Sustainable Nitrogen Removal by Involving Anammox Process

Xiaojin (Jim) Li, Ph.D. Candidate
Zhen (Jason) He, Associate Professor

Environmental and Water Resources Engineering Program
Virginia Polytechnic Institute and State University

March 23, 2017, Abingdon, VA
AGENDA

1. Overview
2. Fundamentals of AMX
3. Evolution of BNR
4. BNR 3.0-Sidestream Application
5. BNR 3.X-Mainstream Development
Overview - Background

Eutrophication

Energy Consumption

GHG Emission

http://www.erc.uic.edu/energy-efficiency/illinois-energy-now-programs/waste-water-treatment-facilities-program
Overview - N-cycle

Mineralization & Ammonification

Organic N → NH₄⁺ → N₂ (Fixation)

Nitritation (oxic bacteria)

N₂O → NO → NO₃⁻ → NO₂⁻ (Denitrification)

Assimilation (algae + bacteria)

Denitrification (anoxic bacteria)

Anammox (anoxic bacteria)

N₂ Fixation

• Atmospheric
• Industrial
• Bacterial

Oxidation state

-3 -2 -1 0 +1 +2 +3 +4 +5

N₂ → N₂O → NO → NO₃⁻ → NO₂⁻
Overview - Pathways involved in BNR

Comammox: Complete Ammonia Oxidation; DNRA: Dissimilatory Nitrate Reduction to Ammonium

AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; NXR, Nitrite oxidoreductase; NIR, nitrite reductase; NAR, nitrate reductase; NOR, nitric oxide reductase; N\(_2\)OR, nitrous oxide reductase; HZS, Hydrazine synthesis; HZO, hydrazine dehydrogenase.


Comammox: Complete Ammonia Oxidation; DNRA: Dissimilatory Nitrate Reduction to Ammonium
• **Prediction:**
  Based on thermodynamics and evolution, Broda (1977) predicted the existence of a "missing" chemolithoautotrophic organism capable of oxidizing ammonia anaerobically.

• **Discovery:**
  Pilot denitrifying fluidized bed reactor in **Gist-Brocades** yeast factory, Delft, Netherlands. “unexpected nitrogen loss”
  Arnold Mulder/Gijs Kuenen

  **Astrid vande Graaf, Mike Jetten, Marc Strous, Michael Wagner, Mark van Loosdrecht, Marcel Kuypers, Boran Kartal…..**
• **Catabolism (Imprecise reaction):**
  \[
  \text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O} \quad \Delta G = -357 \text{ kJ/mol Energy generation}
  \]

• **The stoichiometry of metabolism:**
  \[
  \text{NH}_4^+ + 1.32 \text{NO}_2^- + 0.066 \text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02 \text{N}_2 + 0.256 \text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03 \text{H}_2\text{O}
  \]

• **Anabolism (Carbon fixation):**
  \[
  \text{CO}_2 + 2\text{NO}_2^- + \text{H}_2\text{O} \rightarrow \text{CH}_2\text{O} + 2\text{NO}_3^-
  \]

• **Isotopic labelling:**
  \[
  \text{N}_2 \text{ comes from NH}_4^+ \text{ and NO}_2^-, \text{ N in biomass comes from NH}_4^+
  \]

CH$_2$O$_{0.5}$N$_{0.15}$ vs. C$_5$H$_7$O$_2$N
• Color: red
• Chemolitho-autotrophic organisms affiliated to phylum Planctomycetes, family Brocadiaceae.
• Identified species: ~20 species divided over 5 genera
• Unique components:
  o Anammoxosome: organelle-like cell compartment bound by a single curved membrane
  o Ladderane lipids: membrane lipids with either three or five cyclobutane rings
• Temperature: 30-35 °C; pH: neutral range
• Growth rate: very slow
  o Biomass yield: 0.05 kg VSS kg\(^{-1}\) N
  o Conversion rate: 1 kg N kg\(^{-1}\) VSS d\(^{-1}\)
  o Doubling time: 7~11 days
  o Specific growth rate (\(\mu_m\)): 0.069 d\(^{-1}\) (nitrifiers \(\mu_m = 0.85\) d\(^{-1}\)), requires long SRT (30-50+ days)

A three-step process:

1. \( \text{NO}_2^- + 2\text{H}^+ + \text{e}^- \rightarrow \text{NO} + \text{H}_2\text{O} \) (\( E^\circ = +0.38 \text{V} \)) nitrite reductase (NIR)

2. \( \text{NO} + \text{NH}_4^+ + 2\text{H}^+ + 3\text{e}^- \rightarrow \text{N}_2\text{H}_4 + \text{H}_2\text{O} \) (\( E^\circ = +0.06 \text{V} \)) hydrazine synthase (HZS)

3. \( \text{N}_2\text{H}_4 \rightarrow \text{N}_2 + 4\text{H}^+ + 4\text{e}^- \) (\( E^\circ = -0.75 \text{V} \)) hydrazine dehydrogenase/oxidoreductase (HDH/HZO)

Evolution of BNR

- **BNR 1.0**
  - Conventional Nitrification/Denitrification
  - SRT, Nitrate Recycle, Organic Carbon

- **BNR 2.0**
  - Modified from conventional process
  - SND, Shortcut BNR, Nitritation-Denitritation, Nitrite Shunt

- **BNR 3.0**
  - Established with caveats
  - SHARON-ANAMMOX, OLAND, CANON, DEMON

- **BNR 3.X**
  - Emerging
  - Mainstream Nitritation-Anammox
Evolution of BNR

**BNR 1.0 - Conventional BNR**

**Nitrification - Aerobic**
- 1 mol \(\text{NH}_3/\text{NH}_4^+\)
- 25% \(\text{O}_2\)
- Complete Nitrification
- 4.57 g O\(_2\)/g NH\(_4\)-N oxidized
- 2.86 g COD/g NO\(_3\)-N reduced

**Denitrification - Anoxic**
- 0.5 mol N\(_2\)
- 60% Carbon
- 40% Carbon

- AOB Nitrification (Nitritation): \(\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+\)
- NOB Nitrification (Nitration): \(\text{NO}_2^- + 0.5\text{O}_2 \rightarrow \text{NO}_3^-\)
- Heterotrophic Denitrification: \(\text{NO}_3^- + 5e^- + 6\text{H}^+ \rightarrow 0.5\text{N}_2 + 3\text{H}_2\text{O}\)
BNR 2.0-Nitrite Shunt

Nitritation-Aerobic

1 mol NO$_2^-$

75% O$_2$

1 mol NH$_3$/ NH$_4^+$

Denitritation-Anoxic

1 mol NO$_2^-$

60% Carbon

1/2 mol N$_2$

- 25% reduction in oxygen demand
- 40% reduction in carbon (e- donor) demand
- 40% reduced biomass production
Evolution of BNR

BNR 3.0 - Nitritation-Anammox

Autotrophic Aerobic

- AOB
- Nitritation
- Leftover

- 37% O₂
- 1 mol NH₄⁺
- 0.5 mol NO₂⁻

Autotrophic Anoxic

- Anammox
- 0.1 NO₃⁻ + 0.5 mol N₂

N₂O
**Evolution of BNR**

**Comparison between BNR 1.0 & 3.0**

### Conventional Ammonium Removal

**Nitrification:**
\[ 2 \text{NH}_3 + (3+1) \text{O}_2 \rightarrow 2 \text{NO}_3^- + 2 \text{H}^+ + 2 \text{H}_2\text{O} \]

**Denitrification:**
\[ 2 \text{NO}_3^- + 8\text{g COD} + 2 \text{H}^+ \rightarrow \text{N}_2 + 3\text{g SLUDGE} \]

\[ 2 \text{NH}_3^- + 4 \text{O}_2 + 8\text{g COD} \rightarrow \text{N}_2 + 3\text{g SLUDGE} \]

### Autotrophic Ammonium Removal

**Nitritation:**
\[ \text{NH}_3 + 1.5 \text{O}_2 \rightarrow \text{NO}_2^- + \text{H}^+ + \text{H}_2\text{O} \]

**Anammox:**
\[ \text{NO}_2^- + \text{NH}_3 + \text{H}^+ \rightarrow \text{N}_2 + 2 \text{H}_2\text{O} \]

\[ 2 \text{NH}_3^- + 1.5 \text{O}_2 \rightarrow \text{N}_2 \]

### Nitrification/Denitrification

- **NH}_4^+ \rightarrow \text{NO}_3^- \rightarrow \text{N}_2**
  - aeration (2.8 kWh/kg N)
  - methanol (3 kg/kg N) sludge
  - 3-5 €/kg N
  - >4.7 ton CO\text{\_2}/ton N

### Nitritation/Anammox

- **NH}_4^+ \rightarrow \text{NO}_2^- \rightarrow \text{N}_2**
  - aeration (1 kWh/kg N)
  - methanol (0 kg/kg N) no sludge
  - 1-3 €/kg N
  - 0.7 ton CO\text{\_2}/ton N

- **63% reduction in oxygen demand (energy)**
- **Nearly 100% reduction in carbon demand**
- **80% reduction in biomass production**
- **No additional alkalinity required**
Metabolic Pathways

