NITROGEN RECOVERY FROM URINE IN SPACE:

A CASE FOR NITRIFICATION

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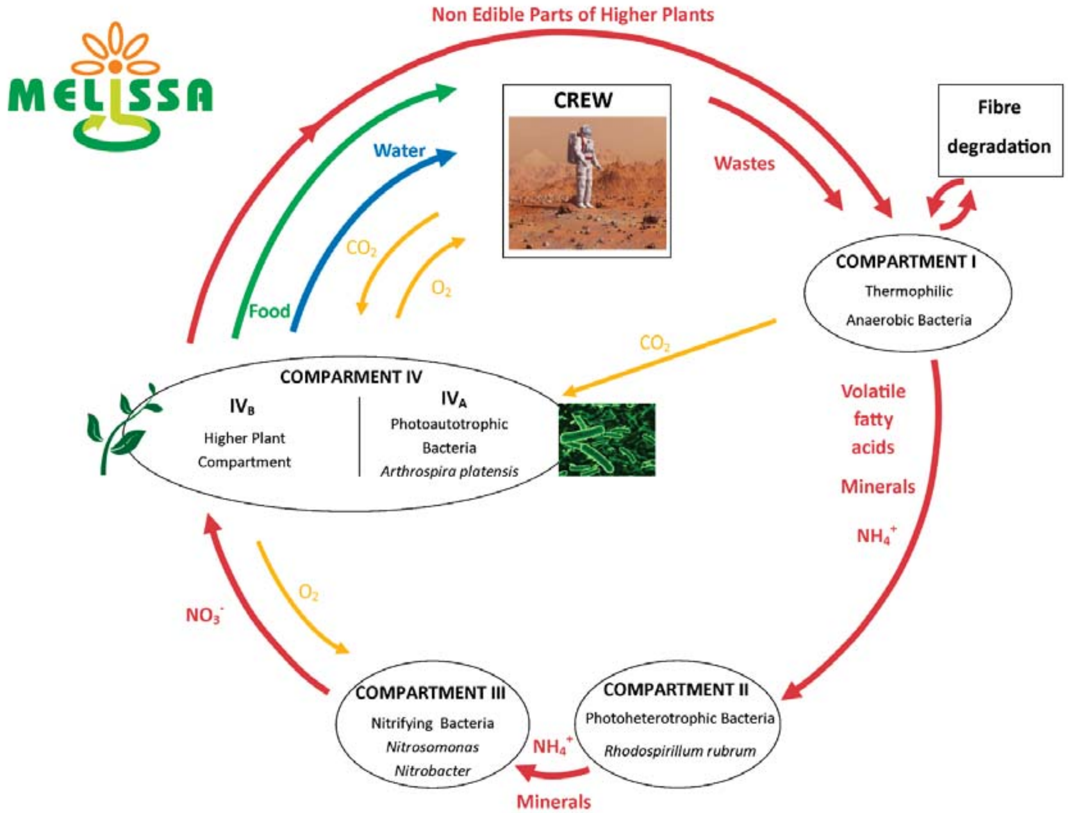
Agrospace/MELiSSA workshop, 16/05/2018

MELiSSA – urine nitrification

- Closing the cycle: waste → food
- ~80% of the nitrogen flux: urine
- $N_{org} \rightarrow NH_4^+/NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$



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Urine nitrification: strategy towards demonstration in Space

<u>Other challenges</u>:

