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 NH4+ and NO2- 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 (NH₄⁺)
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 (NO₂⁻)
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
NaNO₂ 2
DI H₂O

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.

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