Biofilm control by antibiotics and probiotics: a mathematical description

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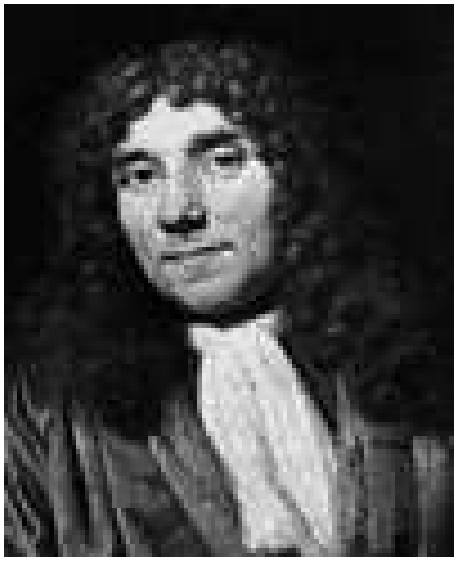
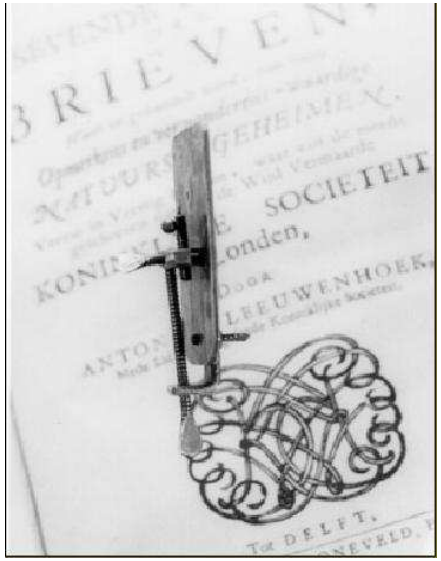
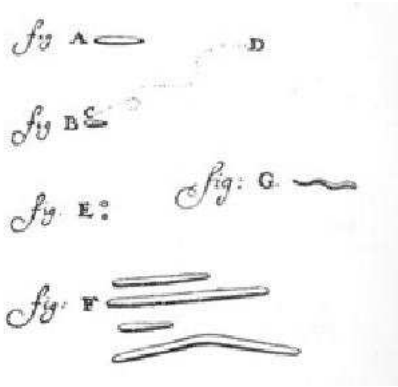
- **How the story began...**







”I then most always saw, with great wonder, that in the said matter there were many very little living animalcules, very prettily a-moving....”

Antonie van Leeuwenhoek (1632-1723)

- **... what we know now, but Antonie did not know then:**

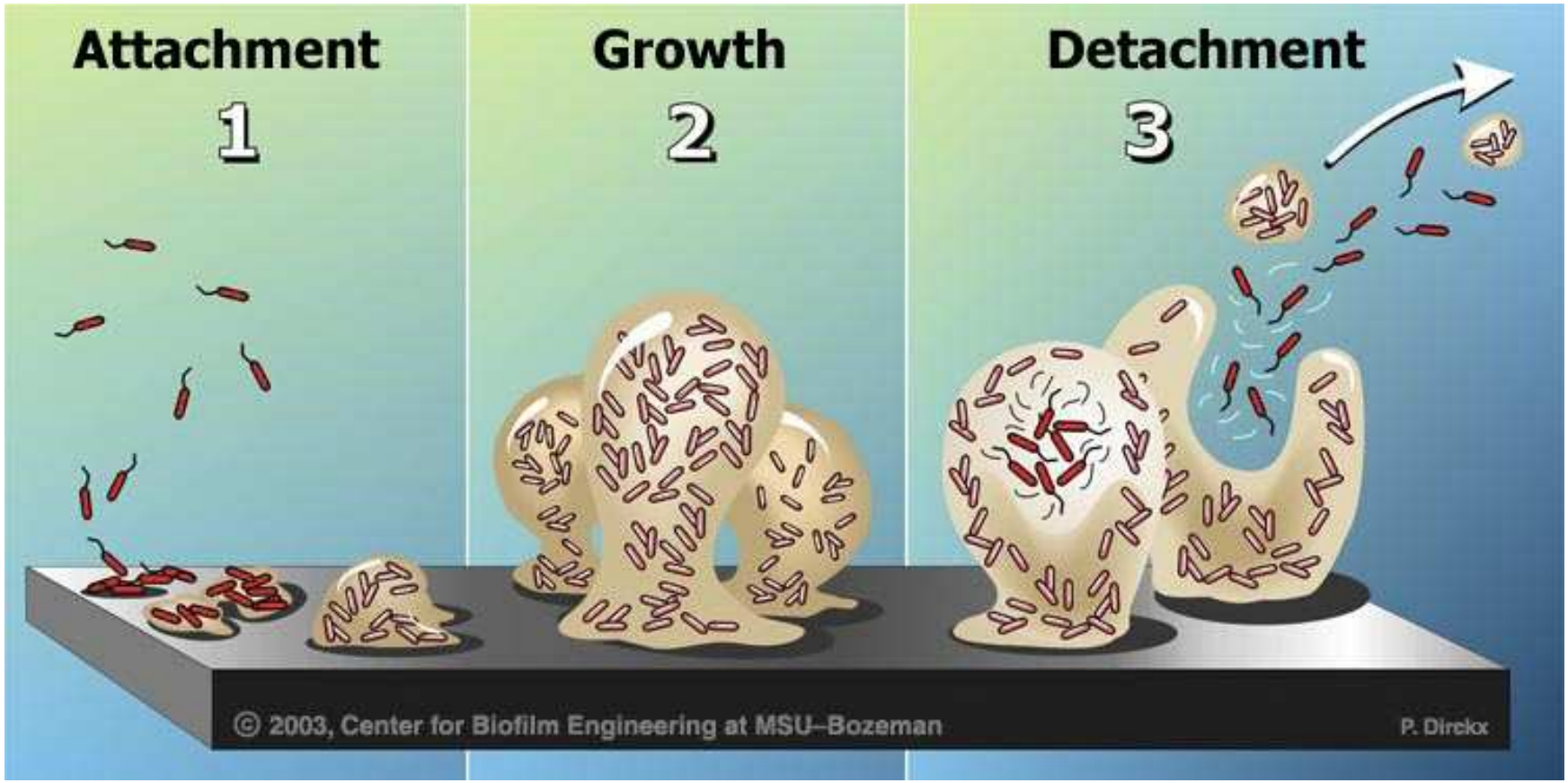
  - most of the animalculi (*a.k.a.* bacteria) live in microbial communities, so-called biofilms

  - biofilms settle on surfaces in aquatic systems

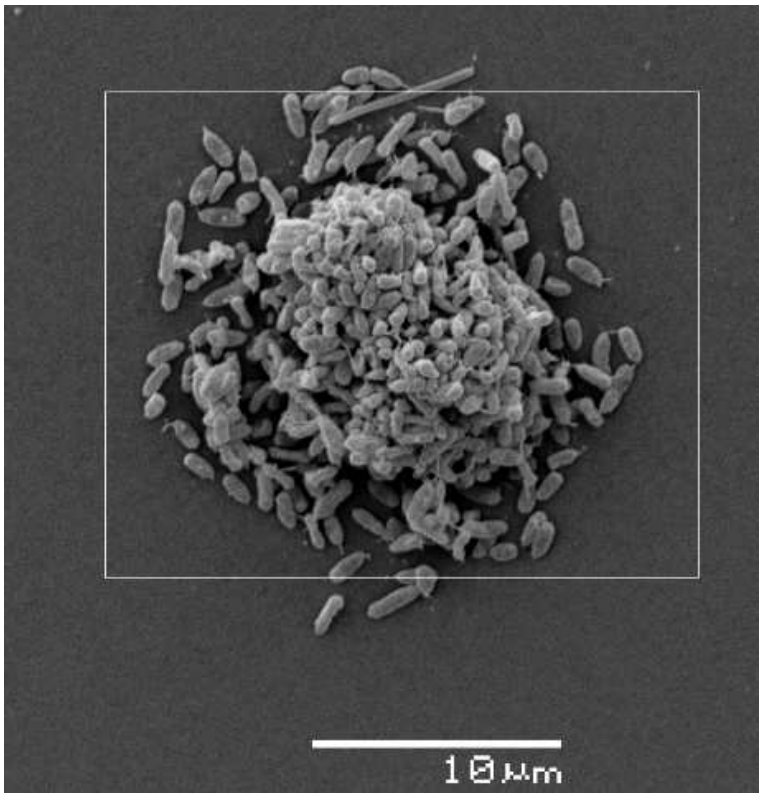
  - despite their name, biofilms are **not thin films** but form rather complicated morphological structures

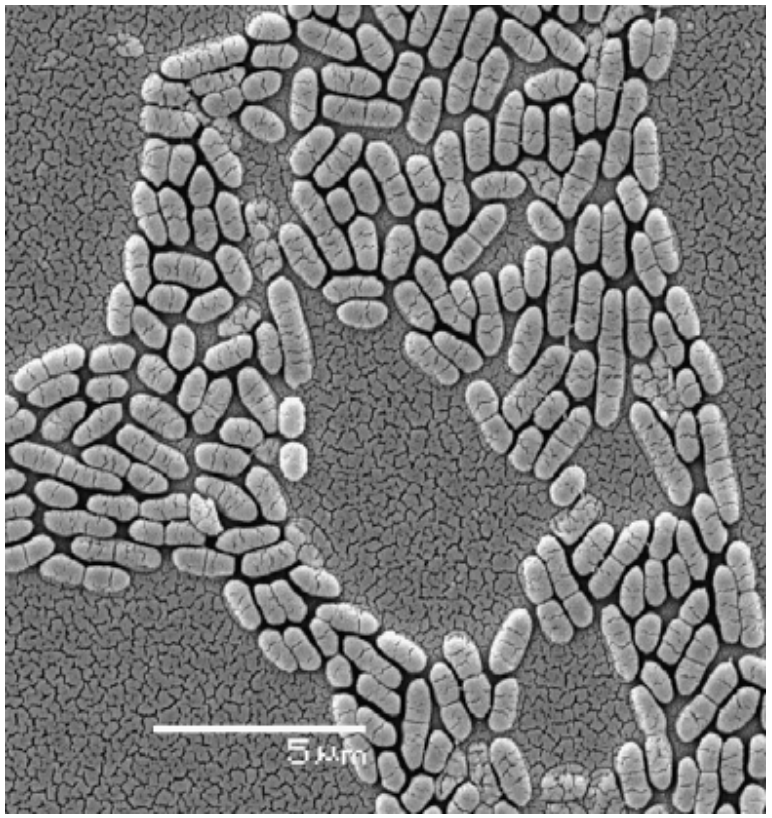
  - biofilm communities respond differently to external stimuli than planktonic communities: resistance against antibiotics, protection against washout, development of ecological niches