- Salts
- Organic compounds
- Micropollutants
- Microbial stabilization

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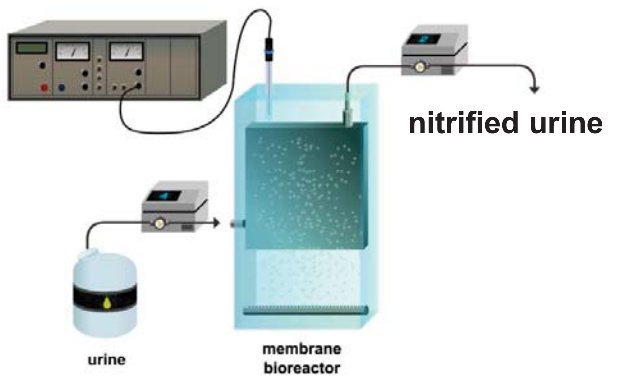
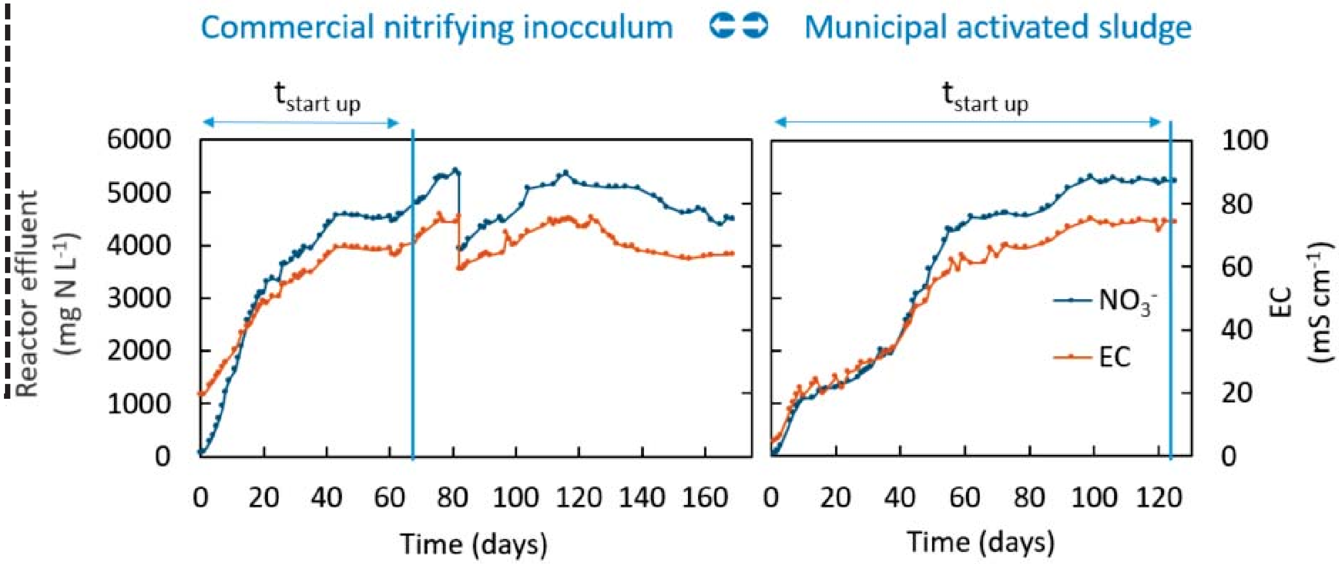
Urine **nitrification** in a MBR

Earth → ~~Space~~
~~Synthetic urine~~ → Real urine
Open community → ~~Synthetic community~~

Urine nitrification





- **First demonstration of urine nitrification with undiluted urine (high EC)**
- **N conversion efficiency > 95% (rate 0.4 g N L^{-1} d^{-1})**
- **COD removal efficiency >95%**

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Coppens et al. (2016) Bioresource Technology, 211, 41–50

