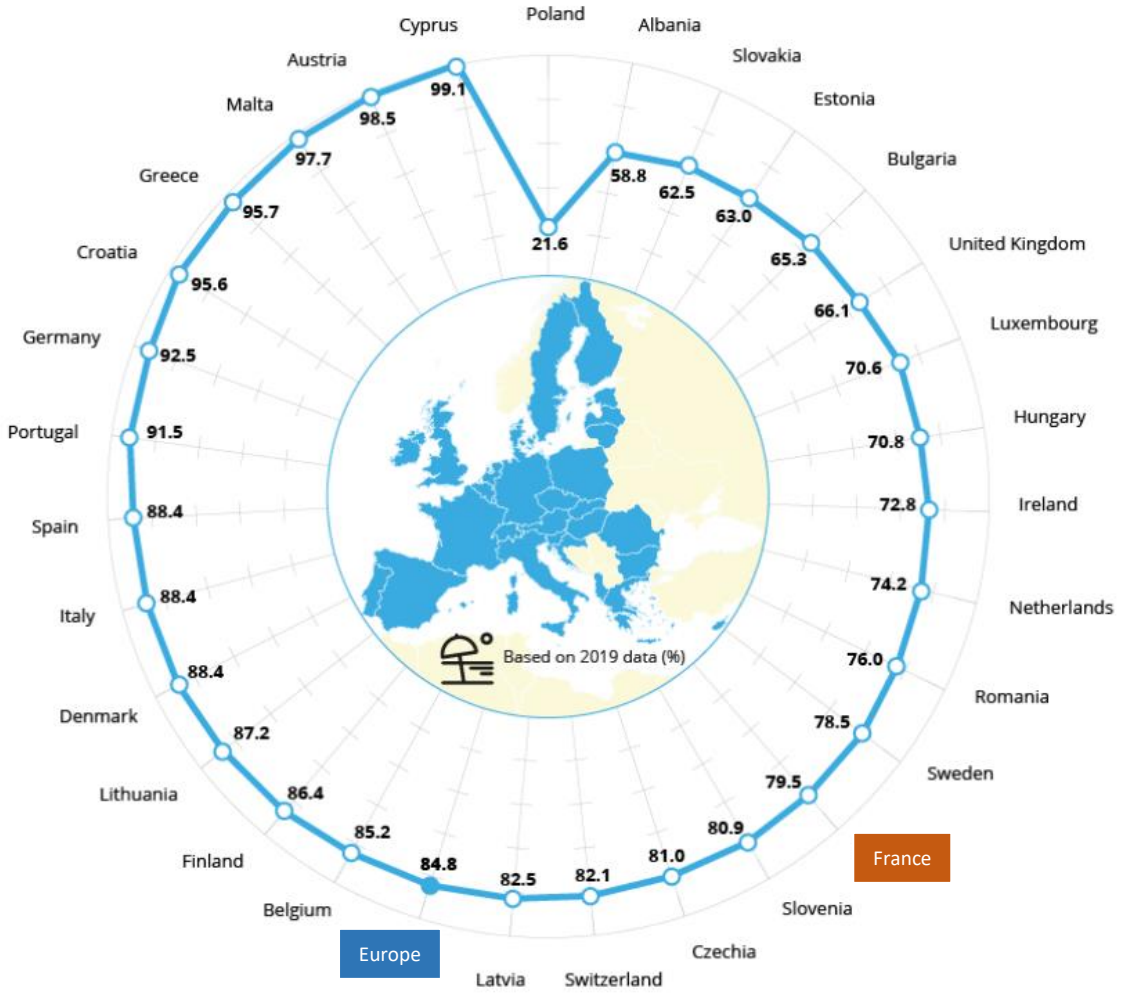
Freeze-casting as a technique to enhance the encapsulation of bacteria for biodegradation applications

<u>Corentin ESCHENBRENNER</u>. Advisor: Francisco M. FERNANDES

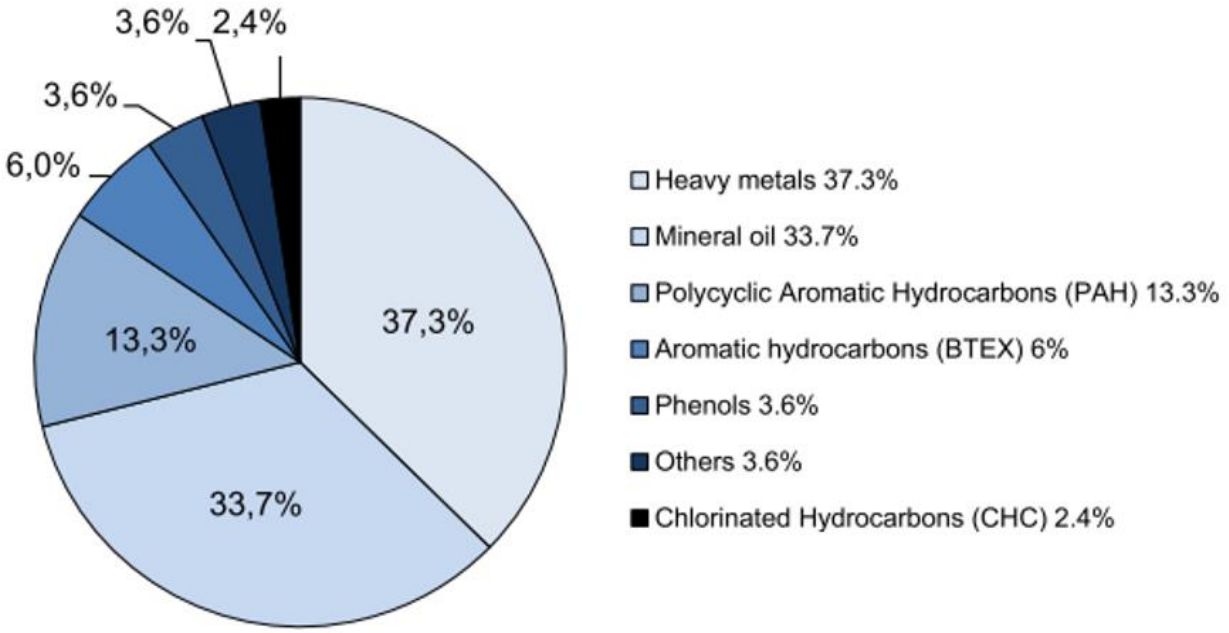
26/10/2020



Proportion of bathing waters with excellent quality in European countries in 2019