- **Schematic of Biofilm Formation**



- **How do they really look like: Young Biofilms I**

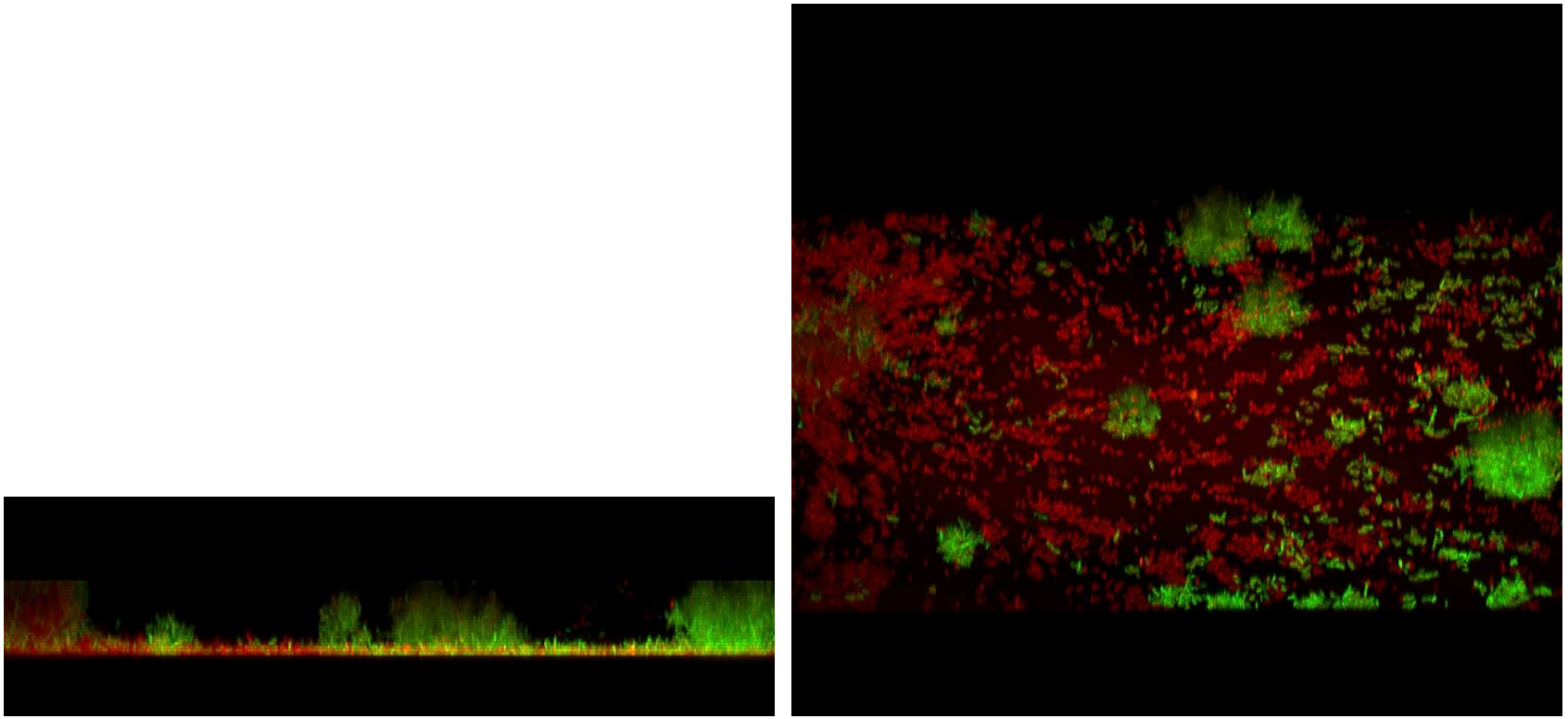




SEM of a *pseudomonas putida* and of a *listeria monocytogenes* biofilm

courtesy Dr. H. Schraft, Lakehead University

- **Young Biofilms II**

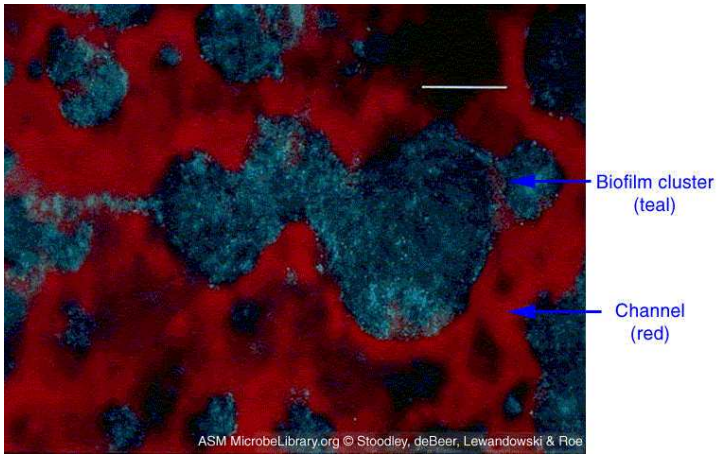


CLSM of *pseudomonas putida* (green) and *listeria monocytogenes* (red) biofilm

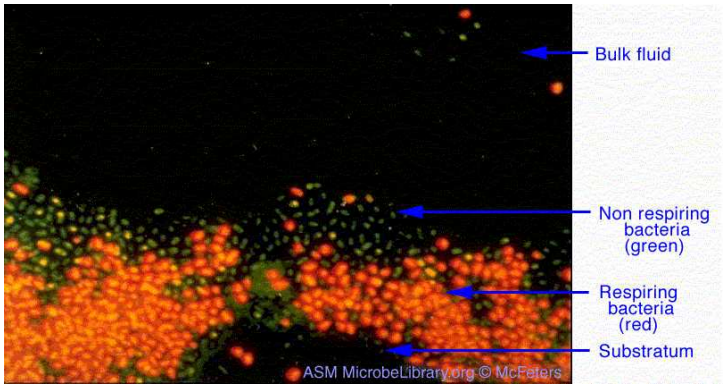
courtesy Dr. H. Schraft, Lakehead University

- **More Biofilms: Length Scales** *pictures from ASM Microbe Gallery*

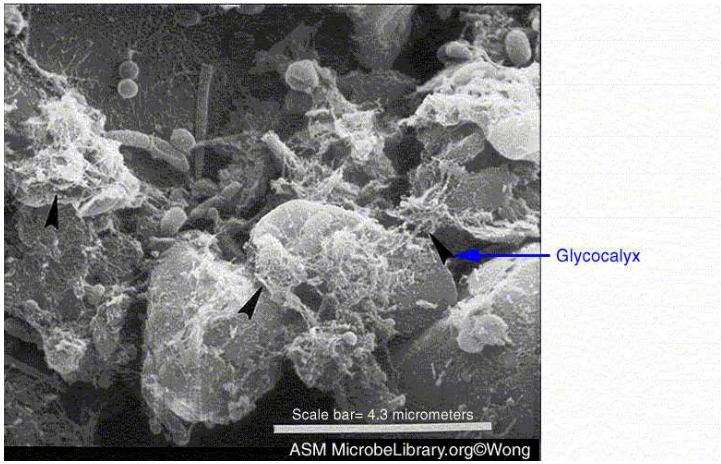
macro/reactor



meso/biofilm

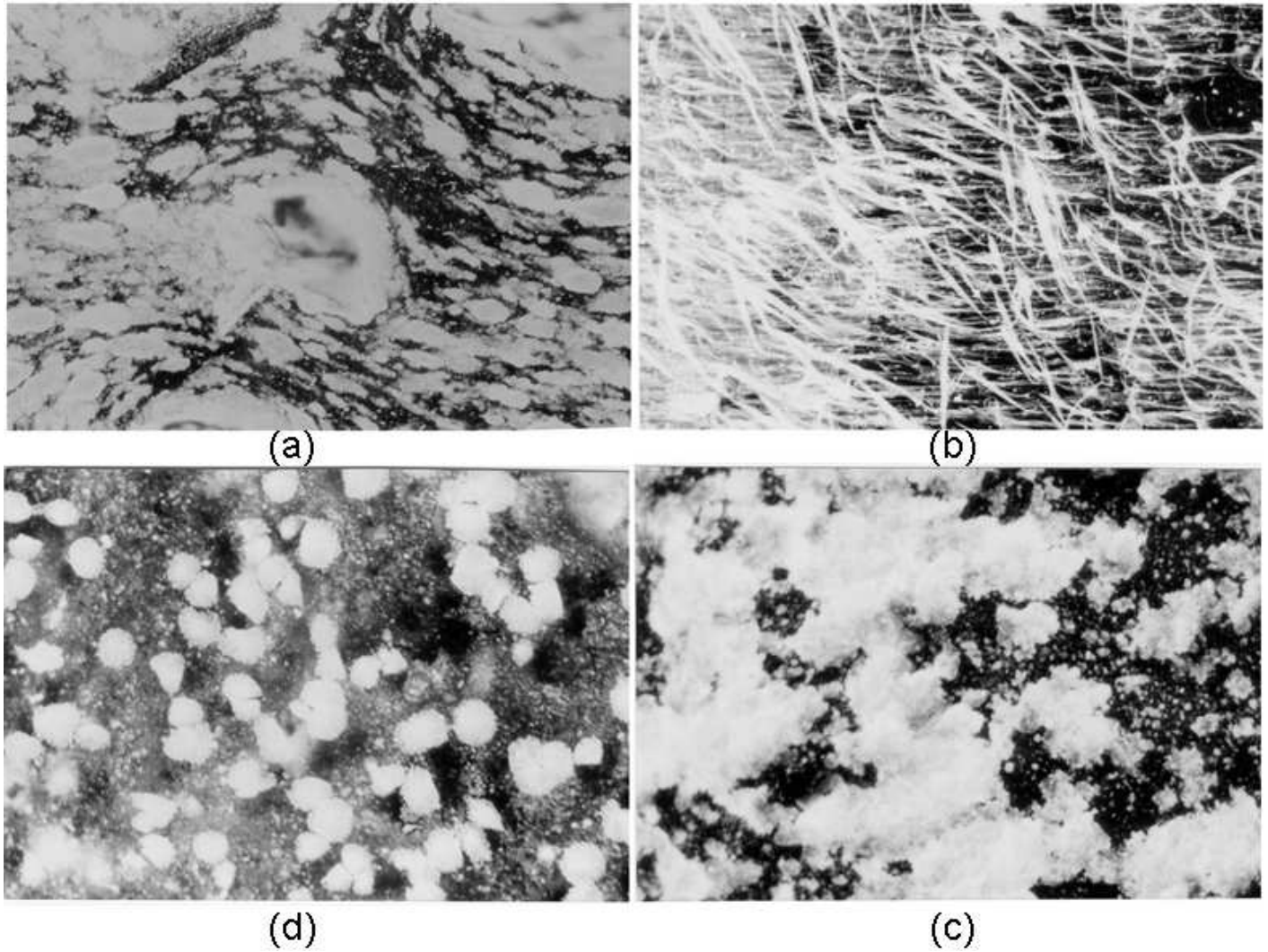


meso/biofilm



micro/cell

- **Biofilm Heterogeneities On The Meso-scale**

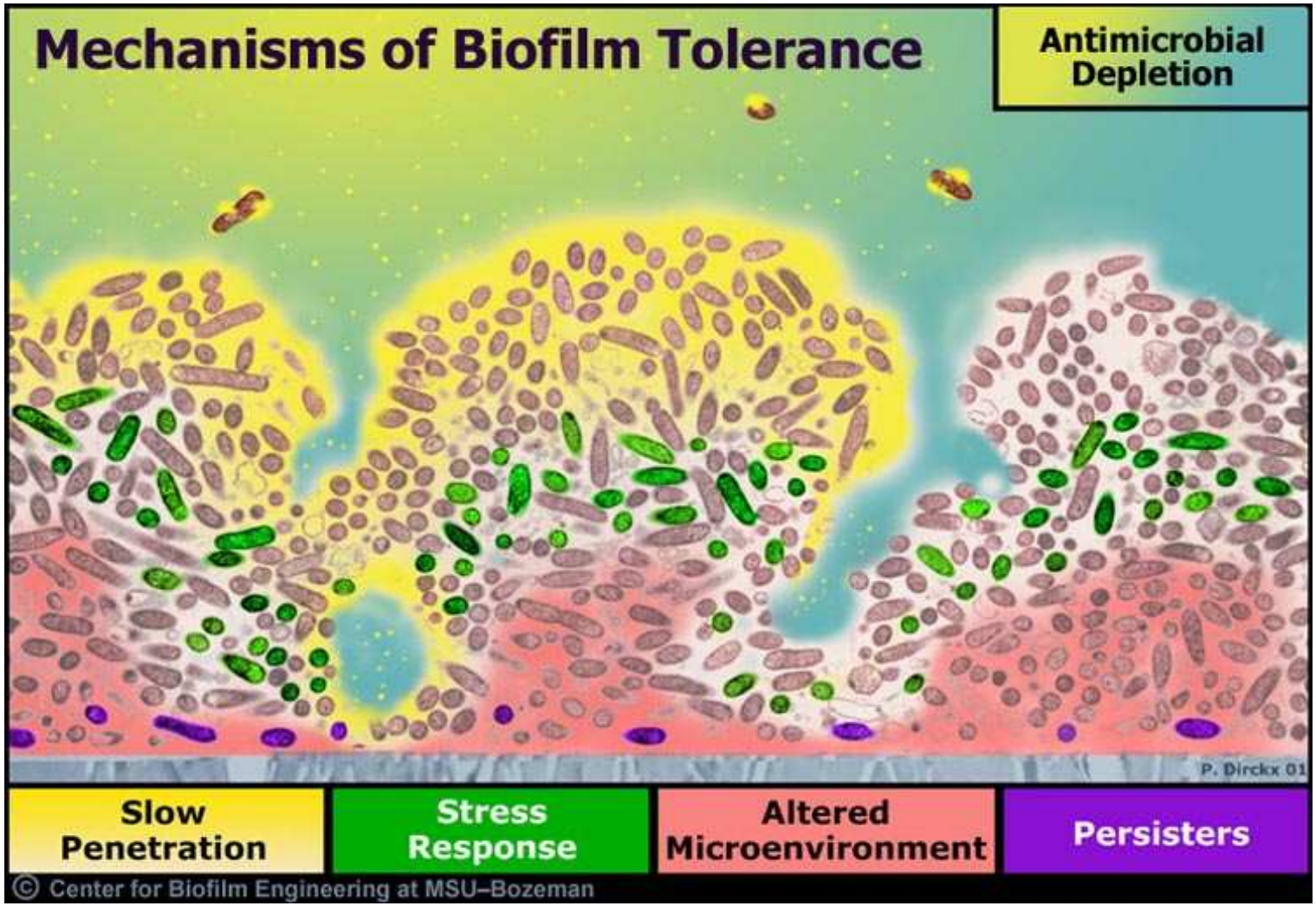


from: Kluyver Lab., Delft UT; each photography covers 0.5 cm × 0.3 cm

- **Medical biofilms**
  - biofilms are responsible for many bacterial infections:
    cystic fibrosis pneumonia, musculoskeletal infections, dental caries, periodontitis, middle ear infections
  - biofilms colonise on artificial surfaces in the body:
    contact lenses, urinary catheters, IUDs, central venous catheders, orthopedic devices, ...
  - biofilm infections are more complicated to treat than other bacterial infections
  - pathogens are embedded in EPS matrix and thus protected

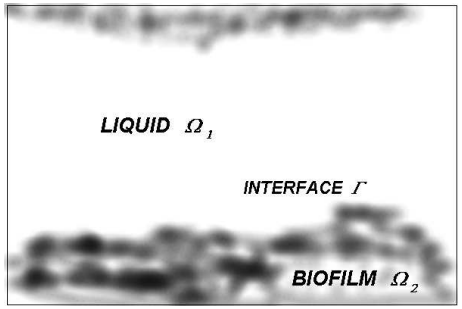
- **Biofilm control I: Antibiotics**

  four mechanisms of biofilm protection against antibiotics are suggested:
  diffusive resistance, reactive resistance, quorum sensing, persister cells



- **Biofilm control II: Probiotics as a novel alternative to antibiotics**
  - *Probiotics:* live microorganisms used as dietary supplements that confer health benefits to the hosts
  - typically administered as a functional food/nutraceutical (e.g. in dairy products)
  - save and stable transport to site of action is a problem
  - ecological principle: adding probiotics (and/or prebiotics) to the microflora (e.g. gut) means to modulate and manipulate the microbial ecology to the benefit of the host system.
  - application areas: irritated bowel syndrom, diarrhea; treatment of urinogenital infections; re-establishment of the natural microflora subsequent to a conventional antibiotic therapy; lowering blood pressure and cholesterol; increased immune response; alleviation of lactose intolerance;
  - several methods of probiotic action possible
  - we focus on lactic acid bacteria: lowering of ph to make it more difficult for pathogens (spoilage bacteria) to grow or even to remove pathogenic biofilms

- **Biofilm modeling: A continuum approach**



  - bulk liquid and biofilm are continua in $\Omega \subset \mathbb{R} \times \mathbb{R}^d$
    $\Omega_1(t) = \{(t,x), M(t,x) = 0\}$, $\Omega_2(t) = \{(t,x), M(t,x) > 0\}$
  - basic processes to be included:
    nutrient transport: diffusion ($\Omega_{1,2}$)
    kinetics: substrate consumption, biomass production (reactions, Ω_2)
    biomass spreading: growth (changes of Ω_2)
  - main problem: spatial spreading of biomass (development of Ω_2)

- **Some properties that a biofilm model should have**

  - "sharp" interface between biofilm Ω_2 and surrounding liquid Ω_1
  - the biomass density does not exceed a physically possible maximum
  - the biofilm does not spread remarkably at low density
  - it should be possible to use the model with reaction kinetics that have been developed for classical 1D biofilm models

    $\Longrightarrow$ *upper bound on biomass density does not come from reaction terms but must come from spatial spreading mechanism*

- **Prototype diffusion-reaction model for biofilm formation**
  E, Parker, van Loosdrecht, J. Theor. Medicine 3(3), 2001

$$\begin{aligned}
&\textit{substrates} && C_t = \nabla_x \left(D_1(M)\nabla_x C\right) - f(C,M) \\
&\textit{biomass} && M_t = \nabla_x \cdot (D_2(M)\nabla_x M) + g(C,M) \\
&\textit{kinetics} && f(C,M) = k_1 CM/(k_2 + C) \\
& && g(C,M) = \tfrac{k_3}{k_1} f(C,M) - k_4 M
\end{aligned}$$

suggestion for $D_2(M)$:

$$D_2(M) = d_2 M^b/(1-M))^a, \quad a, b \geq 1, d_2 > 0$$

- the evolution equation for M is a density-dependent:
  degeneracy as well as fast diffusion
- diffusion coefficient $D_1(M)$ bounded between two positive constants:
  non-critical

- **Some results for the biomass spreading model**
  Efendiev, E, Zelik, 2002

  – for $\frac{k_3}{k_2+1} - k_4 > 0$ one obtains:
  the prototype diffusion-reaction model with boundary conditions

$$C\,|_{\partial\Omega}= 1, \quad M\,|_{\partial\Omega}= 0$$

  and initial conditions

$$C\,|_{t=0}= C_0, \quad M\,|_{t=0}= M_0$$

$$C_0, M_0 \in L^\infty(\Omega), \quad 0 \le C_0(x) \le 1, \quad 0 \le M_0(x) \le 1, \quad x \in \Omega$$

  has a unique solution in the class of functions

$$\begin{cases} 1. & C, M \in L^\infty(\mathbb{R}_+ \times \Omega) \cap \mathcal{C}([0,\infty), L^2(\Omega)) \\ 2. & C, \int_0^M \frac{m^b}{(1-m)^a} dm \in L^\infty(\mathbb{R}_+, H^1(\Omega)) \cap \mathcal{C}([0,\infty), L^2(\Omega)) \\ 3. & 0 \le C(t,x), M(t,x) \le 1, \|M\|_{L^\infty(\mathbb{R}_+ \times \Omega)} < 1 \end{cases}$$

Proof: long and technical

key idea:

– auxiliary problem: replace $D_2(M)$ by

$$f_R(M) = \begin{cases} (M + 1/R)^b/(1-M)^a & \text{if } M \leq 1 - 1/R \\ R^a & \text{if } M > 1 - 1/R \end{cases}$$

and consider $R \longrightarrow \infty$

- **Some results** (*cont.*)

  – in the case of homogeneous Dirichlet conditions or mixed homogeneous Dirichlet/Neumann conditions for M

$$mes\{x \in \Omega : M(t,x) = 1\} = 0, \quad \forall t > 0$$

  – in the case of purely Neumann conditions for M there exist initial data $0 \leq C_0(x) < 1$, $0 \leq M_0 < 1$ such, that there exists $T = T(C_0, M_0)$ with

$$\langle M(t) \rangle < 1, \quad \forall t < T, \quad \text{and} \quad \lim_{t \to T^-} \langle M(t) \rangle = 1$$

  where

$$\langle M(t) \rangle := \frac{1}{\mid \Omega \mid} \int_\Omega M(t) dx$$

  – existence of a global attractor: semigroup $\mathcal{S}_t$ is continuous with respect to initial data; $\mathcal{S}_t$ posseses a compact attracting (even absorbing) set

- **Some results (*cont.*)** (*Duvnjak, E, 2006*)

  for first order kinetics with abundant nutrient supply, i.e.

  $$(*) \qquad M_t = \nabla_x \cdot (D_2(M) \nabla_x M) + kM$$

  – existence of Lyapunov functional

  $$J(M) = \frac{1}{2} \int_\Omega \mid \nabla \Phi(M) \mid^2 dx - k \int_\Omega dx \int_0^M D(s) s ds$$