Urine **nitrification** in a MBBR

Earth → ~~Space~~
~~Synthetic urine~~ → Real urine
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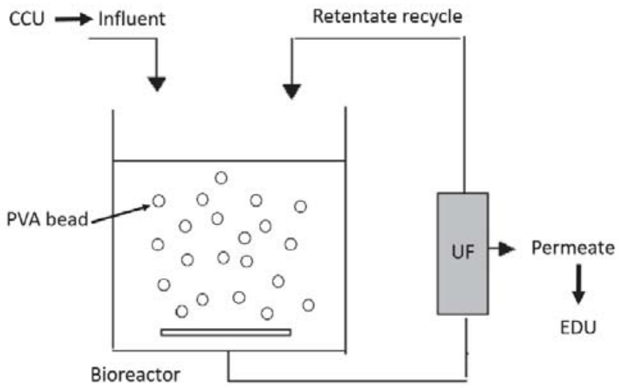
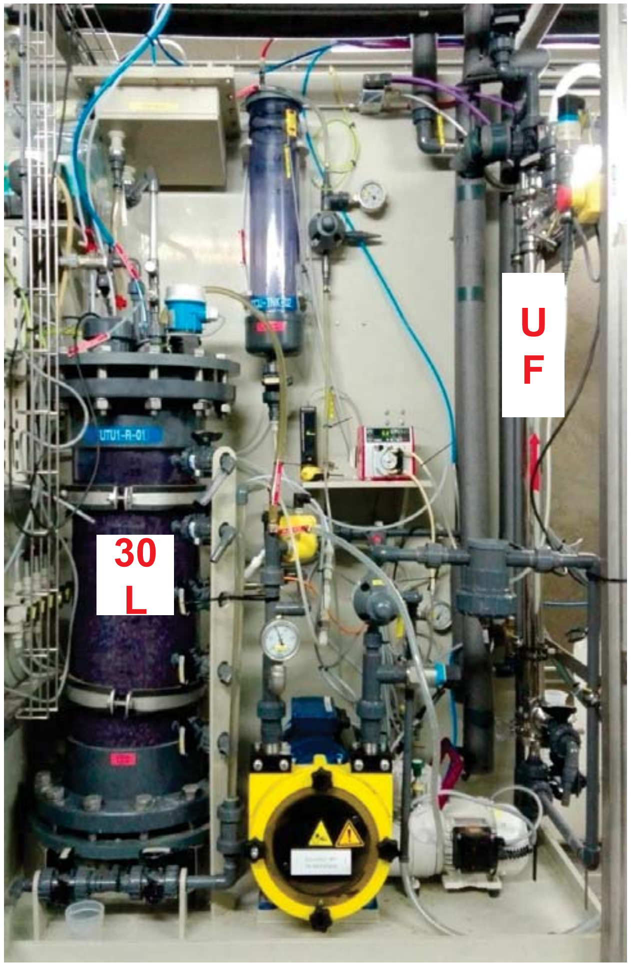
→ PVA beads as biomass carriers
→ From labscale to Breadboard (WTUB)
→ $\text{TRL}_{\text{earth}}$ 6 ($\text{TRL}_{\text{space}}$ 4)





QinetiQ Space nv
vito vision on technology

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De Paepe et al. (submitted)
Lindeboom et al. (in preparation)

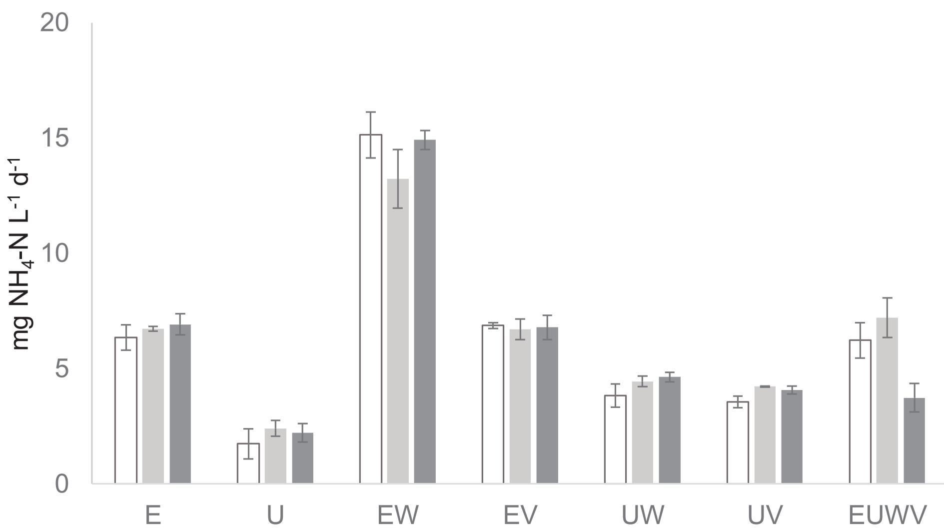
Strain selection for a synthetic community

