NITROGEN RECOVERY FROM URINE IN SPACE:

A CASE FOR NITRIFICATION


Agro-space/MELiSSA workshop, 16/05/2018
- Closing the cycle: waste → food
- ~80% of the nitrogen flux: urine
- $N_{org} \rightarrow \text{NH}_4^+/\text{NH}_3 \rightarrow \text{NO}_2^- \rightarrow \text{NO}_3^-$
Urine nitrification: strategy towards demonstration in Space

Other challenges:
- Salts
- Organic compounds
- Micropollutants
- Microbial stabilization
Urine nitrification in a MBR

- 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%

Coppens et al. (2016) Bioresource Technology, 211, 41–50
Urine nitrification in a MBBR

→ PVA beads as biomass carriers
→ From labscale to Breadboard (WTUB)
→ TRL\textsubscript{earth} 6 (TRL\textsubscript{space} 4)

De Paepe et al. (submitted)
Lindeboom et al. (in preparation)
Strain selection for a synthetic community

- *Nb winogradskyi* enhances 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

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

*Ureolysis up to 4 g N/L.h (Vibrio)*

*Vibrio* suppresses nitrification activity?

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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)

- Synthetic microbial community
- Membrane ultrafiltration
- Bubble aeration

- Synthetic urine ✓
- Real urine ✓

Christiaens et al. (in preparation)
Demonstration of urine nitrification in the MELiSSA Pilot Plant (UAB)

- The MELiSSA demonstrator (Spain)
- High level requirements
- Integration of MELiSSA compartments
Reactivation of nitrifiers (Foton Flight)

Cultivation on Earth → Transport to Launch site → Terrestrial control (G23 and G4) → Flight (F) sample recovery

FOTON M4 Flight
- Flight duration: 44 days
- Altitude: 258 - 571 km
- Gravity: $10^{-3}$ - $10^{-4}$ g (estimated)
- Radiation: Extra dose in flight: $524 \mu$Gy/d

Lindeboom/Ilgrande et al. (submitted)
Comparison rates after reactivation: **Flight** vs. Ground 23°C control

<table>
<thead>
<tr>
<th>Composition</th>
<th>Symbol</th>
<th>Microbial characterization</th>
<th>Ureolysis</th>
<th>Ammonia oxidation</th>
<th>Nitrite oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Urea → NH₄⁺</td>
<td>NH₄⁺→NO₂⁻</td>
<td>NO₂⁻→NO₃⁻</td>
</tr>
<tr>
<td>Defined</td>
<td>C</td>
<td><em>Cupriavidus pinatubonensis</em></td>
<td>=</td>
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</tr>
<tr>
<td></td>
<td>Ns</td>
<td><em>Nitrosomonas europaea</em></td>
<td></td>
<td>X</td>
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<tr>
<td></td>
<td>Nb</td>
<td><em>Nitrobacter winogradskyi</em></td>
<td></td>
<td></td>
<td>=</td>
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<tr>
<td></td>
<td>NsNb</td>
<td><em>Nitrosomonas europaea</em> +</td>
<td></td>
<td>↑</td>
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<td><em>Nitrosomonas europaea</em> +</td>
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<td><em>Nitrobacter winogradskyi</em></td>
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</tr>
</tbody>
</table>

+ mixed communities + anammox
Reactivation of nitrifiers (ISS)

Cultivation on Earth → Transport to Launch site → Terrestrial control (G23 and G4) → Flight (F) sample recovery → Flight duration: 10d (6d) → Altitude: ~400 km → Radation: 2.8mGy

Synthetic urine → Real urine

Open community → Synthetic community

Ilgrande et al. (in preparation)
Comparison rates after reactivation: **Flight** vs. Ground 23°C control

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<th>Strain/consortia</th>
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<th>Ammonia oxidation</th>
<th>Nitrite oxidation</th>
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</thead>
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<tr>
<td></td>
<td>Urea -&gt; NH$_4^+$</td>
<td>NH$_4^+$-&gt;NO$_2^-$</td>
<td>NO$_2^-$-&gt;NO$_3^-$</td>
</tr>
<tr>
<td><em>Cupriavidus pinatubonensis strain 1245</em></td>
<td>=</td>
<td>=</td>
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</tr>
<tr>
<td><em>Nitrosomonas europaea strain ATCC 19718</em></td>
<td>=/↑</td>
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</tr>
<tr>
<td><em>Nitrobacter winogradskyi strain ATCC 2539</em></td>
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<td>↓</td>
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</tr>
<tr>
<td><em>Nitrosomonas europaea + Nitrobacter winogradskyi</em></td>
<td>↑</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td><em>Nitrosomonas europaea + Nitrobacter winogradskyi - coculture</em></td>
<td>↑</td>
<td>=</td>
<td></td>
</tr>
<tr>
<td><em>Nitrosomonas europaea + Nitrobacter winogradskyi + Cupriavidus pinatubonensis</em></td>
<td>=/↓</td>
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</tr>
</tbody>
</table>

- 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

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

Earth → Space
Synthetic urine → Real urine
Open community → Synthetic community
**Urine nitrification in a bioreactor (ISS?): URINIS B**

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