

MODELLING DIFFUSIVE SIGNALLING IN ASPERGILLUS SPP. GERMINATION INHIBITION

LITERATURE REVIEW AND FIRST EXPERIMENTS

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OUTLINE



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The *Aspergillus* genus

Hypothesis

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INTRODUCTION



The *Aspergillus* genus

- » Well studied species of fungi with relevance as **molecular factories, food spoilers and pathogens**.
- » Their main method of reproduction is through asexual spores (**conidia**).
- » This study focusses on *A. niger*, but a lot of the phenomena apply to *A. fumigatus*, *A. flavus*, *A. oryzae*, *A. nidulans*, *A. terreus* and others.

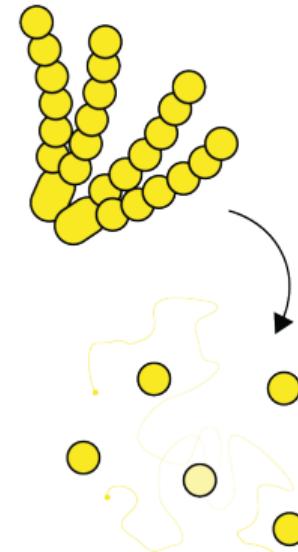


INTRODUCTION



Hypothesis

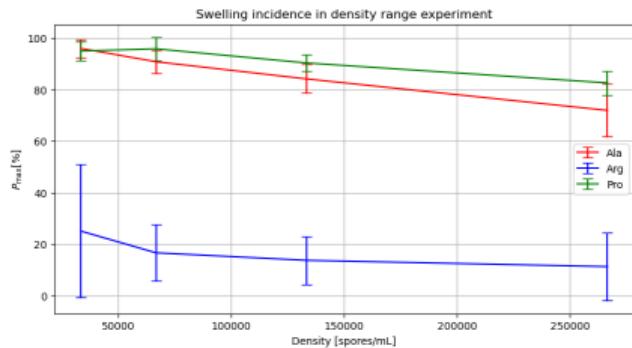
- » Upon inoculation in an aqueous medium, a germination inhibitor diffuses away from the conidium.
 - Experiments have highlighted **1-octen-3-ol** as a likely candidate [3, 4, 5]
 - Unknown heat-labile peptides may also act as inhibitors [9]
- » Once its concentration at the spore falls below a certain threshold, the conidium breaks dormancy and enters a swelling phase.
 - It has been observed that in an **externally non-inhibited** spore swelling begins after about **4 hours** from inoculation



INTRODUCTION

Hypothesis

- » An increased density of spores drives the local inhibitor concentration high, reducing germination probability.
 - Densities above 10^5 spores/mL exhibit germination inhibition (approx. 20% less spores germinate) [5, 7]
- » This could be due to a reduced concentration gradient between the spore and the medium.



INTRODUCTION

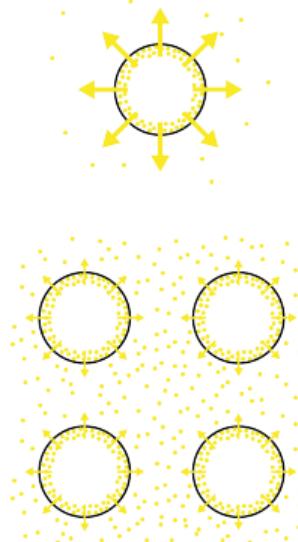
Experiment overview

» Single-spore experiments

- **Goal:** estimate the properties of the inhibitor based on its diffusion time
 - use knowledge of existing molecules;
 - fit sensible parameters.

» Multi-spore experiments

- **Goal:** given a coherent rate of inhibitor release, replicate increased inhibition dependent on spore density.
 - is it only the general density that matters?
 - or also the local clustering due to spore aggregation?



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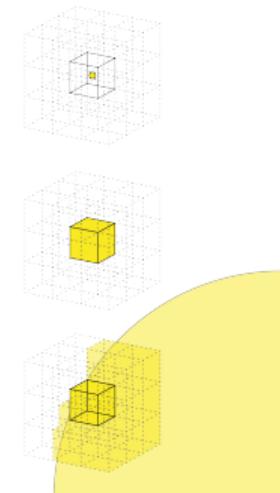
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MODEL ASSUMPTIONS



General approach

- » The experiments consist of:
 - time-dependent **numerical solutions** of the diffusion equation over a discrete lattice
 - **analytical solutions** of the concentration at the spore (when applicable and easy to derive) - used for **verification** and **calibration** of the numerical scheme
- » The consistency of the numerical solutions over several **discretisation scales** is tested:
 - Spore scale (mid-resolution)
 - Super-spore scale (low resolution)
 - Cell wall scale (high resolution)
- » So far, most of the experiments carried out are at the **spore scale**.



MODEL ASSUMPTIONS



Mid-resolution simulation space

» Discrete lattice

- N lattice subdivisions
- Discrete blocks of size $\delta x = \delta y = \delta z = 5 \mu\text{m}$ (1 spore diameter)
- Lattice side length of $L = N\delta x$
- Assuming water as extracellular medium

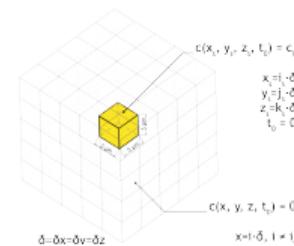
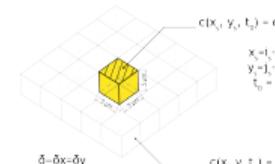
» Spore \equiv single block in the centre of the lattice, inhibitor source