Earth → ~~Space~~

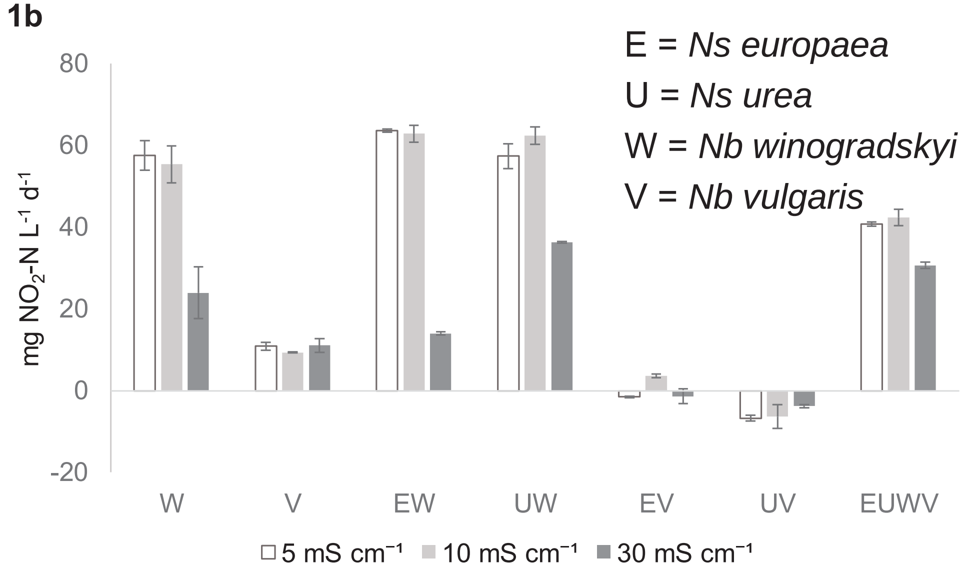
Synthetic urine → ~~Real urine~~

Open community → **Synthetic community**

1a



1b



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- *Nb winogradskyi* enchances ammonia oxidation activity in *Ns europaea* and *Ns urea* in a broad conductivity range
- Nb winogradskyi more sensitive to higher conductivity
- Proteomic analysis: response mechanisms of ***Ns europaea & Nb winogradskyi*** to high salt concentration

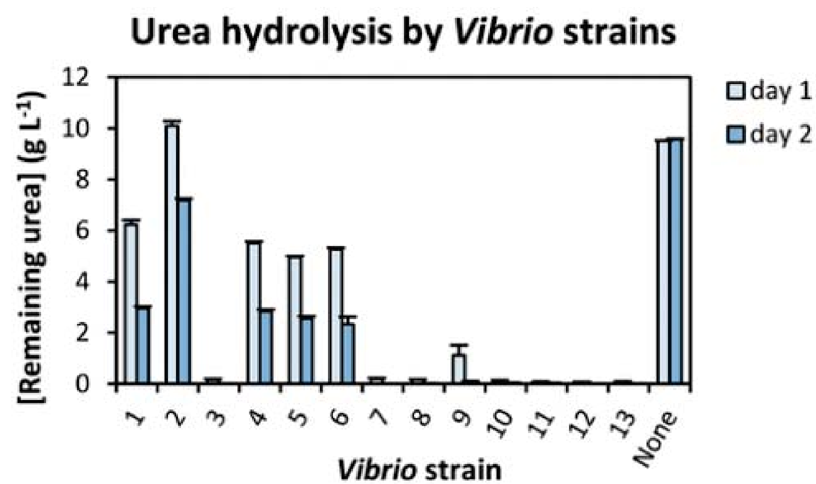
Ilgrande et al. (in preparation)

Strain selection for a synthetic community

Earth → ~~Space~~
Synthetic urine → ~~Real urine~~
Open community → **Synthetic community**

- Ureolysis up to 4 g N/L.h (*Vibrio*)
- *Vibrio* suppresses nitrification activity?

- *Heterotroph(s): ureolysis & COD removal*
- *Ns europaea + Nb winogradkyi + …*
- Selection of heterotroph(s):
  - *Pseudomonas fluorescens*
  - *Acidovorax delafieldii*
  - *Delftia acidovorans*
  - *Comamonas testosteroni, …*



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Defoirdt et al. (2017) ES&T, 51, 13335–13343
Ilgrande et al. (in preparation)

