Recent Progress in Mainstream Deammonification
A Potential Low-Energy Option for Nitrogen Removal
The N Cycle: Too Much of a Good Thing

Present: Human alterations have more than doubled the rate of input to the terrestrial N cycle.

1913: Haber-Bosch process for synthetic N fixation

Why control ammonia & reactive N levels?

- Ammonia toxicity to aquatic life
- High oxygen demand
- Eutrophication and resulting hypoxia in N-limited systems
- Emissions of the potent greenhouse gas N$_2$O
- Public Health Concerns:
  - Methemoglobinemia
  - Cyanobacterial toxins
Make solar energy economical
Provide energy from fusion
Develop carbon sequestration technologies
Manage the nitrogen cycle
Provide access to clean water
Restore and improve urban infrastructure
Advance health informatics
Engineer better medicines
Reverse-engineer the brain
Prevent nuclear terror
Secure cyberspace
Enhance virtual reality
Advance personalized learning

Conventional biological wastewater treatment (particularly N removal bioprocesses) are highly **energy intensive**

Wastewater treatment accounts for ~3% of nationwide electricity use (~15 GW)

Conversely, organic-rich domestic, industrial, and animal wastewater contains potential energy equivalent to ~17 GW of power (Logan et al. 2012)
A Paradigm Shift towards Resource Recovery

**Current**

Wastewater

- BOD
- Nutrients (N, P)

“Misplaced Resources” (Rittmann 2013)

**Centralized WWTP**

- Energy, Money

**Future**

“Used” water

- BOD
- Nutrients (N, P)

**“Biorefinery”**

- Energy, Materials

**Disposal to receiving water body**

**Energy Positive Wastewater Treatment** by rerouting “misplaced resources” and closing the engineered water cycle

**Clean Water for Reuse**
A Paradigm Shift towards Resource Recovery

Current

Wastewater
BOD
Nutrients (N, P)

“Misplaced Resources” (Rittmann 2013)

Future

Energy Positive Wastewater Treatment by rerouting “misplaced resources” and closing the engineered water cycle

Given that conventional nutrient removal processes are highly energy intensive, *it is unlikely that energy positive wastewater treatment targeting resource recovery can be achieved without new innovations in N removal bioprocesses*
Our Agenda For Today
Anammox and the Quest for Mainstream Deammonification

I. New Microbial Players
Tang 2013 J of Haz Mat 250-251: 1-8

II. Innovative Bioprocesses for low energy N removal
Paque BV
Our Agenda For Today

Anammox and the Quest for Mainstream Deammonification

I. New Microbial Players

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II. Innovative Bioprocesses for low energy N removal

Paque BV
The Changing N cycle

<table>
<thead>
<tr>
<th>Oxidation state of N</th>
<th>NITRIFICATION (aerobic)</th>
<th>AMMONIFICATION</th>
<th>DENITRIFICATION (anoxic)</th>
<th>N FIXATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>+5</td>
<td>NO₃⁻</td>
<td>NH₂OH</td>
<td>NO₂⁻</td>
<td>Diazotrophic Bacteria</td>
</tr>
<tr>
<td>+4</td>
<td></td>
<td></td>
<td>NO</td>
<td></td>
</tr>
<tr>
<td>+3</td>
<td></td>
<td></td>
<td>N₂O</td>
<td></td>
</tr>
<tr>
<td>+2</td>
<td></td>
<td></td>
<td>N₂</td>
<td></td>
</tr>
<tr>
<td>+1</td>
<td></td>
<td></td>
<td>NH₄⁺</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>Org-N</td>
<td></td>
</tr>
<tr>
<td>-1</td>
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<td></td>
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<tr>
<td>-2</td>
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</tr>
</tbody>
</table>

Primarily heterotrophic denitrifying bacteria

Oxidation state of N
The Changing N cycle

Nitrite-oxidizing Bacteria
-Primarily heterotrophic denitrifying bacteria

Diazotrophic Bacteria

Ammonia-oxidizing Archaea

Könneke et al. 2005, Francis et al. 2005

Nitrite-driven anaerobic methane oxidation
Ettwig et al. 2010

Nitrification (aerobic)

Denitrification (anoxic)

Oxidation state of N

Ammonification

NO$_3^-$

Missing lithotroph identified as new planctomycete

Marc Strous*, John A. Fuerst†, Evelien H. M. Kramer*, Susanne Logemann*, Gerard Muyzer‡, Katinka T. van de Pas-Schoonen*, Richard Webb†, J. Gijs Kuenen* & Mike S. M. Jetten


Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor

A. Mulder a,1, A.A. van de Graaf b,2, L.A. Robertson b, J.G. Kuenen b,*
The Changing N cycle

**NITRIFICATION** (aerobic)

- Nitrite-oxidizing Bacteria
- Ammonia-oxidizing Bacteria
- Ammonia-oxidizing Archaea