- Volume $V_s = 125 \mu\text{m}^3$
- Surface area $A_s = 150 \mu\text{m}^2$

» 2D lattice \equiv thin layer of medium between 2 impermeable sheets (e.g. water film)

- Top and bottom side of spore are sealed

» 3D lattice: more natural scenario, but heavier for computation



MODEL ASSUMPTIONS



Inhibitor properties

- » Assumed to have known molecular properties of 1-octen-3-ol:

- Molecular weight: $m_M^{\text{oct}} = 128.21 \text{ g/mol}$
- Density: $\rho^{\text{oct}} = 0.837 \text{ g/mL}$
- Octanol-water partition coefficient: $K^{\text{oct}} \approx 316.23$



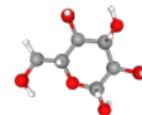
- » Assumed to have similar diffusion properties as glucose:

- Diffusion constant in water at temperature $T = 293.15 \text{ K}$:
 $D^{\text{glu/w}} = 6 \times 10^{-6} \text{ cm}^2/\text{s}$ [6] (in agar only 5% lower [11])
- Diffusion constant in a cellulose film: $D \approx 1.7 \times 10^{-9} \text{ cm}^2/\text{s}$ [2]
- Permeation constant in an artificial lipid bilayer:
 $P_s^{\text{glu/lip}} = 1.9 \times 10^{-10} \text{ cm/s}$ (avg from [8] and [1])

Figure: 1-octen-3-ol

- » The documented threshold for 1-octen-3-ol inhibition is

$$10 \text{ mM} = 0.01 \text{ mol/L}$$
 [9].



- » Once below this level, it is assumed **swelling begins immediately**

Figure: D-Glucose

MODEL ASSUMPTIONS



Inhibitor concentration

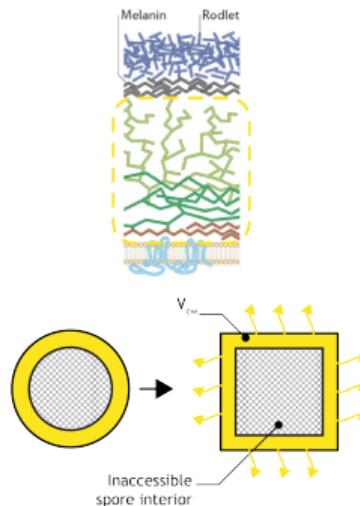
- » At $t = 0$, all the inhibitor is contained in the cell wall, behind a barrier with a permeation coefficient P_s

- Cell wall thickness: $d_{cw} \approx 130 \text{ nm}$ (*A. fumigatus*) [10]
- Total cell wall volume: $V_{cw} \approx 6 \cdot 25 \mu\text{m}^2 \cdot 0.13 \mu\text{m} = 19.5 \mu\text{m}^3$
- Under homogeneous distribution, standard temperature and pressure, 1-octen-3-ol has a concentration of

$$c_{\max} = \frac{\rho^{\text{oct}}}{m_M^{\text{oct}}} = \frac{0.837 \text{ g/mL}}{128.21 \text{ g/mol}} \approx 6.52 \text{ mol/L} \quad (1)$$

- Given the **fraction of the cell wall volume occupying the entire cell volume**, the maximum possible concentration is:

$$c_{cw} = \frac{\rho^{\text{oct}} V_{cw}}{m_M^{\text{oct}} V_s} = 6.52 \text{ mol/L} \cdot 0.156 = 1.018 \text{ mol/L} \quad (2)$$



MODEL ASSUMPTIONS



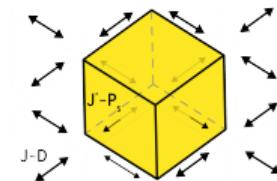
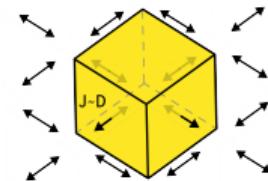
Material data

Property	Inhibitor			Medium	Barrier			Initial inhibitor environment
	1-octen-3-ol	glucose (analogue)	heat-labile peptide	water	cell wall outer layers	lipid bilayer (analogue)	cellulose (analogue)	cell wall inner layers
					rodlet layer (RodA)	melanin		chitin glucans
								α -glucans β -glucans
General	less polar (1 OH-group)	more polar (many OH-groups)	larger molecule		amyloid fibre network; permeability depends on pore size; 10 nm thick	cross-linked to chitin and glucans		
Molecular mass	128.21 g/mol	180.16 g/mol	1.0×10^4 g/mol					504.4 g/mol
Density	0.837 g/mL	1.54 g/mL	1.45 g/mL					1.37 g/mL
Viscosity				0.89 mPa · s				
Diffusion constant	6.22×10^{-6} cm ² /s (water)	6×10^{-6} cm ² /s (water)	1.75×10^{-6} cm ² /s (water)					
		1.7×10^{-7} cm ² /s (cellulose)						
Octanol-water partition coefficient		316.23 (log K = 2.5)	5×10^{-4} (log K = -3.3)					
Permeation constant		1.9×10^{-10} cm/s (lipid bilayer)						
		8.52×10^{-5} cm/s (cellulose)						
Initial concentration in spore	1.5×10^{-5} M		0.25 μ M - 25 μ M					

MODEL ASSUMPTIONS

Inhibitor diffusion

- » Initial concentrations on lattice $c(\vec{x}, t = 0)$:
 - At spore: $c(\vec{x}_s, t = 0) = c_0 \equiv c_{cw}$
 - Elsewhere: $c(\vec{x}, t = 0) = 0$
 - Assumed non-replenishable during process
- » Two modes of diffusion:
 - **direct diffusion** at the interface between spore and medium
 - **slow release** through a semi-permeable barrier



