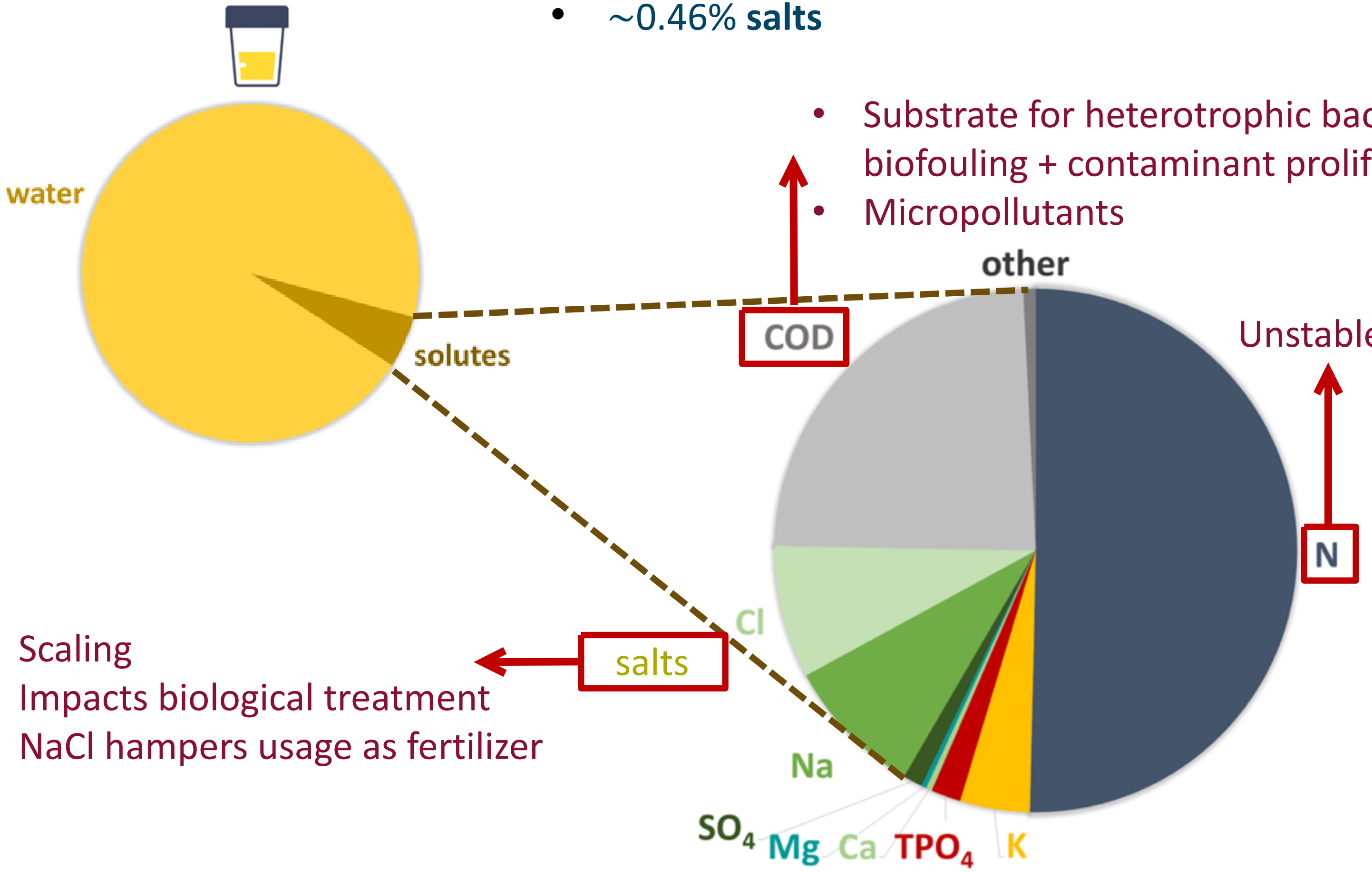
Nitrify for life: Sustainable solutions for Space and Earth

Siegfried Vlaeminck, Marc Spiller, Marijn J. Timmer,
Patricia Gutiérrez Lozano & Tim Van Winckel

Urine: 91-96% **water** + …

- ~0.92% **N:** most in urea
- ~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

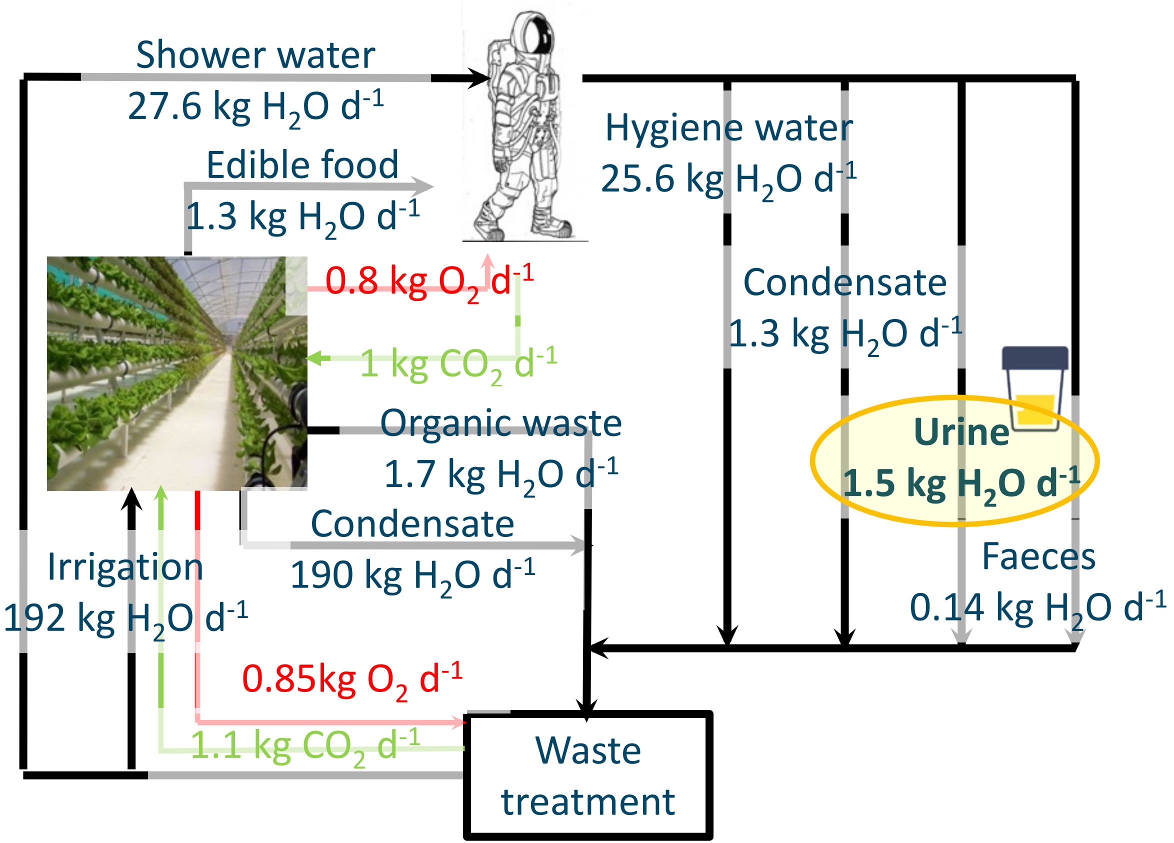
Short missions:

- **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.)
- …



(After Hu et al. 2010)

