MELiSSA
MICRO-ECOLOGICAL
LIFE SUPPORT SYSTEM
ALTERNATIVE

CREATING
A CIRCULAR
FUTURE

Urine and life support: Some nitrification-based MELiSSA solutions

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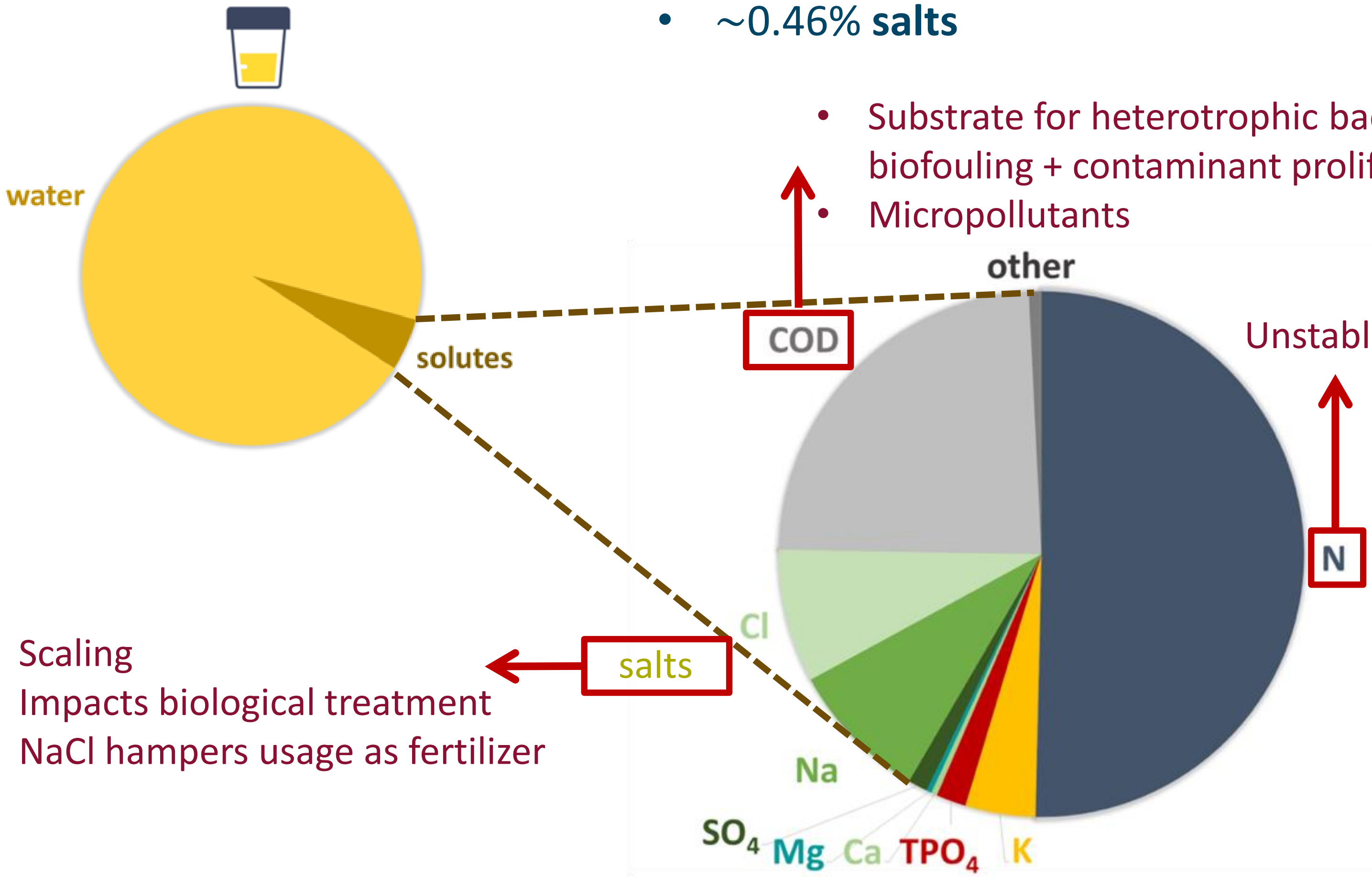
Urine: 91-96% **water** + …

- ~0.92% **N**
- ~0.66% **C:** ~half in urea; ~half in other organics
- ~0.46% **salts**



