Microbial Processes in Constructed Tidal Wetlands for Removal of Nitrogen from Urban Wastewaters

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Charlottesville, VA
Goals for Urban Wastewater Treatment

• Removal of BOD

• Removal of nutrients, particularly nitrogen

• Disinfection to kill pathogens

Typically done in large, central wastewater treatment plants, then water is discharged to the environment

Water **could** be reused for secondary purposes!
Designed for new construction
Living Machine:
Capacity = 5,000 gallons day$^{-1}$
Nitrogen processes important in wastewater renovation

Anammox

Organotrophic reduction (including denitrification)

after Keunen, 2008
Tidal reactors remove nitrogen and reduce BOD efficiently and inexpensively

Not sure what processes are responsible for N removal

Not sure how the microbes are distributed spatially within a reactor

How to optimize operation so as to exploit the microbes to greatest capacity
### Smaller Columns

<table>
<thead>
<tr>
<th></th>
<th>High N</th>
<th>Low N</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄ (g)</td>
<td>13.74</td>
<td>0.491</td>
</tr>
<tr>
<td>COD (g O₂)</td>
<td>1.3</td>
<td>1.3</td>
</tr>
</tbody>
</table>
Hydraulic operation of tidal columns

<table>
<thead>
<tr>
<th>Inundation Frequency (cycles day(^{-1}))</th>
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<tbody>
<tr>
<td>24</td>
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<tr>
<td>16</td>
</tr>
<tr>
<td>8</td>
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<td>4</td>
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<tr>
<td>24</td>
</tr>
</tbody>
</table>
Nitrogen Cycling in Wastewater Treatment

**Ammonium Oxidizing Bacteria (AOB)**

\[
\text{NH}_4^+ \rightarrow \text{NO}_2^- \\
\Delta G = -64.7 \text{ kcal (mol NH}_4^+\text{)}^{-1}
\]

**Nitrite Oxidizing Bacteria (NOB)**

\[
\text{NO}_2^- \rightarrow \text{NO}_3^- \\
\Delta G = -18.5 \text{ kcal (mol NO}_2^-\text{)}^{-1}
\]

**Anaerobic Ammonium Oxidizing Bacteria (AMX)**

\[
\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 \\
\Delta G = -85.5 \text{ kcal (mol NH}_4^+\text{)}^{-1}
\]
Fluorescent *In Situ* Hybridization (FISH)

1) Bacterial cells are made permeable

2) Labeled DNA probes are added

3) Probes pass through the cell wall

4) Probes bind with the 16S region of the ribosome

5) Unbound probe is rinsed away

6) Labeled cells are visible under fluorescence microscopy
In situ hybridization of a wastewater biofilm

Extract Cells

1. Combine 1 g LESA with 2 mL PBS
2. Vortex for 5 min
3. Transfer to microcentrifuge tube.

Preserve Cells

1. Preserve cells in methanol
2. Transfer to ethanol
3. Store @ -20° C

Adhere to Slides

1200 samples to count!
AOB after hybridization with Cy3-labeled oligonucleotides
Abundance of N-cycle organisms in tidal reactors

24 cycles day\(^{-1}\)

**Low N**

- Total Cells
- AOB
- NOB
- AMX

**High N**

- Total Cells
- AOB
- NOB
- AMX

Abundance (cells g\(^{-1}\) LESA)
Abundance of N-cycle organisms in tidal reactors

8 cycles day$^{-1}$

**Low N**

**High N**

- Total Cells
- AOB
- NOB
- AMX

Abundance

5 6 7 8 9 10

Abundance

5 6 7 8 9 10

Low N

High N
Abundance of N-cycle organisms in tidal reactors
4 cycles day$^{-1}$

**Low N**

**High N**

Abundance (cells g$^{-1}$ LESA)

- Total Cells
- AOB
- NOB
- AMX

Abundance (cells g$^{-1}$ LESA)
Ammonium concentration in tidal columns
Nitrite concentrations in tidal columns

Date
Nitrate (ppm N)
0
2000
4000
6000
8000
10000
12000

24i               24             16        8         4         24        f
Nitrate concentrations in tidal columns

Date
Oct  Dec  Feb  Apr  Jun  
Nitrate (ppm N)
0
2000
4000
6000
8000
10000
12000
Nitrogen removal in tidal columns

Nitrogen Removed (ppm N)
-10000
0
10000
20000

Time (months)
Oct  Dec  Feb  Apr  Jun

Nitrogen Removed (ppm N)
24i               24             16        8         4         24        f
Conclusions

• The dominant reaction sequence for N removal from wastewater in tidal reactors is denitrification

• Anammox capable organisms are present, but at insignificant numbers

• Under optimal conditions, AMX may become as plentiful as NOB

• The optimal cycle rate for reactors of the size used in this study is 4 cycles day\(^{-1}\)
Living Machine Demonstration: Tema, Ghana