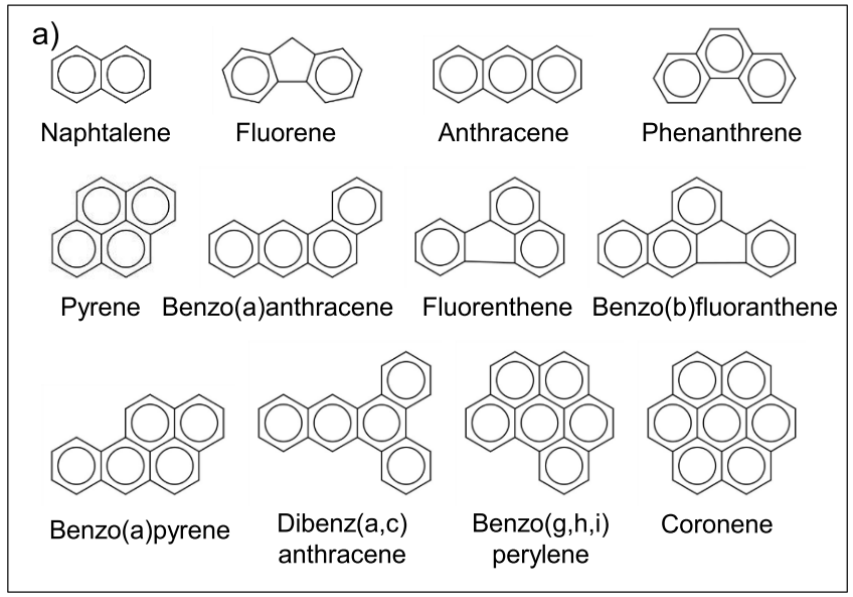
Pollutants in European aquifere:

- Heavy metals: 37 %
- Mineral oils: 34%
- PAH (polycyclique Aromatic Hydrocarbons) & BTEX (Aromatic hydrocarbons): 19%