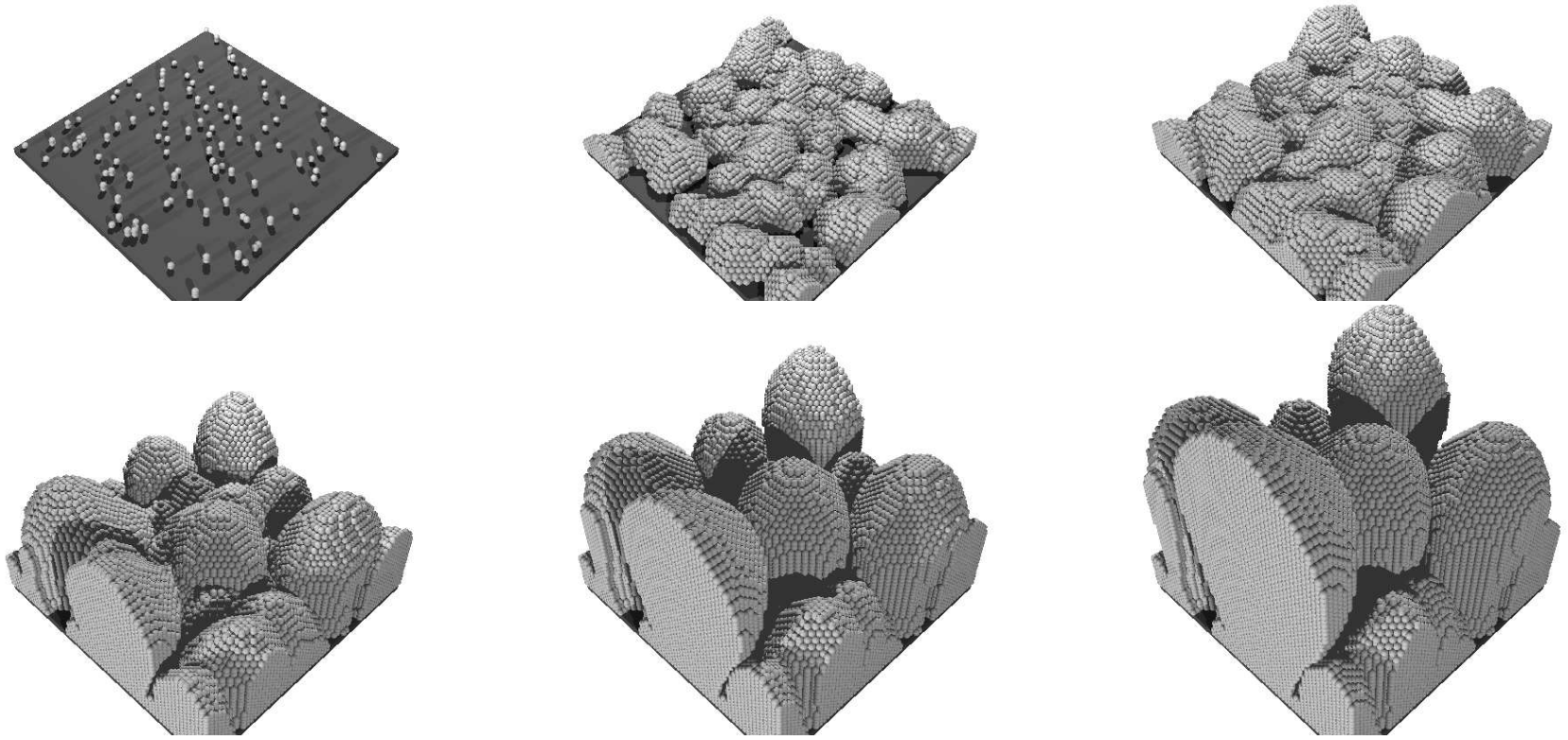
  with $\Phi(M) = \int_0^M D(m) dm$

  – time integration: transformation $N := \Phi(M)$ leads to

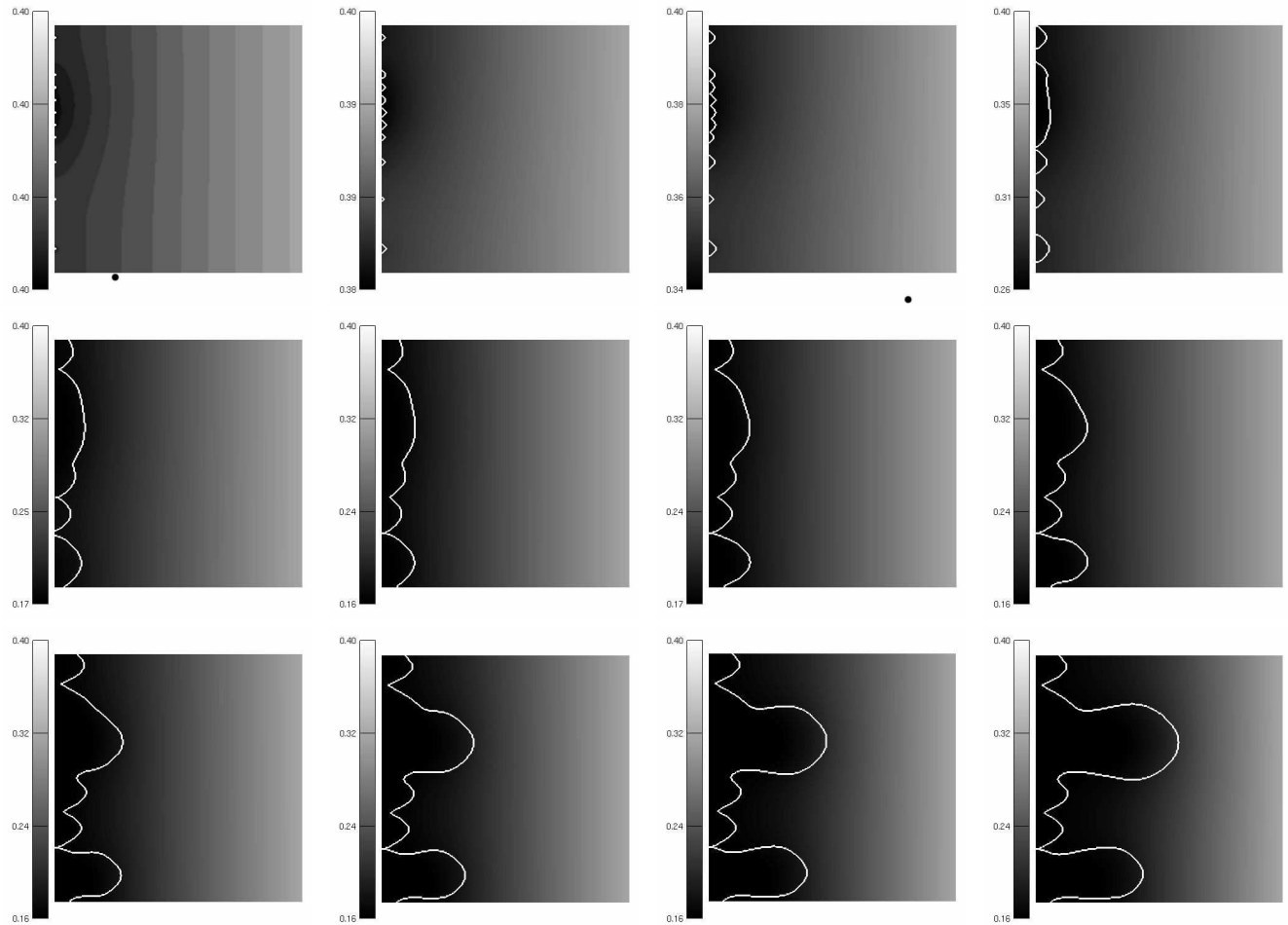
  $$(**) \qquad (\beta(N))_t - \Delta N = k\beta(N)$$

  where $\beta = \Phi^{-1}$. The backwards Euler method for $(**)$ has a solution and converges for $1 > k\Delta t$.

- **Model simulation: biofilm formation in time**

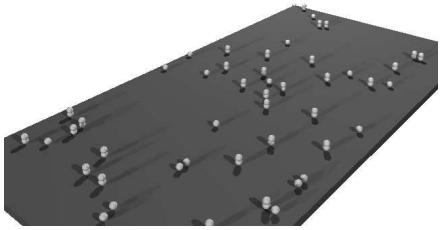


- **... or in 2D with substrates displayed**

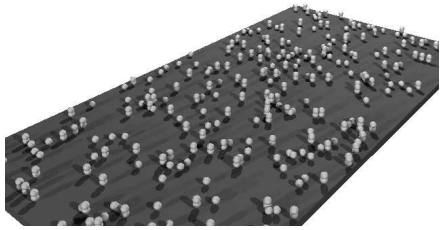


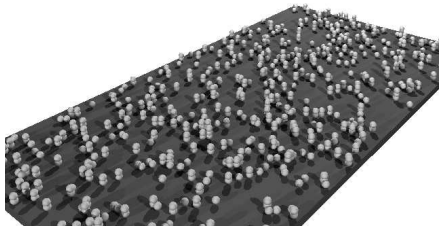
Formation of a cluster-and-channel biofilm morphology (top left to bottom right): Shown are the biofilm/liquid interface and the limiting oxygen concentration $c_2(t, x)$ in time [days]: $t = 0, 4, 6, 9, 19, 39, 79, 119, 159, 199, 229, 249$

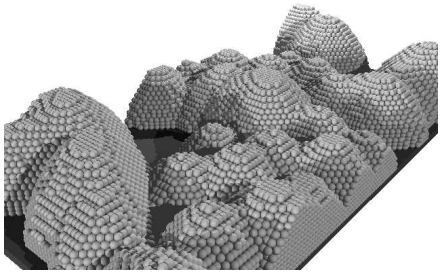
- **Model simulation: dependence on $G = \frac{\text{mass conversion}}{\text{nutrient supply}}$**



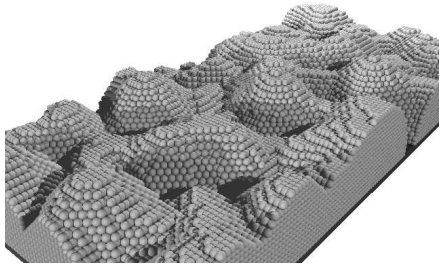
inoculum ($t = 0$)

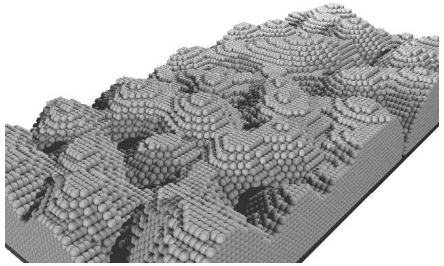


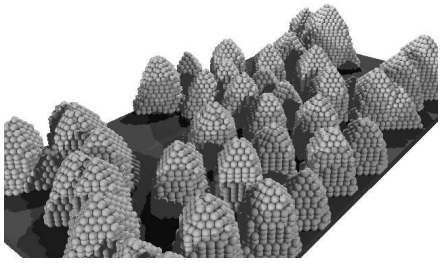




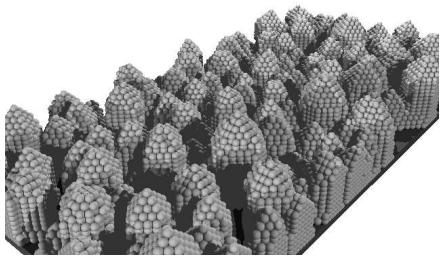
low G-number

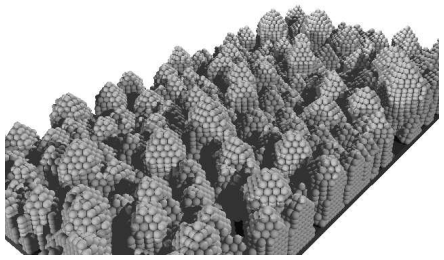






high G-number





- **Main result of spatial biofilm development models**

  Several models based on the "environmental conditions hypothesis" only show qualitatively similar results (individual based modelling, cellular automata, diffusion-reaction, viscuous fluid models):

  Environmental conditions like nutrient availability are sufficient to create spatially heterogeneous biofilm architectures as observed in the laboratory.

