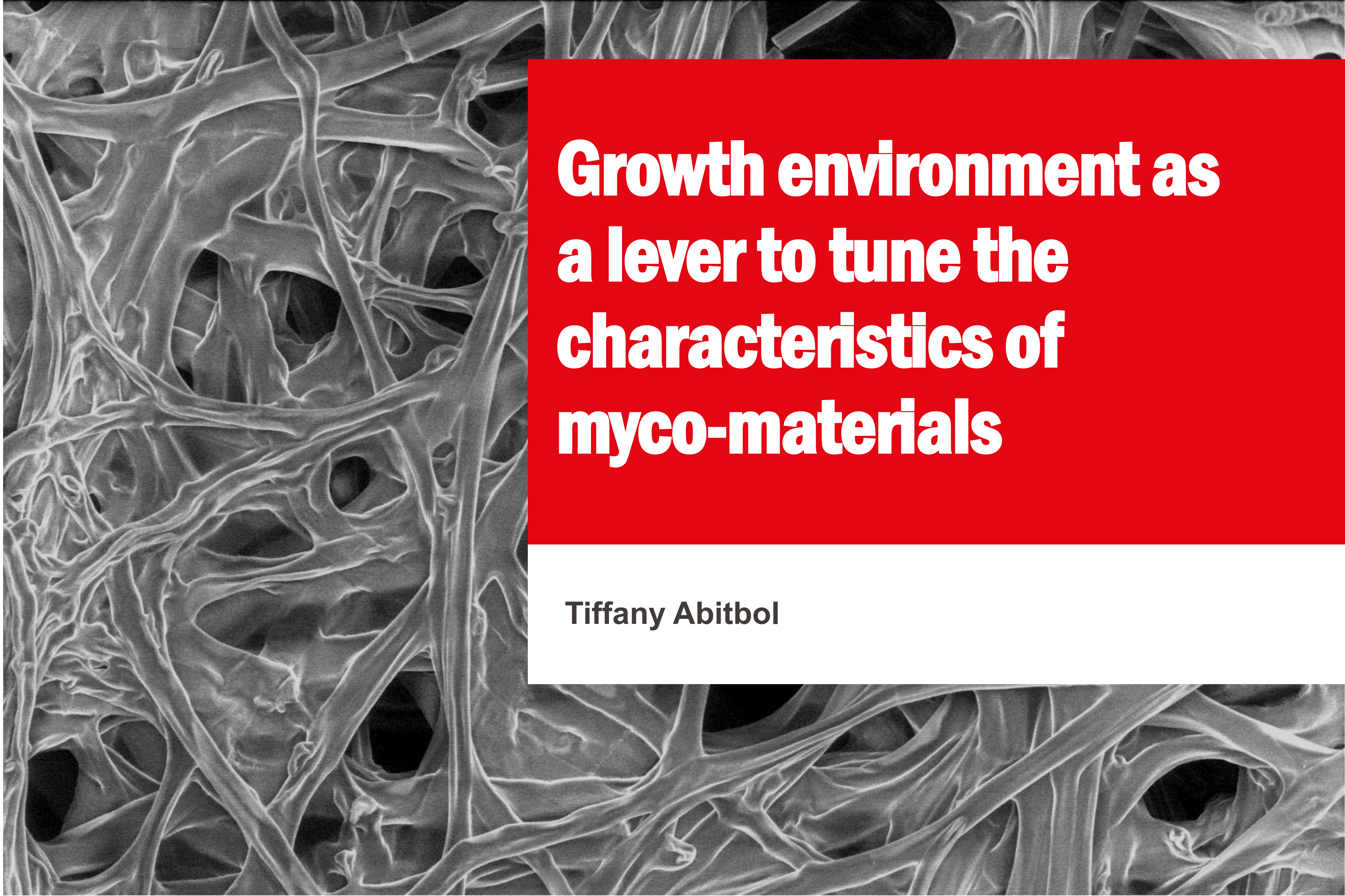


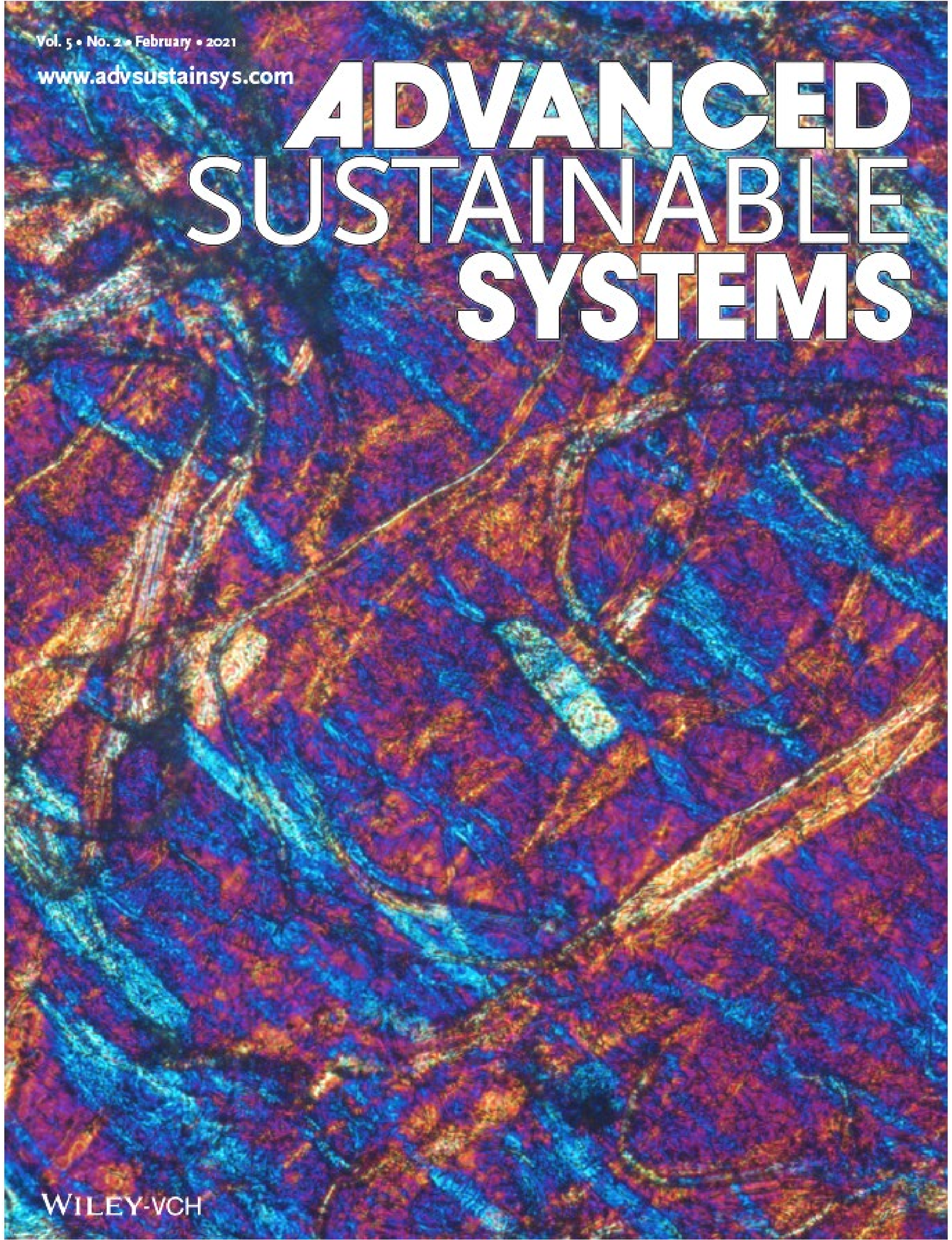
Growth environment as a lever to tune the characteristics of myco-materials

Tiffany Abitbol

MSE 493

Biofabrication of Nanocellulose–Mycelium Hybrid Materials

Attias & Abitbol, et al., Advanced Sustainable Systems **5** (2021).



Citation Impact

2023 CiteScore (Scopus):	10.8
2023 Journal Citation Indicator (Clarivate):	1
2023 Journal Impact Factor (Clarivate):	6.5

Usage

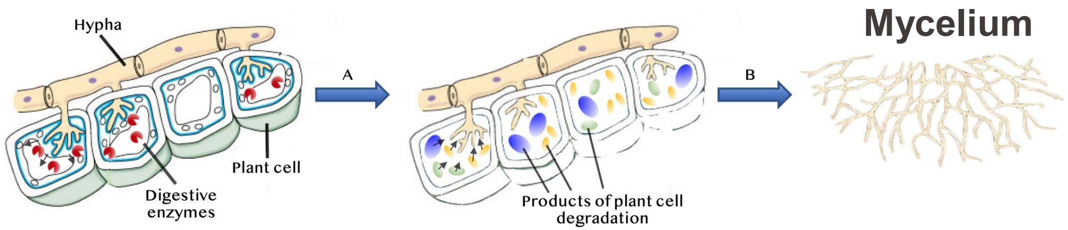
2023 Full Text Views:	305,896

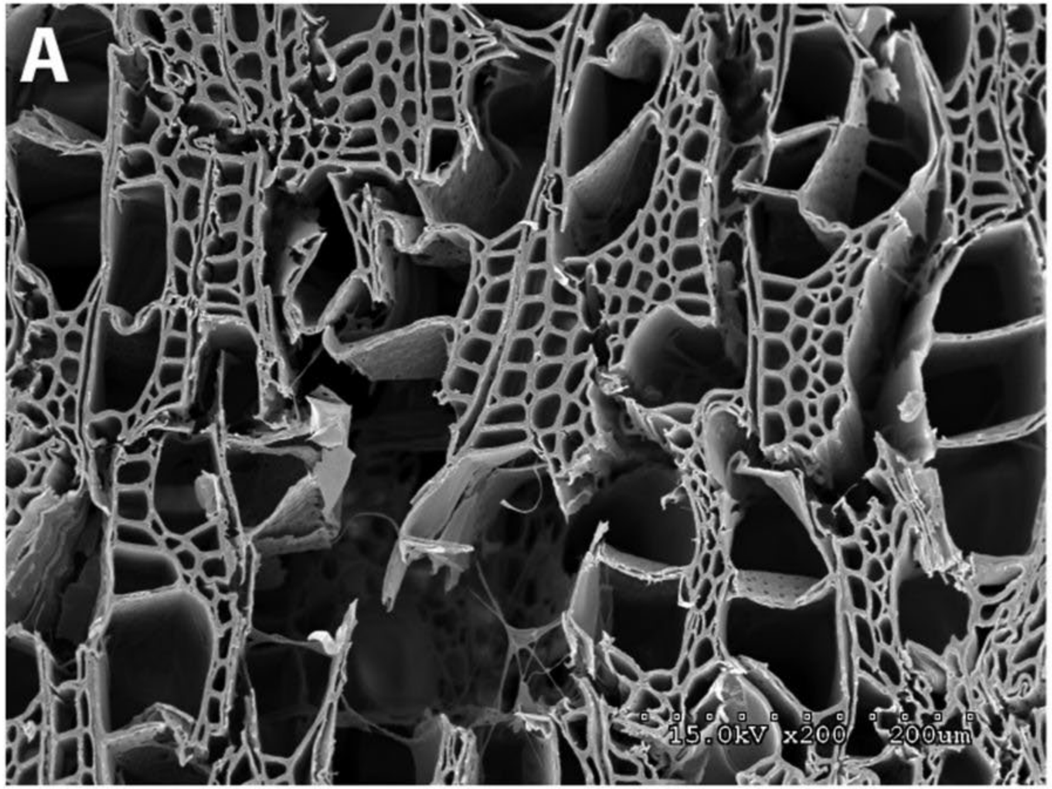
Speed

2023 Acceptance rate:	30%
2023 Submission to first decision:	4 days
2023 Submission to acceptance:	63 days

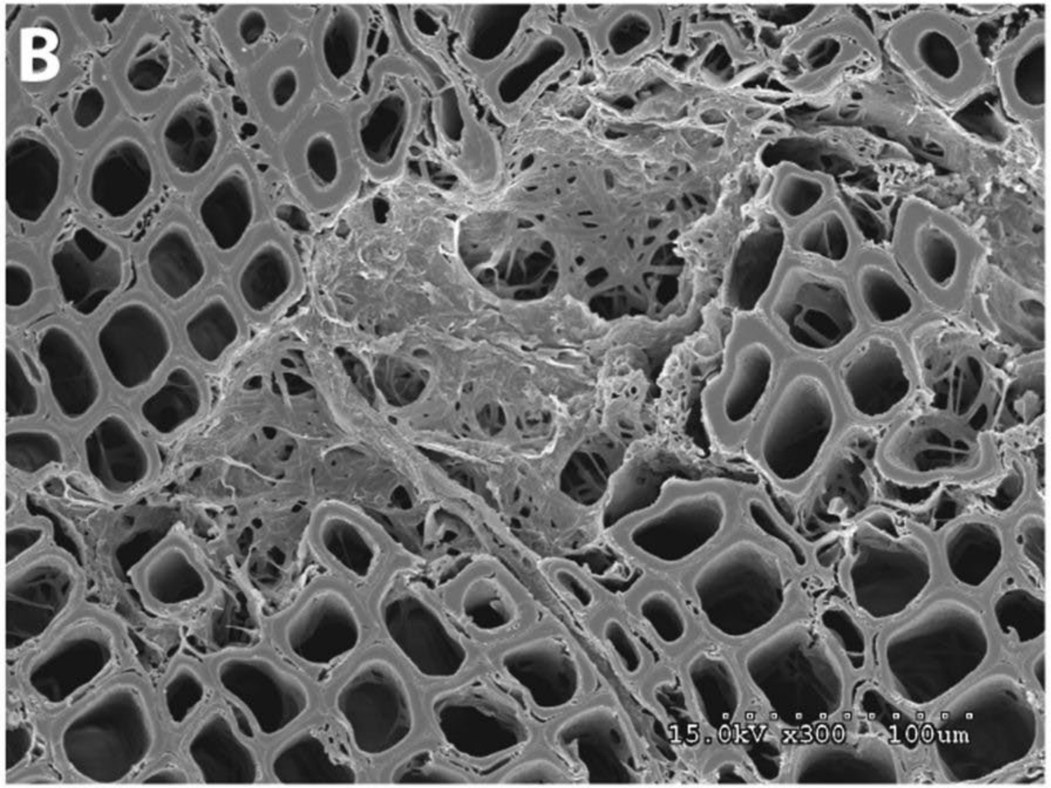
- A sister journal to Advanced Materials… (IF = 27.4)

Wood decaying fungi





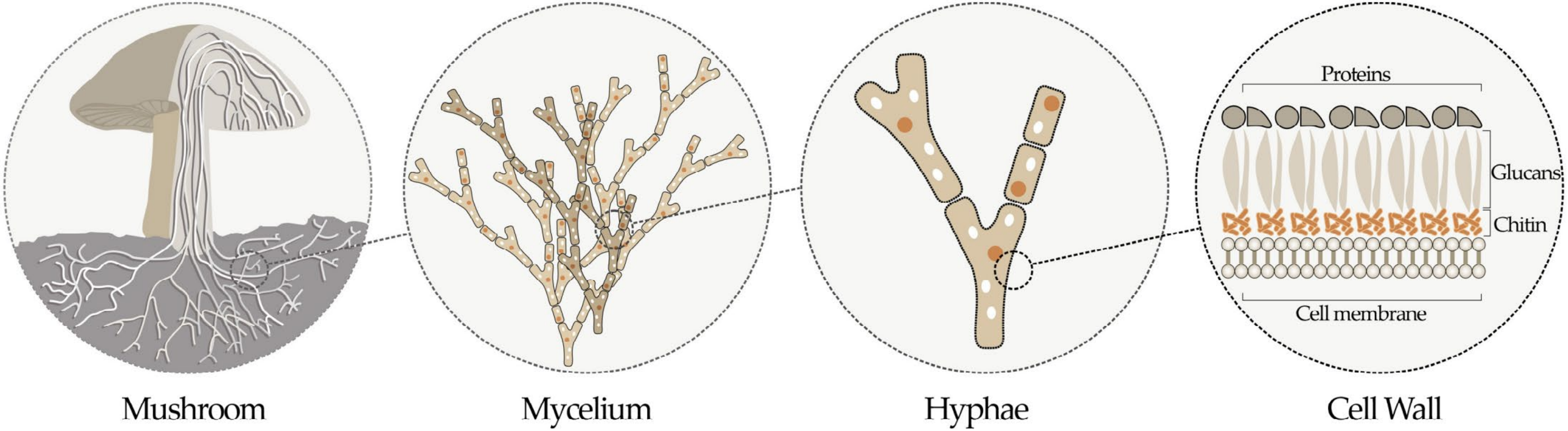
(A) *B. botryosum* on aspen wood with vessel, fiber, and parenchyma cell walls degraded. Mycelia are visible growing through the voids.