MODEL ASSUMPTIONS

Analytical verification

- » Direct diffusion is modelled by a delta pulse at the spore node, leading to the solution:

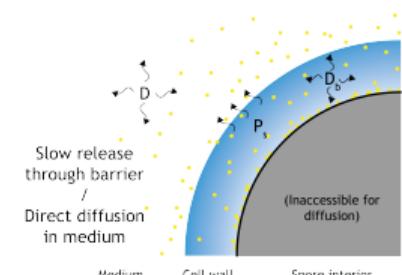
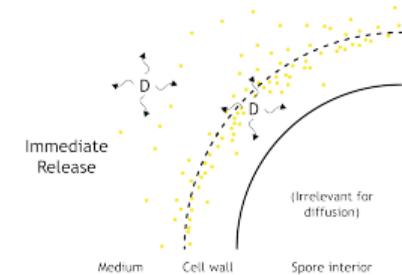
$$c(x, y, z, t) = \frac{V_s c_0}{(4\pi D t)^{3/2}} \exp\left(-\frac{|\vec{r}|^2}{4Dt}\right) \quad (3)$$

- » Slow release leads to a concentration at the spore

$$c_{in}(t) = c_{out} - \Delta c(0) e^{-t/\tau}, \quad (4)$$

where $c_{out} \approx 0$ (diffuses fast) and τ is a decay constant:

$$\tau = \frac{V}{AP_s} \quad (5)$$



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SINGLE-SPORE EXPERIMENTS



Overview

- » Lattice size of $L = 256 \times 5 \mu\text{m} = 1280 \mu\text{m}$
- » Initial concentration of $c_0 = 1.018 \text{ M}$
- » $t_{\max} = 4 \text{ h}$
- » List of experiments
 1. Superficial release with D of **glucose in water** (2D and 3D)
 2. Slow release with P_s of **glucose through a lipid bilayer membrane** (2D and 3D)
 3. Slow release with P_s of **glucose through a CNF film** (2D and 3D)
 4. Slow release with analytically estimated P_s (2D and 3D)
- » Fitted permeation constant is $P_s = 2.675 \times 10^{-8} \text{ cm/s}$, computed by:

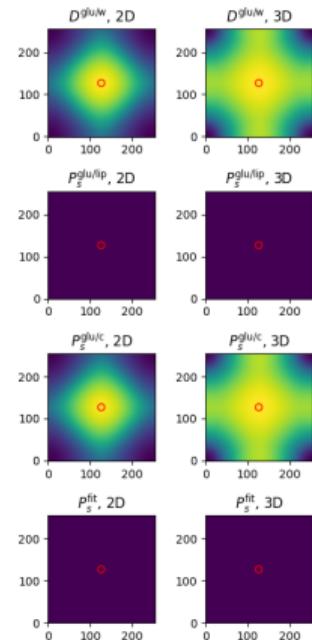
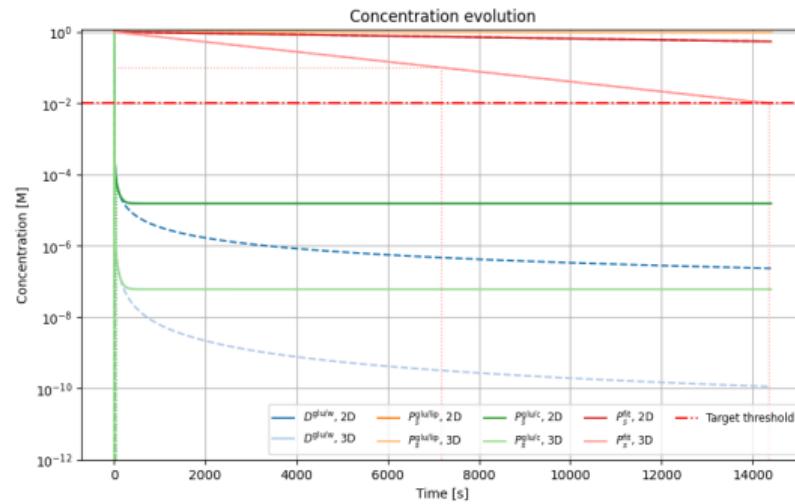
$$P_s = \frac{V_s}{tA} \ln \left(\frac{\Delta c(0)}{\Delta c(t)} \right) \quad (6)$$

SINGLE-SPORE EXPERIMENTS



Results

- »
 - Diffusion in medium is not a limiting factor.
 - Permeation is faster than glucose through a lipid bilayer
 - Permeation is slower than glucose through a cellulose film.



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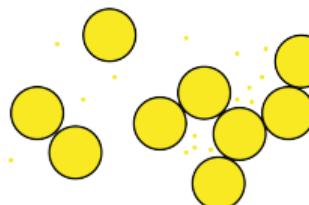
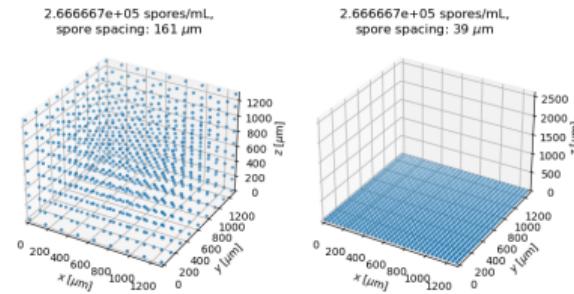
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MULTI-SPORE EXPERIMENTS

Overview

» Three scenarios are tested:

- Spores are **regularly distributed** in a given volume.
- Spores compact at **the bottom of the medium** due to gravity.
- **Clusters of spores** form isolated diffusion traps for the inhibitor.