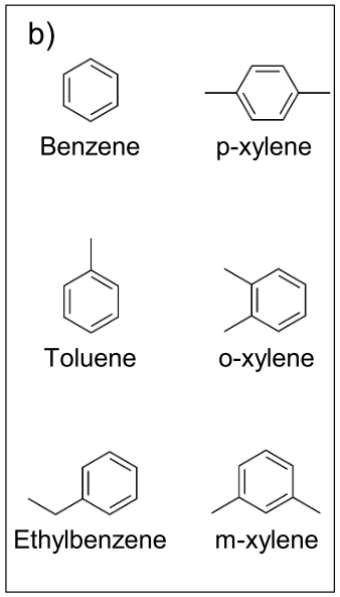


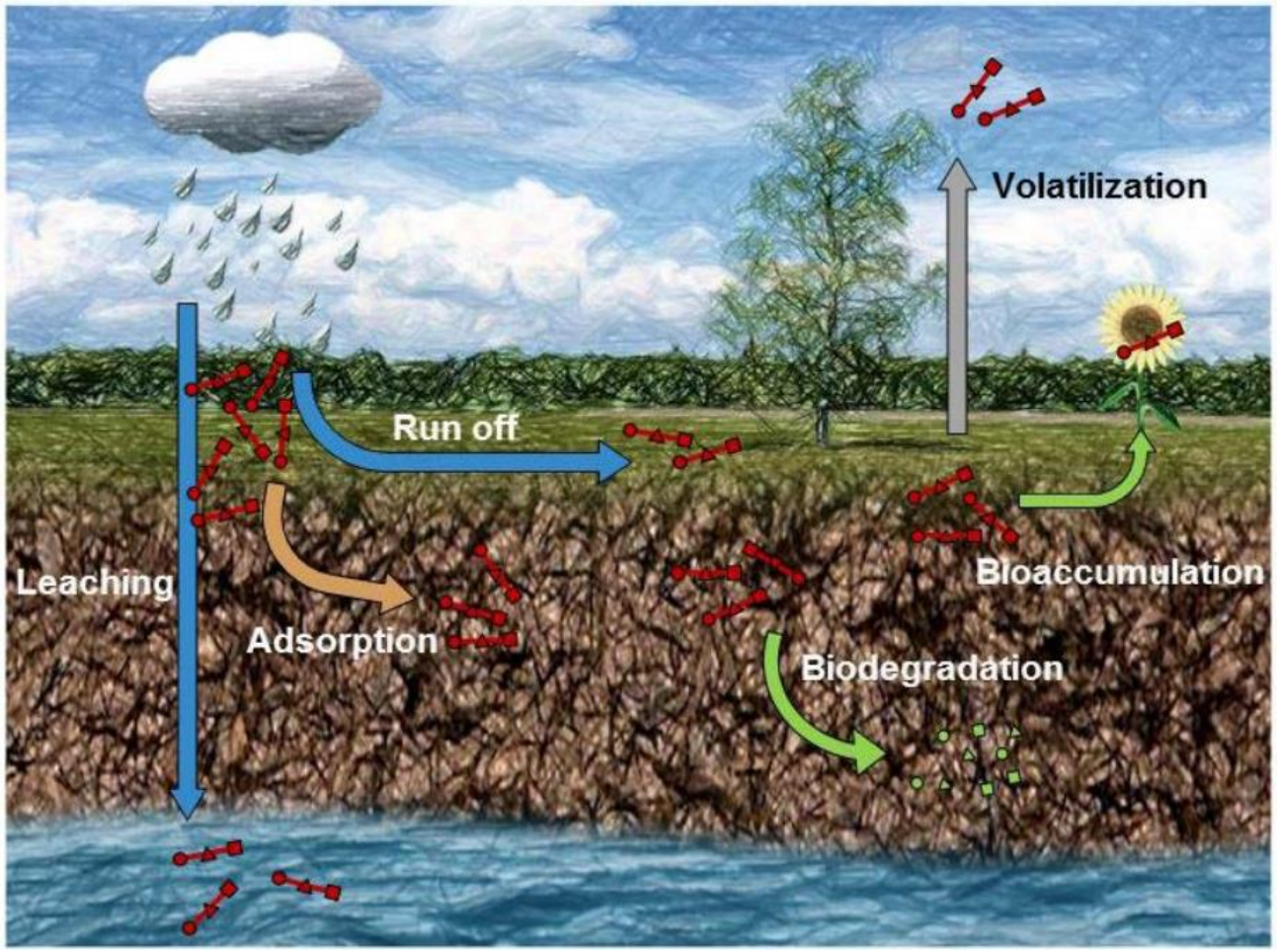
Adapté de European Environmental Agency

PAH



BTEX



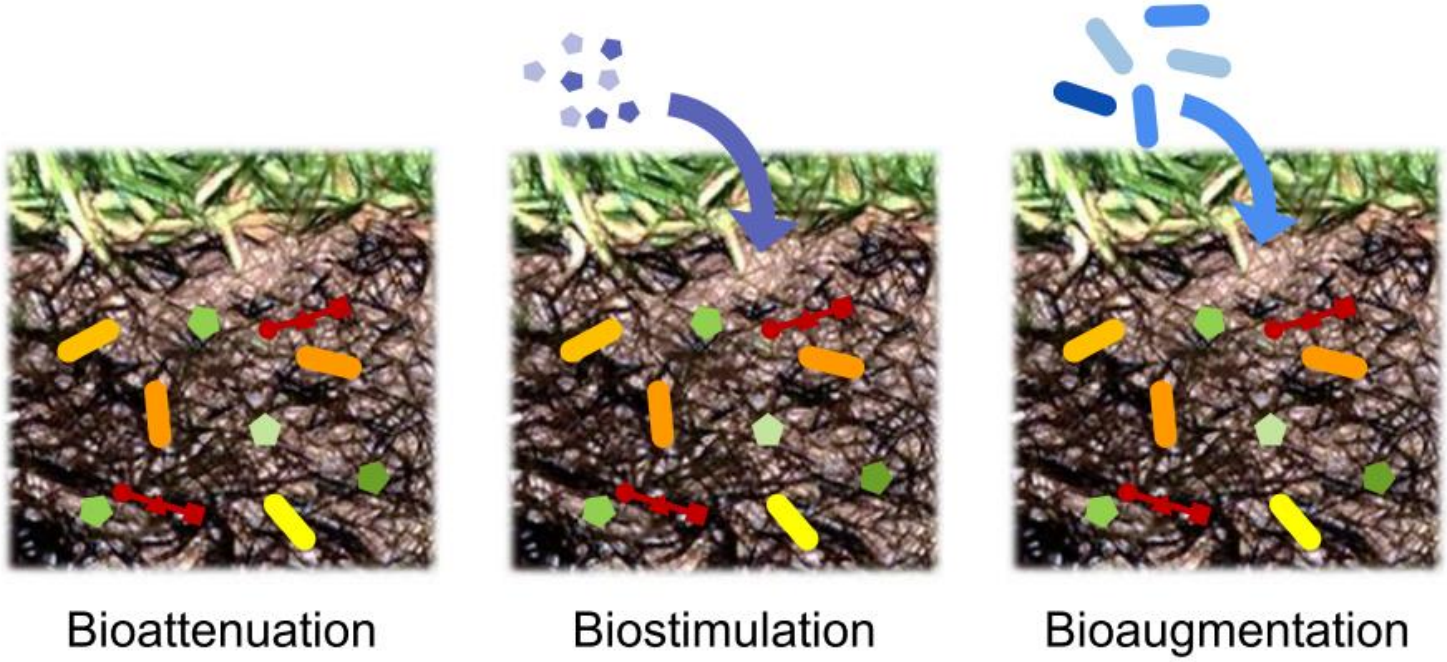


Biodegradation: Natural response against pollutant accumulation

Bioattenuation: Natural response of microorganisms already present, against pollutant accumulation

If too slow, we may help:

- **Biostimulation** → Stimulation of species already present by adding nutrients and other compounds
- **Bioaugmentation** → Introduction of **exogenous microorganisms** specifically degrading the targeted pollutants



Biodegradation

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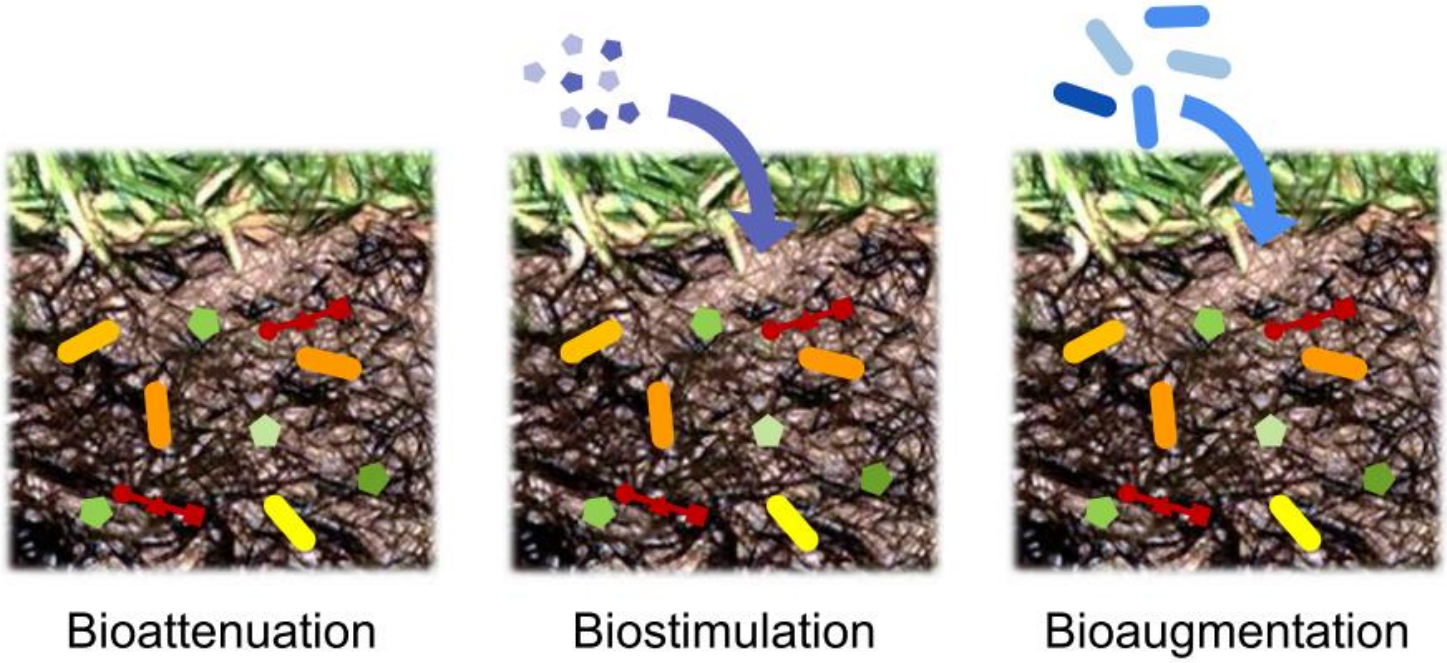
Encapsulation in matrices:

- Protection of exogenous microorganisms
- Limiting their release into the environment



