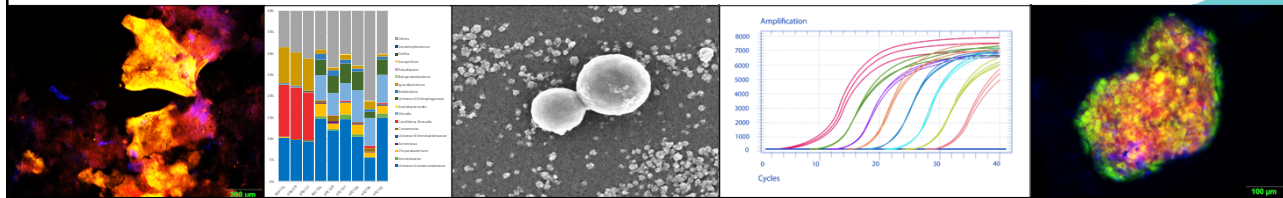


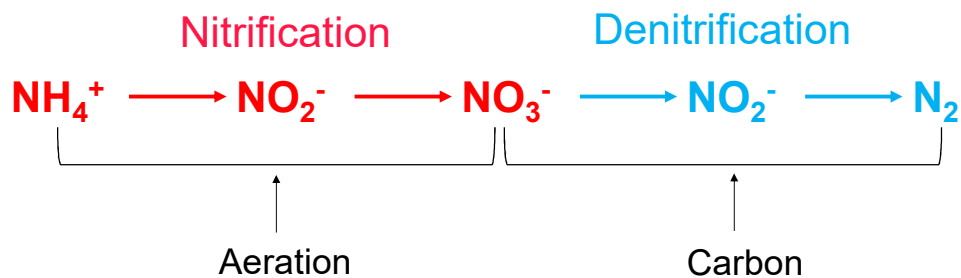
MOLECULAR TOOLS FOR OPTIMIZATION OF MAINSTREAM NITROGEN REMOVAL WITH ANAMMOX SELECTING MEMBRANE BIOREACTORS

