Assessing the Effects of Antibiotic Loading on Bacterial Communities in AnMBRs

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Introduction and Objectives

Antibiotic resistance is a threat to public health. Wastewater treatment plants receive waste with considerable amounts of antibiotics, which could contribute to the selection and proliferation of antibiotic resistant bacteria in the environment.

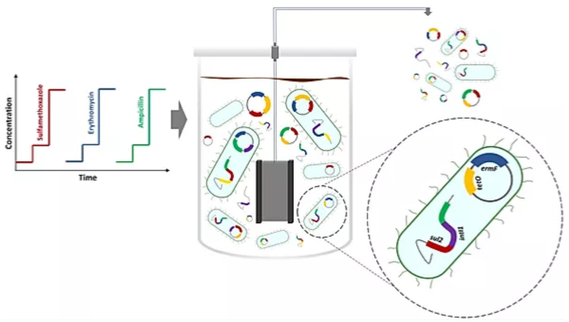

Figure 1. AnMBR treating synthetic wastewater with antibiotics[1].

Understanding the makeup of wastewater microbial communities could help assess the risks of AnMBRs as a potential biotechnology for mitigating antibiotic resistance propagation.

Detail of Research

An AnMBR was operated under two conditions: one with only synthetic wastewater and another with synthetic wastewater with antibiotics. DNA extracts were taken under both conditions from the biosolid and effluent. The v4 region of the 16S rRNA gene were amplified using polymerase chain reaction. Illumina MiSeq[2] (Figure 2) was used to sequence the amplicons. A bioinformatics tool, mothur, was used to process the sequencing data.

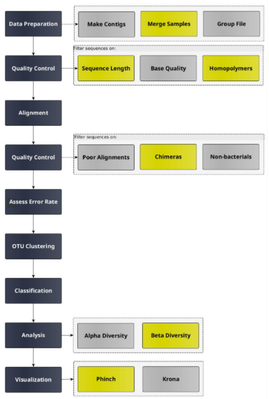

Figure 2. Illumina MiSeq SOP flowchart[3].

Results

The effluent samples were better represented and had less OTUs than biosolid samples (Figure 2).

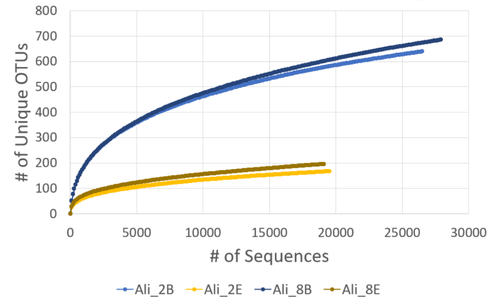

Figure 2. Rarefaction curve[4].

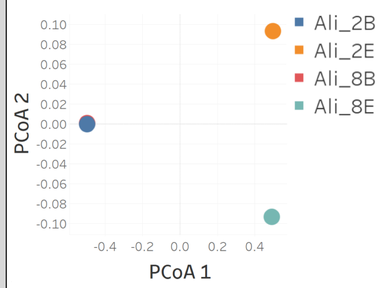

Figure 3. Principal coordinate analysis plot[4].

Distinct microbial communities were visible between the biosolid and effluent. Upon antibiotic loading, the biosolid samples remained similar to each other whereas the effluent samples showed significant divergence (Figure 3).

Phyla (Figure 4)
- Biosolid had more unclassified bacteria and greater abundance of minor phyla than effluent
- Upon antibiotic loading
  - Increase in lower abundant phyla for biosolid and effluent
  - Decrease in proteobacteria in effluent

Genera (Figure 5)
- Biosolid showed greater diversity and presence of less abundant genera than the effluent
- Upon antibiotic loading
  - biosolid remained unchanged
  - increase in unclassified bacteria in effluent

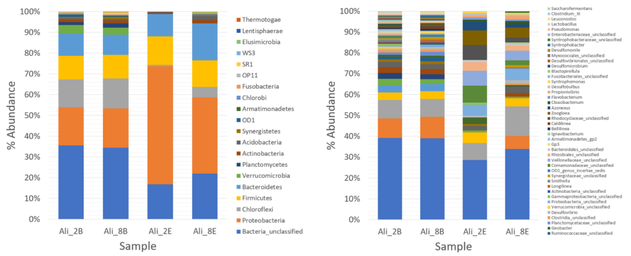

Figure 4. Relative abundance graph by phyla[4].

Figure 5. Relative abundance graph by genera[4].

Skills Learned

- Process data with mothur (Figure 6)
- Visualize data with Excel and Tableau
- Illumina MiSeq
- Python libraries (scipy, numpy, pandas)
- Read and analyze research papers
- Lab safety

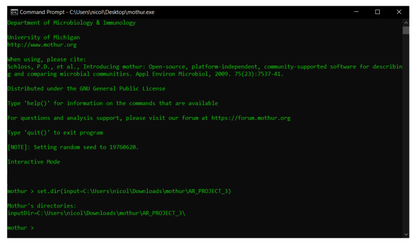

Figure 6. Instance of mothur[4].

How This Relates to My STEM Coursework and Next Steps

Going into the SHINE program, I already knew I wanted to go into a field related to biology as it was my favorite subject and something that really interested me. I hadn't really thought much about the environmental aspect of engineering but after learning more in depth about some of the problems we have in society pertaining to water, I grew fascinated with it. Not only was it something that appealed to me, but I realized how closely it aligned with my interests in biology. Now, when I think about what I want to do in the future, environmental engineering is now something I consider.

Advice for Future SHINE Students

- Try to take away as much as you can. Your mentors are very knowledgeable and have so much to teach, so absorb as much information as possible.
- Have fun! You will be in a lab with a professor, PhD students, and post-docs so it may feel overwhelming. Remember, you are a high school student! Just enjoy the experience!

Figure 7. Full-scale AnMBR in wastewater treatment plant[5].

Acknowledgements

I would like to thank Bianca Costa and Phillip Wang for being awesome mentors, Professor Smith and Dr. Mills for providing me with this amazing opportunity, and Joseph Chen, my lab partner, as well as the rest of the SHINE cohort for making these past seven weeks so memorable.

Citations

[1]Zarei-Baygi, A., Harb, M., Wang, P., Stadler, L., Smith, A. (2019). Evaluating Antibiotic Resistance Gene Correlations with Antibiotic Exposure Conditions in Anaerobic Membrane Bioreactors. *ACS Publications*. https://pubs.acs.org/doi/10.1021/acs.est.9b00798

[2]Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. (2013): Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology. 79(17):5112-20.

[3]Saskia Hiltemann, Bérénice Batut, Dave Clements, 2020 16S Microbial Analysis with mothur (extended) (Galaxy Training Materials). /training-material/topics/metagenomics/tutorials/mothur-miseq-sop/tutorial.html Online; accessed Sun Jul 26 2020.

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