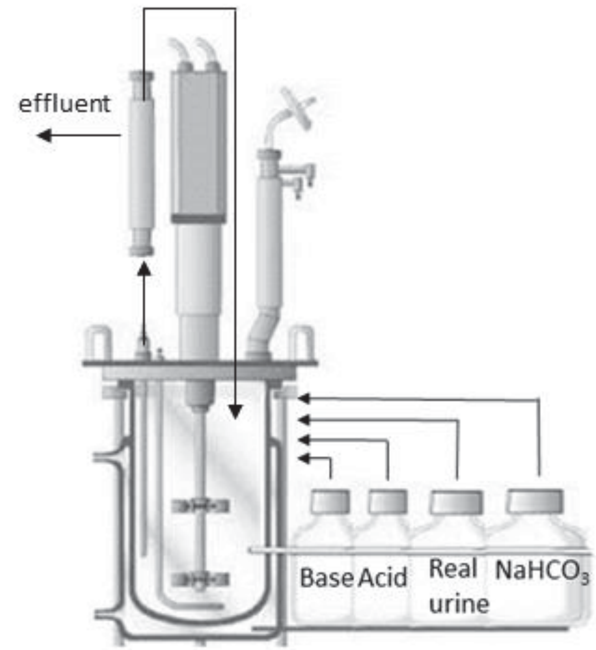
Synthetic microbial community in a membrane bioreactor (MBR)

Earth → ~~Space~~
~~Synthetic urine~~ **→ Real urine**
~~Open community~~ **→ Synthetic community**

- Synthetic microbial community
- Membrane ultrafiltration
- Bubble aeration

↓

- Synthetic urine √
- Real urine √



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Christiaens et al. (in preparation)

Demonstration of urine nitrification in the MELiSSA Pilot Plant (UAB)

Earth → ~~Space~~
Synthetic urine → ~~Real urine~~
~~Open community~~ **→ Synthetic community**

- The MELiSSA demonstrator (Spain)
- High level requirements
- Integration of MELiSSA compartments

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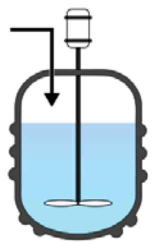
UAB Universitat Autònoma de Barcelona

Reactivation of nitrifiers (Foton Flight)

~~Earth~~ → **Space**

~~Synthetic urine~~ → ~~Real urine~~

~~Open community~~ → **Synthetic community**



Cultivation on Earth

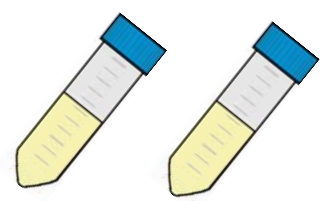
Transport to Launch site

FOTON M4
Flight

- Flight duration: 44 days
- Altitude: 258 - 571 km
- Gravity: 10^{-3} 10^{-4} g (estimated)
- Radiation: Extra dose in flight: 524 μGy/d

Terrestrial control (G23 and G4)

Flight (F) sample recovery



Reactivation

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Lindeboom/Ilgrande et al. (submitted)

Comparison rates after reactivation: **Flight** vs. Ground 23°C control

Composition	Symbol	Microbial characterization	Ureolysis	Ammonia oxidation	Nitrite oxidation
			Urea -> NH_4^+	NH_4^+->NO_2^-	NO_2^-->NO_3^-
Defined	C	*Cupriavidus pinatubonensis*	=		
	Ns	*Nitrosomonas europaea*		X	
	Nb	*Nitrobacter winogradskyi*			=
	NsNb	*Nitrosomonas europaea* + *Nitrobacter winogradskyi*		↑	↑
	CNsNb	*Cupriavidus pinatubonensis* + *Nitrosomonas europaea* + *Nitrobacter winogradskyi*	=	↑	↑

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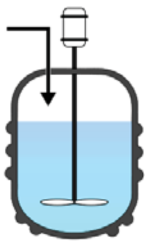
+ mixed communities + anammox

Reactivation of nitrifiers (ISS)

~~Earth~~ → **Space**

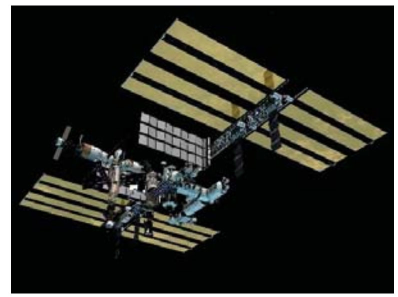
~~Synthetic urine~~ → ~~Real urine~~

~~Open community~~ → **Synthetic community**



Cultivation on Earth

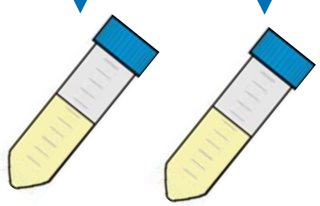
Transport to Launch site



Flight duration: 10d (6d)
Altitude: ~400 km
Radation: 2.8mGy

Terrestrial control (G23 and G4)

