# Examining the Impact of Co-digestion on Antibiotic Resistance Genes during Anaerobic Digestion of Wastewater Treatment Plant Sludge

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## Introduction

Wastewater management is a field in rapid evolution. Issues such as the ecological impacts of effluent water, as well as the increasing amount of antibiotic resistant bacteria (ARB) produced, have caused growing concerns about the impacts of sewage treatment (Rizzo 2013). The Smith Research Group seeks to not only mitigate some of the problems with treatment, but to turn the process into one that benefits our environment, through use of the biogas producing anaerobic digestor.

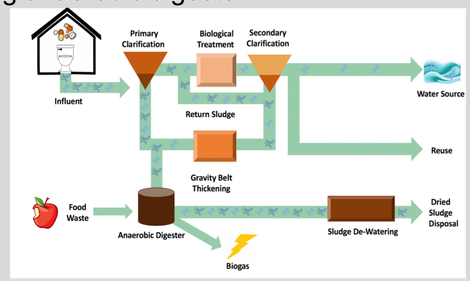


**Figure 1. Wastewater treatment with co-digestion of FW and sludge**

## Research Project

My experiment focused on the potential of co digestion to reduce proliferation ARB in wastewater management. Wastewater treatment plants (WWTP) are a potential hotspot for ARB, due to the large amount of antibiotics in influent wastewater.

**Figure 2. Respirometer**

We hypothesize that the co-digestion of food waste and WWTP sludge, will reduce risk of ARB dispersal, by increasing microbial diversity.

## Methods

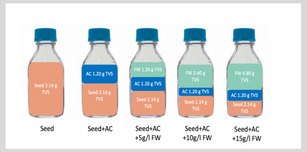


**Figure 3. Batch reactor setup**

Five samples with varying compositions of anaerobic sludge (seed), activated sludge (AC), and food waste (FW) were run in a respirometer for 4 weeks. Every sample, except for the control, was run in duplicate. Gas chromatography was used to monitor the methane levels in the biogas. DNA was extracted from biomass samples were taken from each reactor at the beginning and end of run. The ARGs *Sul1* was analyzed using quantitative polymerase chain reaction (q-PCR).

## Performance Results

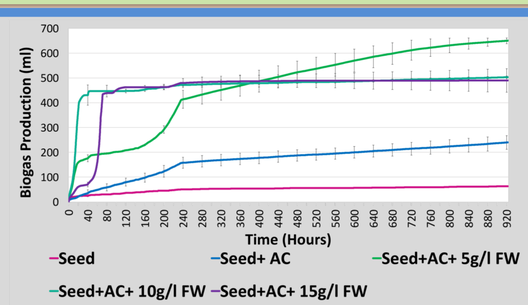


**Figure 4. Biogas production**

Co-digestion of FW increased biogas production. However, the highest performance was not observed in the feeding condition with the highest organic loading rate (OLR). This could be due to inhibition of microbial communities at high OLRs.

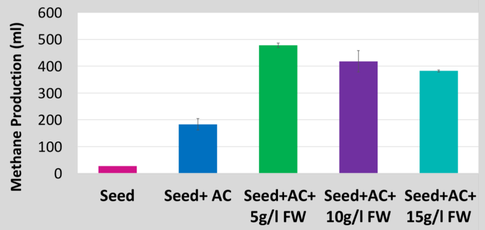


**Figure 5. Final methane production**

## ARG Results

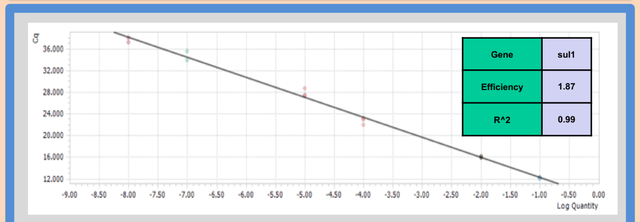


**Figure 6. Standard curve for qPCR**

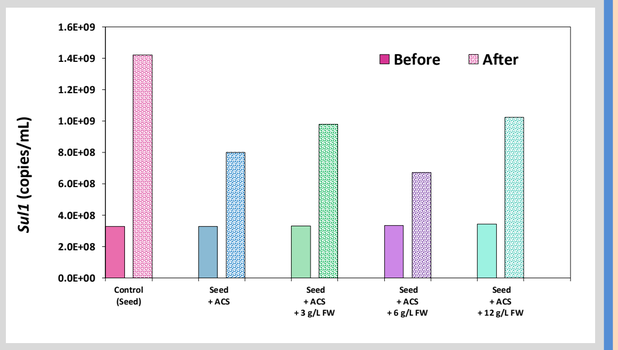


**Figure 7. ARG Sul1 comparative results**

As expected, copies of the ARG *Sul1* grew rapidly during the experiment in all batch reactors due to the horizontal gene transfer (HGT). Co-digestion with FW significantly decreased the growth of *Sul1* gene, showing FW to have an inhibitory effect on spread of ARGs through HGT. However, different OLR of FW didn't significantly change the effect of co-digestion on ARGs. Further research such as DNA sequencing could elucidate the reason for this.

## Improvements

- Running each feeding vessel in triplicate would lead to increased accuracy
- Lower overall production of methane compared to theoretical calculations points to a potential inaccuracy with the respirometer, thus a recalibration may be needed
- The gas chromatography columns need to be replaced due to aging, which reduced the accuracy of results. This analysis should be repeated for future work
- More ARGs should be targeted in future work

## Skills Learned

- Chemical Oxygen Demand (COD)
- DNA extraction
- q-PCR
- Reactor Maintenance
- Gas Chromatography
- Scholarly Literature
- MATLAB
- Lab Safety
- Troubleshooting
- Ammonia testing
- TSS/ TVS testing
- Communication of Results

## Advice to Future SHINE Students

- **Learn from Your Mistakes,** There will be times when you mess up, but that's okay! Think about why it happened and try to prevent it from happening again.
- **Be Efficient,** Lab work can be repetitive. Learn to optimize experiments to save time and increase the quality of the results.
- **Don't Be Intimidated,** Your lab will be working with complex science, but don't be scared! They didn't bring you in expecting a PhD student. Chances are you'll be working a in specific area of knowledge. Focus your attention on learning those particular concepts.
- **Learn from the PhD students,** The people working in your lab are the world's experts on their specific research project. Ask questions and learn what you can from them!

## Acknowledgements

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## References

Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dago, C., Ploy, M.C., & Michael, I. (2013).Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment:A review.*Science of the Total Environment.*