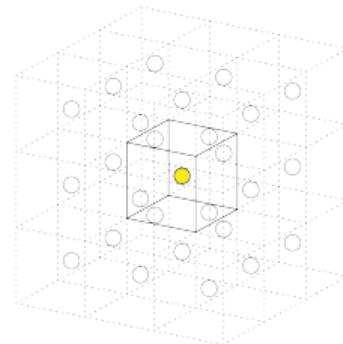


MULTI-SPORE EXPERIMENTS



Uniform spore grid: assumptions

- » Spores assumed to be arranged in a regular three-dimensional grid.
- » Assuming a large inoculum, in which the central part exhibits characteristic germination behaviour and the boundaries are irrelevant.
- » Therefore, the grid can be considered infinite.
- » Since the spores **repeat periodically**, it suffices to simulate a **single spore** in a **volume of variable size L** and a triply periodic boundary.
- » The size L of the simulation volume corresponds to the spore-to-spore distance.

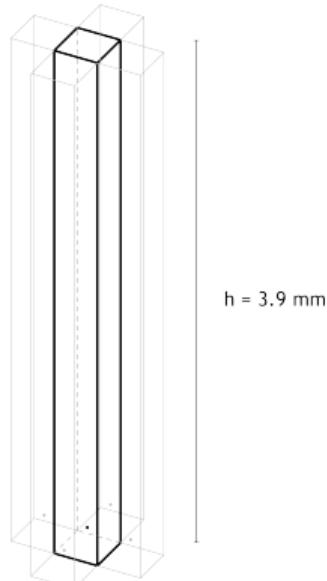


MULTI-SPORE EXPERIMENTS



Bottom spore grid: assumptions

- » Spores sink to the bottom of the medium due to gravity.
- » Typically[7] it takes 1 h for spores to sink to the bottom of a well ($150\mu\text{L}$ medium $\rightarrow h = 3.9 \text{ mm}$ of medium).
- » It is assumed that while spores settle the inhibitor diffuses homogeneously like in the 3D grid scenario, so the simulations of the 2D lattice bottom array start with a concentration $c_0 = c(t = 3600\text{s})$.
- » The lattice is periodic along the x and the y dimensions but has a Neumann boundary condition at $z = 0$ and $z = h$, (zero derivative of $c(x, y, z)$ normal to the boundary).
- » Spore densities from the first multi-spore experiment (5000, 10 000, 20 000, and 40 000 spores per $150\mu\text{L}$)

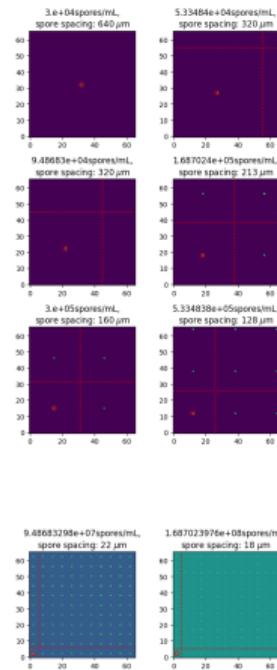
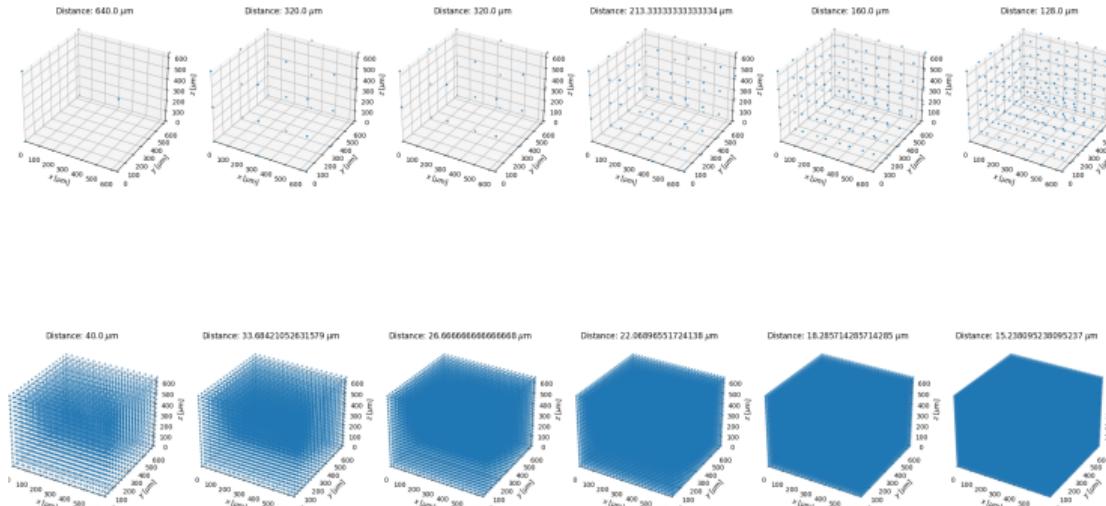


