MOLECULAR TOOLS FOR OPTIMIZATION OF MAINSTREAM NITROGEN REMOVAL WITH ANAMMOX SELECTING MEMBRANE BIOREACTORS

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CONVENTIONAL VS. ANAMMOX PROCESS

Nitrification

\[
\begin{align*}
\text{NH}_4^+ & \rightarrow \text{NO}_2^- \\
\text{NO}_2^- & \rightarrow \text{NO}_3^-
\end{align*}
\]

Aeration

Denitrification

\[
\begin{align*}
\text{NO}_2^- & \rightarrow \text{NO}_2^- \\
\text{NO}_2^- & \rightarrow \text{N}_2
\end{align*}
\]

Carbon
CONVENTIONAL VS. ANAMMOX PROCESS

**Anaerobic ammonia oxidation**

\[
\begin{align*}
\text{NH}_4^+ & \rightarrow \text{NO}_2^- \\
\text{NO}_2^- & \rightarrow \text{NO}_3^- \quad \times \quad \text{NO}_3^- \\
\text{NO}_3^- & \rightarrow \text{NO}_2^- \rightarrow \text{N}_2 \\
\end{align*}
\]

Aeration Aeration Carbon

Aerobic ammonia oxidation (AOB)

MORE COST EFFECTIVE PROCESS

![Bar charts showing energy consumption, COD to N ratio, and sludge production for conventional and Anammox processes.](chart.png)

Source: Crawford and Sandino 2010
MAINSTREAM IMPLEMENTATION CHALLENGES

- Anammox microorganisms are limited by:
  - Slow growth rate
  - Temperatures below 30 ºc
  - High carbon concentrations
  - Dissolved oxygen concentrations over 1%
  - Require high levels of influent $\text{NH}_4^+$
  - Hard to stop nitrification at $\text{NO}_2^-$

SIDESTREAM SUCCESS

- WARM

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$

- NH$_4^+$
IDEAL ANAMMOX MICRO-ENVIRONMENT

ZEOLITE

ZEOLITE

Faujasite

(Rhodes, 2010)
ZEOLITE MEMBRANE AERATED BIOREACTOR

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

MABR
HOW WOULD WE USE MOLECULAR METHODS TO DESIGN AND OPERATE THIS SYSTEM?

- Sequencing – who’s there, untargeted
- qPCR – how many, genes (dead and alive)
- FISH – spatial and physical, genes
- RT-qPCR – how many are active, genes (alive)

EXPERIMENTAL STAGES

STAGE 1 – ZEOLITE OPTIMIZATION

25 mL Synthetic Mainstream Wastewater

+ \( \text{NO}_2^- \)

Inoculum: Activated and anammox sludge

STAGE 2 – OXYGEN OPTIMIZATION

25 mL Synthetic Mainstream Wastewater

Inoculum: Activated and anammox sludge

AIR
STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES

2. VARYING ZEOLITE MASS

3. ZEOLITE COATED VS. UNCOATED MEMBRANES

x4 (3 g each)
ZEOLITE VS. GLASS PARTICLES

30 Day Average Removal

Effluent Ammonia

Effluent Total Nitrogen

Zeolite vs. Glass Particles - Sequencing

Day 30

Sequencing hits:
- Candidatus Brocadia
- Candidatus
- Anammoximicrobium
- Uncultured Planctomycetes
- Unknown Planctomycetes
ZEOLITE VS. GLASS PARTICLES - qPCR

Percent Anammox Gene on Particles

0.00% 0.01% 0.02% 0.03% 0.04% 0.05% 0.06%

# Amx copies
# 16S copies

Day
1 15 29 43

STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES

2. VARYING ZEOLITE MASS

3. ZEOLITE COATED VS. UNCOATED MEMBRANES
VARYING ZEOLITE MASS

Effluent Ammonia

Days

NH₄⁺ mg-N/L

Days

TN mg-N/L

VARYING ZEOLITE MASS

Effluent Ammonia

Days

NH₄⁺ mg-N/L

Days

TN mg-N/L

VARYING ZEOLITE MASS

Effluent Ammonia

Days

NH₄⁺ mg-N/L

Days

TN mg-N/L
VARYING ZEOLITE MASS

Effluent Ammonia

Effluent Total Nitrogen

NITRITE AND NITRATE EFFLUENT
VARYING ZEOLITE MASS - qPCR

Day 30

* Indicates p-value < 0.05

VARYING ZEOLITE MASS - FISH

1.0 g reactor

0.05 g reactor

All bacteria
Anammox
NOB
ACTIVITY MEASUREMENTS
RNA ANALYSIS (RT-qPCR)

STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES

2. VARYING ZEOLITE MASS

3. ZEOLITE COATED VS. UNCOATED MEMBRANES

x3 (Equivalent to 0.5 g of zeolite)
**MEMBRANE TECHNOLOGY**

Growth on surface  
Attachment to surface  
Embedment into matrix

*zeolite*  
*membrane*


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**SORPTION DATA**

![Graph showing sorption data](attachment:image)

Growth on surface  
Attachment to surface  
Embedment into Matrix
MEMBRANES - QPCR

CONCLUSIONS

• Zeolite increased ammonia and TN removal
• Amx gene quantities are not always greater
• Molecular techniques allow us to better understand what microbial processes are occurring and why
SOURCES

CRAWFORD, G., & SANDINO, J. (2010). ENERGY EFFICIENCY IN WASTEWATER TREATMENT IN NORTH AMERICA: A COMPREHEND OF BEST PRACTICES AND CASE STUDIES OF NOVEL APPROACHES. RETRIEVED FROM WWW.WERF.ORG

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QUESTIONS?

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