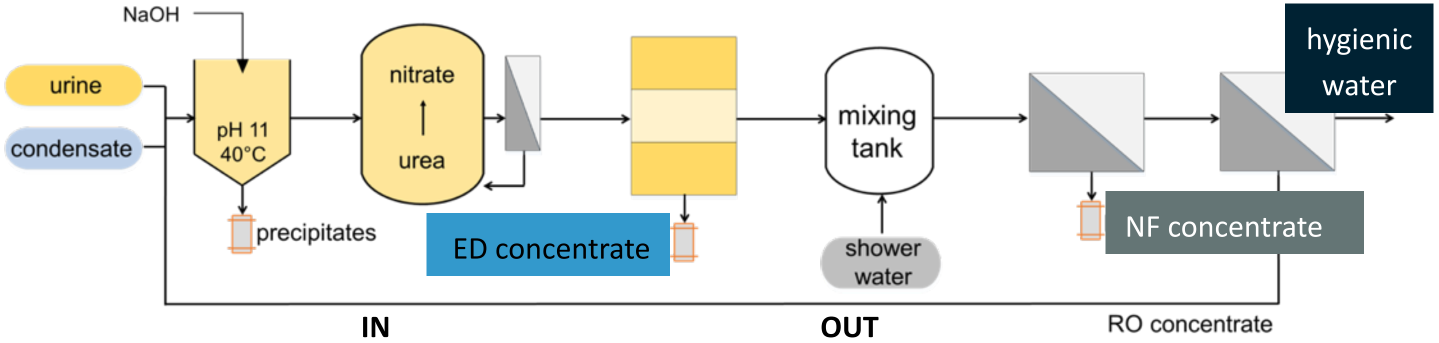
Water recovery from urine in Space?

In 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

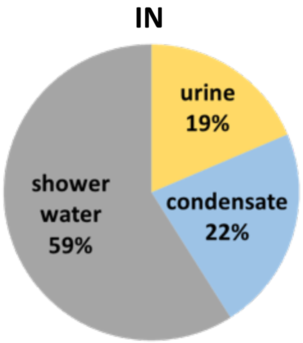
Racks for the ISS water recovery system (Carter et al. 2011)

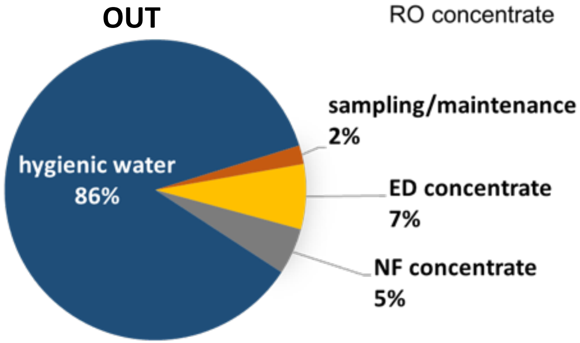
Belgian astronaut Frank De Winne repairing the Urine Processor Assembly (UPA)

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



Ralph Lindeboom



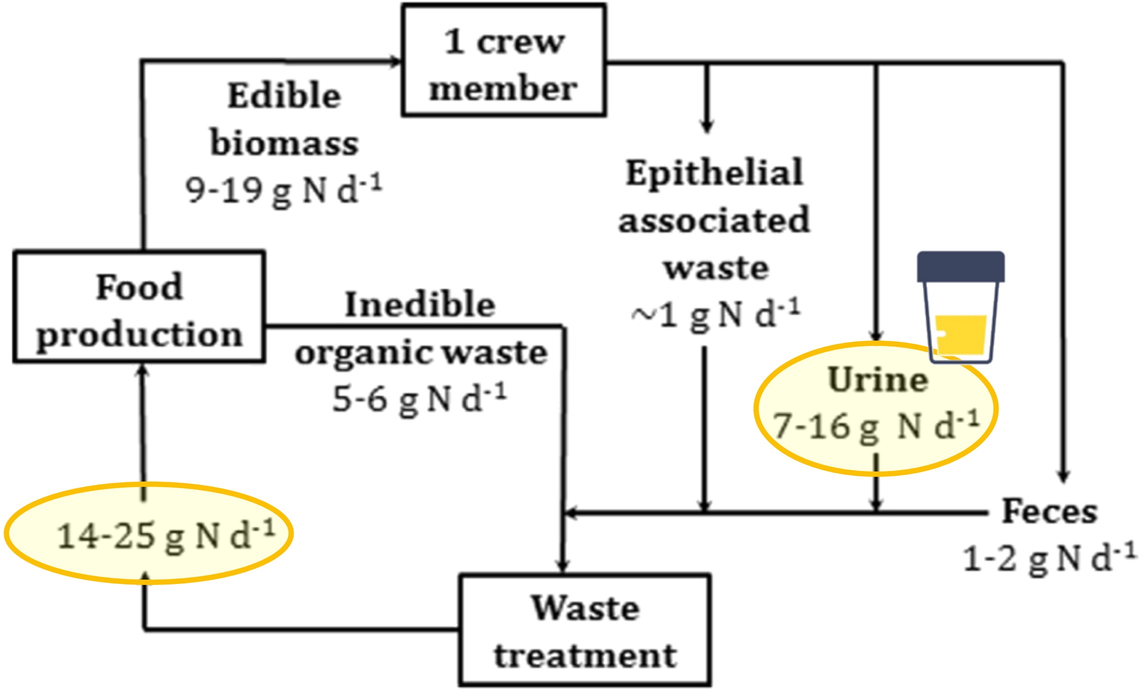


- Results of a 4-months breadboard operation campaign: total water recovery of 86%
- Reduced scaling potential with anti-scalant addition
- Stable but biofouling-limited RO permeability (0.5 L/m^2/h/bar)

(Lindeboom et al., 2020)

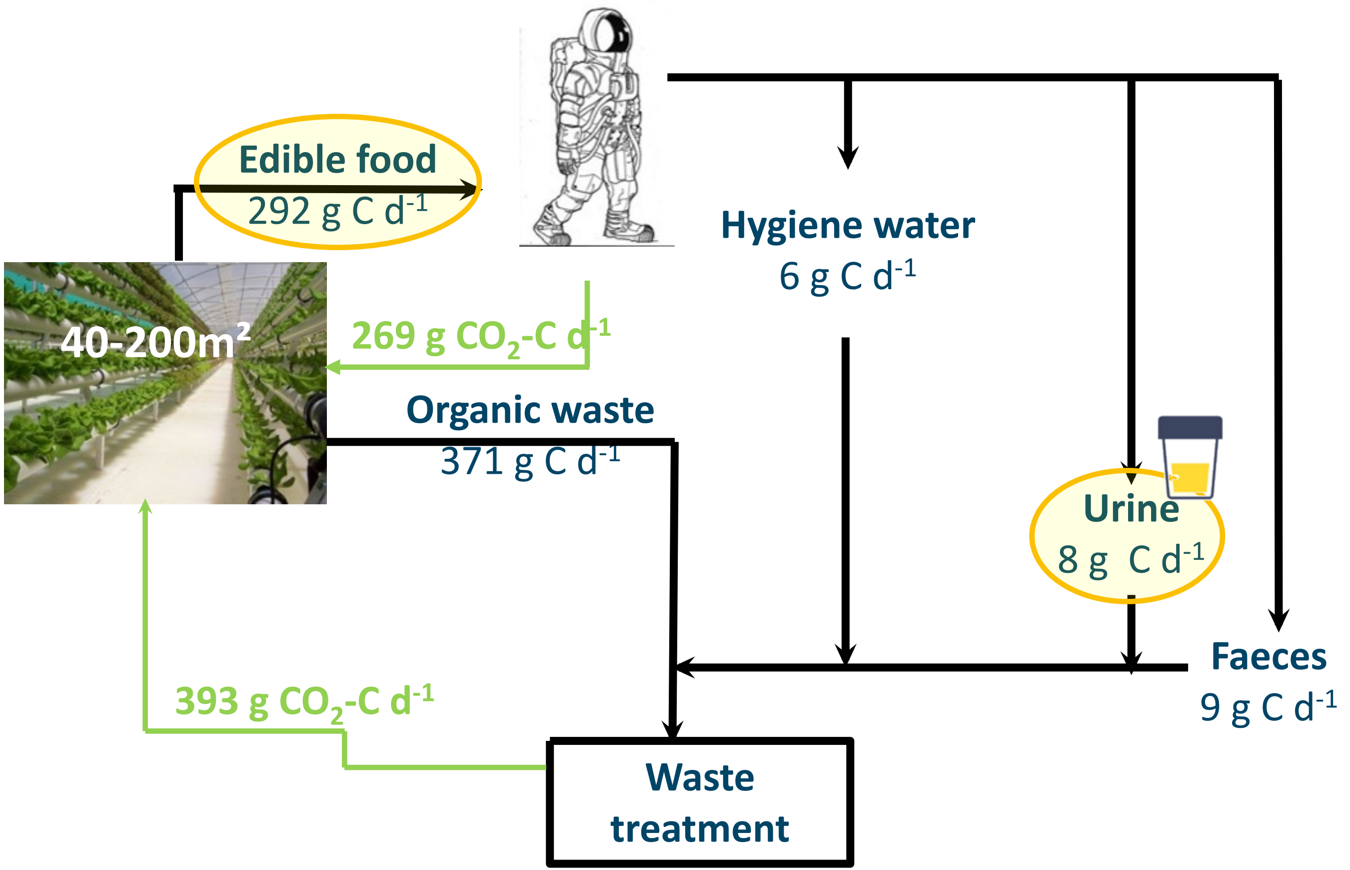
Nitrogen in Space? -> **Urine** as **major flow**