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

Unstable -> NH_3 volatilisation

- Scaling
- Impacts biological treatment
- NaCl hampers usage as fertilizer

Risks & challenges

Water in Space? Urine as major flow in missions without grey water

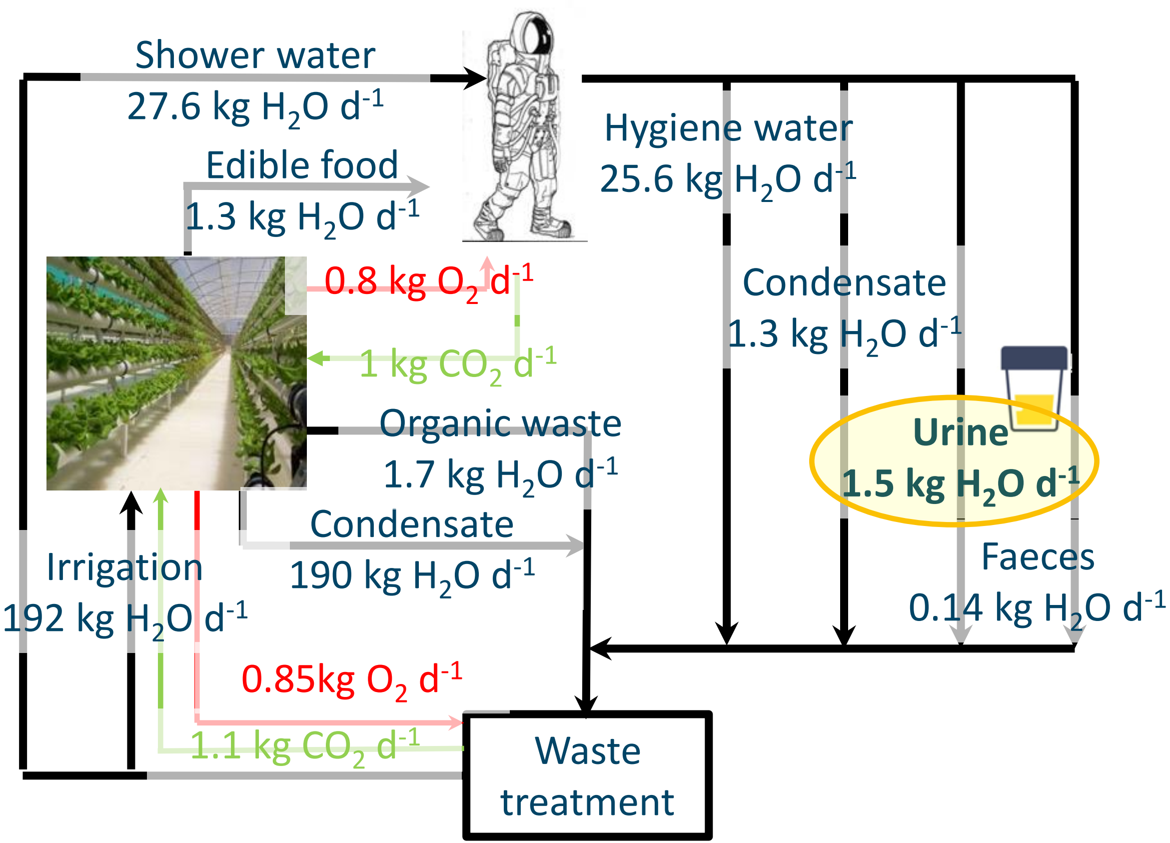
Short missions:

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

Sabatier water, as a function of the CO_2 management system

Additional flows in longer missions:

- **Grey** water from hygiene activities (e.g. shower)
- **Transpiration** water (food production with plants)
- **Black** water (from toilet flush)
- **Grey** water from service activities (laundry, dish-washer, etc.)
- …



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

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

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

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

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

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

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

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

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

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

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

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

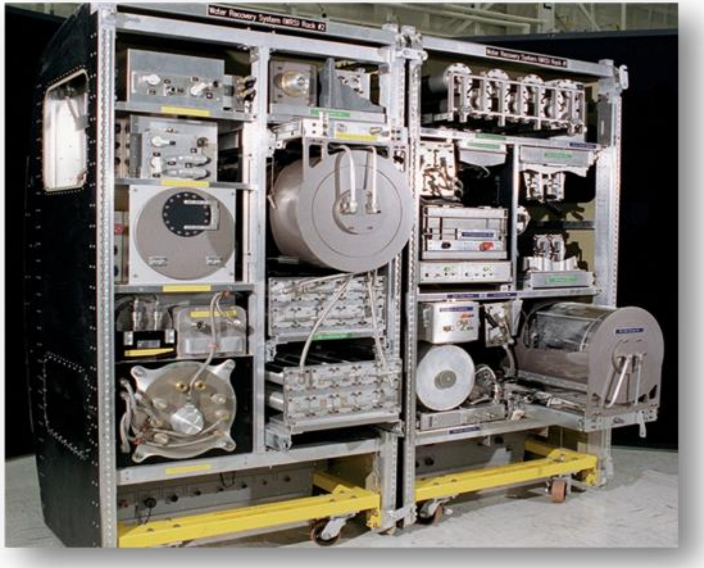
Faeces
0.14 kg $H_2O\ d^{-1}$

Waste treatment

(After Hu et al. 2010)

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

Belgian astronaut Frank De Winne: ‘In Space, we drink the **same coffee every day’**
-> Water recycling from urine is a no-brainer for human exploration

