WELCOME TO THE JULY EDITION OF THE 2011 M&R SEMINAR SERIES
BEFORE WE BEGIN

• SILENCE CELL PHONES & PAGERS

• QUESTION AND ANSWER SESSION WILL FOLLOW PRESENTATION

• SEMINAR SLIDES WILL BE POSTED ON MWRD WEBSITE AT (www. MWRD.org)

• Home Page ⇒ (Public Interest) ⇒ more public interest ⇒ M&R Seminar Series ⇒ 2011 Seminar Series
Professor Kartik Chandran

Ph.D. (Environmental Engineering) University of Connecticut
B.S. (Chemical Engineering) Indian Institute of Technology

Present         Associate Professor, Department of Earth and Environmental Engineering, Columbia University
2005-2010       Assistant Professor, Department of Earth and Environmental Engineering, Columbia University
2004           Research Associate, Virginia Polytechnic Institute and State University
2001-2004       Research Technical Associate, Chief Engineer’s Research Group, Metcalf & Eddy

Research Interests

• Environmental microbiology, microbial N-cycling,
• sustainable sanitation and wastewater treatment,
• global climate impacts of engineered wastewater treatment practice
• microbial ecology of engineered biological waste and water treatment reactors
• elucidation of microbial biochemical degradation pathways

Selected activities and honors

• Water Environment Research Foundation Paul L. Busch Award (2010)
• AEESP accompanying keynote lecture at WEFTEC, New Orleans, LA (2010)
• Nominated to the Board of Trustees, Water Environment Federation (2010)
• National Science Foundation Early Faculty Career Development Award, CAREER (2009)
• Visiting Professor, Delft University of Technology, hosted by Prof. Mark van Loosdrecht,
• National Research Council, National Academies of Science Summer Faculty Fellowship award, hosted by the United States Environmental Protection Agency Headquarters, Cincinnati, OH, (Summer 2007).
Wastewater treatment and climate change
Inventories and mechanisms of biogenic nitrous oxide

Kartik Chandran
Columbia University

Metropolitan Water Reclamation District of Greater Chicago
July 29th, 2011
### Table 8-1: Emissions from Waste (Tg CO₂ Eq.)

<table>
<thead>
<tr>
<th></th>
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<td>151.0</td>
<td>148.1</td>
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<td>24.0</td>
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<td>Composting</td>
<td>0.3</td>
<td>0.7</td>
<td>1.3</td>
<td>1.3</td>
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<td>1.6</td>
<td>1.6</td>
<td>1.6</td>
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<tr>
<td>N₂O</td>
<td>6.6</td>
<td>7.7</td>
<td>8.9</td>
<td>9.2</td>
<td>9.0</td>
<td>9.3</td>
<td>9.6</td>
<td>9.7</td>
<td>9.9</td>
</tr>
<tr>
<td>Domestic Wastewater</td>
<td>6.3</td>
<td>6.9</td>
<td>7.6</td>
<td>7.8</td>
<td>7.6</td>
<td>7.7</td>
<td>7.8</td>
<td>8.0</td>
<td>8.1</td>
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<td>Treatment</td>
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</tr>
<tr>
<td>Composting</td>
<td>0.4</td>
<td>0.8</td>
<td>1.4</td>
<td>1.4</td>
<td>1.4</td>
<td>1.6</td>
<td>1.7</td>
<td>1.7</td>
<td>1.8</td>
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<tr>
<td><strong>Total</strong></td>
<td>179.6</td>
<td>176.8</td>
<td>155.6</td>
<td>152.1</td>
<td>154.5</td>
<td>160.3</td>
<td>157.7</td>
<td>158.7</td>
<td>161.0</td>
</tr>
</tbody>
</table>

Note: Totals may not sum due to independent rounding.

