

# Why the ANAMMOXity?

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## Big Picture:

Anammox provides a cheap, less energy intensive Nitrogen removal process for wastewater treatment.  
Why remove nitrogen? Eutrophication!

## What We Did

1. Made media
2. Made standard curves for NH<sub>4</sub><sup>+</sup> and NO<sub>2</sub><sup>-</sup> and determined concentrations for standard (y=mx+b) with colorimetry.
3. Dilute influent to lower concentration to linear range.
4. Measured flow rate (Q) mL/min to determine mass removed.

All in order to treat wastewater and save energy

## Ammonium (NH<sub>4</sub><sup>+</sup>)

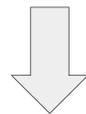
### Procedure:

- Add 25 uL of sample
- Add 175 uL of Citrate reagent
- Let sit for 1 minute
- Add 50 uL of 2-Phenylphenol-Nitroprusside reagent
- Add 25 uL of Buffered Hypochlorite reagent
- Complete these steps for each of the absorbance levels
- Put into 37 degree Celsius incubator for 15 minutes
- Measure absorbance at 660 nm
- Put waste into labeled amber bottle
- Standard curve of data is graphed below

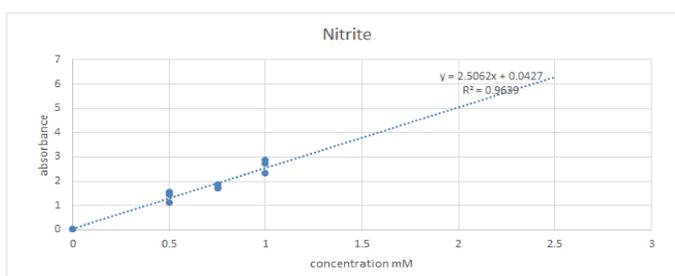
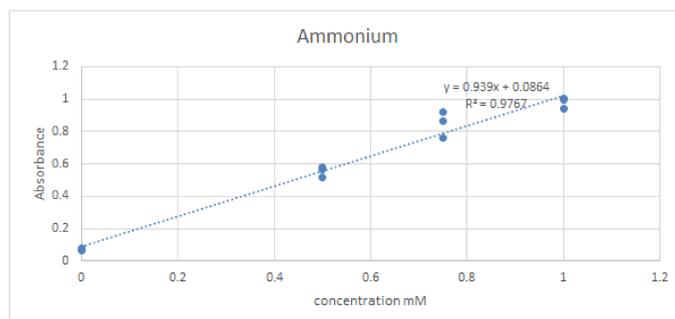
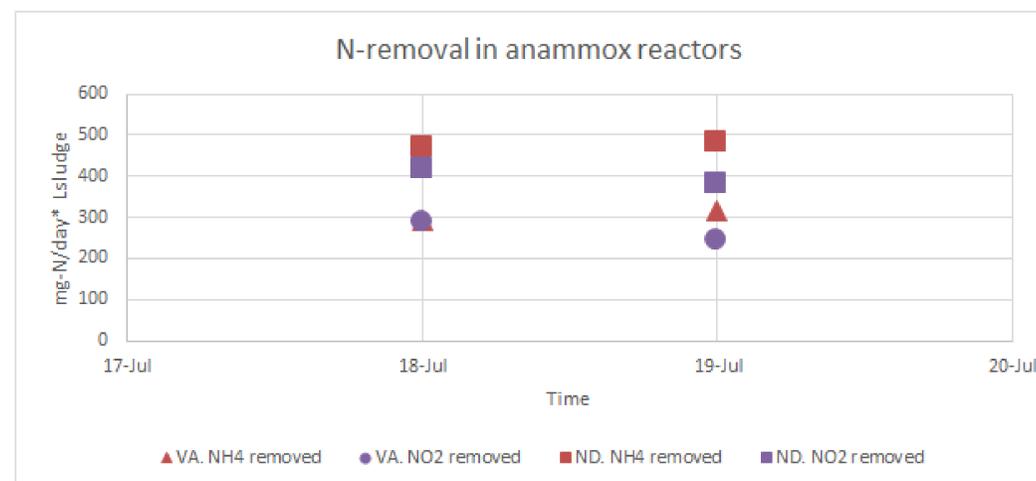
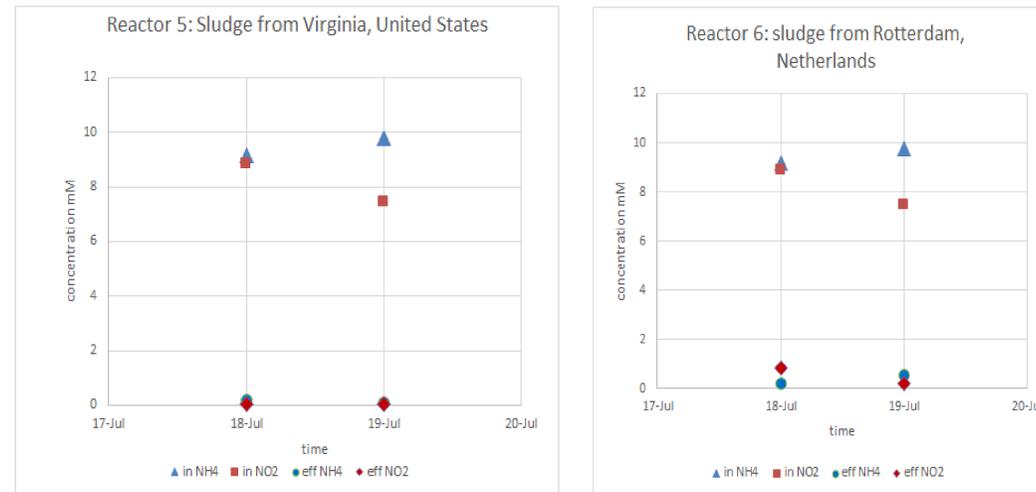
## Nitrite (NO<sub>2</sub><sup>-</sup>)

