

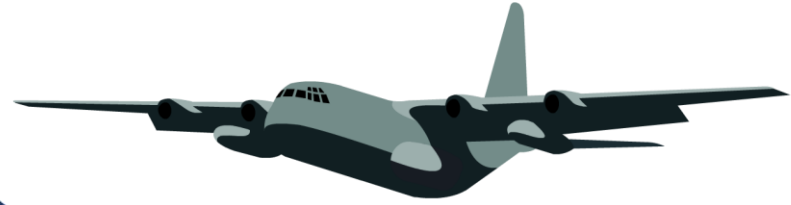


WILL BE THREE A MULTITUDE IN THE NITRIFYING COMPARTMENT ?

**FIRST STEPS TOWARDS THE CHARACTERIZATION OF A NOVEL SYNTHETIC COMMUNITY
BY USING FLOW CYTOMETRY AND ATOMIC FORCE MICROSCOPY.**

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The future of humanity



In space no one can hear you scream



Long distance trips



1 light year = 9.46×10^{12} km

No restock sources

No spacial travel assistance

Exposure to radiation

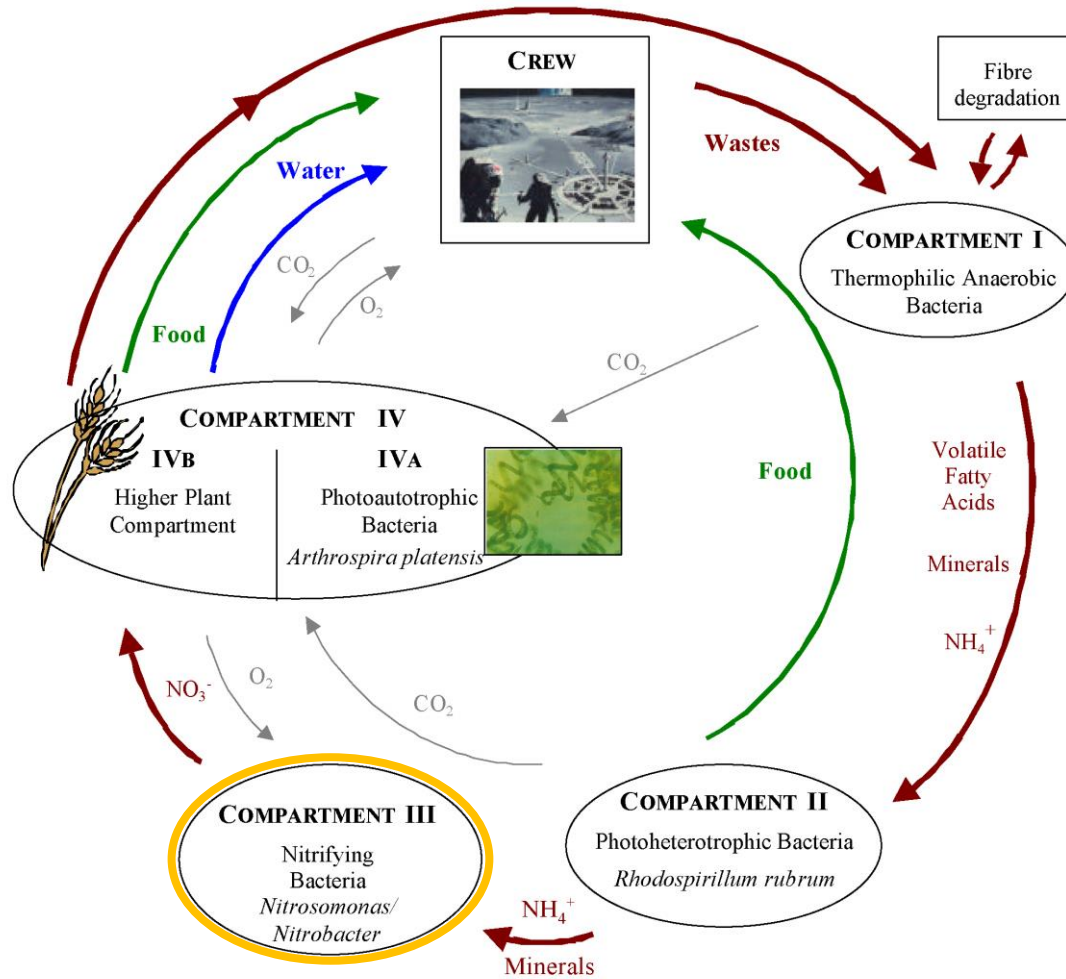
No gravity



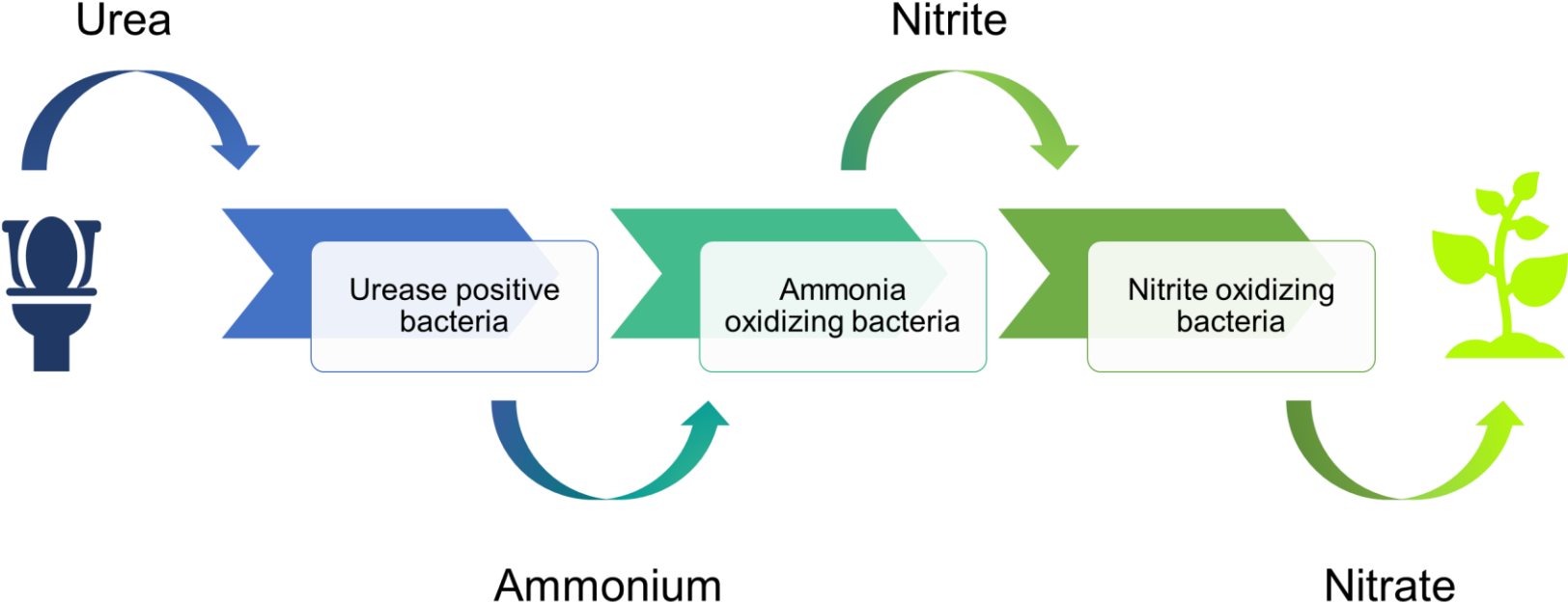
How are we going to survive under these conditions?



