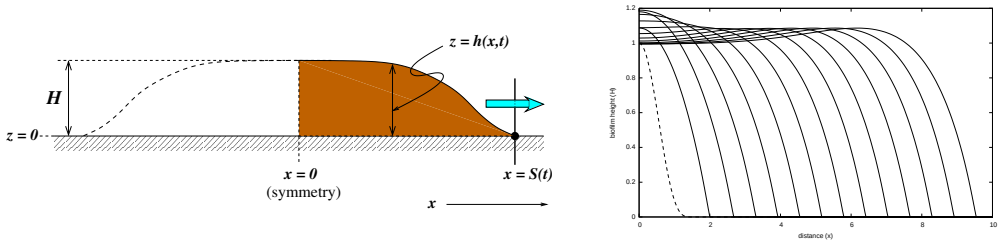
- Poorly developed Biofilm
- Grow up to O(10 µm) in depth
- Easy to remove
  (by antibiotics and immune system)

Biofilm maturation

- Bacteria produce Exopolysaccharides (EPS)
- Mature biofilms: ~95% water, ~3% bacteria
- Depth can be O(mm), typically O(100 µm)
- Highly resistant to antimicrobial agents

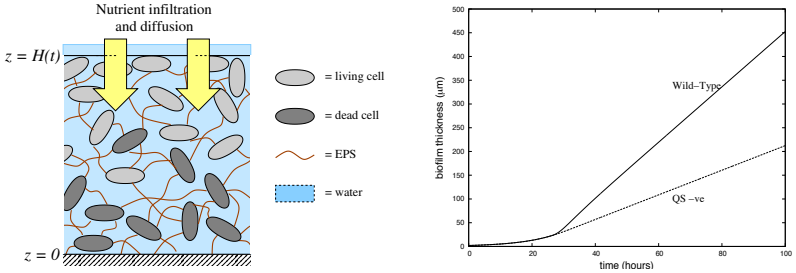
Problems examined - all have moving boundaries of some sort

Early growth



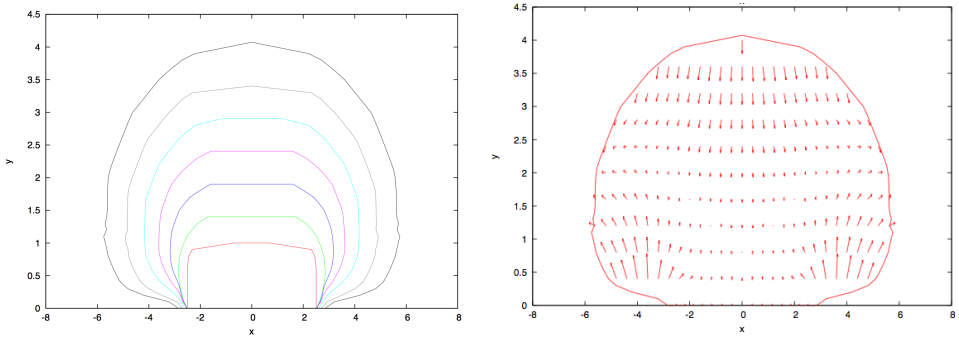
- Surface spread of homogeneous structured "viscous" biofilm.
- Assumed height/width ratio $\to 0 \Rightarrow$ thin-film limits ...
- using free-and no-slip conditions at biofilm-surface interface.
- Used to investigate necessary conditions for QS activity.

1-D continuum modelling



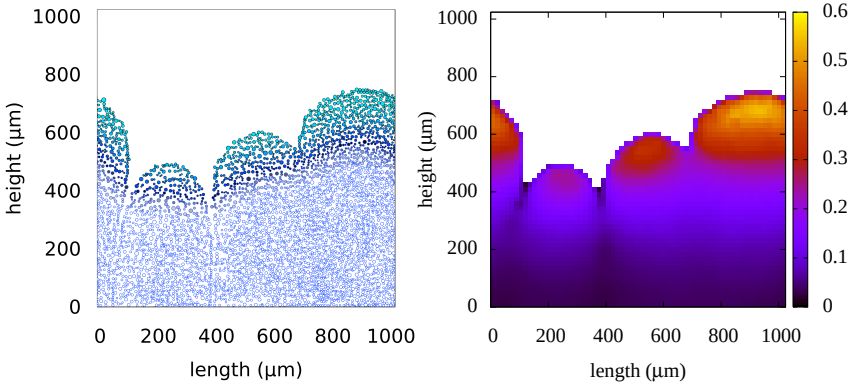
- Depth-based continuum model; conservation of mass of, fluid, solid and EPS phases, diffusion of nutrients.
- Used to investigate effect of agents that inhibit growth and quorum sensing.

