MSc Computational Science
joint programme UvA/VU

MODELLING DIFFUSIVE SIGNALLING IN *ASPERGILLUS* SPP. GERMINATION INHIBITION

INTERMEDIATE PRESENTATION - MAY

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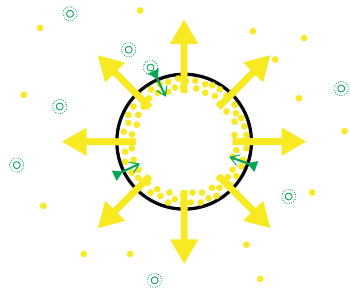
OUTLINE

INTRODUCTION

Considerations for parameter fitting

- There are **two germination-determining mechanisms**:
  - auto-inhibition
  - carbon sensing
- We have a model proposal for the **auto-inhibition**.
- But how does **carbon sensing** enter the picture?
  - Do carbon-source molecules permeate slowly or equilibrate quickly across the rodlet layer?
  - How do inhibition and induction interact?
- **Parameter fitting on germination rate data** requires the germination probability to be expressed in terms of both processes.



INTRODUCTION

Inducer assumptions

- Carbon sources (glucose, amino acids) are **germination inducers** that bind to receptors in the cell wall.
- Experiments have shown that pyrene [7] and 1-octen-3-ol [4] can easily pass through the hydrophobin layer, but these are **much more hydrophobic** molecules than glucose (positive $\log K$).

carbon source	$\log K$
glucose	-3.2
arginine	-3.88
proline	-2.55
alanine	-0.6

Table: Octanol-water partition coefficient of different carbon sources.

INTRODUCTION

Inducer assumptions

- Carbon signals are received by **G-proteins** and **RasA** proteins, which are potentially linked to the activation of the cAMP-PKA pathway upon break of dormancy.
- These proteins are usually membrane-bound but can extend into the inner cell wall polysaccharide layer.
- If the rodlet layer is like a **dense membrane**, carbon sources need to **partition into it** - then the access to receptor proteins would be **slowed down**.
- If the rodlet layer is like a **porous mesh**, then glucose should be able to permeate about **as easy as 1-octen-3-ol**.

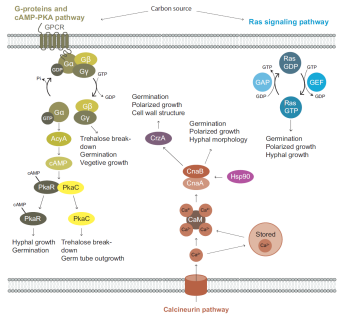


Figure: Carbon signalling pathways [1]

INTRODUCTION

Inducer permeation

- **Does a dense membrane model even allow for carbon signalling?**
- The outside inducer concentration $c_{\mathrm{cs}}^{\mathrm{out}}$ can be considered constant.
- The initial concentration around the carbon receptors can be considered zero.
- The concentration at the carbon receptors follows the permeation law

$$c_{\mathrm{cs}}(t) = c_{\mathrm{cs}}^{\mathrm{out}}(1 - e^{-t/\tau_{\mathrm{cs}}}), \tag{1}$$

where

$$\tau_{\mathrm{cs}} = \frac{V_{\mathrm{cw}}}{P_{\mathrm{cs}} A_s}, \quad V_{\mathrm{cw}} = 0.24\,\frac{4}{3}\pi[(\xi - d_{\mathrm{hp}})^3 - (\xi - d_{\mathrm{hp}} - \kappa)^3], \tag{2}$$

which considers 24% porosity of the cell wall volume V_{cw}, based on *Lycoperdon pyriforme* studies [6], and a randomly varying polysaccharide layer thickness κ.

- Using the P_{cs} of glucose through a lipid bilayer and an external carbon source concentration of 10 mM [3], one arrives at $c_{\mathrm{cs}}(4\,\mathrm{h}) \approx 3.7\,\mathrm{M}$ inside the cell wall, which is **significant**, but still **unequilibrated**.