(B) *J. argillacea on pine showing an area where the fungus has caused a localized simultaneous decay of the cells. Residual cell wall material and mycelia fill the degraded zone.*

Riley et al., Biological Sciences **111** (2014).

Mycelium

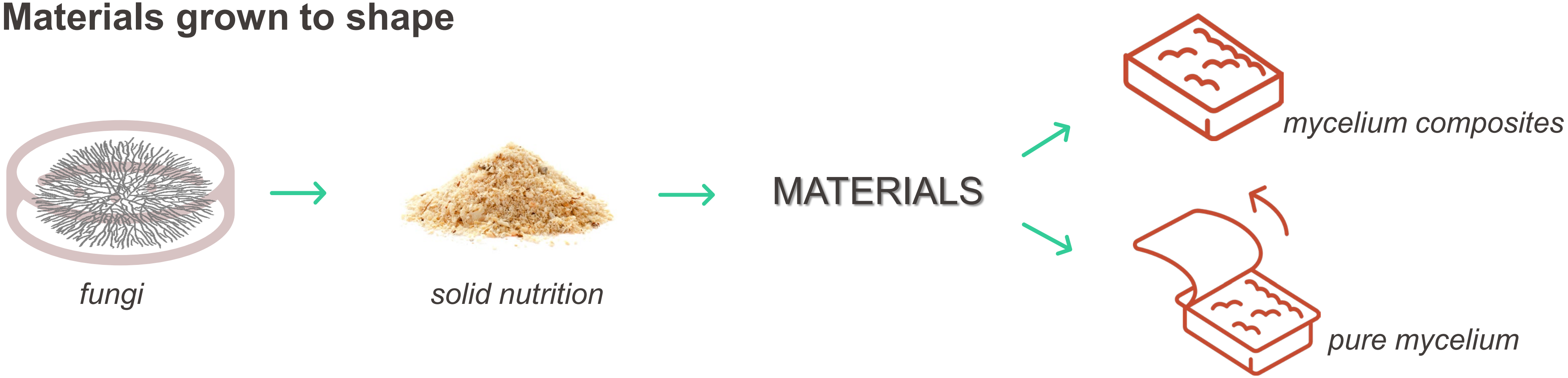


Mohseni et al., Biomimetics **14** (2023).

Fungi for materials

Attias et al., Journal of cleaner production **246** (2019).

Solid Fermentation:
Materials grown to shape



“MyForest Foods” by Ecovative
Bacon in just 9 days

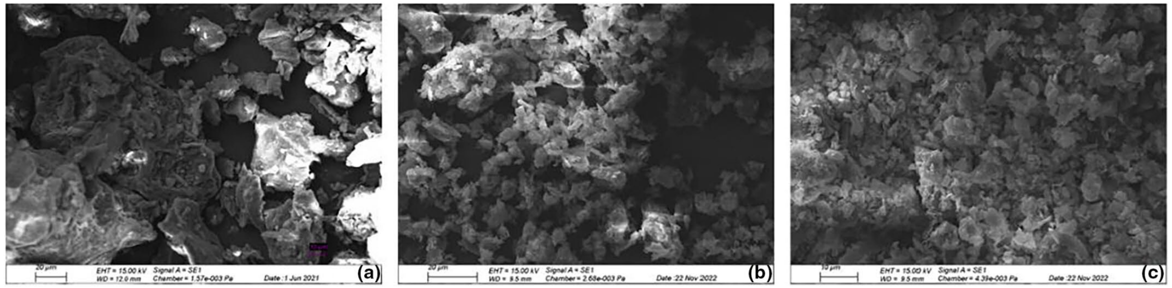
MycoWorks × Hermès

Bolt Threads × Adidas

Food waste is converted to new food:

From EU "Smart Protein" project (2020).

Upcycling cocoa pod husks into a fiber & protein-rich ingredient:



Bickel Haase et al., Food Science & Nutrition **12** (2024).

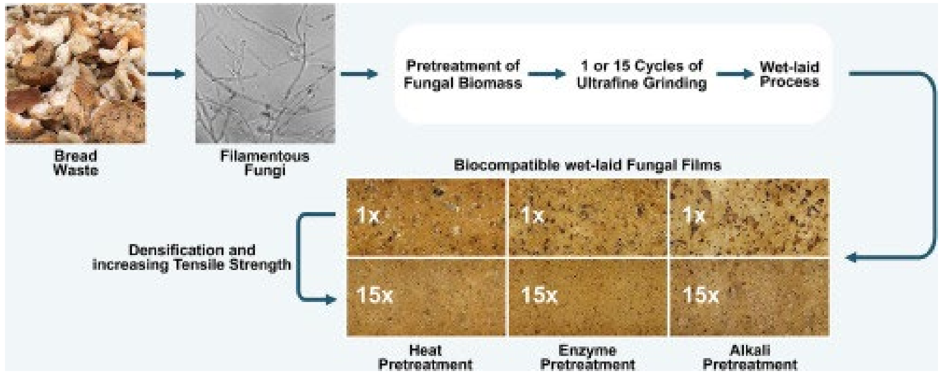
Submerged Fermentation:

fungi → *nutrition* → *biomass + secretome* → MATERIALS

↗ FOOD

↘ PACKAGING

Wet-laid sheets from bread waste:

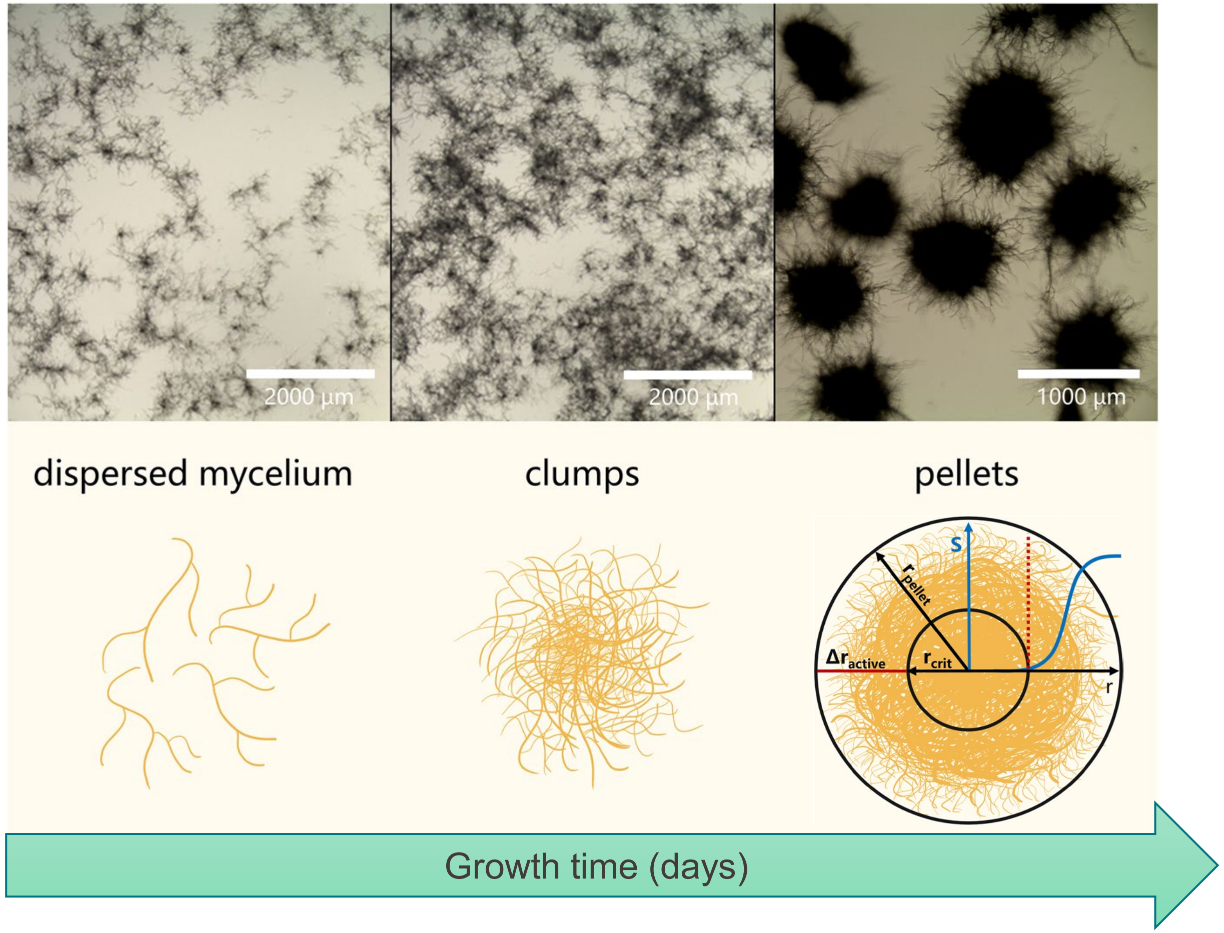


Köhnlein et al., Materials & Design **216** (2022).

Packaging-relevant films from nanocellulose:

Attias & Abitbol, et al., Advanced Sustainable Systems **5** (2021).

Basics of mycelium growth in submerged fermentation



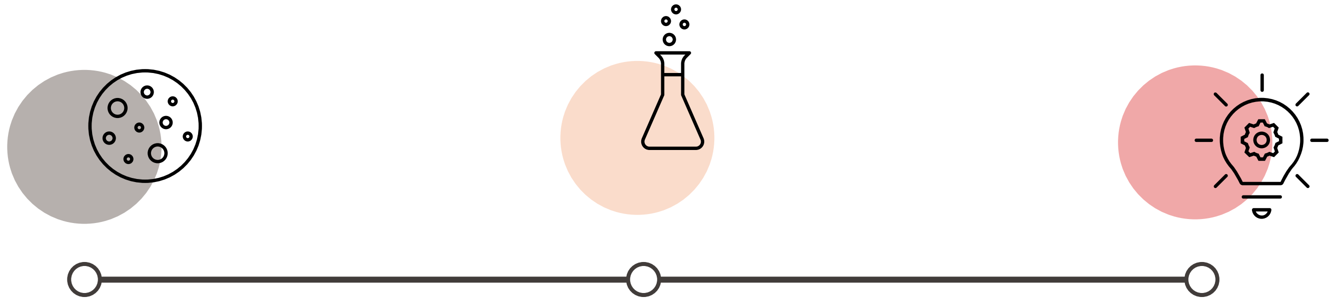
Dinius, et al., Physical Sciences Reviews **9** (2023).

After designated growth time:

- Mycelium pellets
- Media that is depleted in nutrition
- Media that is enriched in exopolysaccharides (EPS)

Our approach to mycelium materials

Nature	Nurture	Nature vs. Nurture
Species	**Adaptive growth**	**Materials**
Encompasses all aspects encoded in genetic script	Encompasses all aspects of growth environment	Leveraging nature and nurture toward new materials

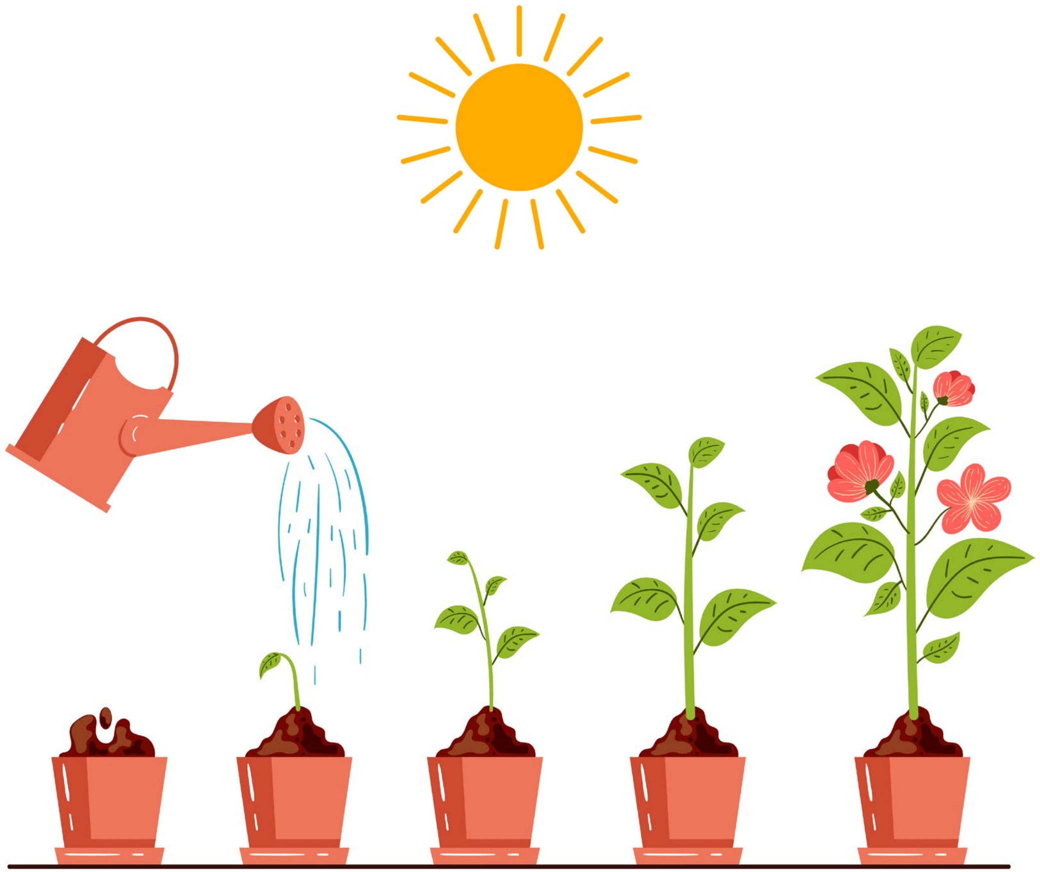


→ **Adaptive growth for materials science relevant outcomes**

Nature vs. nurture? You are what you eat?