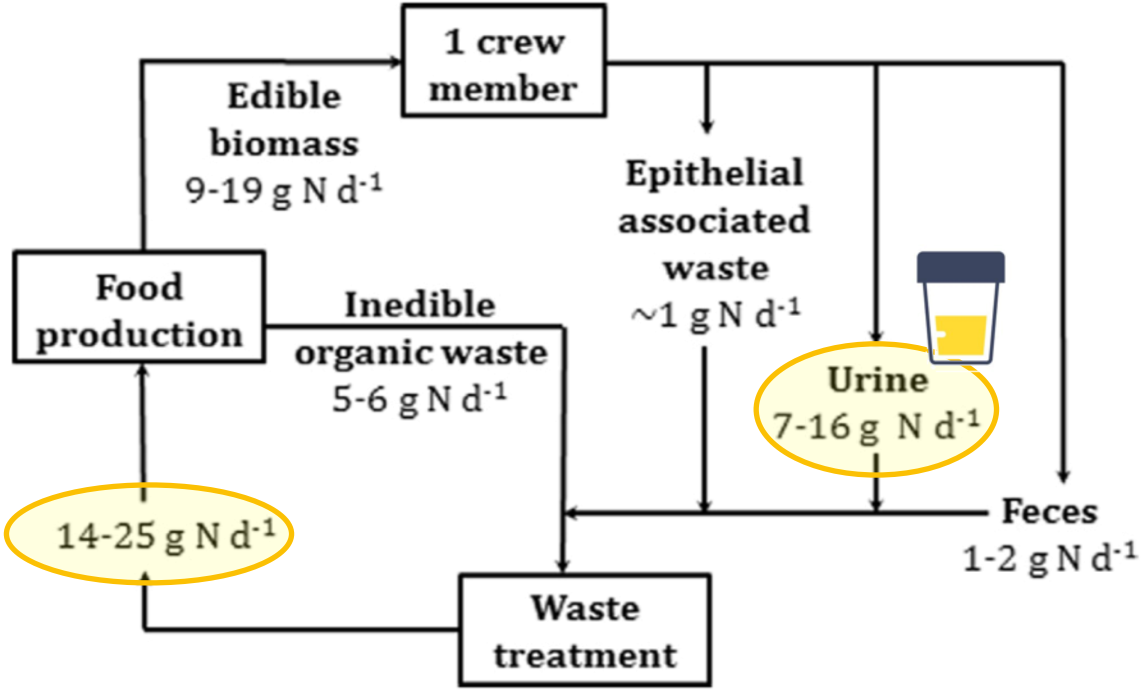


Racks for the ISS water recovery system
(Carter et al. 2011)

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

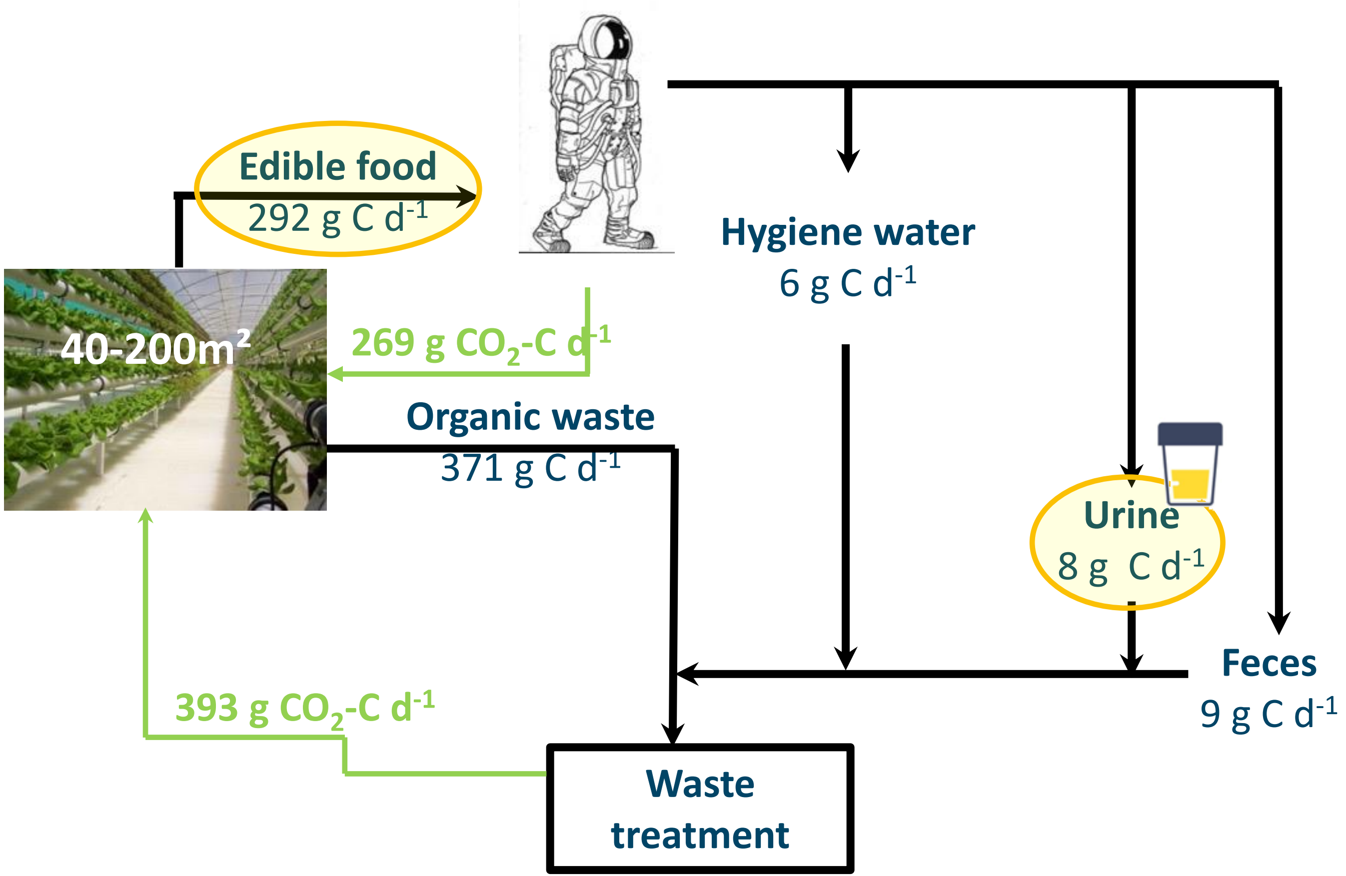
Nitrogen in Space? -> Urine as major flow