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



(Clauwaert et al., 2017)

Carbon in Space? -> Urine as negligible flow



(After Hu et al. 2010)

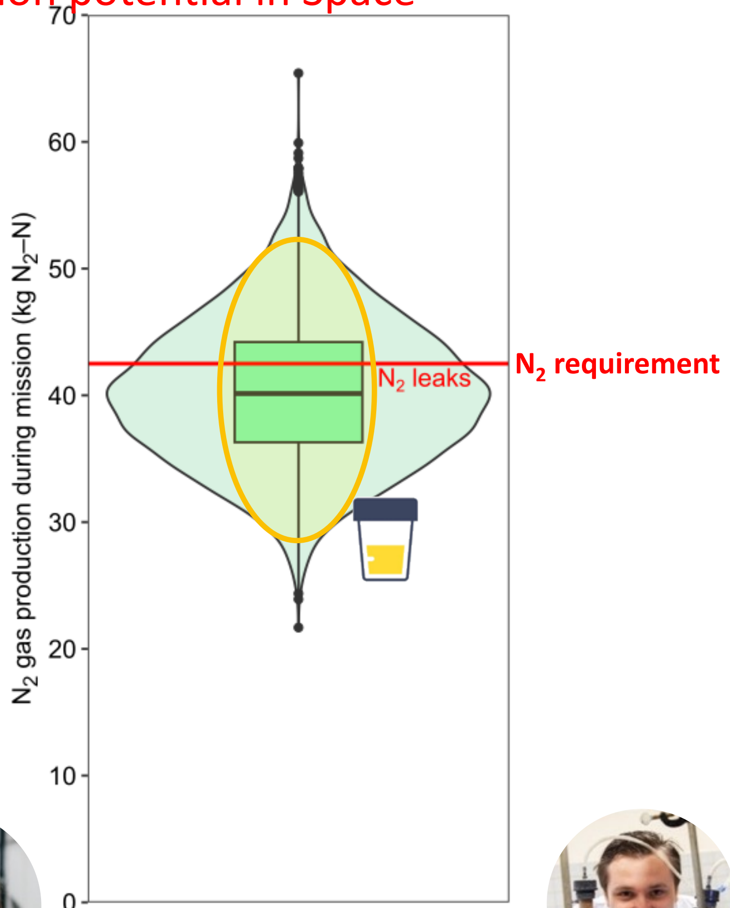
Nitrogen gas (N_2) requirements and production potential in Space

- N_2 **requirement**
  - To maintain a pressurised cabin atmosphere, counteracting losses due to extravehicular activities, structural leakages,…
  - **42.5 kg N** needed

- **Production** potential of N_2 from urine
  - Through partial nitritation/anammox
  - **40.3 kg N** produced on average

- N_2 recovery from urine **offsets on average 95% of the N_2 gas needs** (65% of the stochastic runs can offset all losses)

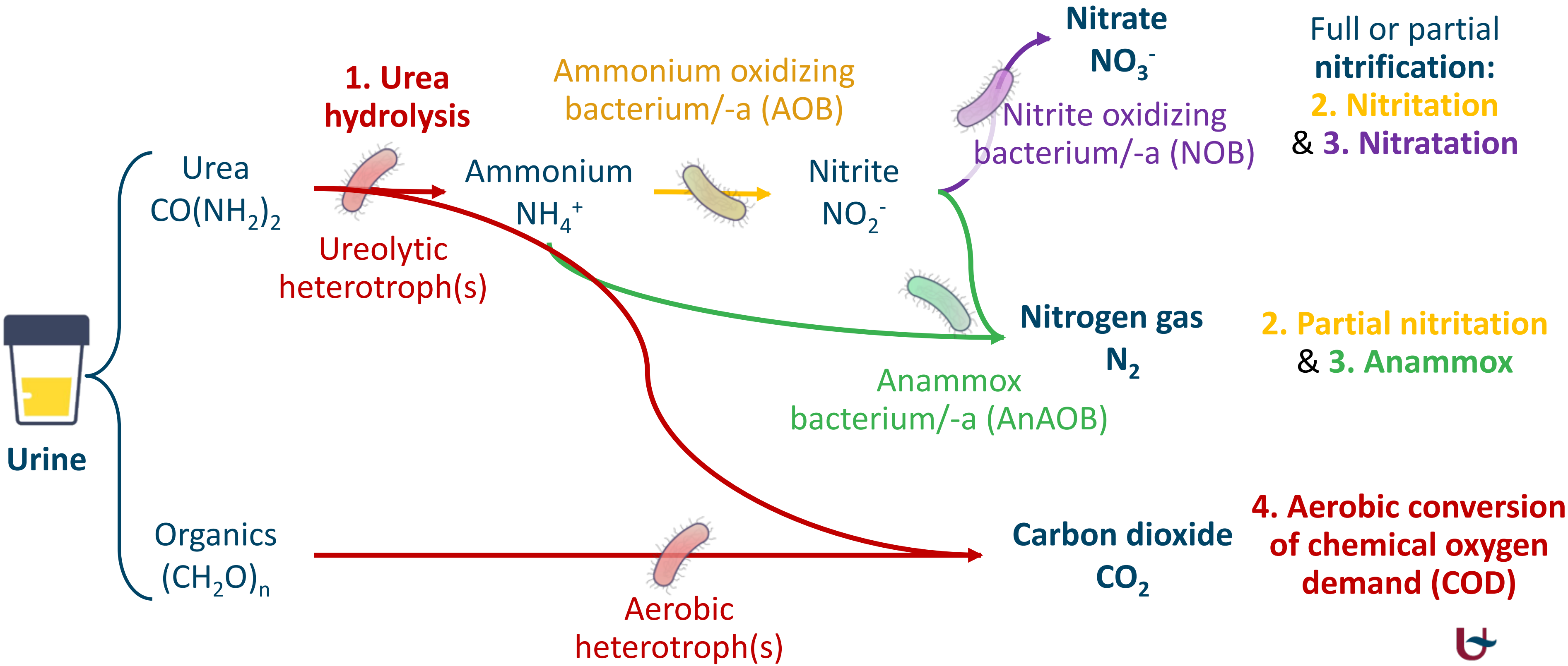
- Assumptions: Mars transit mission, 4 crew members, 850 days