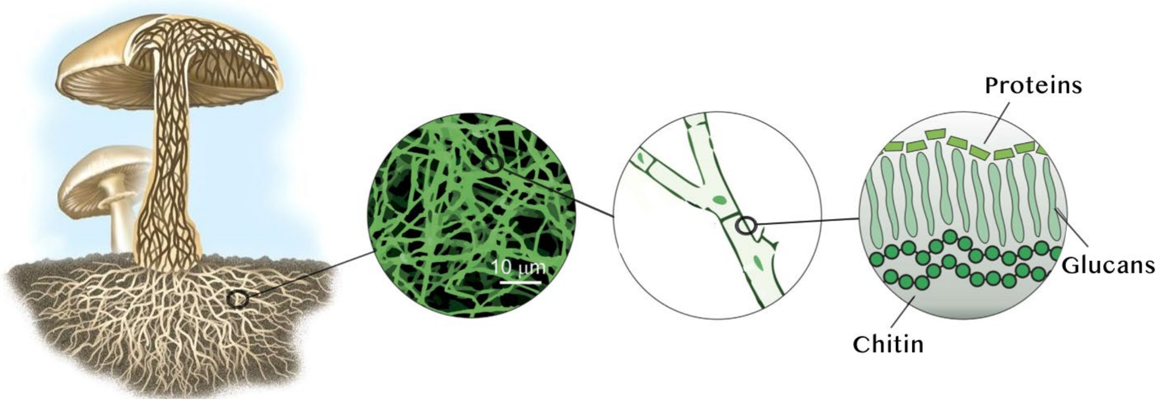
Tune biomass **quantity and quality**, by modifying conditions of growth:

- ✓ Humidity
- ✓ Temperature
- ✓ Time
- ✓ Stressors
- ✓ Nutrition



Vivas Hernando et al., Global Challenges **8** (2023).

Inspiration



- ✓ Species
- ✓ Humidity
- ✓ Temperature
- ✓ Time
- ✓ Nutrition

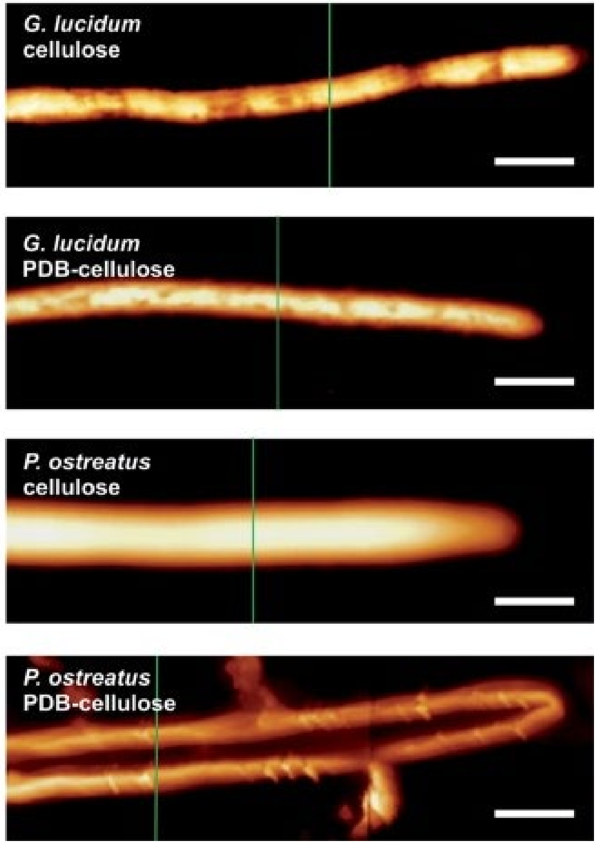
- Pure mycelium mats by solid fermentation
- Mats grown on films of microcrystalline cellulose (MCC) or 1:1 potato dextrose (PDB) with MCC
- 2 species: *P. ostreatus*, *G. lucidum*

Simple vs. complex nutrition: composition & properties

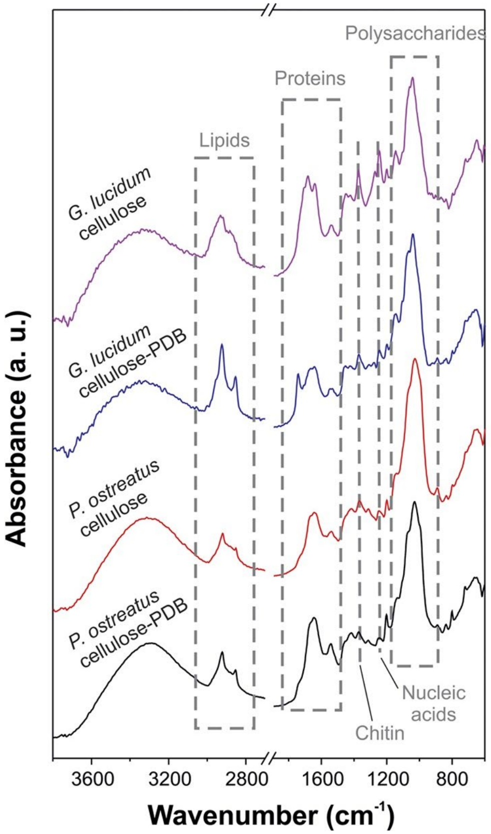
Haneef, M. et al. *Sci. Rep.* 7, (2017).

Inspiration

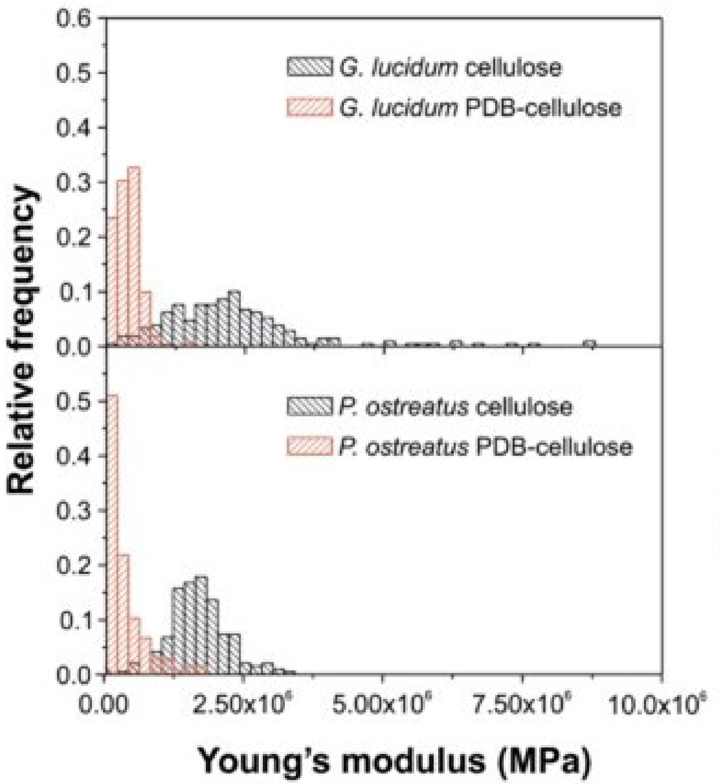
Morphology



Composition



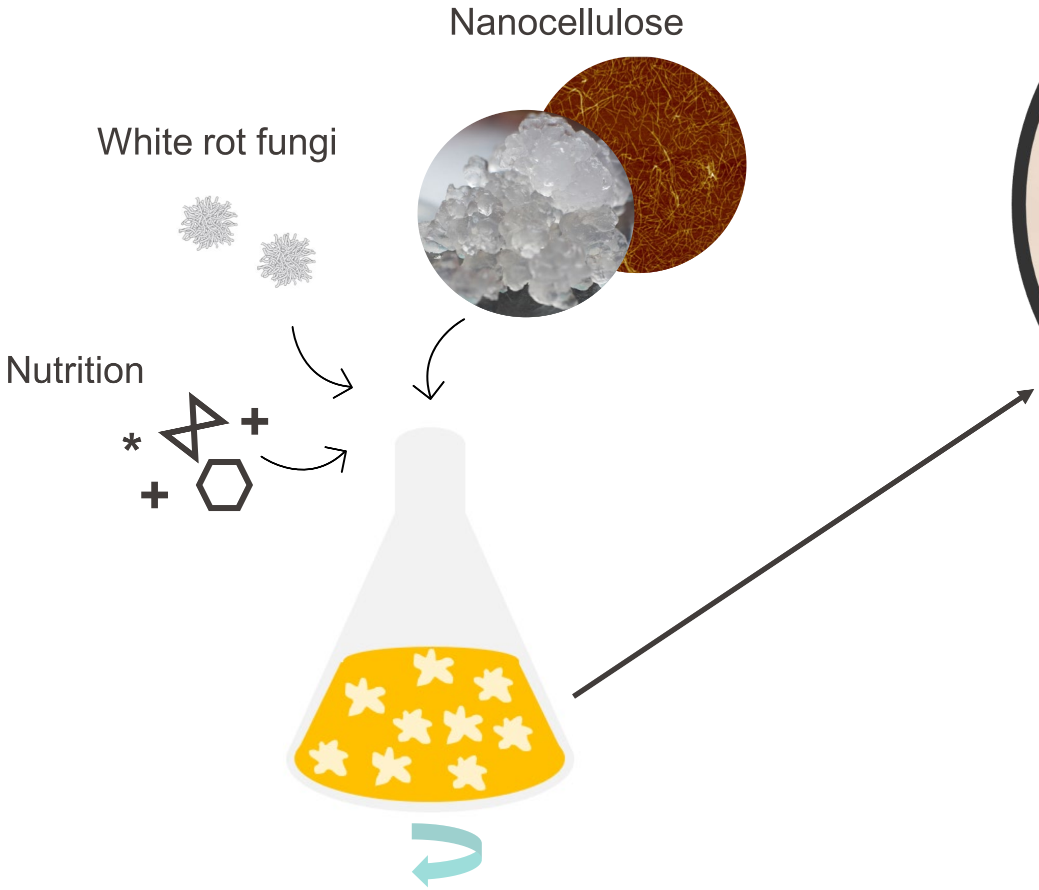
Properties

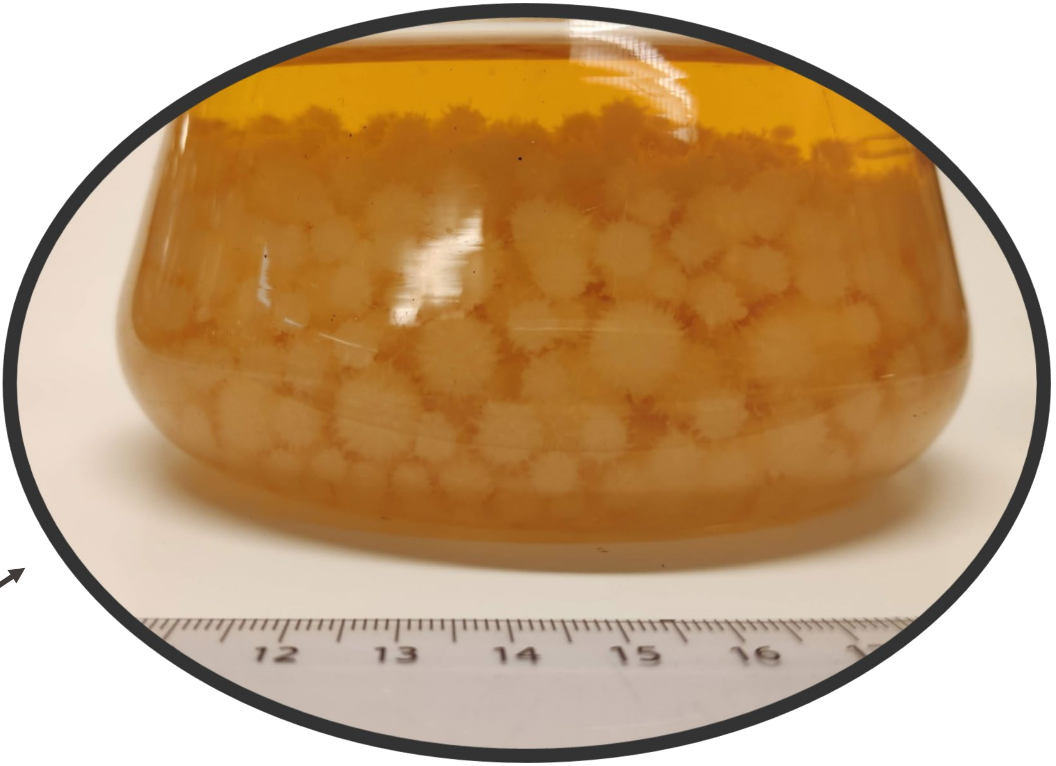


Haneef, M. et al. *Sci. Rep.* 7, (2017).

Nanocellulose as an additive, not a nutrient

Submerged fermentation

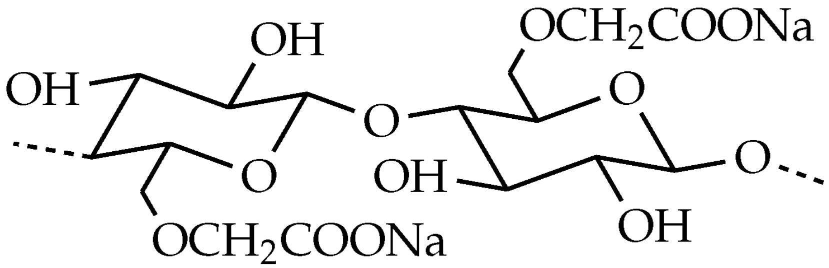




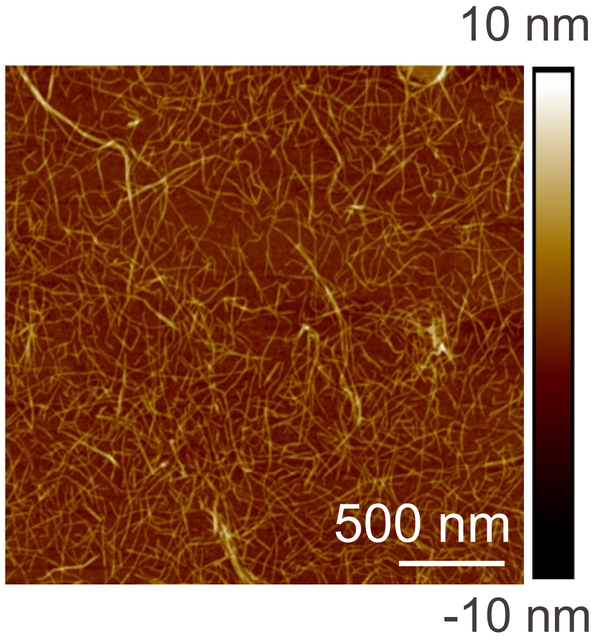
- ✓ Species – *T. ochracea*
- ✓ Humidity – 100%
- ✓ Temperature – 25 °C
- ✓ Time – 14 days
- ✓ Nutrition – protein and sugar-rich media
- ✓ Additive – nanocellulose

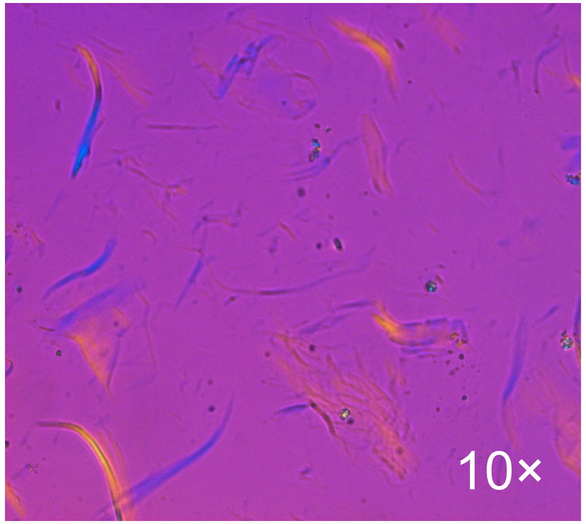
Attias & Abitbol, et al., Advanced Sustainable Systems **5** (2021).