- **Nitritation**: $\text{NH}_4^+ + 1.5\text{O}_2 \rightarrow \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+$
- **Anammox**: $\text{NH}_4^+ + 1.32\text{NO}_2^- + 0.066\text{HCO}_3^- + 0.13\text{H}^+ \rightarrow 1.02\text{N}_2 + 0.256\text{NO}_3^- + 0.066\text{CH}_2\text{O}_{0.5}\text{N}_{0.15} + 2.03\text{H}_2\text{O}$

**Total**: $\text{NH}_4^+ + 0.85\text{O}_2 \rightarrow 0.13\text{NO}_3^- + 0.435\text{N}_2 + 1.4\text{H}^+ + 1.3\text{H}_2\text{O}$
Configuration Selection

Two-stage:

- Nitritation and AMX step can be optimized individually
- Lower risk for anammox to be outcompeted by heterotrophs
- Smaller inoculum needed
- No risk for oxygen inhibition on AMX

Single-stage:

- Investment costs are significantly lower
- Process control is less complicated
- Lower risk for nitrite inhibition
- Less N₂O emissions: 0.4-1.3 vs 2.3-6.6% of N-load
Abbreviations

- **SHARON**: Stable High rate Ammonia Removal Over Nitrite
- **OLAND**: Oxygen-limited Autotrophic Nitrification/Denitrification
- **CANON**: Completely Autotrophic Nitrogen-removal Over Nitrite
- **SNAD**: Simultaneous Partial Nitrification, ANAMMOX and Denitrification
- **DEMON**: Deammonification

Single reactor High activity Ammonia Removal Over Nitrite)
Evolution-BNR 3.0

SHARON

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
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</thead>
<tbody>
<tr>
<td>1997</td>
<td>Delft University Pilot Evaluations</td>
</tr>
<tr>
<td>1998</td>
<td>First Publications</td>
</tr>
<tr>
<td></td>
<td>Full-scale Demonstration in Netherlands</td>
</tr>
<tr>
<td>1999</td>
<td>Process is Patented</td>
</tr>
<tr>
<td>2000</td>
<td>Four Full scale Installations in The Netherlands</td>
</tr>
<tr>
<td>2005</td>
<td>First USA Installation – New York</td>
</tr>
<tr>
<td>2007</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Installation</th>
<th>Start-up</th>
<th>Loading (lbs/day)</th>
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<tbody>
<tr>
<td>Utrecht</td>
<td>1997</td>
<td>1,980</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>1999</td>
<td>1,870</td>
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<tr>
<td>Zwolle</td>
<td>2003</td>
<td>900</td>
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<tr>
<td>Beverwijk</td>
<td>2003</td>
<td>2,640</td>
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<tr>
<td>Groningen</td>
<td>2005</td>
<td>5,280</td>
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<tr>
<td>The Haque</td>
<td>2005</td>
<td>2,860</td>
</tr>
<tr>
<td>New York City</td>
<td>2007</td>
<td>14,500</td>
</tr>
</tbody>
</table>
• Single reactor
• AOB convert NH$_4^+$ to NO$_2^-$ with O$_2$ as the electron acceptor (nitritation)
• AMX bacteria subsequently oxidize NH$_4^+$ with NO$_2^-$ as the electron acceptor.
• NH$_4^+$ + 0.85 O$_2$ → 0.435 N$_2$ + 0.13 NO$_3^-$ + H$_2$O + 1.4 H$^+$
• Developed at University of Nijmegen

• Single reactor
• NH$_4^+$ is autotrophically oxidized to N$_2$ with NO$_2^-$ as electron acceptor under oxygen-limiting conditions
• Both bacteria groups (AOB, AMX) are present in biofilm.
• Developed at Ghent University
Evolution-BNR 3.0

History and Status

- **1988**: First observation @ Gist Brocades
- **1992**: Start development @ TU Delft
- **1995**: First publication
- **1996**: Cultivation of AMX
- **1998**: Description of AMX Bacteria
- **1999**: Worldwide licence Paques BV
- **2002**: 1st full-scale AMAMMOX®
- **2005**: 2nd full-scale AMX
- **2006**: 1st full-scale CANON
- **2014**: >100 full-scale installations

The new species illustrate the versatility of AMX bacteria.

