Quantifying methanogens in an anaerobic baffled reactor

Energy and resource recovery

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Re-Inventing the Nation's Urban Water Infrastructure (ReNUWIt)

Background

A variety of different microbes can aid in wastewater treatment. Determining which microbes are performing specific functions can provide insight into treatment performance. The process of quantitative polymerase chain reaction (q-PCR) amplifies targeted DNA sequences to identify what microbes are present. At the Mines Park Testbed, an anaerobic baffled reactor (ABR)) – anaerobic fixed film reactor (AFFR) system treats wastewater from student dorms. Figure 1 shows a schematic of the ABR-AFFR system at Colorado School of Mines.

Goals:

- Quantify methanogens in different ABR compartments over time
- Compare absolute methanogen concentration to relative abundance determined by 16S rRNA gene sequencing
- Relate methanogen quantity to reactor performance

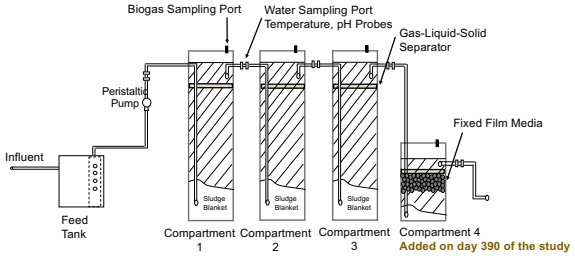

Figure 1: Mines Park ABR

Approach

Step 1: Sludge samples were collected from compartments over time and stored in a freezer. Three replicate samples taken from sludge in reactor compartment 1 on 2/13/18 (day 812 of reactor operation) were analyzed. The sludge in compartment 1 is consistently around 30 inches deep.

Step 2: DNA was extracted from sludge samples using DNeasy PowerLyzer PowerSoil Kit. DNA concentrations were determined with a Qubit fluorometer.

Step 3: q-PCR reaction mix: Making the reaction mix for q-PCR involved mixing the ingredients in specific amounts (Table 1).

Table 1: q-PCR Reaction Mix Composition

Component	Volume (µL)
Extracted DNA	2
mcrA primer left	1
mcrA primer right	1
Nuclease-free water	7
q-PCR master mix	10

Step 4: q-PCR reaction: All q-PCR analyses were performed on a LightCycler 480 II instrument (Roche Applied Sciences). The q-PCR reaction was conducted using the following temperature cycling: denature 95˚C for 3.5 min.; 45 cycles of denature 95˚C for 30s, anneal 55˚C for 45s, extend 72˚C for 60s; final extension 72˚C for 7 min.

Results

Table 2: DNA Concentrations Extracted from Sludge

Sample Number	DNA Concentration (ng/mL)
1	5600
2	1790
3	1420

- Different concentrations of DNA extracted from replicate sludge samples (collected from same compartment on same day), which suggests lack of homogenization of sludge

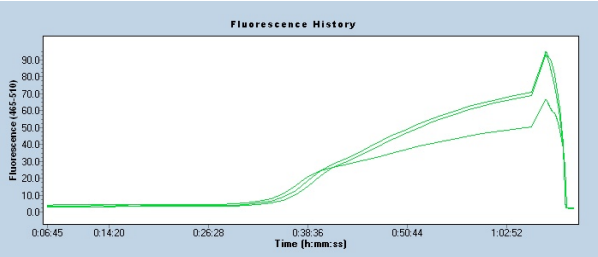

Figure 2: Sample 2 Results

- Sample replicates behaved similarly
- Three reactions nearly mirror each other with similar cycle threshold (Ct)

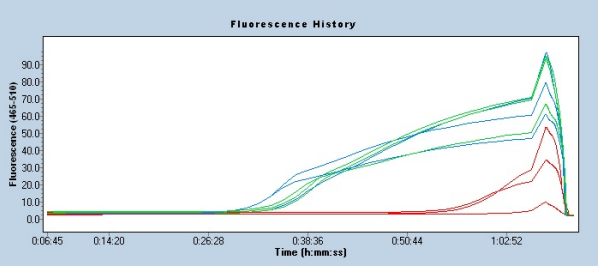

Figure 3: Measured Fluorescence of Mines Park Samples

- Sample replicates behaved similarly
- $Ct_1 < Ct_2 < Ct_3$, suggesting that sample 1 has the highest concentration of methanogens and sample 3 has the lowest, perhaps reflecting extracted DNA concentration

Conclusions

I can successfully perform q-PCR with good within-sample replicability to quantify methanogens. While limited samples have been analyzed to date, the results show that we can distinguish concentrations of mcrA in different samples. The results also suggest a correlation between mcrA concentration and DNA concentration.

Take Away Points:

- $Ct_1 > Ct_2 > Ct_3$
- Correlation between overall DNA and methanogen concentration

Next Steps

The analyses done so far are only the preliminary steps to the overall research project. Quantifying the methanogens in different samples to determine how much are present on a relative basis is a good start to correlating methanogen concentration with reactor performance. In the long run, absolute quantification in each basin will ultimately determine the true relationship between methanogens and reactor performance.

Next Steps:

- Develop standards for quantification
- Identify key samples for analysis
- Run absolute quantification
- Determine correlation between methanogens and reactor performance
- Do similar process for acetogens in sludge basins

Acknowledgements

I would like to thank my mentors Junko Munakata-Marr, Gary Vanzin, and Carolyn Coffey. They have really made my current research enjoyable. I look forward to doing lots of research this summer as I will continue working in the reactor lab under the direction of Junko.

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Supported by the National Science Foundation at: