Fungal biomass analytics: how to measure fungal growth in 3D

Prof. Dr. Patrick A. Rühs
Eidgenössische Technische Hochschule Zürich
Institut für Lebensmittelwissenschaften,
Ernährung und Gesundheit
Lebensmittelstrukturverfahrenstechnik

Ciatta Wobill, Natalie Nussbaum, Peter Fischer and Patrick A. Rühs

Fungal growth in 3D is crucial for engineered living materials [1] and food applications, and it depends on substrate properties [2] and nutrient density [3]. Quantifying fungal biomass in three dimensions is challenging, due to the limitations of currently available methods.

We assessed fungal growth and proliferation by measuring ergosterol [4] and interfacial shear rheology [5], and compared our methods to conventional methods such as optical density and colony diameter measurements. We assessed fungal growth by measuring ergosterol, focusing on extensive growth along the substrate surface and invasive growth into the substrate. Extensive growth contributed significantly more to global biomass accumulation than local growth. Altering nutrient density and stiffness of host materials showed that extensive growth drives biomass accumulation, challenging the assumption that radial extension corresponds to biomass increase.

Furthermore, we used interfacial shear rheology (ISR) to investigate fungal film formation, identifying growth phases by measuring film viscoelasticity. Modifying sugar concentration in liquid growth media delayed germination while enhancing exponential propagation speed. Combining ISR with spectrophotometry and colony diameter measurements provided a comprehensive picture of transient film formation, elucidating germination, exponential growth, and stationary phases through qualitative and quantitative measures. With ergosterol and interfacial shear rheology as methods to quantify fungal growth, we have developed a toolbox to quantify fungal growth in solid-state fermentation.

[1] Gantenbein, S.; Colucci, E.; Käch, J.; Trachsel, E.; Coulter, F. B.; Rühs, P. A.; Masania, K.; Studart, A. R. Three-Dimensional Printing of Mycelium Hydrogels into Living Complex Materials. *Nat. Mater.* 2023, *22* (1), 128–134.

[2] Wobill, C.; Azzari, P.; Fischer, P.; Rühs, P. A. Host Material Viscoelasticity Determines Wrinkling of Fungal Films. *ACS Biomater. Sci. Eng.* 2024. (3)

[3] Nussbaum, N.; von Wyl, T.; Gandia, A.; Romanens, E.; Rühs, P. A.; Fischer, P. Impact of Malt Concentration in Solid Substrate on Mycelial Growth and Network Connectivity in Ganoderma Species. *Sci. Rep.* 2023, *13* (1).

[4] Nussbaum, N.; Balmelli, L.; Fischer, P.; Rühs P.A. (in review)

[5] Wobill, C.; Steffen, S.; Macken, D.; Fischer, P.; Rühs, P. A. (in review)