  Note: This does not exclude contributions of further processes, e.g. quorum sensing

- **Biofilm control by antibiotics**

  - biofilm protection mechanism: diffusive-reactive resistance
  - two dissolved substrates: nutrients and antibiotics (B, C)
  - two biomass fractions: viable and inert biomass (X, Y)
  - both biomass fractions are shifted around together
  - reaction kinetics from literature (Stewart *et al* 1994, 1995, 1996)
  - biofilm/antibiotics model

$$
\begin{aligned}
B_t &= \nabla \cdot (D_B \nabla B) - \beta B X \\
C_t &= \nabla \cdot (D_C \nabla C) - \gamma X C/(k + C) \\
X_t &= \nabla \cdot (D_M(X + Y)\nabla X) + \xi_1 X C/(k + C) - \xi_2 X B - \xi_3 X \\
Y_t &= \nabla \cdot (D_M(X + Y)\nabla Y) + \xi_2 X B
\end{aligned}
$$

with
$D_B = D_B(X + Y) \gg 0$, $D_C = D_C(X + Y) \gg 0$
$D_M(X + Y) = d_2(X + Y)^b/(1 - X - Y)^a$

- **Theorem: Existence of Solutions**

  The initial-boundary value problem with Dirichlet conditions

$$X\big|_{\partial\Omega} = Y\big|_{\partial\Omega} = 0, \quad B\big|_{\partial\Omega} = B_r(x), \quad C\big|_{\partial\Omega} = C_r(x), \quad x \in \partial\Omega$$

  and non-negative initial data

$$B(0,\cdot) = B_0,\ C(0,\cdot) = C_0,\ X(0,\cdot) = X_0,\ Y(0,\cdot) = Y_0$$

$$B_0, C_0, X_0, Y_0 \in L^\infty(\Omega), \quad 0 \le X_0 + Y_0 \le 1 - \delta, \quad 0 < \delta < 1$$

  possesses a solution

$$(B, C, X, Y) \in L^\infty(\mathbb{R}_+ \times \Omega) \times L^\infty(\mathbb{R}_+ \times \Omega) \times L^\infty(\mathbb{R}_+ \times \Omega) \times L^\infty(\mathbb{R}_+ \times \Omega)$$

  with $\|X + Y\|_{L^\infty} \le 1$

Proof:

- conducted in several steps, using the existence proof of prototype model
- we note that

$$(X+Y)_t = \nabla\left(D(X+Y)\nabla(X+Y)\right) + \xi_1 \frac{CX}{\kappa + C} - \xi_3 X$$

- again, we first consider a family of related non-degenrate problems, show their existence and pass to the degenrate limit
- boundedness: solution of prototype model can by used to construct an upper bound on $X + Y$

- **... additional remarks**

  – existence result carries over to other sets of boundary conditions, in particular mixed Dirichlet/Neumann

  – in case of Neumann conditions, using a time-scale argument, the divergence theorem yields the following simple lumped version

$$\frac{d\Xi}{dt} = -\frac{\xi_2}{\beta} J_B(t) + \frac{\xi_1}{\gamma} J_C(t) - \xi_3 \Xi$$

$$\frac{d\Upsilon}{dt} = \frac{\xi_2}{\beta} J_B(t)$$

  Ξ, Υ: total viable and inert biomass
  $J_B(t)$, $J_C(t)$: fluxes of substrate into the system (specified as B.C.s)
  ODE model breaks down when reactor is filled

  – model and existence theorem can be extended to:
  ◇ reduced antibiotic efficiency in regions with oxygen limitation
  ◇ adaptation of viable biomass to become less resistant

- **some model extensions I**
  - reduced antibiotic efficiency in regions with oxygen limitation

$$
\begin{aligned}
B_t &= \nabla(D_B \nabla B) - \beta B X C/(\kappa + C) \\
C_t &= \nabla(D_C \nabla C) - \gamma X C/(k + C) \\
X_t &= \nabla\left(D_M(X+Y)\nabla X\right) + (\xi_1 X - \xi_2 X B)C/(\kappa + C) - \xi_3 X \\
Y_t &= \nabla\left(D_M(X+Y)\nabla Y\right) + \xi_2 X B C/(\kappa + C)
\end{aligned}
$$

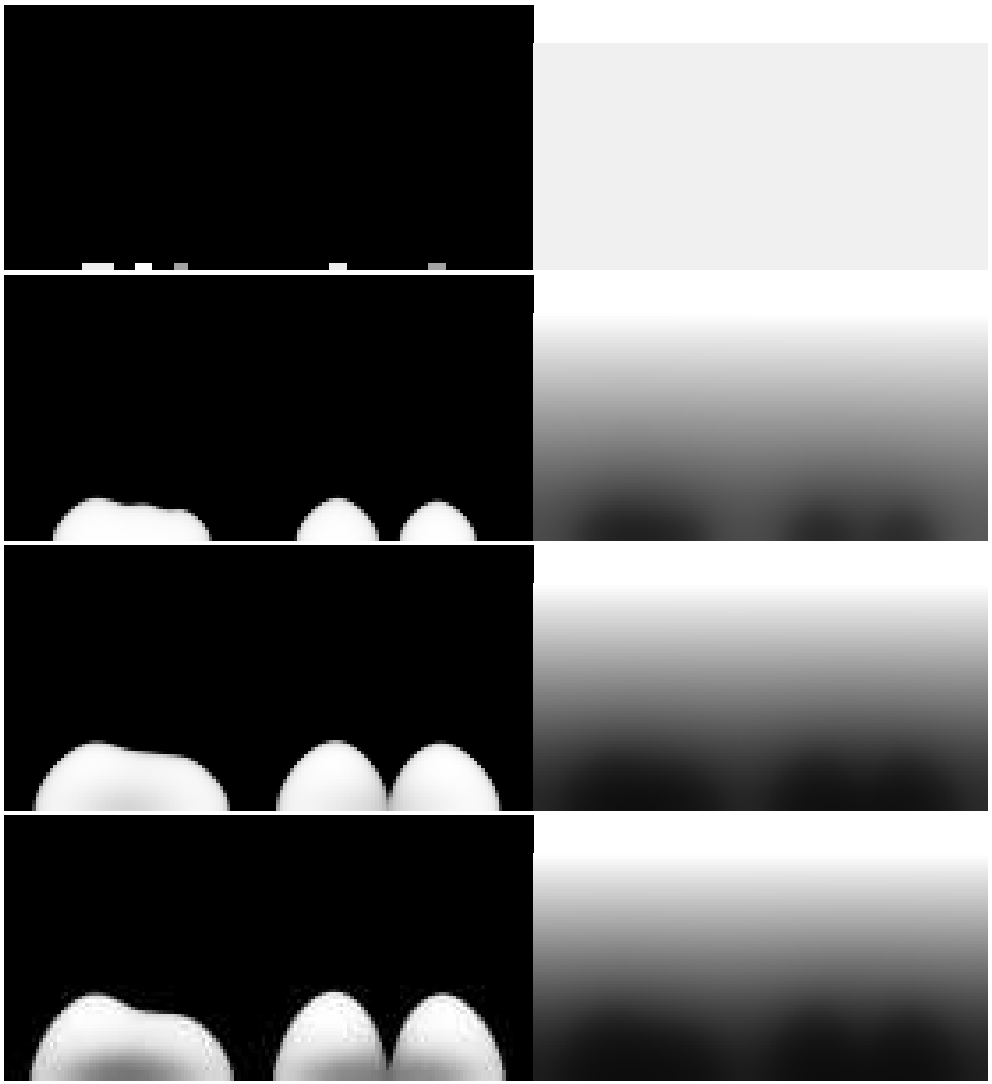
- **some model extensions II**
  - adaptation of viable biomass to become more resistant

$$
\begin{aligned}
B_t &= \nabla(D_B \nabla B) - \beta B X \\
C_t &= \nabla(D_C \nabla C) - \gamma X C/(k+C) \\
X_t &= \nabla\left(D_M(M)\nabla X\right) + \xi_1 X C/(k+C) - \xi_2 X B - \xi_3 X - r(B)X \\
Y_t &= \nabla\left(D_M(M)\nabla Y\right) + \xi_2 X B \\
\tilde{X}_t &= \nabla\left(D_M(M)\nabla \tilde{X}\right) + \xi_1 \tilde{X} C/(k+C) - \tilde{\xi}_2 \tilde{X} B - \tilde{\xi}_3 X + r(B)X \\
\tilde{Y}_t &= \nabla\left(D_M(M)\nabla \tilde{Y}\right) + \tilde{\xi}_2 \tilde{X} B
\end{aligned}
$$

with
- $M = X + Y + \tilde{X} + \tilde{Y}$
- $\xi_2 > \tilde{\xi}_2 \geq 0$
- $r(B) \geq 0, \quad r(0) = 0, \quad r'(B) \geq 0$

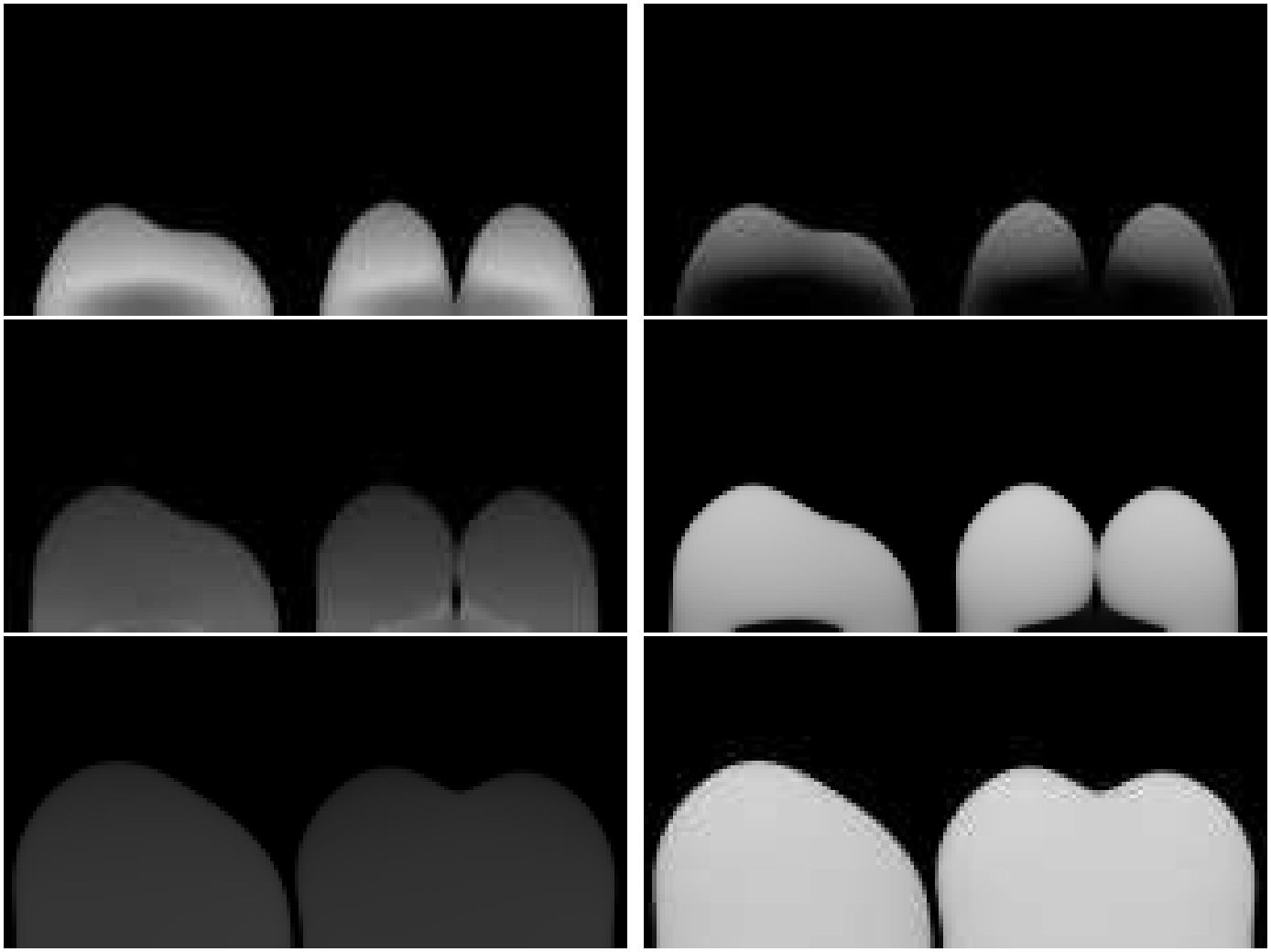
- **Simulation study I: an illustration**
  - conducted in 2D on a regular grid
  - numerical method: Non-standard Finite Difference Scheme
    ◇ non-local (in time) discretisation of nonlinear diffusion operator
  - inoculum seeded randomly on substratum (5 pockets):
    ◇ only viable, no inert biomass
  - boundary conditions:
    ◇ X and Y: no-flux
    ◇ C: constant concentration on top boundary, no-flux everywhere else
    ◇ B: non-negative flux on top boundary, no flux everywhere else
  - initially (12 days) only growth, no disinfection:
    produces a mature biofilm
  - then antibiotics are added to the system to disinfect biofilm