ANNDEE HUFF, PAIGE NOVAK, MARC HILLMYER, MICHAEL TSAPATIS, KIWON EUM

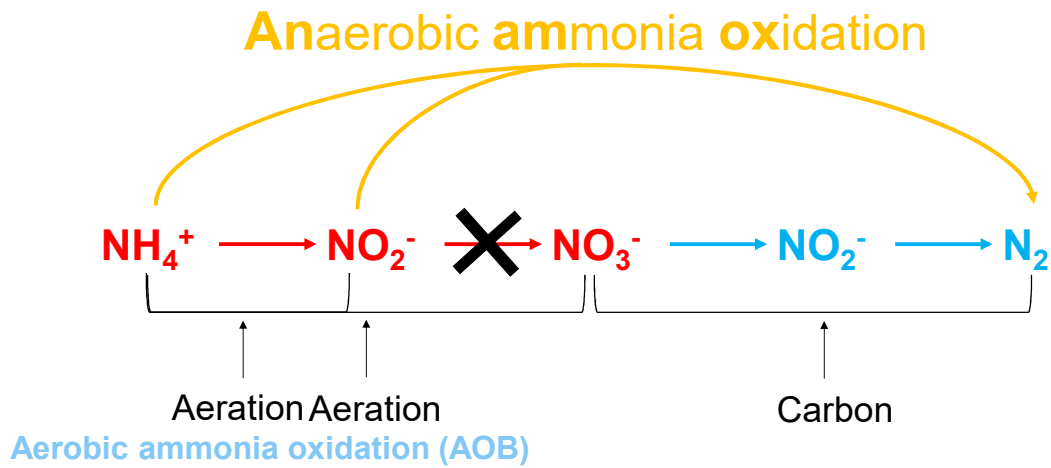
SEPTEMBER 25, 2019



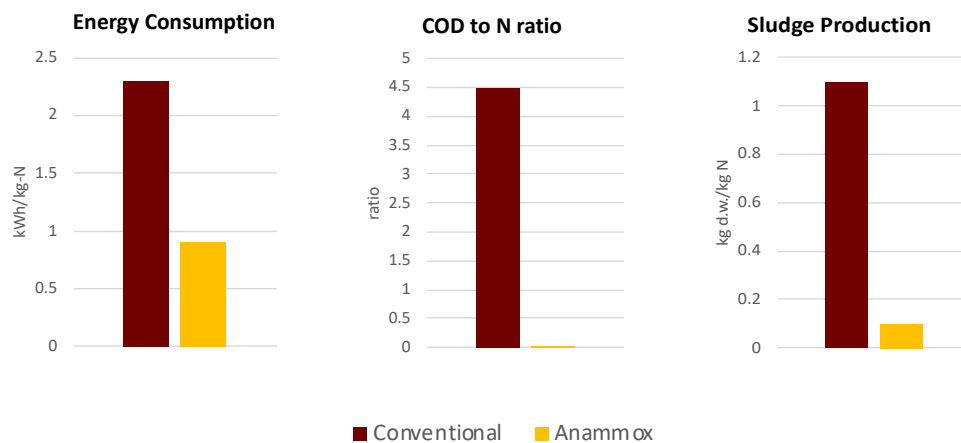
CONVENTIONAL VS. ANAMMOX PROCESS



CONVENTIONAL VS. ANAMMOX PROCESS



MORE COST EFFECTIVE PROCESS

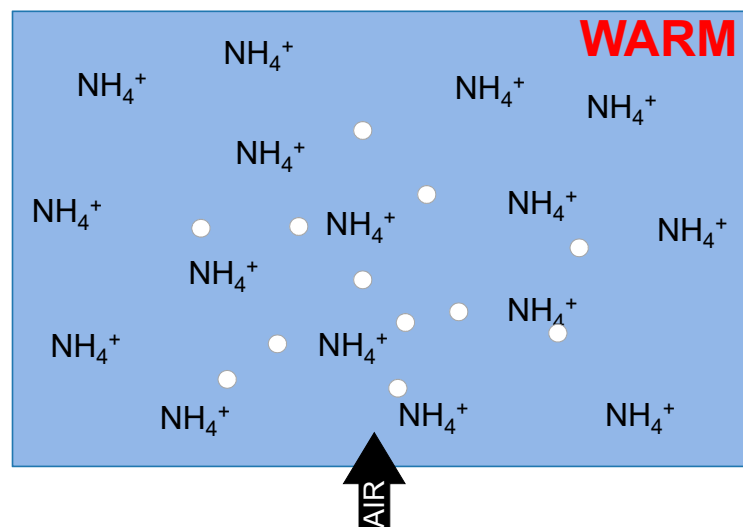


Source: Crawford and Sandino 2010

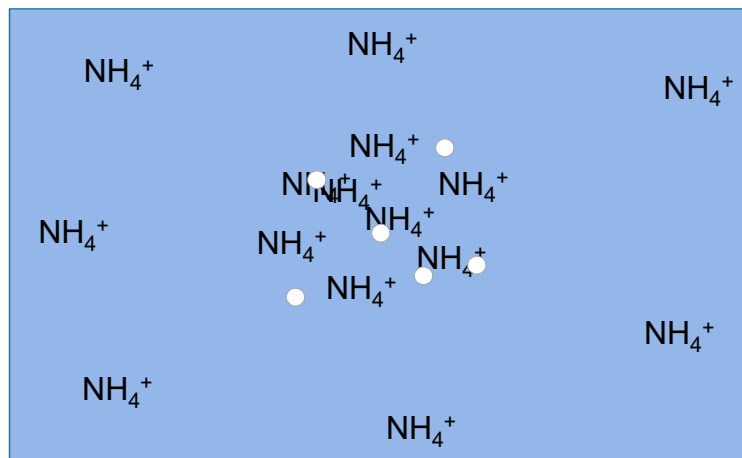
MAINSTREAM IMPLEMENTATION CHALLENGES

- Anammox microorganisms are **limited** by:
 - Slow growth rate
 - Temperatures below 30 °c
 - High carbon concentrations
 - Dissolved oxygen concentrations over 1%
- Require high levels of influent NH_4^+
- Hard to stop nitrification at NO_2^-

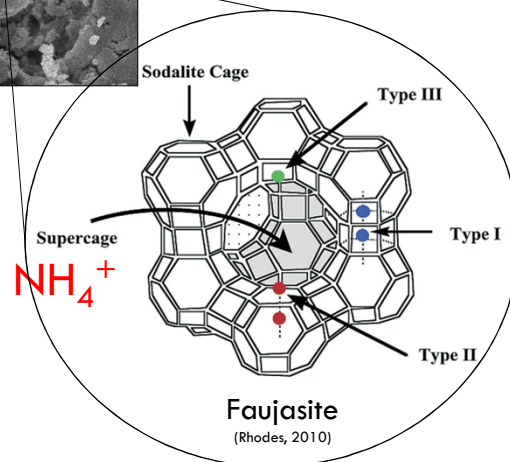
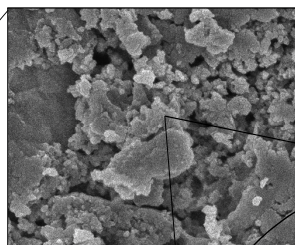
SIDESTREAM SUCCESS