Biodegradation

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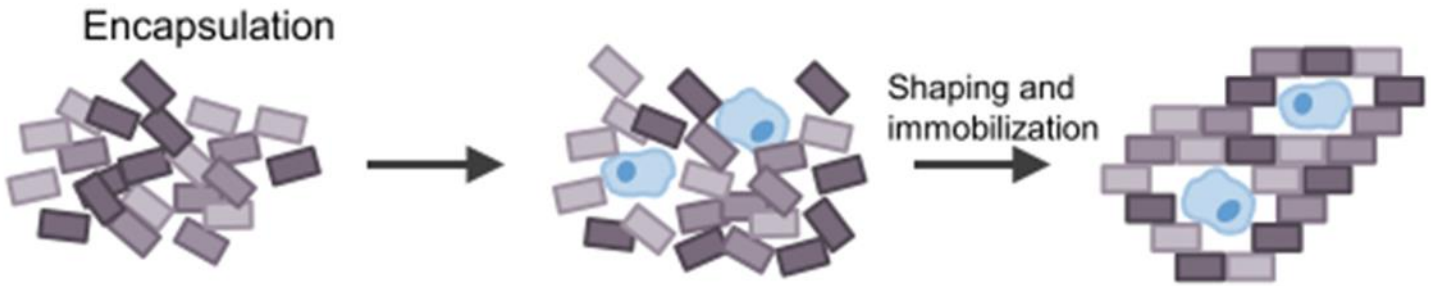
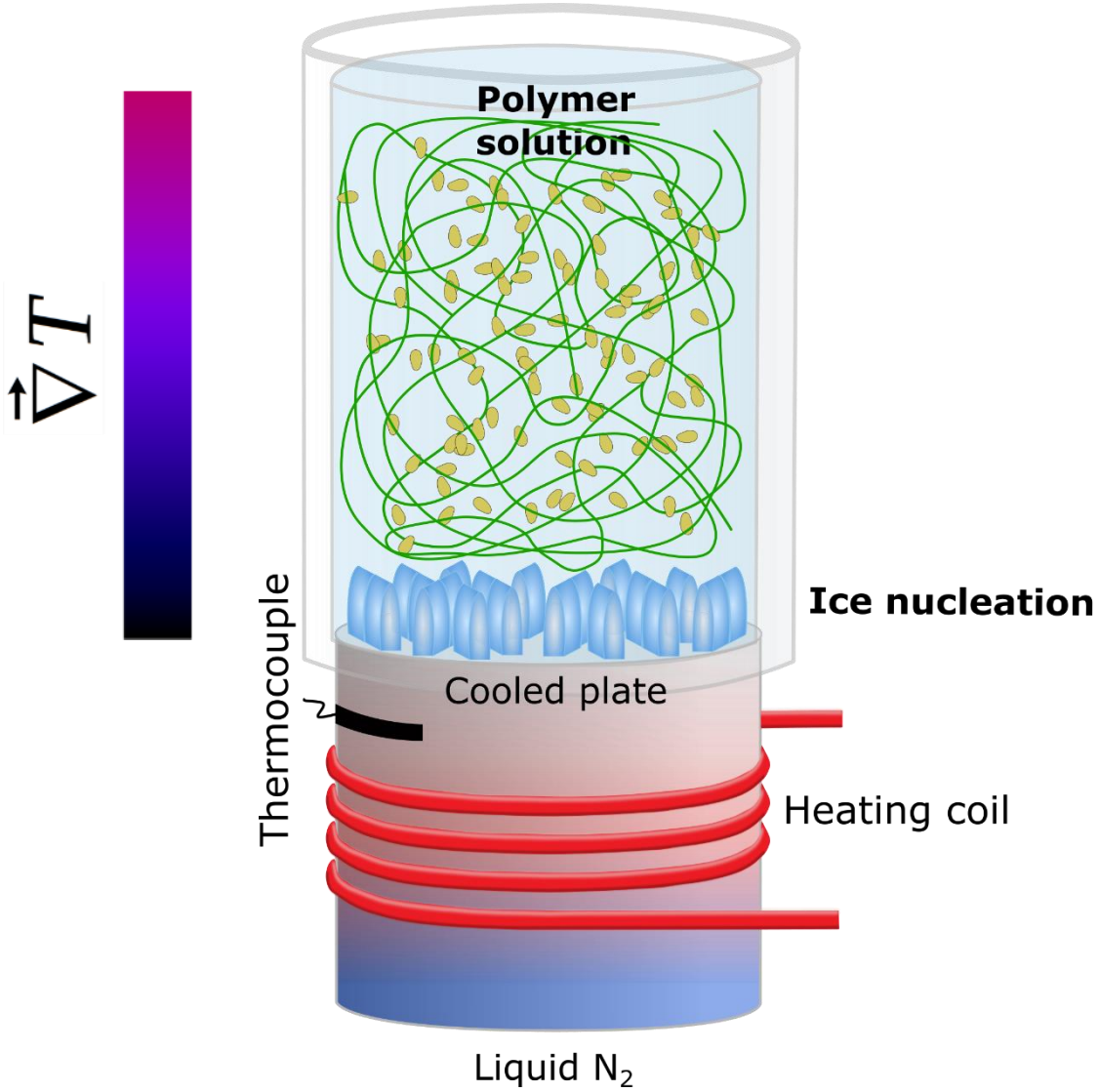


Shapping strategy:



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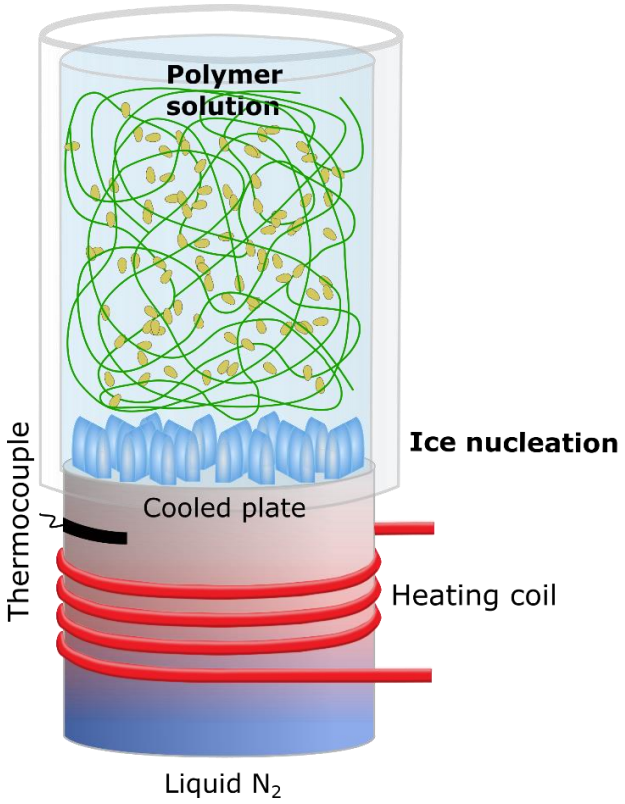

