Ecogenomics reveals distributed metabolic networks in anammox bioreactors

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Anaerobic ammonium oxidation (Anammox): Short-cutting the Nitrogen cycle

Advantages:
- Efficient N removal
- ~ 60% less oxygen
- No organic carbon
- Large energy savings

Challenges:
- Prevent NO$_2^-$ oxidation
- Bacteria grow slowly
Anammox process configurations

SHARON-Anammox Process:

SHARON (Nitritation)

1 NH$_4^+$N
1 HCO$_3^-$

ANAMMOX (SBR or UASB)

0.5 N$_2$

0.5 NH$_4^+$N
0.5 NO$_2^-$N

DEMON Process:

PN-ANAMMOX (SBR)

1 NH$_4^+$N
1 HCO$_3^-$

0.5 N$_2$
Anammox bioreactors employ “granular” or “attached” growth modes
Engineering practices focus on out-selecting nitrite oxidizing bacteria (NOB).
What do other microorganism do?
Understanding microbial interactions in anammox bioreactors

Research objectives:

1. Does bioreactor growth mode (granular vs. attached) select for different microbial communities?

2. What is the metabolic potential of microorganisms in anammox bioreactors?

3. How do microorganisms interact in anammox bioreactors?
Experimental Setup
Attached growth bioreactor seeded with suspended sludge

SHARON (Nitritation)

1 NH₄-N → 0.5 NH₄-N → 0.5 NO₂-N

Both bioreactors sampled after 200 days operation

ANAMMOX (Suspended)

0.5 N₂

ANAMMOX (Attached)

0.5 N₂

0.5 N₂
**Metagenome.** Total genetic material from environmental sample.
Does bioreactor growth mode select for different microbial communities?
Both bioreactors contained similar taxonomic groups. Commonly detected neighboring organisms are abundant.
Binning. Grouping contigs into operational units based on abundance and sequence composition.
5 near-complete population genomes recovered from metagenomic contigs

<table>
<thead>
<tr>
<th>Phylogenetic Affiliation</th>
<th>Genome size (Mbp)</th>
<th>GC content</th>
<th>Gene count</th>
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<tr>
<td>Planctomycetaceae sp.</td>
<td>4.30</td>
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<tr>
<td>Ignavibacteriales sp.</td>
<td>2.63</td>
<td>37.6</td>
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<td>Planctomycetaceae sp.</td>
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<td>Anaerolineae sp.</td>
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<td>Rhodocyclaceae sp.</td>
<td>2.83</td>
<td>66.4</td>
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</table>
What is the metabolic potential of microorganisms in anammox bioreactors?
Organisms are Metabolically Versatile
Anammox bacteria may provide AA’s for heterotrophic bacteria.

Ignavibacteria missing hydrophobic AA’s biosynthesis pathways.

### Amino Acid Metabolism

<table>
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<tr>
<th></th>
<th>Biosynthesis</th>
<th>Degradation</th>
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<td>Glutamate</td>
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<td>Selenocysteine</td>
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</tbody>
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**Legend:**
- Plancto
- Ignavi
- Anaerol
- Rhodo
Could iron acquisition shape microbial interactions?

Anammox bacteria

Ignavibacteria
How do microorganisms interact in anammox bioreactors?
What do other microorganism do?
Possible interactions?
Summary

1. Granular and attached anammox bioreactors select for similar microbial communities.

2. Neighbouring community members include Ignavibacteriales, Rhodobacteraceae, Anaerolineae.

3. Novel opportunities for metabolic cooperation between community members mediated through SMPs exchange and nutrient cycling.
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