MULTI-SPORE EXPERIMENTS



Uniform spore grid: density vs. inter-spore distance

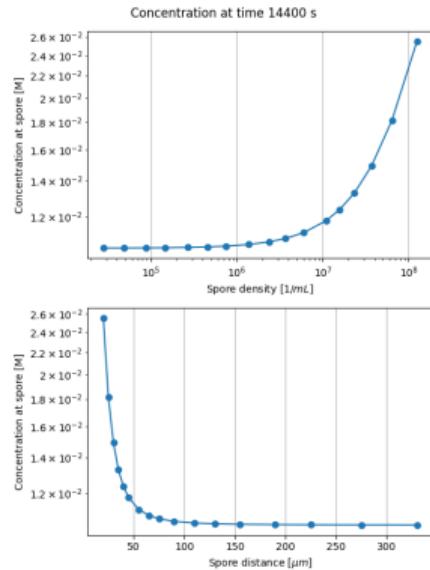
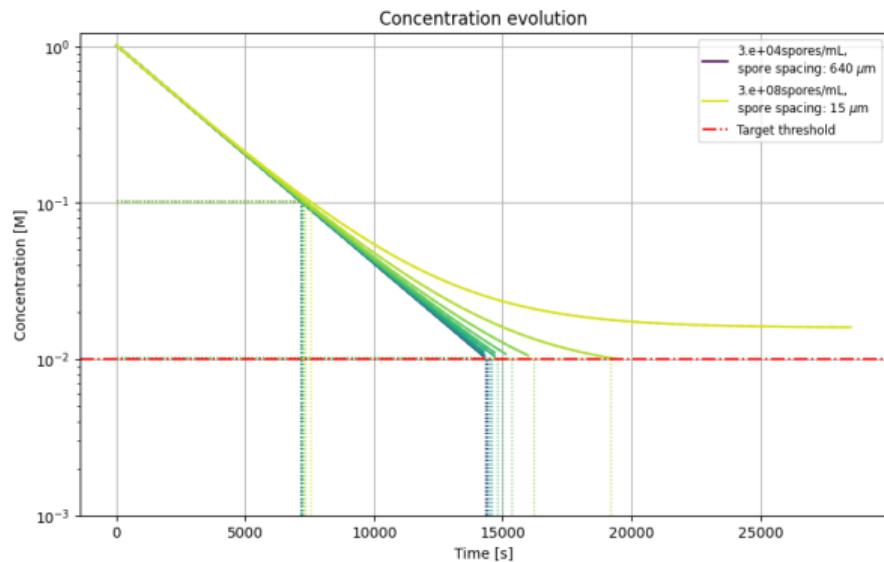
- » Densities from 3×10^4 to 3×10^8 spores/mL are simulated.



MULTI-SPORE EXPERIMENTS



Uniform spore grid: results

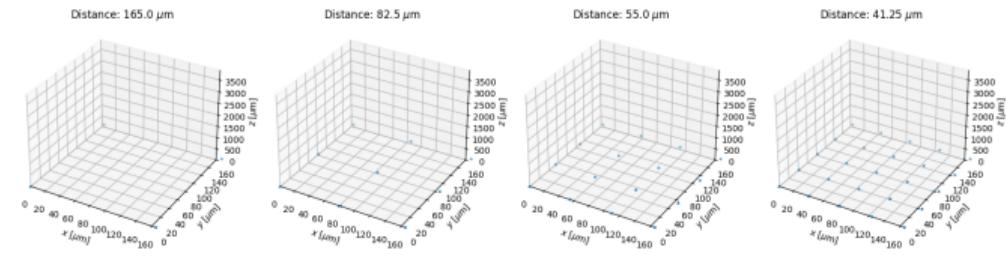


MULTI-SPORE EXPERIMENTS



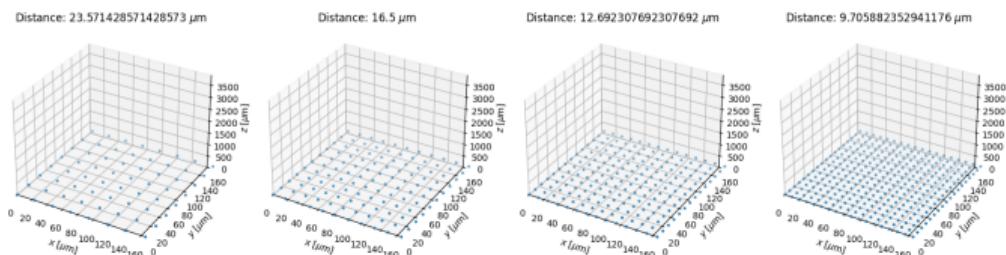
Bottom spore grid: density vs. inter-spore distance

- » Densities from 3×10^4 to 3×10^6 spores/mL are simulated.



3.e+04spores/mL;
spore spacing: 165 μm

5.33484e+04spores/mL;
spore spacing: 82 μm



9.486833e+04spores/mL;
spore spacing: 55 μm

1.687024e+05spores/mL;
spore spacing: 41 μm

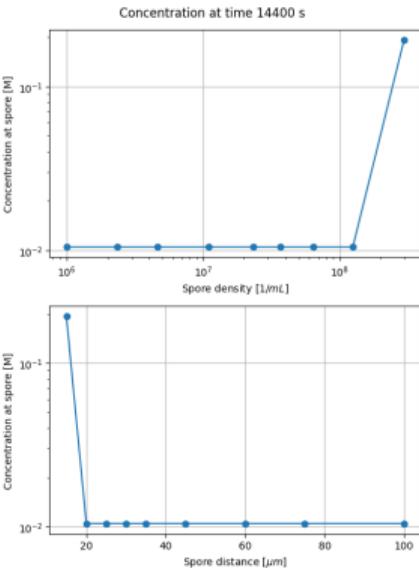
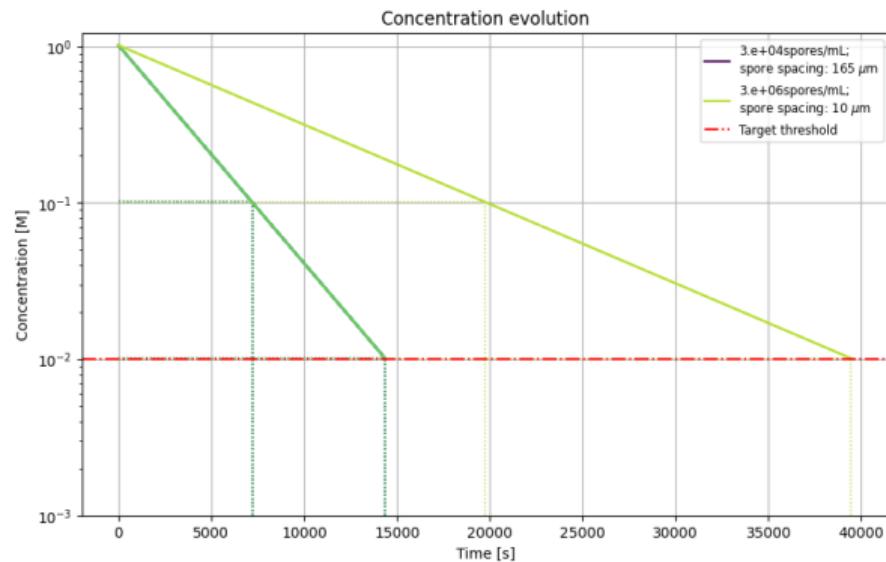
9.486833e+05spores/mL;
spore spacing: 16 μm

1.687024e+06spores/mL;
spore spacing: 13 μm

MULTI-SPORE EXPERIMENTS



Bottom spore grid: results

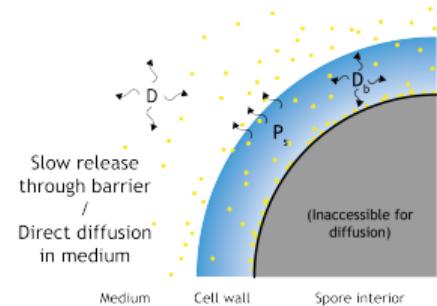


MULTI-SPORE EXPERIMENTS



Discussion: analytical verification

- » Numerical simulations are **slow** and lack **functional relationships**.
- » Analytical derivations have previously helped **verify** and **replicate** numerical schemes.
- » But these have handled **direct diffusion** and **slow release** separately.
- » In dense spore systems, **both regimes matter** (c_{out} cannot be assumed constant). But how to combine the formulas?



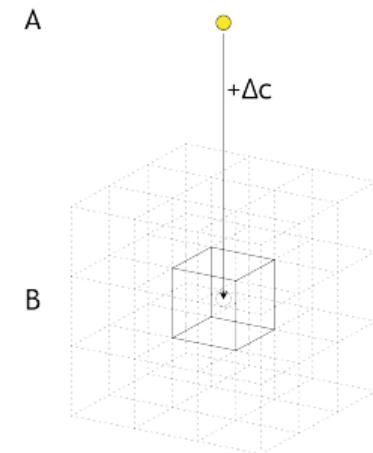
MULTI-SPORE EXPERIMENTS

Discussion: analytical verification

- » If the spore is viewed as much smaller than the lattice resolution, it will be a **negligible obstacle** for diffusion through the medium.
- » This way it can be considered **only as a source** (A) adding new concentration to the medium space (B).
- » An analytical solution for a point with position \vec{r} among M spore sources is:

$$c(\vec{r}, t) = \frac{AVP_s c_0^2}{(4\pi D)^{3/2}} e^{-\frac{t}{\tau}} \sum_{i=0}^M \int_0^t t'^{-3/2} \exp\left(-\frac{t'}{\tau} - \frac{|\vec{r}_i|^2}{4Dt'}\right) dt'. \quad (7)$$

- » This term potentially simplifies under a periodic spore assumption.



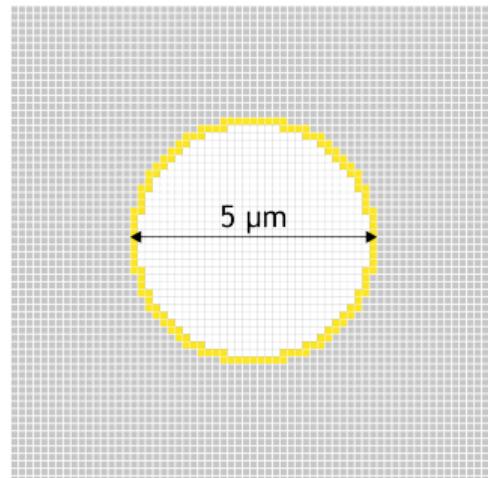
MULTI-SPORE EXPERIMENTS



Discussion: fine-graining

- » At the other extreme, taking a **fine-grain model** allows the observation of **diffusion traps in spore clusters**.
- » This time, the **cell wall thickness** becomes the scaling unit.
- » Cell wall nodes allow diffusion between each other and with the medium, but not with the spore interior.

$\delta = 0.15 \mu\text{m}$

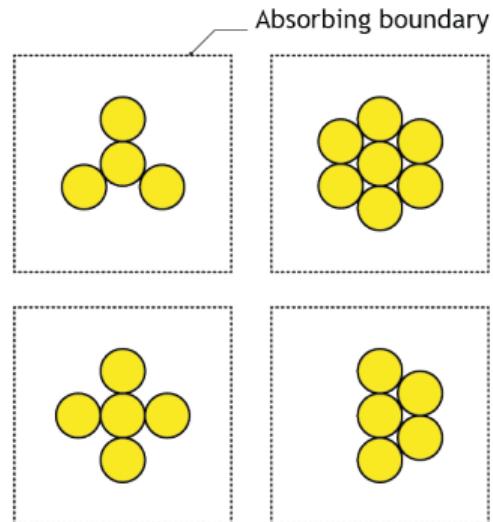


MULTI-SPORE EXPERIMENTS



Discussion: fine-graining

- » A measure of exposure to the medium can be established, e.g. based on the **number of directly adjacent spores** or their **projected volumes** onto the measured spore.
- » Local diffusion effects can be examined
 - Are there regions where inhibitor molecules linger?
- » The interplay between **spore exposure** and **cluster distances** can be studied with respect to **inhibitor diffusion lag**.