- **Simulation study I: growth period**



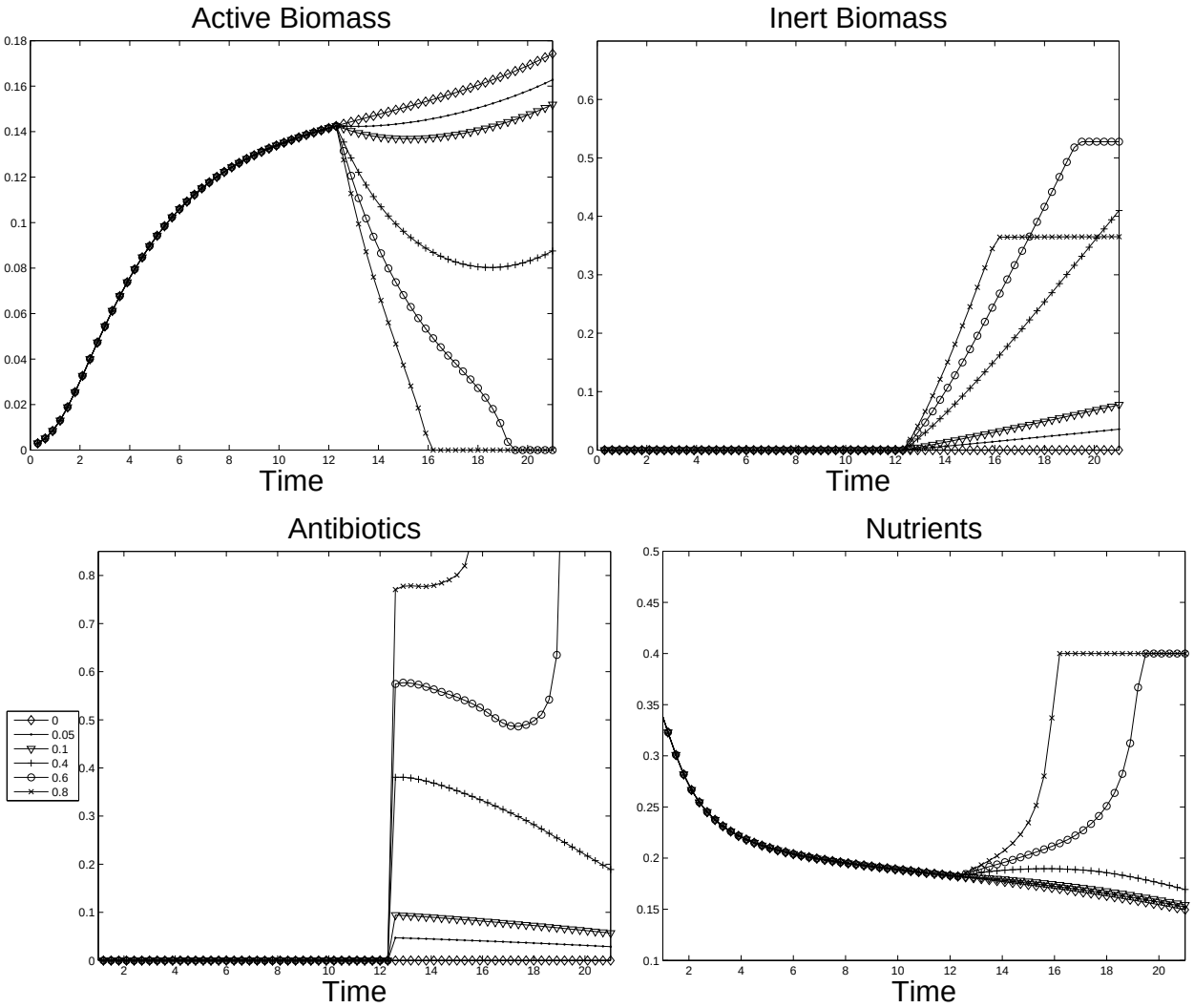
shown are X and C for $T = 0, 3, 8, 12d$.

- **Simulation study I: disinfection period**



shown are X and Y for $T = 13.5, 16.5, 20d$.

- **Simulation study I: various antibiotics intensities**



shown are lumped data for X, Y, B, C

- **Simulation study II: comparison of disinfection strategies**

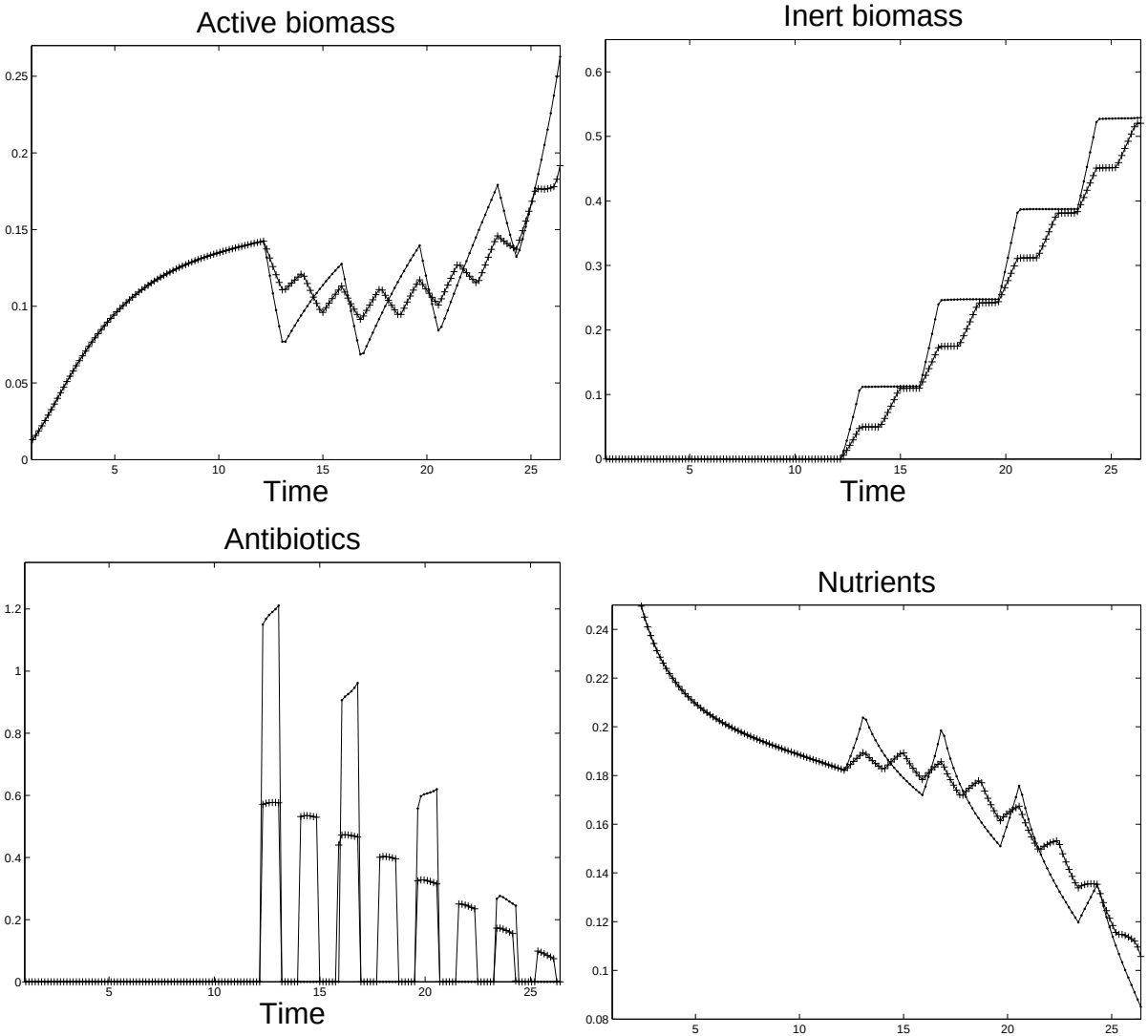
  – set-up and methods as in study I
  – growth phase as in Study I

  – disinfection phase:
    ◇ periodic, alternating between constant a and 0

$$A_B(t) = \begin{cases} \alpha, & t - t_0 \in [k\pi, \tau + k\pi], \quad k \in IN \\ 0, & \text{else} \end{cases}$$

  – two strategies are compared:
    ◇ same average intensity of antibiotics,
    ◇ different intensities and periods ($a_1 = 2a_2, \pi_1 = 2\pi_2$)
    ◇ mimicking, e.g. 12h vs. 24 treamtent

- **Simulation study II: lumped results for X, Y, B, C**



Main result: *initially the more intense strategy is better, eventually the milder dosage option leads to lower active biomass*

- **Initial penetration of antibiotics into the biofilm (1D study)**
  - substrate processes much faster than biomass processes
    $\Rightarrow$ *quasi-steady state assumption*
  - rescaled 1D model for homogeneous biofilm:

$$0 = \begin{cases} b'' - \theta_b^2 b & \text{for } x \le \lambda := L_f/L_z \\ b'' & \text{for } \lambda \le x \le 1 \end{cases}$$