Urine: 50-64% in closed system with food production



(Clauwaert et al., 2017)

Carbon in Space? -> Urine as **negligible** flow



Edible food
292 g C d^{-1}

40-200m^2

269 g CO_2-C d^{-1}

Hygiene water
6 g C d^{-1}

Organic waste
371 g C d^{-1}

Urine
8 g C d^{-1}

Feces
9 g C d^{-1}

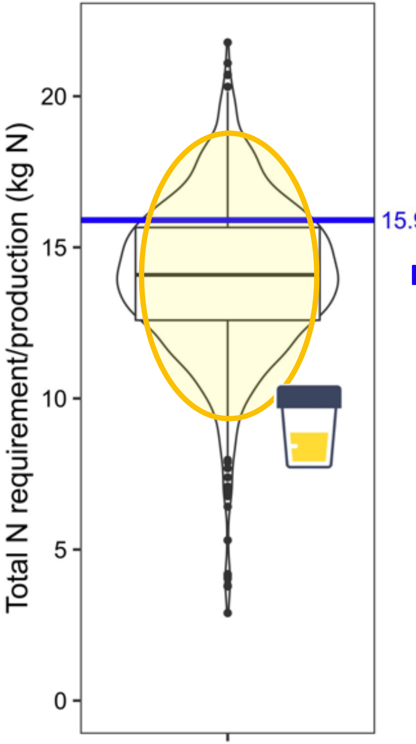
393 g CO_2-C d^{-1}

Waste treatment

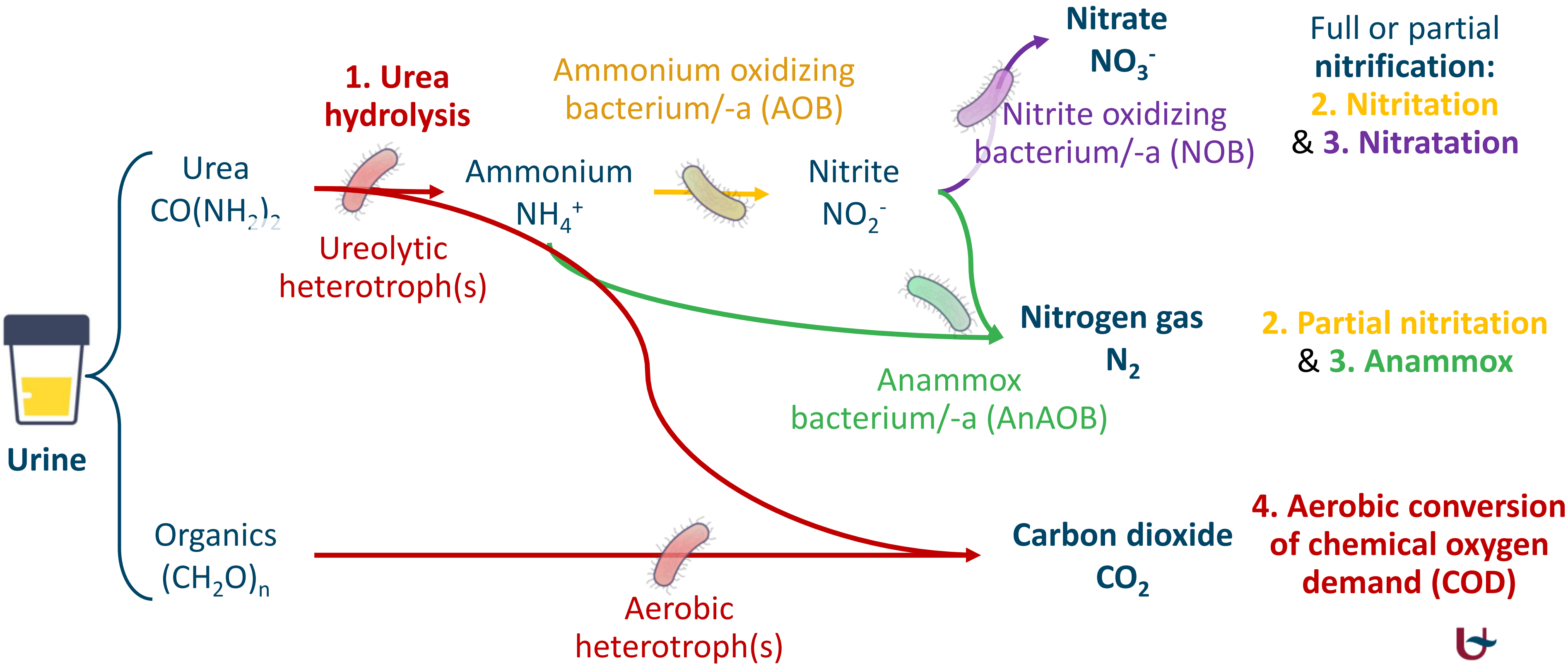
(After Hu et al. 2010)

