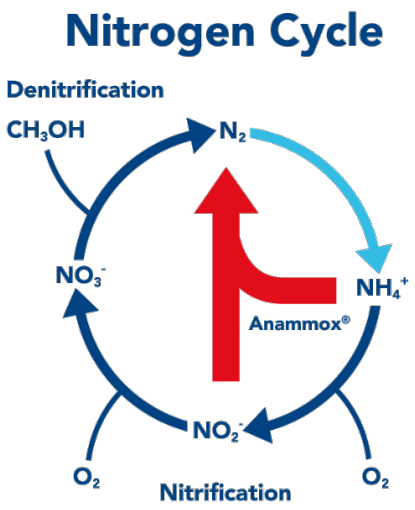
Introduction of Reseach

Prof Dr. Mari Winkler is a world expert in using anaerobic ammonium oxidizers (anammox) for removing ammonium from wastewater as nitrogen gas (deammonification). The process relies on a symbiotic relationship between ammonium oxidizers and anammox, and a current thrust in her research group is replacing ammonium oxidizing bacteria (AOB) with ammonium oxidizing archaea (AOA), and combining anammox and nitrate-dependent methane oxidizing archaea (NDAMO). Currently there are three opportunities for projects furthering this work under direct supervision of Postdoctoral Research Associate Levi Straka (Project I & II) and PhD. student Ting Xie (Project III). The first is to start up a pure culture AOA bio-reactor to study growth kinetics and cultivation of AOA. The second is to run an AOA/anammox biofilm flow cell studying the penetration depth of the organisms in a biofilm. The third is to run an Anammox/NDAMO moving bed biofilm bioreactor to evaluate its feasibility and efficiency of simultaneous ammonium and nitrate removal.

Background

Deammonification is a strategy for nitrogen removal from wastewater where ammonium oxidizers (AOB) convert ammonium (NH_4^+) to nitrite (NO_2^-; nitritation) and then anaerobic ammonium oxidizers (anammox) convert ammonium and nitrite to nitrogen gas (N_2). This process requires up to 60% less energy for aeration, produces less sludge, and emits less CO_2 and N_2O (greenhouse gases) when compared to a conventional nitrification/denitrification biological nitrogen removal system. Application in wastewater treatment is currently limited, however. Anammox bacteria must compete with nitrite oxidizing bacteria (NOB) that oxidize the nitrite to nitrate (NO_3^-), which is inaccessible to anammox. NOB activity has been successfully limited in side-stream treatment where the ammonium concentrations and temperatures are high. However, at the low temperatures and ammonium concentrations present in mainstream wastewater treatment, NOB activity cannot be limited, and anammox are outcompeted.



Previous attempts have suggested operating at low DO concentration in the mainstream to suppress NOB, but this results in poor ammonium removal because AOB have a low affinity for ammonium (0.5-0.7 mgN/L) and oxygen (0.25-0.5 mg/L). Therefore, operating at low DO means that AOB activity is limited, and anammox do not have an adequate source of nitrite. By replacing AOB with AOA, a microaerophilic organism, there is potential to drive ammonium concentrations much lower. AOA are a good fit for anammox because both organisms share remarkably high affinity for ammonium and oxygen. Anammox affinities are (0.05 mgN/L) for NH_3-N and (0.03 mg/L) for nitrite. AOA have been shown to have a remarkably low affinity for both oxygen (0.01 mg/L) and ammonium (0.001 mg/L). These affinities suggest that AOA and anammox could achieve a high removal of total nitrogen at low DO and low ammonium concentrations. AOA and anammox have been found together in the cold oxygen

minimum zones of the ocean, suggesting the potential synergism between these two organisms.

In side-stream deammonification systems, conditions favor AOB due to high levels of ammonium and oxygen, and it is our goal to understand conditions to favor AOA over AOB, and ultimately improve the deammonification system for use in mainstream wastewater treatment.

Project I: AOA Bioreactor project

Approach

We want to cultivate pure culture AOA in a sterile bioreactor with automated oxygen control, controlled ammonium input, and a membrane to control biomass retention. The reactor will be used to characterize the kinetics of the AOA strain, and used to cultivate seed cultures for AOA/Anammox systems. Target findings include:

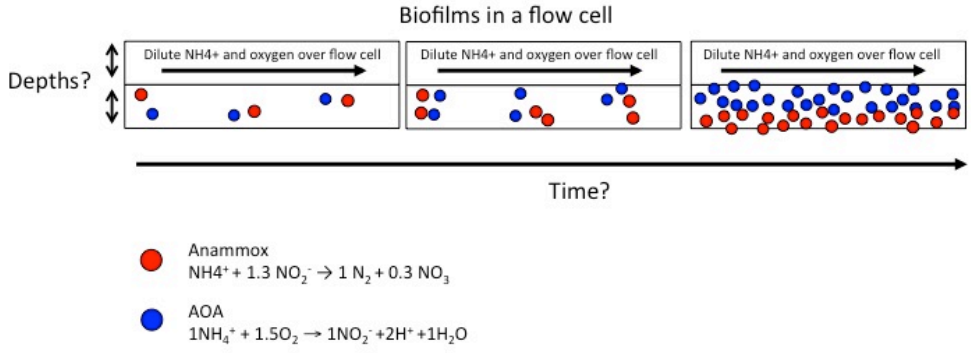
- Culture density in excess of 1 g/L
- Culture kinetic constants for maximum specific growth rate, affinity constants for oxygen and ammonium, and nitrite toxicity

Required Methods and Protocols:

- Sterile culture techniques
- Microbial community analysis using staining and microscopy.
- Colorimetric analytical methods for nitrite, ammonium, and protein analysis
- Gas chromatography for oxygen and nitrogen species analysis
- Biomass concentration
- System automation

Project II: AOA/Anammox Biofilms project

Project Title: Characterization of Archaea and Anammox in flow cells



Approach:

AOA and Anammox will be inoculated into a flow cell. After which, synthetic wastewater will flow through the flow cell, providing a food source to the cultures. This project will characterize these biofilms to determine:

- Ideal biofilm thicknesses to enable a good gas liquid transfer (better in thin biofilms) while offering different redox potentials (better in thicker biofilms)

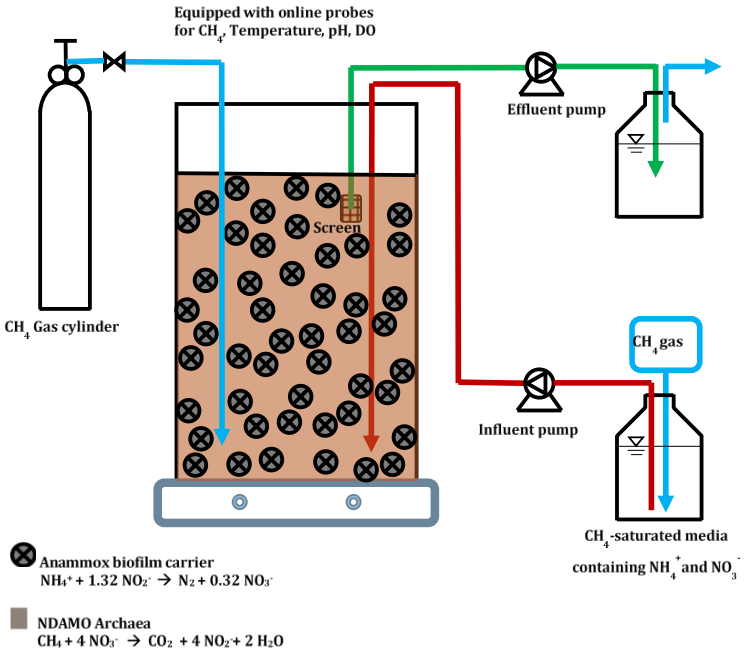
- Length of time required to develop healthy synthetic biofilms
- Nutrient Removal Capacities as a function of biofilm thickness

Required Methods and Protocols:

- Measuring metabolite concentrations of flow cell effluent via spectrophotometry, gas chromatography.
- Measuring metabolite diffusion across via microprofiling probes
- Slicing biofilms with a cryomicrotome
- Using microbial analysis techniques to evaluate species abundance and distribution across the biofilms via fluorescent in situ hybridization (FISH)
- Other imaging techniques such as introduction of fluorescent lectins into the culture
- Flow cell inoculation techniques
- Flow cell operation & monitoring protocols

Project III: NDAMO/Anammox Project

Project Title: Simultaneous Ammonium and Nitrate Removal by Coupling Anammox and Nitrate-Dependent Anaerobic Methane Oxidation (NDAMO) Process in a Moving Bed Biofilm Bioreactor



Motivation:

Nitrate-Dependent Anaerobic Methane Oxidizing archaea (NDAMO) has been recently discovered with ability to utilize methane as an electron donor and nitrate as acceptor to generate nitrite. The resulting nitrite is then accessible to the Anammox, to produce N_2 gas which will escape the reactor leaving low nitrogen effluent. Successful implementation of this process in mainstream wastewater treatment would improve

water quality and bypass the problem of nitrification at cold temperatures, while simultaneously reduce methane emissions, a potent greenhouse gas.

This process has already been applied to wastewater treatment research, and anammox and NDAMO have been shown to grow together in sediments, freshwater, and groundwater. However, the ideal operating conditions of a NDAMO/anammox reactor for optimum nitrogen removal have yet to be shown in practice.

Objective:

This research will tune operation and monitor nitrogen removal efficiency of a NDAMO/anammox bioreactor at lab scale (3L). This will provide key insight into the implementation of anammox and DAMO in full scale wastewater treatment systems.

Approach:

A bioreactor inoculated with healthy anammox biofilm carriers and suspended NDAMO culture. Synthetic wastewater will be supplied to the reactor as influent, and pH will be controlled online. The reactor will then be operated anaerobically and will be monitored to evaluate:

- Nitrogen removal efficiency of the system as with varied control parameters (i.e. Metabolite influent concentrations? Temperature?)
- Microbial ecology community dynamics of these cultures in a built environment over time (i.e. will NDAMO be incorporated into the biofilm carriers over time?)

Required Methods & Protocols:

1. Sterile anaerobic culture handling
2. Measuring metabolite concentrations of bioreactor effluent via spectrophotometry and gas chromatography.
3. Bioreactor sample collection protocols
4. VSS Determination for biomass activity to evaluate nitrogen removal efficiency
5. Microbial analysis techniques to evaluate species abundance and distribution within the bioreactors via fluorescent in situ hybridization (FISH) and quantitative polymerase chain reaction (qPCR)

General matter:

No funds will be available for salary or living expenses. University of Washington ranks 14th among worlds universities. The Mercer Quality of Living Survey 2015 ranked Seattle on place 44 in the world.

Contact:

Mari Winkler
assistant Professor
University of Washington, Civil and Environmental Engineering
Email: mwinkler@uw.edu
www.winklerlab.com