AN ABSTRACT OF THE THESIS OF

Ana A. Aranda for the degree of Baccalaureate of Science in BioResource Research presented on May 29th, 2019. Title: Biogas inhibition of Fats, Oils, and Greases with varying ammonia levels.

Abstract Approved: ____

Tyler Radniecki

Biogas can be used to generate energy from a wide variety of sources. Wastewater treatment sludge is an effective source of waste to be used for generation of biogas to make energy which can be used to heat homes and run businesses. This would help decrease the use of fossil fuel for energy, but also give access to clean water, as wastewater treatment plants re-introduce purified water back into rivers and streams. High levels of ammonia cause inhibition of biogas, especially when using fats, oils, and greases as a feed source. Using gas chromatography, and colorimetric assays, the effects of increasing amounts of ammonia on biogas production when fats, oils, and greases were present in the feedstock was examined. The ammonia sensitivity of sludge from the Corvallis and Gresham wastewater treatment plants were compared. The Corvallis sludge was more sensitive to ammonia changes than the Gresham sludge, which is speculated to be because of different microbial community composition due to different feed sources at the respective full-scale facilities.

Biogas inhibition of Fats, Oils, and Greases with varying ammonia levels

by

Ana A. Aranda

A PROJECT

submitted to

Oregon State University

BioResource Research

in fulfillment of

the requirements of

the degree of

Baccalaureate of Science in BioResource Research, Bioenergy Option

Presented on May 29, 2019

Commencement on June 15, 2019

Baccalaureate of Science in BioResource Research project of Ana A. Aranda presented on May 29, 2019.

Approved:

Tyler Radniecki, Chemical, Biological, and Environmental Engineering, College of Engineering

---

Katherine Field, Bioresource Research, College of Agricultural Sciences

---

Ashley Berninghaus, Environmental Engineering, College of Engineering

I understand that my project will become part of the permanent collection of the Oregon State University Library and will become part of the Scholars Archive collection for BioResource Research. My signature below authorizes release of my project and thesis to any reader upon request.

Ana Aranda Date

Introduction

Wastewater has become a topic of interest, as there is a shortage of usable drinking water and there is a need to dispose of waste. One of the most promising efforts to stem the global water crisis is industrial and municipal water reclamation and reuse [12]. A proposed method of reusing waste is biogas co-digestion, in which organic matter is broken down into methane and carbon dioxide [13]. The microbes responsible for this decomposition are living in anaerobic environments, conditions similar to that of the human digestive system. These microbes consume everyday wastes ranging from cow manure to fryer grease from fast food restaurants. The lipidic nature of FOG causes higher biogas production due to its higher convertibility (94.8%) to biogas compared to carbohydrate (50.4%)- and protein (71%)-rich substrates [14]. This is because of long fatty acid chains that are further produced into methane. Since, protein rich biomass has higher biomethane potential, it makes for a better biomass product [3,4]. This research focuses on is the use of wastes consisting of fats, oils, and greases (FOG), as a way create energy.

The waste generated from the wastewater treatment plants (WWTP) has a high capacity to create energy. Activated waste from WWTP can produce gas energy, which can recover 20-40 % of the energy requirement [1]. However, with the addition of FOG, studies have shown that biogas can increase by 30 % [1]. This can lead to WWTP to providing half of their energy needs with anaerobic digestion [1]. However, not every WWTP uses their waste for biogas, nor does every WWTP with a biogas digester actually use their gas for energy. Of the 1,269 wastewater treatment plants using an anaerobic digester in the United States, only around 860 use their biogas [2]. This leads to more carbon dioxide and methane being released into the atmosphere instead of burning the methane to be used for energy. If all the facilities that currently use anaerobic digestion—treating over 5 million gallons each day—were to install an energy recovery facility, the United States could reduce annual carbon dioxide emissions by 2.3 million metric tons—equal to the annual emissions from 430,000 passenger vehicles [2].

In Oregon, the biggest biogas production sites are on WWTP. Approximately 34% of WWTP are currently using anaerobic digesters to generate biogas; however, there is a potential for 33% more to establish biogas facilities [8]. If fully realized, these biogas systems could produce enough electricity to power 13,553 homes (233 million kWh) or enough renewable natural gas to fuel 33.825 vehicles. This would also reduce greenhouse gas emissions by the equivalent of 4.6 trillion tons of carbon dioxide, the same as growing 4.1 million tree seedlings for ten years or the amount 139,803 acres of U.S. American forest sequester each year [8]. The economic value of adding these additional biogas plants would generate $201 million in capital investment, and create 1,675 short-term construction jobs, 134 long term jobs, and numerous industry-supporting jobs. This proves that more work needs to be done overall to develop biogas into a large source of energy in Oregon.

The steps of anaerobic digestions are; hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 1). During hydrolysis, complex organic compounds, such as proteins, are broken down into simple organic compounds like amino acids [10]. Then in the acidogenesis step, simple organic compounds are further broken down into smaller fatty acid chains [11]. The third process, acetogenesis, creates acetate while

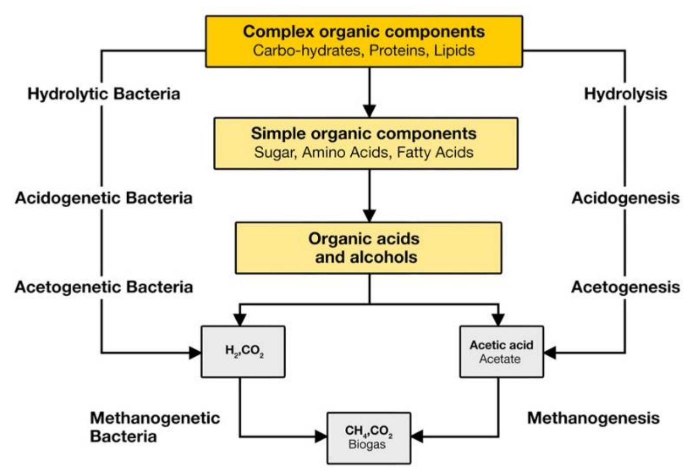

Figure 1: Biochemical pathway for biogas generation
https://biogas.me-le.de/en/technology

simultaneously releasing hydrogen and carbon dioxide. Acetate is then used in the methanogenesis step to generate carbon dioxide and methane [15].

High protein levels correlate to elevated ammonia concentrations, which can become harmful to the microbial communities involved in the biogas process [5]. Although there are sometimes species of microbes that are tolerant of high ammonia, the result can still be degraded methane levels which is not ideal [6, 7]. High concentrations of ammonia could lead to the failure of AD as a result of inhibited microbial activities [9].

While the effects of ammonia inhibition on anaerobic digestion has been significantly studied in the past, it is unclear how the presence of FOG would influence the sensitivity of the anaerobic digestion process. This research used batch anaerobic digesters with varying ammonia concentrations in with the presence and absence of fats, oils and greases to determine how FOG influences ammonia inhibition of biogas production.

Methods

FOG Source

The FOG mixture acquired from the Gresham WWTP (Gresham, OR) was sourced from local restaurants in the area. This facility has a FOG receiving station where food establishments can drop off these unwanted biproducts. The products then undergo a thickening process where water is isolated and removed at room temperature by segregating the settled FOG from the water residue.

Corvallis Ammonia and FOG

Table 1: Experimental set up using Corvallis sludge. 5 mL of FOG was added on Day 21.

Digestate Volume (mL)	Ammonia Concentration Added (ppm)	Initial Ammonia Concentration (ppm)	Final Ammonia Concentration (ppm)
100	0	816	1310
100	125	817	1515
100	250	776	1600
100	500	862	1824
100	750	964	2113
100	1000	1002	2233

Table 1 shows different ammonia added to sludge digestate. The seed digestate was from the City of Corvallis WWTP from which, 100 mL of sludge was added to each bottle and ammonia and pH testing was done once a week, referenced in the Ammonia Determination and Liquid Sampling sections. The bottles were then sparged with nitrogen gas using a gas needle and a 3 mL needle was injected as a way for excess gas to be released. The bottles were then placed in a shaker table at 37° C. The light conditions were not monitored as the shaker table was placed in a room where both light and dark were exposed. The column described as “Initial ammonia composition” was taken Day 0 when the experiment was assembled, after additional ammonia concentrations were added. Gas sampling and gas composition determination was done three times a week, referenced in the Gas Sampling section. Each ammonia concentration was repeated in triplicate. After 21 days, the bottles were spiked with 5 mL of FOG from the City of Gresham WWTP. The experiment ended after 52 Days.

Gresham and FOG

Batch bottles were assembled using four conditions; each condition had 5 replicates, having a total of 20 bottles. These groups of consisted of a control (no ammonia), 500 ppm NH_4^+, 1000 ppm NH_4^+, and 1500 ppm NH_4^+. The seed digestate was from the City of Gresham WWTP from which, 100 mL of sludge was added to each bottle and ammonia and pH testing was done once a week, referenced in the Ammonia Determination and Liquid Sampling sections. The bottles were then sparged with nitrogen gas using a gas needle and a 3 mL needle was injected as a way for excess gas to be released. Gas sampling and gas composition determination was done three times a week, referenced in the Gas Sampling section. The bottles were then placed in a shaker table at 37° C. The light conditions were not monitored as the shaker table was placed in a room where both light and dark were exposed. The experiment ran for 27 days.

Another 20 bottles were assembled using the same conditions, but 5 ml of FOG was added to this group to determine the difference in overall biogas production when both ammonia (NH_4^+) and FOG were present. The FOG source was from the City of Gresham WWTP.

Corvallis and FOG

Batch bottles were assembled using four conditions; each condition had 5 replicates, having a total of 20 bottles. These groups of consisted of a control (no ammonia), 500 ppm NH_4^+, 1000 ppm NH_4^+, and 1500 ppm NH_4^+. The seed digestate was from the City of Corvallis WWTP from which, 100 mL of sludge was added to each bottle and ammonia and pH testing was done once a week, referenced in the Ammonia Determination and Liquid Sampling sections.

The bottles were then sparged with nitrogen gas using a gas needle and 3 mL needle was injected as a way for excess gas to be released. Gas sampling and gas composition determination was done three times a week, referenced in the Gas Sampling section. The bottles were then placed in a shaker table at 37° C. The light conditions were not monitored as the shaker table was placed in a room where both light and dark were exposed. The experiment ran for 27 days.

Another 20 bottles were assembled using the same conditions, but 5 ml of FOG was added to this group to determine the difference in overall biogas production when both ammonia (NH_4^+) and FOG were present. The FOG source was from the City of Gresham WWTP.

Organic Acids

The following experiment was done to determine which organic acids are most sensitive to ammonia in biogas production. For this, different of concentrations acetate, propionate, and butyrate were used. Each experimental condition was tested in quantities of 5. The seed digestate was from the City of Corvallis WWTP from which, 100 mL of sludge was added to each bottle and ammonia and pH testing was done once a week, referenced in the Ammonia Determination and Liquid Sampling sections. The bottles were then sparged with nitrogen gas using a gas needle and a 3 mL needle was injected as a way for excess gas to be released. Gas sampling and gas composition determination was done three times a week, referenced in the Gas Sampling section. The bottles were then placed in a shaker table at 37° C. The light conditions were not monitored as the shaker table was placed in a room where both light and dark were exposed.

Table 2: Experimental set up for acetate, propionate, and butyrate.

Bottle Label	Digestate Volume [mL]	Ammonia Solution Added (ppm)	Final Ammonium Concentration-Acetate (mg NH_4-N/L)
Control	100	--	961
Acid control	100	--	873
Acid +500	100	500	1051
Acid +1000	100	1000	1559
Acid +1500	100	1500	1833

The table states only "acid", however the same set up and concentrations were completed with the all the organic acids. Each organic acid experiment ran for 15 days.

Liquid Sampling

Sludge and gas sampling were done three times a week. Once a week, pH was tested using a pH probe; 3 mL of sludge was removed, this was the quantity that was pulled in order to get an accurate reading. Then, 2 mL was spun down using a centrifuge for 5 minutes at 13000 rpm. Only 2 mL of the sample was saved because of freezer space and the available microcentrifuge vials were 2 mL size. Finally, 1.5 mL of supernatant was saved, stored in clean centrifuge tubes, and frozen to avoid further metabolic processes including, ammonia testing and high-performance liquid chromatography analysis.

Gas Sampling

Gas composition was determined using an HP-5890 GC thermal conductivity detector (TCD) with argon gas at a flow rate of 20 mL/min. The samples were run at 220 °C. 100 µL of each bottle was sampled using a gas tight Hamilton syringe (1700 series) and was manually injected into the TCD. This was done by injecting the syringe into the rubber stopper located on the cap of each bottle. Standard curves were generated for N_2, CH_4, and CO_2. The composition of each gas was determined using peak areas given after TCD integration and compared to the mass of the known elements.

Gas volume was taken using a 200 mL syringe and injecting each bottle at the same time each day it was sampled. The displacement of the syringe determined how much biogas was made. From there, the volumes were recorded, and methane and carbon dioxide volumes were determined by multiplying the overall volume by the percent given by the TCD.