Mycelium growth with added nanocellulose



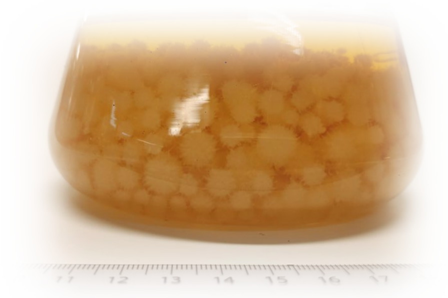
CARBOXYMETHYLATED
CNF DS0.1



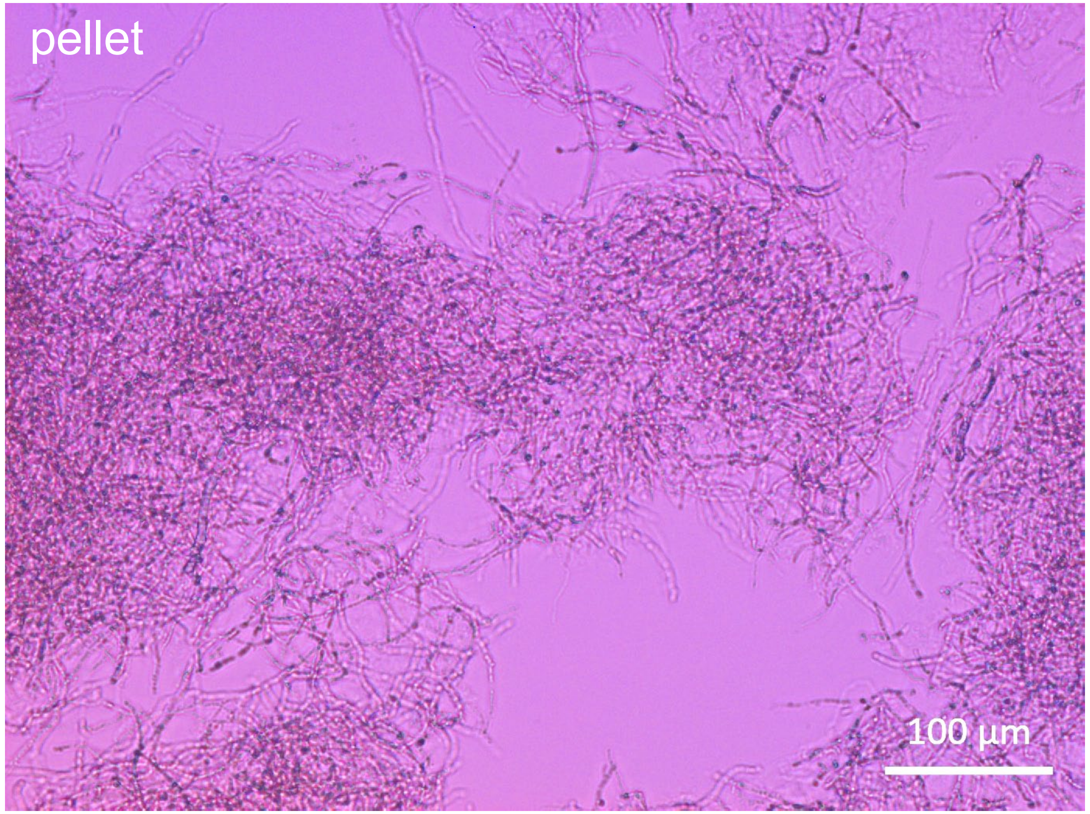


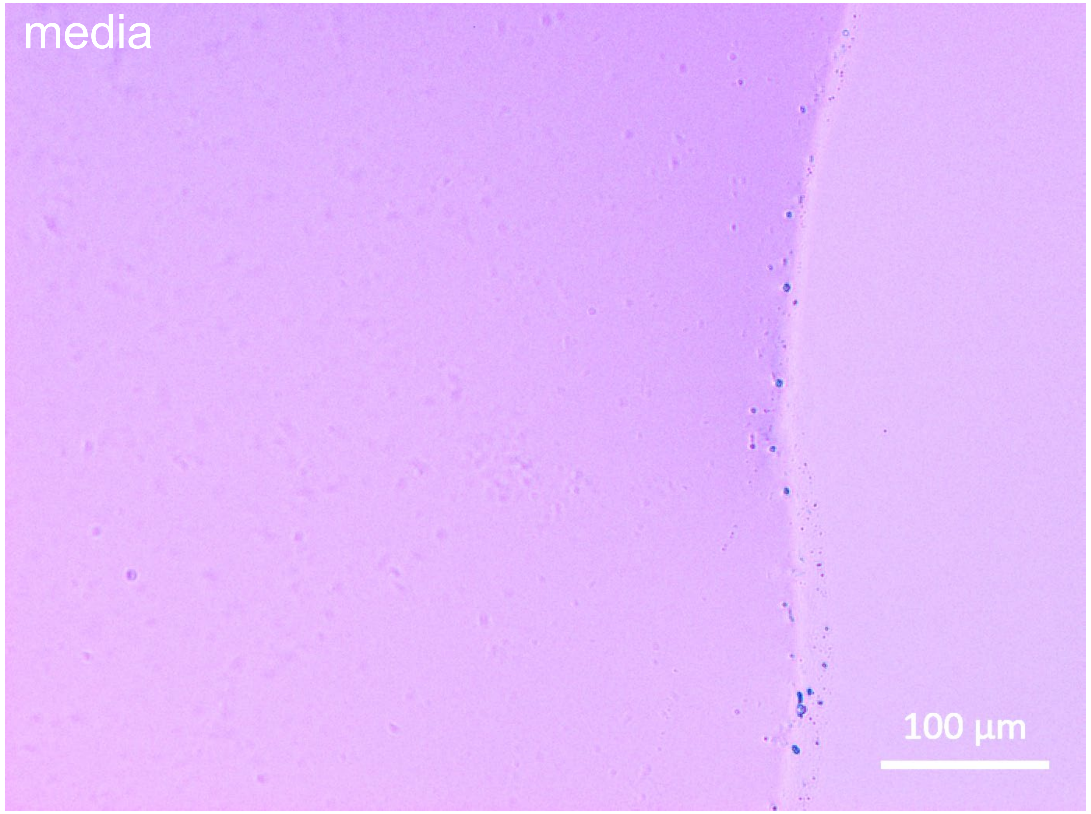
Nano-fraction (ca. 25%+)

Mycelium growth without added nanocellulose

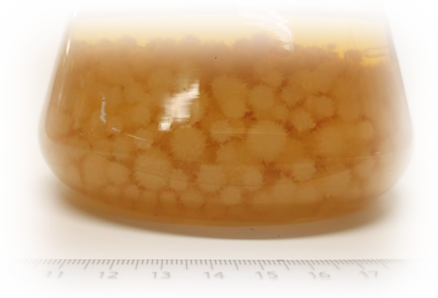


- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (EPS)

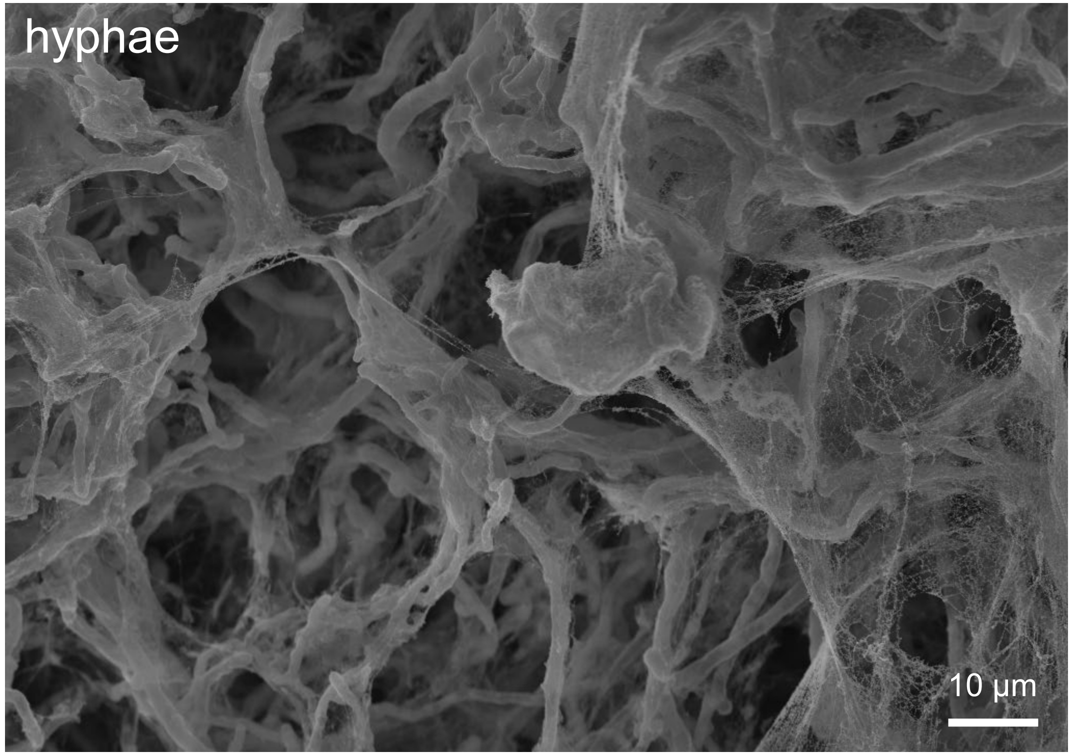


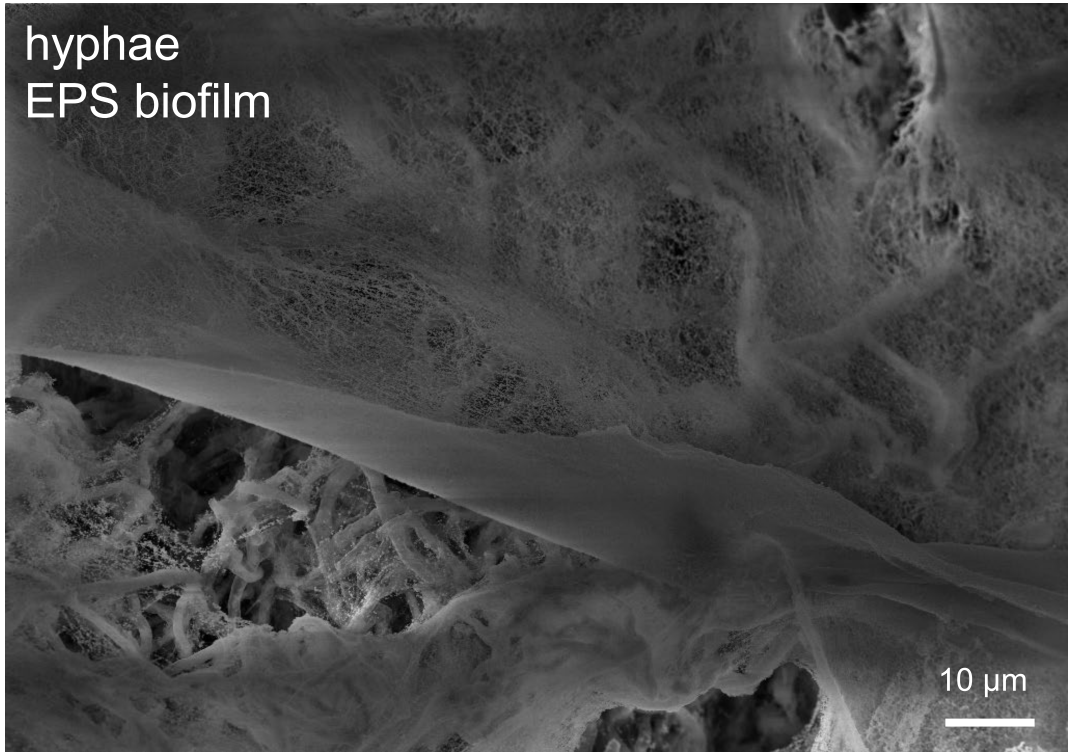


Mycelium growth without added nanocellulose

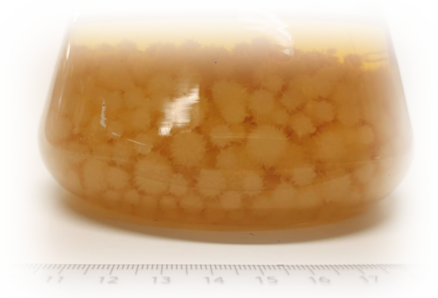


- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (EPS)