Nitrogen gas (N_2) requirements and production potential in Space

- N_2 **requirement**
  - To maintain a pressurized cabin atmosphere, counteracting losses due to extravehicular activities, structural leakages,…
  - 15.9 kg N needed
- **Production** potential of N_2 from urine
  - Through partial nitritation/anammox
  - 14 kg N produced on average
- N_2 recovery from urine can **offset on average 88% of the N_2 gas need** (25% of the stochastic runs can offset all losses)
- Curious for more? **Talks on Thursday** (urine & nitrification session 3/3, room 2):
  - Technology R&D: **Marijn** Timmer
  - Mission scenarios: **Tim** Van Winckel
- Assumptions: Mars transit mission, 4 crew members, 650 days



Microbial conversion routes for N and C in urine: 2 routes, having 3 out of the 4 steps in common:
'Nitrification-based'
= Ureolysis **AND** (Partial) Nitritation **AND** Nitratation **OR** Anammox **AND** COD conversion



Urine

Urea
$CO(NH_2)_2$

Organics
$(CH_2O)_n$

1. Urea hydrolysis

Ureolytic heterotroph(s)

Ammonium
NH_4^+

Ammonium oxidizing bacterium/-a (AOB)

Nitrite
NO_2^-

Nitrite oxidizing bacterium/-a (NOB)

Nitrate
NO_3^-

Nitrogen gas
N_2

Anammox bacterium/-a (AnAOB)

Aerobic heterotroph(s)