$x := \frac{z}{L_z}$, $b = \frac{B}{B_0}$, $b'(0) = 0$, $b(1) = 1$, $\theta_b^2 = \frac{\beta X_0 L_f^2}{D_B(X_0)}$, $\tau_b = \frac{D_B(X_0)}{D_B(0)}$

  - closed solution in the biofilm $x < \lambda$:

$$b(x) = b(\lambda)\frac{\cosh(\theta_b x)}{\cosh(\theta_b \lambda)}, \quad b(\lambda) = \frac{b_0}{1 + (1-\lambda)\tau_b \theta_b \tanh(\lambda \theta_b)}$$

  - b declines fast at the interface $x \approx \lambda$
  - $b(0)$ depends strongly on L_f; for realistic values: $b(0) < 10^{-2}$

$\Longrightarrow$ antibiotic penetration into biofilms is seriously hampered by diffusive resistance and reactions at the interface

- **An a priori criterion for disinfection**

  - under the assumption $C_0 \ll k$, the equation for C can be approximated by a linear equation, the solution of which is as for B:

$$0 = c'' - \theta_c^2 c$$

  - based on initial data steady-state analysis and the assumption $X \equiv X_0$ for $x \leq \lambda$, production of new viable biomass is slower than disinfection if

$$1 < \mathcal{D} := \frac{\int_0^\lambda (\xi_2 b + \xi_3) dz}{\int_0^\lambda \frac{\xi_1}{k} c dz}$$

  - **Question 1:** Is the $\mathcal{D}$-criterion valid if $C_0 \not\ll k$?
  - **Question 2:** Is the $\mathcal{D}$-criterion valid for the transient case? (*i.e.* $X(z,t) \neq const., L_f = L_f(t) \neq const.$)

- **Analysis for Monod kinetics (still steady state)**

  Let $\tilde{c}$ be the solution with regard to Monod kinetics and

$$\mathcal{D}_{Monod} := \frac{\int_0^\lambda (\xi_2 b + \xi_3) dz}{\int_0^\lambda \frac{\xi_1 \tilde{c}}{k+\tilde{c}} dz}$$

  then

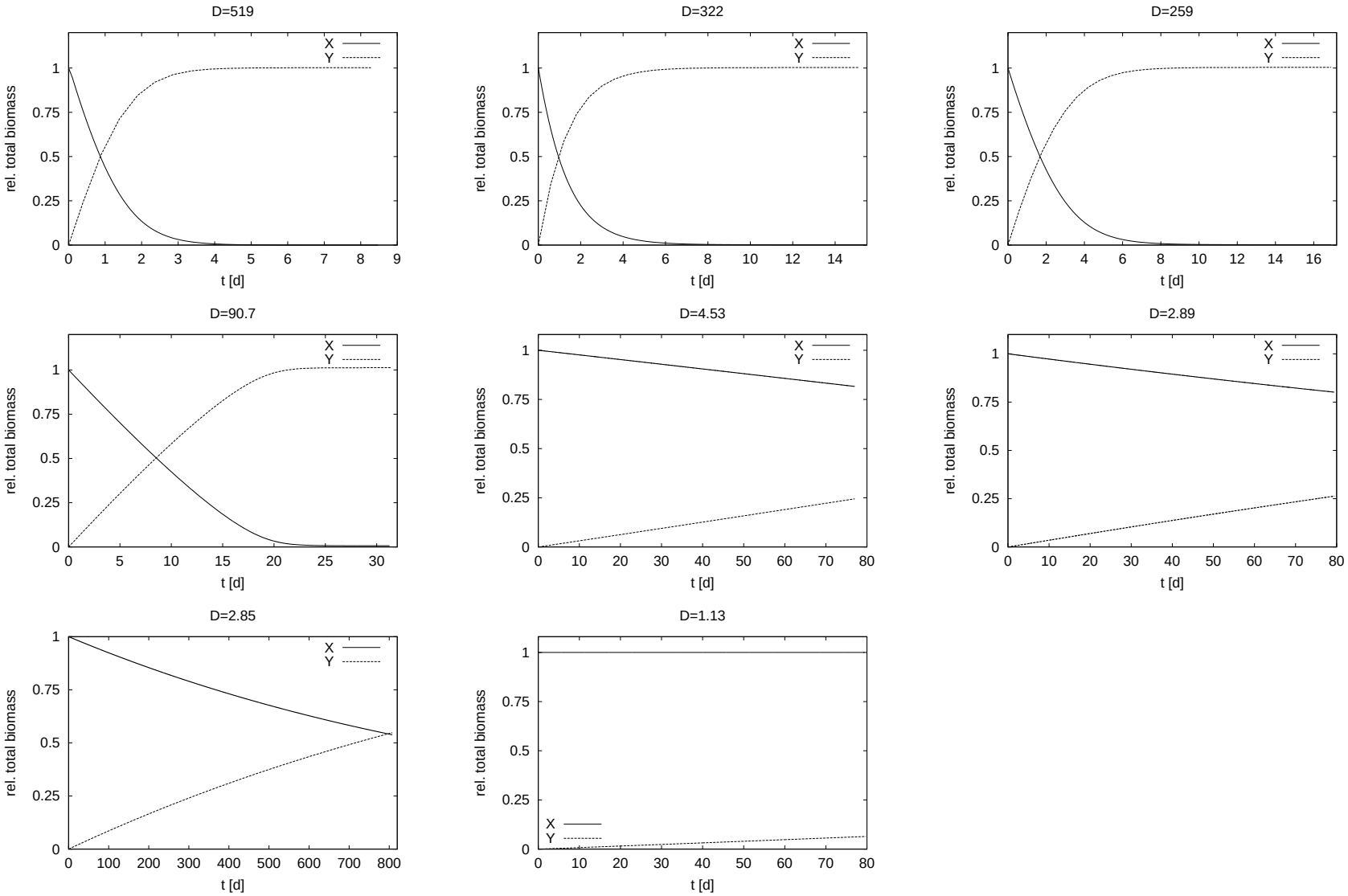
$$\mathcal{D}_{Monod} \geq \mathcal{D}.$$

  Note: $\tilde{c}$ not known $\Rightarrow \mathcal{D}_{Monod}$ cannot be evaluated *a priori*

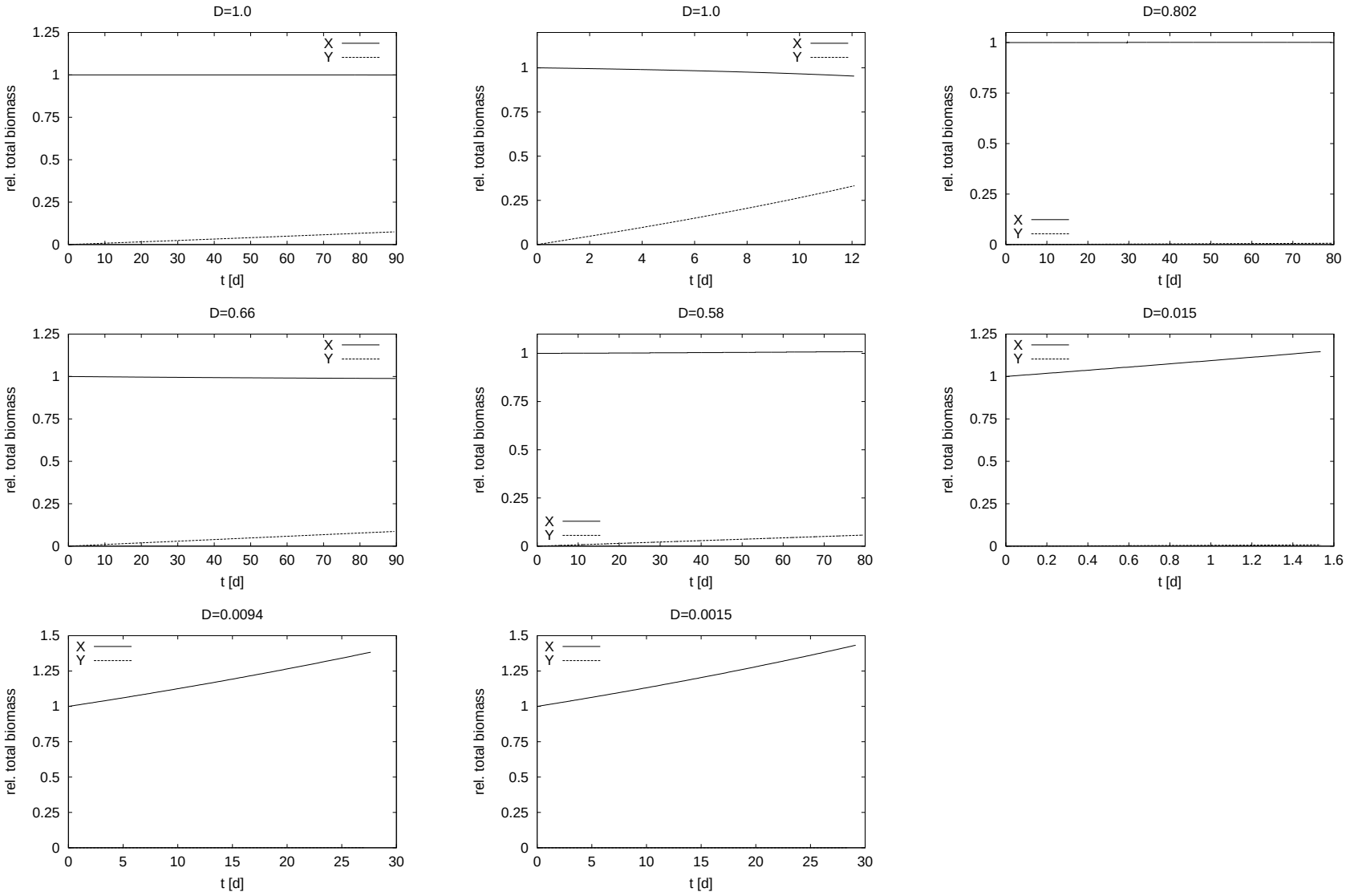
  proof: use comparison theorem for two-point boundary value problems

Thus: $\mathcal{D} > 1 \Rightarrow$ disinfection
$\mathcal{D} < 1 \not\Rightarrow$ growth of the biofilm

• The Transient Case By Simulations



• The Transient Case By Simulations (cont.)