Determination of Ammonia Concentrations

Dilutions of ammonium were made using a 100x diluted solution of 990 µL of deionized water and 10 µL of thawed and mixed supernatant sample. The samples were frozen supernatants from the liquid sampling. The mixer used was a VWR benchtop vortex mixer. A 96- well plate was used; each well was filled in the following order; 25 μL of diluted sample, 175 μL of citrate reagent, 50 μL of 2-phenylphenol nitroprusside reagent, and 25 μL of buffered hypochlorite reagent. Ammonia levels were measured using a BioTek Synergy microplate reader. The plate was heated to 37°C and held for 15 minutes at an absorbance of 660 nm. After that, it was shaken for 30 seconds. Once the test was complete, ammonium concentrations were determined by using a 5-point calibration standard curve.

Results

Corvallis Ammonia and FOG data sets

The purpose of this study was to determine the sensitivity of the Corvallis anaerobic digestate to ammonia in the presence and absence of FOG. The City of Corvallis WWTP currently, uses only sludge as a feed source for their anaerobic digesters which may account for some sensitivity when FOG is present.

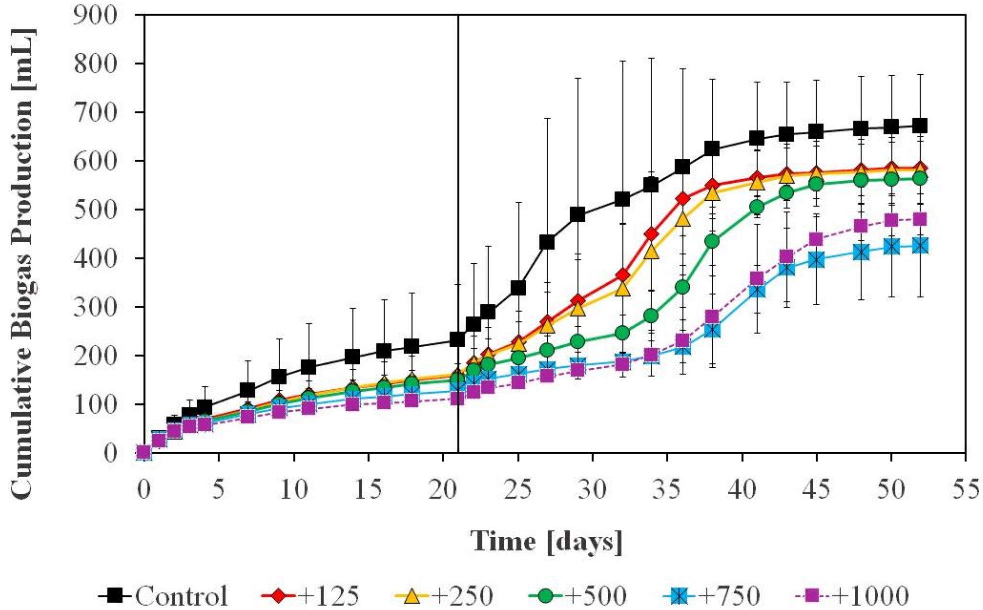

Figure 2: Overall biogas accumulation during Corvallis Ammonia and FOG experiment. On Day 21, 5 mL of FOG was injected displayed by the black line. The error bars shown on the graph show a 95% confidence interval, meaning the value is 95% likely to fall within that range.

There was significant ammonia inhibition when FOG was added on day 21 (**Figure 2**); this was when 5 mL of FOG was injected. It is displayed as the black thick line on day 21. Different amounts of gas production are shown on the graph. The bottles with a higher ammonia content were slower to make biogas, while the control with no ammonia had the fastest biogas production. Inhibition within the first 21 days was seen but not very drastic since they all made relatively the same amount of gas. Once FOG was present the amount of biogas created was more widespread, meaning that at higher ammonia levels, less biogas was made. According to **Figure 2**, the highest ammonia level made around 170 mL by Day 28, while the control which had no ammonia, made nearly 500 mL of biogas over the same time period. The City of Corvallis WWTP only uses sludge during co digestion when using their anerobic digester.

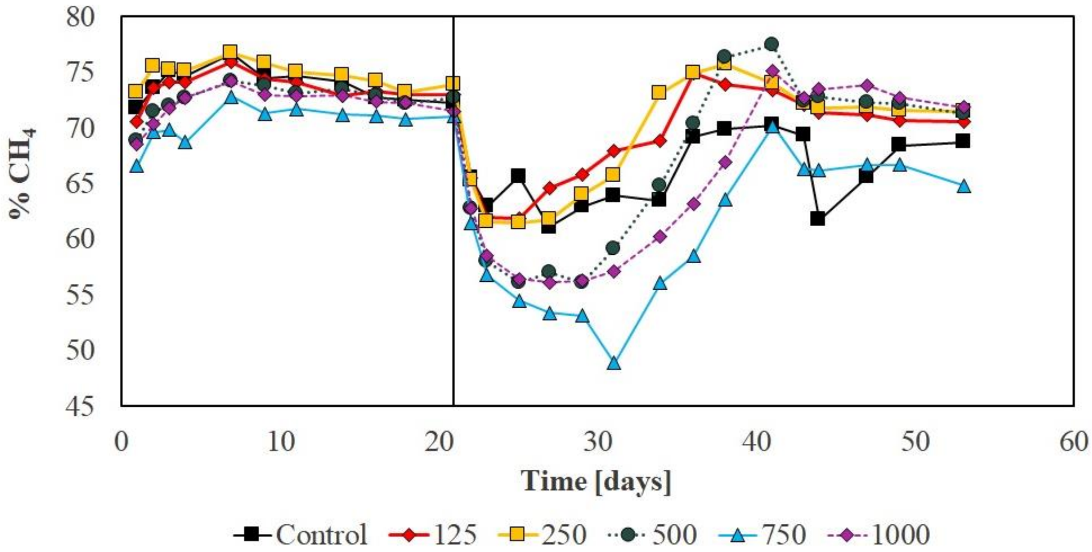

Figure 3: CH_4 percent by weight during Corvallis Ammonia and FOG experiment. The black line on Day 21 indicates when 5 mL of FOG was added to the system.

Figure 3 depicts percent methane produced for each level of ammonia. A drastic drop in methane percentage is shown when FOG is added to the bottles. This agrees with the hypothesis that biogas production is most sensitive to increase ammonia concentrations. The highest ammonia concentrations are the lowest methane percentages, as there is most inhibition seen at those levels. For example, on Day 20 before FOG was added the methane percent was around 70-75 % across all ammonia levels, which is considered normal within anaerobic digestion. On Day 21, the methane fell to around 55 % at the higher ammonia levels, displaying that there was some sensitivity shown to FOG within Corvallis sludge.