From denitrification in anoxic or non-aerated zones

This is equivalent to 900,000 passenger cars added each year

Source: USEPA GHG Sources and Sinks Inventory, 2008
Domestic wastewater \( \text{N}_2\text{O} \) emission estimates

\[
\begin{align*}
\text{N}_2\text{O}_{\text{TOTAL}} &= \text{N}_2\text{O}_{\text{PLANT}} + \text{N}_2\text{O}_{\text{EFFLUENT}} \\
\text{N}_2\text{O}_{\text{PLANT}} &= \text{N}_2\text{O}_{\text{NIT/DEMIT}} + \text{N}_2\text{O}_{\text{WOUT/NIT/DEMIT}} \\
\text{N}_2\text{O}_{\text{NIT/DEMIT}} &= [(\text{US}_{\text{POP}}) \times \text{EF}_2 \times \text{F}_{\text{IND-COM}}] \times 1/10^9 \\
\text{N}_2\text{O}_{\text{WOUT/NIT/DEMIT}} &= \{(\text{US}_{\text{POP}} \times \text{WWTP}) - \text{US}_{\text{POP}} \times \text{F}_{\text{IND-COM}}\} \times \text{EF}_1 \times 1/10^9 \\
\text{N}_2\text{O}_{\text{EFFLUENT}} &= \{(\text{US}_{\text{POP}} \times \text{Protein} \times \text{F}_{\text{FPR}} \times \text{F}_{\text{NON-CON}} \times \text{F}_{\text{IND-COM}}) - \text{N}_{\text{SLUDGE}}\} \times \text{EF}_3 \times 44/28 \times 1/10^6
\end{align*}
\]

- \( \text{EF}_1 = 3.2 \text{ g N}_2\text{O}/\text{PE}/\text{year} \)
- \( \text{EF}_2 = 7.0 \text{ g N}_2\text{O}/\text{PE}/\text{year} \)
- \( \text{EF}_3 = 0.005 \text{ kg N}_2\text{O} \cdot \text{N}/\text{kg sewage-N produced} \)

Source: USEPA GHG Sources and Sinks Inventory, 2008
This presentation focuses on

• $\text{N}_2\text{O}$ emissions from different wastewater treatment process configurations

• Insights to molecular phenomena linked with $\text{N}_2\text{O}$ and NO production in *N. europaea*

• Impact of partial nitrification OR organic carbon source on $\text{N}_2\text{O}$ production via denitrification
Role of nitrification and denitrification in $\text{N}_2\text{O}$ emissions

- Based on known mechanisms, significantly higher emissions from aerated zones expected.

- How does this influence the way we have been thinking about $\text{N}_2\text{O}$ emissions from WWTPs?

**Diagram:**

- **Aerobic**
  - $\text{N}_2\text{O}$ production mainly
  - High $\text{N}_2\text{O}$ emission expected

- **Anoxic**
  - $\text{N}_2\text{O}$ production and consumption
  - Low $\text{N}_2\text{O}$ emission expected
Development of a standardized protocol for measurement

- Protocol has been reviewed by US EPA and is now being implemented nationwide
- Shared with other teams around the globe via GWRC