... and now for something completely different:

- **Biofilm control by probiotics**
  - biofilm control by modulation of pH
  - three biomass fractions: probiotic, pathogen, inert biomass (N_1, N_2, Y)
  - two dissolved substrates: carbonated acids and proton ion concentration (C, P)
  - reaction kinetics taken from literature (Breidt and Fleming (1998))

- **Probiotics biofilm model**
  - governing equations

$$\begin{aligned}
\partial_t C &= \nabla \cdot (D_C \nabla C) - u \nabla C + \alpha_1 N_1 (k_1 - C) + \alpha_2 N_2 (k_2 - C) \\
\partial_t P &= \nabla \cdot (D_P \nabla P) \nabla P - u \nabla P + \alpha_3 C (k_3 - P) \\
\partial_t N_1 &= \nabla \cdot (D_M(M) \nabla N_1) + \mu_1 g_1(C,P) N_1 \\
\partial_t N_2 &= \nabla \cdot (D_M(M) \nabla N_2) + \mu_2 g_2(C,P) N_2 \\
\partial_t Y &= \nabla \cdot (D_M(M) \nabla Y) \\
&\quad - \min(0, \mu_1 g_1(C,P) N_1) - \min(0, \mu_2 g_2(C,P) N_2)
\end{aligned}$$

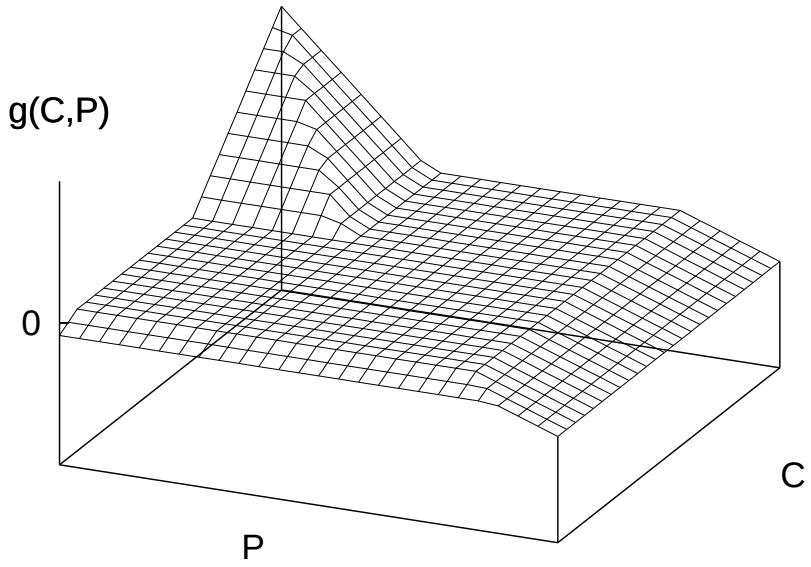
$$D_M(M) = \epsilon \frac{M^a}{(1-M)^b}, \quad M = N_1 + N_2 + Y$$

  - we include convective transport terms for C and P as a mechanism of substrate supply
  - flow velocity u calculated analytically from an approximation of the Stokes equations

- **Probiotics biofilm model** (*cont.*)

  – bacterial population ...
  ... *grow if* C *and* P *small*
  ... *decay if one of* C *or* P *is large*

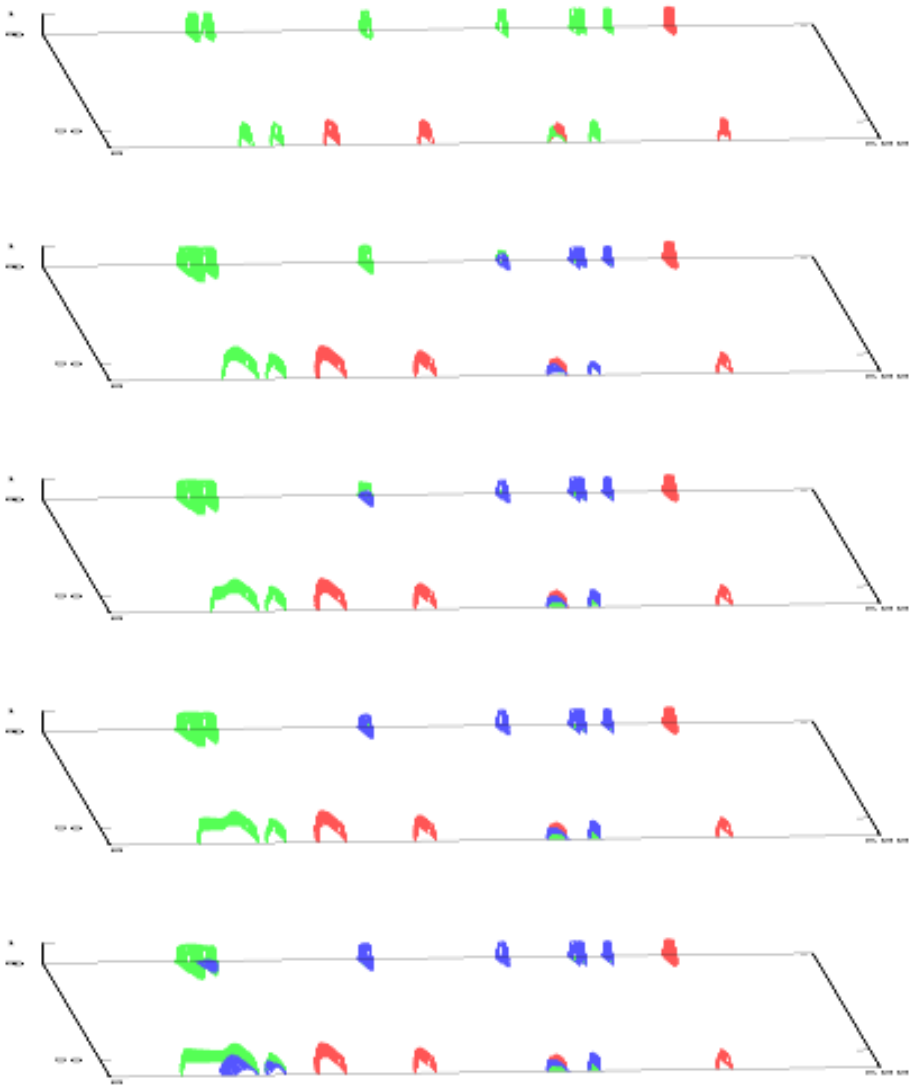
  – piecewise first order kinetics



  – probiotics grow long and decay later than pathogen:
  $g_1(C, P) \geq g_2(C, P)$

- **Simulation setup**
  - 2D on a regular grid, simulating a long skinny flow channel
  - numerical method: Non-standard Finite Difference Scheme
    ◇ non-local (in time) discretisation of nonlinear diffusion operator
  - inoculum seeded randomly on substratum (5 pockets):
    ◇ only viable probiotics and pathogens, no inert biomass
  - boundary conditions:
    ◇ N_1, N_2, Y and Y: no-flux
    ◇ C, P: constant concentration on inflow, no-flux everywhere else
  - creeping flow conditions: $Re = 10^{-3}, Pe \approx 1$
  - we compare the effect of initial conditions (biomass distribution)

- **Simulation results: biofilm formation and control in time**



$t = 1$

$t = 10$

$t = 20$

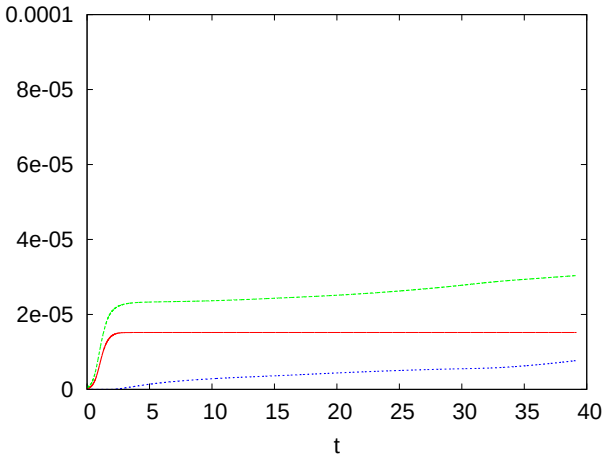
$t = 30$

$t = 40$

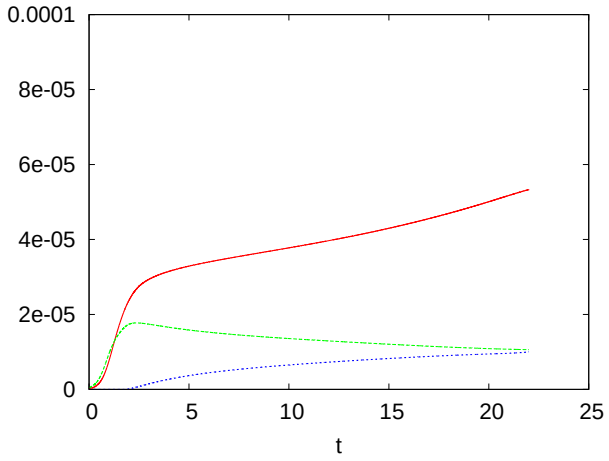
$\Longrightarrow$ *creeping flow* $\Longrightarrow$

- **Simulation results: lumped data (4 runs)**

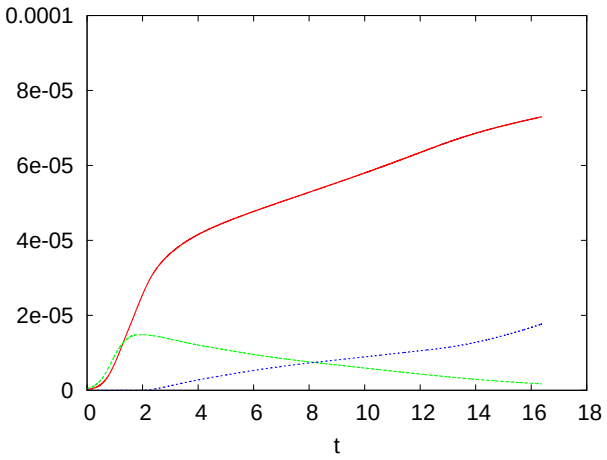
(a)



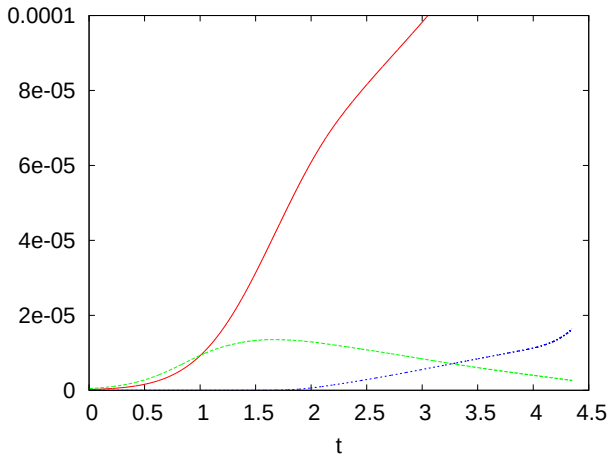
(b)



(c)



(d)



biomass of probiotic (red), pathogen (green), inerts (blue)
(a), (b), (c): random inoculations
(d): some probiotics placed upstream

- **Probiotics: Preliminary conclusions**
  - no upstream control effect (even at creeping flow)
  - efficiency of probiotic control is sensitive to site of attachment of probiotics and pathogens
  - cells in deeper regions of the biofilm are not better protected than the outer layers (no flow regime; maximum principle)
  - much more work and data evaluation needed
  - are combined antibiotic-probiotic control strategies possible?

- **Take Home**
  - biofilms are omnipresent and bad in the medical context
  - we presented a modeling framework for spatio-temporal biofilm formation with some unique mathematical features
  - model is able to predict spatially organised biofilms, e.g. mushroom morphologies
  - we extended the model to simulate biofilm control with antibiotics and probiotics
  - the antibiotics model could be analyzed, the probiotics model not (yet)
  - the antibiotics model reproduces our intuitive expectations
  - probiotics are an emerging area of research in food science and medicine
  - we presented a first step toward a mathematical formulation of probiotic theory, taking an ecological view