Non Edible Parts of Higher Plants

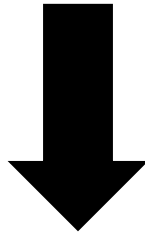


Key players



URiNIS

URiNe Nitrification In Space

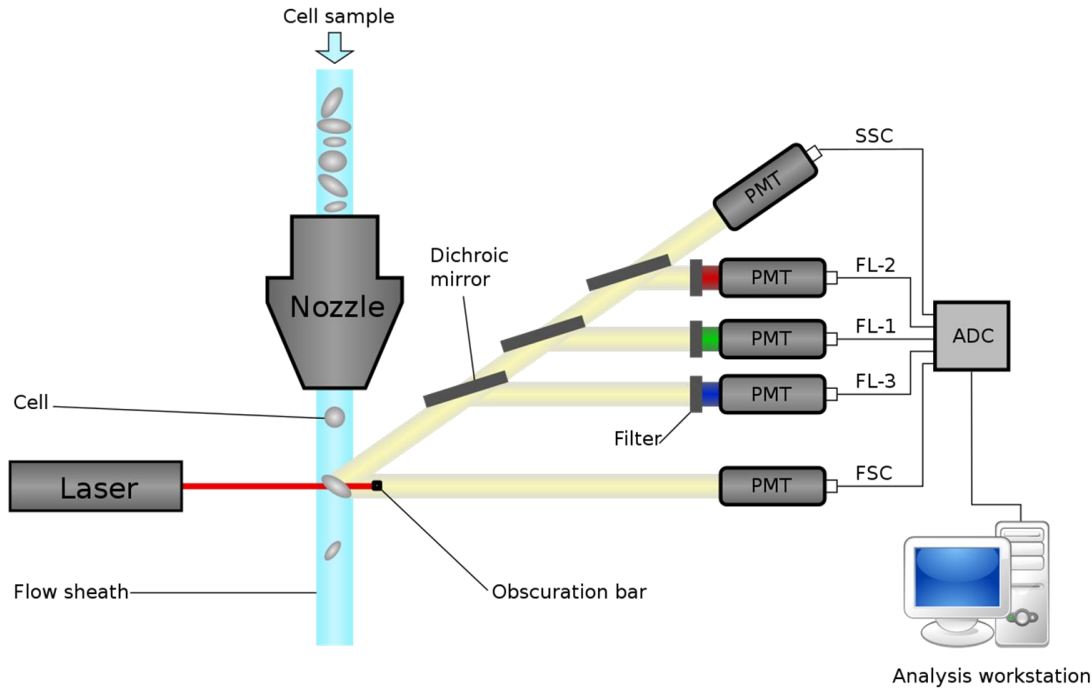


GOAL: To proof that nitrification in space is possible.

Single cell level

Community level

Flow cytometry for single cell characterization



Total cell count

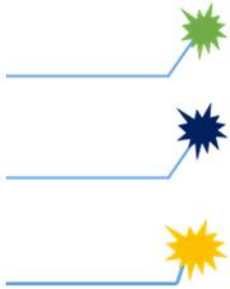
Distinction between
intact and damage cells

Phenotype fingerprinting

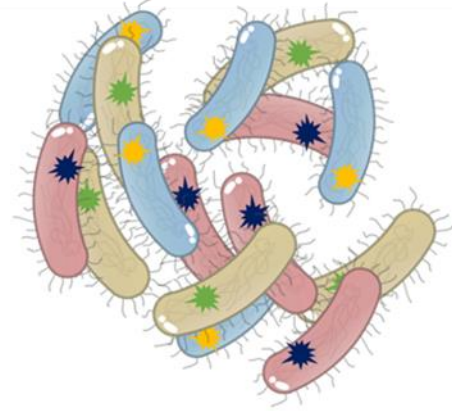
Detection of metabolic
activity

Fluorescent *In Situ* Hybridization (FISH)

Labelled probe targeting
rRNA



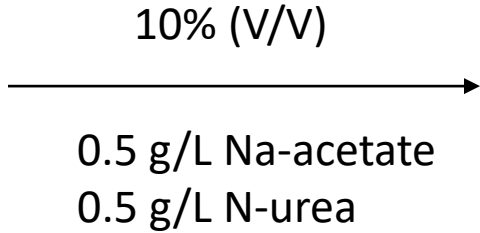
Labelled bacteria



<i>C. testosteroni</i>	→	CteA/COM1424
<i>N. europaea</i>	→	Nse1472
<i>N. winogradskyi</i>	→	NIT3