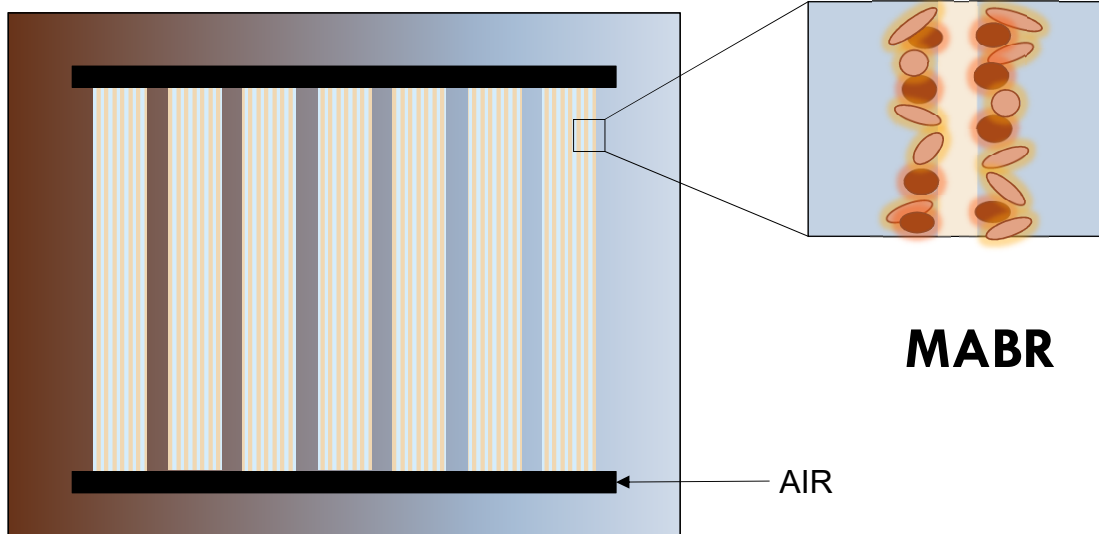
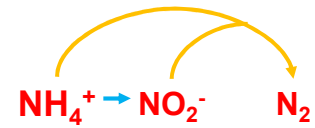
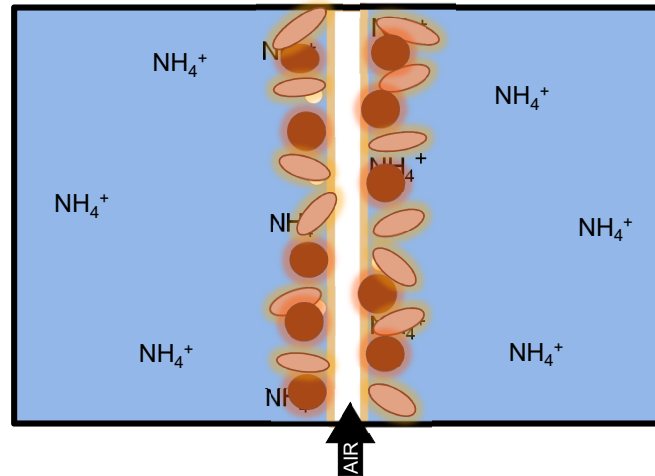
IDEAL ANAMMOX MICRO-ENVIRONMENT



ZEOLITE



ZEOLITE MEMBRANE AERATED BIOREACTOR



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

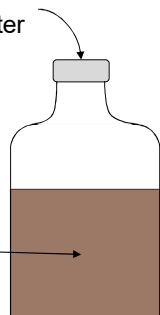
STAGE 1 – ZEOLITE OPTIMIZATION

25 mL Synthetic
Mainstream Wastewater

+

NO_2^-

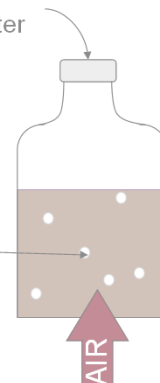
Inoculum:
Activated and
anammox sludge



STAGE 2 – OXYGEN OPTIMIZATION

25 mL Synthetic
Mainstream Wastewater

Inoculum:
Activated and
anammox sludge



STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES



2. VARYING ZEOLITE MASS



3. ZEOLITE COATED VS. UNCOATED MEMBRANES



STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES



x4 (3 g each)

