MSc Computational Science
joint programme UvA/VU

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

FIRST EXPERIMENTS

Presented by Boyan Mihaylov

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Supervisor: Prof. dr. Han Wösten, Utrecht University

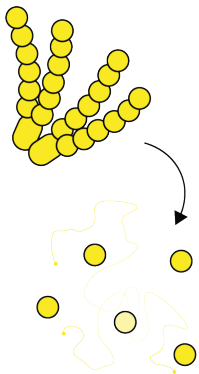
Examiner: Dr. Jaap Kaandorp, University of Amsterdam

OUTLINE


INTRODUCTION

Hypothesis

- Upon inoculation in an aqueous medium, a germination inhibitor diffuses away from the conidium.
  - Experiments have highlighted **1-octen-3-ol** as a likely candidate [2, 3, 4]
- Once its concentration at the spore falls below a certain threshold, the conidium breaks dormancy and enters a swelling phase.
  - It has been observed that in a **externally non-inhibited** spore swelling begins around **4 hours** from inoculation
- An increased density of spores drives the local inhibitor concentration high, reducing overall germination.
  - Densities above 10^5 sporesl/mL exhibit germination inhibition[4, 6]

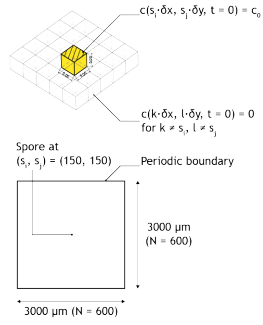


OUTLINE


GENERAL ASSUMPTIONS

Simulation space

- 2D lattice ≡ 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 extracellular medium
- Spore ≡ 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: $c(x, y, t = 0) = 0$
  - Assumed non-replenishable during process



GENERAL ASSUMPTIONS

Inhibitor

- Assumed to have known molecular properties of 1-octen-3-ol:
  - Molecular weight: $m_M = 128.21\,\mathrm{g/mol}$
  - Density: $\rho = 0.837\,\mathrm{g/mL}$
- Assumed to have similar diffusion properties as glucose:
  - Diffusion constant in water at temperature $T = 293.15K$: $D = 600\,\mu\mathrm{m}^2/\mathrm{s}$ [5] (in agar only 5% lower[12]) (i.e. a molecule wanders on average $\langle x \rangle = \sqrt{6 \cdot D} = 60\,\mu\mathrm{m}$ per second)
  - Permeation constant in an artificial lipid bilayer: $P_s = 1.9 \times 10^{-6}\,\mu\mathrm{m/s}$ (average from [7] and [1], close to [11])
  - Octanol-water partition coefficient: $K \approx 10^{-3}$
- The documented threshold for 1-octen-3-ol inhibition is $10\,\mathrm{mM} = 0.01\,\mathrm{mol/L}$[8].
- Once below this level, it is assumed swelling begins immediately

Figure: 1-octen-3-ol

Figure: D-Glucose

OUTLINE

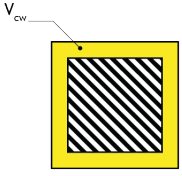
EXPERIMENT 1

Inhibitor 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.
  - Cell wall thickness: $d \approx 130$ nm (*A. fumigatus*)[10]
  - Total cell wall volume: $V_{\text{cw}} \approx 6 \cdot 25\,\mu\text{m}^2 \cdot 0.13\,\mu\text{m} = 19.5\,\mu\text{m}^3$
  - The maximum possible concentration is:

$$c_{\text{cw}} = \frac{\rho V_{\text{cw}}}{m_M V_s} = 6.5\,\text{mol/mL} \cdot 0.156 = 1.014\,\text{mol/mL} \quad (1)$$

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



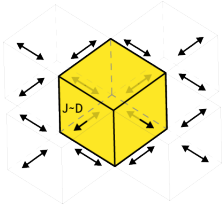
EXPERIMENT 1

Diffusion assumptions

- Diffusion is simulated using the diffusion equation (Fick's Law):

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

- Simulation run for $t_{\max} = 4\,\text{h} = 14\,400\,\text{s}$
- The times for reaching threshold concentrations of $c_t = kc_0$ ($k = \{10^{-1}, 10^{-2}, ..., 10^{-6}\}$) are recorded.
- To prevent numerical error, the concentration in the simulations is normalised by a factor $\lambda = \frac{1}{c_0}$.



EXPERIMENT 1

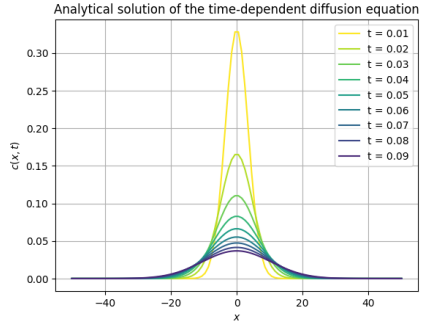
Analytical verification

- 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[9]:

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

- At the spore site, this equation becomes:

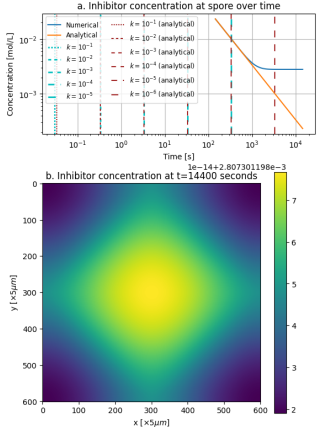
$$c(x_0, y_0, t) = \frac{(V_s c_0)^{2/3}}{4\pi Dt} \quad (4)$$



EXPERIMENT 1

Results

- 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), threshold of 10 mM reached within a few minutes
- Diffusion is not a limiting factor



OUTLINE

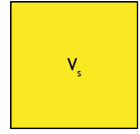
EXPERIMENT 2

Inhibitor assumptions

- All the inhibitor is contained within the cell wall volume, separated from the medium by a membrane having the properties of an artificial lipid bilayer.
- Maximum concentration limit
  - Under homogeneous distribution, standard temperature and pressure:

$$c_{\max} = \frac{\rho}{m_M} = \frac{0.837\,\mathrm{g/mL}}{128.21\,\mathrm{g/mol}} \approx 6.5\,\mathrm{mol/mL} \qquad (5)$$

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



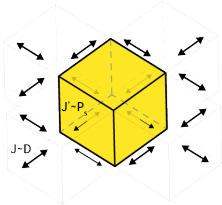
EXPERIMENT 2

Diffusion assumptions

- Diffusion through the medium as before
- Diffusion at the interface follows the flux equation

$$J' = -P_s \Delta c \tag{6}$$

  where J' is the flux through the cell wall and Δc is the concentration difference across the membrane.
- Simulation run for $t_{\max} = 4\,\text{h} = 14\,400\,\text{s}$
- The times for reaching threshold concentrations of $c_t = kc_0$ ($k = \{10^{-1}, 10^{-2}, ..., 10^{-6}\}$) are recorded.



EXPERIMENT 2

Analytical verification

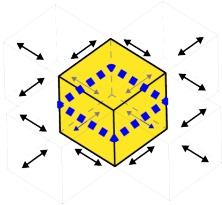
- Since the concentration outside (c_{out}) the spore with a volume V and a surface A diffuses much faster than inside (c_{in}), $c_{\text{out}} \approx \text{const}$.
- Then, an analytical solution is:

$$c_{\text{in}}(t) = c_{\text{out}} - \Delta c(0) e^{-t/\tau}, \tag{7}$$

where τ is a decay constant:

$$\tau = \frac{V}{AP_s} \tag{8}$$

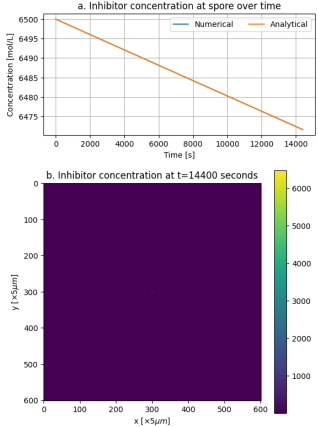
- Only 2D communication in the numerical simulation $\rightarrow A$ is actually the **circumference** of the cross section $\times 1\ \mu$m!



EXPERIMENT 2

Results

- Results after 4 hours
- Numerical vs. analytical
  - Good alignment of numerical and analytical solution (because medium does not saturate in the given time)
- Almost linear decrease in concentration
- Threshold of 10 mM never reached, neither is one tenth of the initial concentration



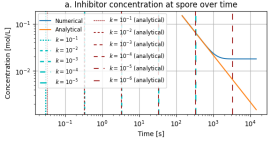
OUTLINE

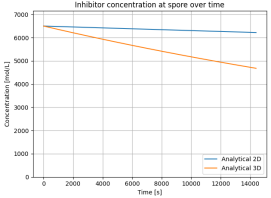


DISCUSSION

Validity of results

- Known threshold for 1-octen-3-ol inhibition (10 mM)
  - **Experiment 1:** threshold reached within minutes
  - **Experiment 2:** threshold not reached within 4 h
- The resulting concentrations can be rescaled to different c_0:
  - **Experiment 1:** If the starting concentration is based on the entire spore volume V_s, then 1-octen-3-ol threshold reached within 30 minutes. More reasonable result, but unrealistic c_0 and permeability assumptions.
  - **Experiment 2:** If c_0 is only slightly above threshold, it can be reached within 4 h.
- The truth lies in between:
  - Permeation of cell wall is between that of membrane and water
  - If permeating through the membrane, 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

Tuning the permeation coefficient

- The analytical solution can be rewritten for P_s:

$$P_s = \frac{V_s}{tA} \ln\left(\frac{\Delta c(0)}{\Delta c(t)}\right) \tag{9}$$

- Assuming the inhibitor stops being effective below $c_t = 10\,\text{mM}$
- Keeping the assumptions about c_0, d, K, V_s and A_s
- A hypothetical permeation constant P_s can be computed for a diffusion of 4 hours
- This results in $P_s = 0.001\,174\,\mu\text{m/s}$
- Remains to be interpreted in terms of physical properties

DISCUSSION

Future outlook

- Focus on 3D systems
- Investigating the relationship between inhibitor concentration and spore density
- Investigating effects on the outward flux when the medium is locally saturated

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