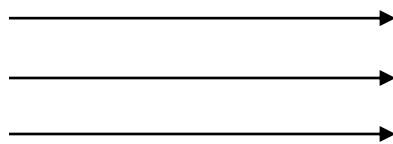
Flow-FISH for characterization of the community

C. testosteroni
N. europaea
N. winogradskyi



28°C
Shaker 120 RPM
Darkness
32 Days

Ion chromatography
Flow-FISH
qPCR



N-NH₄⁺, N-NO₂⁻, N-NO₃⁻

Active metabolism

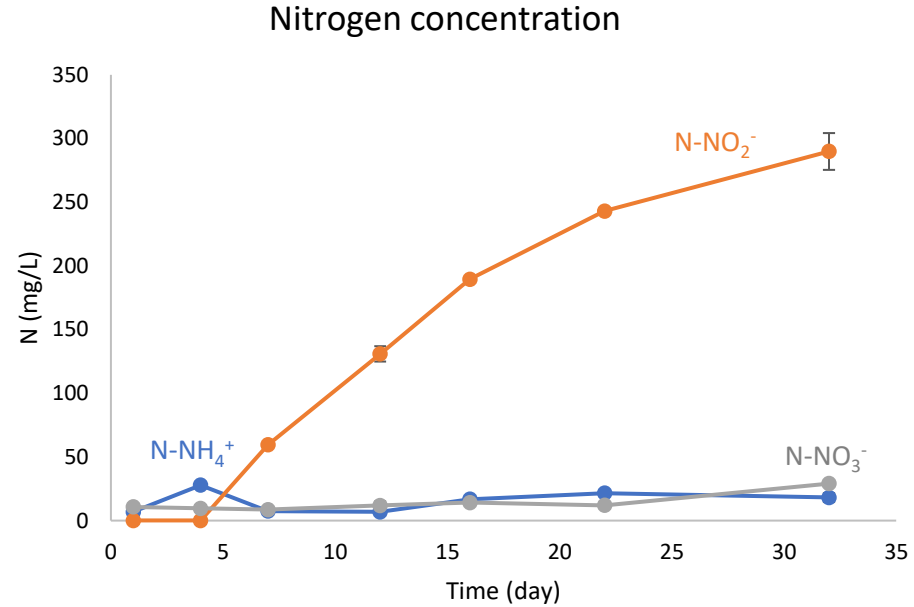
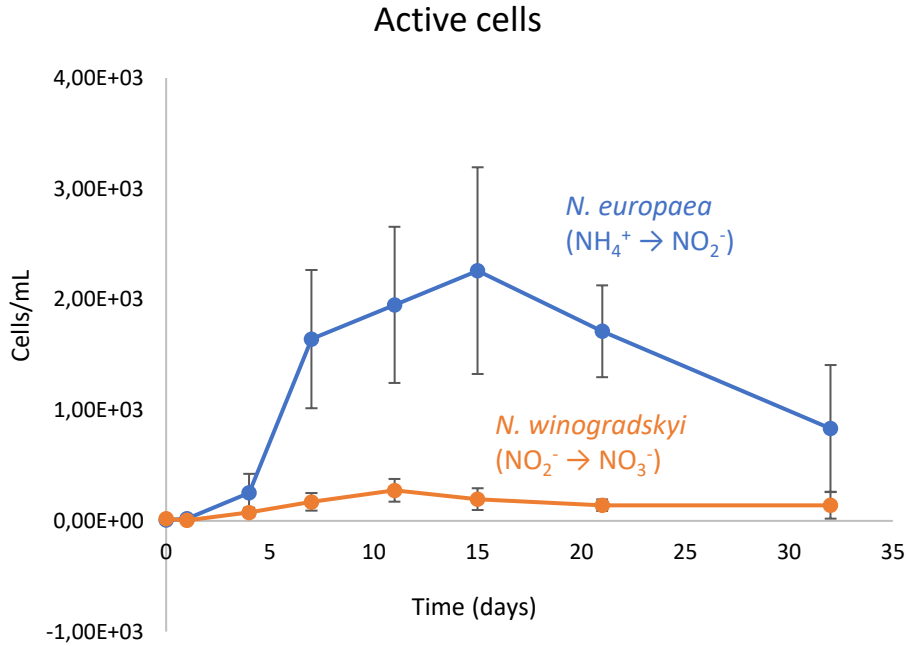
Benchmark technique (ongoing)