2. VARYING ZEOLITE MASS



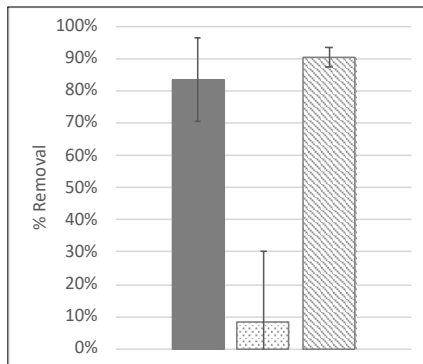
3. ZEOLITE COATED VS. UNCOATED MEMBRANES



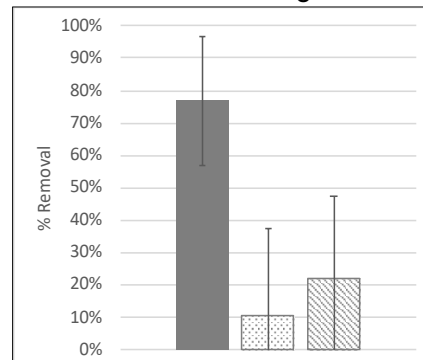
ZEOLITE VS. GLASS PARTICLES

30 Day Average Removal

Effluent Ammonia



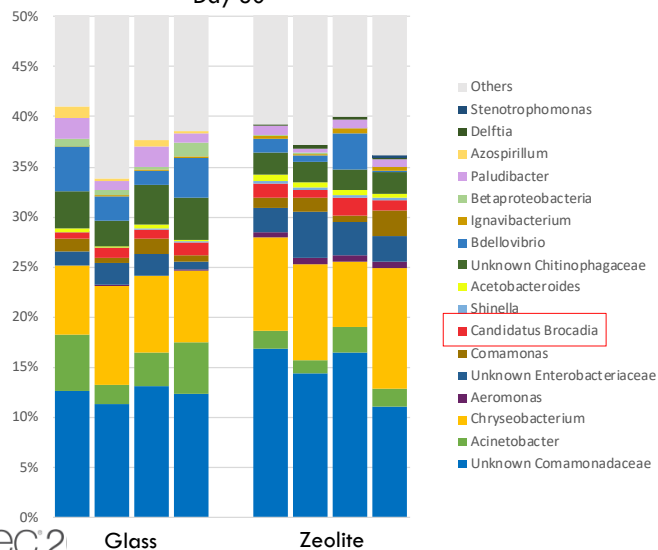
Effluent Total Nitrogen



■ Zeolite ▨ Glass ▩ Abiotic Zeolite

ZEOLITE VS. GLASS PARTICLES - SEQUENCING

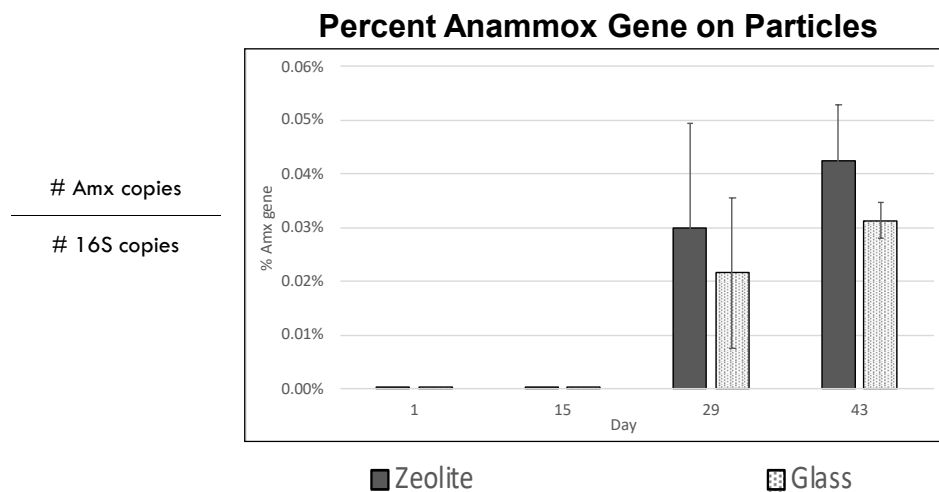
Day 30



Sequencing hits:

- *Candidatus Brocadia*
- *Candidatus Anammoximicrobium*
- *Uncultured Planctomycetes*
- *Unknown Planctomycetes*

ZEOLITE VS. GLASS PARTICLES - qPCR



STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES



2. VARYING ZEOLITE MASS



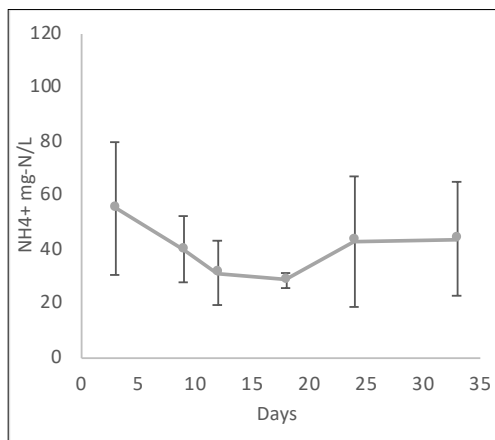
x3 (1g, 0.5g, 0.1g, 0.05g)