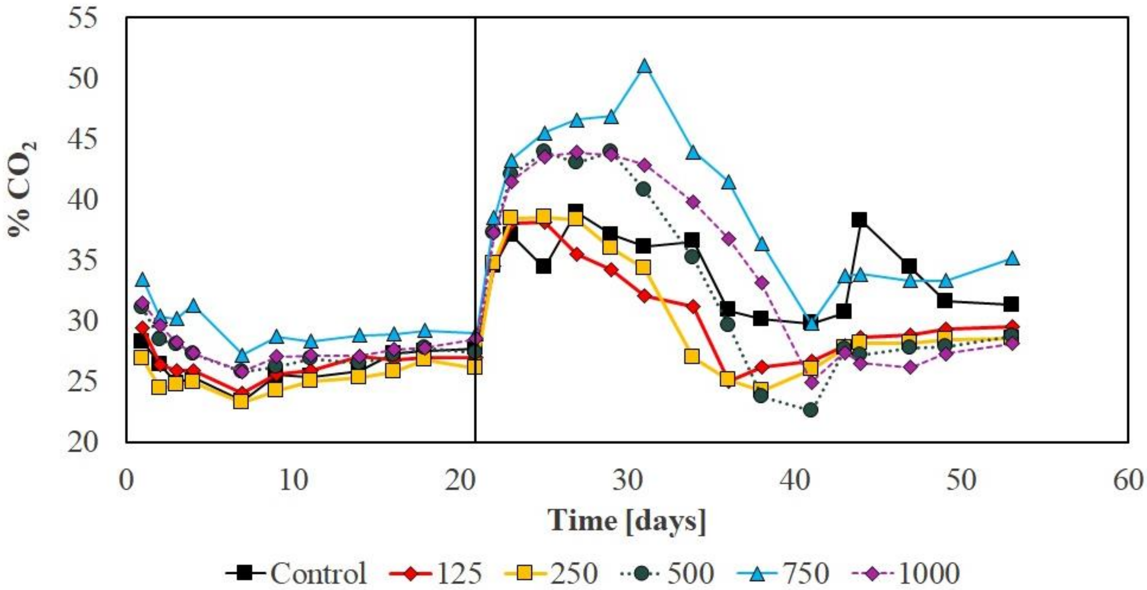

Figure 4: CO_2 percent by weight during Corvallis Ammonia and FOG experiment. The black line on Day 21 indicates when 5 mL of FOG was added to the system.

Figure 4 shows the CO_2 percent by weight before and after FOG was injected. The rising CO_2 rates shown after the 20 days is a result of ammonia inhibition, which is found in the higher ammonia concentrations (1000 and 750 ppm). On Day 20 the bottles were all within 25-30 % carbon dioxide, while after Day 21, the carbon dioxide content was as high as 46% found in the higher levels of ammonia.

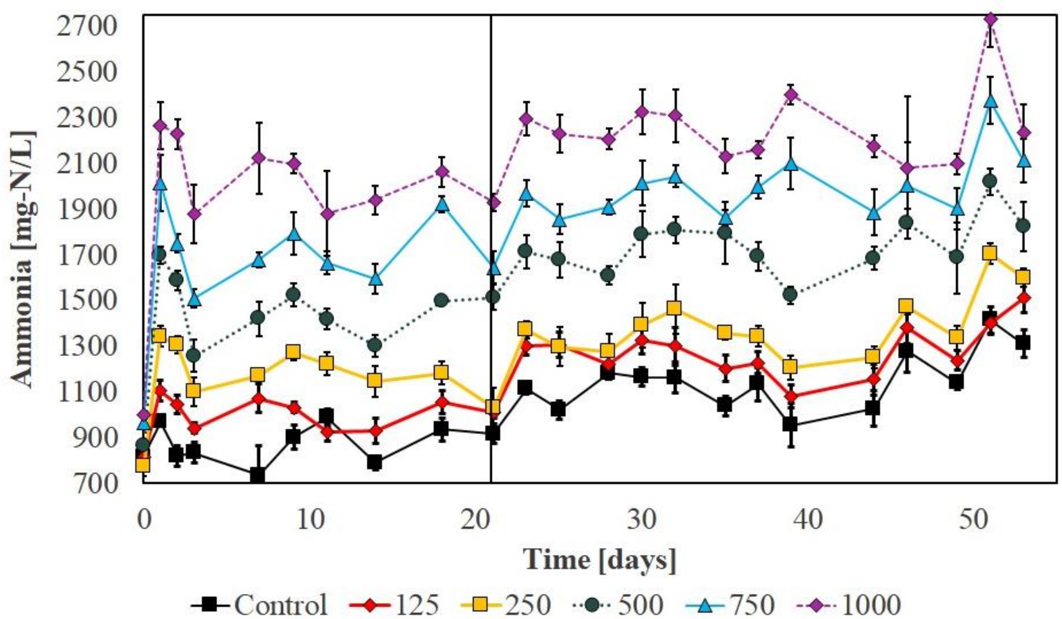

Figure 5: Ammonia content during Corvallis Ammonia and FOG experiment. The black line on Day 21 indicates when 5 mL of FOG was added to the system. Error bars show 95% confidence.

Ammonia concentrations from each condition (**Figure 5**) displayed overall separations between the conditions. When each group is separate this ensures that the ranges found do not overlap values with the other condition. This step is done to ensure that ammonia levels stay around the same value in which the experiment began. On Day 0 when the ammonia levels were sampled, all the conditions started out between 800-1000 mg N/L, however on Day 1 when the samples were tested again, the digesters spiked to a higher ammonia content. For example, the highest ammonia level began at 1000 mg N/L on Day 0 and on became 2300. From then on, the ammonia levels stayed around the 2300 mg N/L level. The final day the ammonia content was also 2300 mg N/L, showing that ammonia levels can fluctuate and that our samples were consistent with the number it started with on Day 1, even with increases and decreases along the way.