2-D continuum modelling



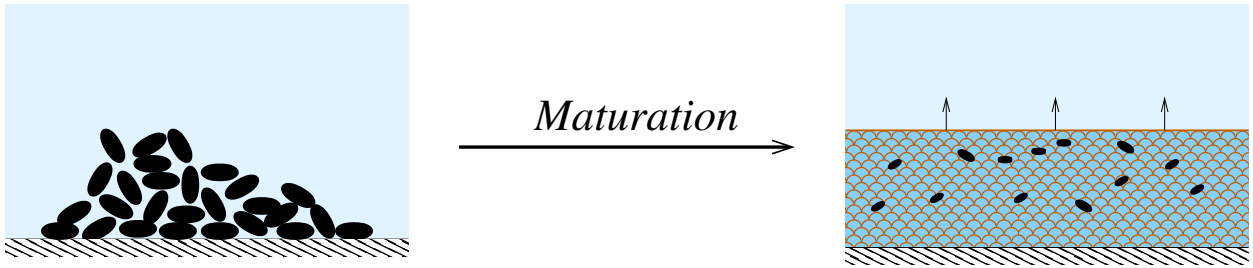
- Extended 1D continuum model to viscous biofilm with no load
- Used to investigate how flow patterns in 2-D structures help or hinder anti-microbial delivery.

Hybrid individually-based model



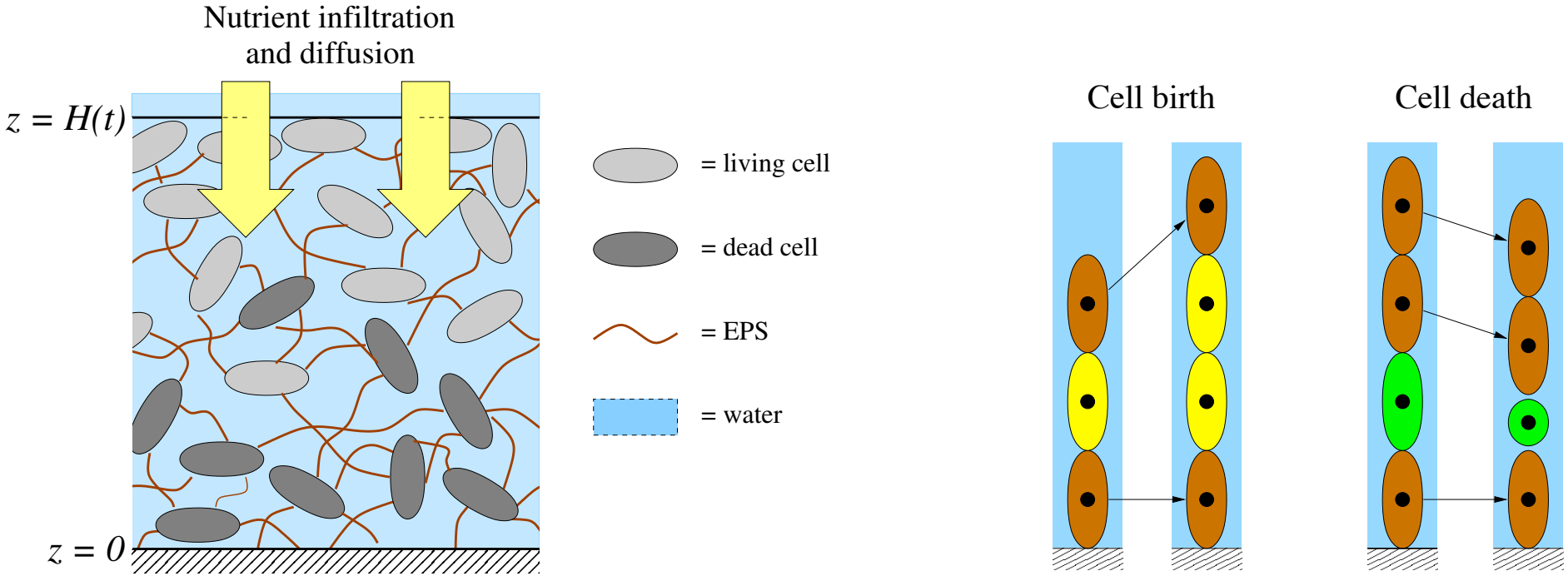
- Hybrid discrete-continuum approach: bacteria form particles, whilst EPS, nutrients etc. form a continuum.
- Used to simulated action of cold-plasma products.