Chandran, 2011
## Summary of emissions

<table>
<thead>
<tr>
<th>Plant Configuration</th>
<th>Temp(°C)</th>
<th>Avg. reactor influent TK N load (g-N/day)</th>
<th>Avg. reactor effluent TN load (g-N/day)</th>
<th>Q (MGD)</th>
<th>% influent TKN emitted as N₂O</th>
<th>% removed TKN emitted as N₂O</th>
<th>Emission factor (g N₂O/PE/yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Separate-stage BNR</td>
<td>15 ± 0.48</td>
<td>1.8 x 10⁶</td>
<td>3.6 x 10⁵</td>
<td>23</td>
<td>0.03 ± 0.00</td>
<td>0.03 ± 0.01</td>
<td>1.2 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>23 ± 0.28</td>
<td>2.3 x 10⁶</td>
<td>4.3 x 10⁵</td>
<td>27</td>
<td>0.01 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.28 ± 0.13</td>
</tr>
<tr>
<td>Four-stage Bardenpho</td>
<td>14 ± 0.26</td>
<td>8.6 x 10⁵</td>
<td>1.7 x 10⁵</td>
<td>7.8</td>
<td>0.16 ± 0.10</td>
<td>0.19 ± 0.12</td>
<td>9.8 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>23 ± 0.20</td>
<td>7.4 x 10⁵</td>
<td>7.6 x 10⁴</td>
<td>8.1</td>
<td>0.60 ± 0.29</td>
<td>0.66 ± 0.32</td>
<td>33 ± 16</td>
</tr>
<tr>
<td>Step-feed BNR 1</td>
<td>19 ± 0.22</td>
<td>3.1 x 10⁶</td>
<td>1.4 x 10⁵</td>
<td>29</td>
<td>1.6 ± 0.83</td>
<td>2.9 ± 1.5</td>
<td>92 ± 47</td>
</tr>
<tr>
<td></td>
<td>25 ± 0.28</td>
<td>2.9 x 10⁶</td>
<td>9.4 x 10⁵</td>
<td>30</td>
<td>0.62 ± 0.27</td>
<td>0.90 ± 0.39</td>
<td>33 ± 14</td>
</tr>
<tr>
<td>Step-feed non-BNR</td>
<td>17 ± 0.12</td>
<td>8.6 x 10⁵</td>
<td>4.4 x 10⁵</td>
<td>71</td>
<td>0.18 ± 0.18</td>
<td>0.37 ± 0.36</td>
<td>13 ± 13</td>
</tr>
<tr>
<td></td>
<td>26 ± 0.81</td>
<td>8.9 x 10⁵</td>
<td>4.2 x 10⁵</td>
<td>93</td>
<td>1.8 ± 0.79</td>
<td>3.3 ± 1.5</td>
<td>97 ± 43</td>
</tr>
<tr>
<td>Separate centrate</td>
<td>30 ± 2.3</td>
<td>8.8 x 10⁶</td>
<td>5.5 x 10⁵</td>
<td>2.0</td>
<td>0.24 ± 0.02</td>
<td>0.63 ± 0.06</td>
<td>590 ± 53</td>
</tr>
<tr>
<td></td>
<td>34 ± 0.32</td>
<td>8.5 x 10⁶</td>
<td>4.2 x 10⁵</td>
<td>1.6</td>
<td>0.54 ± 0.16</td>
<td>0.96 ± 0.32</td>
<td>1600 ± 500</td>
</tr>
<tr>
<td>Plug-flow 1</td>
<td>11 ± 0.20</td>
<td>1.8 x 10⁶</td>
<td>1.0 x 10⁵</td>
<td>18</td>
<td>0.40 ± 0.14</td>
<td>0.92 ± 0.32</td>
<td>23 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>23 ± 0.46</td>
<td>1.8 x 10⁶</td>
<td>7.3 x 10⁵</td>
<td>15</td>
<td>0.41 ± 0.14</td>
<td>0.70 ± 0.24</td>
<td>28 ± 9.6</td>
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<tr>
<td>Plug-flow 2</td>
<td>11 ± 0.41</td>
<td>6.3 x 10⁵</td>
<td>4.0 x 10⁵</td>
<td>8.7</td>
<td>0.62 ± 0.15</td>
<td>1.7 ± 0.41</td>
<td>26 ± 6.4</td>
</tr>
<tr>
<td></td>
<td>22 ± 0.58</td>
<td>6.6 x 10⁵</td>
<td>4.0 x 10⁵</td>
<td>6.6</td>
<td>0.09 ± 0.03</td>
<td>0.22 ± 0.06</td>
<td>5.0 ± 1.4</td>
</tr>
<tr>
<td>MLE 1</td>
<td>22 ± 0.28</td>
<td>7.3 x 10⁵</td>
<td>1.3 x 10⁵</td>
<td>4.0</td>
<td>0.44 ± 0.37</td>
<td>0.54 ± 0.45</td>
<td>47 ± 39</td>
</tr>
<tr>
<td></td>
<td>26 ± 1.8</td>
<td>6.8 x 10⁵</td>
<td>1.9 x 10⁵</td>
<td>4.0</td>
<td>0.07 ± 0.04</td>
<td>0.09 ± 0.05</td>
<td>6.8 ± 3.5</td>
</tr>
<tr>
<td>MLE 2</td>
<td>21 ± 0.72</td>
<td>5.9 x 10⁵</td>
<td>1.2 x 10⁵</td>
<td>3.3</td>
<td>0.07 ± 0.02</td>
<td>0.09 ± 0.02</td>
<td>7.4 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>26 ± 0.17</td>
<td>6.9 x 10⁵</td>
<td>1.5 x 10⁵</td>
<td>4.1</td>
<td>0.06 ± 0.02</td>
<td>0.07 ± 0.03</td>
<td>5.4 ± 2.0</td>
</tr>
<tr>
<td>Step-feed BNR 2</td>
<td>29 ± 0.18</td>
<td>2.2 x 10⁵</td>
<td>2.9 x 10⁵</td>
<td>14</td>
<td>1.5 ± 0.02</td>
<td>1.7 ± 0.02</td>
<td>140 ± 1.2</td>
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<tr>
<td>Oxidation ditch</td>
<td>14 ± 0.58</td>
<td>3.7 x 10⁵</td>
<td>1.8 x 10⁵</td>
<td>3.3</td>
<td>0.10 ± 0.03</td>
<td>0.19 ± 0.06</td>
<td>6.1 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>19 ± 0.58</td>
<td>3.9 x 10⁵</td>
<td>4.3 x 10⁴</td>
<td>3.4</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>1.8 ± 0.77</td>
</tr>
<tr>
<td>Step-feed BNR 3</td>
<td>20 ± 1.8</td>
<td>4.5 x 10⁵</td>
<td>7.3 x 10⁵</td>
<td>40</td>
<td>0.14 ± 0.02</td>
<td>0.17 ± 0.03</td>
<td>9.3 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>24 ± 0.78</td>
<td>7.8 x 10⁵</td>
<td>8.6 x 10⁵</td>
<td>57</td>
<td>0.05 ± 0.03</td>
<td>0.06 ± 0.03</td>
<td>4.1 ± 2.2</td>
</tr>
</tbody>
</table>