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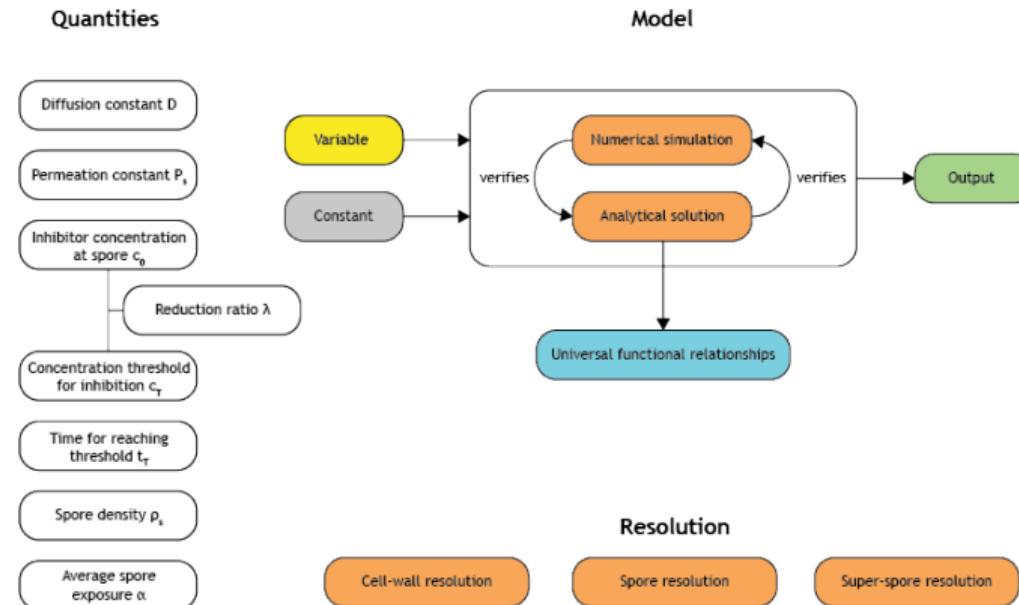
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SUMMARY



Diffusive signalling model

- » Objective: find functional relationships between the diffusion parameters.

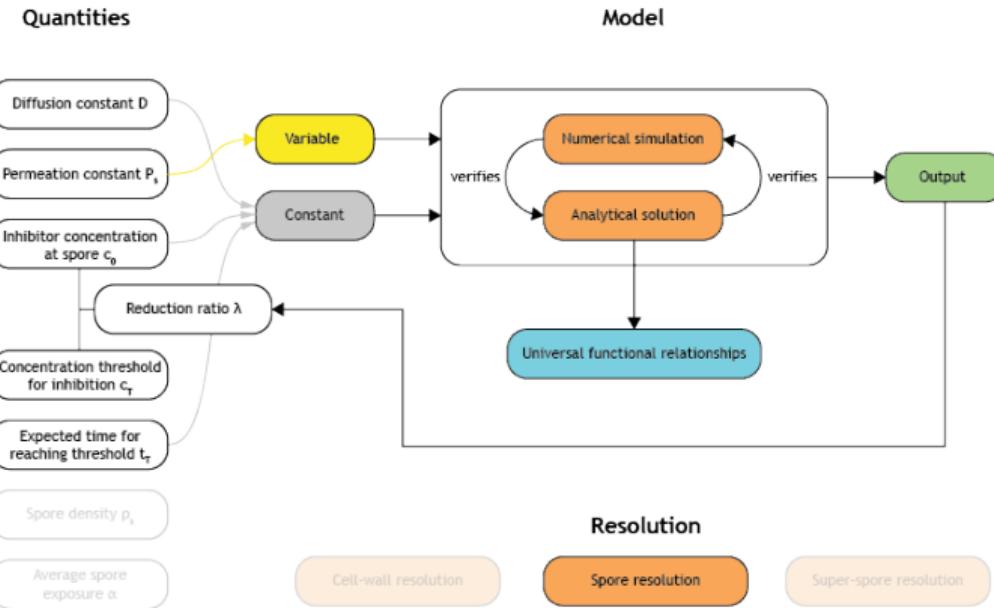


SUMMARY



Diffusive signalling model

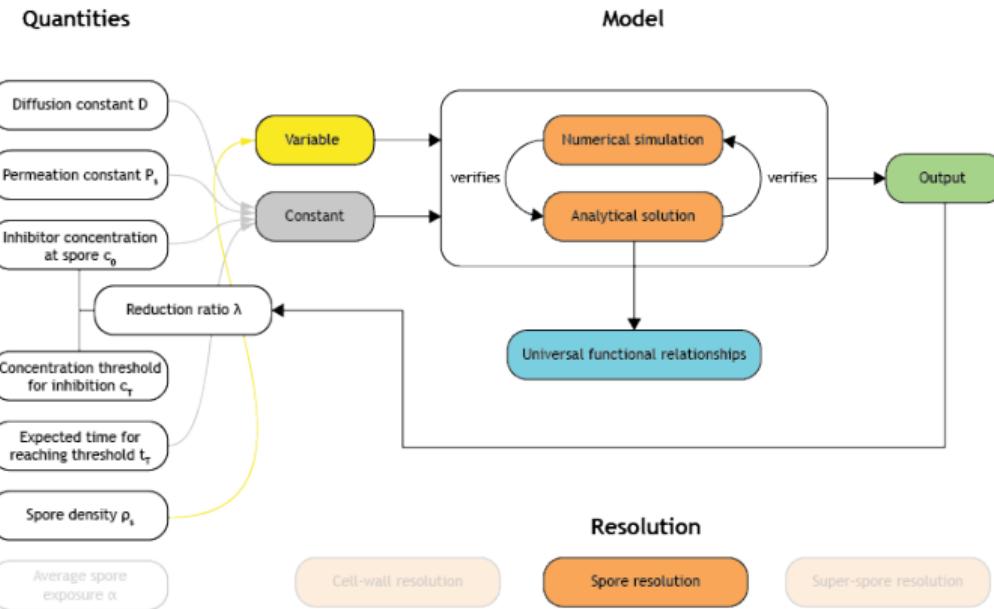
» Single-spore experiments: How do different (known) permeation coefficients affect the inhibitor concentration at $t = 4 \text{ h}$?