3. ZEOLITE COATED VS. UNCOATED MEMBRANES

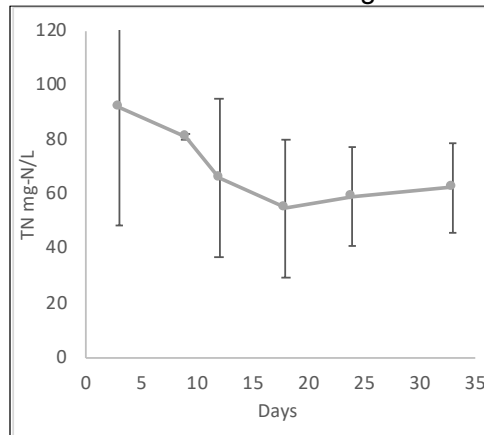


VARYING ZEOLITE MASS

Effluent Ammonia



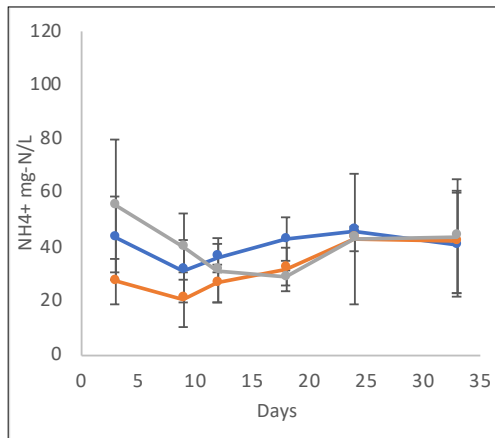
Effluent Total Nitrogen



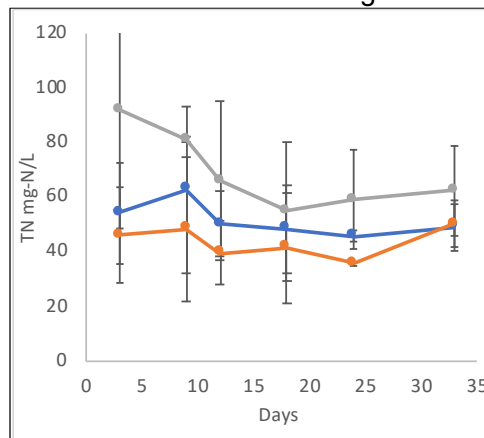
(g) — 0.05 — 0.1 — 0.5 — 1 — Control

VARYING ZEOLITE MASS

Effluent Ammonia



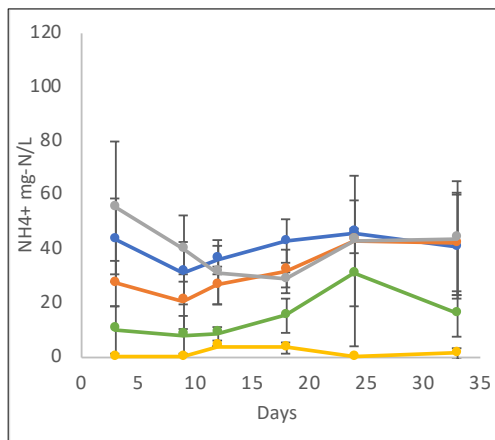
Effluent Total Nitrogen



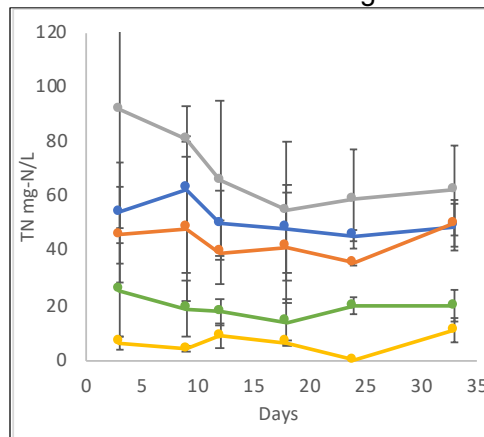
(g) — 0.05 — 0.1 — 0.5 — 1 — Control

VARYING ZEOLITE MASS

Effluent Ammonia

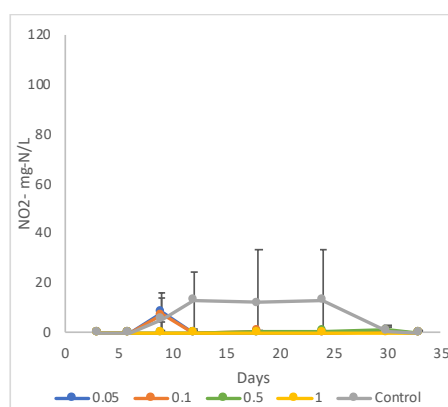
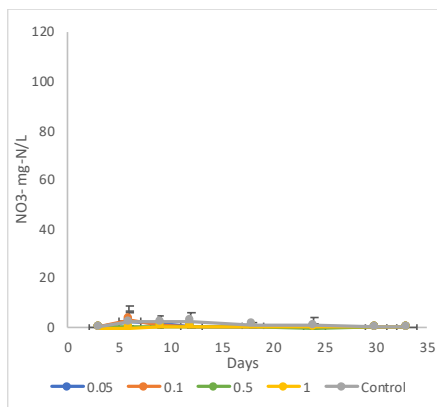


