MSc Computational Science
joint programme UvA/VU

A DIFFUSION-BASED MODEL OF SPATIAL INTERACTIONS IN *ASPERGILLUS* SPP. GERMINATION

FIRST EXPERIMETNS

Presented by Boyan Mihaylov

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Supervisor: Prof. dr. Han Wösten, Utrecht University

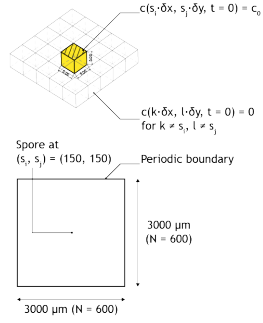
Examiner: Dr. Jaap Kaandorp, University of Amsterdam

OUTLINE

GENERAL ASSUMPTIONS

Simulation space

- 2D lattice $\equiv$ thin layer of medium clamped between 2 impermeable sheets
  - L = 3000 μm
  - Discrete blocks of size $\delta x = \delta y = \delta z = 5\ \mu$m
  - $N = 600$ subdivisions
  - Lattice volume $V_L = 4.5 \times 10^7\ \mu\text{m}^3 = 4.5 \times 10^{-5}$ mL
  - Assuming water as medium
- Spore $\equiv$ single block in the center of the lattice, inhibitor source
  - Volume $V_s = 125\ \mu\text{m}^3$
  - Top and bottom side of spore are sealed
- Inhibitor - initial concentrations $c(x, y, t = 0)$:
  - At spore: $c(x_s, y_s, t = 0) = c_0$
  - Elsewhere: $c(x, y, t = 0) = 0$



GENERAL ASSUMPTIONS

Inhibitor

- Initial concentration is assumed to be contained within the cell wall
  - Assumed cell wall thickness: $d = 10\,\text{nm}$ [6]
  - Total cell wall volume: $V_{\text{cw}} \approx 6 \cdot 25\,\mu\text{m}^2 \cdot 0.01\,\mu\text{m} = 1.5\,\mu\text{m}^3$
  - This concentration is non-replenishable.
- Assumed to have known molecular properties of 1-octen-3-ol:
  - Molecular weight: $m_M = 128.21\,\text{g/mol}$
  - Density: $\rho = 0.837\,\text{g/mL}$
- Assumed to have similar diffusion properties as glucose:
  - Diffusion constant in water at temperature $T = 293.15K$: $D = 600\,\mu\text{m}^2/\text{s}$ [2] (i.e. a molecule wanders on average $\langle x \rangle = \sqrt{6 \cdot D} = 60\,\mu\text{m}$ per second)
  - Permeability constant in an artificial lipid bilayer: $P_s = 1.9 \times 10^{-6}\,\mu\text{m/s}$ (average from [3] and [1], close to [8])
  - Octanol-water partition coefficient: $K \approx 10^{-3}$

Figure: 1-octen-3-ol

Figure: D-Glucose

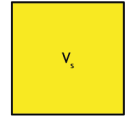
GENERAL ASSUMPTIONS

Initial concentration check

- Full spore volume
  - Homogeneous distribution, standard temperature and pressure:

$$c_{\max} = \frac{\rho}{m_M} = \frac{0.837\,\mathrm{g/mL}}{128.21\,\mathrm{g/mol}} \approx 6.5\,\mathrm{mol/mL} \qquad (1)$$

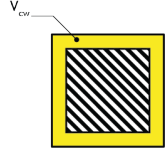
  - Equivalently, $N = N_A c_{\max} V_s = 4.89 \times 10^{14}$ molecules.



- Cell wall volume only
  - The maximum possible concentration is:

$$c_{\mathrm{mcw}} = \frac{\rho V_{\mathrm{cw}}}{m_M V_s} = 6.5\,\mathrm{mol/mL} \cdot 0.012 = 0.078\,\mathrm{mol/mL} \qquad (2)$$

  - Equivalently, $N = N_A c_{\mathrm{mcw}} V_{\mathrm{cw}} = 7.04 \times 10^{10}$ molecules.
  - The experiments use an initial concentration $c_0 = c_{\max,\,\mathrm{cw}}$.



OUTLINE

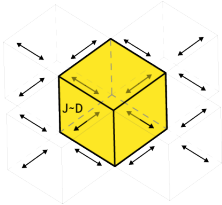
EXPERIMENT 1 SETUP

Assumptions

- At $t = 0$, all the inhibitor is contained in a cell wall at the interface between the spore and the medium, having the same diffusion coefficient as water.
- To prevent numerical error, the concentration in the simulations is normalised by a factor $\lambda = \frac{1}{c_0}$.
- Diffusion is simulated using the diffusion equation (Fick's Law):

$$\frac{\partial c}{\partial t} = D\nabla^2 c \tag{3}$$

- Simulation run for $t_{\max} = 3600\,\text{s}$
- The times for reaching threshold concentrations of $c_t = kc_0$ ($k = \{10^{-1}, 10^{-2}, ..., 10^{-6}\}$) are recorded.