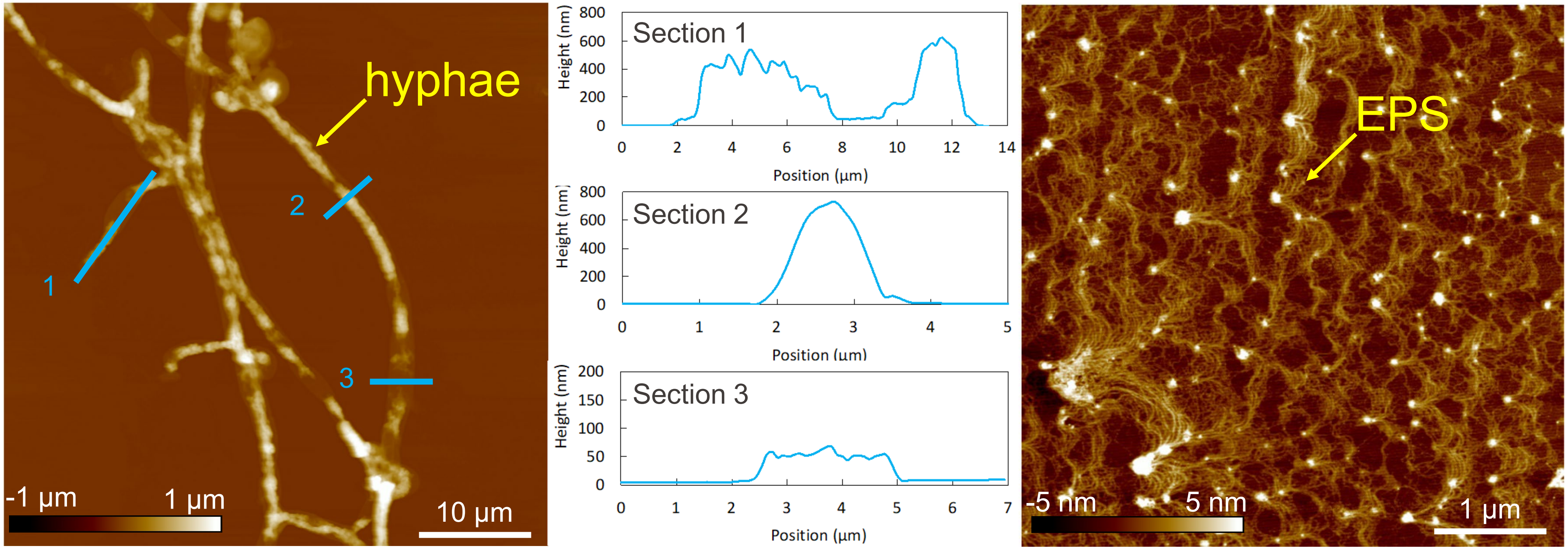




Mycelium growth without added nanocellulose

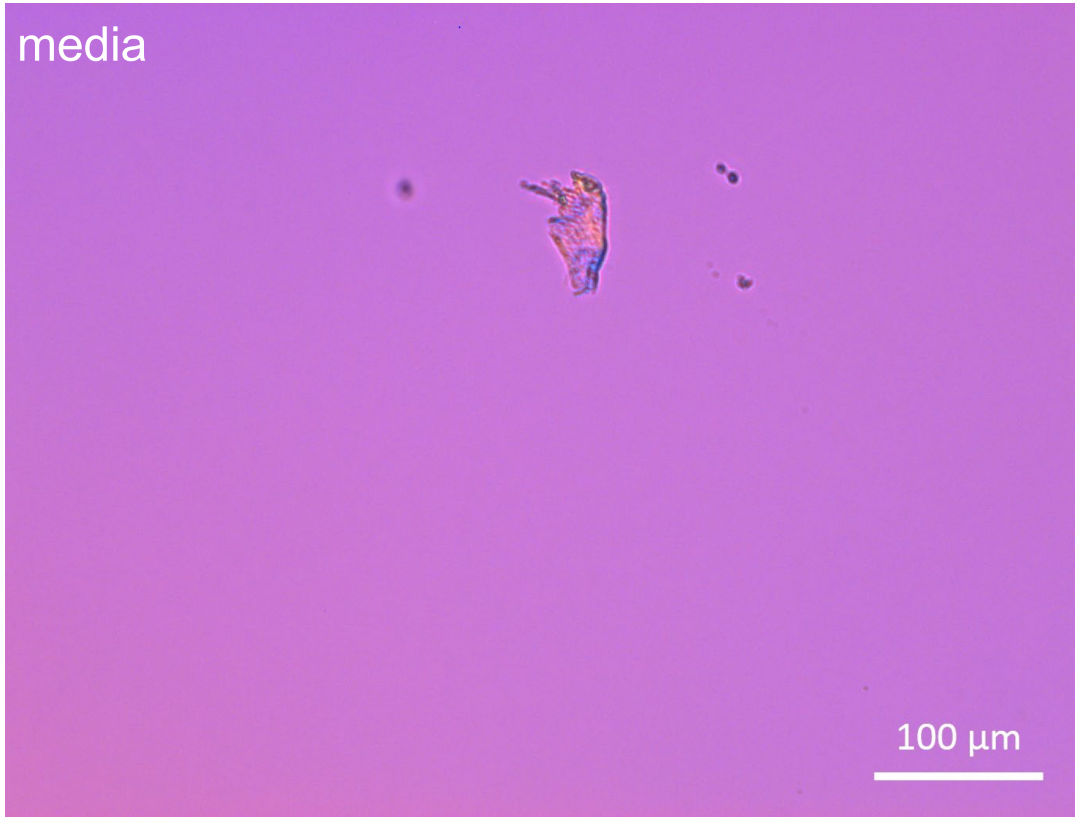


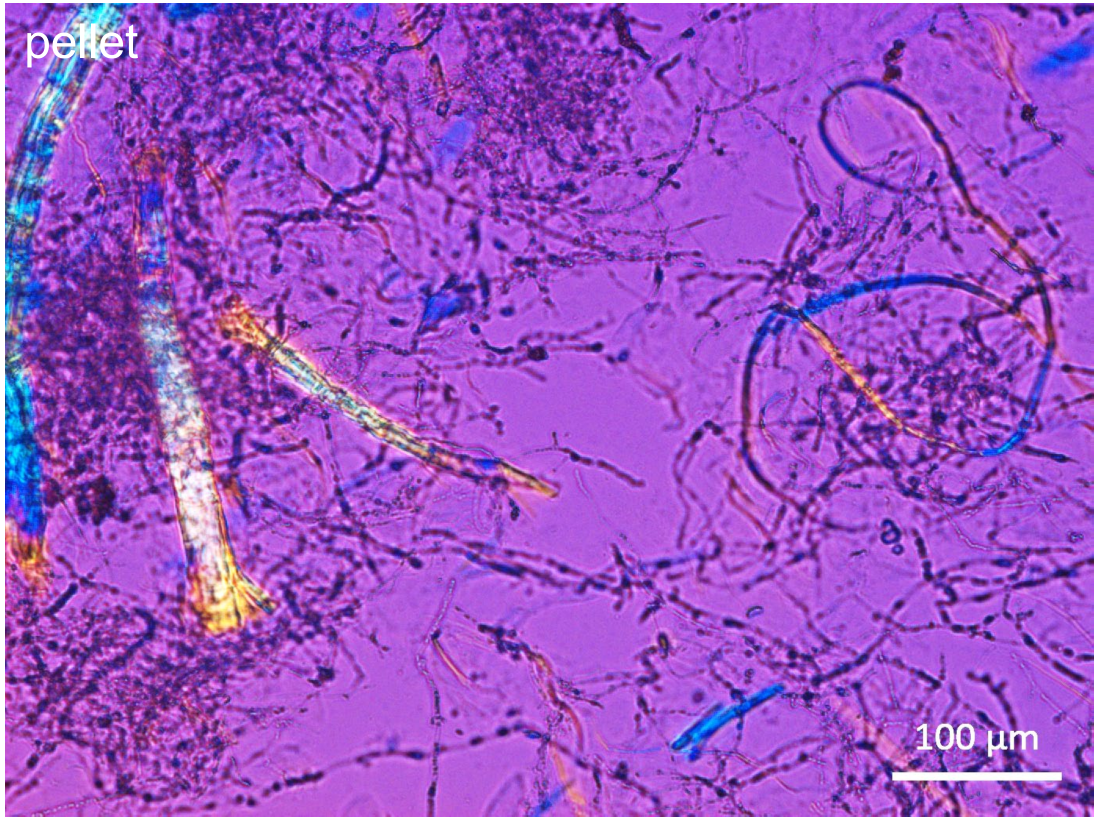
- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (EPS)



Mycelium growth with added nanocellulose

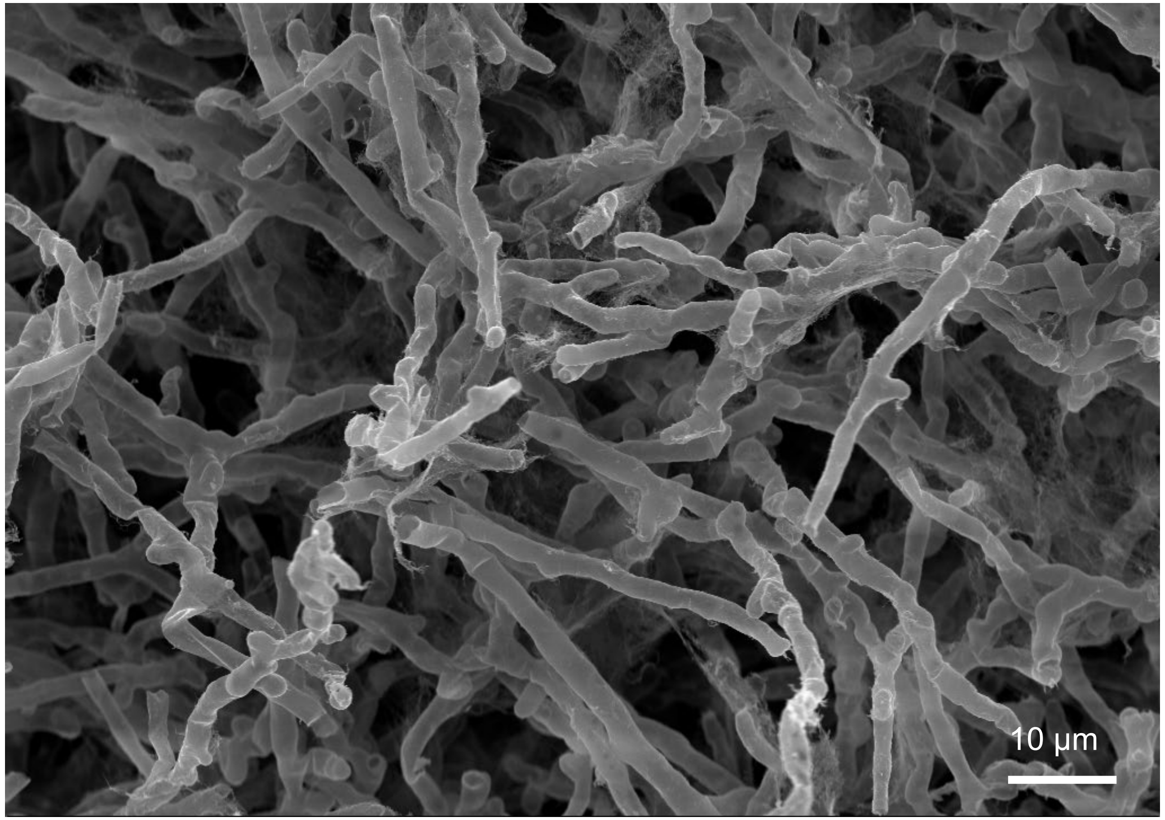
- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (media)
- Where's the nanocellulose?

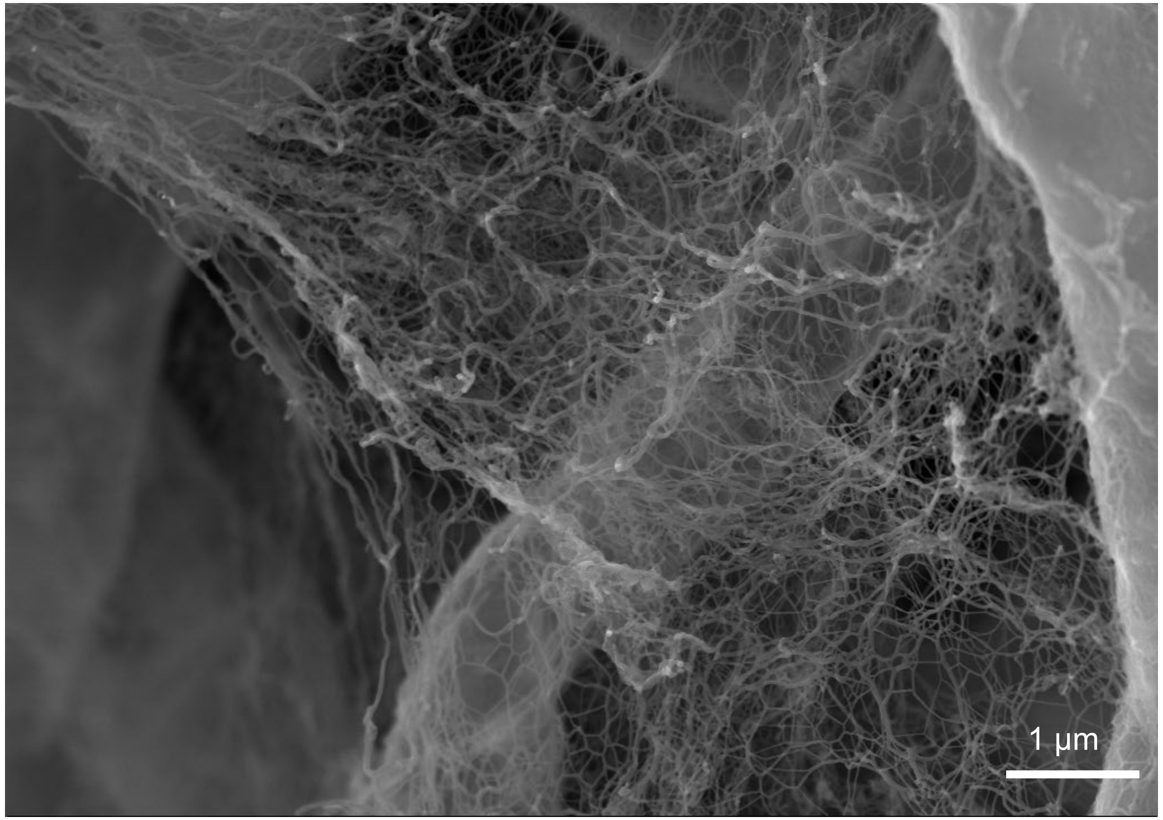




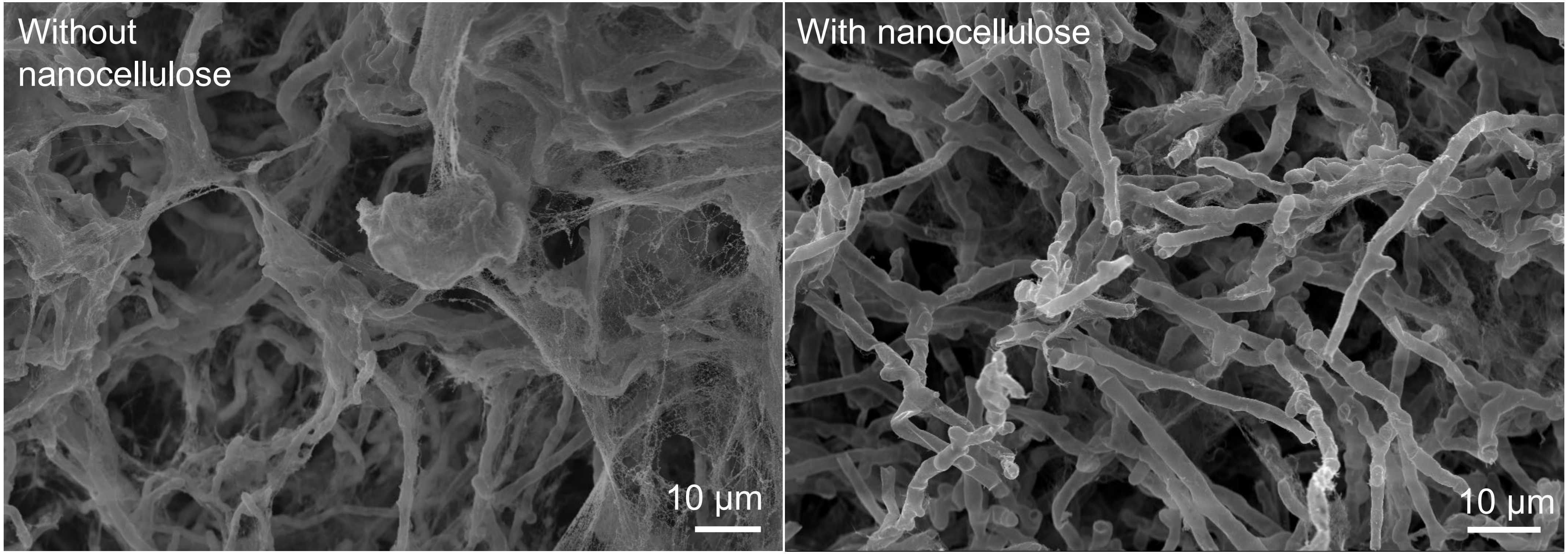
Mycelium growth with added nanocellulose

- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (media)
- Where's the nanocellulose?

