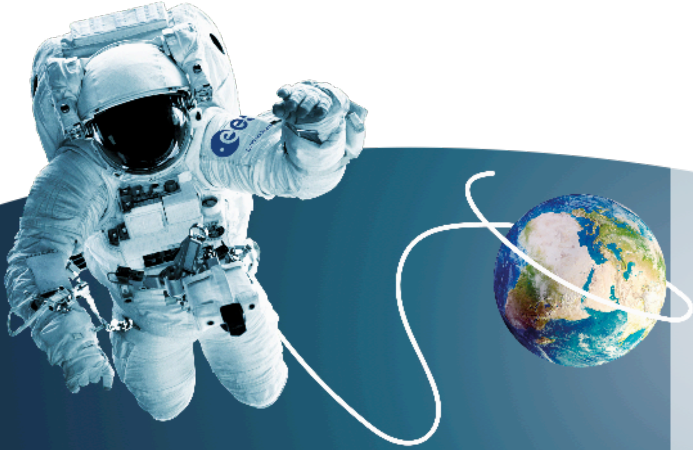
MELiSSA
MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

CREATING
A CIRCULAR
FUTURE

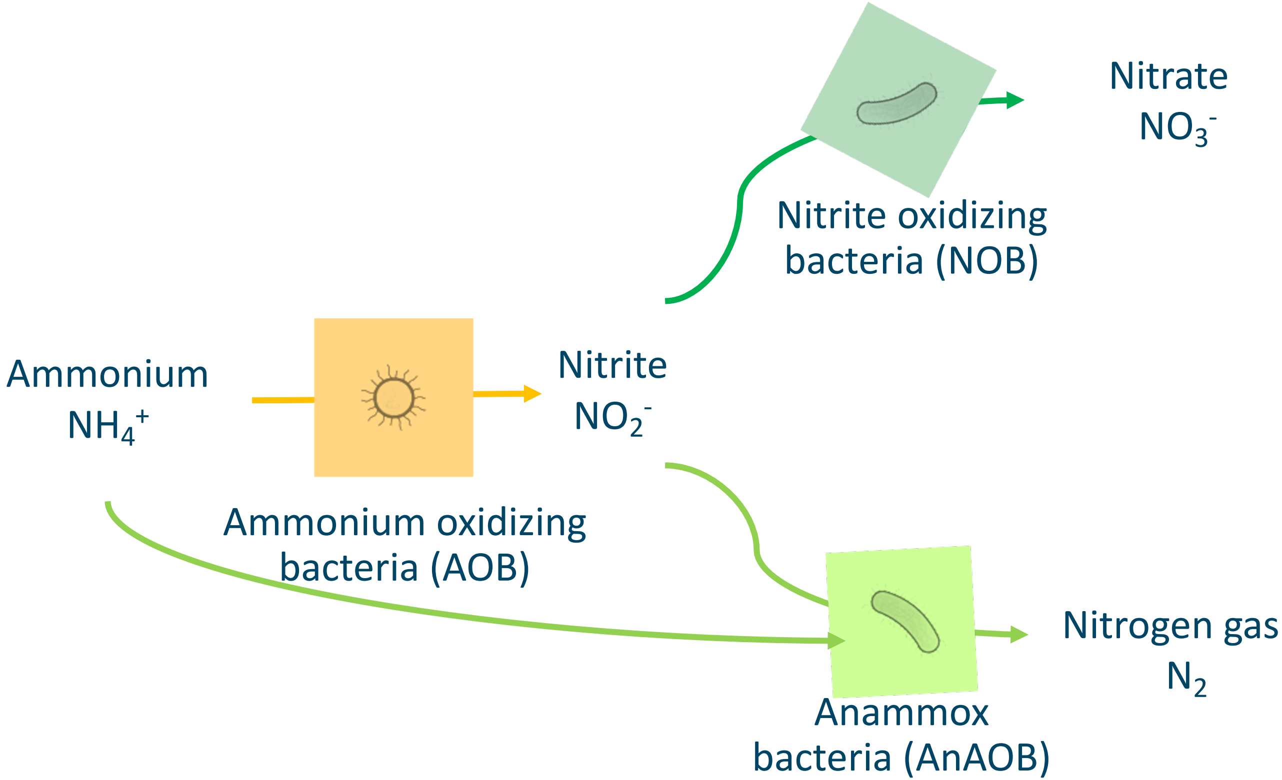
Nitrify to support life:
MELiSSA’s development path of an essential process



Siegfried Vlaeminck &

Spiller, M., Van Malderen, V., Xie, Y., Alloul, A., Udert, K., Faust, V., Crain, G., Demey, D., Leroy, B., Wattiez, R., Sachdeva, N., Dussap, C.-G., Poughon, L., Creuly, C., Gòdia, F., Peiro, E., Arnau, C., Ciurans Molist, C., Barys, J., De Paepe, J., Al-Saadi, A., Boon, N., Rabaey, K., Ganigué, R., Clauwaert, P., Lindeboom, R.E.F., Suters, R., Giurgiu, R.M., Smets, I., Rummens, K., Gerbi, O., Fiani, P., Leys, N., Mastroleo, F., Lamaze, B., Paille, C., Lasseur, C.

Nitrification-based, or more accurately nitritation-based processes start from the aerobic oxidation by autotrophic bacteria:



Ammonium NH_4^+

Ammonium oxidizing bacteria (AOB)

Nitrite NO_2^-

Nitrite oxidizing bacteria (NOB)

Nitrate NO_3^-

Anammox bacteria (AnAOB)

Nitrogen gas N_2

Full or partial nitrification:

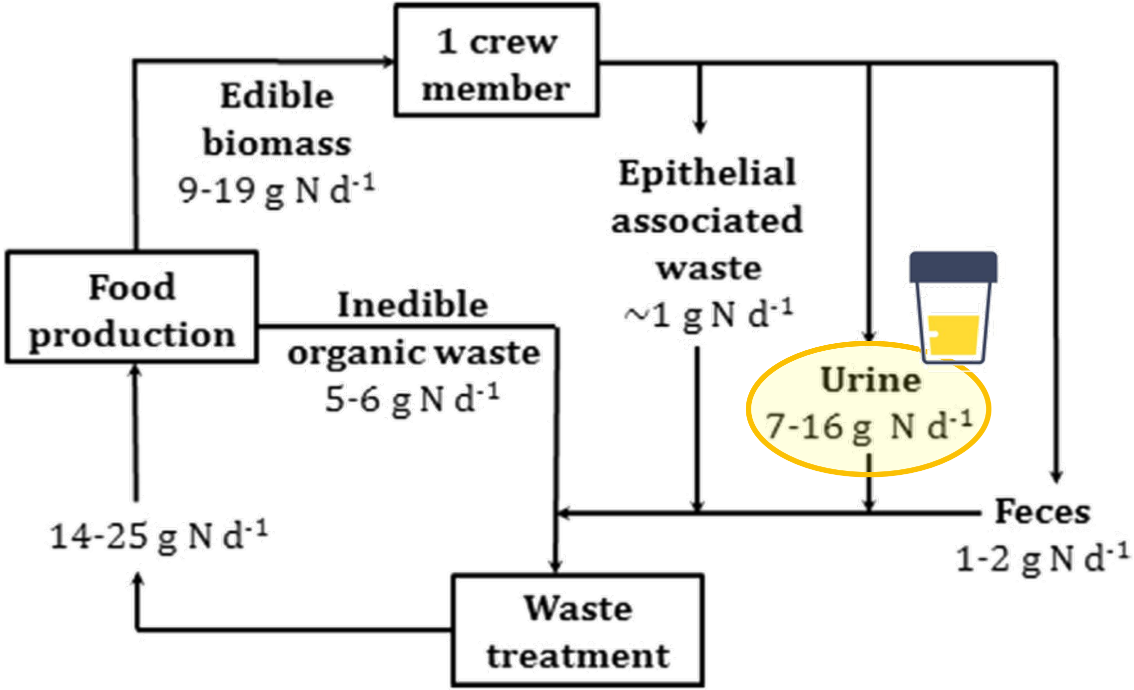
Nitritation + Nitratation

Nitrification bioreactor at the MELiSSA pilot plant

Partial nitritation / Anammox

Nitrogen in Space? -> Urine

Urine as major N flow:
50-64% in closed system with food production



(Clauwaert et al., 2017)

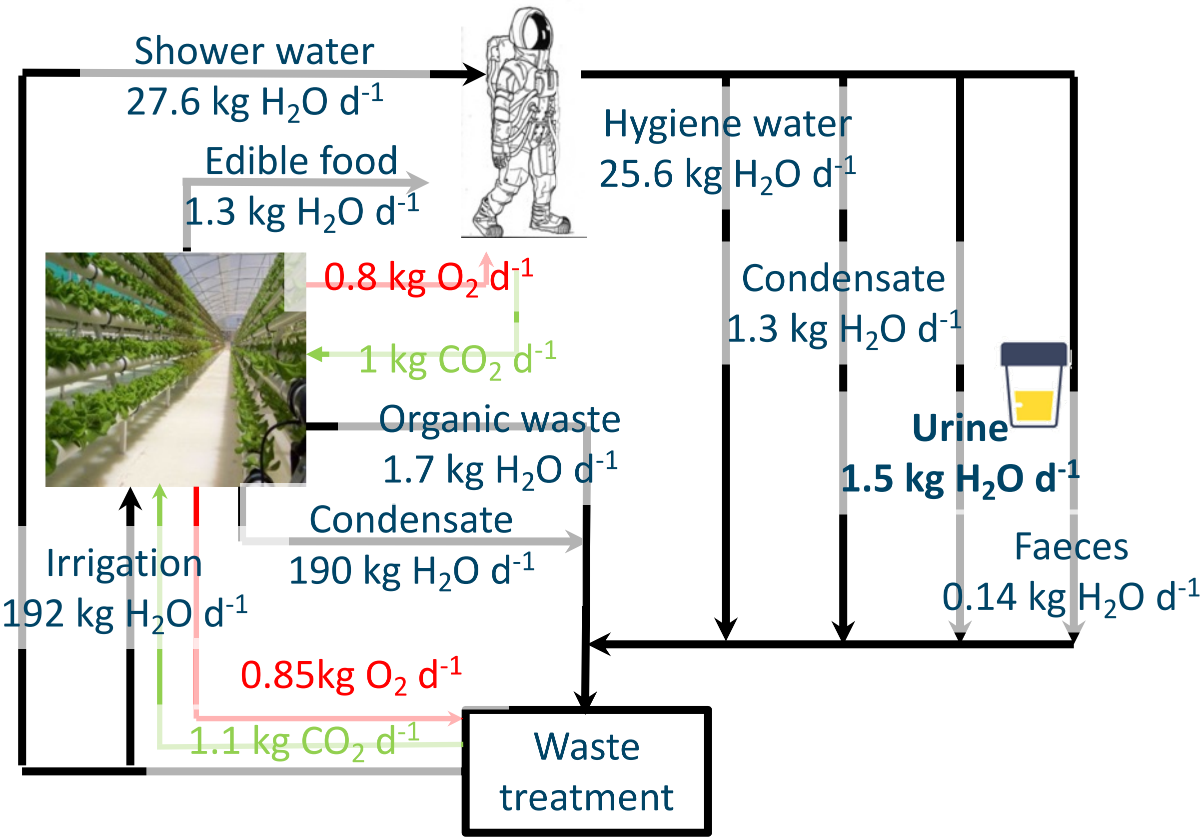
Urine in Space? -> Nitrogen, but also water

Short missions:

- **Yellow water:** Urine + flush water → **around half of the water flow**
- **Condensate:** Respiration and transpiration crew

Additional flows in longer missions:

- **Sabatier** water: From CO_2 removal
- **Grey** water from hygiene activities (e.g. shower)
- **Black** water (from toilet flush)
- **Transpiration** water (food production with plant)
- **Grey** water from service activities (laundry, dish-washer, etc.)