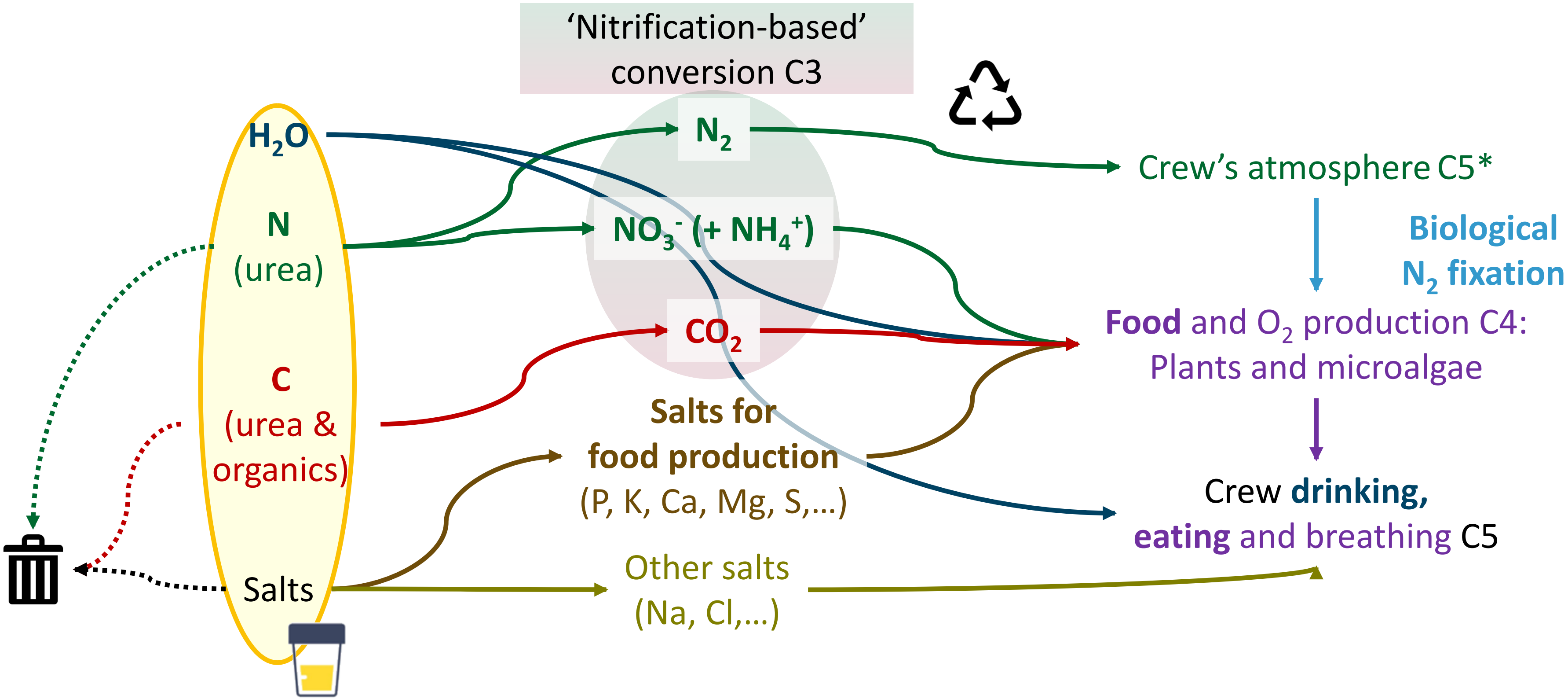
(Van Winckel et al., in prep.)

‘Nitrification-based’ conversions for N and C in urine: 2 routes, having 3 out of the 4 steps in common:

= Ureolysis **AND** (Partial) Nitritation **AND** (Nitratation **OR** Anammox) **AND** COD conversion



Modular and complimentary options to deal with urinary resources



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

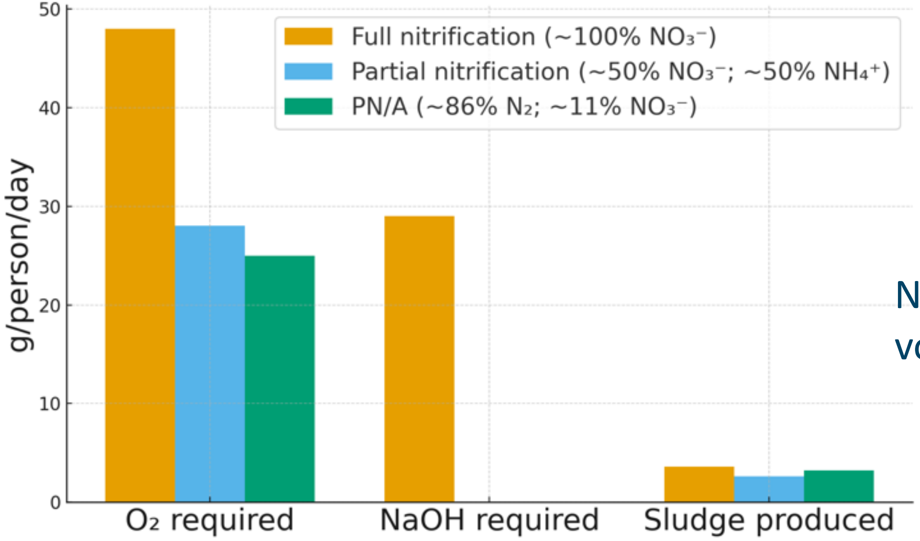
Combined processes	Overall stoichiometry
Ureolysis, full nitrification & C conversion	$0.5\ CO(NH_2)_2 + 0.22\ CH_3COOH + 2.18\ O_2$ $\rightarrow$ **$0.96\ NO_3^-$** $+ 0.036\ C_5H_7O_2N +$ **$0.76\ CO_2$** $+ 1.06\ H^+ + 0.73\ H_2O$
Ureolysis, partial nitritation/anammox & C conversion	$0.5\ CO(NH_2)_2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

- N:
  - Full 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
- C:
  - For both: ~half of CO_2 production from urea; ~half of CO_2 production from COD
  - 17-19% of the incoming C is converted to biomass-C

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

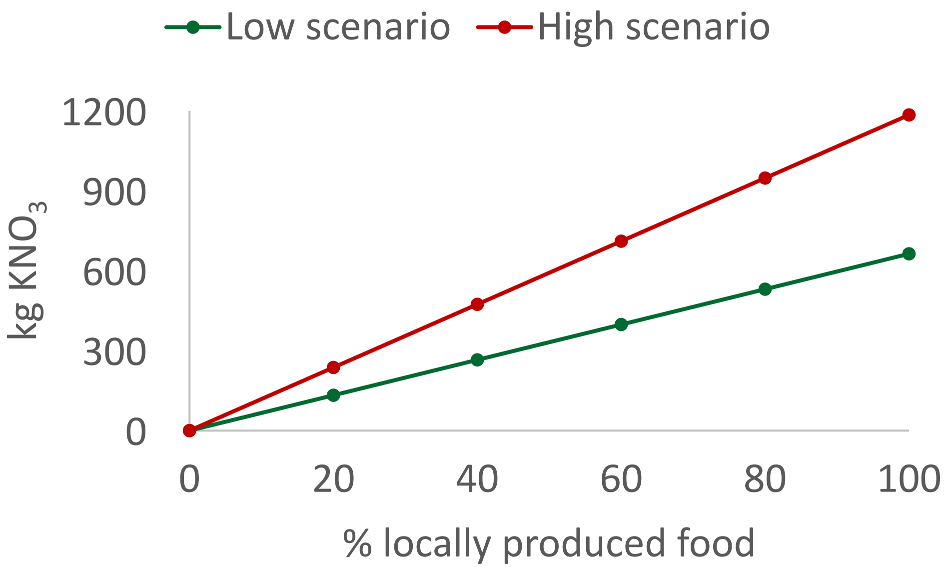


Enrique Peiro (and myself) at the MELiSSA Pilot Plant (UAB)