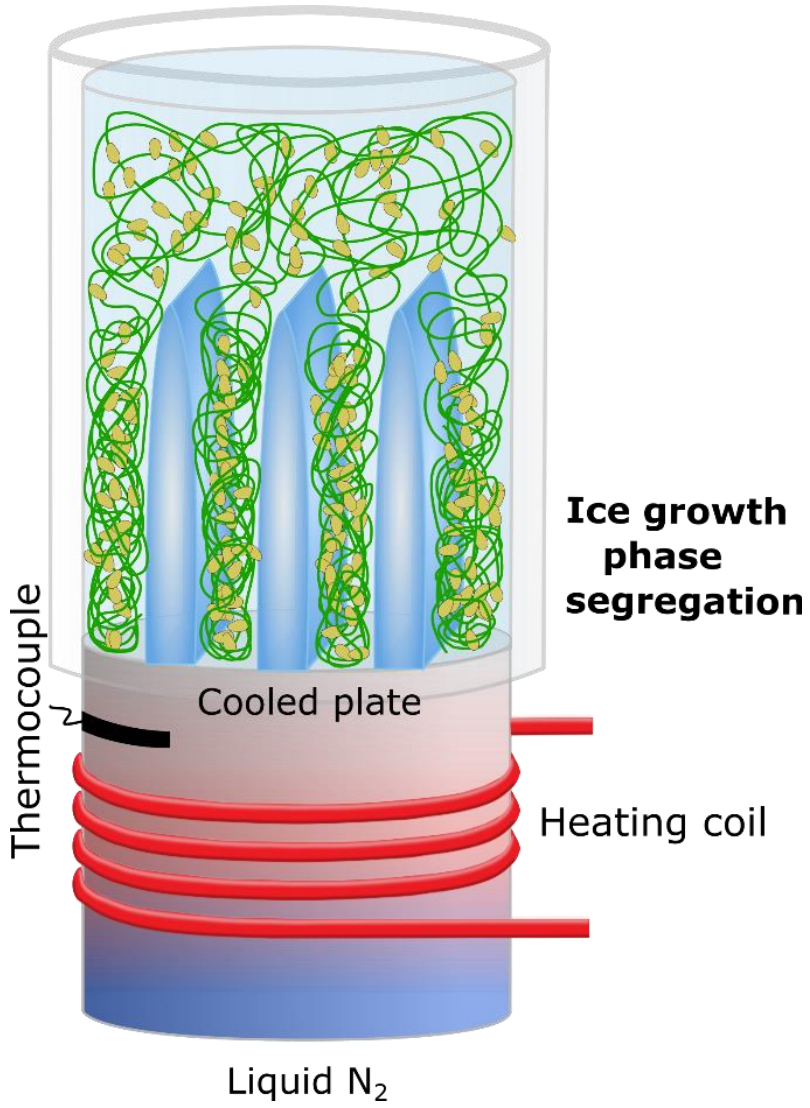

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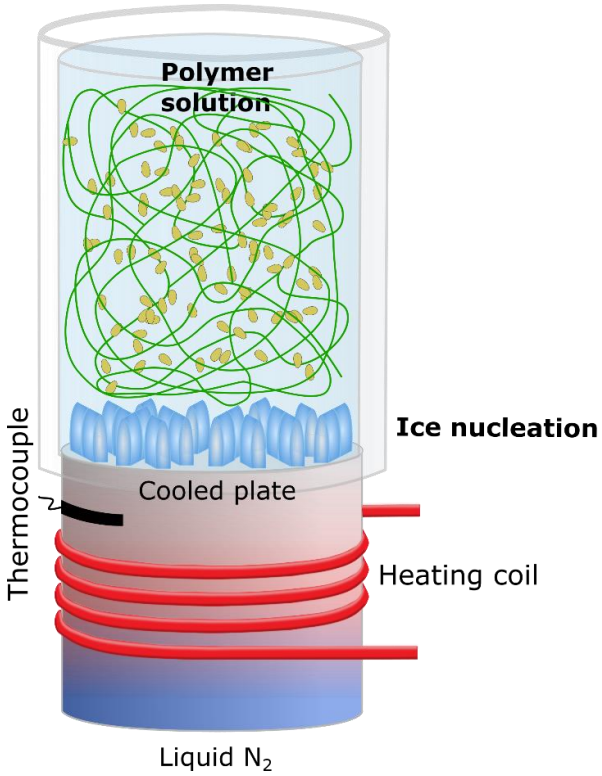

