Nutrient Management: Heterotrophic Nitrification

Team Members: Zac McClellan, Jaden Storrer, Anna Scribner, Jayce Bradley
Project Sponsor: Kirsten Sims – WesTech
Academic Mentor: Dr. Ronald Sims

Background

The removal of nitrogen in the form of ammonia from wastewater is an important yet expensive step in treatment often done in two stages, typically by different autotrophic nitrifiers:

- Nitrification: Ammonia is converted to Nitrite and Nitrate.
- Denitrification: Nitrate is converted to nitrogen gas (N2)

Heterotrophic nitrifiers like *Alcaligenes faecalis* are able to perform both processes and reduce the two step treatment process to one.

Previously, experiments were performed on *Alcaligenes faecalis* in a synthetic wastewater environment to determine the kinetic constants for a substrate-dependent ammonia removal rate (Michaelis Mentin Kinetics).

K_M = 2.09 mg/L V_{MAX} = 0.088 mg/L-min

Growth Method Development

The ammonia removal performance of *A. faecalis* in exponential phase was compared between three growth methods using sythetic wastewater media: suspension culture, rope substrate, and MBBR substrate. The various substrates were used to test for possible biofilm developments.

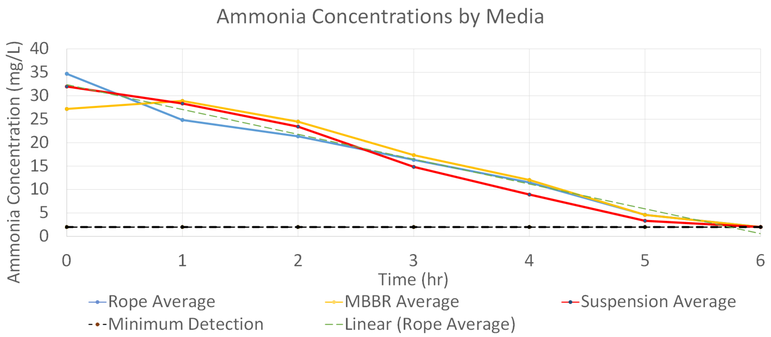

Figure 1. Results from the growth method experiment.

Biofilm Growth Test

A three-week growth test was run to determine if *Alcaligenes faecalis* is capable of forming a viable biofilm on MBBR (Moving Bed Bioreactor) plastic media and test the ability of *A. faecalis* to perform in a competitive community environment. Cultures were grown in both synthetic and real wastewater.
The MBBR was tested for biofilm presence at the end of the three-week growth period.

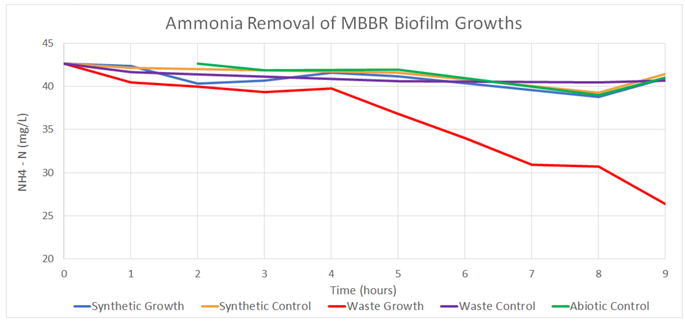

Figure 2. Ammonia removal for various biofilm growths; only the wastewater growth samples showed consistent ammonia removal within 9 hours.

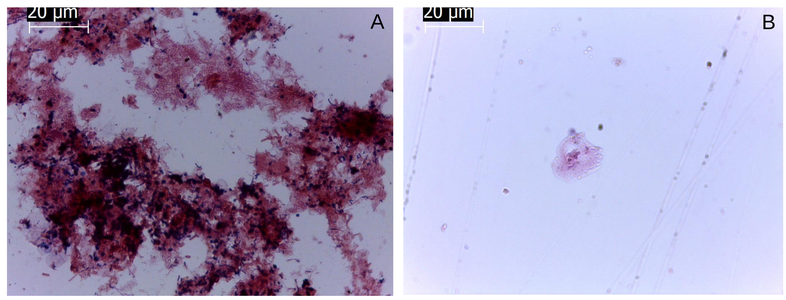

Figure 3. Microscopy of gram-stained scrapings taken from MBBR pieces. *A)* Wastewater growth sample; biofilm-evident structure implies symbiotic relationship between *A. faecalis* (gram-negative) and an organism present in the wastewater sample.
B) Synthetic growth sample; some structures are present.

Growth Flasks with MBBR Media

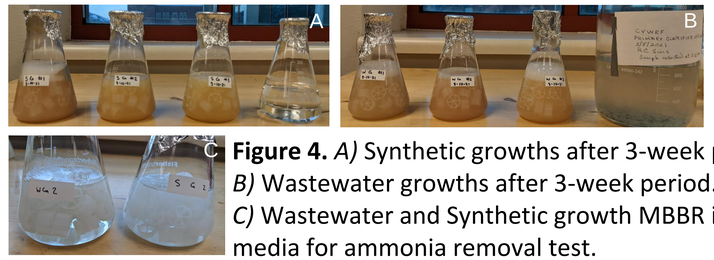

Figure 4. *A)* Synthetic growths after 3-week period.
B) Wastewater growths after 3-week period.
C) Wastewater and Synthetic growth MBBR in new media for ammonia removal test.

Notable Results

- *A. faecalis* can take up ammonia, removing it from solution, at a zero order reaction rate for typical ammonia levels in wastewater (>5 mg/L NH4-N). This is supported by the low K_M value of 2.09 mg/L found by a previous senior design group; for most of the NH4-N concentration range, the bacteria are performing at V_{MAX}.
- Although it is unclear whether or not *A. faecalis* is able to grow a biofilm on its own, preliminary results seem to suggest that it can form a symbiotic relationship with other organisms found in wastewater in order to grow a biofilm (see figure 3A).

Future Work

- More experimentation on biofilm capabilities could be done to find if *A. faecalis* is able to form a biofilm on its own, given time and ideal conditions for biofilm formation.
- Research to better understand what temperature ranges are optimal for *A. faecalis'* removal of ammonia.
- Experimentation to determine the kinetic constants for ammonia removal by *A. faecalis* in wastewater, to compare to previous work done in synthetic wastewater.

National IBE Conference | Apr 9-10, 2021
Biological Engineering Department
College of Engineering, Utah State University
engineering.usu.edu

WesTech®

Utah State University
BIOLOGICAL ENGINEERING

College of Engineering
Utah State University