- Since the carbon source binds to receptor proteins, the inducer concentration may affect the germination signal via **Michaelis-Menten kinetics**:

$$s(c_{\mathrm{cs}}) = s_{\max} \frac{c_{\mathrm{cs}}}{K_{\mathrm{cs}} + c_{\mathrm{cs}}}, \tag{3}$$

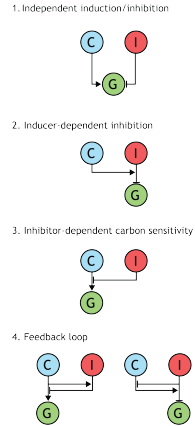
where $s_{\max}$ is a signal saturation level and K_{cs} is a half-saturation constant.

OUTLINE

INDUCER-INHIBITOR INTERACTIONS

Overview

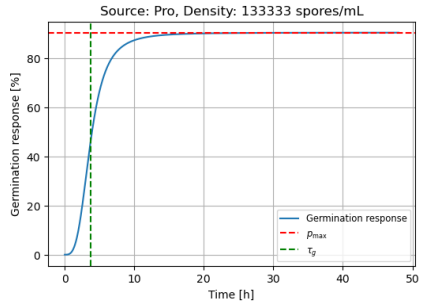
- The following types of interactions are possible:
  - Independent induction/inhibition
  - Inducer-modulated inhibition
    - Inducer shifts inhibition threshold (1)
    - Inducer increases inhibitor permeation (2)
  - Inhibitor-modulated carbon sensitivity
    - Inhibitor shifts signal threshold (3)
    - Inhibitor attenuates signal strength (4)
  - Feedback loop
    - Combinations of the above.
    - Too complex to be handled in the present study.



INDUCER-INHIBITOR INTERACTIONS

Overview

- The models are fitted to the *A. niger* germination data [3] using an adaptive differential evolution algorithm [8].
- The data contains the Dantigny model [2] parameters $P_{\max}$, τ_g and ν, which allow the reconstruction of **time-dependent germination rates**.
- Thus, the model can be fitted not only to the final germination percentages, but also to **intermediate time points**.
- Since the data-points from the arginine-rich medium are noisy / do not produce a correct Dantigny model, only the **alanine and proline** data are used.



INDUCER-INHIBITOR INTERACTIONS

Independent induction/inhibition - **constant induction**

- In the simplest scenario, the two mechanisms can be viewed as **mutually independent**, causing germination when both conditions are fulfilled simultaneously:

$$c_{\text{in}} < c_T \quad \text{and} \quad s > s_T, \tag{4}$$

- Like the inhibition threshold c_T, dependent on the fluctuating variable γ, the induction threshold s_T can be modelled by a normally distributed random variable ω.

- The combined germination probability is

$$p = P(\beta < \gamma \cap \omega < s) = \Phi\left(\frac{s-\mu_\omega}{\sigma_\omega}\right) \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi)-\mu_\gamma}{\sigma_\gamma}\right)\right] \frac{1}{\sqrt{2\pi}\sigma_\xi} \exp\left(-\frac{(\xi-\mu_\xi)^2}{2\sigma_\xi^2}\right) d\xi. \tag{5}$$

INDUCER-INHIBITOR INTERACTIONS

Independent induction/inhibition - **constant induction**

» The model parameters are

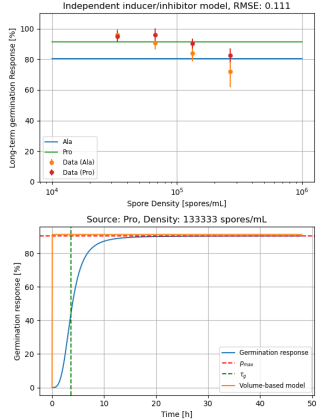
Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$	Inhibitor permeation constant	$\in [10^{-5}, 10^{-2}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-5}, 10^{-1}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-12}, 10^{-2}]$	–
s^*	inducing signal strength	$\in [10^{-12}, 1 \times 10^{2}]$	–
μ_ω	induction threshold mean	$\in [10^{-12}, 1 \times 10^{2}]$	–
σ_ω	induction threshold standard deviation	$\in [10^{-12}, 1 \times 10^{2}]$	–

*inducer-specific

INDUCER-INHIBITOR INTERACTIONS

Independent induction/inhibition - **constant induction**

» If the inducer concentration is considered constant, the model fails to approximate the data.