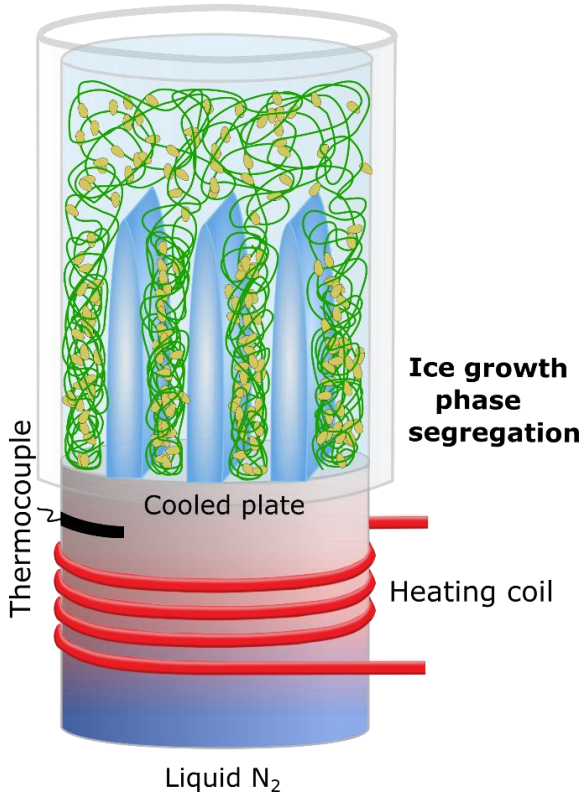

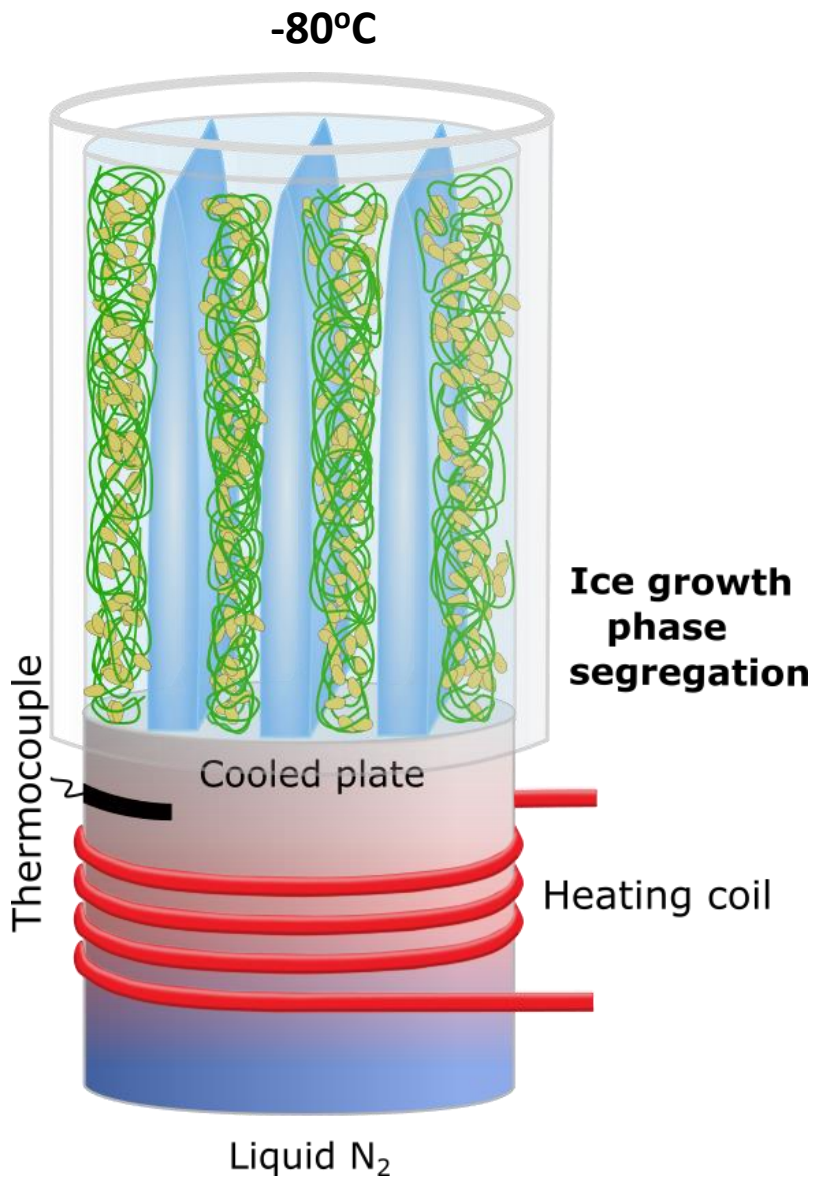

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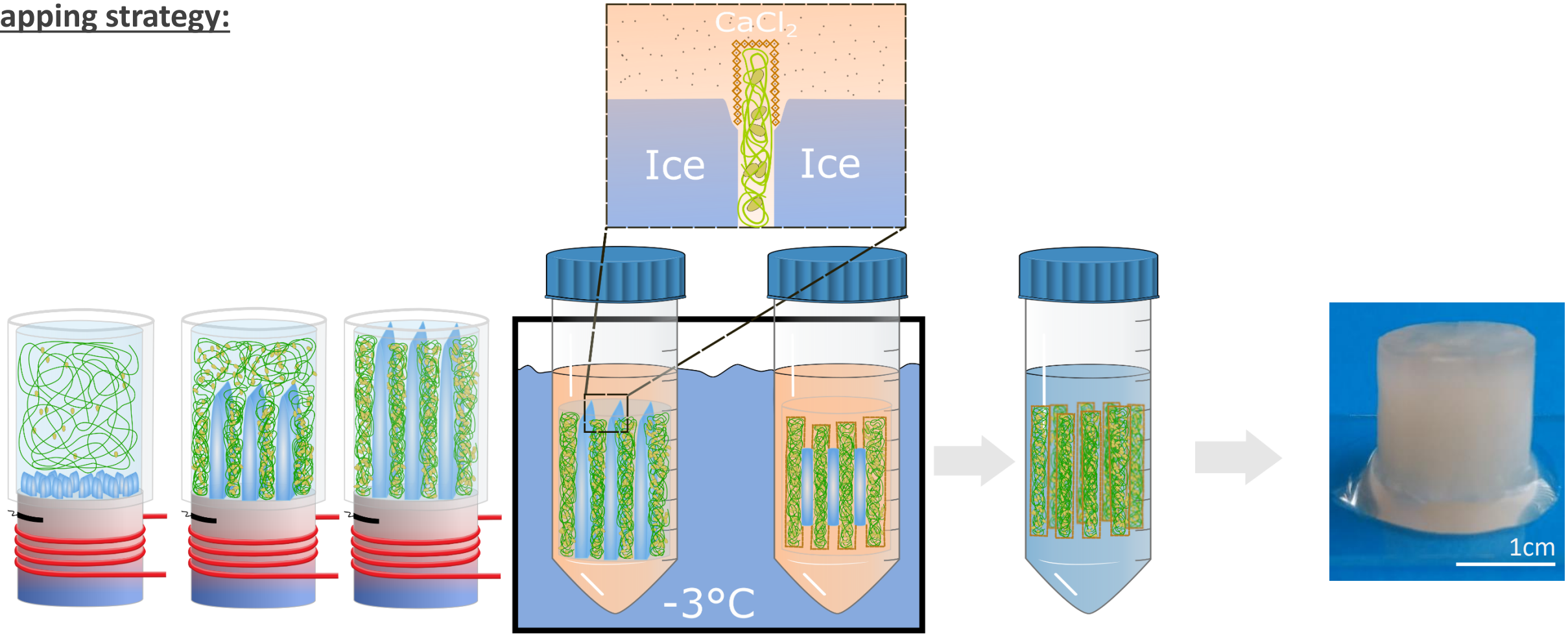
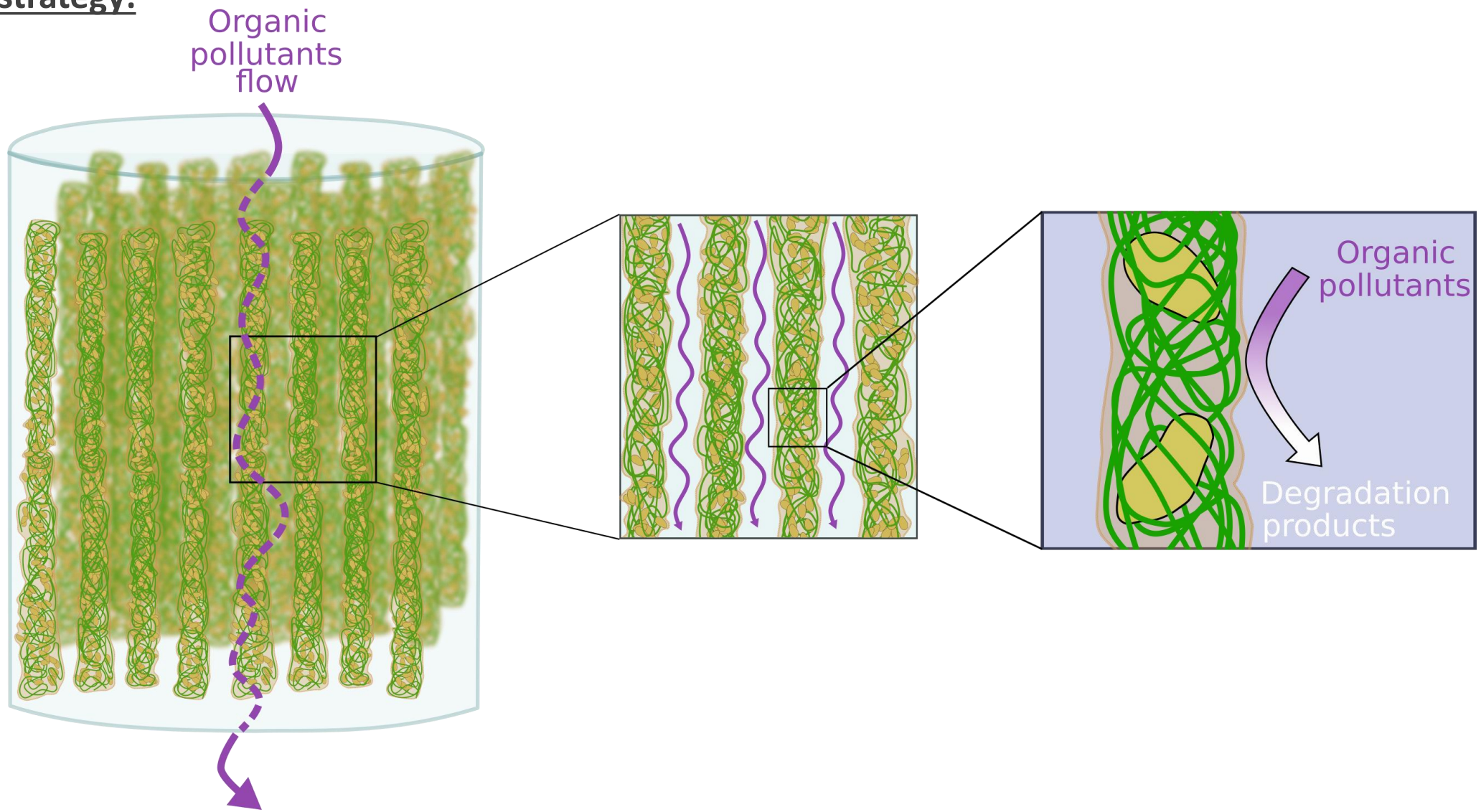
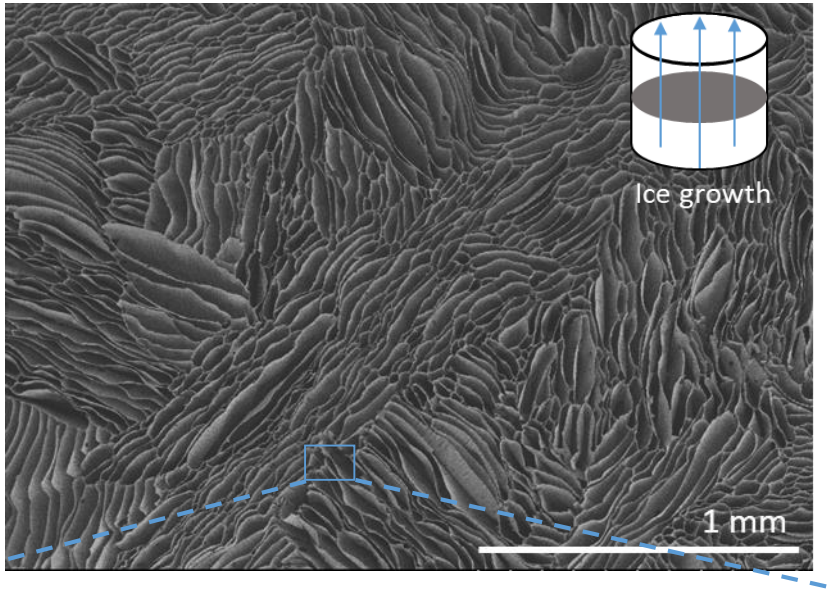