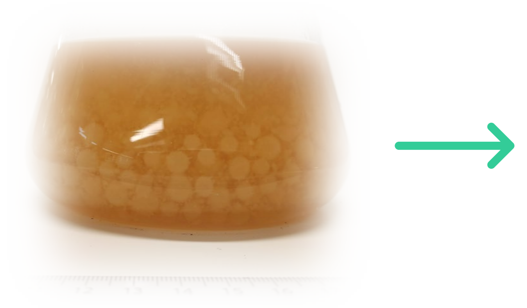




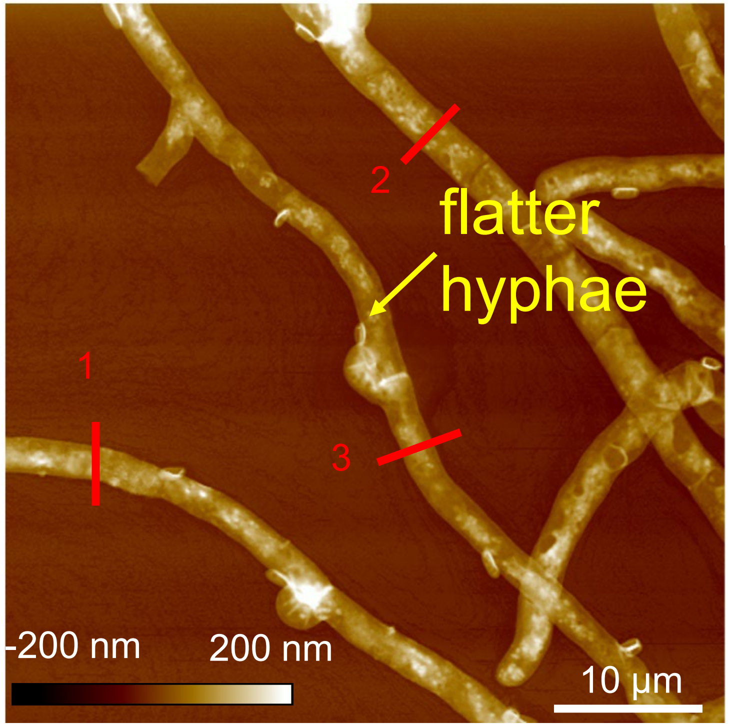
Side by side SEM

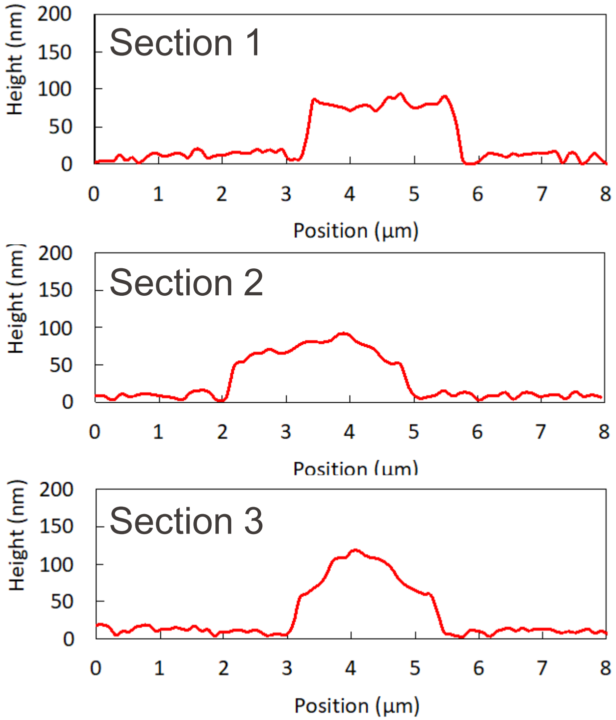


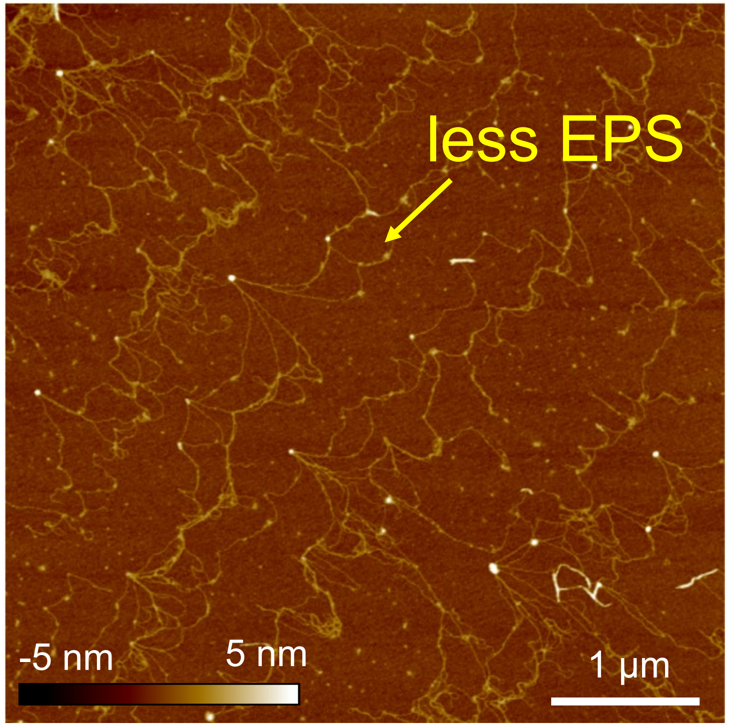
Mycelium growth with added nanocellulose



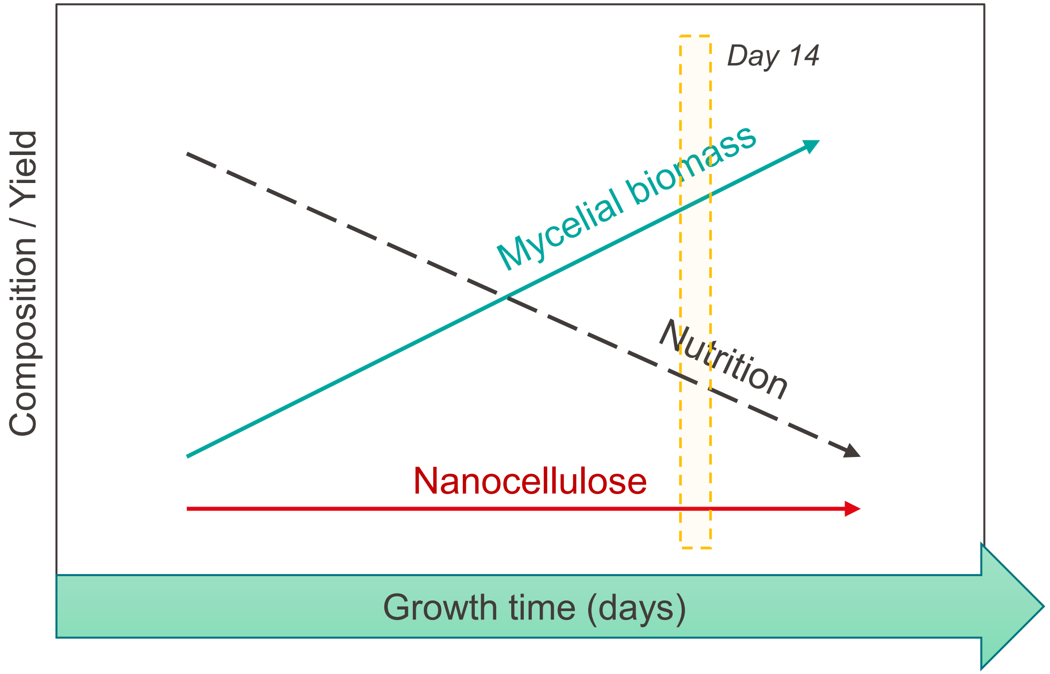
- Deactivate
- Dialyze (12-14 kDa)
- Solid part (mycelium) and liquid part (media)
- Where's the nanocellulose?







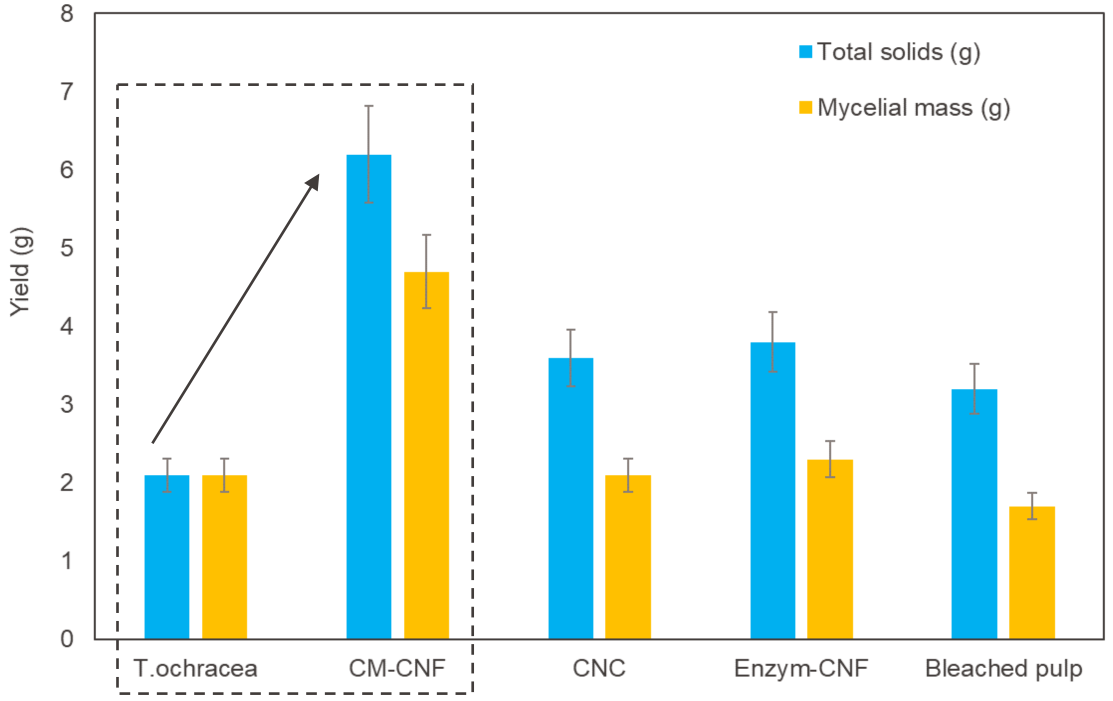
Schematic yield and composition during growth



A + B → C ?

- Biomass composition depends on when growth is stopped
- Under conditions of ample nutrition, nanocellulose content is fixed
- Whether nanocellulose is located in the pellets or in the surrounding medium depends on growth time
- After 14 days, all nanocellulose is in the pellets

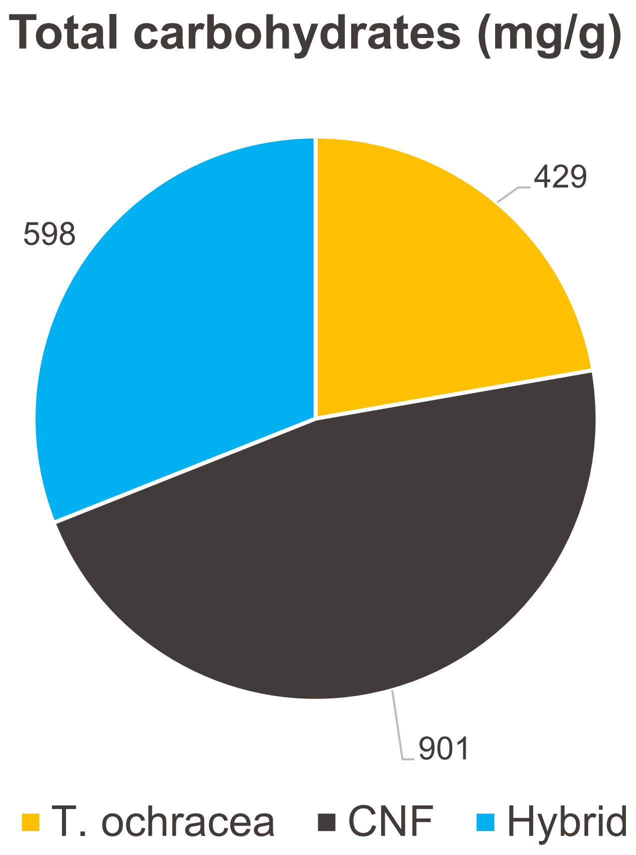
Yield after day 14



Yield:

- Total solids includes mycelium, EPS, and added nanocellulose
- Mycelial mass (mycelium+EPS) corrects for added nanocellulose
- Increase in total solids with carboxymethylated CNF