Shower water $27.6\ kg\ H_2O\ d^{-1}$

Edible food $1.3\ kg\ H_2O\ d^{-1}$

Hygiene water $25.6\ kg\ H_2O\ d^{-1}$

$0.8\ kg\ O_2\ d^{-1}$

$1\ kg\ CO_2\ d^{-1}$

Condensate $1.3\ kg\ H_2O\ d^{-1}$

Organic waste $1.7\ kg\ H_2O\ d^{-1}$

Urine **$1.5\ kg\ H_2O\ d^{-1}$**

Condensate $190\ kg\ H_2O\ d^{-1}$

Irrigation $192\ kg\ H_2O\ d^{-1}$

Faeces $0.14\ kg\ H_2O\ d^{-1}$

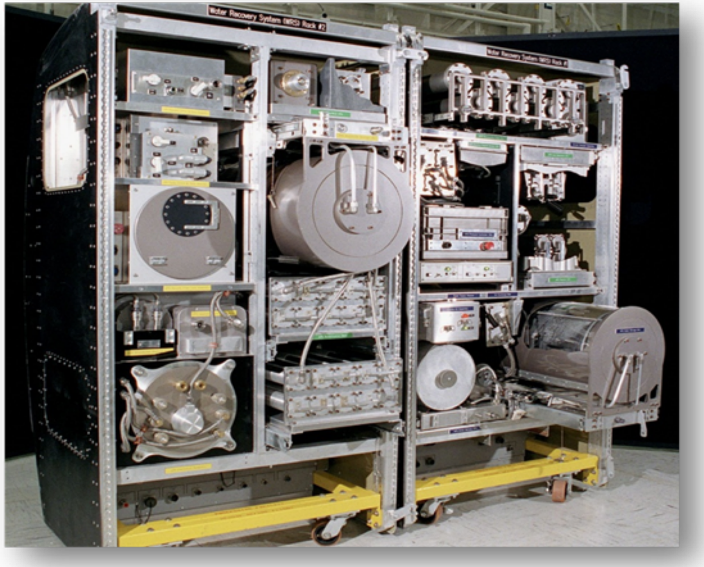
$0.85kg\ O_2\ d^{-1}$

$1.1\ kg\ CO_2\ d^{-1}$

Waste treatment

(After Hu et al. 2010)

Water recovery from urine at the International Space Station (ISS)

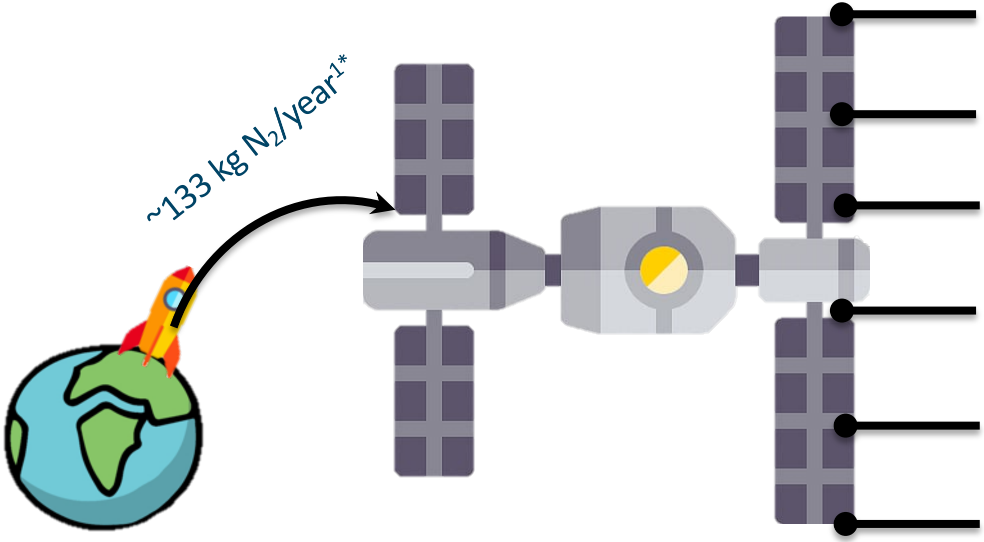


Racks for the ISS water recovery system
(Carter et al. 2011)

Belgian astronaut Frank De Winne repairing
the Urine Processor Assembly (UPA)

Frank De Winne: ‘In Space, we drink the same coffee every day’

Needs for N_2 as neutral gas for pressurization and main artificial atmosphere constituent



~133 kg N_2/year[1*]

EMU Maintenance:
4.9 kg N_2/year

Payload Usage:
17.7 kg N_2/year

TOC Analyzer Usage:
6.24 kg/year N_2

US EVA Air Loss:
0.7 kg N_2/EVA

Russia EVA Air Loss:
12 kg N_2/EVA

Structural leak rate
74.4 kg N_2/year
(0.2 kg/day)

~ 117 kg N_2 per year[1]

Could go up to 1.3 kg/day or more when there is a physical “hole” in ISS[2,3]

Credits: Tim Van Winckel (UAntwerp)

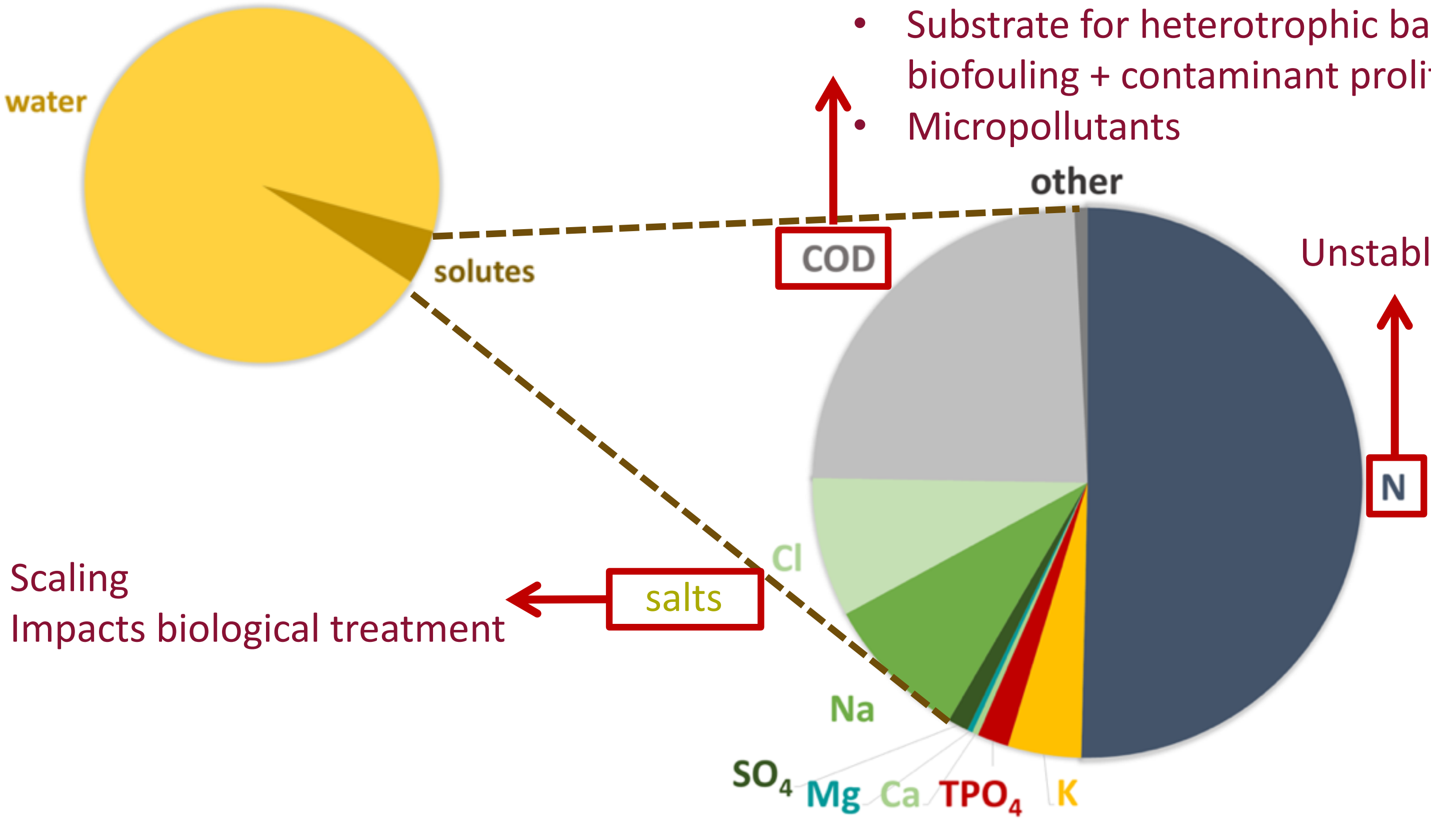
* Between 2008-2015

[1] Schaezler R. N. and Schaezler J. A. 2015, **Report on ISS O2 Production, Gas Supply & Partial Pressure Management,** 45th International Conference on Environmental Systems, 12-16 July 2015, Bellevue, Washington

[2] NASA communications

[3] https://www.space.com/space-station-air-leak-cosmonaut-update-oxygen-supply

Challenges for urine treatment



- Substrate for heterotrophic bacteria -> stimulates biofouling + contaminant proliferation
- Micropollutants

Unstable -> NH_3 volatilisation

- Scaling
- Impacts biological treatment

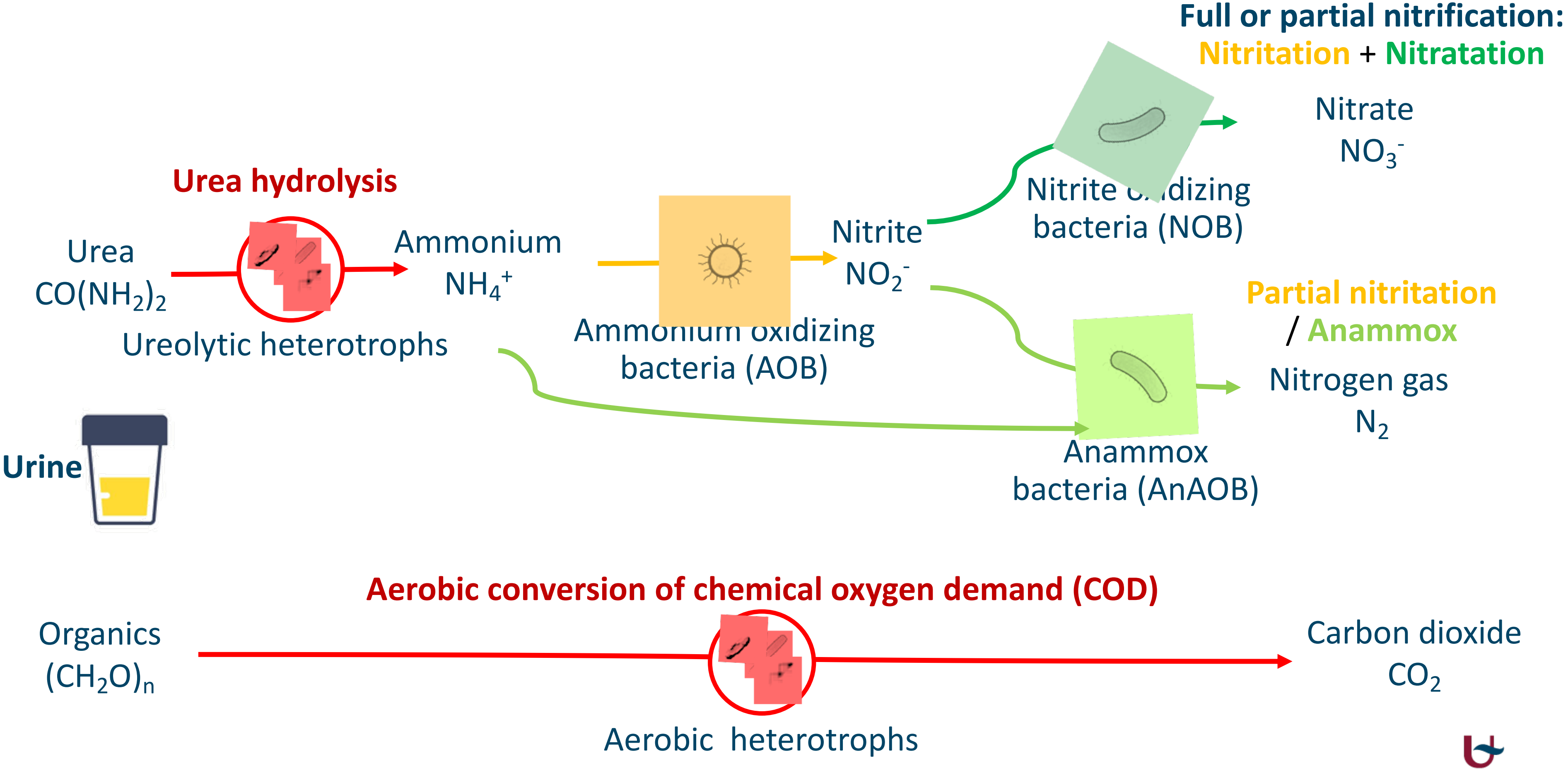
Solutions based on biological oxidation of nitrogen and organics

(No suitable other technologies for N conversion available)

(Credits: De Paepe)

Goals for treatment of urine (and/or MELiSSA effluent from CI or CII)	Nitrogen process: **Resulting product(s)**	
	Full nitrification: **NO_3^-** Partial nitrification: **NH_4^+ and NO_3^-**	Nitrification/denitrification, nitritation/denitritation, or partial nitritation/anammox: **N_2**
Stabilization to mitigate risks: • Controlled ureolysis and pH -> Avoids **NH_3 volatilization** • Removal of organics -> **Prevents biofouling** & removes carbonaceous biological oxygen demand	**Yes**	**Yes**
Enable subsequent **water recovery** with reverse osmosis (without NH_4^+)	**Yes** (for full nitrification)	**Yes**
Production of N_2 as **neutral gas** to pressurize and compensate losses and leaks	No	**Yes**
Production of **liquid fertilizer** for food production (plants, microalgae), containing macro- and micronutrients	**Yes**	No

Microbial production of nitrate or nitrogen gas on urine



Full or partial nitrification:
Nitritation + **Nitratation**

Urea hydrolysis

Urea
$CO(NH_2)_2$

Ureolytic heterotrophs

Ammonium
NH_4^+

Ammonium oxidizing bacteria (AOB)

Nitrite
NO_2^-

Nitrite oxidizing bacteria (NOB)

Nitrate
NO_3^-

Partial nitritation / Anammox

Anammox bacteria (AnAOB)

Nitrogen gas
N_2

Urine

Aerobic conversion of chemical oxygen demand (COD)

Organics
$(CH_2O)_n$

Aerobic heterotrophs

Carbon dioxide
CO_2

Urine nitrification stoichiometry

Assumptions:

- Urine: ~1 g COD/g N -> equivalent to ~0.22 mol acetic acid/mol N
- Only aerobic COD conversion (no denitrification)
- Aerobic COD conversion at 21-days mean cell retention time (sludge age)

Stoichiometries:

- Nitrification: ~96% N recovered as nitrate (~4% present in biomass)
- PN/A: ~86% N recovered as nitrogen gas (~11% as nitrate; ~3% present in biomass)
- ~half of CO_2 production from urea; ~half of CO_2 production from COD

Process	Stoichiometry
Ureolysis	$0.5\ CO(NH_2)_2 + 0.5\ H_2O + H^+ \rightarrow NH_4^+ + 0.5\ CO_2$
C conversion	$0.22\ CH_3COOH + 0.016\ NH_4^+ + 0.36\ O_2$ $\rightarrow 0.016\ C_5H_7O_2N + 0.36\ CO_2 + 0.016\ H^+ + 0.41\ H_2O$
Nitrification	$0.98\ NH_4^+ + 1.83\ O_2 + 0.1\ CO_2 \rightarrow 0.96\ NO_3^- + 0.02\ C_5H_7O_2N + 2.05\ H^+ + 0.82\ H_2O$
Partial nitritation/ anammox (PN/A)	$0.98\ NH_4^+ + 0.78\ O_2 + 0.08\ CO_2$ $\rightarrow 0.43\ N_2 + 0.11\ NO_3^- + 0.016\ C_5H_7O_2N + 1.1\ H^+ + 1.36\ H_2O$
Ureolysis, Nitrification & C conversion	$0.5\ CO(NH_2)_2 + 0.22\ CH_3COOH + 2.18\ O_2$ $\rightarrow \mathbf{0.96\ NO_3^-} + 0.036\ C_5H_7O_2N + 0.76\ CO_2 + 1.06\ H^+ + 0.73\ H_2O$
Ureolysis, PN/A & C conversion	$0.5\ CO(NH_2)_2 + 0.22\ CH_3COOH + 1.14\ O_2$ $\rightarrow \mathbf{0.43\ N_2 + 0.11\ NO_3^-} + 0.032\ C_5H_7O_2N + 0.78\ CO_2 + 0.11\ H^+ + 1.26\ H_2O$

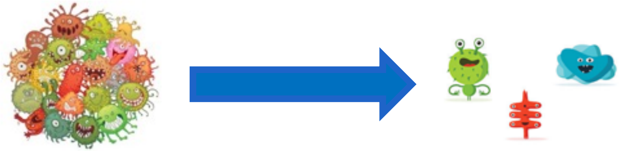
Preliminary nitrification dimensioning and input

- 9.6 g N/crew member/d in urine
- Volume nitrification or PN/A unit, conservatively assuming 0.4 g N/L/d:
  - ca. 24 L active reactor volume/crew member
  - **ca. 96 L reactor+instrumentation/crew member**
- Oxygen demand: Majority for N conversion
- Sludge production: Similar for N and COD conversion
- Base demand: only for full nitrification (~1 mol OH^-/mol N)

Numbers for 1 crew member	**Oxygen required**			**NaOH**	**Sludge produced**		
	For N	For COD	Total	**required**	From N	From COD	Total
	g O_2/d	g O_2/d	**g O_2/d**	**g/d**	g TSS/d	g TSS/d	**g/d**
Full nitrification (~100% NO_3^-)	40	8	**48**	**29**	2.0	1.6	**3.6**
Partial nitrification (~50% NO_3^-; ~50% NH_4^+)	20	8	**28**	**0**	1.0	1.6	**2.6**
Partial nitritation/anammox (~86% N_2; ~11% NO_3^-)	17	8	**25**	**0**	1.6	1.6	**3.2**

Urine nitrification: The MELiSSA strategy towards demonstration in Space

Open and dynamic
-> 'natural' selection



Defined and thus biosafe
-> 'manmade' selection

Synthetic: Progressively increasing complexity

Real: Full complexity (organics, salts, micropollutants)

Flask incubation and process characterization

Reactor operation, modelling, automation, control

Bioreactor

Fully integrated treatment pipeline

1 g

Reduced gravity **(< 1 g)**
-> gas/liquid mass transfer challenges

Radiation protected
(magnetic field)

Higher solar and cosmic radiation
-> effects on biology (including crew)

+ Further **decision making and optimization** based on **ALiSSE** (advanced life support system evaluator)
criteria: mass and energy requirements, reliability, and crew time and safety

Conclusions on type of matrix and microbial community

- Development maturity **nitrification > ureolysis > COD conversion > partial nitritation/anammox**
- Development maturity with **open** communities **> defined** communities

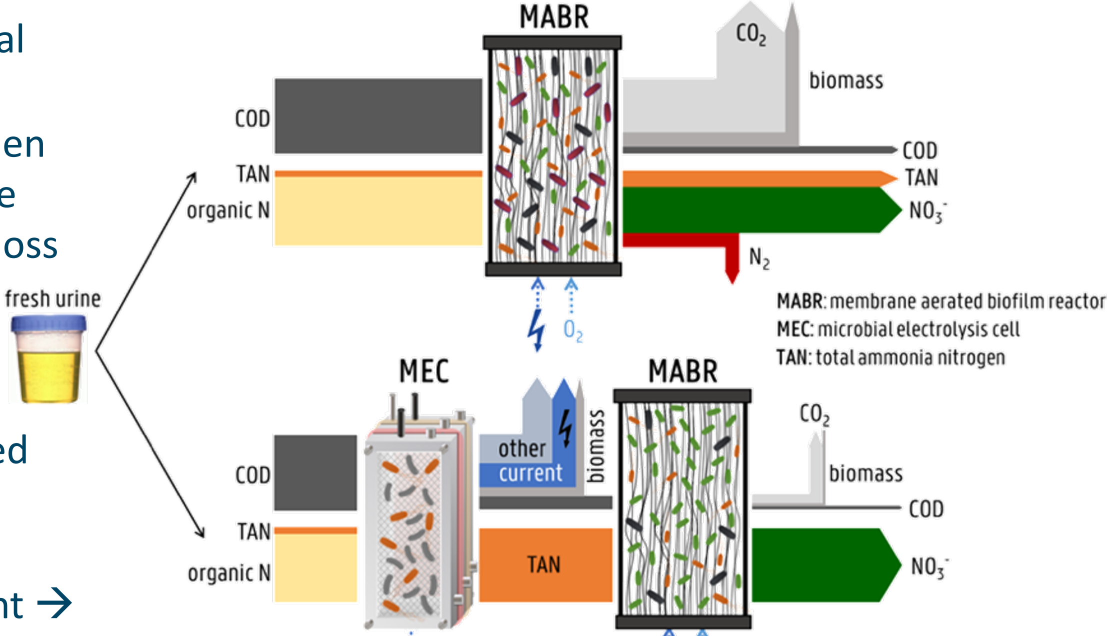
Process	**Medium**			**Community**	
	NH_4^+ w/o COD	Synthetic urine	Real urine	Open	Defined
Nitrification	+++				+++
			+++	+++	
		++	++		++
Ureolysis			+++	+++	
		++	++		++
COD conversion			+++	+++	
		+	+		+
Partial nitritation/anammox		In prep	In prep	In prep	

Key achievements open communities:

- Demonstrated at salinities of undiluted urine with open community (Coppens et al., 2016)
- pH control:
  - Full nitrification: Chemical OH^- addition (a.o. Coppens et al., 2016)
  - Full nitrification: Electrochemical OH^- addition (PhD De Paepe, 2020)
  - Partial nitrification: pH-based feeding, no OH^- addition (POMP Faust)
- Particle-free effluent through use of membrane bioreactors (microfiltration Coppens et al., 2016; ultrafiltration De Paepe et al., 2018)
- Bubbleless aeration: membrane-aerated biofilm reactor on real urine (De Paepe et al., 2020b)

Urine nitrification in a membrane-aerated biofilm reactor (MABR), with or without COD pretreatment

- ✓MABR -> Microgravity compatible aeration for nitrification
- ✓Optional pre-removal of COD in a microbial electrolysis cell (MEC)
- ✓Full nitrification with closed N balance when MABR was operated on MEC-treated urine
- ✓Partial nitrification (70-75%) and ~20% N loss directly operated on raw urine (denitrification)
- ✓MEC:
  - ✓prevents denitrification in highly loaded MABR → full N recovery
  - ✓No oxygen demand for COD removal
  - ✓Oxidation of organics generates current → energy recovery



(De Paepe et al., 2020)

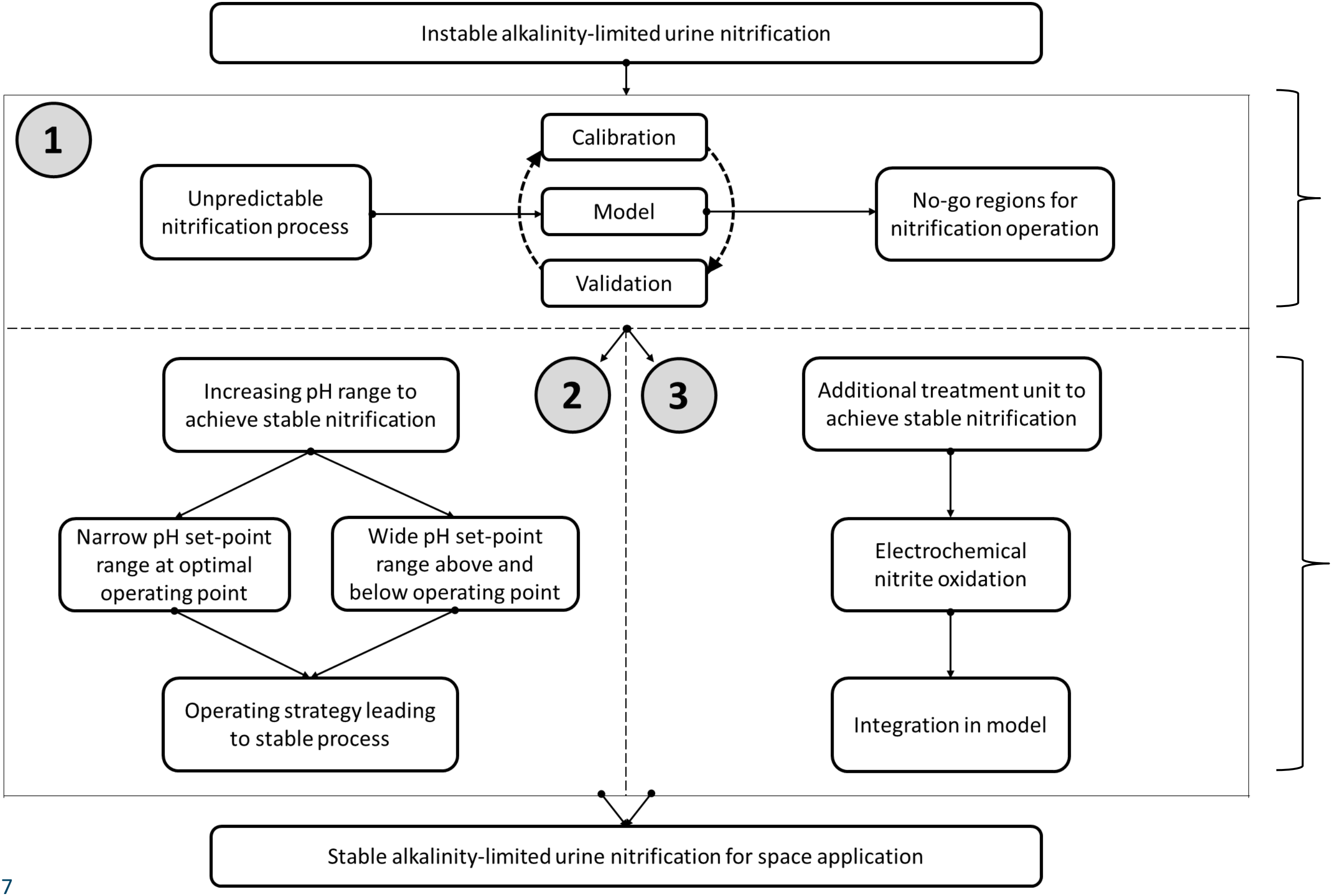
Partial vs. full nitrification

Partial nitrification (endogenous alkalinity only)	Full nitrification (endogenous + exogenous alkalinity)
Produces an ammonium-nitrate fertilizer	Produces a nitrate fertilizer
⊕ 50% less oxygen ⊕ No base addition	⊕ Process stability
⊖ Process stability (nitrite, acid-tolerant AOB)	⊖ Oxygen consumption ⊖ Base addition

POMP Faust

-> See presentation Valentin Faust

Control and modelling for stable partial nitrification



Instable alkalinity-limited urine nitrification

1

Unpredictable nitrification process

Calibration

Model

Validation

No-go regions for nitrification operation

2

3

Increasing pH range to achieve stable nitrification

Narrow pH set-point range at optimal operating point

Wide pH set-point range above and below operating point

Operating strategy leading to stable process

Additional treatment unit to achieve stable nitrification

Electrochemical nitrite oxidation

Integration in model

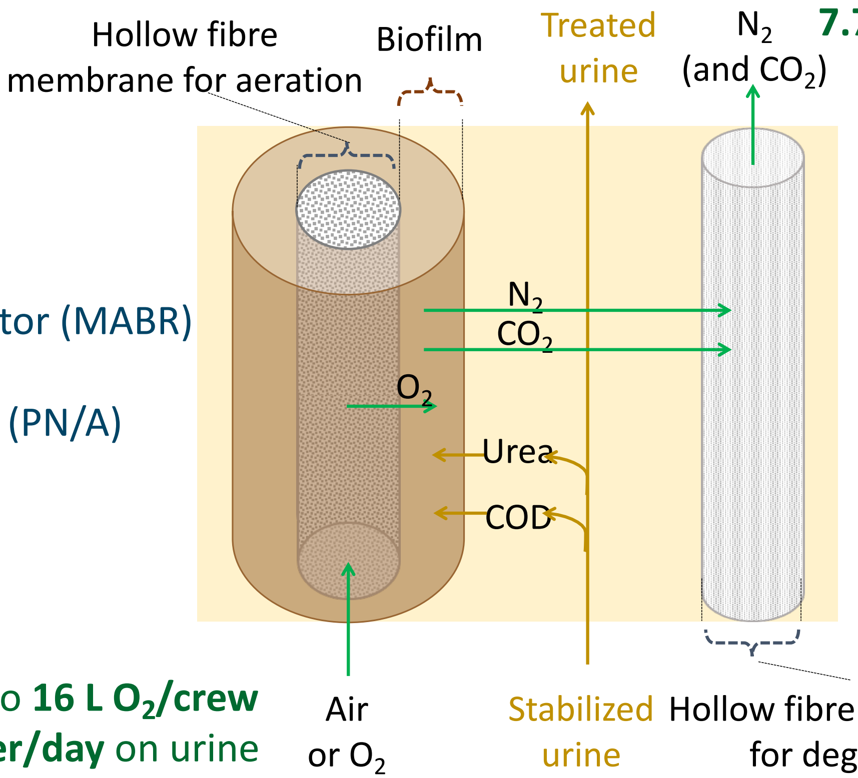
Stable alkalinity-limited urine nitrification for space application

Prevent acid-tolerant AOB
+
Prevent complete cessation of nitrification

Prevent nitrite accumulation

First exploration on N_2 production from urine in the project **Nitrogenisor**

Membrane-aerated biofilm reactor (MABR)
for
partial nitritation/anammox (PN/A)



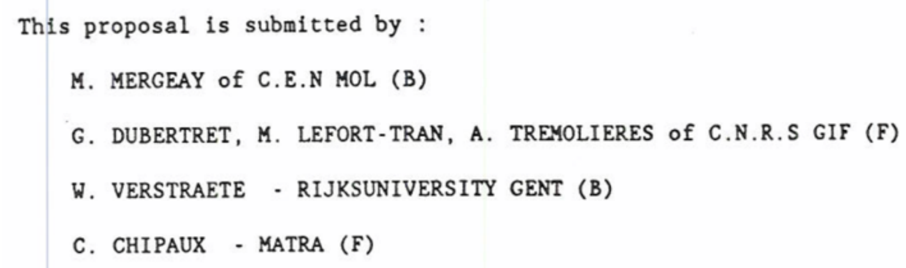
7.7 L N_2/crew member/day
(and down to **15 L CO_2)**

Down to **16 L O_2/crew member/day** on urine

Marijn Timmer
Jolien De Paepe
Philipp Markus

A defined nitrifier community: Once upon a time… (1/2)

Research proposal (1988)



This proposal is submitted by :

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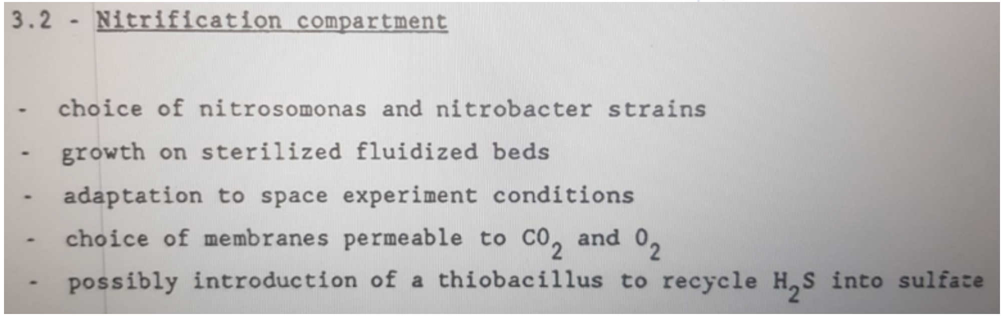
Vélizy,
March 7th, 1988.



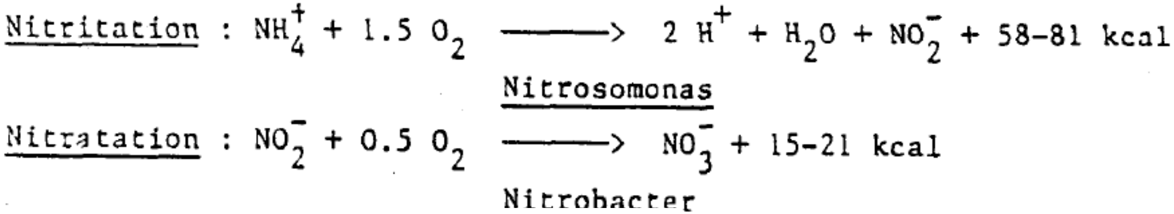
3.2 - Nitrification compartment

- choice of nitrosomonas and nitrobacter strains
- growth on sterilized fluidized beds
- adaptation to space experiment conditions
- choice of membranes permeable to CO_2 and O_2
- possibly introduction of a thiobacillus to recycle H_2S into sulfate

MELiSSA concept (1988): Mergeay M., Verstraete W., Dubertret G., Lefort-Tran M., Chipaux C., Binot R.A. (1988) "MELISSA" - A micro-organism-based model for "CELSS" development. Proceedings of the 3rd European Symposium on Space Thermal Control and Life Support Systems, Noordwijk (The Netherlands) (ESA SP-288), p.65-69.

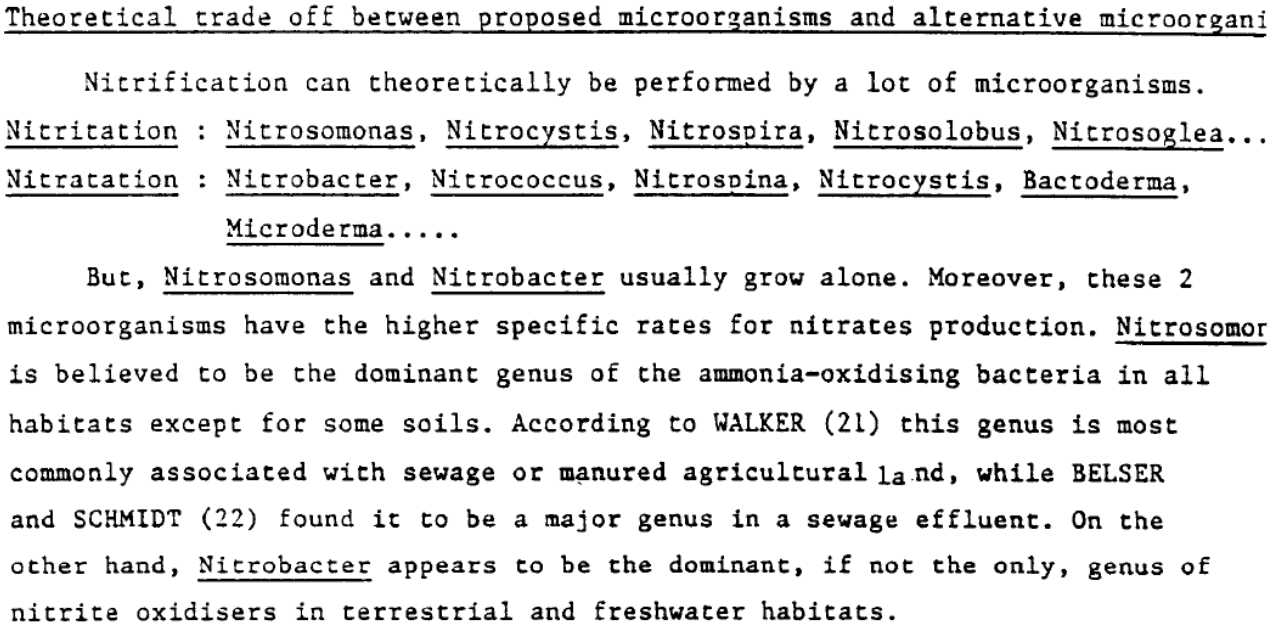
A defined nitrifier community: Once upon a time… (2/2)

MELiSSA technical note 1 (1989):



Nitritation : $NH_4^+ + 1.5\ O_2 \xrightarrow[\text{Nitrosomonas}]{} 2\ H^+ + H_2O + NO_2^- + 58\text{–}81\ \text{kcal}$

Nitratation : $NO_2^- + 0.5\ O_2 \xrightarrow[\text{Nitrobacter}]{} NO_3^- + 15\text{–}21\ \text{kcal}$



Theoretical trade off between proposed microorganisms and alternative microorgani

Nitrification can theoretically be performed by a lot of microorganisms.

Nitritation : Nitrosomonas, Nitrocystis, Nitrospira, Nitrosolobus, Nitrosoglea...

Nitratation : Nitrobacter, Nitrococcus, Nitrospina, Nitrocystis, Bactoderma, Microderma.....

But, Nitrosomonas and Nitrobacter usually grow alone. Moreover, these 2 microorganisms have the higher specific rates for nitrates production. Nitrosomor is believed to be the dominant genus of the ammonia-oxidising bacteria in all habitats except for some soils. According to WALKER (21) this genus is most commonly associated with sewage or manured agricultural land, while BELSER and SCHMIDT (22) found it to be a major genus in a sewage effluent. On the other hand, Nitrobacter appears to be the dominant, if not the only, genus of nitrite oxidisers in terrestrial and freshwater habitats.

30+ years later:
Nitrosomonas europaea* and *Nitrobacter winogradskyi are **corroborated** to be very suitable nitrifiers for MELiSSA

Key achievements defined communities

With focus on the nitrifiers:

- Predictive **modeling** available for nitrification (Cruvellier et al., 2016)
- Very high volumetric conversion **rates** for nitrification: 1.7-2.5 g N/L/d (100-54% efficiency) (Cruvellier et al., 2017)
- Deep **proteomic understanding of salt** effects on nitrification (Ilgrande et al., 2018)
- Reactor operation demonstrated at **salinity of undiluted urine** for nitrification (Christiaens et al., 2019)

With focus on the heterotrophic bacteria (ureolysis; COD conversion):

- Selection of **ureolytic, salt-tolerant** heterotrophs with batch tests (Ilgrande et al., 2018)
- **First reactor** treatment of **real urine** achieved with defined nitrifiers (Christiaens et al., 2019)
- Finetuned selection of **ureolytic, COD-degrading heterotrophs** with batch tests, and first reactor treatment with synthetic urine (POMP Barys –> Marcel Vilaplana, MPP)

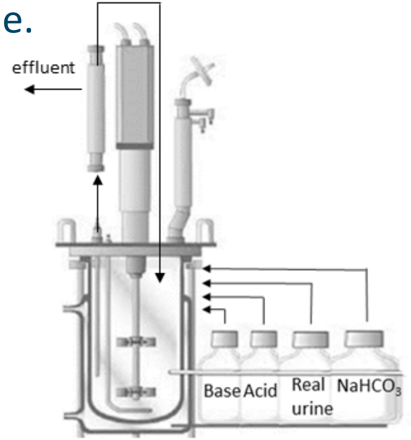
Overview heterotrophs for ureolysis and COD conversion

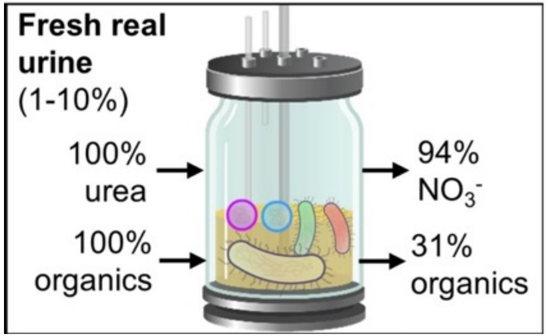
Project/test	Heterotrophs	COD removal	Comments
UNICUM (Chiara Ilgrande; Marlies Christiaens)	*Cupriavidus necator* *Vibrio campbellii* *Comamonas testosteroni* ***Pseudomonas fluorescens*** ***Acidovorax delafieldii*** ***Delftia acidovorans***	Insufficient	• All strains screened in batch: Combination of *C. necator* and *V. campbellii* with nitrifiers did not work (*C. testosteroni* yielded incomplete nitrification) • **Bold:** introduced to the reactor • **Bold & underlined:** dominant in the reactor
POMP Barys -> Marcel Vilaplana	***Cupriavidus necator*** ***Comamonas testosteroni*** ***Pseudomonas fluorescens*** ***Acidovorax delafieldii*** *Delftia acidovorans* *Acinetobacter venetianus* *Pseudomonas putida*	Tested in synthetic and real urine	• Synthetic urine: Creatinine, citric acid, hippuric acid and 4 amino acids • Medium COD removal in synthetic urine; low COD removal in real urine • **Bold:** gave best ureolysis and COD removal rates combined with nitrifiers

Urine nitrification consortium **(UNICUM),** Pool of MELiSSA PhDs **(POMP)**

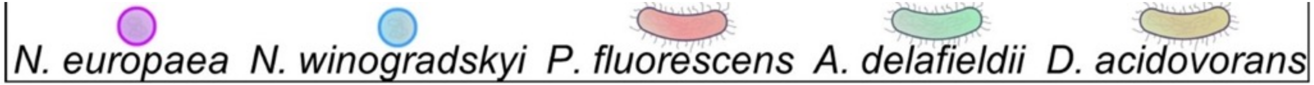
Synthetic microbial community in a membrane bioreactor (MBR)

- Membrane bioreactor (ultrafiltration)
- The full community achieved 29 ± 3 mg NO_3^-–N L^{-1} d^{-1} for 10% fresh real urine.
- Organics removal in the reactor (69 ± 15%) should be optimized.
- *D. acidovorans* dominated the community, suppressing invasive strains.

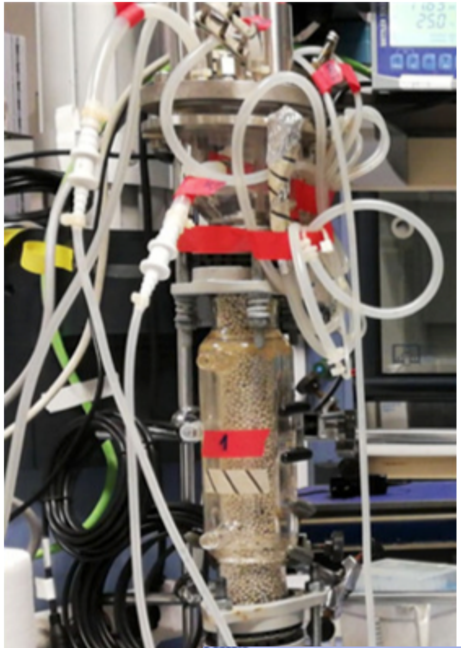




Christiaens et al. (2019)



Bioreactor start-up strategy
POMP Barys -> Marcel Vilaplana



- Four heterotrophs:
  1. *Cupriavidus necator*
  2. *Comamonas testosteroni*
  3. *Pseudomonas fluorescens*
  4. *Acidovorax delafieldii*
- Three strategies -> three reactors:
  - I. First nitrifiers, then heterotrophs
  - II. Nitrifiers and heterotrophs together
  - III. First heterotrophs, then nitrifiers

Synthetic urine treatment by a defined bacterial consortium for urea hydrolysis, nitrification and COD removal in an up-flow packed bed reactor

Vilaplana M.[1], Dolz M.[1], Barys J.[1], Garcia D.[1], Arnau C.[1], Peiro E.[1], Gòdia F.[1]

(1) MELiSSA Pilot Plant – Claude Chipaux Laboratory. Universitat Autònoma de Barcelona, Spain

Key pipeline/integration achievements

- Pre-treatment:
    - Chemical alkalinization (a.o. De Paepe et al., 2018)
    - Electrochemical alkalinization (De Paepe et al., 2020a)
    - Spontaneous maturation (POMP Faust)
    - Bio-anodic COD removal (De Paepe et al., 2020b)
- Co-treatment of urine and:
    - Black water, organic waste (BWTB, KULeuven)
    - Shower water, condensate (Lindeboom et al., 2020)
- Post-treatment or valorization:
    - Water production
    - Food and oxygen production
        - Microalgae
        - Plants
- Automation/control development

Treating faeces, organic waste and urine in the Black water treatment breadboard (BWTB)

Concept:

- Anaerobic liquefaction sub-system (ALSS):
  - Fermentation of faeces and organic waste
  - With or without urine
- Nitrification sub-system (NSS):
  - ALSS effluent and urine if not entered in ALSS
  - Nitrification and conversion of volatile fatty acids (VFA) and other COD

Overall outcome: promising results

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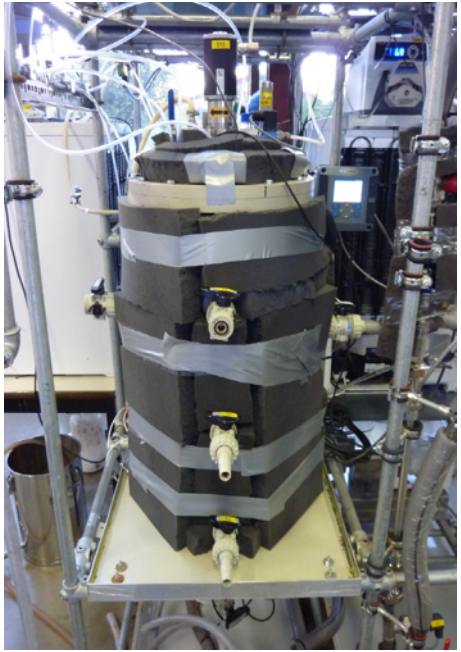
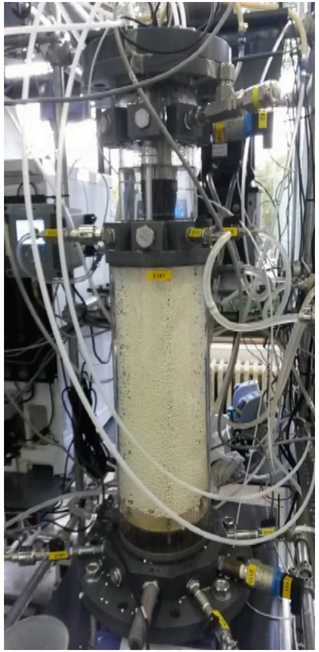


Anaerobic liquefaction sub-system

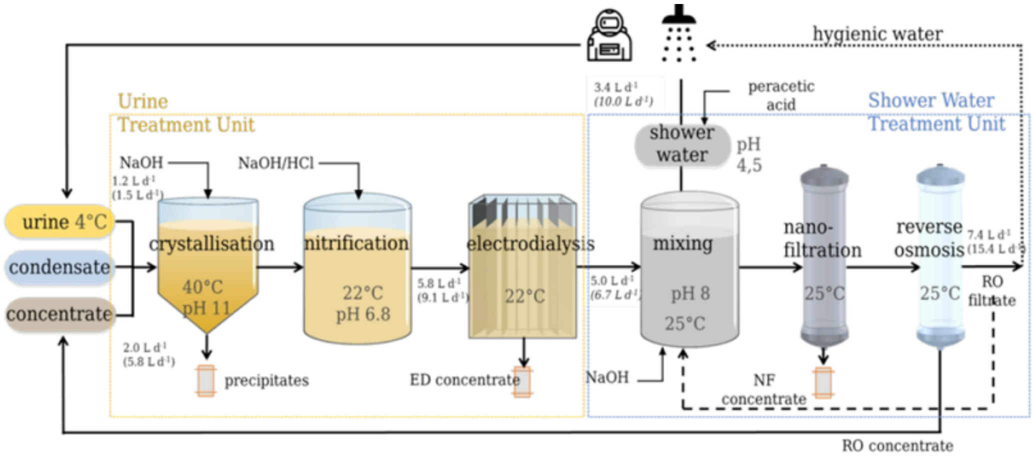


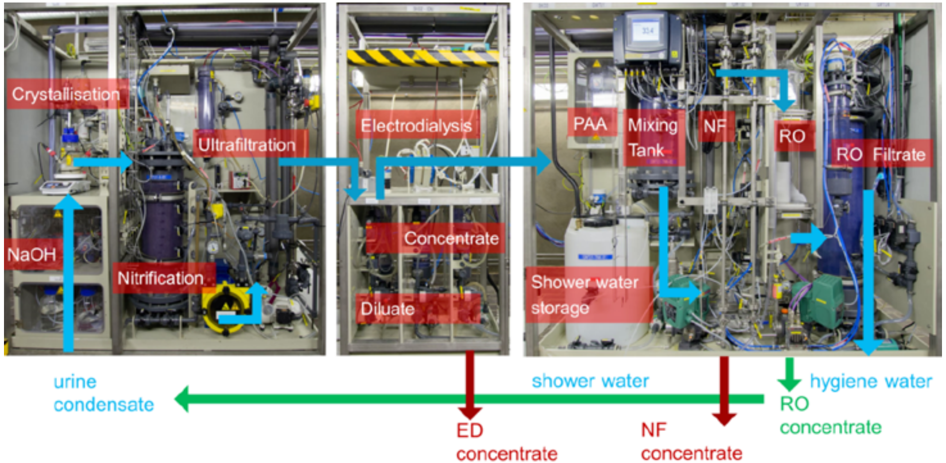
Nitrification sub-system

Water recovery enabled by nitrification: Water treatment unit breadboard (WTUB)

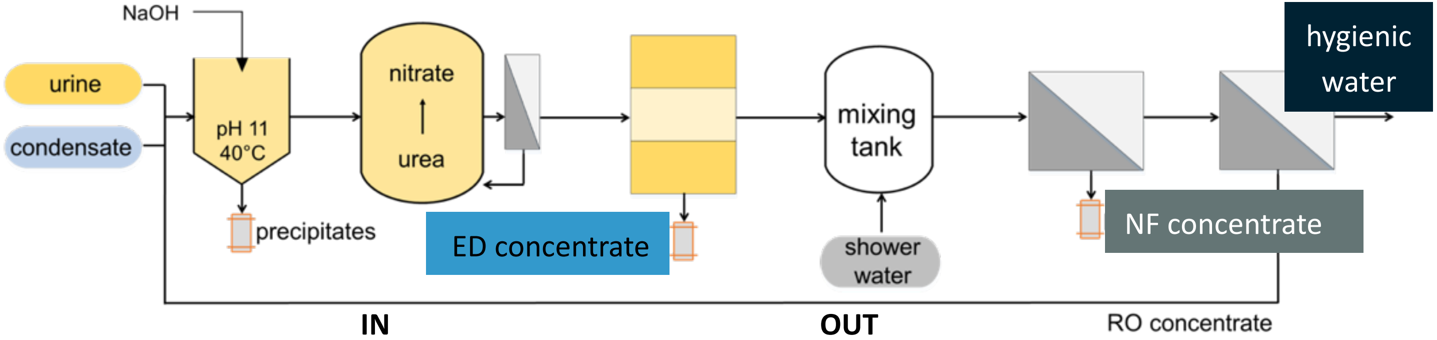
- A new 5-stage approach based on membrane filtration (nanofiltration and reverse osmosis), with urine pre-treatment based on crystallization, nitrification and electrodialysis
- Breadboard sized for 1 person
- Avoiding scaling: crystallisation to remove hardness + ED to lower EC
- Lowering biofouling: removal of organics urine in the nitrification unit + use of peracetic acid to stabilize shower water

(Lindeboom et al., 2020)





Performance water treatment unit breadboard (WTUB)

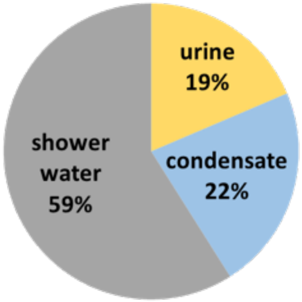


hygienic water

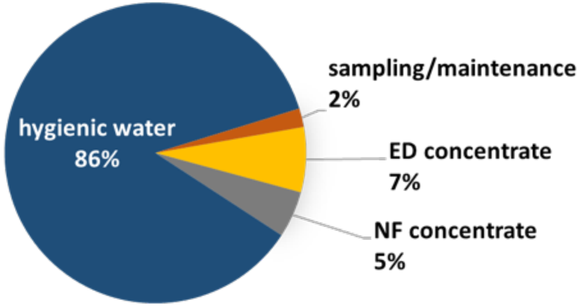
ED concentrate

NF concentrate

IN



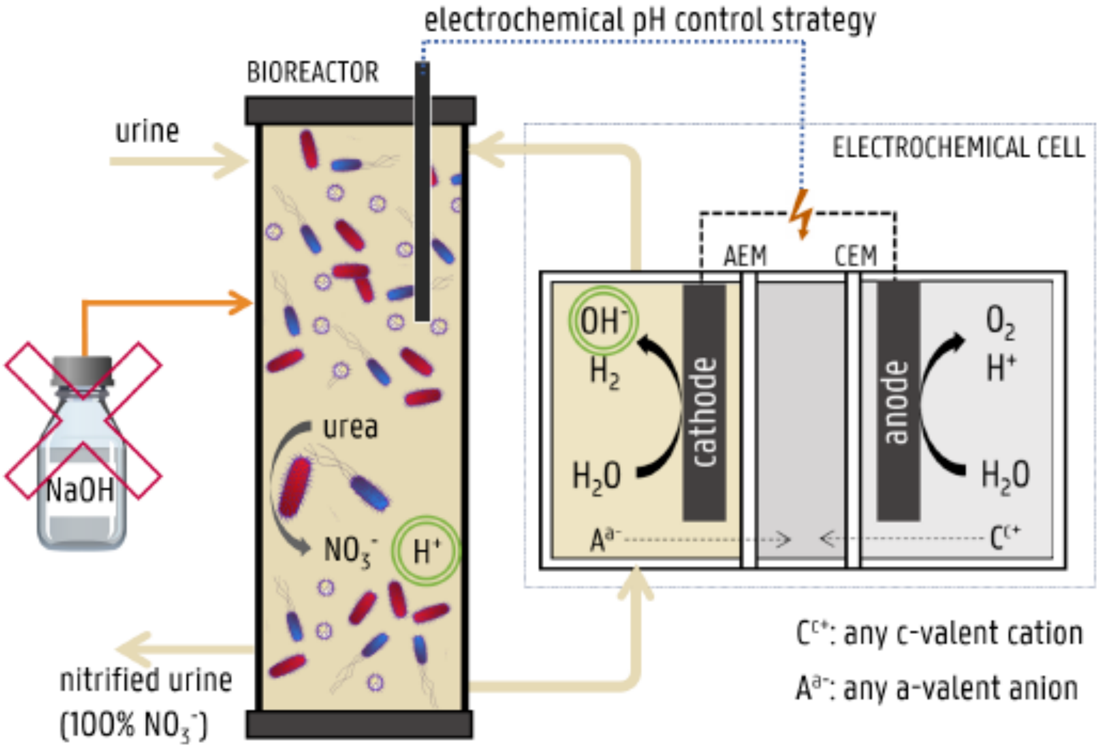
OUT



- Results of a 4-months breadboard operation campaign: total water recovery of 86%
- Reduced scaling potential with anti-scalant addition
- Stable but biofouling-limited RO permeability (0.5 L/m^2/h/bar)

(Lindeboom et al., 2020)

Electrochemical *in-situ* pH control enables chemical-free full urine nitrification with concomitant nitrate extraction

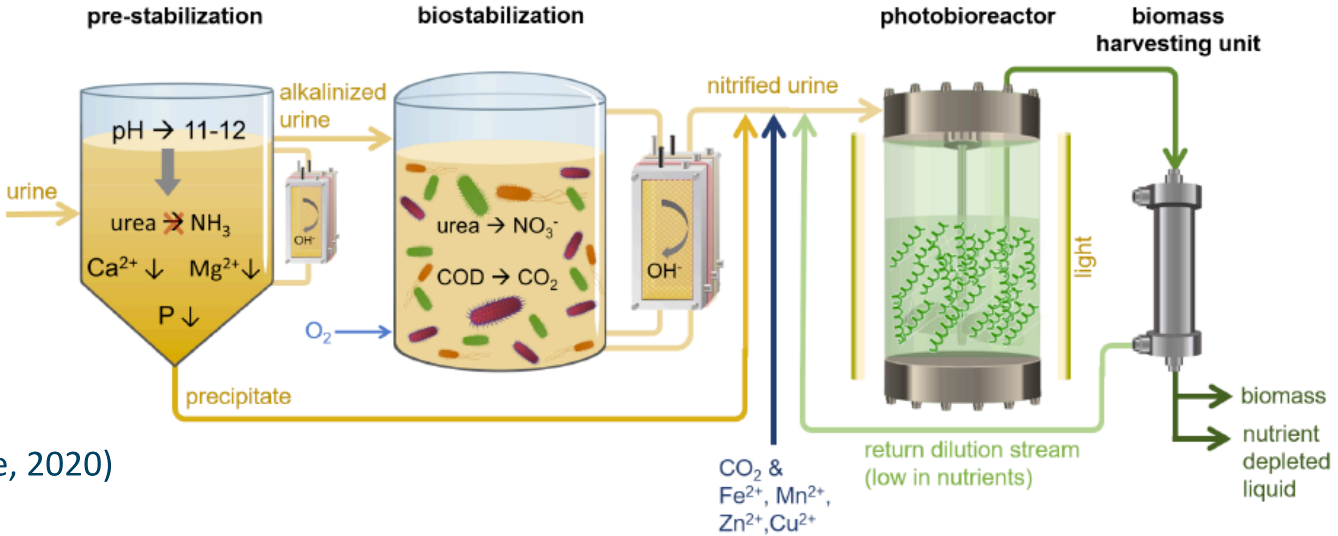


(PhD De Paepe, 2020)

Microalgae cultivation (CIVa) on a nitrified/urine matrix (1/2)

“Limited **supplementation** maximizes nitrogen recovery
from **nitrified urine** through continuous cultivation of **microalgae**”

- Batch tests: Nitrified urine supplemented with **limited amounts of P, Ca, Mg, Fe and EDTA:** as effective as modified Zarrouk medium for biomass production, nutrient uptake and protein yield
- Urine precipitates formed by alkalinization could in principle supply enough P, Ca and Mg, requiring only **external addition of iron, trace minerals and inorganic carbon.**
- Photobioreactor tests: suitability of supplemented nitrified urine as culture medium confirmed



(PhD De Paepe, 2020)

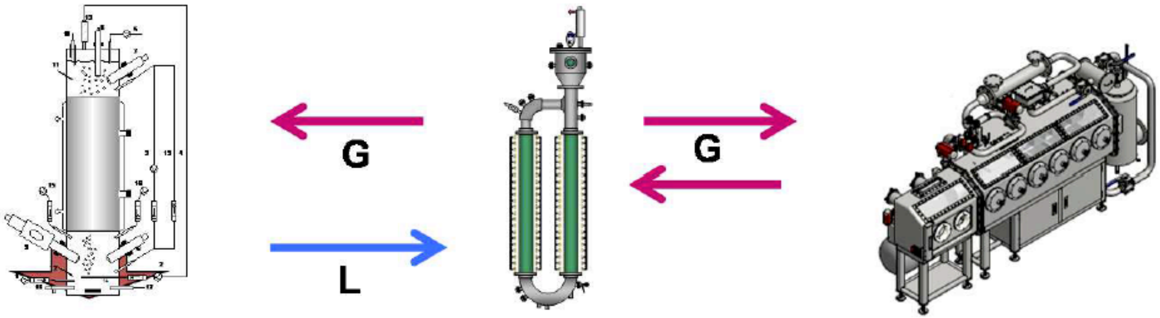
Microalgae cultivation (CIVa) on a nitrified/urine matrix (2/2)

- Biorat 2: Impact of incomplete nitrification or absence of nitrification on *Limnospira* cultivation
  - Effects specific N and COD compounds
  - See presentations UMons (Neha Sachdeva) and UAntwerp (Veerle Van Malderen)

UMONS
Université de Mons

Universiteit Antwerpen

- MELiSSA pilot plant (MPP): direct coupling between:
  - Liquid connection from nitrification (CIII) to *Limnospira* cultivation (CIVa)
  - Gas connection from *Limnospira* cultivation (CIVa) to nitrification (C3) and crew compartment (CV)
  - Demonstrated for ammonium-based medium -> shift to synthetic urine planned
  - See presentations UAB



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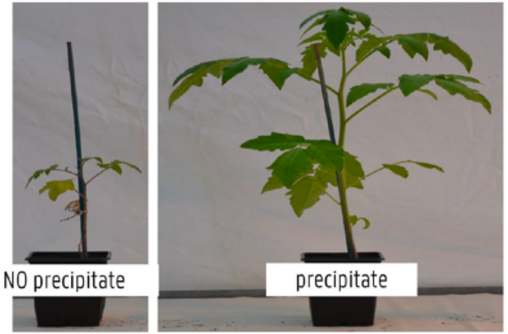
Plant production on products from a urine treatment line with nitrification