SUMMARY

Diffusive signalling model

- » **Multi-spore experiments:** Given a fixed permeation constant, how does increased spore density affect the inhibitor concentration at $t = 4 \text{ h}$?

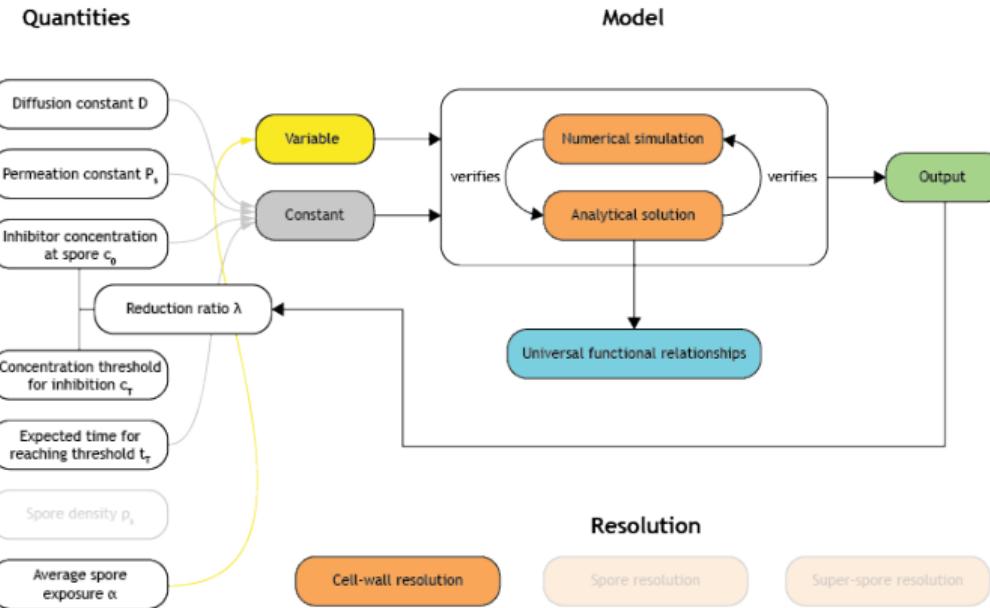


SUMMARY



Diffusive signalling model

» [Future experiment]
Spore cluster experiments: How does the exposure of the spore influence the slow release?

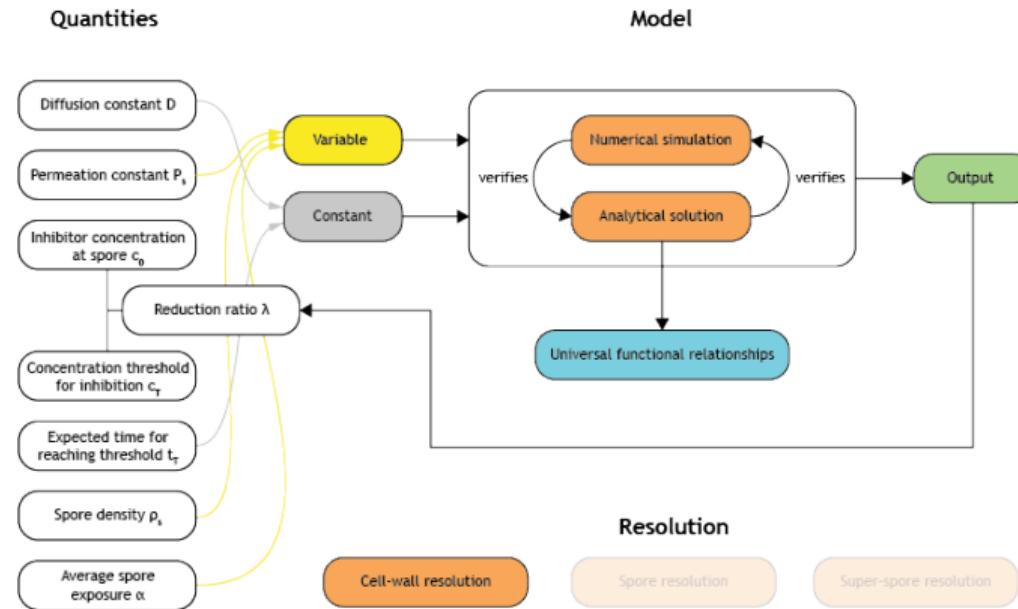


SUMMARY

Diffusive signalling model

» [Future experiment]

Parameter fitting:
Which combinations
of permeation
constant, spore
density and spore
exposure (within
reasonable ranges)
result in 20% less
germinated spores at
 $t = 4 \text{ h}$?



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