Effluent Total Nitrogen



(g) — 0.05 — 0.1 — 0.5 — 1 — Control

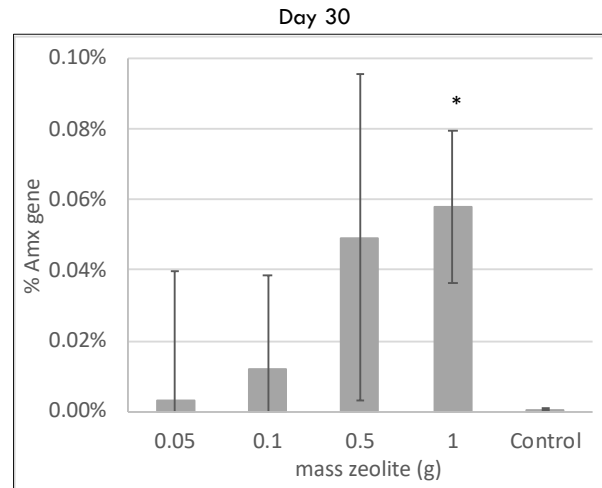
NITRITE AND NITRATE EFFLUENT



VARYING ZEOLITE MASS - qPCR

Amx copies

16S copies

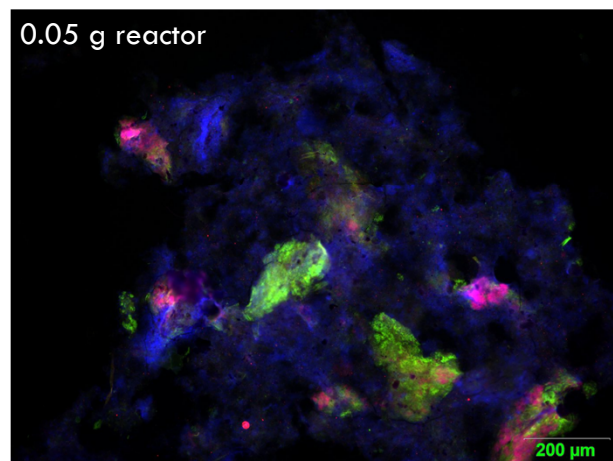
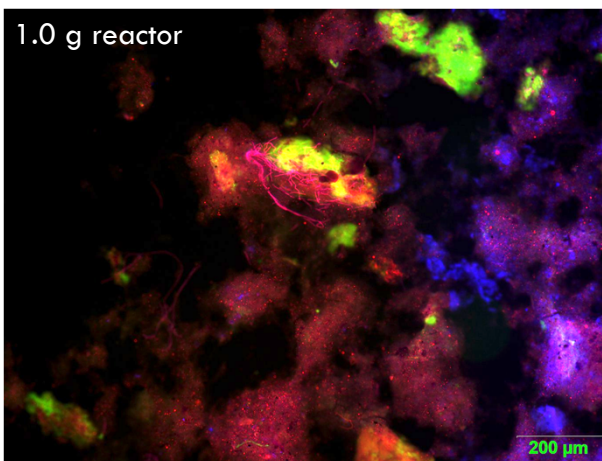


* Indicates p-value < 0.05

TECHNICAL SESSIONS

VARYING ZEOLITE MASS - FISH

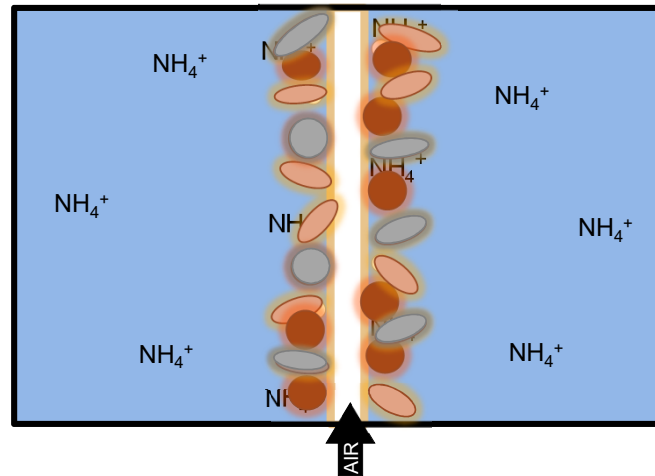
■ All bacteria
■ Anammox
■ NOB



TECHNICAL SESSIONS

ACTIVITY MEASUREMENTS

RNA ANALYSIS (RT-qPCR)