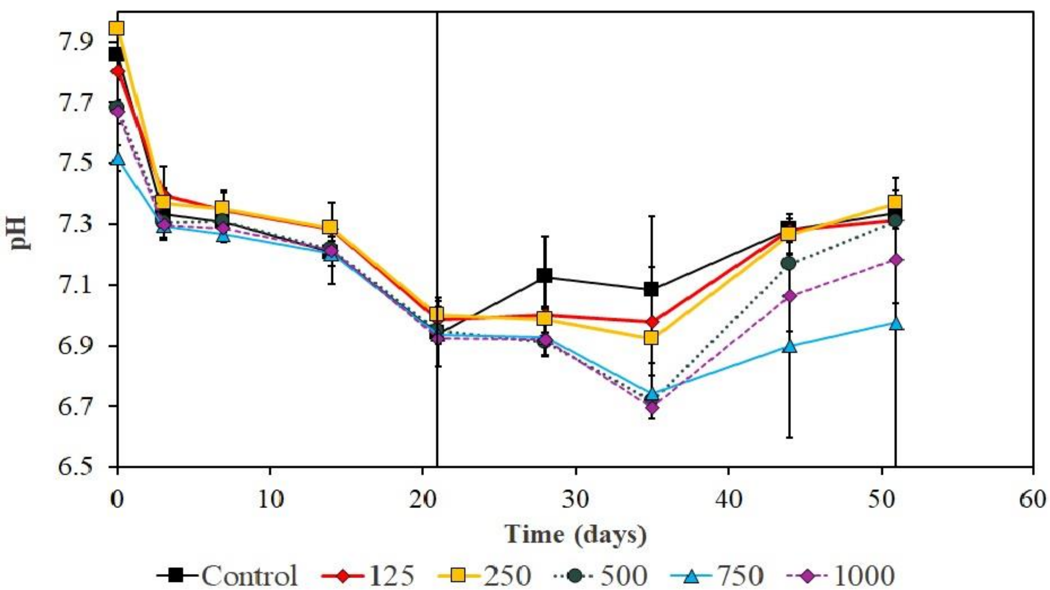

Figure 6: pH levels during Corvallis Ammonia and FOG experiment. The black line on Day 21 indicates when 5 mL of FOG was added to the system. Error bars show 95% confidence.

Figure 6 depicts pH level at the different ammonia concentrations. A decrease in pH is shown when FOG is added to the bottles, except the control bottle. The highest ammonia concentrations are the lowest pH levels, as there is most inhibition seen at those levels. For example, on Day 14 a week before FOG was added the pH was around 7.2- 7.3 across all ammonia levels. On Day 30, the methane fell to 6.7 at the higher ammonia levels. It should also be noted that the scale of the graph is shown in .2 increments, over the course of the experiment the change in ammonia was only seen between 1.3 ; highest was 8, lowest was 6.7.

Gresham and Corvallis data set

The purpose of this study was to determine the sensitivity of the City of Gresham anaerobic digester sludge to ammonia in the presence and absence of FOG. It should be noted that the City of Gresham sludge has been adapted to FOG loading in the full-scale reactor.

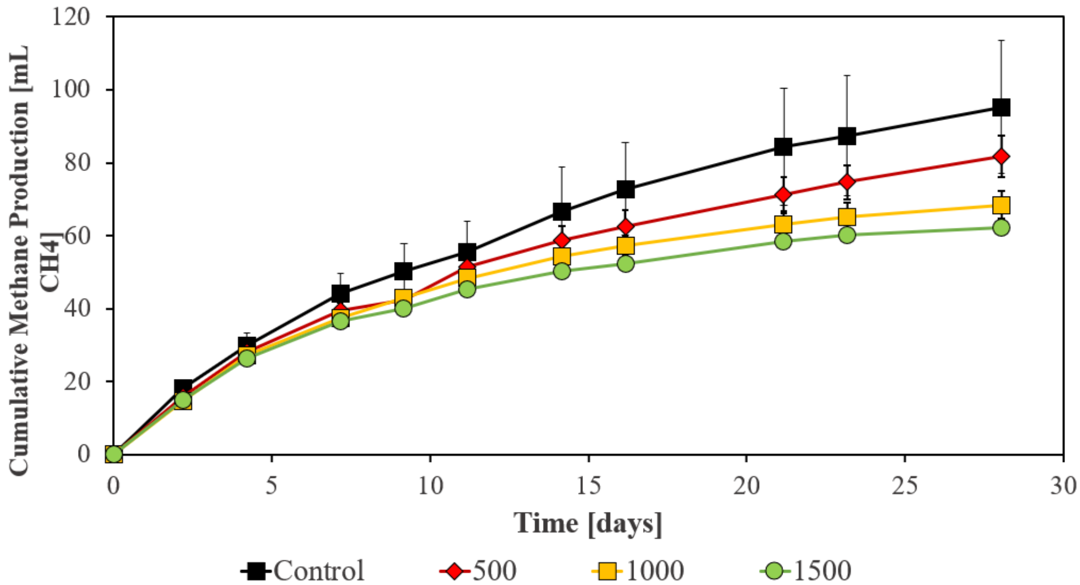

Figure 7: Corvallis sludge, methane content without FOG.

The digestate in this section is coming from the City of Corvallis WWTP. FOG was retained from the City of Gresham WWTP. The graph without FOG in this section displays the total methane by volume of control, 500 ppm, 1000 ppm, and 1500 ppm ammonia samples. The control did slightly better in this study; however, all the bottles initially had the same rate of production.

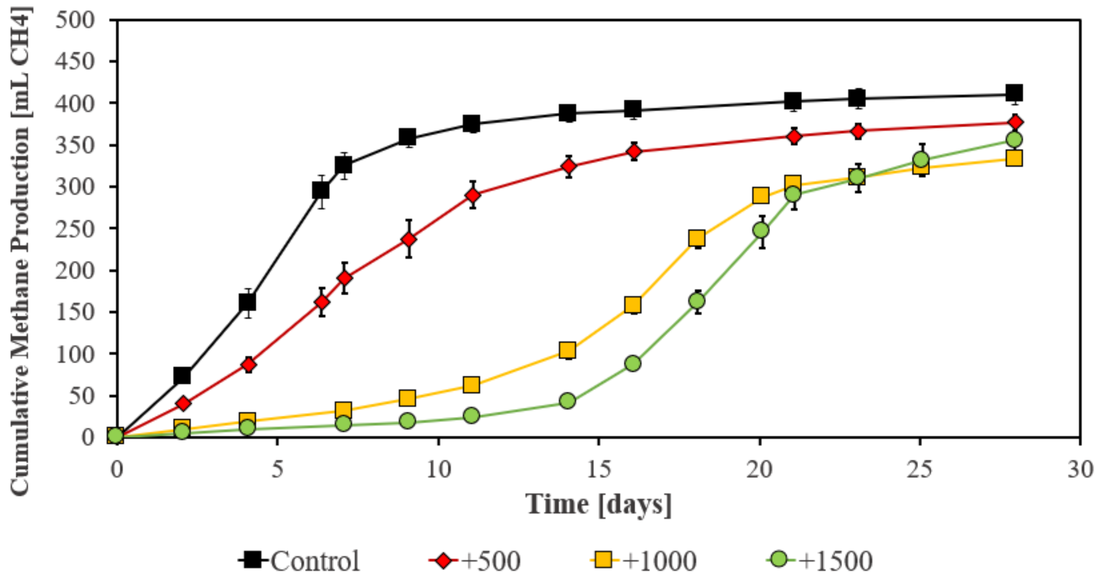

Figure 8: Corvallis sludge, methane content with 5ml of FOG added at Day 0.

