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On the three-dimensional morphology and substrate-diffusion in filamentous fungal pellets

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Morphology and productivity

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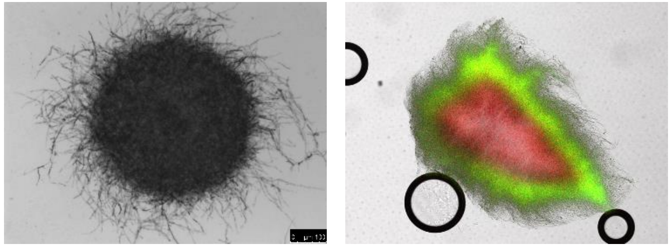
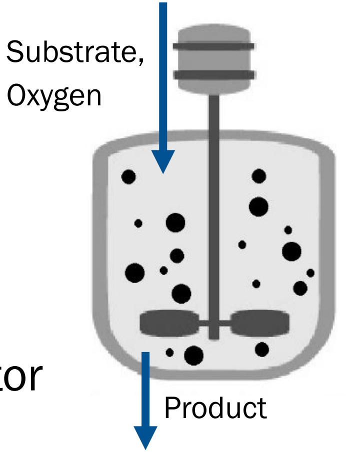
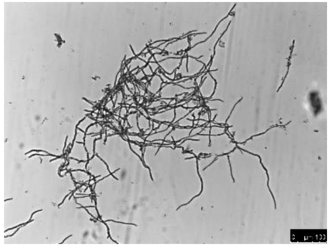
Pellet





Disperse mycelia



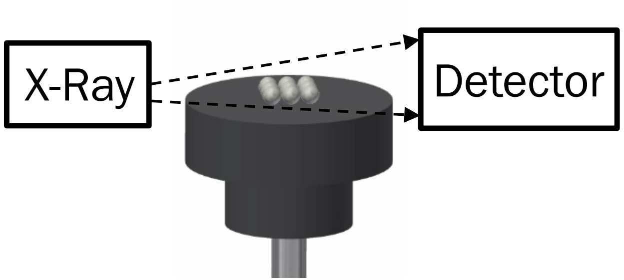
+ O_2-uptake and mixability of reactor
– Inactive core possible

+ No inactive core
– O_2-uptake and mixability of reactor

Strong correlation between morphology and productivity

→ **How to measure (micro-) morphology?**

→ **How to compute the substrate-transport into pellets?**



3D image analysis of a fungal pellet

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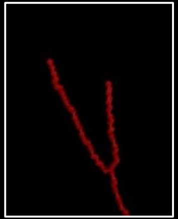