Bulk composition after day 14

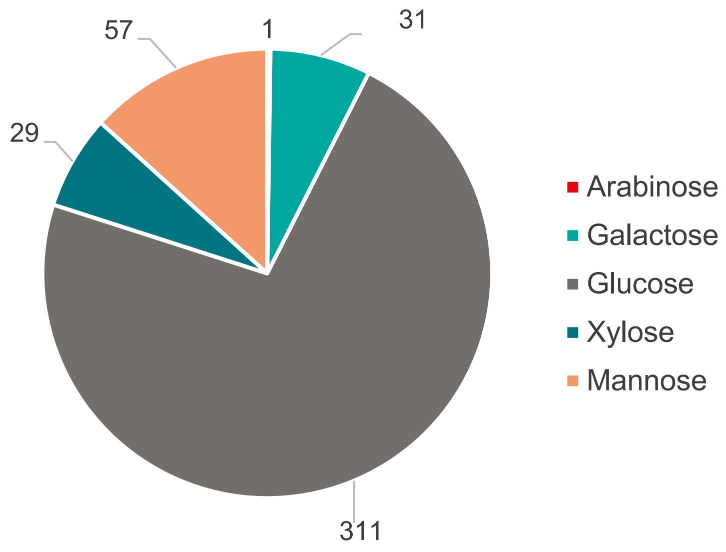


- Hybrid and pure mycelium also contain **lipids** and **protein**
- CNF is basically all carbohydrate

Bulk composition after day 14

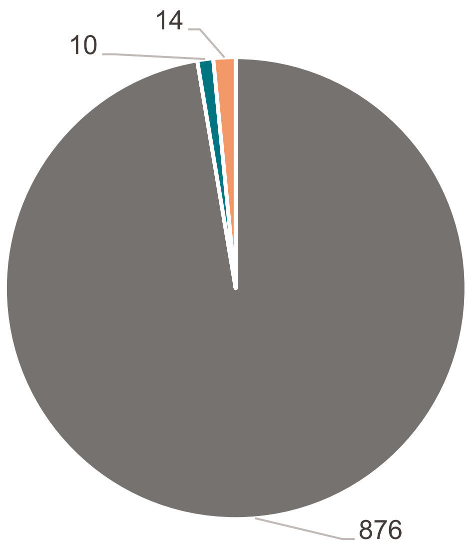
Detailed sugar analysis:

T. ochracea



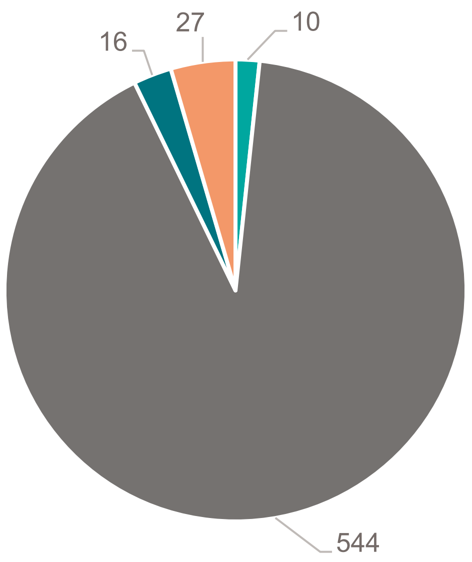
Mostly glucose & mannose

CNF



Mostly glucose

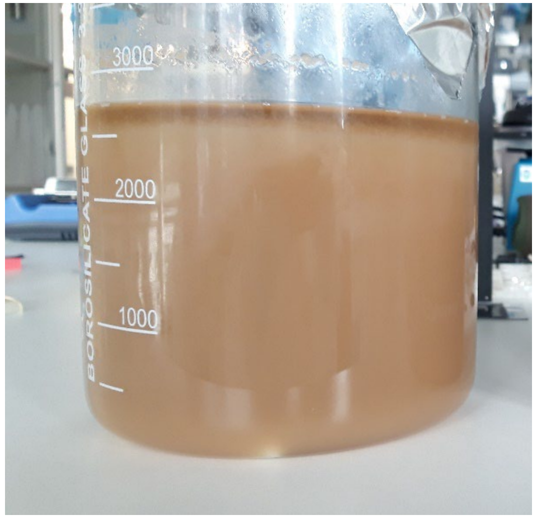
Hybrid



Mostly glucose & mannose

Materials: Dewatering into films

Mycelium-CNF slurry

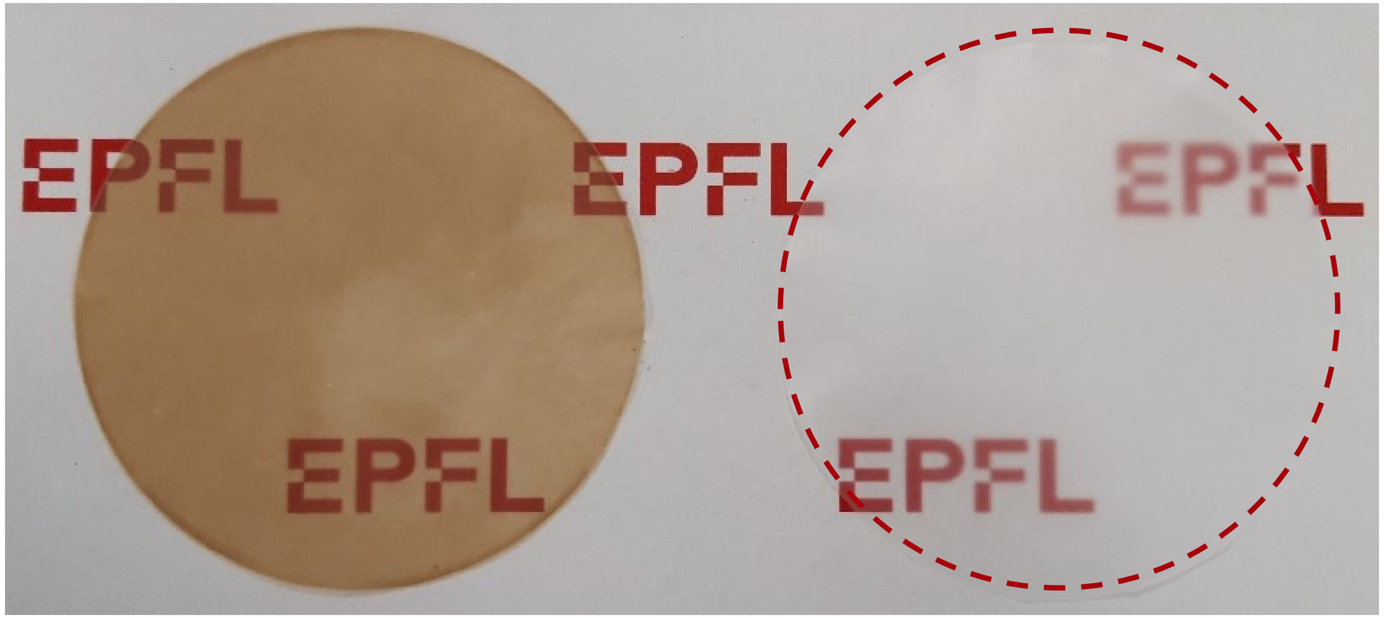


Homogenized:
Solid + liquid

Vacuum filtration

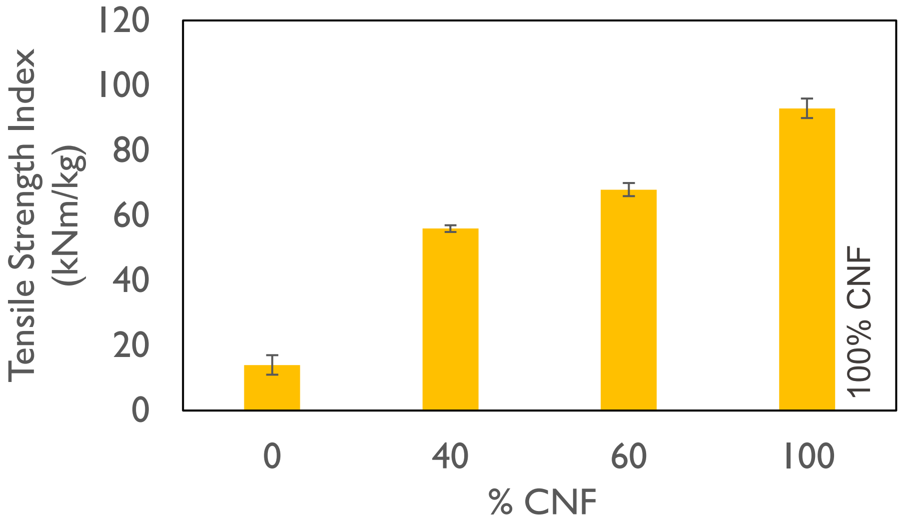
Mycelium-CNF hybrid

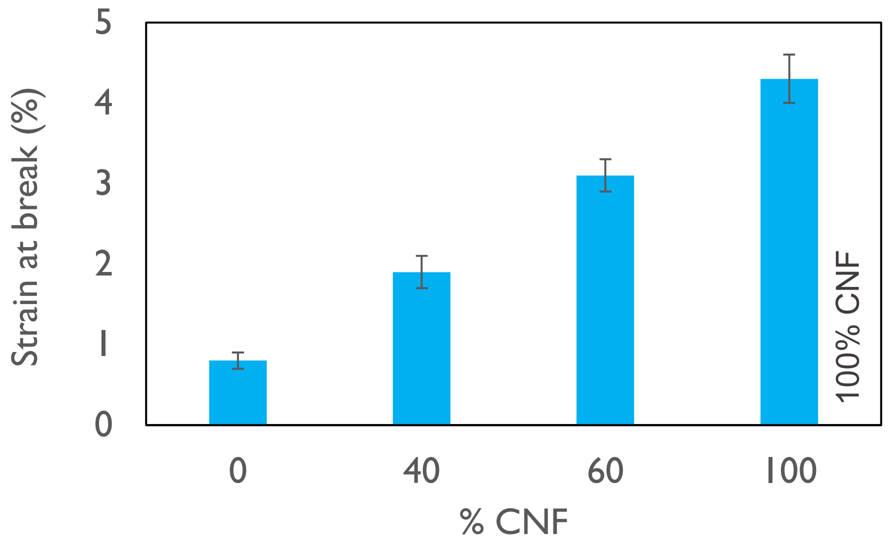
Pure CNF



- Approximately 30 g/m^2
- Drainage more efficient with hybrid due to clustered CNF, bound up in mycelium network

Summary of film properties: mechanical

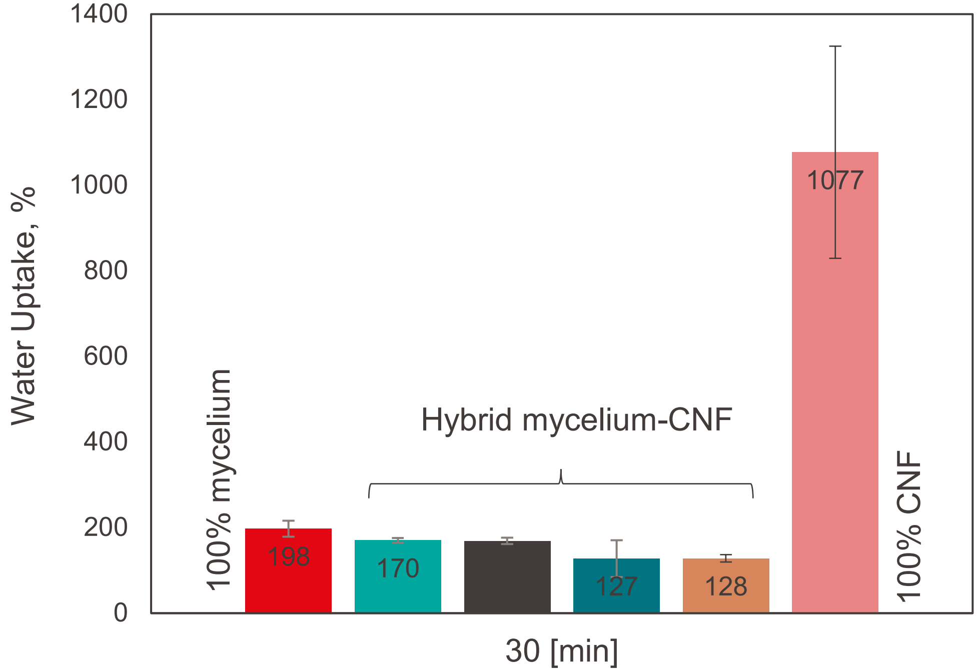


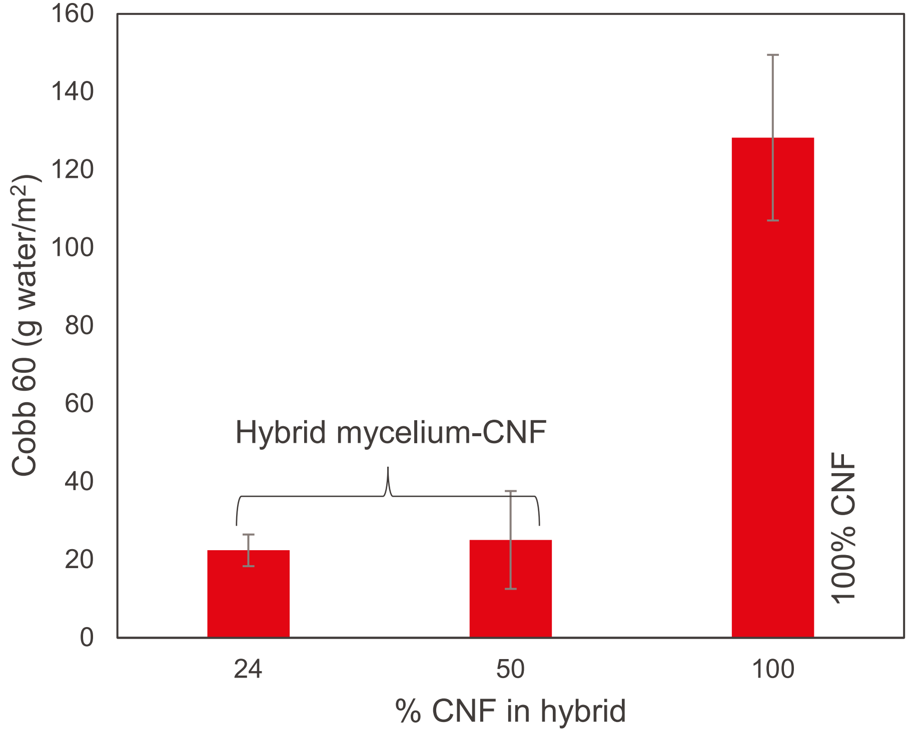


Mechanical properties depend on growth derived composition

%CNF can be changed by adding more CNF (or changing growth time)

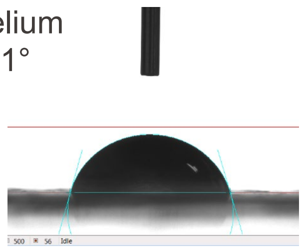
Summary of film properties: interactions with water