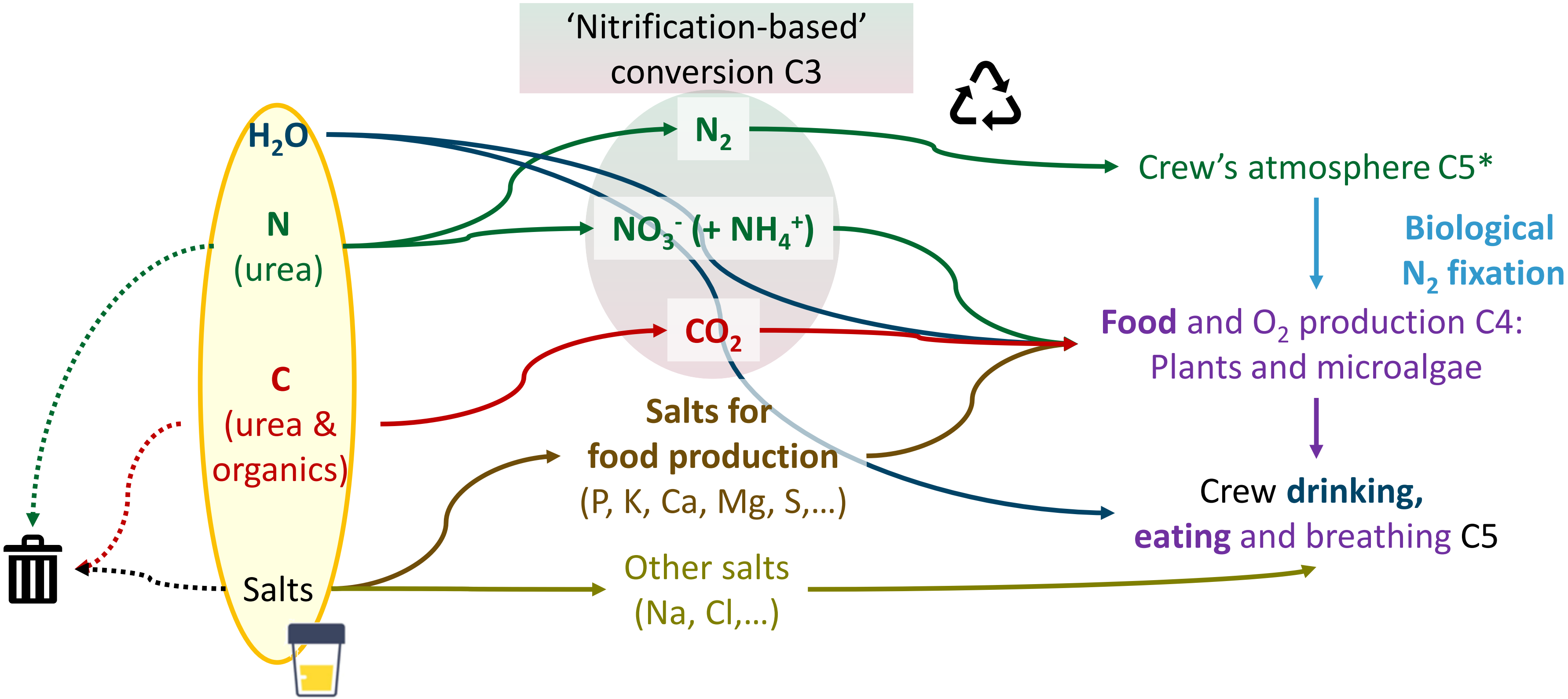
Carbon dioxide
CO_2

Full or partial **nitrification:**
2. Nitritation
& **3. Nitratation**

2. Partial nitritation
& **3. Anammox**

4. Aerobic conversion of chemical oxygen demand (COD)

Modular and complimentary options to deal with urinary resources



‘Nitrification-based’ conversion C3

H_2O

N (urea)

C (urea & organics)

Salts

N_2

NO_3^- (+ NH_4^+)

CO_2

Salts for food production

(P, K, Ca, Mg, S,…)

Other salts (Na, Cl,…)

Crew’s atmosphere C5*

Biological N_2 fixation

Food and O_2 production C4: Plants and microalgae

Crew **drinking, eating** and breathing C5

Note: **N_2 production** can be an elegant solution to uncouple undesired salinity (NaCl) from water and useful nutrients for food production driven by **biological N_2 fixation**

N and C conversion stoichiometries in urine

Combined processes	Overall stoichiometry
Ureolysis, full nitrification & C conversion	$0.5\ CO(NH_2)_2 + 0.22\ CH_3COOH + 2.18\ O_2$ $\rightarrow \mathbf{0.96\ NO_3^-} + 0.036\ C_5H_7O_2N + \mathbf{0.76\ CO_2} + 1.06\ H^+ + 0.73\ H_2O$
Ureolysis, partial nitritation/anammox & C conversion	$0.5\ CO(NH_2)_2 + 0.22\ CH_3COOH + 1.14\ O_2$ $\rightarrow \mathbf{0.43\ N_2} + 0.11\ NO_3^- + 0.032\ C_5H_7O_2N + \mathbf{0.78\ CO_2} + 0.11\ H^+ + 1.26\ H_2O$

Conclusions:

- Nitrification: ~96% N recovered as nitrate, ~81% C converted to CO_2
- Partial nitritation/anammox: ~86% N recovered as nitrogen gas, ~83% C converted to CO_2
- For both: ~half of CO_2 production from urea; ~half of CO_2 production from COD

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)

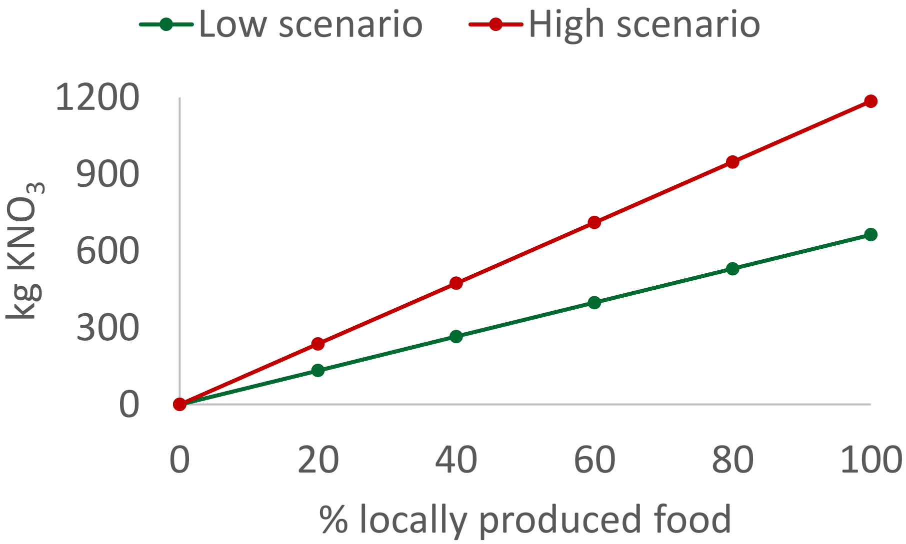
Preliminary dimensioning and input/output assessment

- Volume nitrification or PN/A unit, assuming a loading rate of 0.5-1 g N/L/d (and a crew of 6):
  - 58-120 L active reactor volume
  - **ca. 230-460 L reactor + skid/instrumentation/…**
- Input/output:
  - Oxygen demand: Majority for N conversion
  - OH^- demand: Only for full nitrification (~1 mol OH^-/mol N)
  - Sludge production: Similar for N and COD conversion
- Assumption: 9.6 g N/crew member/d in urine

Numbers for 1 crew member	**O_2** required g O_2/d	**NaOH** required g/d	**Sludge** produced g/d
Full nitrification (~100% NO_3^-)	48	29	3.6
Partial nitrification (~50% NO_3^-; ~50% NH_4^+)	28	0	2.6
Partial nitritation/anammox (~86% N_2; ~11% NO_3^-)	25	0	3.2