For the trial with FOG, 5 ml of FOG was added on Day 0, the graph depicts higher production rates in the control, while the higher ammonia concentrations showed inhibition, leading to the slower rate of production.

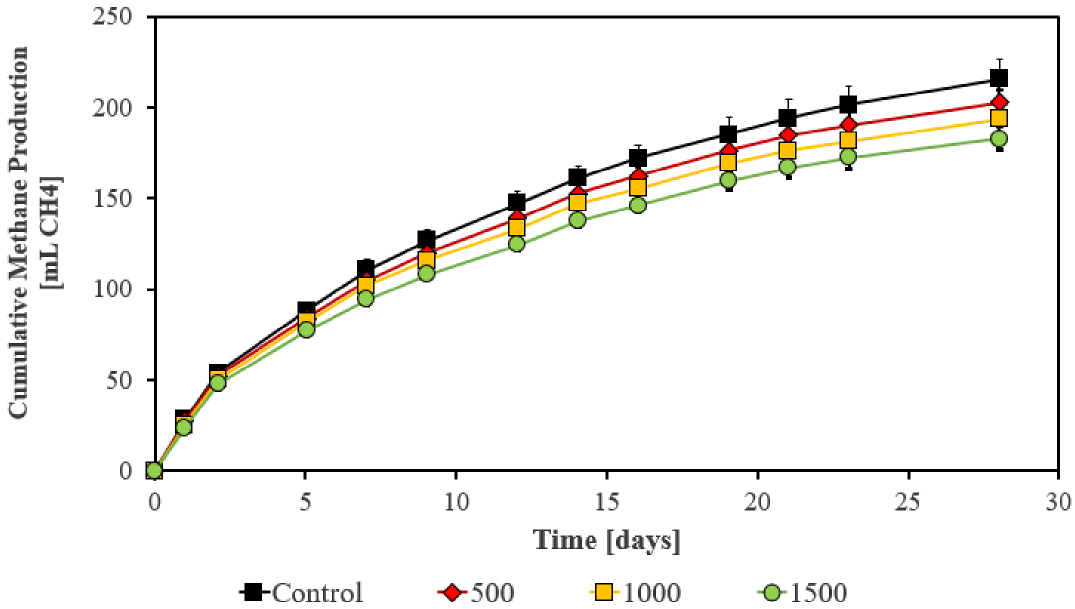

Figure 9: Gresham digestate without FOG.

The digestate and FOG in this section is from the City of Gresham WWTP. The graph without FOG in this section displays the total methane by volume of the control, 500 ppm, 1000 ppm, and 1500 ppm ammonia samples. Based on the graph, the control did slightly better in this study, compared to the higher ammonia levels. However, all the bottles initially had the same rate of production and ended at around the same volume. It should be noted that the trial without FOG also had generated more methane volume than the Corvallis without FOG.

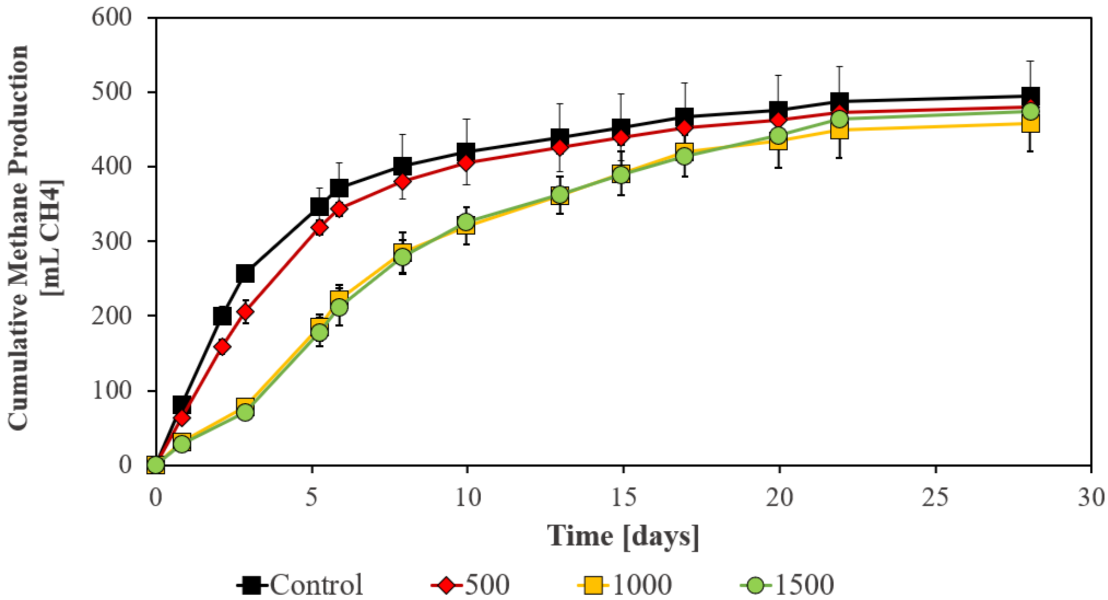

Figure 10: Gresham digestate with 5ml of FOG added at Day 0.

For the FOG trial, 5 ml of FOG was added on Day 0. The graphs depict higher production rates in the control and 500 ppm bottles, while the higher ammonia concentrations showed inhibition, leading to the slower rate of production.

Organic acid data sets

The purpose of this experiment is to determine which group of microorganisms are the most sensitive to ammonia addition, the microorganisms that break down butyrate, propionate, or acetate. The sludge in this experiment was from the City of Corvallis WWTP.