Note: sludge expressed in volatile suspended solids (VSS)

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



Big bag (1200 kg fertilizer)

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

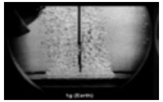
Open, biodiverse and dynamic -> 'natural/stochastic' selection → Defined, axenic and always biosafe -> 'curated/controlled' selection

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

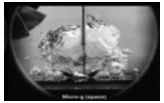
Flask incubation and process characterization → Reactor operation, modelling, automation, control

Storage, bioreactors and other unit processes → Fully integrated treatment pipelines/systems

1 g

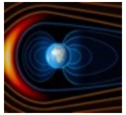


→



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

Radiation protected (magnetic field)



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

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 **N conversions > COD conversions**
- To date, no 100% pure anammox cultures are available

Matrix realism \ Microbiome definition	Open	Axenic
Real	Ureolysis +++ Full or partial nitrification +++ Partial nitritation/anammox +++ COD conversion +++	Ureolysis ++ Full or partial nitrification +++ COD conversion **+**
Synthetic	(not relevant)	Ureolysis +++ Full or partial nitrification +++ COD conversion +

Key achievements **open** communities

- Full nitrification (and COD conversion) demonstrated for **undiluted urine** (Coppens et al., 2016)
- **pH control** variations:
  - Full nitrification:
    - Alkalinization to stabilize in the collection/storage tank, at no surplus base 'cost' (a.o. De Paepe et al., 2018)
    - Chemical OH^- addition (a.o. Coppens et al., 2016)
    - Electrochemical OH^- addition (De Paepe et al., 2021)
  - Partial nitrification on 'spontaneously' fermented/ureolysed: pH-based feeding, no OH^- addition (e.g. Faust et al., 2023)
- **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 (MABR) on real urine
  - Nitrification (De Paepe et al., 2020b)
  - Partial nitritation/anammox (Timmer et al., 2024)

Microbiomes for nitrification-based treatment solutions

- **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 confirmed for urine treatment:
- ***Nitrosomonas** europaea*
- ***Nitrobacter** winogradskyi*

(e.g. Ilgrande et al., 2018)

- **Anammox bacteria** in an open microbiome: *"Candidatus* **Brocadia** sp."
(in an open/mixed community; Timmer et al., 2024)

- **Heterotrophs:** The previously established **consortium** of four **is currently being reconsidered** (tested in UAB/MPP and UAntwerp): **good for ureolysis, but limited for COD conversion**
    1. ***Cupriavidus** necator*
    2. ***Comamonas** testosteroni*
    3. ***Pseudomonas** fluorescens*
    4. ***Acidovorax** delafieldii*

Talks later in this session (2.2):
Queralt Farras: 16h30
Patricia Gutiérrez: 16h45

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 the **salinity of undiluted urine** for nitrification (Christiaens et al., 2019)

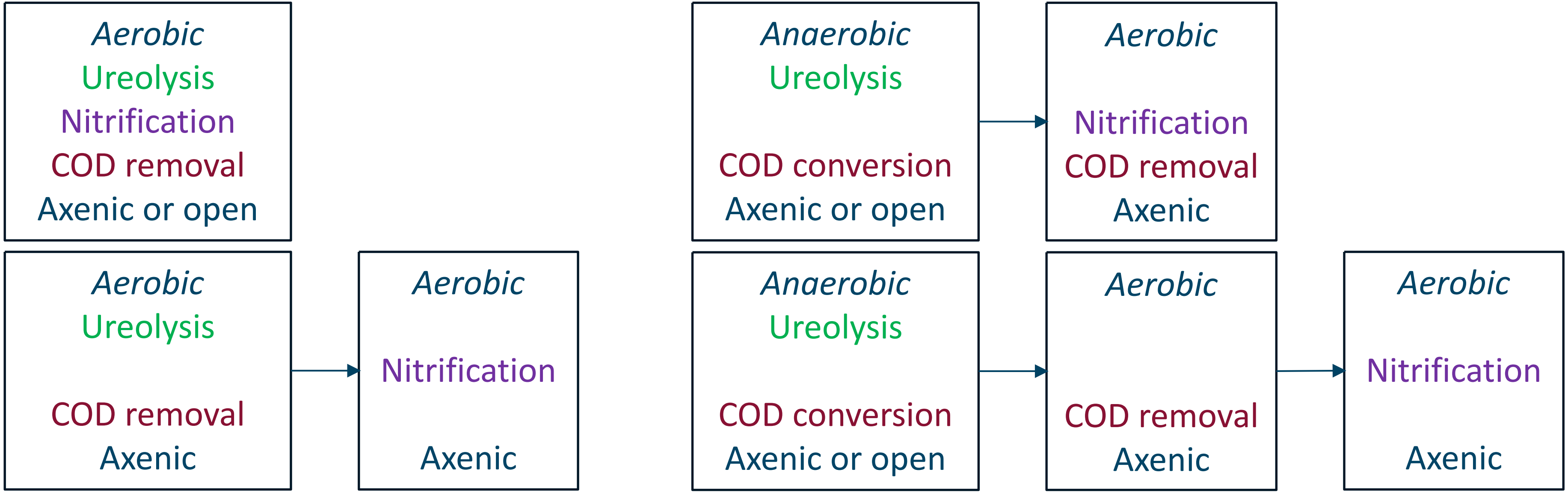
With focus on the heterotrophic bacteria (ureolysis; COD conversion):

- 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 real urine at **high nitrate production rates** (>0.5 g N/L/d) (Queralt Farras/Marcel Vilaplana/Carolina Arnau, MPP/UAB)

Best route to integrate conversion of urinary organics with nitrification?

Aerobic COD conversion: 1- or 2-stage?

Anaerobic & aerobic COD conversion: 2- or 3-stage?



- Ureolysis separated from nitrification increases the pH -> need to manage volatile NH_3
- Anaerobic pre-treatment (fermentation):
  - Yields volatile fatty acids (mainly acetic acid), which easing axenic aerobic conversion
  - Can replace the collection/storage tank and the alkalinization/stabilization
  - Can be integrated with organic waste treatment scenarios
- Axenic or (biosafe) open? – Function of treatment/recovery goal and mission scenario

Key pipeline/integration/system achievements

- Pre-treatment:
  - **Alkalinization:** Electrochemical (De Paepe et al., 2020a) or chemical (a.o. De Paepe et al., 2018)
  - Spontaneous **'maturation':** ureolysis (Udert et al., 2003) and organics fermentation (Eawag, POMP Nele Kirkerup)
  - **COD removal:** Bio-anodic (De Paepe et al., 2020b) or aerobic (Heusser et al., 2024)
- Co-treatment of **urine** and:
  - **Black** water, **organic** waste (BWTB, KULeuven)
  - **Grey** water (shower), **condensate** (Lindeboom et al., 2020)
- Post-treatment, valorization and integration:
  - **Water** recovery – integrated process (Lindeboom et al., 2020)
  - **Concentrated liquid fertilizer** production (incl. removal of micropollutants and water) (Eawag/VUNA)
  - **Food and oxygen** production on urine with microalgae and plants (C4a and C4b) (UAB/MPP; CIRiS: Øyvind Jakobsen)
- **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 (Appiah-Twum et al., 2025)

Talks later in this session (2.2):

Øyvind Jakobsen: 15h45

Nele Kirkerup: 16h15

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)

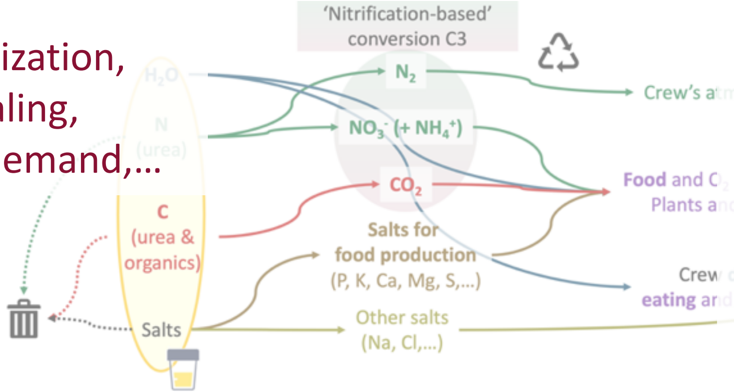
MELiSSA's EC(R)LSS view on **'nitrification-based'** urine treatment: Win-win-win-…

-> 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 +/- …)

What about MELiSSA-inspired nitrification solutions for Earth?

Earthrise (1968, Apollo 8 mission)

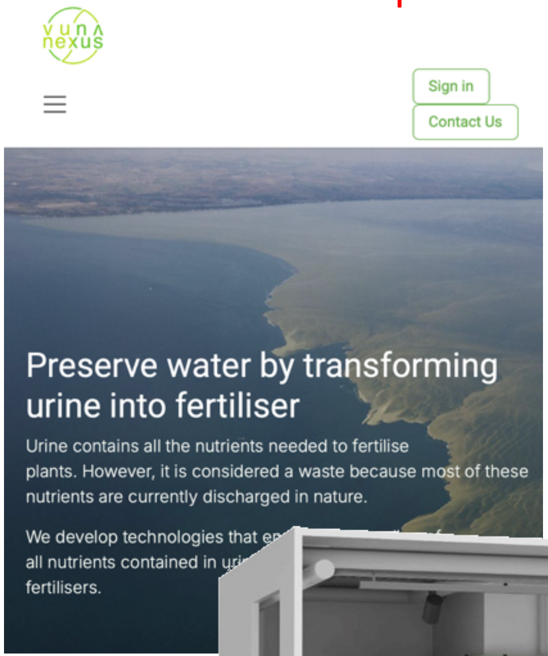
Tapping into human and animal urine?

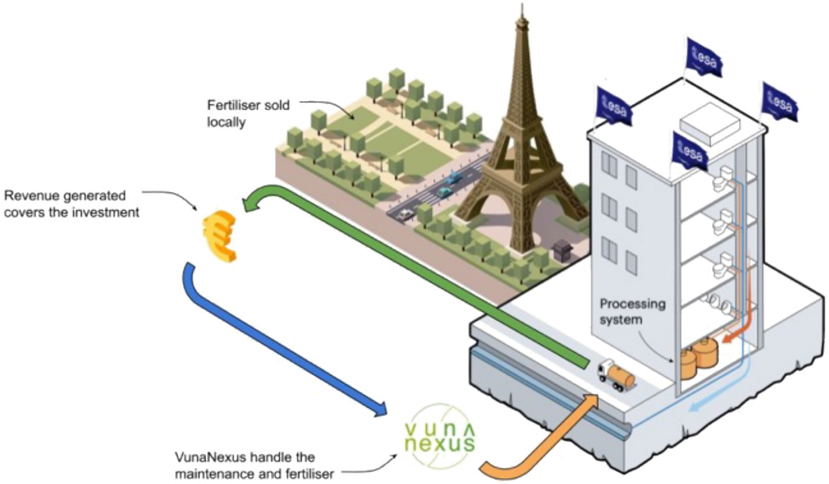
Manneken Pis
(1619)

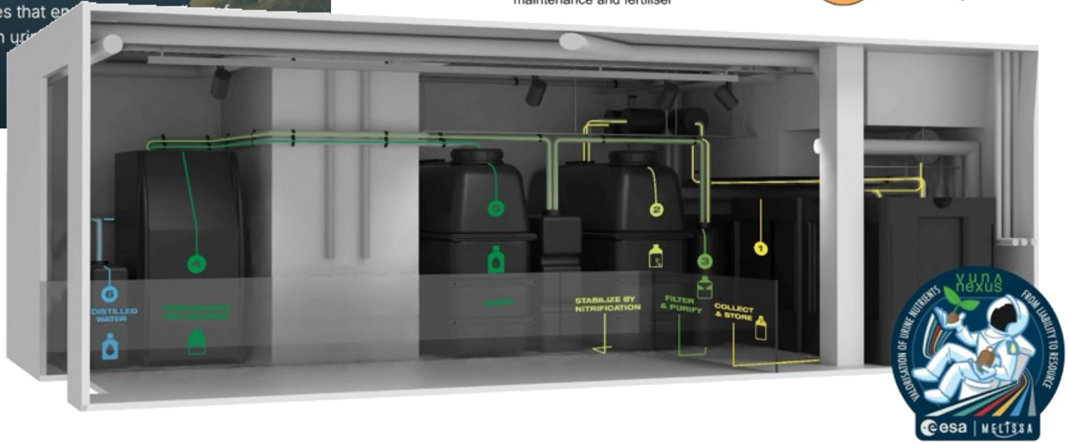
Jeanneke Pis
(1987)

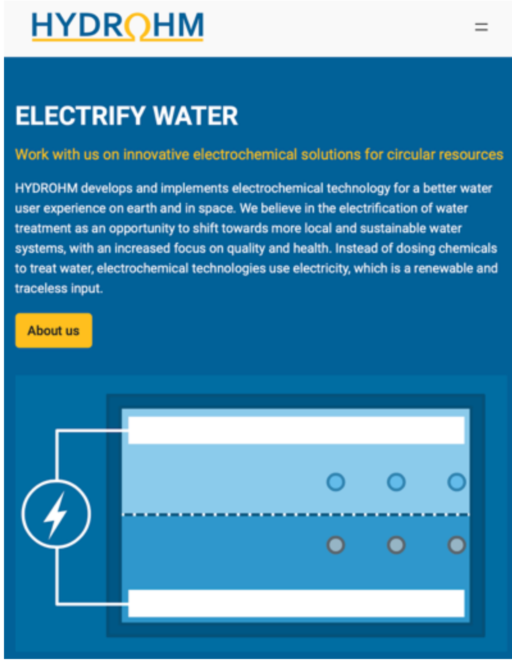
Zinneke Pis
(1999)