Thought exercise – Bring your N fertilizer from home

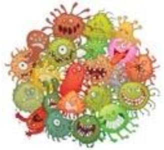
- Mission: 3 years, 6 astronauts
- Food production with ‘conventional hydroponics’: KNO_3 as N source, 14 (‘low’) -> 25 (‘high’) g N needed/person/day
- N fertilizer need: up to **660-1200 kg KNO_3**



Big bag (1200 kg fertilizer)

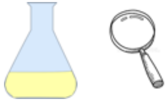
- Note: **Not recycling water** (12 L/person/day) requires about **79,000 kg H_2O** -> 66-120x more mass

Nitrification-based urine treatment: The MELiSSA strategy towards demonstration in Space

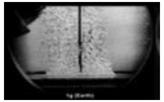


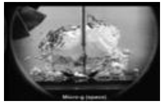
Open and dynamic -> ‘natural/stochastic’ selection	Defined and thus biosafe -> ‘curated/controlled’ selection

Synthetic: Progressively increasing complexity	Real: Full complexity (organics, salts, micropollutants)

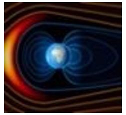


Flask incubation and process characterization	Reactor operation, modelling, automation, control

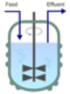


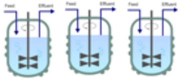


1 g	Reduced gravity **(< 1 g)** -> gas/liquid mass transfer challenges



Radiation protected (magnetic field)	Higher solar and cosmic radiation -> effects on biology (including crew)





Bioreactors and other unit processes	Fully integrated treatment pipelines/systems

Ground demonstration	Space demonstration

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

Overview maturity microbiome and matrix types

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

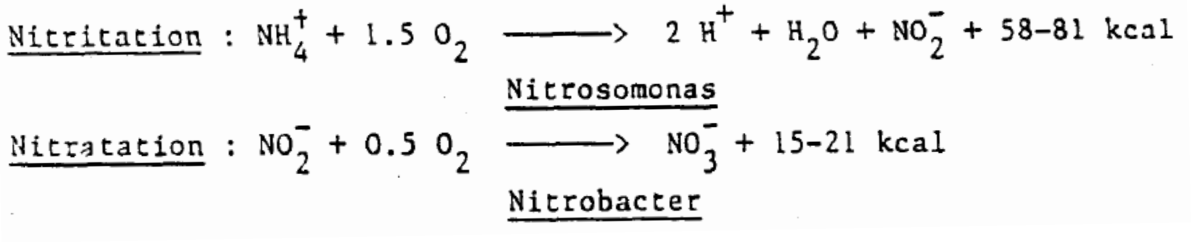
Process	**Microbiome**		**Type of urine**	
	Open	**Defined**	Synthetic	**Real**
Ureolysis	+++			+++
		+	+	+
Nitrification	+++			+++
		++	++	++
Partial nitritation/anammox	+++			+++
COD conversion	+++			+++
		+	+	+

Key achievements **open** communities

- Demonstrated at salinities of **undiluted urine:** Coppens et al. (2016)
- **pH control** variations:
  - 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 (Eawag POMP Valentin Faust)
- **Particle-free effluent** through use of membrane bioreactors (Coppens et al., 2016; De Paepe et al., 2018)
- First insights into **N_2O emissions** and optimization potential (0.4-1.2% of N load) (Faust et al., 2022a)
- Insights into understanding and avoiding ingrowth of novel **acid-tolerant ammonia oxidizer** "*Candidatus* Nitrosoacidococcus urinae" (Faust et al., 2022b)
- **Gravity-independent (bubbleless) aeration:** membrane-aerated biofilm reactor on real urine
  - Nitrification (De Paepe et al., 2020b)
  - Partial nitritation/anammox (Timmer et al., in prep.)

A microbiome for nitrification-based urine treatment

Nitrifiers in a defined/synthetic microbiome: Once upon a time (1989): MELiSSA technical note 1



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

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

30+ years later: Suitability ***Nitrosomonas*** *europaea* and ***Nitrobacter*** *winogradskyi* confirmed for urine treatment

Anammox bacteria in an open microbiome: *“Candidatus* **Brocadia** sp.”
(in an open/mixed community; Timmer et al., in prep.)