However, these do not convey the complete picture.
Relative emissions from aerated and non-aerated zones

- Aerated zones contributed more to emissions than non-aerated zones
## Spatial variability in $\text{N}_2\text{O}$ emissions

<table>
<thead>
<tr>
<th>Zone3 (Aerobic)</th>
<th>Zone2 (Aerobic)</th>
<th>Zone1 (Anoxic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ammonia (ppm-N)</strong></td>
<td>1.5 ±0.71</td>
<td>11.5 ±4.95</td>
</tr>
<tr>
<td><strong>Nitrite (ppm-N)</strong></td>
<td>0</td>
<td>0.003 ±0.001</td>
</tr>
<tr>
<td><strong>Nitrate (ppm-N)</strong></td>
<td>10.15 ±0.21</td>
<td>2.65 ±0.35</td>
</tr>
<tr>
<td><strong>DO (mg-O$_2$/L)</strong></td>
<td>4.2</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>ORP (mV)</strong></td>
<td>55.9</td>
<td>-10</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.1</td>
<td>7.12</td>
</tr>
<tr>
<td><strong>Temp (°C)</strong></td>
<td>29.5</td>
<td>29.3</td>
</tr>
<tr>
<td><strong>Aqueous $\text{N}_2\text{O}$ (ppb-N$_2$O)</strong></td>
<td>572.55</td>
<td>192.16</td>
</tr>
<tr>
<td><strong>Gaseous $\text{N}_2\text{O}$ (ppm-N$_2$O)</strong></td>
<td>22.8 ±0.67</td>
<td>16.47 ±0.27</td>
</tr>
</tbody>
</table>
Diurnal variability in N$_2$O emissions

- Significant diurnal variability in N$_2$O(g) and N$_2$O (l) conc. in aerobic zones
- Near perfect correlation with diurnal NH$_3$, NO$_2^-$ and NO$_3^-$ conc.
Summary

• High-degree of variability in emissions observed

• $\text{N}_2\text{O}$ emissions from aerobic zones were consistently higher than from anoxic zones

• Based on multivariate regression and data mining
  – High ammonia, nitrite and DO conc. positively correlated with $\text{N}_2\text{O}$ fluxes
  – High DO and nitrite conc. together correlated positively with $\text{N}_2\text{O}$ fluxes

• $\text{N}_2\text{O}$ emissions are related to inadequate design and operation of BNR processes
  – There is no conflict between water quality and air quality, rather they go hand in hand
  – $\text{N}_2\text{O}$ emissions can be used as an indicator of process upsets
What are the mechanisms linked to N$_2$O and NO generation by nitrifying bacteria?