Acetate

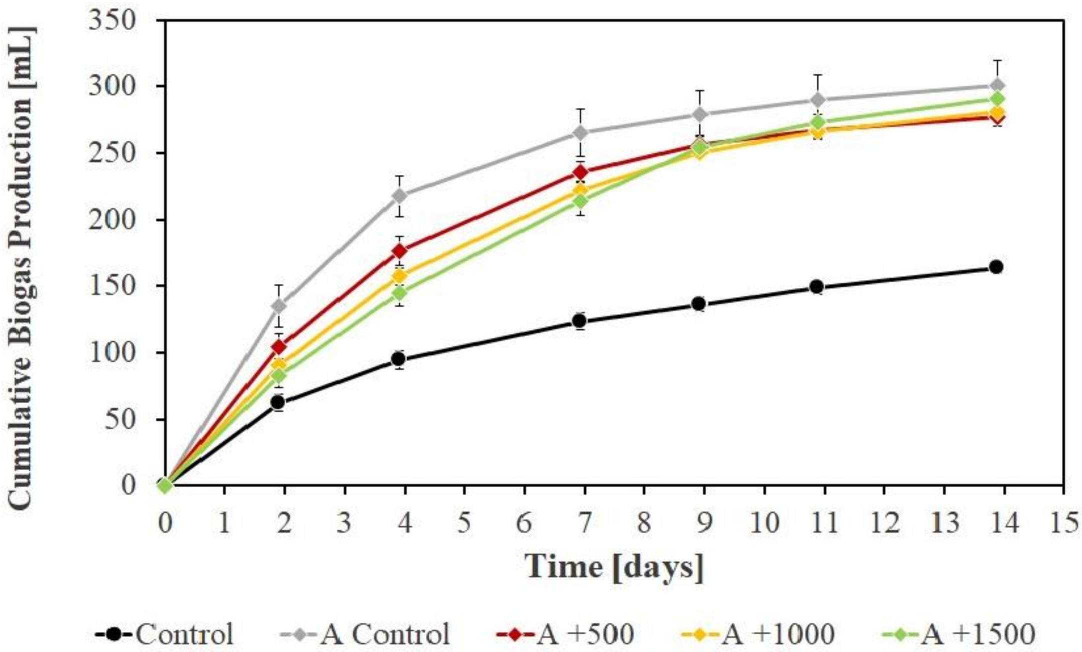

Figure 11: Overall biogas production during acetate experiment.

The biogas production shown above displayed some ammonia inhibition (**Figure 11**). The rates were relatively all around the same time, excluding the control. This is evidence that ammonia inhibition was lightly seen within the 500, 1000, and 1500 ppm samples.

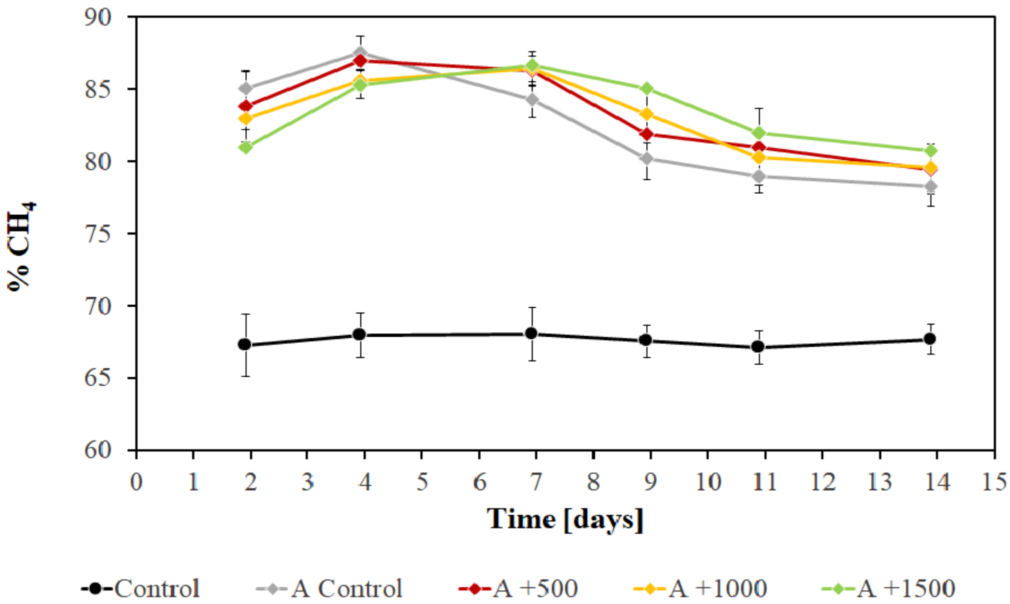

Figure 12: CH_4 percent production during acetate experiment.

Cumulative methane percentages were lower with the increasing ammonia concentrations (**Figure 12**). The acetate control bottle had the highest methane production rate overall. The control had the worst methane production, as it is known that acetate is the easiest organic acid to be used in an anaerobic digester. Based on the graph, there was some inhibition among the higher ammonia levels.

Propionate

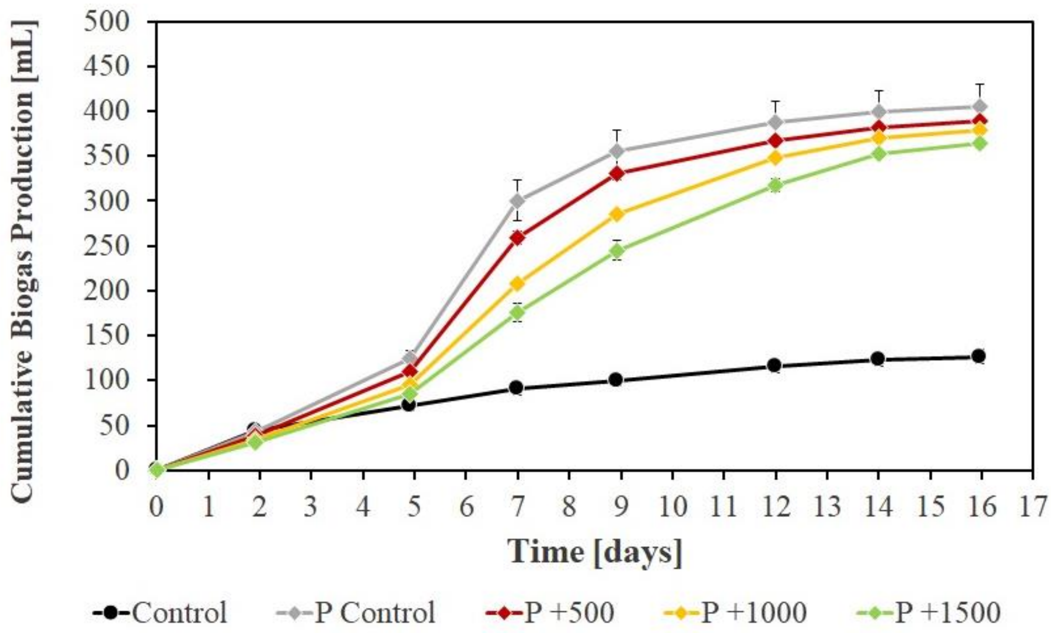

Figure 13: Overall biogas production during propionate experiment.