A 1-D model for biofilm maturation



Model of mature phase biofilm growth (Ward 2008)

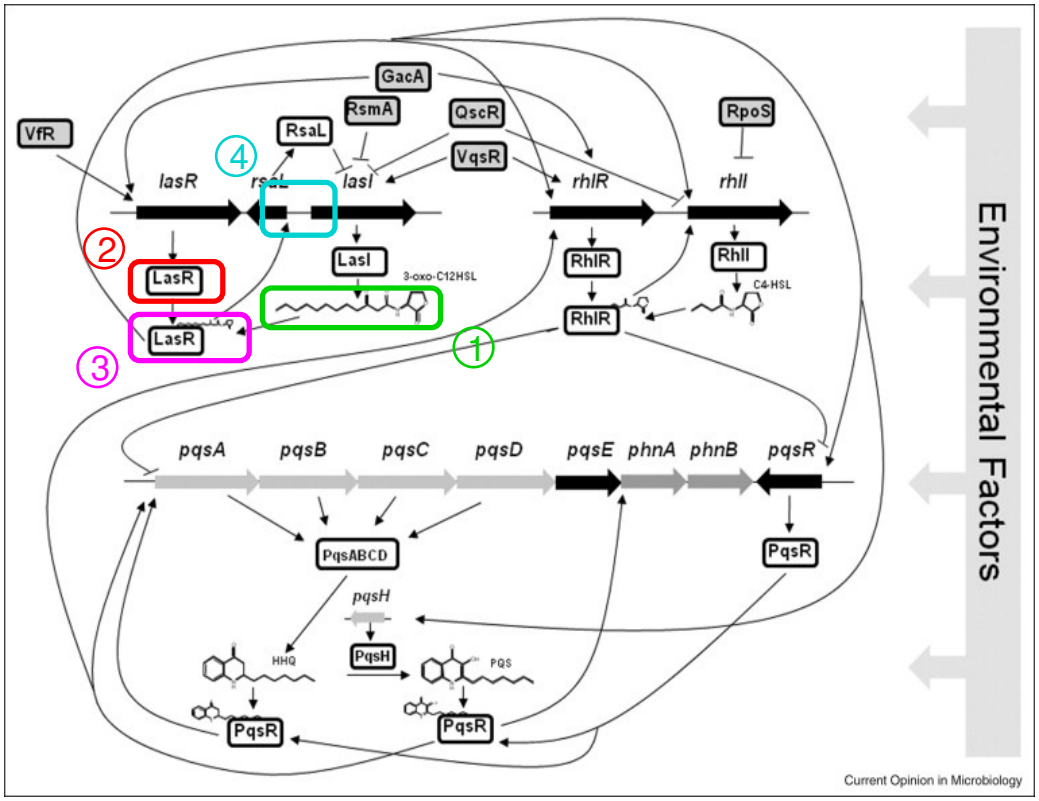
- Schematic of biofilm (left) and role of growth on movement within biofilm (right):



- Consider a 1-D depth based model with a moving boundary at $z = H(t)$.
- Movement by cell birth and death (at nutrient dependent rates) are described by a velocity field.
- Nutrients disperse via diffusion and advection from source at $z = H(t)$.
- Quorum sensing regulates enhanced EPS production.

Modelling quorum sensing (*P. aeruginosa*)

- Quorum sensing is regulated by numerous molecules in and multiple feedback loops – a biologists viewpoint:



- **Key components are:** (1) a signalling molecule (autoinducer made by enzyme LasI), (2) a protein LasR, (3) a LasR-autoinducer complex which can bind to (4) an activation site on the DNA called a *lux*-box, such that
  - Empty *lux*-box $\implies$ cell is **down-regulated** (ϕ_d) $\implies$ **Low** EPS and autoinducer production
  - Occupied *lux*-box $\implies$ cell is **up-regulated** (ϕ_u) $\implies$ **High** EPS and autoinducer production
- We thus assume the following reaction describing quorum sensing

$$\textbf{Down-regulated cell} + \textbf{Autoinducer} \rightleftharpoons \textbf{Up-regulated cell}$$

Model Variables

- **Biofilm structure variables:**

$\Phi_L(z,t)$	Live cell volume fraction
$\Phi_D(z,t)$	Dead cell volume fraction
$\Phi_E(z,t)$	EPS volume fraction
$\Phi_W(z,t)$	Volume fraction of water

where

$$\boldsymbol{\Phi_L + \Phi_D + \Phi_E + \Phi_W = 1}$$

- **Biofilm growth variables**

$v(z,t)$	Local cellular (or solid phase) velocity
$u(z,t)$	Local water velocity
$H(t)$	Biofilm depth

- **Nutrients:**

$c(z,t)$	Nutrient concentration

- **Quorum sensing variables:**

$\Phi_d(z,t)$	Down-regulated cell volume fraction
$\Phi_u(z,t)$	Up-regulated cell volume fraction
$A(z,t)$	Autoinducer concentration

where

$$\boldsymbol{\Phi_d + \Phi_u = \Phi_L}$$

Dimensionless equations (1-D)