Flight (F) sample recovery



Reactivation

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Ilgrande et al. (in preparation)

Comparison rates after reactivation : **Flight** vs. Ground 23°C control

Strain/consortia	Ureolysis	Ammonia oxidation	Nitrite oxidation
	Urea -> NH_4^+	NH_4^+->NO_2^-	NO_2^-->NO_3^-
Cupriavidus pinatubonensis strain 1245	=		
Nitrosomonas europaea strain ATCC 19718		=/↑	
Nitrobacter winogradskyi strain ATCC 2539			↓
Nitrosomonas europaea + Nitrobacter winogradskyi		↑	=
Nitrosomonas europaea + Nitrobacter winogradskyi - coculture		↑	=
Nitrosomonas europaea + Nitrobacter winogradskyi + Cupriavidus pinatubonensis	=/↓	↑	=

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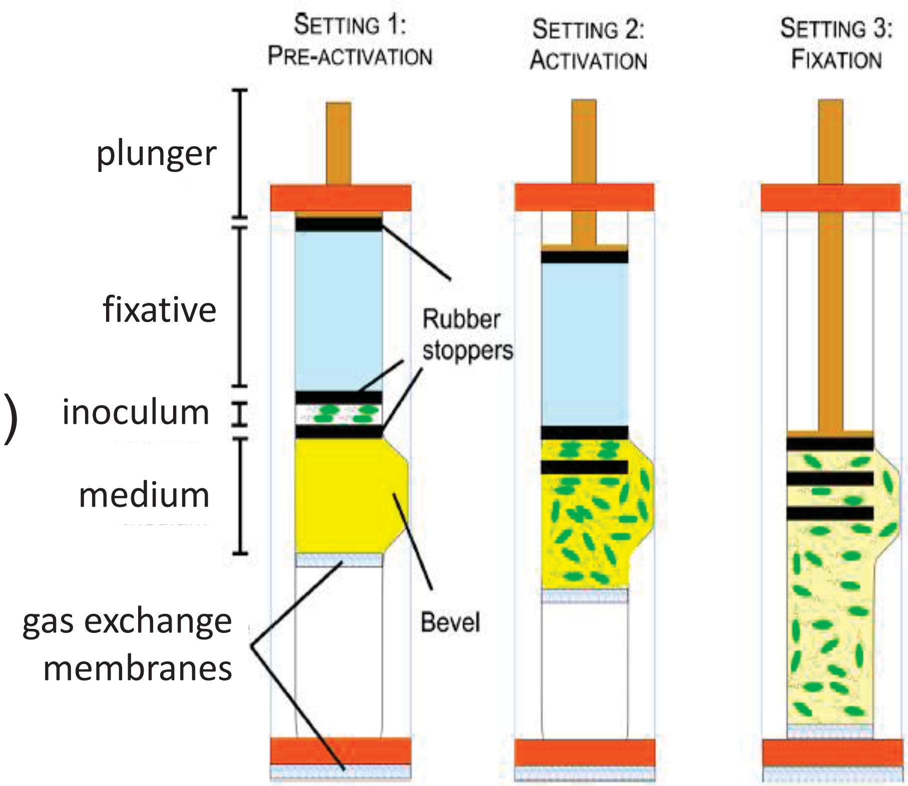
- All nitrifying strains could be reactivated
- Synthetic communities: higher ammonia oxidation rate after space flight?
- Negative impact of space flight on *N. winogradskyi* undone in synthetic microbial community?

Nitrification activity tests (ISS): URINIS A

~~Earth~~ → **Space**
Synthetic urine → ~~Real urine~~
~~Open community~~ → **Synthetic community**

- Gravity independent aeration
- Effect of microgravity on:
  - biofilm structure/formation
  - nitrification rate
  - metabolism (transcriptomics/proteomics)
- ISS (<2020?)