Heterotrophs: A consortium of four is currently considered (tested in UAB/MPP):

1. ***Cupriavidus*** *necator*
2. ***Comamonas*** *testosteroni*
3. ***Pseudomonas*** *fluorescens*
4. ***Acidovorax*** *delafieldii*

-> Talk Carolina Arnau on Thursday in Urine session 3/3

Key achievements **defined** communities

With focus on the nitrifiers *(Nitrosomonas* and *Nitrobacter):*

- Predictive **modeling** (Cruvellier et al., 2016)
- Very high nitrification **rates:** 1.7-2.5 g N/L/d (100-54% efficiency) (Cruvellier et al., 2017)
- **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)
- First reactor treatment with synthetic urine at **high nitrate production rates** (>0.5 g N/L/d) (Marcel Vilaplana/Carolina Arnau, MPP/UAB)

Key pipeline/integration/system achievements

- Co-treatment of **urine** and:
  - **Black** water, **organic** waste (BWTB, KULeuven)
  - **Grey** water (shower), **condensate** (Lindeboom et al., 2020)
- Pre-treatment:
  - **Alkalinization:** Electrochemical (De Paepe et al., 2020a) or chemical (a.o. De Paepe et al., 2018)
  - Spontaneous **'maturation':** ureolysis and organics fermentation (Eawag, POMP Nele Kirkerup)
  - **COD removal:** Bio-anodic (De Paepe et al., 2020b) or membrane aeration (Eawag, PhD Aurea Heusser)
- Post-treatment, valorization and integration:
  - **Water** recovery – integrated process (Lindeboom et al., 2020)
  - **Concentrated liquid fertilizer** (water removal) – integrated process (Eawag/VUNA)
  - **Food and oxygen** production
    - Microalgae – integrated process liquid/gas (UAB/MPP)
    - Plants – off-line combination
- **System aspects**
  - Automation and control (a.o. Sherpa Engineering; Université Clermont Auvergne)
  - Stochastic Space mission scenario analyses (Van Winckel et al., in prep.)
  - Preliminary establishment ALiSSE (advanced life support system evaluator) metrics
  - Terrestrial environmental sustainability assessment (UAntwerp & Eawag, in prep.)

Nitrification-based processes in Space

Experiment	**BiSTRO**	**Nitrimel**
Research topic	Reactivation potential of stored microbes (executed)	
Conversions	Nitrification	Nitrification, ureolysis, denitrification, anammox
Activity determination	Pre- and post-flight batch reaction	Post-flight batch reaction
	(Ilgrande et al., 2019)	(Lindeboom et al., 2018)

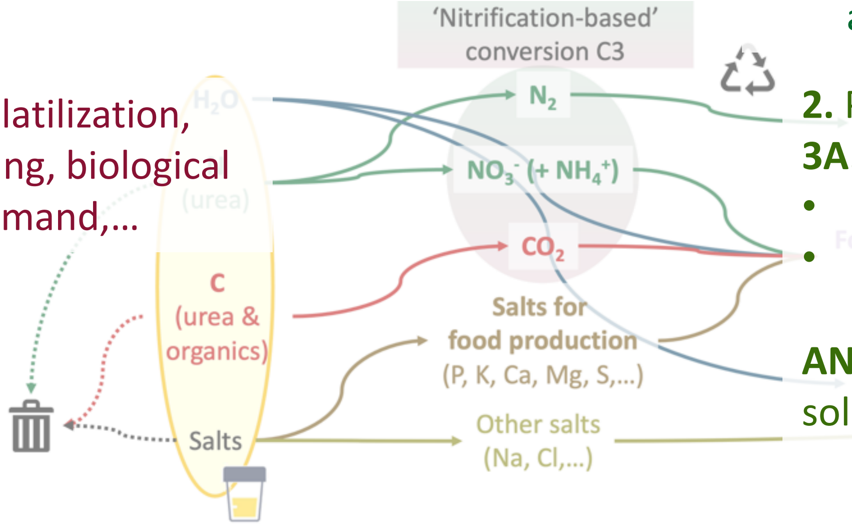
MELiSSA's EC(R)LSS view on **'nitrification-based'** urine treatment: Win-win-win-…

-> Nitrification-based processes **modularly and flexibly fit in many EC(R)LSS 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, biological oxygen demand,…



2. Produce water

3A. Produce N_2 gas
- for atmosphere provision
- for biological nitrogen fixation for food/O_2 production

AND/OR 3B. Produce a mineral nutrient solution for food/O_2 production

For all goals, the processes are feasible **for 'just' urine** (+ condensate) but also any **more complex waste treatment effluents or MELiSSA cycle** (faeces +/- organic waste +/- grey water +/- …)

esa

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

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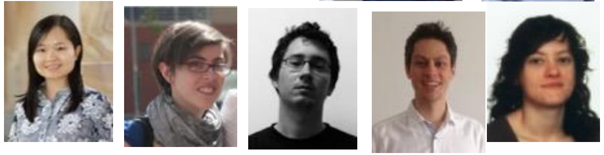

Nico Boon, Ramon Ganigué, Peter Clauwaert, Korneel Rabaey, Ralph Lindeboom, Jolien De Paepe, Celia Alvarez Fernandez,...

KU LEUVEN

Ilse Smets, Koen Rummens,...

QinetiQ

Dries Demey,...

eawag aquatic research

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

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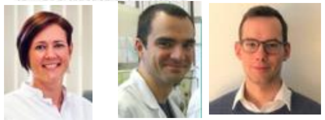

Natalie Leys, Felice Mastroleo, Tom Verbeelen,...

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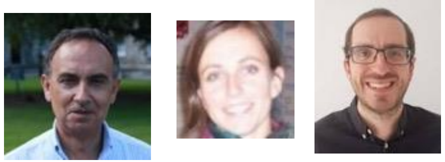

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