Companies for urine treatment solutions: Vuna Nexus & Hydrohm

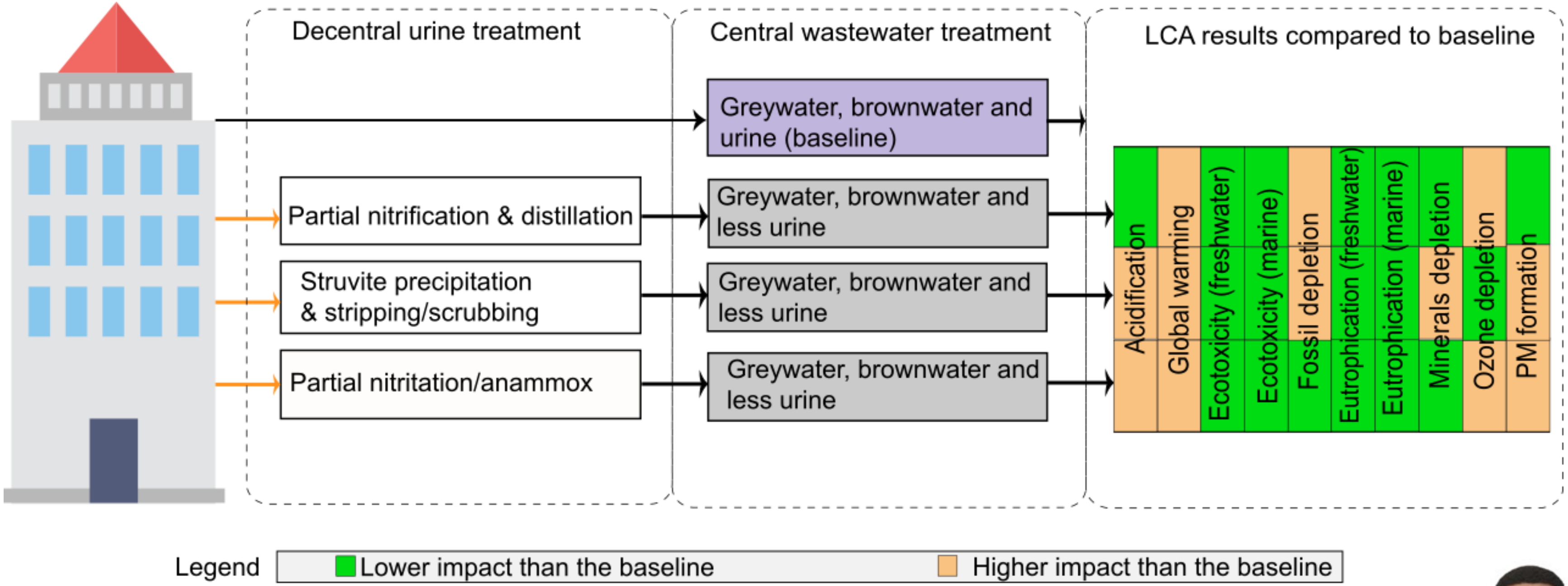








Environmental impact **centralized <-> decentralized urine** collection and treatment



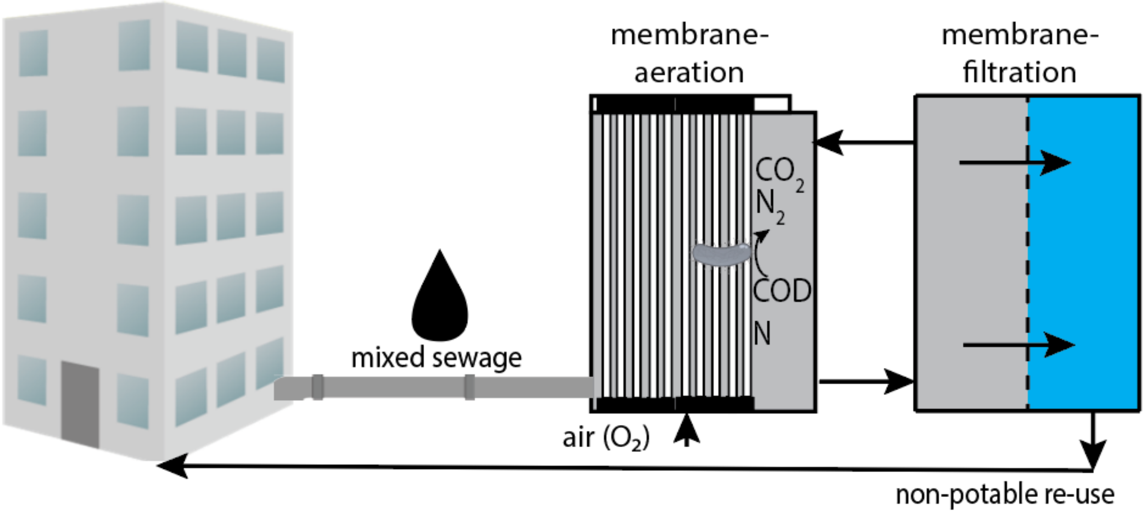
- Hybrid scenarios showed increased impacts on global warming compared to the baseline
- Hybrid scenarios performed better in 9 out of 10 categories.
- Hybrid scenarios have a lower global warming impact at higher central WWTP N_2O emissions and electricity use
- Urine alkalinization showed a higher impact in 7 out of 10 categories

(Appiah-Twum et al., 2025, Water Research)

Hanson Appiah-Twum

TwinMemBio: COD and N removal in a membrane-aerated membrane bioreactor (MA-MBR) for **resource-efficient local water reuse**

Marijn Timmer



Compared to conventional bubble-aerated MBR (BA-MBR):

- **Energy savings**
- **Space savings**

✔ Successful **1-stage simultaneous nitrification/denitrification**

✔ Stable treatment sewage with **COD/N ratio 5-20**

✔ Oxygen demand (=TN+COD) loading rates of > **4 g O_2 L^{-1} d^{-1}** vs. 2 in bubble-aerated MBR

✔ Effluent quality adheres to **strictest domestic re-use standards** COD, BOD_5, NH_4^+ and TN

CAPTURE

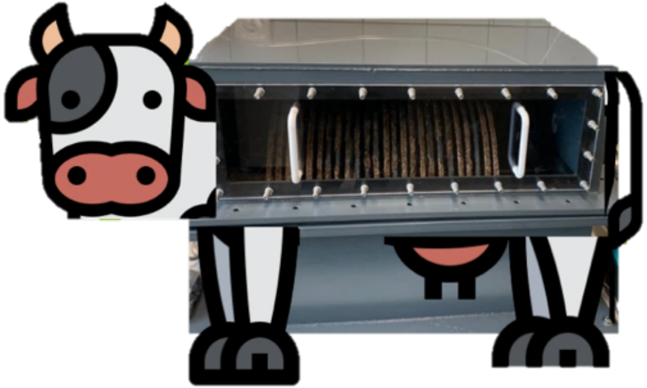
fwo Research Foundation Flanders Opening new horizons

(Timmer et al., 2025)

Lowering nitrogen emissions from dairy farms: Treating cow urine

CowToilet (Hanskamp)

- Simple and robust biofilm reactor technology: Rotating biological contactor (RBC)
- Cow urine:
  - Relatively high COD/N (~5); N ~ 4 g N/L (undiluted)
  - Partially ureolyzed, despite source separation
- Pre-treatment: Alkalinization <-> Acidification
- Operation at ~33% and ~50% urine:
  - pH ~7.4; EC ~30 mS/cm
  - High N and COD removal efficiencies (>90%)
  - Removal rates around 100 mg N/L/d and 500 mg COD/L/d (20°C)
- Next step: transition to undiluted cow urine