INDUCER-INHIBITOR INTERACTIONS

Independent induction/inhibition - **time-dependent induction**

» If the carbon source slowly permeates the cell wall, the germination probability becomes

$$p = \int_0^\infty \int_0^\infty \Phi\left(\frac{s(\xi,\kappa) - \mu_\omega}{\sigma_\omega}\right)\left[1 - \Phi\left(\frac{\beta(\xi) - \mu_\gamma}{\sigma_\gamma}\right)\right] f_\kappa(\kappa) f_\xi(\xi)\, d\kappa\, d\xi. \tag{6}$$

» The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Independent induction/inhibition - **time-dependent induction**

» This adds several new parameters:

Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$	inhibitor permeation constant	$\in [10^{-5}, 10^{-2}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-5}, 10^{-1}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-12}, 10^{-2}]$	–
c_0^{cs}	initial inducer concentration outside the cell wall	0.01 [3]	M
d_{hp}	hydrophobin layer thickness	0.01	µm
μ_κ	polysaccharide layer thickness mean	0.2	µm
μ_κ	polysaccharide layer thickness standard deviation	0.05	µm
$s_{\max}$*	maximum inducing signal strength	$\in [1 \times 10^{-12}, 1 \times 10^{2}]$	–
K_{cs}*	inducer half-saturation constant	$\in [1 \times 10^{-10}, 1 \times 10^{3}]$	–
$P_{\text{eff}}^{\text{cs}}$*	inducer permeation constant	$\in [10^{-6}, 10^{-1}]$	µm s^{-1}
μ_ω	induction threshold mean	$\in [10^{-12}, 1 \times 10^{2}]$	–
σ_ω	induction threshold standard deviation	$\in [10^{-12}, 1 \times 10^{2}]$	–

*inducer-specific

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibition

- In the following scenarios, the germination starts solely upon depletion of the inhibitor, but the inducer **increases the rate** of depletion or **lifts the threshold** at which the inhibitor is considered depleted.
- As derived previously, the germination probability is

$$P(\beta(\xi) < \gamma) = \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi, P_{\text{eff}}) - \mu_\gamma}{\sigma_\gamma}\right)\right] \frac{1}{\sqrt{2\pi}\sigma_\xi} \exp\left(-\frac{(\xi - \mu_\xi)^2}{2\sigma_\xi^2}\right) d\xi, \tag{7}$$

where γ is a normally distributed random variable.

- At first, a **constant inducer concentration over time** is assumed for simplicity.

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibition - **constant induction**

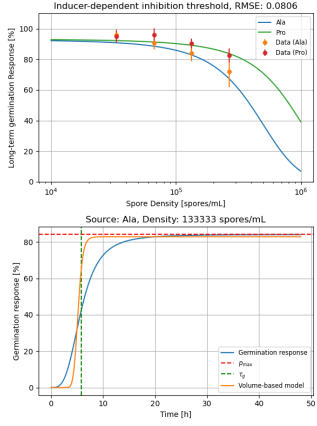
- In the case of a constant inducer concentration, all parameters from the inducer model can be **absorbed** into the variations of γ or P_{eff}.
- The remaining parameters are

Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$ *	Inhibitor permeation constant	$\in [10^{-5}, 10^{-2}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-5}, 10^{-1}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-12}, 10^{-2}]$	–

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibition threshold - **constant induction**

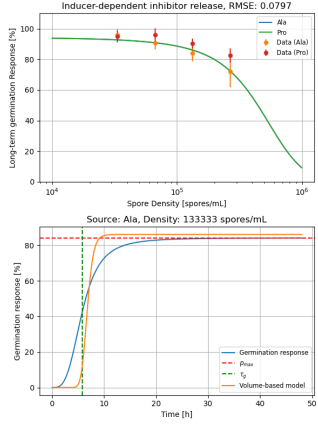
- If the inducer concentration is considered constant and **modifies the inhibition threshold** (μ_γ and σ_γ allowed to vary between Ala and Pro), there is a considerable fit.
- The onset of germination over time is still quite steep compared to the Dantigny model.



INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibitor permeation - **constant induction**

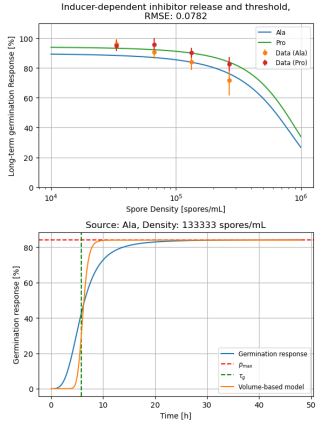
- If the inducer concentration is considered constant and **modifies the inhibitor permeation** (P_{eff} allowed to vary between Ala and Pro), there is a somewhat representative fit in the time-evolution, but not difference in germination is noticeable between Ala and Pro.
- This makes sense, since the permeability dictates the *approach* to the maximum germination, but not the saturation limit itself.
- Therefore, this scenario is less likely than the previous one.



INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibitor (combined) - **constant induction**

- If the inducer concentration is considered constant and **modifies both the inhibition threshold the inhibitor permeation** (all parameters allowed to vary between Ala and Pro), the fit is most accurate so far (both shifts in the saturation *time* and the saturation *limit* captured).
- Still, the curves deviate from the data.



INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibition - **time-dependent induction**

» If the inducer **permeates the cell wall slowly**, the parameters are

Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$	inhibitor permeation constant	$\in [10^{-5}, 10^{-1}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-6}, 10^{-1}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-20}, 10^{-1}]$	–
c_0^{cs}	initial inducer concentration outside the cell wall	0.01 [3]	M
d_{hp}	hydrophobin layer thickness	0.01	µm
μ_κ	polysaccharide layer thickness mean	0.2	µm
μ_κ	polysaccharide layer thickness standard deviation	0.05	µm
$s_{\max}{}^*$	maximum inducing signal strength	$\in [1 \times 10^{-12}, 1 \times 10^{2}]$	–
$K_{\text{cs}}{}^*$	inducer half-saturation constant	$\in [1 \times 10^{-10}, 1 \times 10^{3}]$	–
$P_{\text{eff}}^{\text{cs}}{}^*$	inducer permeation constant	$\in [10^{-12}, 10^{-2}]$	µm s^{-1}

*inducer-specific

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibition threshold - **time-dependent induction**

- The number of parameters increases, because the signal cannot be absorbed into γ or P_{eff} and must therefore be explicitly included
- For an inducer-dependent inhibition threshold, $\gamma = \gamma_0 + s$.
- This results in the probability

$$p = \int_0^\infty \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi, P_{\text{eff}}) - s(\xi, \kappa, t) - \mu_{\gamma_0}}{\sigma_{\gamma_0}} \right) \right] f_\kappa(\kappa) f_\xi(\xi) \, d\kappa \, d\xi. \tag{8}$$

- The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibitor permeation - **time-dependent induction**

- A time-dependent inducer may modulate the inhibitor release by scaling the permeation constant: $P_{\text{eff}} = sP_{\max}$
- Hence, the permeation constant is increased from zero (no release) to a maximum limit (fast release) depending on the inducing signal.
- The probability is thus

$$p = \int_0^\infty \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi, P_{\text{eff}}^{\max}, s) - \mu_{\gamma_0}}{\sigma_{\gamma_0}}\right)\right] f_\kappa(\kappa) f_\xi(\xi)\, d\kappa\, d\xi, \tag{9}$$

where

$$\beta = \phi + (1-\phi) e^{-s(\xi,\kappa,t) P_{\text{eff}}^{\max} \frac{A_s}{V_s(1-\phi)} t}. \tag{10}$$