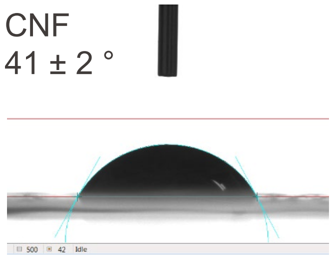


Static contact angle

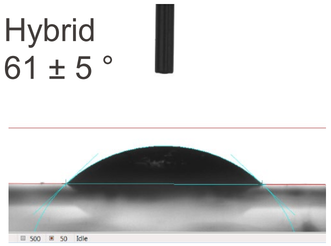
Mycelium
$73 \pm 1°$



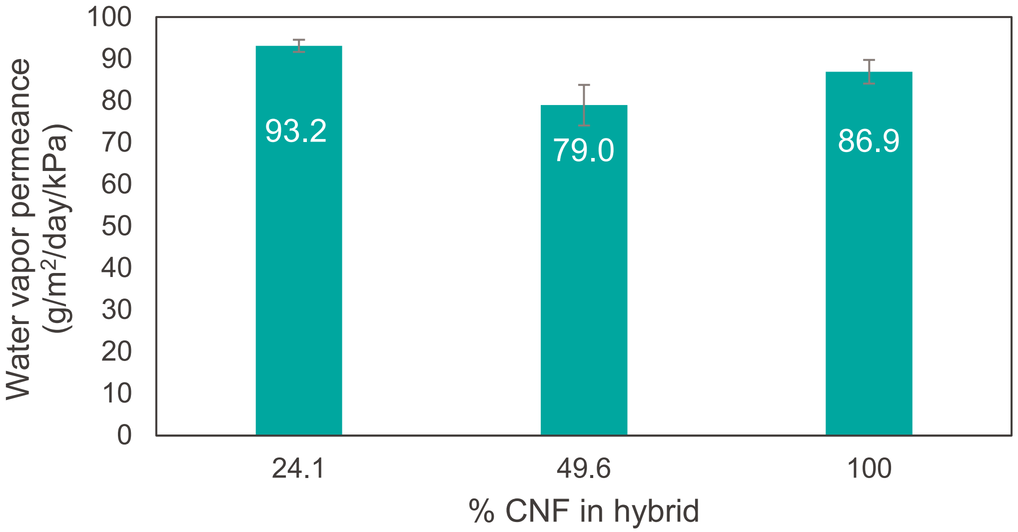
CNF
41 ± 2 °

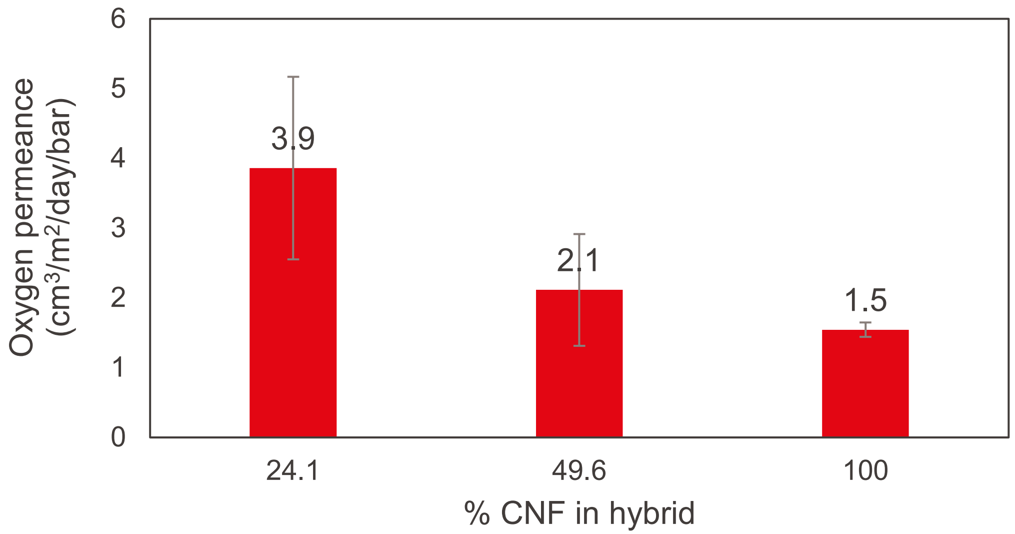


Hybrid
61 ± 5 °



Summary of film properties: barrier



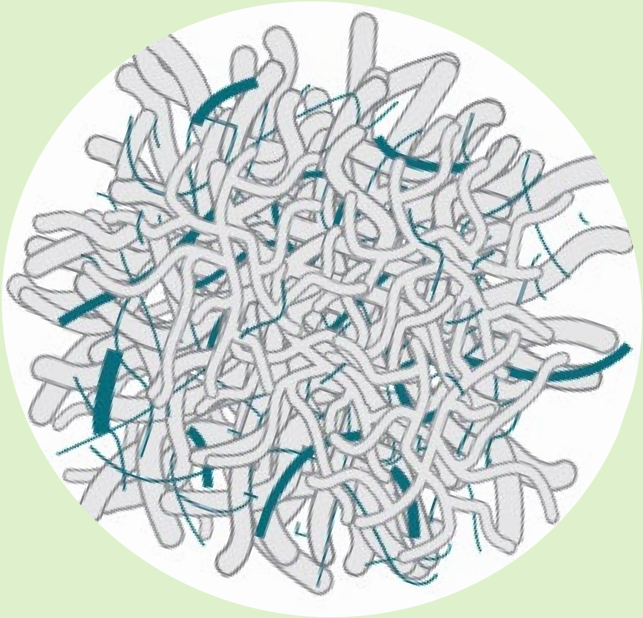


→ **Water vapor permeance of hybrid is similar to pure nanocellulose**

→ **Oxygen permeance of hybrid is similar to pure nanocellulose**

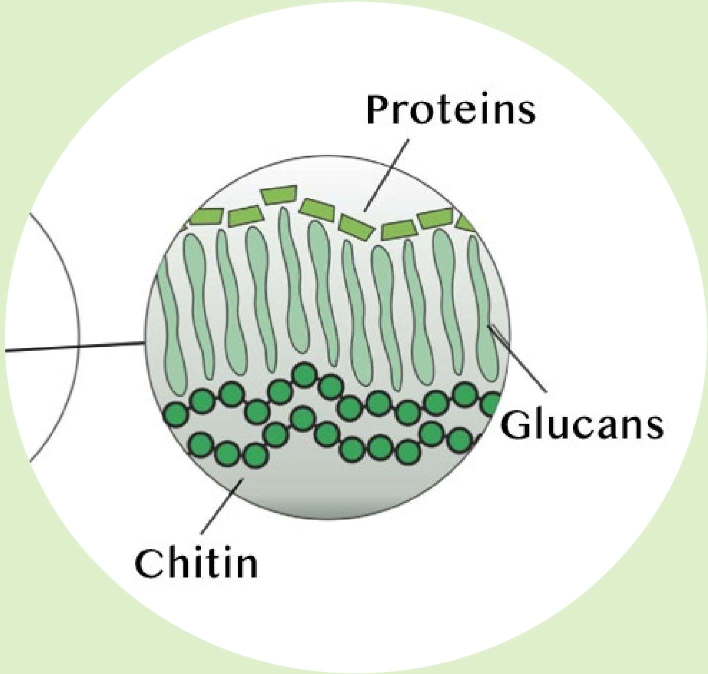
Recap: Nanocellulose and fungal mycelium

At the mm-µm scales:



Nanocellulose is concentrated within the fungal mycelium pellets

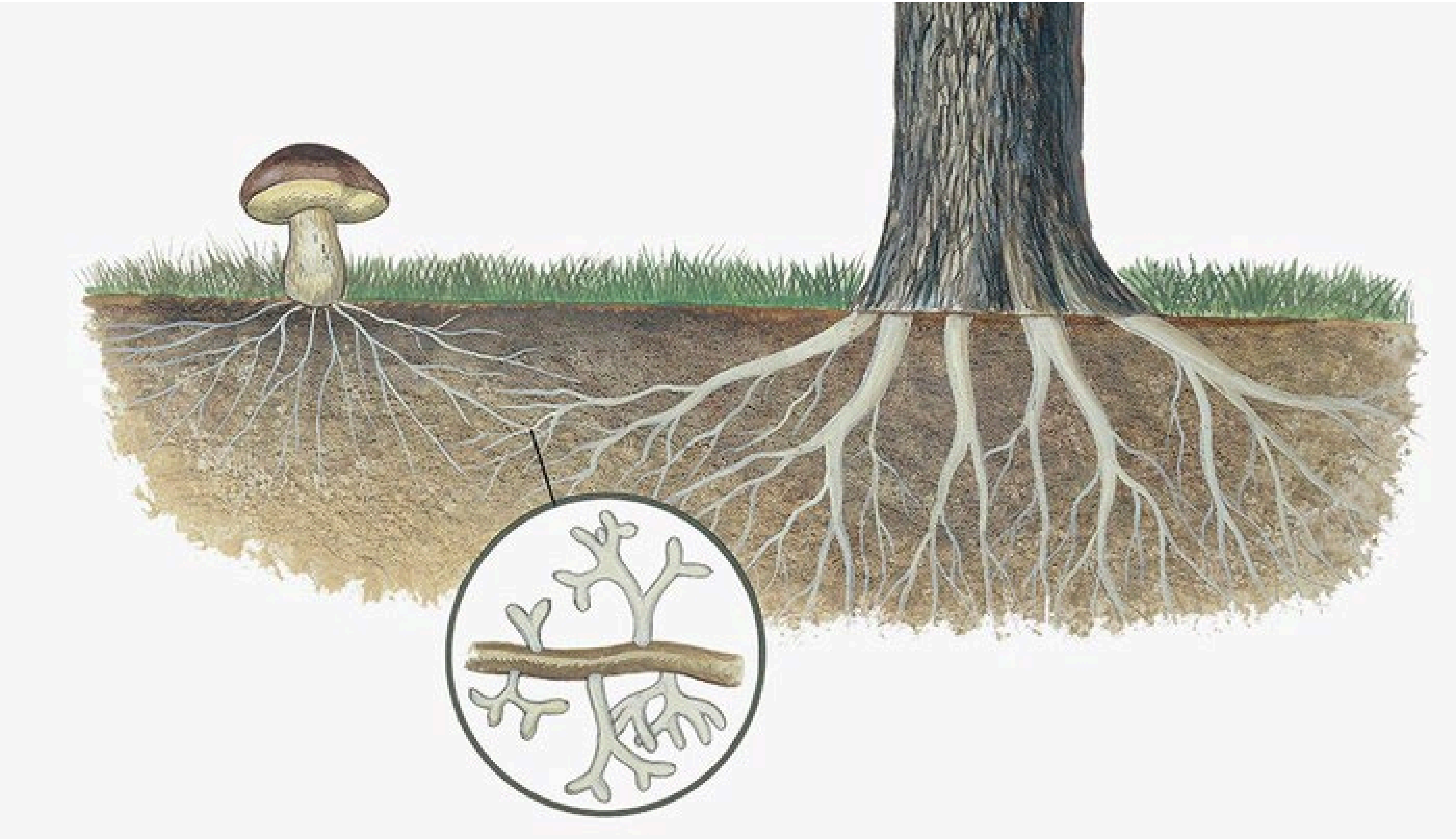
At the nm scale:



NC integrated within the hyphal cell wall/more chitin/replacing glucans?

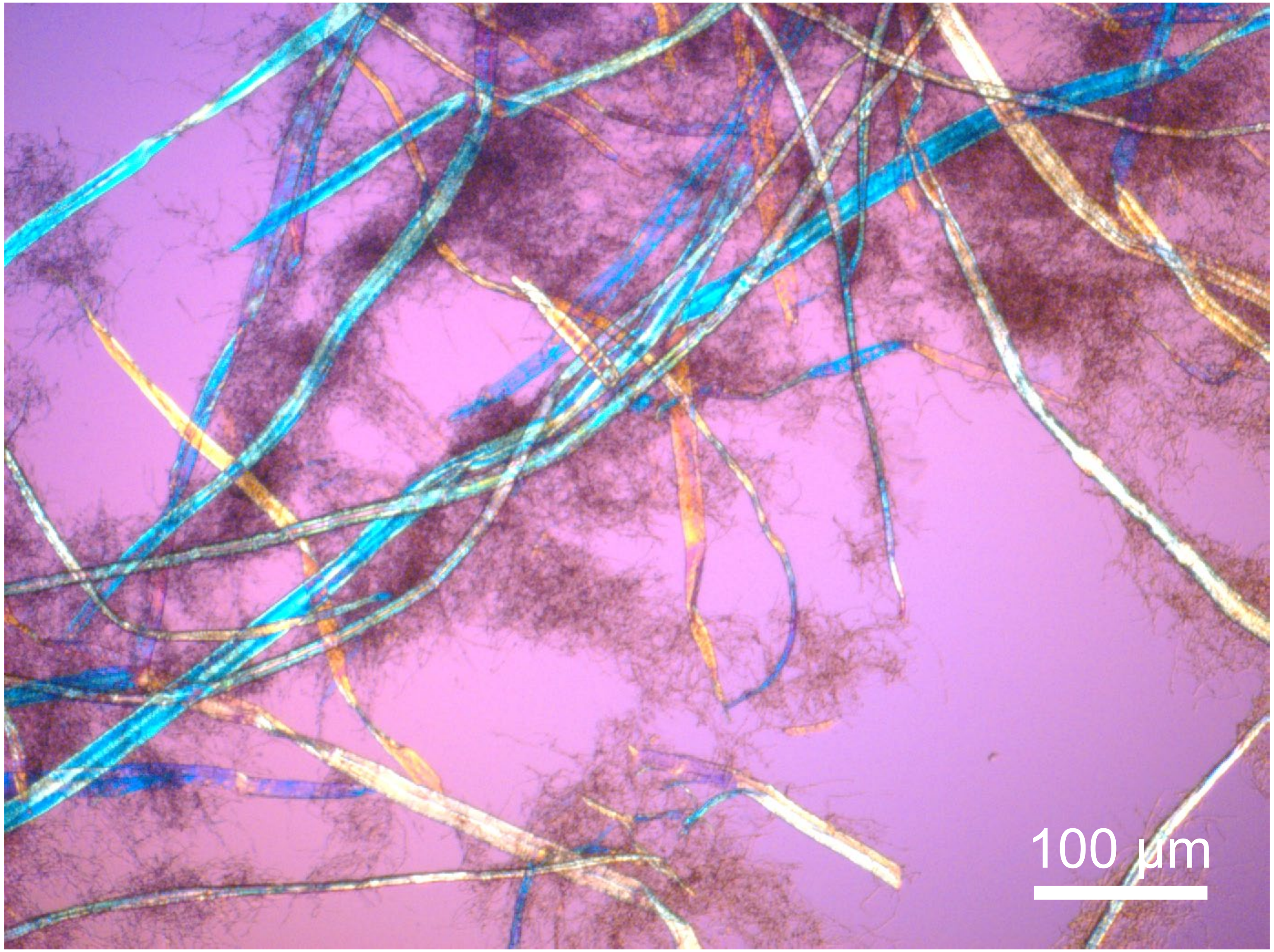
EPFL

Inspiration from mycorrhizae



https://www.sciencefocus.com/nature/mycorrhizal-networks-wood-wide-web/

Lab-grown mycorrhiza mimic ?



Conclusions

Fungal modification of nanocellulose:

- Changes yield, secretome (biofilm), and mycelium cell wall
- Masks surface interactions of cellulose
- Makes nanocellulose less nano
- Improves or maintains “bulk” properties expected from nanocellulose
- Is a way to grow hybrids, where growth conditions change material composition and properties