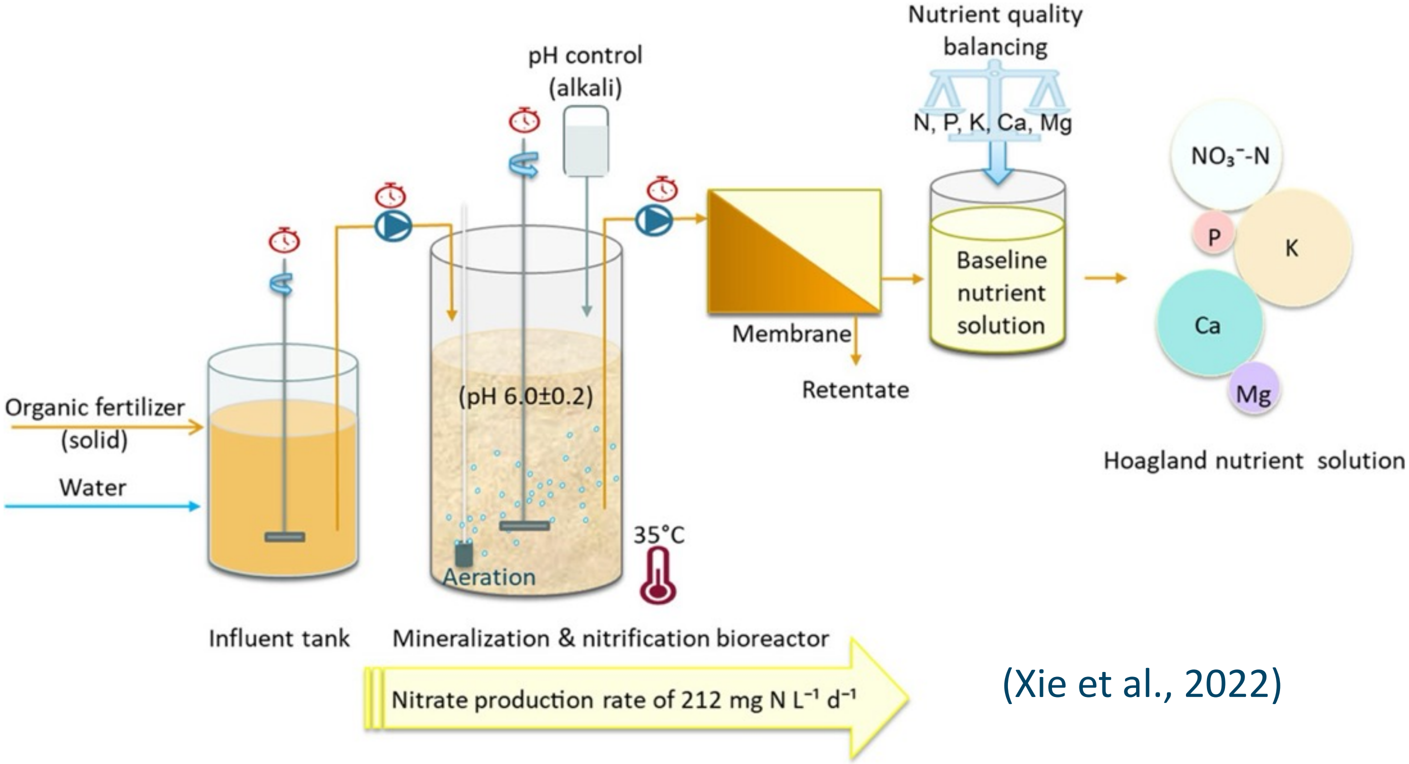


Patricia Gutierrez

Iris De Corte

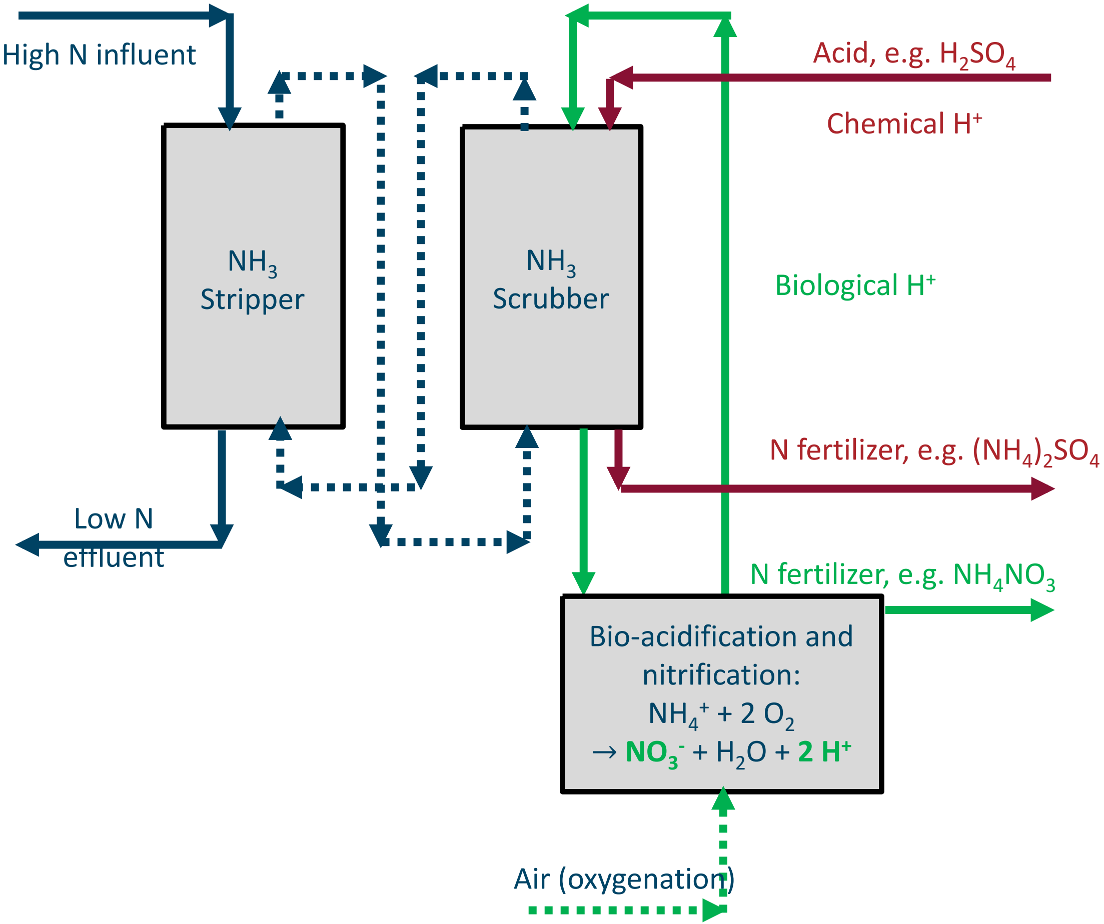
Transforming solid organic fertilisers to nitrate-based liquid fertiliser solutions for horticulture

- Mineralized and nitrification of solid organic fertilizers in an aerobic bioreactor
- pH control with $Ca(OH)_2$ or $Mg(OH)_2$ supplements plant macronutrients
- Besides recovered nutrients in solution, also CO_2 can be used for plant production
- The concept has the potential to be economically feasible for hydroponics



(Xie et al., 2022)

The **BioCatcher** concept: A bioscrubber overcoming the challenges of chemical acid scrubbing



Lower costs for chemicals

Less soil acidification

Renure quality NO_3^- fertilizers

Safe for operators

Patricia Gutierrez

Colsen

water, energy & environment

esa

Christophe Lasseur, Chloé Audas, Brigitte Lamaze, Sandra Ortega Ugalde, Christel Paille,...

University of Antwerp

Siegfried Vlaeminck, Patricia Gutiérrez Lozano, Marijn Timmer, Marc Spiller, Tim Van Winckel, Jolien De Paepe, ...

GHENT UNIVERSITY

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

KU LEUVEN

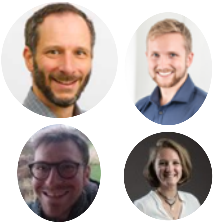
Ilse Smets, Koen Rummens,...

REDWIRE

QinetiQ

Dries Demey,...

eawag aquatic research



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

ETH Zürich

Grace Crain,...

SCK•CEN

STUDIECENTRUM VOOR KERNENERGIE CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

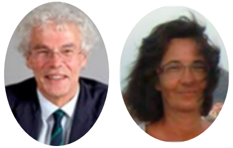
Natalie Leys, Felice Mastroleo, Tom Verbeelen,...

MELiSSA

vito vision on technology

UCA UNIVERSITÉ Clermont Auvergne

EPAS ECO PROCESS ASSISTANCE



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

UAB Universitat Autònoma de Barcelona

Francesco Gòdia, Enrique Peiro, Carolina Arnau, Queralt Farràs Costa, David García, Carles Ciurans, Justyna Barys, Marcel

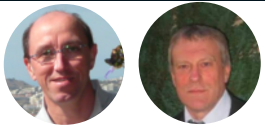
SEMiLLA IPSTAR Circular Systems™

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,...

BLUE HORIZON

NTE SENER

beyond gravity

(Credits: Jolien De Paepe)

Get in touch or follow our activities:

Siegfried.Vlaeminck@UAntwerpen.be

https://www.linkedin.com/in/siegfried-vlaeminck-84678853/

#UAntwerpSUSTAIN

www.uantwerpen.be/sustain

University of Antwerp
SUSTAIN | Biobased Sustainability Engineering