STAGE 1 – ZEOLITE OPTIMIZATION

1. ZEOLITE PARTICLES VS. GLASS PARTICLES



2. VARYING ZEOLITE MASS



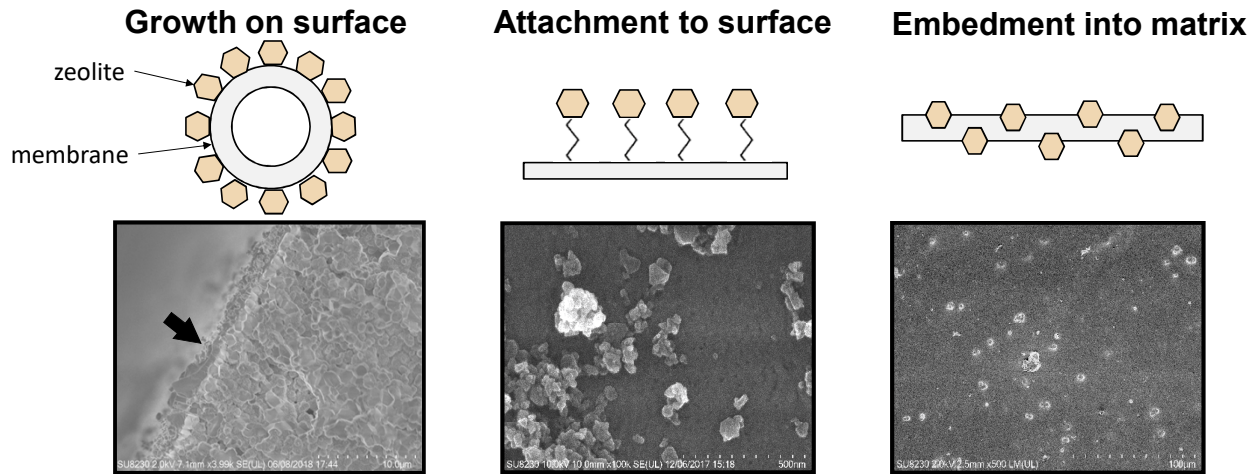
3. ZEOLITE COATED VS. UNCOATED MEMBRANES



x3

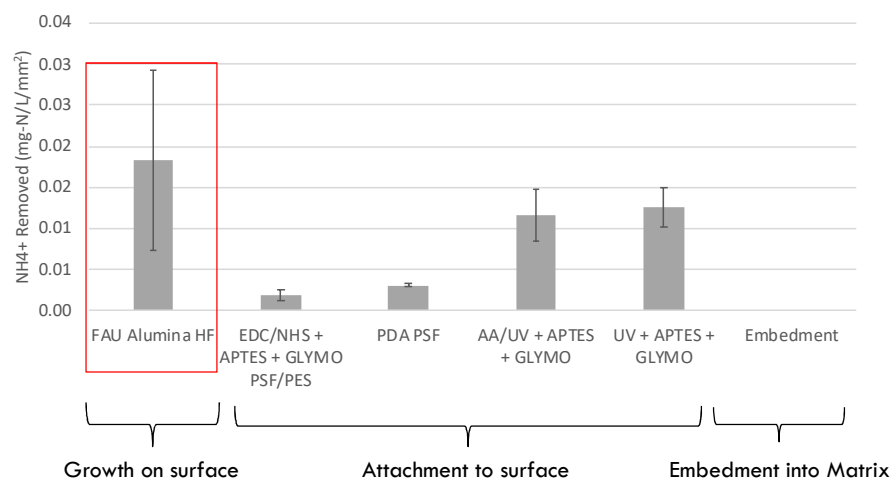
(Equivalent to 0.5 g of zeolite)

MEMBRANE TECHNOLOGY



Patent: Novak, P., Romero-Vargas Castrillón, S., Huff, A., Hillmyer, M. and Tsapatsis, M. U.S. Patent 16/433,915. 2019.