**DENITRIFICATION** (anoxic)

- Primarily heterotrophic denitrifying bacteria
- Nitrite-driven anaerobic methane oxidation

**ANAMMOX**

- Anammox bacteria
  - Mulder et al. 1995, Strous et al. 1999

**AMMONIFICATION**

- A short circuit in the N Cycle
- Oxidation state of N
- NH₂OH
- NO₂⁻
- NO
- N₂O
- N₂
- N₂H₄
- Org-N

**N FIXATION**

- Diazotrophic Bacteria

**Notes:**
- Könneke et al. 2005, Francis et al. 2005
- Ettwig et al. 2010
Anaerobic Ammonia Oxidation (Anammox)

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}
\]

Cytophaga, Flexibacter, Bacteroides
Fibrobacter
Spirochetes
Cyano-bacteria
Planctomycetes
Chlamydia
Gram-positive Bacteria
High G+C
Fusobacteria
Gram-positive Bacteria
Low G+C
Leptospirilli
Deinococci
Green Nonsulfur Bacteria
Thermotogales

Archaea
Crenarchaeota & Thaumarchaeota

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II. Innovative Bioprocesses for Low Energy N removal
Paque BV
Anammox Bioprocesses: A Critical Opportunity for Sustainable Wastewater Treatment

Conventional Biological N Removal*

\[
\begin{align*}
\text{NH}_3 & \xrightarrow{1.5O_2} \text{NO}_2^- \\
\text{NO}_2^- & \xrightarrow{0.5O_2} \text{NO}_3^- \\
\text{N}_2 (g) & \xrightarrow{5e^-} \\
\end{align*}
\]

Nitrification (aerobic)

Denitrification (Anoxic)

AOB: Ammonia-Oxidizing Bacteria
AOA: Ammonia-Oxidizing Archaea
NOB: Nitrite-Oxidizing Bacteria

*Neglecting biomass growth and decay
Anammox Bioprocesses: A Critical Opportunity for Sustainable Wastewater Treatment

Deammonification*

Deammonification processes **decrease** O\(_2\) requirement for N removal by ~60%.

Deammonification processes **decouple C and N removal**, thereby potentially enabling enhanced C removal as biogas or value-added products (bioplastics, platform chemicals, liquid biofuels, etc.).

*Neglecting biomass growth and decay*
Initial Development of Deammonification Processes has focussed on *sidestream* treatment of anaerobic digester supernatant.

**Sidestreams are characterized by:**
- High temperature (~30°C)
- High $\text{NH}_4^+$ (~500-1000 mgN/L)
While challenges remain to be addressed, particularly regarding process stability, *sidestream deammonification* is a rapidly maturing technology.

Pushing the envelope: Can we apply deammonification bioprocesses in the main stream?

The mainstream is characterized by:
- Low temperatures
- Low $\text{NH}_4^+$

The holy grail of anammox environmental biotechnology

Critical Challenges to (Mainstream) Deammonification

1. Process stability and reliability under dynamic conditions expected in the mainstream

2. Robust and stable outcompetition of NOB

3. Maintenance of high levels of anammox biomass and activity under low temperature, low substrate conditions

4. Coupled deammonification and biological P removal
Impact of Aggregate Architecture on Deammonification Process Stability

Alex Rosenthal
David Weissbrodt
Eberhard Morgenroth
Adriano Joss

eawag aquatic research

NORTHWESTERN UNIVERSITY
Our Hypothesis

**Mass transport limitations and aggregate structure in deammonification processes impact process performance and stability**

**Approach:** Side-by-side comparison between two common process variations employing different aggregate types:

- **Biofilm Carriers (MBBR)**
- **Suspended Growth Biomass**
  - Flocs
  - Granules
Process Performance and Stability in Replicated Lab-Scale Reactors
Process Performance and Stability in Replicated Lab-Scale Reactors

3x MBBRs (Reactors R1, R2, R3)

3x Suspended Growth (Reactors R4, R5, R6)
Phases of Operation

1. Baseline
   - Baseline Stable Operation (6 months)

2. Transient Perturbation Scenarios
   - 2A. Temperature Disturbance
   - 2B. ATU pulses

Feed: anaerobic digester centrate
Phases of Operation

1. Baseline
   - Baseline Stable Operation (6 months)

2. Transient Perturbation Scenarios
   - 2A. Temperature Disturbance
   - 2B. ATU pulses

- **MBBRs**: Strong decline in performance ($\text{NH}_4^+$ depletion rate), no accumulation of the key intermediate $\text{NO}_2^-$
- **Suspended Growth**: Moderate decline in performance ($\text{NH}_4^+$ depletion rate), with substantial $\text{NO}_2^-$ accumulation
Deammonification Bioprocesses

Phases of Operation

1. Baseline
   - Baseline Stable Operation (6 months)

2. Transient Perturbation Scenarios
   - 2A. Temperature Disturbance
   - 2B. ATU pulses

- **ATU (Allythiourea):** specific inhibitor of aerobic ammonia oxidation (AOB and AOA)
- Dose: 500-1100 µg/L, expected to only partially inhibit activity
Deammonification Bioprocesses
Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