- Recall $\Phi_L + \Phi_D + \Phi_E + \Phi_W = 1$ **(∗)** and $\Phi_L = \Phi_d + \Phi_u$.
- Biofilm structure equations

$$\begin{aligned}
\partial_t \Phi_L + \partial_z(v\,\Phi_L) &= \Phi_L\,(F_B(c) - F_D(c)),\\
\partial_t \Phi_D + \partial_z(v\,\Phi_D) &= \Phi_L\,\delta\,F_D(c),\\
\partial_t \Phi_E + \partial_z(v\,\Phi_E) &= (E_0\,\Phi_L + \kappa_E\,\Phi_u)\,G_E(c) - \lambda_E\,\Phi_E,\\
\partial_t \Phi_W + \partial_z(u\,\Phi_W) &= -\Phi_L\,(F_B(c) - (1-\delta)F_D(c)).
\end{aligned}$$

- Biofilm growth equations (first equation uses **(∗)**)

$$\begin{aligned}
\partial_z((1-\Phi_w)v + \Phi_w u) &= (E_0\,\Phi_L + \kappa_E\,\Phi_u)\,G_E(c) - \lambda_E\,\Phi_E,\\
\frac{dH}{dt} &= v(H,t).
\end{aligned}$$

- Nutrients

$$0 = \partial_z(D_c(1-\Phi_E)\,\partial_z c) - \rho\,\Phi_L F_B(c).$$

- Quorum sensing equations

$$\begin{aligned}
\partial_t \Phi_u + \partial_z(v\,\Phi_u) &= -F_D(c)\Phi_u + \boldsymbol{\alpha\,A\,\Phi_d} - \boldsymbol{\beta\,\Phi_u},\\
\partial_t \Phi_d + \partial_z(v\,\Phi_d) &= F_B(c)\Phi_L - F_D(c)\Phi_d - \boldsymbol{\alpha\,A\,\Phi_d} + \boldsymbol{\beta\,\Phi_u},\\
0 &= \partial_z(D_A(1-\Phi_E)\,\partial_z A) + \boldsymbol{\Phi_u} + \boldsymbol{\varepsilon\,\Phi_L} - \eta\,A\,\Phi_L - \lambda_A\,A, \qquad \boldsymbol{\varepsilon \ll 1}.
\end{aligned}$$

- $F_B(c) = \dfrac{c}{c_B + c}, \quad F_D(c) = B_D\left(1 - \tau\dfrac{c}{c_D + c}\right), \quad G_E(c) = F_B(c).$

Model closure

- **Constitutive equation:** Relate water to EPS density

$$\boldsymbol{\Phi_W \;=\; \Phi_0 \,+\, \theta\, \Phi_E},$$

  - so that "$1-\Phi_0$" is the maximum packing density of bacteria,
  - and $\Phi_L + \Phi_D + \Phi_E + \Phi_W \;=\; 1 \;\;\Rightarrow\;\; \Phi_L + \Phi_D + (1+\theta)\Phi_E \;=\; 1-\Phi_0,$
  - thus $u(z,t)$ and $v(z,t)$ decouples to give

$$(1-\phi_0)\,\partial_z v \;=\; \Phi_L\,\big(F_B(c) \,-\, (1-\delta)F_D(c)\big) \,+\, (1+\theta)\big((E_0\Phi_L + \kappa_E\Phi_u)\,G_E(c) \,-\, \lambda_E\Phi_E\big),$$

  and

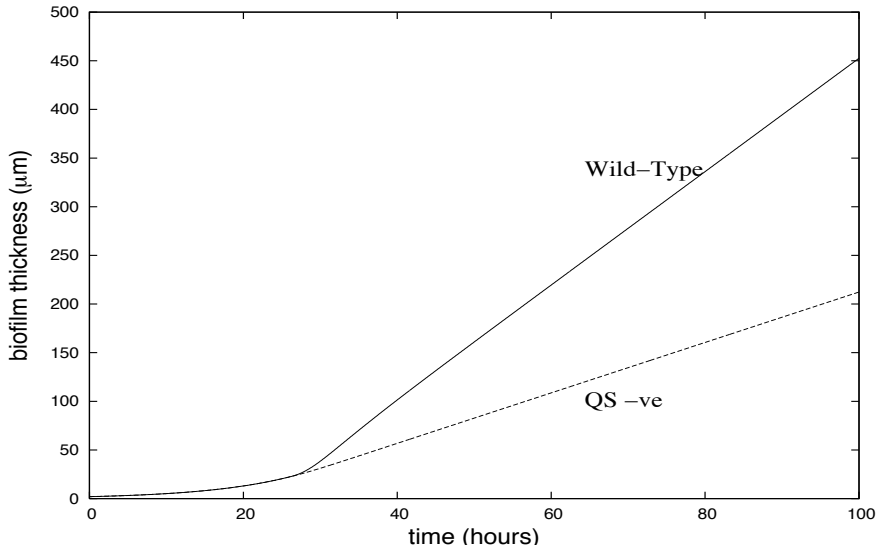
$$u \;=\; -\,\frac{(1-\Phi_W)}{\Phi_W}\,v \,+\, \frac{1}{\Phi_W}\int_0^z (E_0\,\Phi_L + \kappa_E\,\Phi_u)\,G_E(c) \,-\, \lambda_E\Phi_E\,dz'.$$