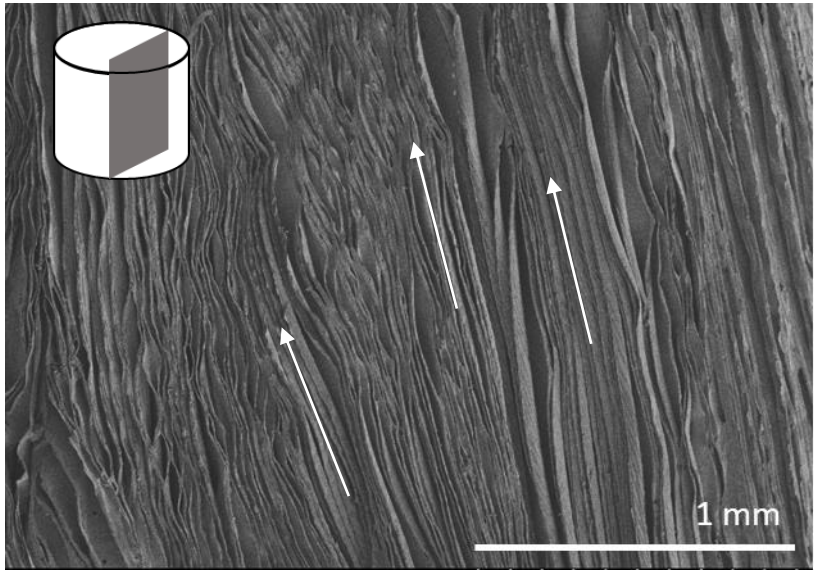
Polymer and bacteria solution → Freeze-casting → Topotactic Cross-linking → Bacteria encapsulated in alginate structured hydrogel

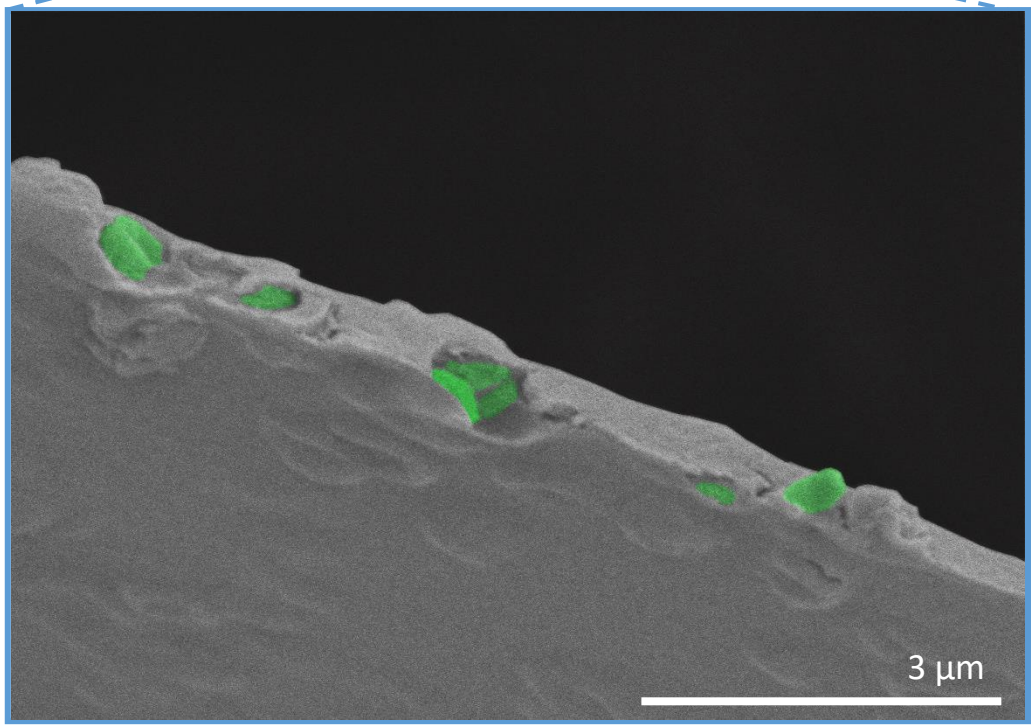
Depollution strategy:

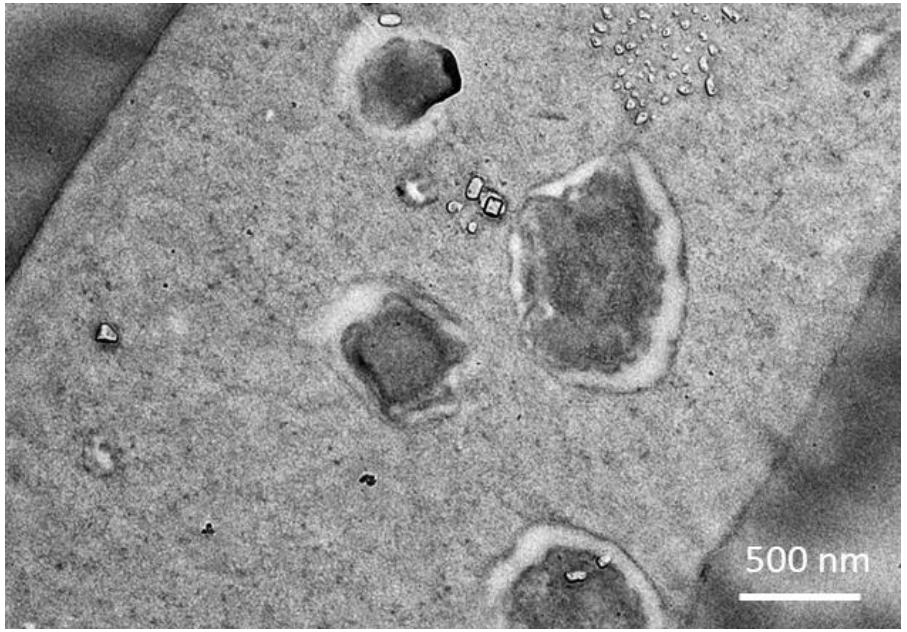


SEM









How bacteria survive to these low temperatures?

How to stabilize matrices to use them in water?

Are bacteria surviving to all process?

What's about depollution?

Thank you!

Poster 9

Freeze casting as a technique to enhance the encapsulation of bacteria for biodegradation application

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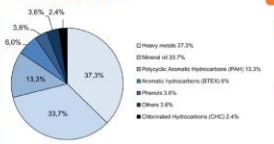
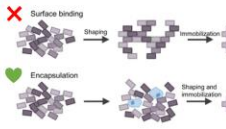
Sorbonne Université, Laboratoire de Chimie de la Matière Condensée, LCMCP, F-75005 Paris, France

Introduction



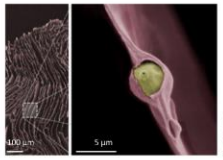
More commons pollutant found in European aquifer are Heavy metals, Mineral oil and PAH.

Strategy to clean-up: Bioremediation



Bacteria are able to clean-up the pollutants of waste water. The best strategy to enhance there activity and protect them is the encapsulation inside matrices during the shaping process.

... but which process is relevant?



Freeze-casting!

This ice-templating process allows the encapsulation of cells at low temperature without using cryoprotectants and kept them alive.

Here is a **yeast cell** (in green) inside alginate wall. But what about bacteria encapsulation for waste water treatment?

Objective

Encapsulate bacteria P. putida alive in stabilised matrices of alginate tuned by freeze-casting

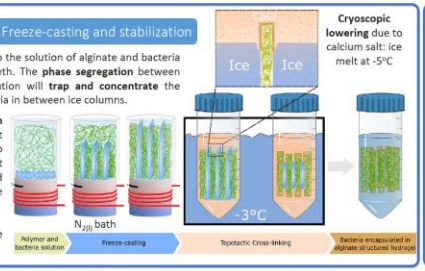
Methodology

Freeze-casting and stabilization

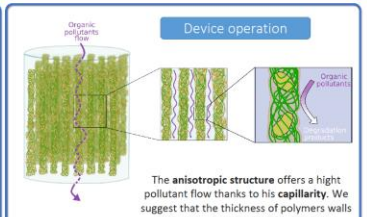
The **thermal gradient** applied to the solution of alginate and bacteria induce an **anisotropic ice** growth. The **phase segregation** between pure ice crystals and the solution will **trap and concentrate** the alginate polymer and the bacteria in between ice columns.

A **stabilizing solution of calcium** at -3°C melt the ice in contact and allow the calcium ions to **cross-link alginate walls**. It stabilizes the matrix and preserves the periodic structure tune by freeze-casting.

Bacteria are among the alginate walls of this **hydrogel**.

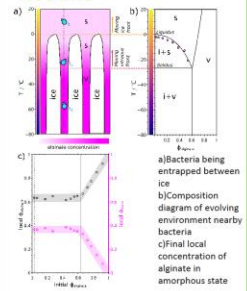


Device operation



The **anisotropic structure** offers a hight pollutant flow thanks to his **capillarity**. We suggest that the thickness of polymers walls allow a quick exchange of water and pollutants toward the bacteria and will optimise the biodegradation efficiency.

Results

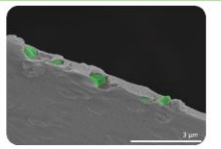


a)Bacteria being entrapped between ice
b)Composition diagram of evolving environment nearby bacteria
c)Final local concentration of alginate in amorphous state

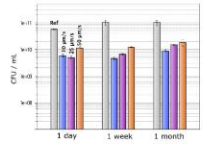
The environment surrounding bacteria change due to temperature lowering and to **phase segregation.** The alginate concentration between ice follows the liquidus and **is fixed** by its junction with the **glass transition** curve. At ~60% the water no longer freeze.

Water around bacteria is thus in **amorphous state and** preserves their viability.

Environment around bacteria during the process



Colorized in green (image), here is **bacteria entrapped** in a wall of alginate. The humping on the wall are also encapsulated bacteria.

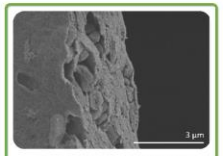


Freeze-casting allows the **storage of bacteria** at -80°C for **long time** (months) **without using any cryoprotectant**. It can be use to store bacteria **before** stabilization and application.

Encapsulation by freeze casting *(without cross-linking)*

The structure obtained is **porous** and **anisotropic**, pores have the shape of ice crystals that calcium removed.
The process of **topotactic-crosslinking preserves** thereby the structure tuned by **freeze-casting** and **stabilises** matrices for within **water application**.

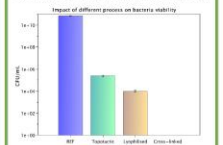
Morphology



Here is bacteria entrapped in a **stabilized** wall of alginate.

Freeze-casting preserves the viability of bacteria but it decrease widely after stabilization.

Topotactic cross-linking allow a **better viability** than the previews process to obtain stabilized matrices. But it may be **still optimized**, lowering the calcium bath time or vary the bath composition.



Entrapped bacteria viability

Conclusion and Prospects

- ✓ Freeze casting has successfully entrapped bacteria
- ✓ Freeze casting technic allows for long time storage at low temperature without any cryoprotectants
- ✓ Topotactic cross-linking stabilizes matrices in hydrogels and preserves the periodic structure tuned by freeze-casting
- ➢ On going work on cross-linking condition to enhance again the bacteria viability
- ➢ Biodegradation experiments are coming soon

Acknowledgments
Kankan Qin for his wonderful work on alginate and on freeze-casting
Francisco Fernandes for his wise advises and his process imagination
ANR for financial support

Qin, K. *et al. ArXiv* (2020). doi:2004.08682 ; Christoph, S., *et al. Macromol. Biosci.* **16**, 182–187 (2016). ; Cassidy, M., *et al. J. Hazard. Mater.* **16**, 79–101 (1996).