First explorations

tomato plants grown on urine precipitates



Laurens De Pryck, UGent, master thesis

GHENT UNIVERSITY

lettuce grown on MABR effluent

Università degli Studi di Napoli Federico II

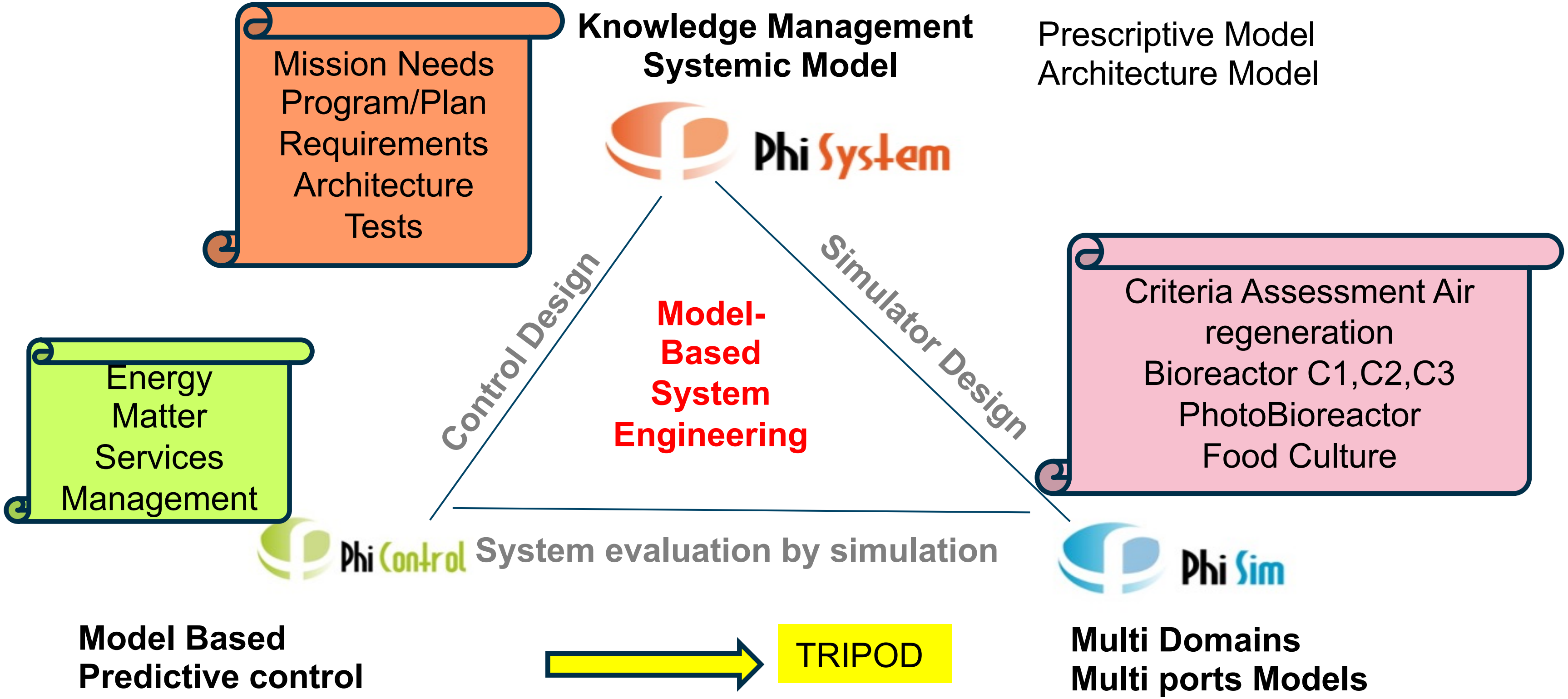
Christophe El Nakhel, Unina, POMP1 MELiSSA

POMP Crain: Production of soybean and *Salicornia* with nitrified urine and precipitates maturation tank

-> see **poster** (soybean)

ETH zürich

To ensure high reliability and minimum crew time: a model-based integrated system approach



Mission Needs
Program/Plan
Requirements
Architecture
Tests

Knowledge Management
Systemic Model

Prescriptive Model
Architecture Model

Phi System

Model-Based System Engineering

Criteria Assessment Air regeneration
Bioreactor C1,C2,C3
PhotoBioreactor
Food Culture

Energy
Matter
Services
Management

Phi Control

System evaluation by simulation

Phi Sim

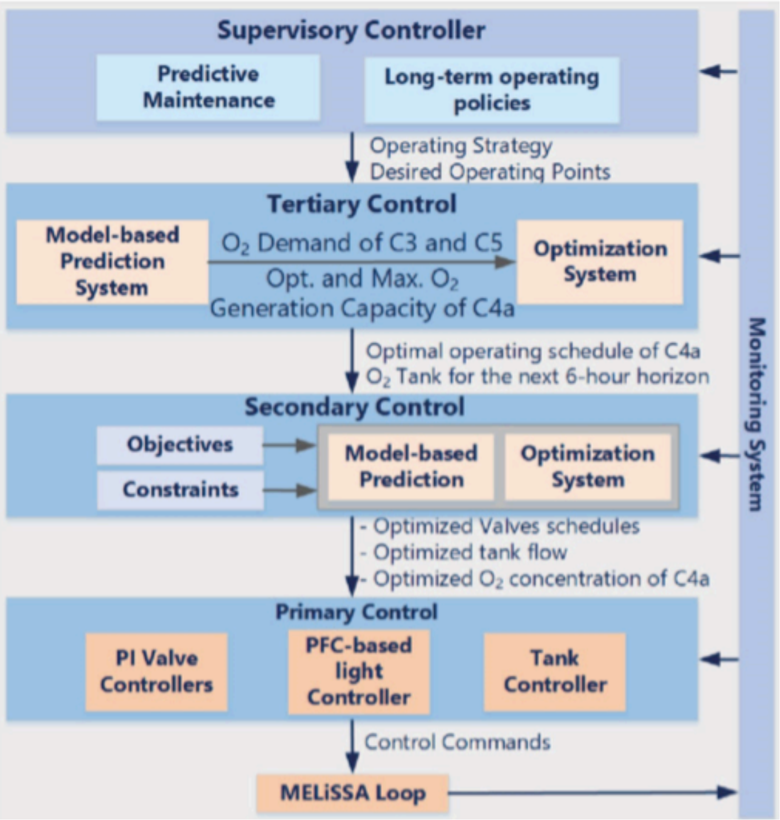
Model Based
Predictive control
Decision & Supervision Model

TRIPOD

Multi Domains
Multi ports Models
Simulation Models

SHERPA ENGINEERING

Overall control loop architecture



See poster

Proposal for MELiSSA Overall Control Loop Architecture

C. Ciurans, N. Bazmohammadi, L. Poughon, O. Gerbi, J.C. Vasquez, JM. Guerrero, CG. Dussap, F. Gòdia

(Ciurans et al., 2020)

Nitrification-based processes in Space

Experiment	**BiSTRO**	**Nitrimel**	**URINIS A**	**URINIS B**
Research topic	Reactivation potential of stored microbes (executed)		In-flight activity (planned)	
Conversions	Nitrification	Nitrification, ureolysis, denitrification, anammox	Nitrification, ureolysis	
Activity determination	Pre- and post-flight batch reaction	Post-flight batch reaction	In-flight batch reaction	In-flight continuous reactor
Destination or spacecraft	International Space Station (ISS)	Foton-M No. 4	ISS	
Altitude	400 km	258-571 km	400 km	
Radiation dose	140x higher than on Earth	250-400x higher than on Earth	To be determined	
Duration	Flight: 7 days Complete storage: 10 days	Flight: 44 days Complete storage: 104 days	To be determined	

(Ilgrande et al., 2019)

(Lindeboom et al., 2018)

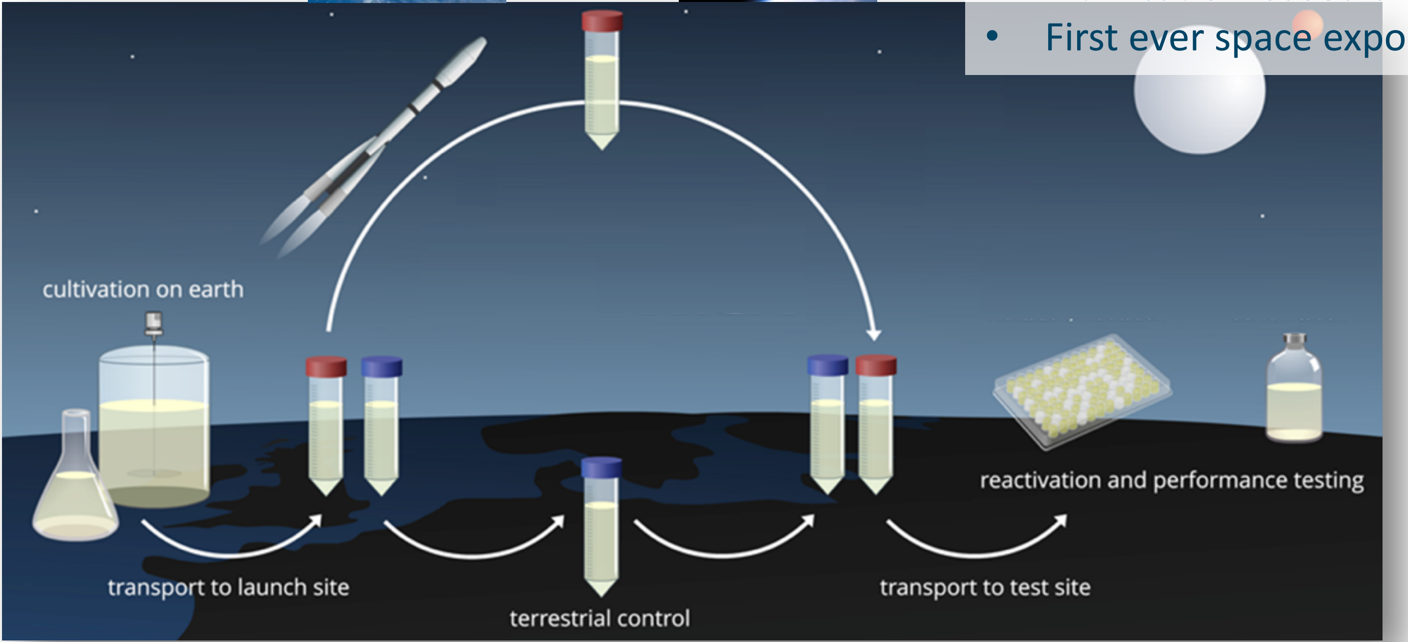
Reactivation of preserved cultures

(Ilgrande et al., 2019)

(Lindeboom et al., 2018)

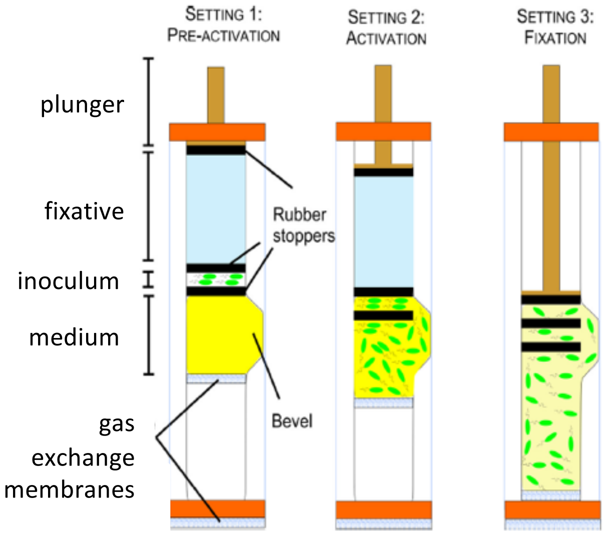
Key achievements:

- Highest radiation: 687 ± 170 µGy (Gray) d^{-1} in a 44-day FOTON-M4 flight
- Reactivation of all key functions needed for nitrification-based urine treatment
- First ever space exposure of anammox bacteria