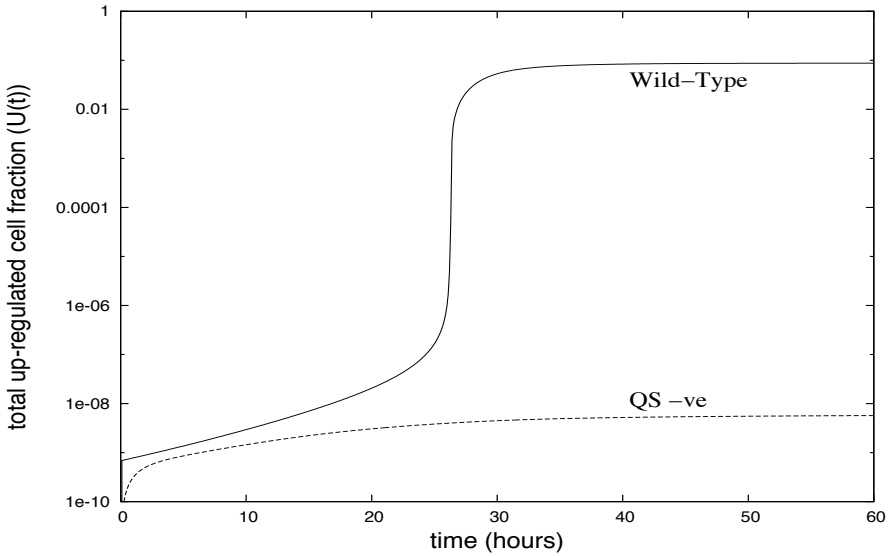
- Boundary and initial conditions used in simulations to follow:

$$\begin{aligned} t \;=\; 0: &\qquad H = H_0,\;\; \Phi_L = 1-\Phi_0,\;\; \Phi_D = 0,\;\; \Phi_E = 0,\;\; \Phi_u = 0,\;\; \Phi_d = \Phi_0. \\ z \;=\; 0: &\qquad v = \partial_z c = \partial_z A \;=\; 0. \\ z = H(t): &\qquad c = 1,\;\; A = 0. \end{aligned}$$

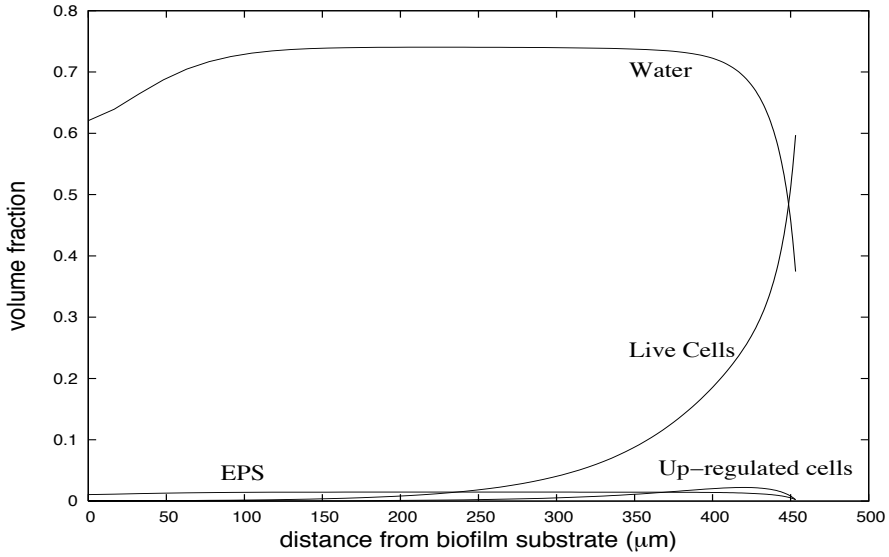
Model simulation

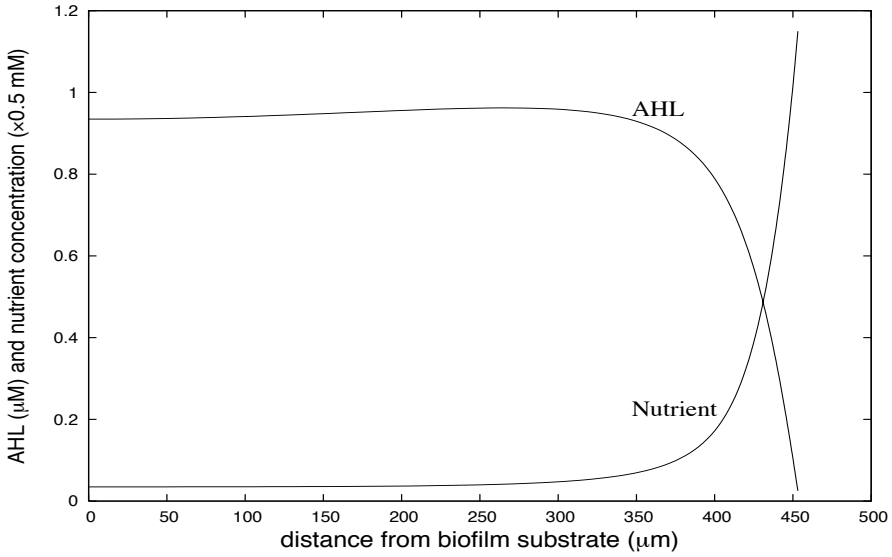
- Biofilm growth, $dH/dt \sim$ constant as t gets large (left) and up-regulated cell fraction (right):