C. testosteroni : 16S rRNA

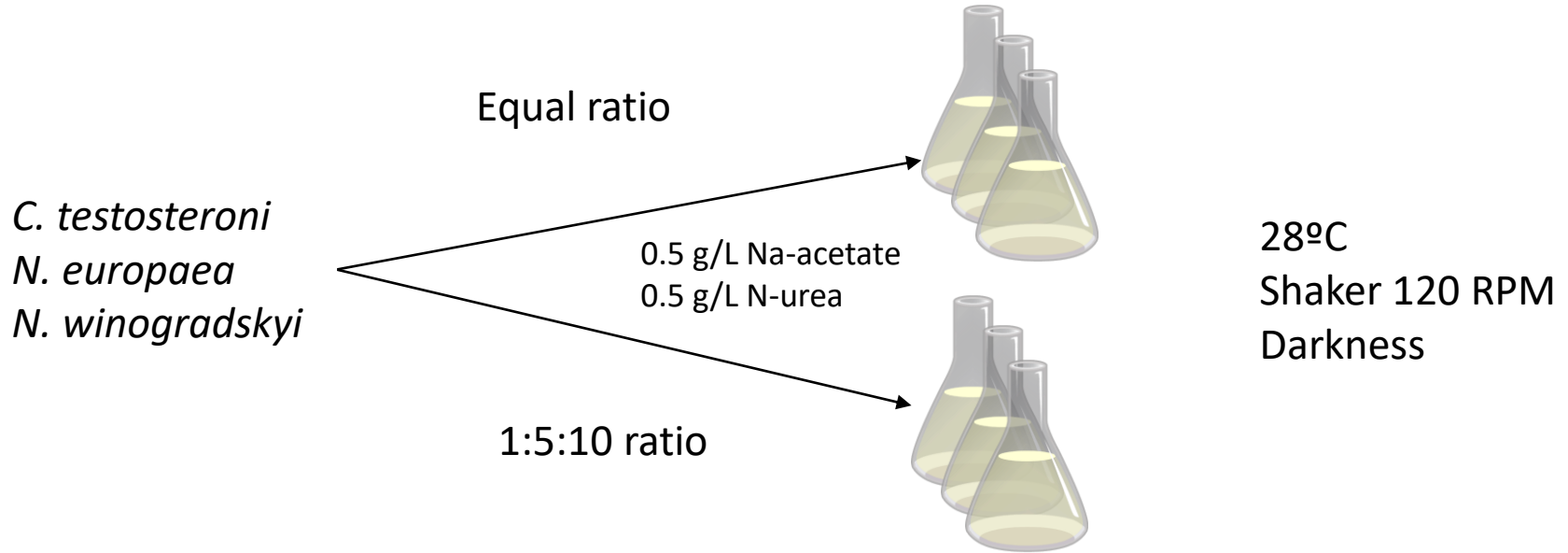
N. europaea : amoA

N. Winogradskyi : nxrA

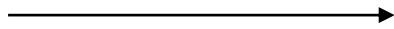
Flow-FISH for characterization of the community



Flow-FISH for characterization of the community PART 2



Ion chromatography



Acetate, N-NH₄⁺, N-NO₂⁻, N-NO₃⁻
(ongoing)

Flow-FISH



Active metabolism (ongoing)