500 µg/L ATU Pulse

NH$_4^+$ Depletion Rate
(2 hour rolling average, kg N/m$^3$-d)

- R1 (MBBR)
- R4 (Suspended Growth)

Day of Operation

Deammonification Bioprocesses
Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

500 µg/L ATU Pulse

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The Amplification Envelope: Borrowing from Ecology to Quantify Stability

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Amplification Envelope

Response Variable

Time

Stability Parameter = Area of Amplification Envelope
Higher stability parameter means greater response to perturbation (e.g. less stability)

Resilience

Resistance

Deammonification Bioprocesses
Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

"Amplification Envelope" of R1 and R4 in response to 500 µg/L ATU perturbation

- R1 (MBBR)
- R4 (Suspended Growth)

Deammonification Bioprocesses
Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

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"Amplification Envelope" of R1 and R4 in response to 500 µg/L ATU perturbation

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Deammonification Bioprocesses

Response to Pulse of Inhibitor of Aerobic Ammonia Oxidation

500 µg/L ATU Pulse

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<th>Reactor</th>
<th>Resilience (d)</th>
<th>Resistance (%)</th>
<th>Stability Parameter (d-%)</th>
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<tr>
<td>R1</td>
<td>0.67</td>
<td>0.86</td>
<td>0.41</td>
</tr>
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<td>R2</td>
<td>0.77</td>
<td>0.92</td>
<td>0.39</td>
</tr>
<tr>
<td>R3</td>
<td>0.66</td>
<td>0.96</td>
<td>0.35</td>
</tr>
<tr>
<td>R4</td>
<td>0.54</td>
<td>0.68</td>
<td>0.17</td>
</tr>
<tr>
<td>R5</td>
<td>0.48</td>
<td>1.00</td>
<td>0.30</td>
</tr>
<tr>
<td>R6</td>
<td>0.49</td>
<td>0.94</td>
<td>0.12</td>
</tr>
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- MBBRs (biofilm systems) displayed significantly increased response to perturbation (higher stability parameter, p<0.05) relative to suspended growth reactors.
In response to transient temperature and ATU disturbances, we observed:

- No excess NO$_2^-$ Accumulation
- Strong decrease in NH$_4^+$ depletion rate
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- Our results suggest that the MBBRs in this study may be strongly limited by AOB activity—and thus may maintain an excess anammox capacity.

While suspended growth systems exhibited an apparent excess AOB capacity that muted the impact of variations in nitrification activity.

**Suspended growth systems** may be more resistant to fluctuations in aerobic ammonia oxidation activity, while **MBBR systems** may be more resistant to perturbations that predominantly impact anammox activity.
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- While **suspended growth** systems exhibited an **apparent excess AOB capacity** that muted the impact of variations in nitrification activity.
Ongoing Work: Enrichment of Deammonification Biofilms under Mainstream Conditions - Linking Mesoscale Aggregate Structure to Emergent Function

**ROTATING ANNULAR REACTOR (RAR)**

250 mg COD/L
30 mg NH$_3$-N/L
15 mg PO$_4^{3-}$-P/L
pH=7
T= 23°C
HRT = 0.2 days
Re$_{rot}$ = 2000

3D rendering of RAR biofilm on day 77 by optical coherence tomography
Ongoing Work: Enrichment of Deammonification Biofilms under Mainstream Conditions: Linking **Mesoscale Aggregate Structure** to Emergent Function

**Rotating Annular Reactor (RAR)**

- 250 mg COD/L
- 30 mg NH$_3$-N/L
- 15 mg PO$_4^{3-}$-P/L
- pH=7
- T= 23°C
- HRT = 0.2 days
- $Re_{rot} = 2000$

Sustained NOB outcompetition and putative (but low) anammox activity under mainstream conditions

**RAR Effluent Nitrite and Nitrate**
Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs?

MBBR:
3 Compartments in Series
Loaded with real primary effluent from the O’Brien WRP

Initial Target:
• Sustained partial nitritation (NOB outcompetition)

Final Target:
• COD removal/ Nitritation in Tank 1
• Full deammonification in Tank 2
• Anammox in Tank 3
Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs with real wastewater?
Ongoing Work: Can deammonification be coupled to C removal in mainstream MBBRs with real wastewater?

MBBR Performance Snapshot 7/28/2015-7/29/2015

Initial results suggest successful NOB outcompetition in a biofilm system (compartments M1 and M2) under mainstream conditions.
Take Away Points

• New understanding of N cycle microbial ecology is leading to emerging sustainable bioprocesses for nutrient removal and recovery of “misplaced resources”

• Mainstream deammonification has extraordinary promise, but is in its infancy, with key remaining challenges to be addressed

• Deammonification process variations harboring different aggregate types display starkly different patterns of performance and stability
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