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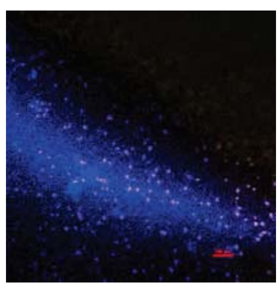
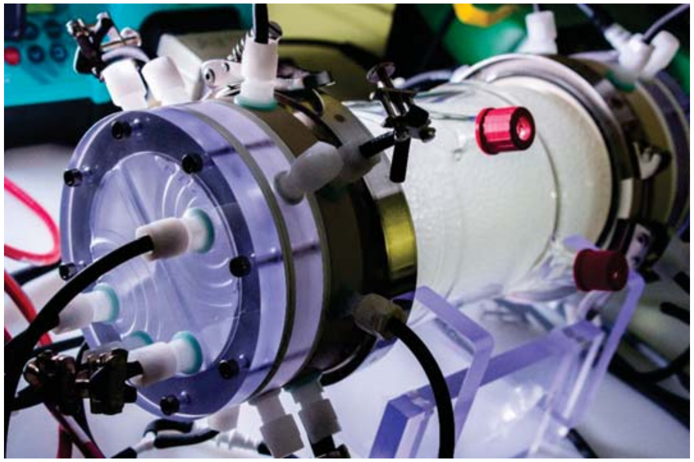
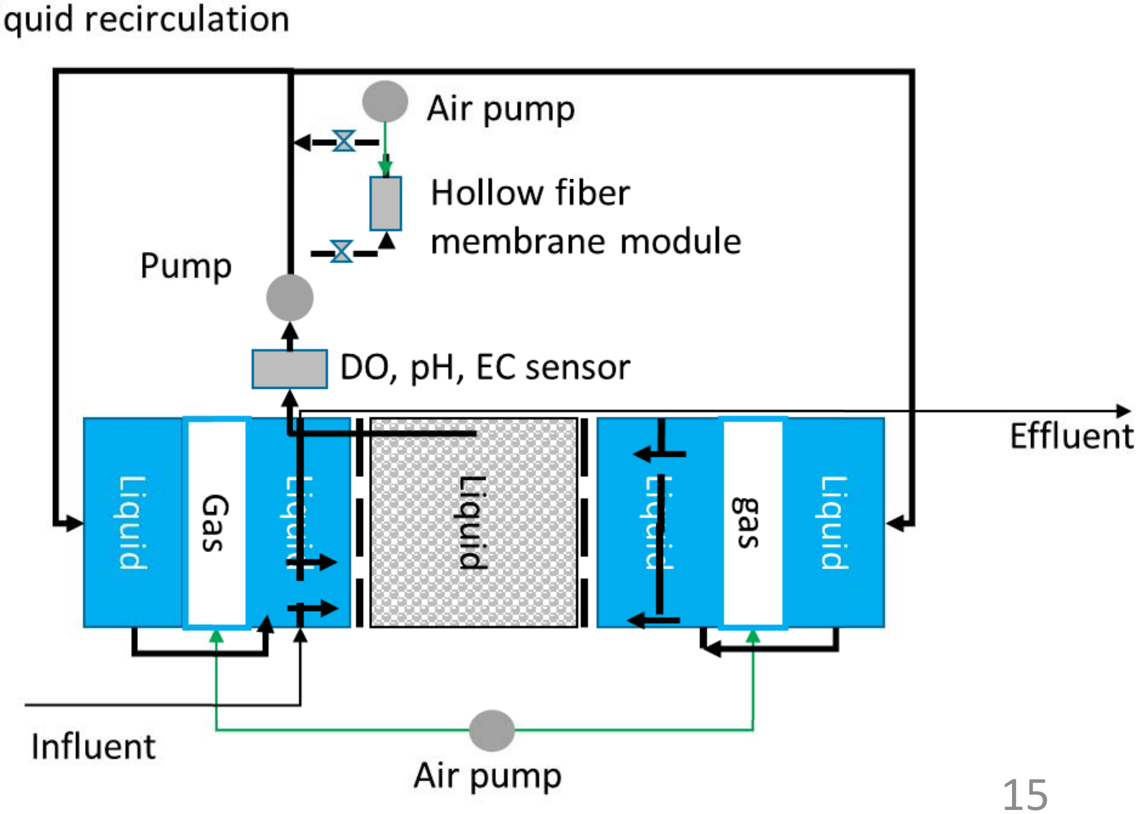


Urine nitrification in a bioreactor (ISS?): URINIS B

~~Earth~~ → **Space**
Synthetic urine → Real urine
~~Open community~~ → **Synthetic community**

Membrane aeration with flat sheet or hollow fiber membranes for gravity independent aeration





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Acknowledgements:

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MELiSSA

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esa