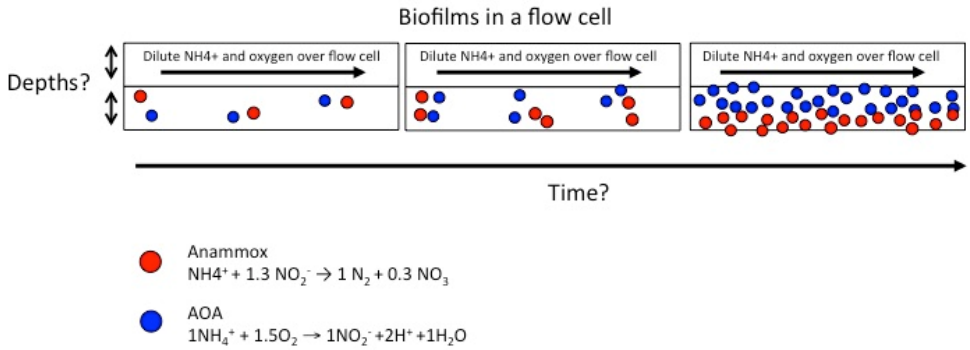
Contact: Prof. Dr. Mari Winkler, Civil and Environmental Engineering, University of Washington, Seattle, USA, Email: mwinkler@uw.edu

Project I Shorthand Name: Biofilms project

Project Title: Characterization of Archaea and Anammox in flow cells



Motivation:

Anammox granular sludge is a major improvement to nitrogen removal within wastewater treatment plants. Anammox take up ammonium and nitrite in a 1:1.3 molar ratio and produce N_2 gas. Anammox granular technology requires 60% less energy for aeration, produces less sludge, and emits less CO_2 and N_2O when compared to a conventional denitrification system. In addition, they can form dense 1-3mm diameter granules (biofilms), which separate easily from flocs, allowing reactors with smaller footprints. However, application of anammox in wastewater treatment is currently limited. They depend on ammonium oxidizing bacteria (AOB) to supply them with nitrite and the anammox must compete with nitrite oxidizing bacteria (NOB), which oxidize the nitrite to nitrate, which inaccessible Anammox under autotrophic conditions. NOB activity has been successfully limited in sidestream 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, 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. This research will explore the use of ammonium oxidizing archaea (AOA), a microaerophilic organism, to supply nitrite to anammox instead of AOB within a biofilm structure. 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 ammonia (0.001 mg/L). These affinities suggest that AOA and anammox would have a would allow for high removal 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. However, these organisms have not yet been shown to grow in a biofilm together, which is essential to the implementation of AOA to anammox granular sludge. This research will characterize the growth of these organisms over time and the penetration depth of these organisms and their metabolites within a flat biofilm in a flow cell. This will provide key insight into the implementation of anammox and AOA into granular sludge for mainstream wastewater treatment.

Approach:

These cultures 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:

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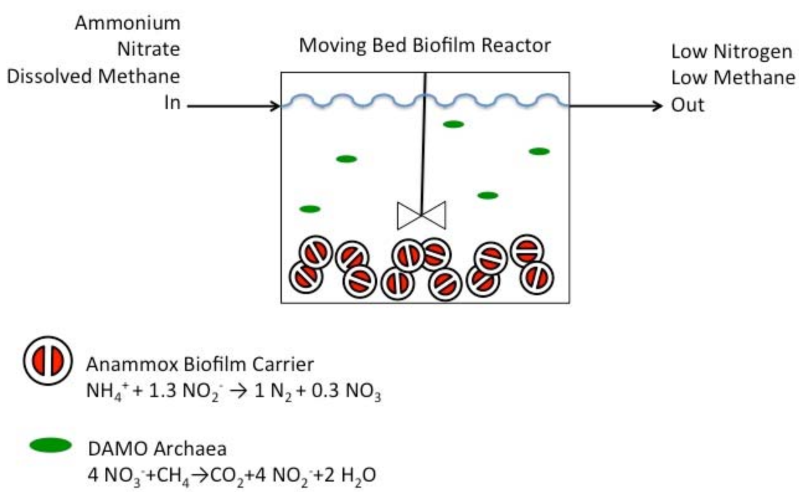
- Ideal biofilm thicknesses to enable a good gas liquid transfer (better in thin biofilms) while offering different redox potentials (better in thicker biofilms)
- Length of time required to develop healthy synthetic biofilms
- Nutrient Removal Capacities as a function of biofilm thickness

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 II: Shorthand Name: NDAMO/Anammox Project

Project Title: Evaluating Nitrogen Removal Efficiencies of Anammox and DAMO Archaea Co-culture in a Moving Bed Biofilm Bioreactor



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nitrate, which inaccessible Anammox under autotrophic conditions. NOB activity has been successfully limited in sidestream 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.

Recently, an organism that can obviate the need to minimize NOB activity has been discovered. Denitrifying anaerobic methane oxidizing archaea (DAMO) are unique because of their ability to utilize methane as an electron donor and nitrate as acceptor to generate nitrate 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 while simultaneously reduce methane emissions, a potent greenhouse gas.

This process has already been applied to wastewater treatment research, and anammox and DAMO have been shown to grow together in sediments, freshwater, and groundwater. However, the ideal operating conditions of a DAMO/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 DAMO/anammox bioreactor at lab scale (2L). 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 DAMO 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 with control parameters (i.e. Metabolite influent concentrations? Temperature?)
- Microbial ecology community dynamics of these cultures in a built environment over time (i.e. will DAMO 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 Setup, Inoculation, and Operation Protocols
4. Bioreactor sample collection protocols
5. VSS Determination for biomass activity to evaluate nitrogen removal efficiency
6. 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 rakes 14th in ranking among worlds universities. The Mercer Quality of Living Survey 2015 ranked Seattle on place 44 in the world.