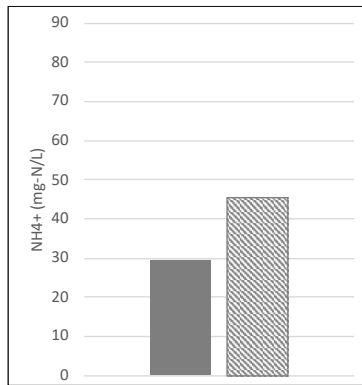
SORPTION DATA



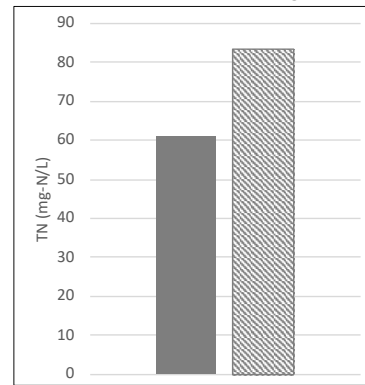
MEMBRANES – ZEOLITE VS. PLAIN

30 Day Average

Effluent Ammonia

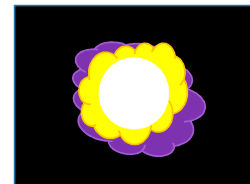
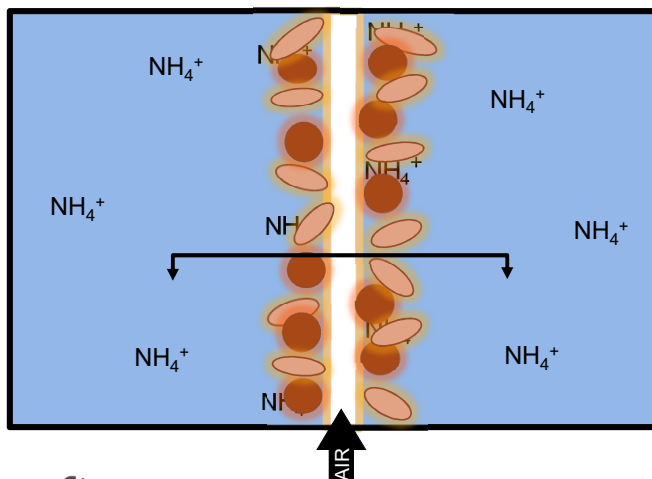


Effluent Total Nitrogen

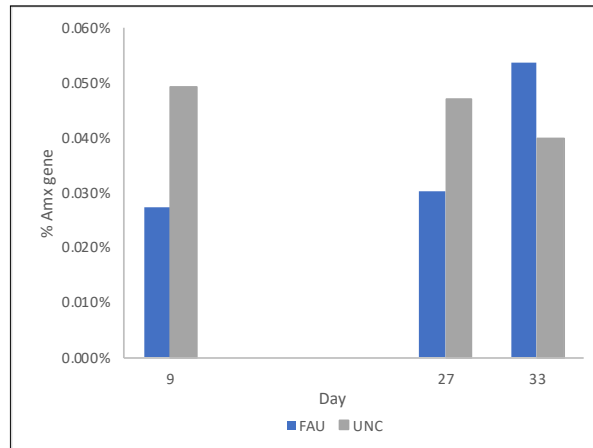


■ Zeolite ▨ Control

FISH FOR MEMBRANES



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

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GOLDSTEIN, R.; SMITH, W. WATER AND SUSTAINABILITY (VOLUME 4): U.S. ELECTRICITY CONSUMPTION FOR WATER SUPPLY AND TREATMENT – THE NEXT HALF CENTURY. ELECTRIC POWER RESEARCH INSTITUTE, INC.: PALO ALTO, CALIFORNIA, 2002.

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KUENEN, J. G. (2008). ANAMMOX BACTERIA: FROM DISCOVERY TO APPLICATION. NATURE REVIEWS MICROBIOLOGY, PUBLISHED ONLINE: 01 APRIL 2008; | DOI:10.1038/NRMICRO1857, 6(4), 320. [HTTPS://DOI.ORG/10.1038/NRMICRO1857](https://doi.org/10.1038/NRMICRO1857)

MULDER, A. (2003). THE QUEST FOR SUSTAINABLE NITROGEN REMOVAL TECHNOLOGIES. WATER SCIENCE & TECHNOLOGY, 48(1), 67–75. RETRIEVED FROM [HTTP://WST.IWAPONLINE.COM.EZP3.LIB.UMN.EDU/CONTENT/PPIWAWST/48/1/67.FULL.PDF](http://wst.iwaponline.com.ezp3.lib.umn.edu/content/ppiwawst/48/1/67.full.pdf)

US EPA, OW, O. (N.D.). THE PROBLEM. RETRIEVED NOVEMBER 6, 2018, FROM [HTTPS://WWW.EPA.GOV/NUTRIENTPOLLUTION/PROBLEM](https://www.epa.gov/nutrientpollution/problem)



TECHNICAL SESSIONS

QUESTIONS?

- Thank you to...

- Dr. Paige Novak
- Novak Lab
- Tsapatsis Lab
- Dr. Santiago Romero-Vargas Castrillón, Dr. Kiwon Eum, Dr. Michael Tsapatsis and Dr. Marc Hillmyer
- Undergrad Researchers Kalie Manke, Doug Shield, and Nikhil Khurana
- LCCMR



TECHNICAL SESSIONS