- The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Inducer-dependent inhibitor (combined) - **time-dependent induction**

- With the two effects combined, the probability is

$$p = \int_0^\infty \int_0^\infty \left[1 - \Phi\left(\frac{\beta(\xi, P_{\max}, s) - ks(\xi, \kappa, t) - \mu_{\gamma_0}}{\sigma_{\gamma_0}}\right)\right] f_\kappa(\kappa) f_\xi(\xi)\, d\kappa\, d\xi, \tag{11}$$

- Here, an extra inducer-specific parameter k serves as a proportionality constant between the effect on the signal and the effect on the threshold.
- The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent carbon sensitivity

- Another possibility is that the **inhibitor suppresses the carbon signalling pathway**, which determines germination.
- Once the inhibition is removed through diffusion, the cAMP-PKA pathway may receive stronger triggers that can switch the germination regime on.
- The inhibitor may act on the carbon signalling pathway by
  - attenuating the signal strength,
  - shifting the signal threshold.

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent signal threshold - **constant induction**

- In the first case, the threshold is linearly shifted from a randomly fluctuating baseline ω_0 by a factor k:

$$\omega(c_{\text{in}}) = \omega_0 + kc_{\text{in}}. \tag{12}$$

- The condition for germination can then be rewritten from $s > \omega_0 + kc_{\text{in}}$ to $s - kc_{\text{in}} > \omega_0$.
- This yields the spore-specific germination probability:

$$P(\omega_0 < s - kc_{\text{in}} \mid c_{\text{in}}) = \Phi\left(\frac{s - kc_{\text{in}} - \mu_{\omega_0}}{\sigma_{\omega_0}}\right) \tag{13}$$

and the general probability

$$P(\omega < s) = \int_0^\infty \int_0^\infty \Phi\left(\frac{s - kc_{\text{in}} - \mu_{\omega_0}}{\sigma_{\omega_0}}\right) d\xi \, d\psi, \tag{14}$$

where ψ is a Gaussian-distributed random variable modelling c_0.

- The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent signal threshold - **constant induction**

» The parameters in this model are

Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$	Inhibitor permeation constant	$\in [10^{-5}, 10^{-1}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-6}, 10^{-1}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-20}, 10^{-1}]$	–
s^*	inducing signal strength	$\in [10^{-12}, 1 \times 10^{2}]$	–
k^*	inhibition susceptibility factor	$\in [10^{-12}, 1 \times 10^{2}]$	–
μ_ω	induction threshold mean	$\in [10^{-12}, 100]$	–
σ_ω	induction threshold standard deviation	$\in [10^{-12}, 100]$	–

*inducer-specific

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent signal strength - **constant induction**

- The inhibitor may attenuate the carbon signal strength by a Hill-type factor:

$$s'(c_{\text{in}}) = s \, \frac{1}{1 + \left(\frac{c_{\text{in}}}{K_I}\right)^n}, \tag{15}$$

  where K_I a half-saturation constant and n is a Hill exponent. The signal strength maximum, $s_{\max}$, can be considered constant for simplicity (the threshold absorbs the variation).

- The germination probability for a specific spore is thus

$$P(\omega < s \mid c_{\text{in}}) = \Phi\left(\frac{s'(c_{\text{in}}) - \mu_\omega}{\sigma_\omega}\right). \tag{16}$$

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent signal strength - **constant induction**

- The two random variables in c_{in} are ξ and ψ. Therefore, the full CDF is

$$P(\omega < s) = \int_0^\infty \int_0^\infty \Phi\left(\frac{s\left[1 + \left(\frac{c_{\text{in}}(\xi,\psi)}{K_I}\right)^n\right]^{-1} - \mu_\omega}{\sigma_\omega} \right) d\xi\, d\psi, \tag{17}$$

where

$$c_{\text{in}}(\xi,\psi) = \left[\phi + (1-\phi)e^{-\frac{t}{\tau(1-\phi)}}\right]\psi. \tag{18}$$

- The data fit is yet to be performed.