### Procedure:

- Add 200 uL of the sulfanilamide solution
- Add 20 uL of sample
- Add 20 uL of 0.1% NED reagent
- Mix by pipetting each well up and down
- Wait 10 minutes
- Measure absorbance at 540 nm
- Standard curve of data is graphed below

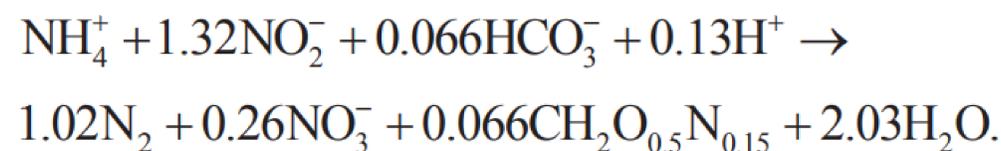


## Results:



## Conclusions:

Nitrogen removal was determined to be **571 mg-N/day\*L sludge** in the Virginia reactor and **875 mg-N/day\*L sludge** in the Netherlands reactor. These results do not account for nitrate in the mass balance as the assay uses a highly corrosive acid. However, these results are consistent with the historical observations for these reactors. From the equation below (van de Graaf et al. 1997) one can see that this represents the production of **22 mmol Nitrogen gas/day\*L sludge** in the Virginia reactor and **34 mmol Nitrogen gas/day\*L sludge** in the Netherlands reactor.



Anammox biomass equation van de Graaf et al. 1997

## Vocabulary to know

- Anammox = ANaerobic AMMonium OXidation
- Media = A liquid designed to support the growth of microorganisms or cells
- Eutrophication = excessive richness of nutrients

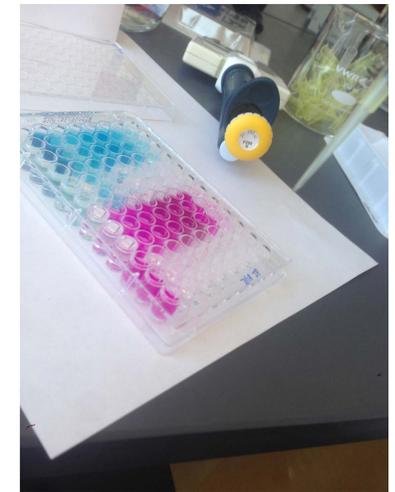
## Materials:

### Ammonium Colorimetric Assay-

- 2-Phenylphenol Tetrahydrate
- Sodium Nitroprusside
- Trisodium Citrate
- Sodium Phosphate
- Sodium Hypochlorite Solution

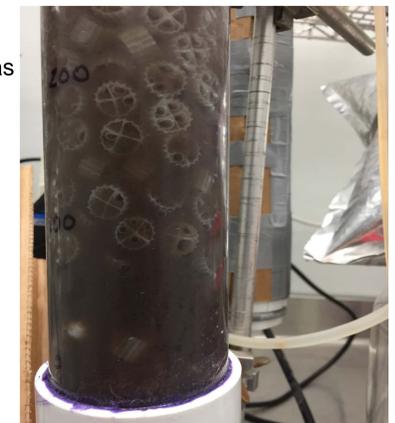
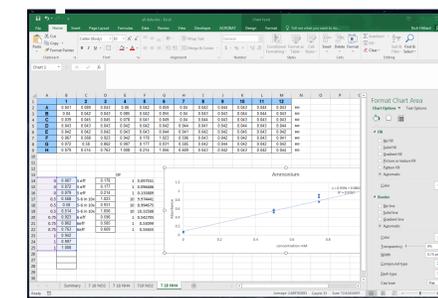
### Nitrite Colorimetric Assay-

- Sulfanilamide
- N-(1- Naphthyl) Ethylenediamine
- Dihydrochloride
- Hydrochloric acid
- NaNO<sub>2</sub>
- DI H<sub>2</sub>O



## Learning Outcomes:

To the right is some sludge where the anammox lives. Try and find the nitrogen gas bubbles!  
Below is our Excel spreadsheet where we analyzed our data.



Left to Right:  
Rich Hilliard,  
Julia Pingel,  
Deven Leon  
Patino posing  
comfortably in  
the walk in  
refrigerator  
while modeling  
proper PPE  
(personal  
protective  
equipment!).

## Acknowledgements:

We would like to thank Oregon State University and Dr. Skip Rochefort