- Volume fracs. (left) and autoind./nut. conc. (right) at $t = 100$. Qualitative agreement with observation.





$F_B(1) > 0 \;\Rightarrow\;$ travelling waves (Anguige *et al.* 2006)

- In the limit $\rho H_0^2/D_A \to 0$, we can show that if $F_B(1) > 0 \;\Rightarrow\; \exists$ constants $C_L, C_U > 0$, such that

$$\boldsymbol{a_L + C_L\, t \;\le\; H(t) \;\le\; a_U + C_U\, t}, \qquad \text{as } t \to \infty.$$

  - As $\rho H_0^2/D_A \to 0$, the equations for ϕ_L, ϕ_D, ϕ_E and v_c are unchanged, but

$$\partial_x(D_c\, \partial_x c) - \psi_c\, c \;=\; 0,$$

    where $0 < D_{min} \le D_c \le D_{max}$ and $0 < \psi_{min} \le \psi_c \le \psi_{max}$.

  - **Lower bound (C_L):** Writing

$$H \;=\; \int_0^H dx \;\ge\; \int_0^H \frac{(\delta\phi_L + \phi_D)}{1+\delta} dx \;=\; a_L + \frac{\delta}{1+\delta}\int_0^t\!\!\int_0^H \phi_L F_B\, dx$$

    where $a_L = \int_0^{H_0} \frac{(\delta\phi_L(x,0)+\phi_D(x,0))}{1+\delta} dx$, using $\partial_t \int_0^H (\delta\phi_L + \phi_D) dx = \int_0^H \phi_L F_B\, dx$.
    In the strip $x \in [H-\delta_0, H]$, in which $F_B - F_D \ge \varepsilon_0 > 0$, comparison methods yields

$$\phi_L \ge \phi_{min} = \varepsilon_0(1-\phi_0)(G_{E_{max}} + 1 - B_D(1-\delta)(1-\sigma)/B_B), \qquad F_B \ge F_B(c_{min}),$$

    where $c_{min} = C_0 \cosh(H-\delta_0)/\cosh(H)$. Thus a lower bound for C_L is $C_L = \frac{\delta}{1+\delta}\phi_{min} F_B(c_{min})$.

  - **Upper bound (C_U):** Integrating the equation for v_c we get

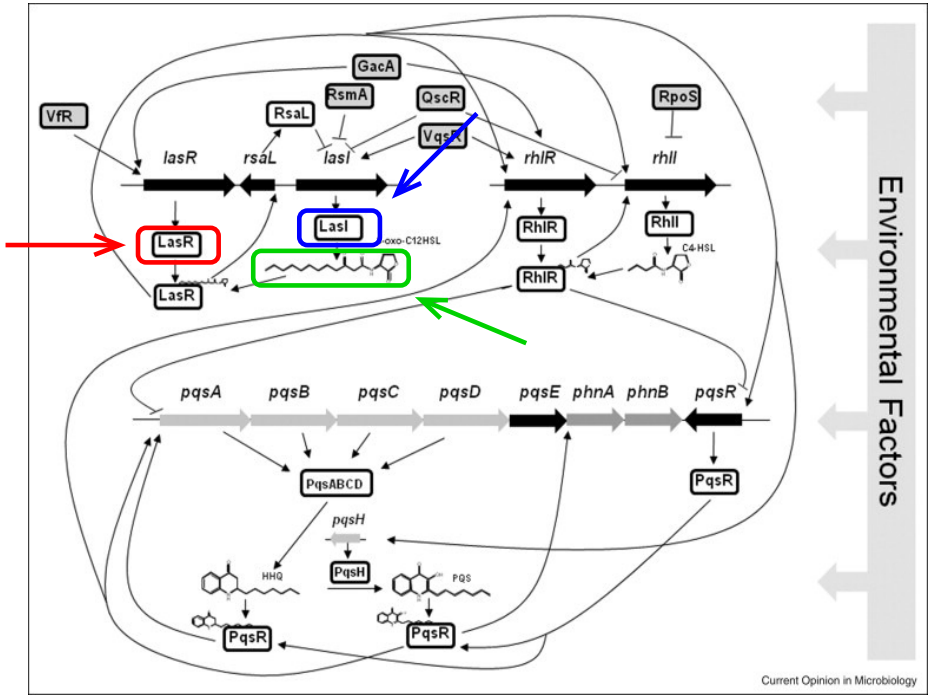
$$\begin{aligned}\frac{dH}{dt} \;=\; v_c(H,t) \;&=\; \frac{1}{1-\phi_0}\int_0^H \phi_L(F_B - (1-\delta)F_D + (1+\alpha)G_E)\, dx \\ &\le\; \frac{1}{1-\phi_0}\left(\frac{1}{c_B} + G_{E_{max}}\right)\left(\frac{D_{max}}{\psi_{min}}\right)^{1/2} C_0 \;=\; C_U,\end{aligned}$$