INDUCER-INHIBITOR INTERACTIONS

Inhibitor-dependent signal strength - **constant induction**

» The parameters in this model are

Parameter	Description	Value	Units
μ_ξ	spore radius mean	2.65 [5]	µm
σ_ξ	spore radius standard deviation	0.3 [5]	µm
$P_{\text{eff}}^{\text{inh}}$	Inhibitor permeation constant	$\in [10^{-4}, 10^{-3}]$	µm s^{-1}
μ_γ	inhibitor depletion fraction mean	$\in [10^{-20}, 10^{-4}]$	–
σ_γ	inhibitor depletion fraction standard deviation	$\in [10^{-20}, 10^{-4}]$	–
K_I*	inhibitor half-saturation constant	$\in [10^{-10}, 10^{10}]$	M
n*	Hill coefficient	$\in [1, 3]$	–
s*	inducing signal strength	$\in [10^{-12}, 10]$	–
μ_ω	induction threshold mean	$\in [10^{-12}, 1]$	–
σ_ω	induction threshold standard deviation	$\in [10^{-12}, 1]$	–

*inducer-specific

INDUCER-INHIBITOR INTERACTIONS


Inhibitor-dependent carbon sensitivity - **time-dependent induction**

- The germination probabilities for the time-dependent case in the inhibitor-modulated carbon signalling scenario are yet to be derived.
- The procedure is similar to before, adding the carbon source permeation parameters to the unknowns.

BIBLIOGRAPHY I

[1] Tim J. H. Baltussen et al. “Molecular Mechanisms of Conidial Germination in Aspergillus spp”. In: *Microbiology and Molecular Biology Reviews* 84 (2019). URL: https://api.semanticscholar.org/CorpusID:208642285.

[2] Philippe Dantigny et al. “A new model for germination of fungi.”. In: *International journal of food microbiology* 146 2 (2011), pp. 176–81. URL: https://api.semanticscholar.org/CorpusID:38620666.

[3] Maryam Ijadpanahsaravi et al. “The impact of inter- and intra-species spore density on germination of the food spoilage fungus Aspergillus niger.”. In: *International journal of food microbiology* 410 (2023), p. 110495. URL: https://api.semanticscholar.org/CorpusID:265268197.

[4] Kana Miyamoto et al. “Formation of 1-octen-3-ol from Aspergillus flavus conidia is accelerated after disruption of cells independently of Ppo oxygenases, and is not a main cause of inhibition of germination”. In: *PeerJ* 2 (2014). URL: https://api.semanticscholar.org/CorpusID:8514039.

BIBLIOGRAPHY II

[5] Henri Müller et al. “From spores to fungal pellets: A new high-throughput image analysis highlights the structural development of Aspergillus niger”. In: *Biotechnology and Bioengineering* 119 (2022), pp. 2182–2195. URL: https://api.semanticscholar.org/CorpusID:248414482.

[6] Zbigniew Ulanowski and Ian Keith Ludlow. “Water distribution, size and wall thickness in Lycoperdon pyriforme spores”. In: *Fungal Biology* 93 (1989), pp. 28–32. URL: https://api.semanticscholar.org/CorpusID:84216118.

[7] X. Wang et al. “The SC3 hydrophobin self-assembles into a membrane with distinct mass transfer properties.”. In: *Biophysical journal* 88 5 (2005), pp. 3434–43. URL: https://api.semanticscholar.org/CorpusID:16739076.

[8] Zhiyong Zhang, Jianyong Zhu, and Feiping Nie. “A novel hybrid adaptive differential evolution for global optimization”. In: *Scientific Reports* 14 (2024). URL: https://api.semanticscholar.org/CorpusID:271950331.