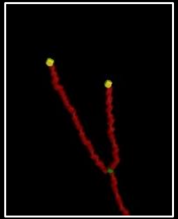

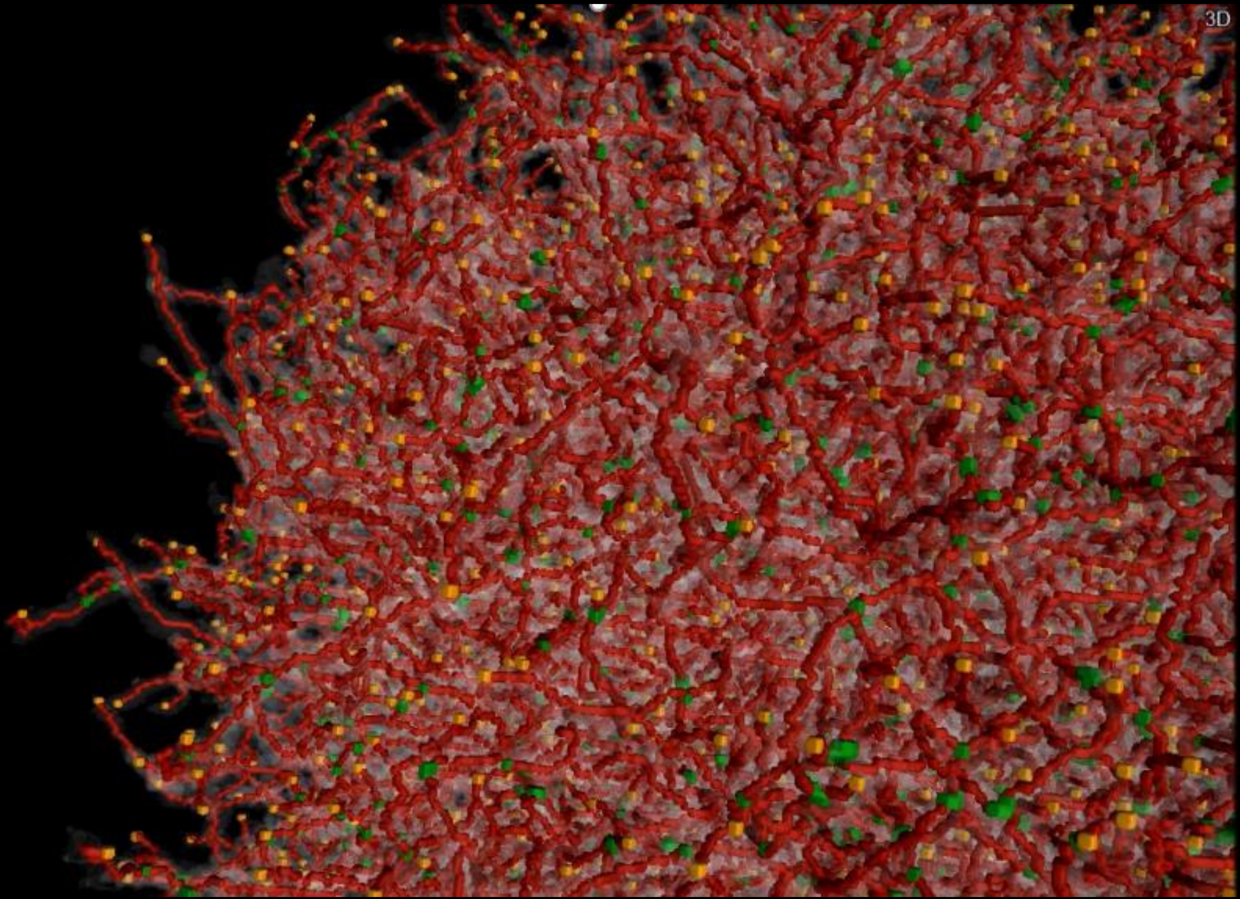

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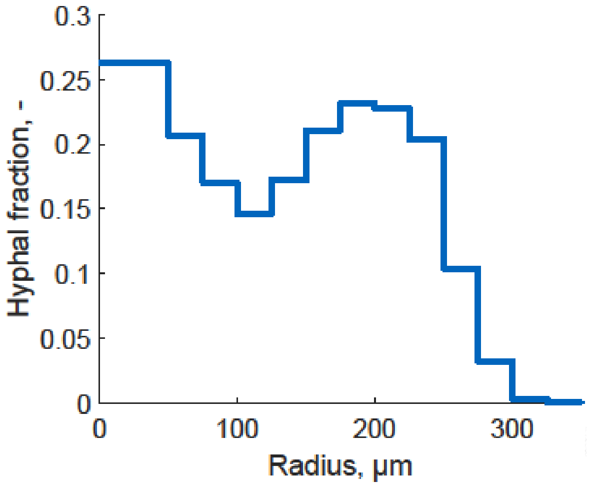
An X-ray microtomography-based method for detailed analysis of the three-dimensional morphology of fungal pellets

Stefan Schmideder[1] | Lars Barthel[2] | Tiaan Friedrich[1] | Michaela Thalhammer[1] | Tijana Kovačević[1] | Ludwig Niessen[3] | Vera Meyer[2] | Heiko Briesen[1]

Morphological Property	*Aspergillus niger*	*Penicillium chrysogenum*	Unit
Hyphal Length	1.472	2.995	m
Number of Tips	15,425	20,000	-
Ø Diameter of Hyphae	3.8	4.1	µm
Diameter of Pellet	0.6	1.1	mm



Location of tips, branches, and hyphal material can be determined within pellets

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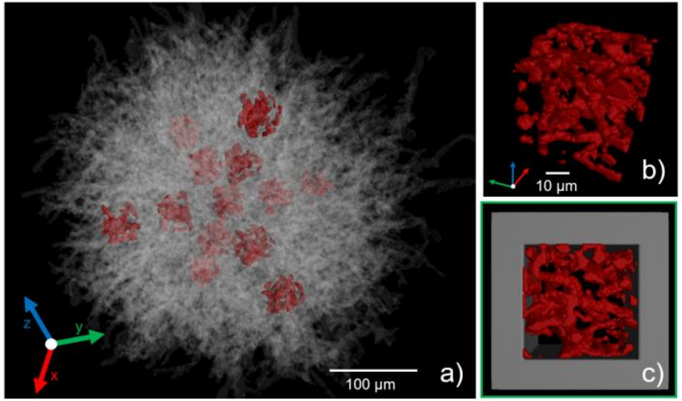
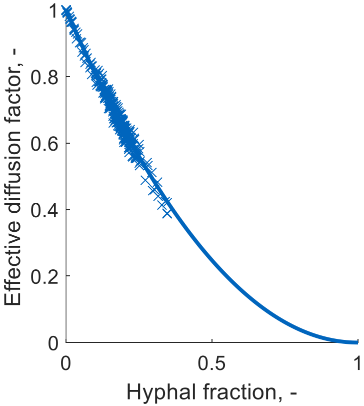
From three-dimensional morphology to effective diffusivity in filamentous fungal pellets

Stefan Schmideder[1] | Lars Barthel[2] | Henri Müller[1] | Vera Meyer[2] | Heiko Briesen[1]