**Hypotheses**

- Anoxic conditions stimulate the co-expression of nir$K$ and nor$B$ in *N. europaea* and thus, NO and N$_2$O production.

- Upon *recovery* back to aerobic conditions, the trends are reversed.

Yu et al., 2010
Chemostat operation

• $V = 4L$
• $HRT = SRT = 2.2d$
• Transient anoxic period = 48h, followed by about 80 h recovery
• $S_{nh,o} = 280 \text{ mg-N/L at steady state}$
• $S_{nh,o} = 28, 140, 280 \text{ mg-N/L during transient state}$
  – To determine the impact of $S_{nh}$ accumulation on response and recovery
Short term change in DO-Nitrification

- $\text{N}_2\text{O}_\text{O}$ production is directional
  - Manifestation of recovery response
Short term change in DO-Nitrification

Yu et al., 2010
Nitrite reductase was by far the most responsive to anoxic-oxic cycling

- $nirK \rightarrow NO$

nirK and norB are not co-expressed

Gene level imbalances are linked to process level $N_2O$ inventories
Adaptation to repeated anoxic-oxic cycling
The quest for cost effective BNR
Engineering microbial communities

NOB
Nitrification step II
0.5 mol O₂/mol N

25% savings in air $

NO₃⁻-N
N(+V)

DNB
Denitrification step I
1.15 mol COD/mol N

40% savings in COD $

NO₂⁻-N
N(+III)

DNB
Denitrification step n
1.71 mol COD/mol N

Nitrogen fixation

NO₂⁻-N
N(+III)

AOB
Nitrification step I
1.5 mol O₂/mol N

NH₄⁺-N
N(-III)

Denitrification step n
1.71 mol COD/mol N

N₂
N(0)
Factors correlating with N$_2$O emissions from nitrification

- **Known triggers for N$_2$O from nitrification**
  - High nitrite concentrations
  - Low DO concentrations and cycling from anoxic to oxic conditions
  - High ammonia concentration transients

**Diagram:**

- NirK
- HAO
- Cyt aa$_3$ oxidase
- AMO
- Nor

Ahn et al., 2011

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Do we need to re-think partial nitrification based N-removal strategies?
Reactor Operation

- $V=11.18 \text{ d}$, $HRT=1.1 \text{ d}$, $pH=7.5 \pm 0.1$, $T=21^\circ \text{C}$
- **Pre-study partial-nitrification phase**
  - $SRT = 3\text{d}$, $DO = 1.5 \pm 0.87 \text{ mg O}_2/\text{L}$
- **Full-nitrification phase**
  - $SRT= 8\text{d}$, $DO = 3.8 \pm 0.38 \text{ mg O}_2/\text{L}$, 104 days
- **Partial-nitrification phase**
  - $SRT= 3\text{d}$, $DO = 1.1 \pm 0.38 \text{ mg O}_2/\text{L}$, 273 days
Performance and kinetics

- Rapid change in N-speciation upon changing operating conditions
- Significant decrease in NOB kinetics during PN
- No change in AOB kinetics
Impact of changing operating conditions on microbial ecology

- PN mode led to significant washout of NOB
- No change in dominant AOB speciation
  - *Nitrosomonas europaea and eutropha* dominant AOB in both phases (not shown)
Impact on $\text{N}_2\text{O}$ and NO emissions

- Highest emissions observed just after switch from full nitrification to partial nitrification
  - However, emissions during PN were not sustained – subsided and stabilized after 80 days
  - Stabilized emissions during PN still statistically higher than during FN ($\alpha=0.05$)
Why does PN result in higher emissions?