    using the fact (not shown) that c is bounded. The result for the upper bound follows.

Modelling anti-quorum sensing treatment

Modelling quorum sensing inhibition stratgies

- Feasible anti-quorum sensing strategies are to target
  - the protein LasR (boxed in red, e.g. halogenated furanones), $\boldsymbol{Q_1}$.
  - the autoinducers (boxed in green, e.g. lactonases), $\boldsymbol{Q_2}$.
  - the protein LasI (boxed in blue, e.g. nothing that I know of), $\boldsymbol{Q_3}$.



- Model modification (noting $\Phi_d = \Phi_L - \Phi_u$)

$$\begin{aligned} \partial_t \Phi_u + \partial_z(v\,\Phi_u) &= -F_D(c)\Phi_u + \frac{\alpha\, A\, \Phi_d}{1+\gamma_1 Q_1} - \beta\,\Phi_u, \\ 0 &= \partial_z(D_A(1-\Phi_E)\,\partial_z A) + \frac{\Phi_u}{1+\gamma_3 Q_3} + \boldsymbol{\varepsilon}\,\Phi_L - \frac{\eta\, A\,\Phi_L}{1+\gamma_1 Q_1} - \lambda_A\, A - \mu_2 Q_2 Q. \end{aligned}$$

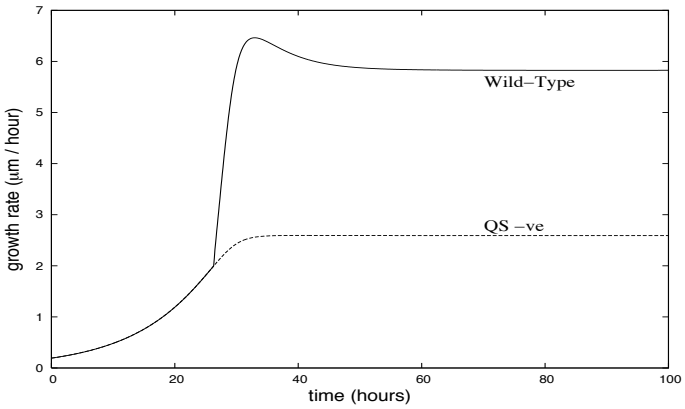
where Q_1, Q_2 and Q_3 are sourced at $z = H(t)$ and diffuse into the biofilm according to

$$0 = \partial_z(D_1\,\partial_z Q_1) - \frac{\mu_1 Q_1 \Phi_L}{1+\gamma_1 Q_1} - \lambda_1 Q_1, \quad 0 = \partial_z(D_2 W \partial_z Q_2) - \mu_2 \nu_2 A W Q_2 - \lambda_2 W Q_2, \quad 0 = \partial_z(D_3\,\partial_z Q_3) - \frac{\mu_3 Q_3 \Phi_L}{1+\gamma_3 Q_3} - \lambda_3 Q_3.$$

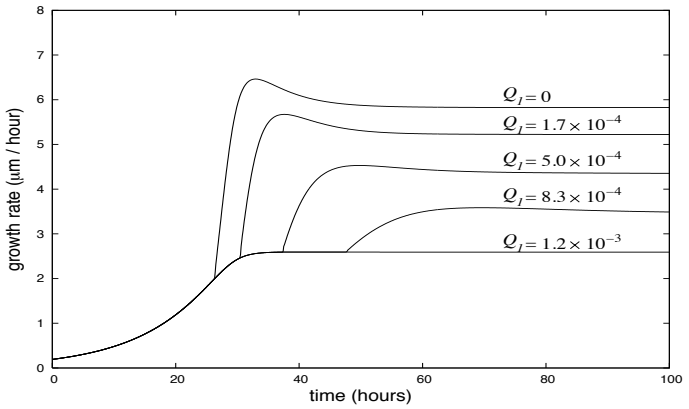
Treatment simulations

- Effect of each of these treatments on the growth rate of the biofilm dH/dt:

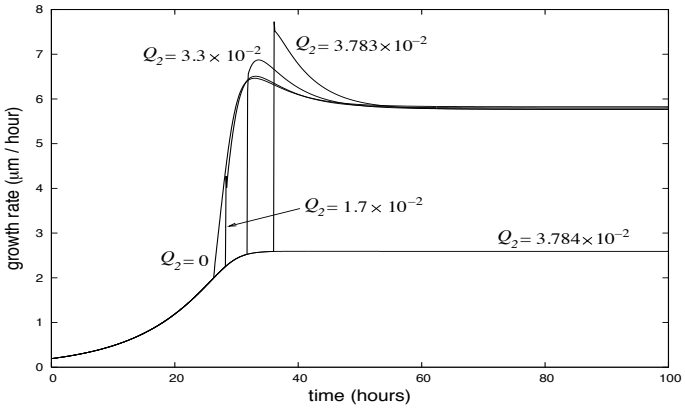
Extreme cases for no treatment



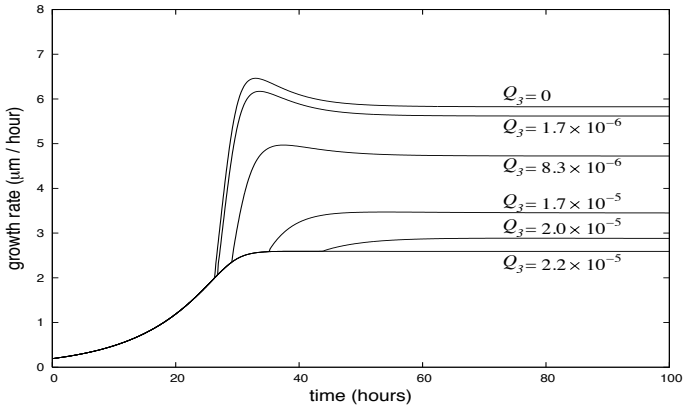
Targeting LasR



Targeting autoinducer (catastrophe!)



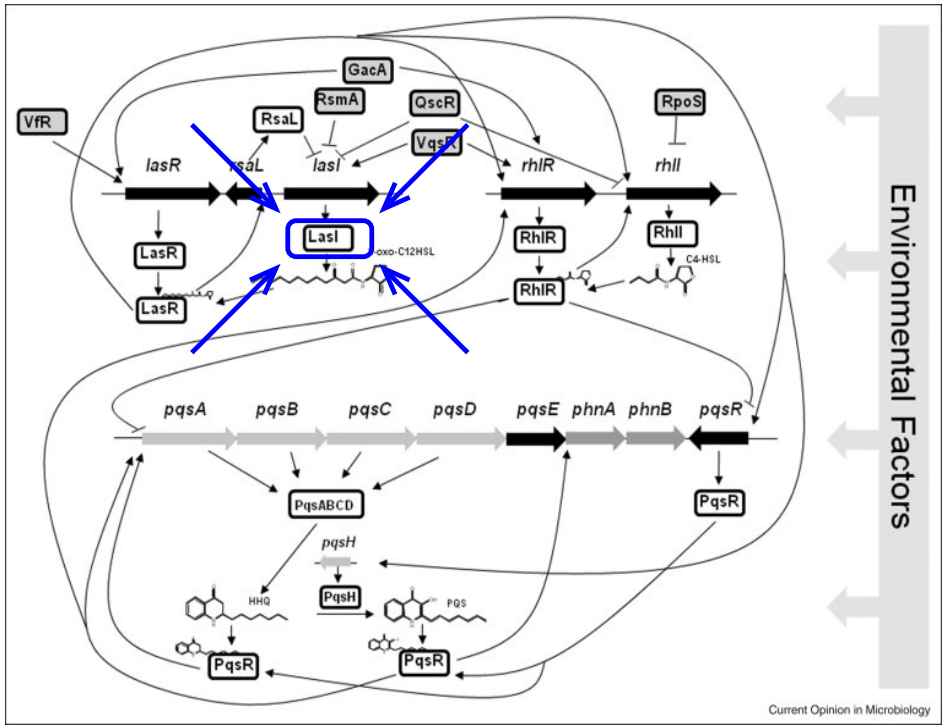
Targeting LasI



- With equivalent parameters being equal, for inhibition of growth: $Q_3(H,t) \ll Q_1(H,t) \ll Q_2(H,t)$.

In summary

- Biofilm growth is a very fertile area for moving/free boundary enthusiasts. E.g.
  - the moving interface between the biofilm and surroundings.
  - contact lines arising from the thin-film models for early biofilm growth.
  - "etching problems" in biofilm treatment (e.g. via H_2O_2, O_3 introduced by cold plasma).
- Regarding anti-QS treatments, I would suggest targeting LasI



  - ... though there are plenty of ifs and buts (including that no such agent is known to exist).