- Diffusion of substrates, especially O_2, is crucial
- Geometrically hindrance of diffusion throuh hyphal network





$$\frac{\partial c_i}{\partial t} = \underbrace{\vec{\nabla}\left(D_{i,eff}(\vec{x})\vec{\nabla}c_i\right)}_{\text{Diffusion}} + \underbrace{q_i(c_i,\vec{x},\vec{r})}_{\text{Consumption}}$$

$$\varepsilon = 1 - Hyphal\ fraction$$

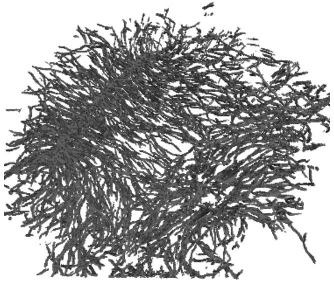
$$D_{i,eff} = Const \cdot \varepsilon^a$$

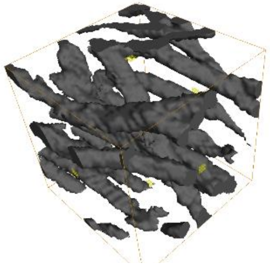
A. niger MF22.4:

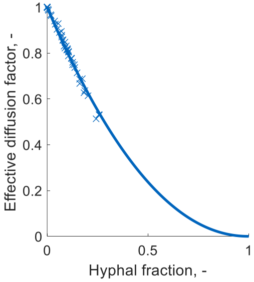
$$a = 2.02 \pm 0.02$$

Diffusion depends only on hyphal fraction/porosity

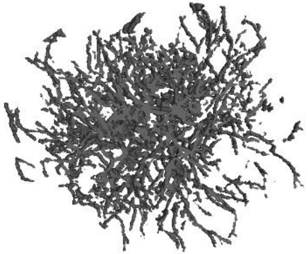
Diffusion for different strains

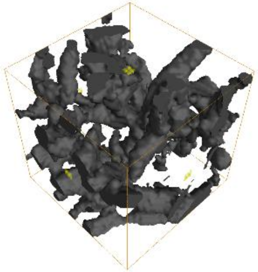


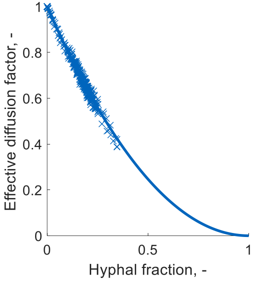




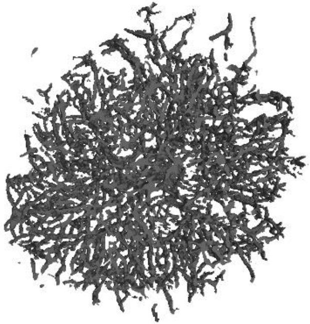
P. chrysogenum
MUM17.85
$a = 2.08 \pm 0.04$

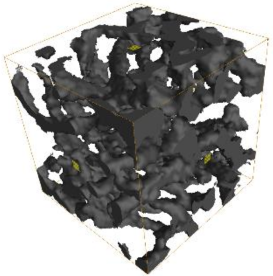


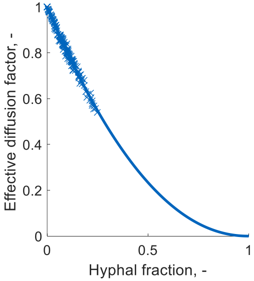




A. niger MF19.5
Parental of MF22.4
$a = 2.10 \pm 0.03$







A. niger MF22.4
Hyperbranching
$a = 2.02 \pm 0.02$

Diffusion depends only on porosity

Is there a generalized law for fungal pellets, such as $D_{i,eff} = C * \varepsilon^2$?

Conclusion

- Analysis of the micro-morphology based on X-ray microcomuted tomography (µCT)
  - First method to locate tips, branches, and hyphal material in whole pellets
  - Limitations: 1-10 pellets per measurement, measurement time (3 h), investment costs for µCT

- Diffusive transport of oxygen and nutrients into pellets based on 3D-morphology
  - First method to compute geometrically hindrance of diffusion
  - 3D morphological input: µCT preferable, CLSM possible
  - Limitations CLSM: penetration depth ≈ 50 µm → Slicing of pellet necessary
  - Effective diffusivity follows easy law: $D_{i,eff} = Const * Porosity^{a}$

Vision: «perfect» pellet

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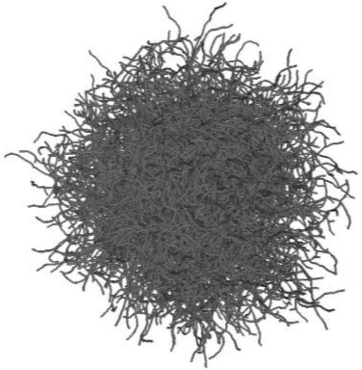
1) Generate pellets through experiments or simulations
2) Compute correlation between porosity and diffusivity of all pellets generated in Step 1
3) Compute substrate-supply of all pellets (e.g. Buschulte et al., 1992)

µCT-measurement

Simulation



Adopted from Celler et al. (2012)

$$\frac{\partial c_i}{\partial t} = \vec{\nabla}\left(D_{i,eff}(\vec{x})\vec{\nabla}c_i\right) + q_i(c_i,\vec{x},\vec{r}) = \text{Diffusion} + \text{Consumption}$$

4) Data base of all pellets including substrate-supply and morphological features
5) Select «perfect» pellet from the data base
6) Realize «perfect» pellet through genetic modifications or process control