Insights from gene expression profiles

- The switch from FN to PN resulted in spikes in expression of *nirK* and *norB*
  - *nirK* → NO  *norB* → N₂O
- Good agreement between gene expression and chemical profiles
Summary

• Statistically higher emissions of $\text{N}_2\text{O}$ and NO during PN than during FN

• Highest emissions close to the point of switching modes from FN to PN
  – Gaseous emissions observed even after rapid change in aqueous N-speciation

• Spikes in gaseous emissions were linked to spikes in expression of genes coding for their production in AOB ($\text{nirK}$ and $\text{norB}$)
  – Microbes tend to adapt!
To put matters in perspective

• PN offers significant benefits in terms of lower operating costs
  – Nitrification as well as downstream removal via denitrification or anammox

• Higher N$_2$O emissions from PN operation for treating streams such as centrate and leachate represents an optimization challenge

• Additional analyses such as LCA could be useful in decision making on a case-specific and site-specific basis
  – Poor performance remains a bigger factor for higher emissions
Role of different electron donors on $\text{N}_2\text{O}$ and NO emissions

- Different electron donors give rise to different $\mu_{\text{max}}$ and $K_S$ for denitrification on
  - Response to different transient stressors needs to be systematically studied
  - Different susceptibilities $\Rightarrow$ different emissions?
Transitient stressors

- Organic carbon limitation COD:N = 2.5 : 1
- Exposure to high nitrite concentration spike: 50mg-N/L
- Oxygen Inhibition
  DO = 2-3 mg/L, 5-6 mg/L, 7-9 mg/L

USEPA reviewed gas phase monitoring protocol
Impact on methanol based denitrification

- Minimal N$_2$O and NO emissions
  - COD limitation: transient NO$_3^-$ accumulation
  - NO$_2^-$ pulse: transient NO$_3^-$ accumulation
  - High DO: permanent NO$_3^-$ accumulation

Lu and Chandran, 2010
Impact on ethanol based denitrification (I)

• Minimal N$_2$O and NO emissions with transient and finite peaks
  – COD limitation: transient NO$_3^-$ accumulation
  – NO$_2^-$ pulse: transient NO$_3^-$ and NO$_2^-$ accumulation

Lu and Chandran, 2010
Impact on ethanol based denitrification (II)

- $N_2O$ and NO emissions increased with DO concentration
- $N_2O$ emission peak: correlated with peak $NO_3^-$ concentration
- Transient accumulation of $NO_3^-$: increased with DO concentration
- Permanent accumulation of $NO_2^-$: increased with DO concentration
Gas emissions from methanol-denitrification

- Approximately 0.12% and 0.05% of influent NO$_3^-$-N load converted to N$_2$O and NO, respectively at steady state
- Statistically similar emissions in
  - Control, carbon limitation, NO$_2^-$-N exposure, O$_2$ inhibition

Lu and Chandran, 2010
Gas emissions from ethanol-denitrification

- Approximately 0.10% and 0.01% of influent NO$_3^{-}$-N load converted to N$_2$O and NO, respectively at steady state
- Statistically similar emissions in
  - Control, carbon limitation, NO$_2^{-}$-N exposure
- Significantly higher N$_2$O and or NO emissions at DO > 5mg O$_2$/L

Lu and Chandran, 2010
Implications

- **Emissions related to denitrification are dependent upon the organic C-sources used**
  - the microbial ecology and kinetics thus fostered
  - relative susceptibility and tolerance to stressors

- **Organic C-limitation and nitrite toxicity played a minor role in emissions from both methanol and ethanol**
  - Partial inhibition resulted in N$_2$O emissions (ethanol)
  - Higher inhibition led to low emission (methanol)
Implications for pre-anoxic zone sizing

- Ethanol bleed out to aerobic zone can result in \( \text{N}_2\text{O} \) and NO emissions
- Lower emissions expected during similar methanol bleed out
Summary of observations

• Started with one or two emission factors in 2008
• N₂O emissions related to recovery from stress response of nitrifying bacteria
  – Similar patterns observed at full-scale
  – Attributed to an imbalance between the expression of specific pathways in AOB
• Next: Based on mechanisms, develop BNR strategies to minimize both aqueous and gaseous N discharges
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**Emissions Credits: Opportunity To Promote Integrated Nitrogen Management in the Wastewater Sector**

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