*Modified from handouts by Tommaso Lotti. CIE4485 Wastewater Treatment.*
Celebrity Effect

- **Installations**
- **Publications**

Year: 1995 to 2015

Ammonium-rich **sidestream** such as sludge dewatering or digester supernatant
# NRR Achieved in Reactor with Various Biomass Types

<table>
<thead>
<tr>
<th>Biomass type</th>
<th>Number of reactors</th>
<th>Names</th>
<th>Nitrogen removal rate (kg-N m(^{-3}) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suspended</td>
<td>1</td>
<td>Single suspended-growth SBR(^a)</td>
<td>0.5*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>NAS(^b)</td>
<td>0.26</td>
</tr>
<tr>
<td>Granular</td>
<td>1</td>
<td>CANON(^c)</td>
<td>1.2*</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SHARON(^{d,e})-anammox</td>
<td>0.6(^i)*</td>
</tr>
<tr>
<td>Hybrid</td>
<td>1</td>
<td>DEMON(^f)</td>
<td>0.6*</td>
</tr>
<tr>
<td>Biofilm</td>
<td>1</td>
<td>ANITA-Mox(^g)</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>DeAmmon(^h)</td>
<td>0.3–0.4*</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>OLAND(^i)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>Aerobic deammonification</td>
<td>1.23(^m)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>SNAP(^j)</td>
<td>0.31–0.45</td>
</tr>
</tbody>
</table>

Biomass Types

- **Flocs & Suspended Sludge**
  - e.g., AS Systems

- **Large AMX Granules**
  - e.g., granular sludge systems

- **Hybrid Suspended & Attached Growth**
  - e.g., IFAS

- **Biofilm**
  - e.g., RBC, MBBR, MABR, Biofilter

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**Increasing diffusivity or mass transfer resistance**
BNR 3.0-Sidestream Application

- 1% Plant Influent Flow
- Rich in N & P
- 10 to 30% Plant Influent TN load
- $\text{NH}_4^+$ Conc. 800 to 2,500 mg N/L
- Centrate TP = 200-800 mg/L
- Temperature 30 - 38°C
- Insufficient Alk. for complete nitrification
- Insufficient carbon for denitrification

PST → Activated Sludge → RAS → WAS → Effluent

- Sidestream Deammonification
- Digestion /CHP
- Centrate
- Dewatering
- Beneficial Reuse of Biosolids

Beverley Stinson, AECOM
Anammox Retention

Types of Mechanisms:

- **Cyclones** - Strass & Glarnarland
- **Sieves** - Blue Plains
- **Membranes** - Singapore PUB, American Water
- **Granules** - Delft TU/Paques
- **Biofilms**
  - MBBR - Veolia, HRSD
  - RBC - Ghent University
Control

- Elevated NH$_3$-N concentrations
- Elevated temperature (30-35 °C)
- Low SRT (1-2 days)
- Low DO (~0.5 mg/L)

Repression Mechanisms

- Free NH$_4^-$-N inhibition: NOB > AOB
- Nitrous acid inhibition: NOB > AOB
- Max growth rate: AOB > NOB at high temp
- DO affinity: AOB > NOB (*high temp.?*)
Commercial Technology Examples

- **SBR + Hydrocyclone Granular Sludge (DEMON)**
  - Strass, Austria + ~20 others
  - Demon GmbH (formly Cyklar-Stulz) – World Water Works, Inc.

- **Upflow Granular Sludge (CANON/ANAMMOX)**
  - Olburgen, Netherlands + ~7 others
  - Paques (NL)

- **Biofilm process (MBBR-style)**
  - ANITA Mox – Malmo, Sweeden
    - AnoxKaldnes – Kruger - Veolia
  - DeAmmon -- Hattingen, Germany & Stockholm
  - Purac
BNR 3.X - Mainstream Development

Limitations of Mainstream Conditions

Barriers/challenges:
- High COD/N ratio
- Lower temperature (activities, growth rate)
- Relatively low ammonium concentrations

Sustainable and Efficient Nitrogen Management

A-Stage: Maximize carbon capture/energy recovery

B-Stage: Minimize carbon & energy demand for N & P removal
BNR 3.X-Mainstream Development

A-Stage
- Option 1: Anaerobic Process
- Option 2: High Rate Activated Sludge
- Option 3: Bioelectrochemical System
- Option 4: Chemically Enhanced Primary Treatment

B-Stage
- Autotrophic Nitrogen Removal
  - Nitritation-anammox

DOMestic Wastewater → COD capture → Energy Recovery

Effluent → Energy Neutral or Positive

Wan et. al. Scientific Reports 6, 2016.
BNR 3.X/Mainstream Development

**Promote AOB and Anammox**
- Bioaugmentation from sidestream deammonification reactor
- SND type reactor with selective AMX retention
- Maintain residual ammonia > 2 mg/L

**Suppress NOB**
- Intermittent on/off aeration, DO level
- Rapid transition to anoxia

**Reduce Heterotrophs**
- A stage pretreatment
- Maintain low influent COD/N ratio for AMX reactor

**Polish Effluent**
- Remove residual ammonia via ammonia based control
- Methanol addition in post-anoxic zone to reduce NOx⁻
Take-home Message

- A fully developed technology, and currently mainly applied in sidestream/industrial wastewater treatment.
- Commercial products are available from several suppliers.
- Mainstream nitritation-anammox is promising but its application could take a while.
Thank you!

Questions?
xjli@vt.edu