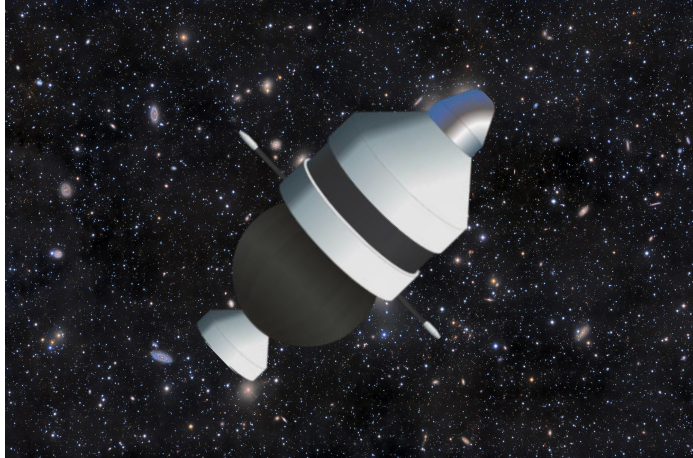
qPCR



Benchmark technique (ongoing)

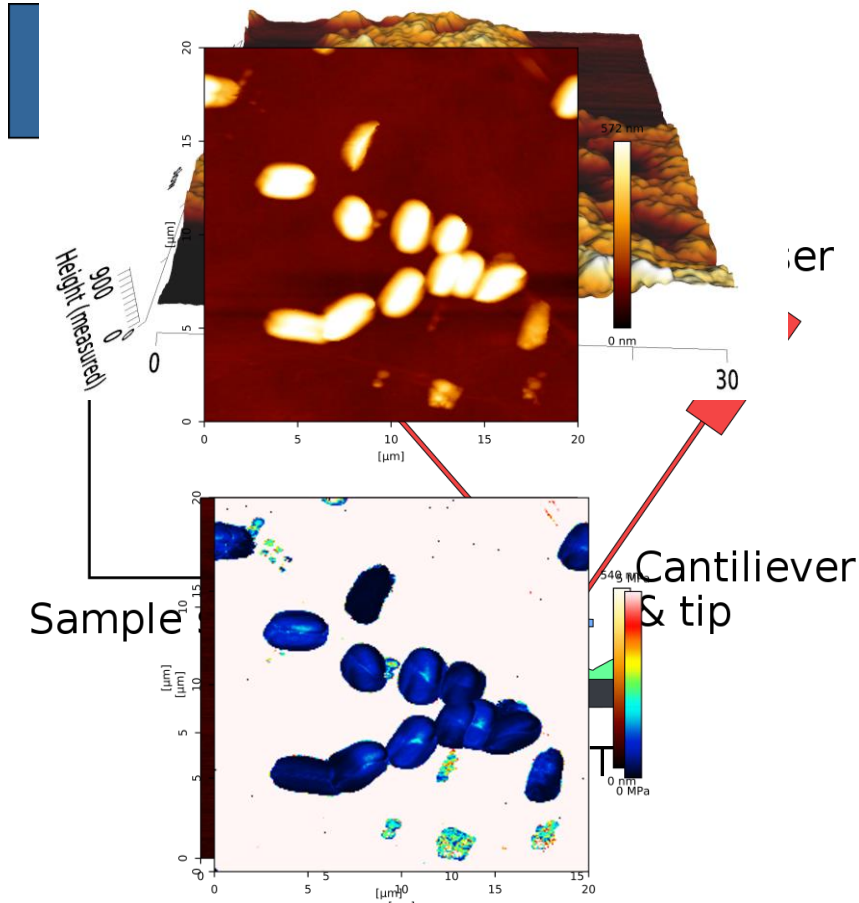
Community interactions - Biofilms

- Nitrifiers biofilms have been observed during the compartment 3 operation
- Several studies reported the enhancement of biofilms under microgravity (S. V. Lynch et al., 2006, W Kim et al., 2013, H. Wang et al., 2016)
- Biofilms development is different between gravity and microgravity conditions (W Kim et al., 2013)



- Biofilm development under both conditions
- Interactions between the different bacteria in the synthetic community

Biofilms under the atomic force microscope



Topography of the biofilms

Single cells

Phenotypic changes

Mechanical properties

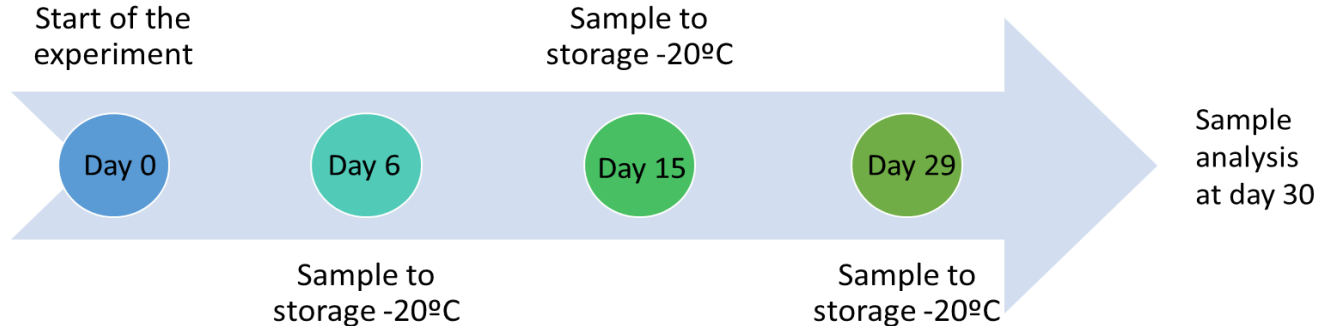
Biofilms under the atomic force microscope

C. testosteroni
N. europaea
N. winogradskyi
Synthetic culture



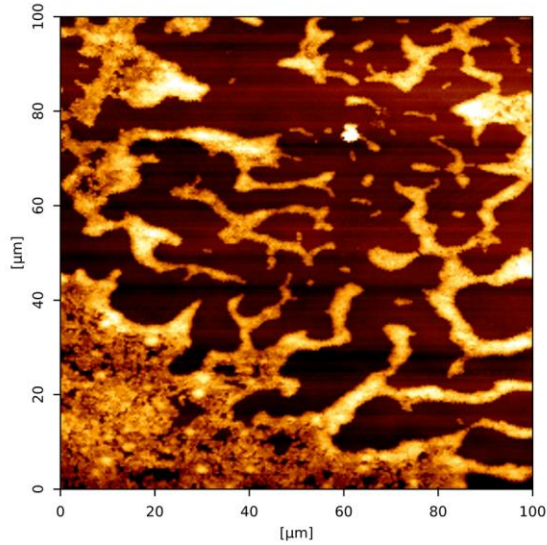
0.5 g/L Na-acetate
0.5 g/L N-source

Polyethylene
28°C
Shaker 120 RPM
Darkness

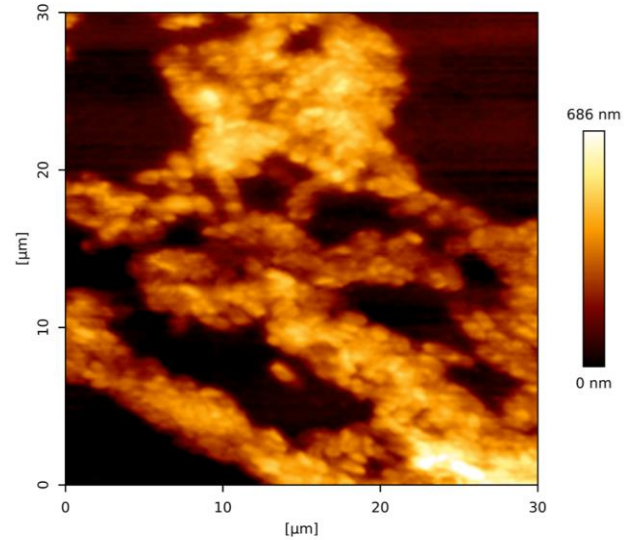


Biofilms under the atomic force microscope

C. testosteroni



Day 6



Day 29

Take home messages

- Flow-FISH: a rapid and solid tool to follow dynamics of metabolic activities in the co-culture
- Different ratios of Heterotrophs:AOB:NOB are being tested
- Atomic force microscope: a powerful tool for biofilms characterization
- *C. testosteroni* can produce biofilms relatively quick

PARTNERS

IN COOPERATION WITH



MELISSA



MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

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THANK YOU.

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