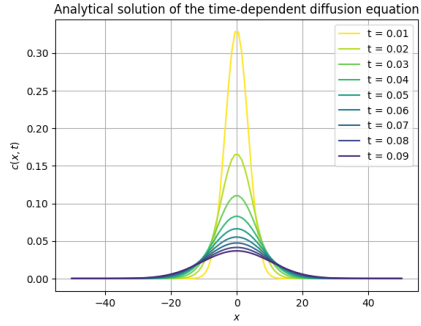
ANALYTICAL VERIFICATION

- Assuming the initial condition is a delta pulse of concentration at coordinates (x_0, y_0)
- The concentration at an arbitrary position (x, y) at time t can also be computed through the analytical formula[5]:

$$c(x, y, t) = \frac{N^{2/3}}{4\pi Dt} \exp\left(-\frac{(x - x_0)^2 + (y - y_0)^2}{4Dt}\right) \quad (4)$$

- At the spore site, this equation becomes:

$$c(x_0, y_0, t) = \frac{(V_s c_0)^{2/3}}{4\pi Dt} \quad (5)$$



OUTLINE



EXPERIMENT 2 SETUP

Assumptions

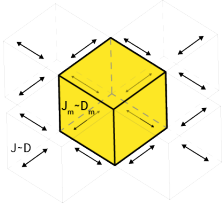
- All the inhibitor is contained within in a cell wall with the properties of a lipid bilayer at the spore-medium interface.
- Diffusion through the medium as before
- Diffusion at the interface follows the flux equation

$$J_m = -D_m \nabla c \tag{6}$$

- where J_m is the flux through the cell wall such that

$$D_m = \frac{d}{K} P_s = 1.9 \times 10^{-5}\ \mu\text{m}^2/\text{s} \tag{7}$$

- Simulation run for $t_{\max} = 7200$ s
- The times for reaching threshold concentrations of $c_t = kc_0$ ($k = \{10^{-1}, 10^{-2}, ..., 10^{-6}\}$) are recorded.



ANALYTICAL VERIFICATION

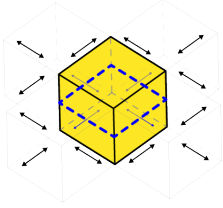
- Since the concentration outside (c_{out}) the spore with a volume V and a surface A diffuses much faster than inside (c_{in}), $c_{\text{out}} \approx \text{const}$.
- Then, an analytical solution is:

$$c_{\text{in}}(t) = c_{\text{out}} - \Delta c(0) e^{-t/\tau}, \tag{8}$$

where τ is a decay constant:

$$\tau = \frac{V}{AD_m} \tag{9}$$

- Only 2D communication in the numerical simulation $\rightarrow A$ is actually the **circumference** of the cross section!



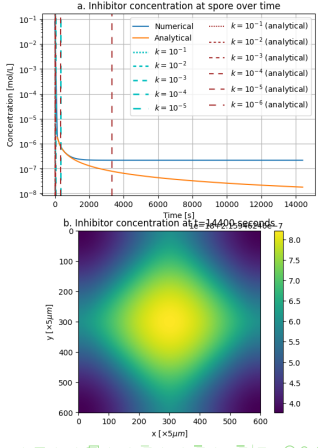
OUTLINE



RESULTS

Experiment 1

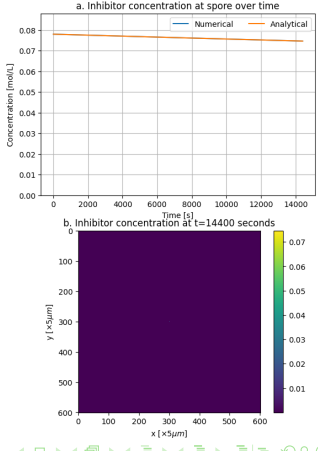
- Results after 4 hours
- Numerical vs. analytical
  - Alignment of numerical and analytical solution up to $t \approx 1000$ s, then finite size starts to take effect.
  - Under the assumed c_0, the concentration in this period is low enough to make this deviation insignificant (few thousands of molecules).
- Threshold times follow a power law
- Under the given c_0 (maximum that the cell wall can fit), the results seem unrealistic $\rightarrow$ diffusion is too fast



RESULTS

Experiment 2

- Results after 4 hours
- Numerical vs. analytical
  - Good alignment of numerical and analytical solution (because medium does not saturate in the given time)
- Linear decrease in concentration
- Diffusion appears too slow, threshold of one tenth never reached



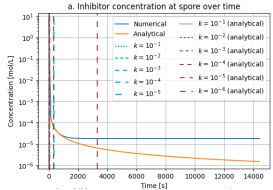
OUTLINE

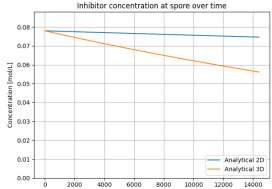


DISCUSSION

Validity of results

- The documented threshold for 1-octen-3-ol inhibition is $10\,\text{mM} = 1 \times 10^{-5}\,\text{mol/mL}$[4].
  - Diffusion through water: threshold immediately reached
  - Diffusion through membrane: threshold not reached
- The resulting concentrations can be rescaled to different c_0:
  - If the starting concentration is based on the entire spore volume V_s, then Experiment 1 would reach the 1-octen-3-ol threshold within 30 minutes.
  - More reasonable result, but unrealistic initial concentration.
  - Perhaps the initial concentration is only slightly above threshold?
- The truth lies in between:
  - Permeation through the membrane is facilitated by proteins (e.g. glucose transporters) $\rightarrow$ increased P_s?
  - Cell wall permeability modulated during transition from dormancy.
  - In three dimensions: more surface area for diffusion





DISCUSSION

Future outlook

- A spore produces relatively little 1-octen-3-ol[4, 7]. Perhaps it is not a baseline inhibitor for a single spore but only adds upon other inhibitors?
- Therefore, a single spore cannot inhibit itself beyond its base germination time with this substance, but many spores can?
- Future experiments could investigate the relationship between inhibitor concentration and spore density.

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