URINIS (Urine nitrification in Space) A: Activity tests @ ISS

- Start URINIS 2: 1/1/2020
- Gravity independent aeration
- Effect of microgravity on:
  - Biofilm structure/formation
  - Nitrification rate
  - Metabolism (transcriptomics/proteomics)
- Consortium:
  - *Comamonas testosteroni* (UMons)
  - *Nitrosomonas europaea* (SCK-CEN)
  - *Nitrobacter winogradskyi* (UGent/UAntwerpen)



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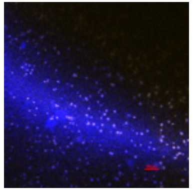
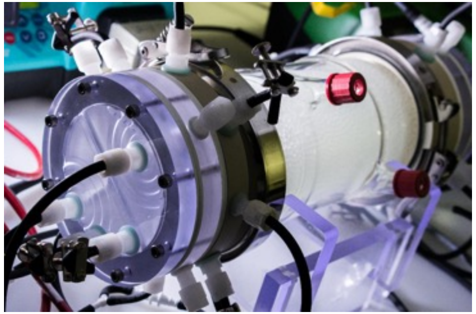
SCK•CEN
STUDIECENTRUM VOOR KERNENERGIE
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE
UMONS
Université de Mons
University of Antwerp



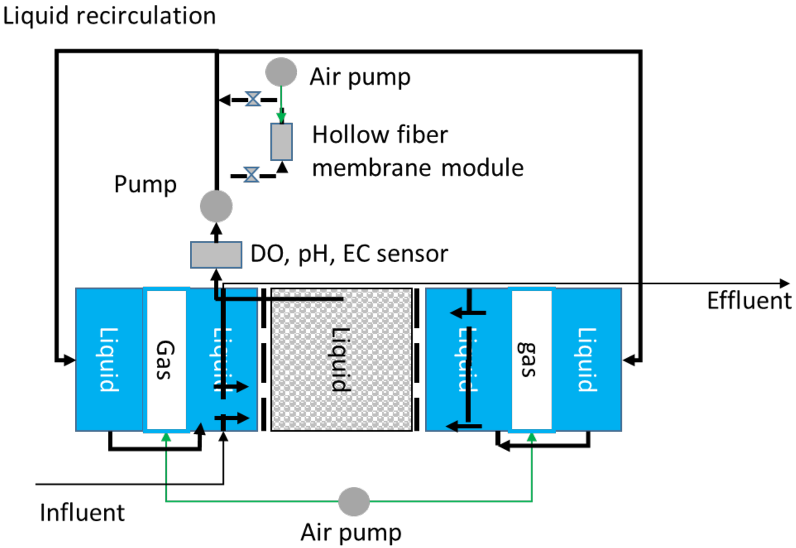
Athraa Al-Saadi Thanh Huy Nguyen
Tom Verbeelen

URINIS (Urine nitrification in Space) B: Nitrifying bioreactor @ ISS

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



QinetiQ Space nv



MELiSSA's ECLSS view on urine nitrification: Go for win-win-win-…

-> Nitrification-based processes **modularly fit in many ECRLSS goals/scenarios:**

1. Environmental control (EC)
-> Waste treatment for risk mitigation

Regenerative life support systems (RLSS)
-> Resource recovery to increase circularity and decrease external dependency

Avoid NH_3 volatilization, biofouling, scaling,…

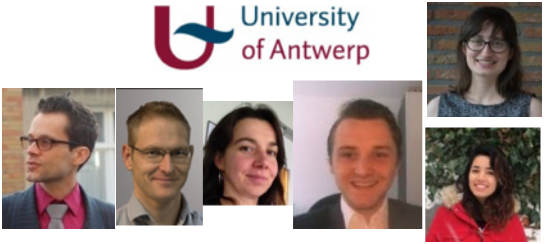
2. Produce water
3A. Produce neutral gas **OR**
3B. Produce nutrients/mineral solution for food and O_2 production

In all cases, feasible **for 'just' urine** (+ condensate) and any **more complex waste treatment or MELiSSA cycle** (faeces +/- organic waste +/- grey water +/-…)
-> At least 12 scenarios

esa

Christophe Lasseur, Brigitte Lamaze, Christel Paille,...

University of Antwerp



Siegfried Vlaeminck, Marc Spiller, Veerle Van Malderen, Tim Van Winckel, Marijn Timmer, Jolien De Paepe, Athraa Al-Saadi,...

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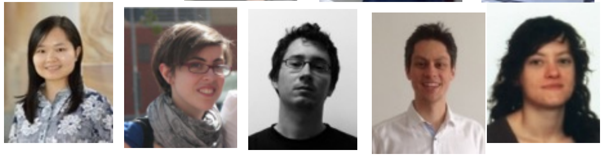

Nico Boon, Ramon Ganigué, Peter Clauwaert, Korneel Rabaey, Ralph Lindeboom, Jolien De Paepe, Athraa Al-Saadi,...

KU LEUVEN

Ilse Smets, Koen Rummens,...

QinetiQ

Dries Demey,...

eawag
aquatic research

Kai Udert, Valentin Faust, Philipp Markus,...

ETH Zürich

Grace Crain,...

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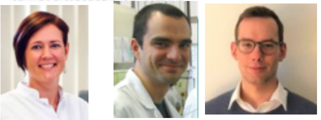

Natalie Leys, Felice Mastroleo, Tom Verbeelen,...

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Francesco Gòdia, Enrique Peiro, Carolina Arnau, David García, Carles Ciurans, Justyna Barys, Marcel Vilaplana,...

MELiSSA

UCA
UNIVERSITÉ Clermont Auvergne

Gilles Dussap, Laurent Poughon, Cathérine Creuly, Nelly Cruvellier,...

EPAS
ECO PROCESS ASSISTANCE

SEMiLLA
IPSTAR Circular Systems™

Rob Suters, Radu Giurgiu,...

UMONS
Université de Mons

Baptiste Leroy, Ruddy Wattiez, Neha Sachdeva, Thanh Huy Nguyen,...

SHERPA ENGINEERING

Philippe Fiani, Olivier Gerbi, Jean Brunet,...

NTE SENER

vito
vision on technology

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MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

THANK YOU.

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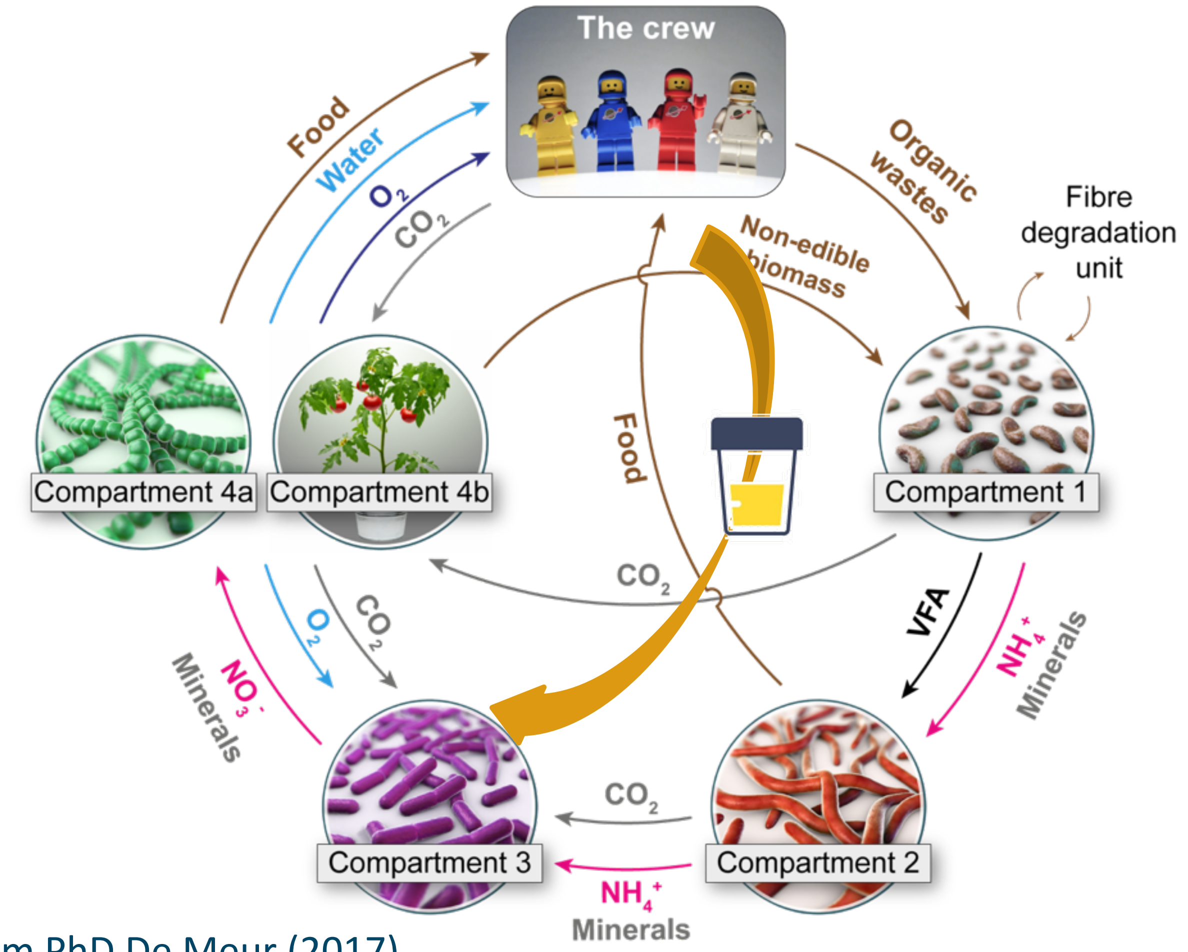

Modified from PhD De Meur (2017)

Nitrification-related projects completed in the past 5 years

Green: MELiSSA's goal for Space use

Project	Consortia (or strains)	Urine (or medium)	Process type	Space-flight	Comments
MBR	Open	Real	Reactor	No	Urine (undiluted); preliminary link to CIVa
WTUB	Open	Real	Reactor	No	Urine, condensate and treated shower water; pretreatment; water recovery and preliminary link to CIVb
BWTB	Open	Synthetic & real	Reactor	No	Urine and fermented faeces and organic waste
UNICUM	Defined	Synthetic & real	Flask & reactor	No	Urine (diluted); focus on salinity and ureolysis
Bistro	Defined	Synthetic	Flask	Yes	ISS flight experiment with preserved cultures
NitriMel	Open & defined	Synthetic	Flask	Yes	Foton-M4 flight experiment with preserved cultures
URINIS 'part 1'	Defined	Synthetic	Flask	No	Preparatory tests to an ISS flight experiment
POMP De Paepe	Open	Real	Reactor	No	Urine (diluted), pretreatment, electrochemical OH^- addition, membrane aeration, preliminary link to CIVa and CIVb
POMP Barys	Defined	Synthetic & real	Flask & reactor	No	Urine (diluted), biofilm carrier material, organics
MPP	Defined	Synthetic	Reactor	No	So far only on NH_4^+ (no urea, no COD); link to CIVa

Membrane bioreactor **(MBR),** Water treatment unit breadboard **(WTUB),** Black water treatment breadboard **(BWTB),** Urine nitrification consortium **(UNICUM),** Urine nitrification in Space **(URINIS),** Pool of MELiSSA PhDs **(POMP)**

Nitrification-related projects currently running/planned

Green: MELiSSA's goal for Space use

Project	Consortia (or strains)	Urine (or medium)	Process type	Space-flight	Comment
POMP Valentin Faust	Open	Real	Reactor	No	Urine (± undiluted), 'spontaneous' maturation (ureolysis, fermentation), partial nitrification, process robustness/control/automation
POMP Grace Crain	Open	Real	Reactor	No	CIVb study using partially nitrified urine (± undiluted) and precipitates from the collection/maturation tank
Biorat 2	Open	Real	Reactor	No	CIVa study on effects of specific nitrogen and COD organics
URINIS 'part 2'	Defined	Synthetic	Flask	No	Continued preparatory tests to an ISS flight experiment
Nitrogenisor	Open	Synthetic & real	Reactor	No	Partial nitritation/anammox for N_2 production; membrane aeration
MPP	Defined	Synthetic	Reactor	No	Shift from NH_4^+-based medium to synthetic urine (10%); link to CIVa

Urine nitrification in Space **(URINIS),** Pool of MELiSSA PhDs **(POMP)**