The propionate biogas production shown in **Figure 13** displays some ammonia inhibition. This is likely because of propionate being a larger molecule than acetate, meaning it will take longer to break down into methane. This would then show a delay in increased production rate, which is seen on Day 5 when the propionate bottles spike in biogas production. Throughout the experiment, the propionate control was shown to have the highest biogas volume, and at higher ammonia levels more inhibition was shown. Methane production for propionate is further under analysis.

Butyrate

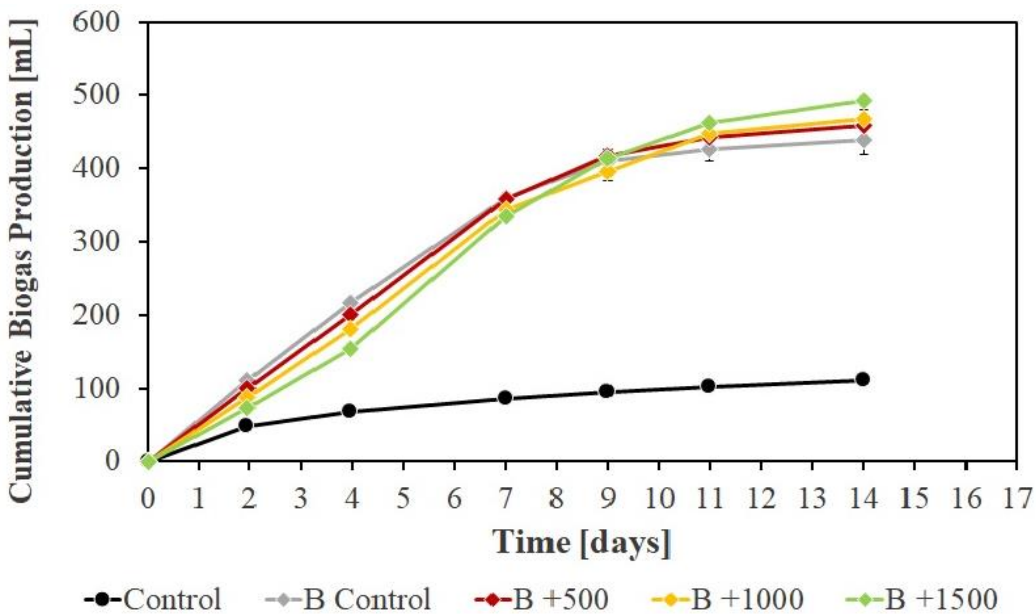

Figure 14: Overall biogas production during butyrate experiment.

The butyrate biogas production shown above in **Figure 14** displays slight ammonia inhibition shown on Day 4. However, the overall trend of production displays to be relatively all the same. The gas production rates were similar, excluding the control. This shows that ammonia inhibition was not seen as drastically as the other organic acids. Butyrate degradation does not seem to be affected by ammonia inhibition. Methane production for propionate is further under analysis.

Discussion

Understanding biogas inhibitions is a key problem in establishing more facilities in Oregon and in the U.S. We examined ammonia inhibition, in conjunction with FOG. The results displayed that Corvallis was most sensitive to ammonia inhibition, while digestate from Gresham was more resistant to increasing ammonia concentrations when FOG was present. This is most likely because Gresham WWTP feeds their biogas digester FOG, and this has given the microbes in the sludge an advantage, since they are more acclimated to the FOG rich environments, while Corvallis sludge is not.

The purpose of the organic acid experiments was to determine which organic acid degradation processes was most inhibited by the addition of ammonia. In literature there aren't solid conclusions, if any, on how ammonia inhibits volatile fatty acids (VFA) like those used in the final experiment (acetate, propionate, butyrate)[3]. The findings from the organic acid experiment were various. Acetate displayed some inhibition, as well as propionate. However, because propionate is a heavier molecule, the microorganisms take longer to generate methane. Butyrate didn't seem to be affected by ammonia inhibition, because of relatively similar rates throughout the trial. Future studies might include repeating the final experiment and adding a second trial with FOG added with each VFA. This would help indicate the step that is most sensitive to ammonia inhibition with FOG.

Conclusion

High ammonia concentrations can have detrimental effects on the microorganisms living in aerobic conditions. From the results of this study, sludge from Corvallis displayed the most biogas inhibition. Sludge from Gresham was shown to be more resistant to the changes in ammonia concentrations when FOG was present. The varying organic acids also play a role in determining ammonia inhibition. Acetate and propionate are more sensitive to ammonia inhibition. Butyrate was somewhat sensitive to ammonia inhibition, but not nearly as much as the acetate and propionate. For WWTP in Oregon that are interested in FOG co-digestion, it is important to put thought into varying ammonia concentrations due to increased sensitivity of biogas digesters. Between Corvallis and Gresham sludge, not much is known about the microbial communities living in the anaerobic digesters, but the difference in sensitivity is likely due to the different feed sources. The Gresham sludge is more resilient to ammonia inhibition, which is likely caused from the continuous feeding of FOG to the digester. This information is crucial considering that Oregon has potential to expand their existing biogas WWTP sites by 33%. This would generate more economic revenue and could decrease the use of fossil fuels, releasing less greenhouse gases.

References

[1] Long, J., Aziz, T., & Ducoste, J. (2012). Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Safety and Environmental Protection, 90*(3), 231-245.

[2] Piccirilli Dorsey, Inc. (2017, October 3). Fact Sheet - Biogas: Converting Waste to Energy. Retrieved from https://www.eesi.org/papers/view/fact-sheet-biogasconverting-waste-to-energy

[3] Ek A., Hallin S., Vallin L., Schnürer A., and Karlsson M. (2011) Slaughterhouse waste co-digestion – experiences from 15 years of full-scale operation. In: Proceedings of World Renewable Energy Congress. Linköping, Sweden.

[4] Nordell E., Moestedt J., and Karlsson M. (2011) Biogas Producing Laboratory Reactor, Application No. 1150954-4. SE.

[5] Moestedt, J., Müller, B., Westerholm, M., & Schnürer, A. (n.d.). Ammonia threshold for inhibition of anaerobic digestion of thin stillage and the importance of organic loading rate. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4767286/

[6] Schnürer A., and Nordberg A. (2008) Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Sci Technol 57: 735–740. [PubMed]

[7] Westerholm M., Levén L., and Schnurer A. (2012) Bioargumentation of syntrophic acetate-oxidizing cultures in biogas reactors exposed to increasing levels of ammonia. Appl Environ Microbiol 78: 7619–7625. [PubMed]

[8] American Biogas Council. Admin. (2018, December 22). State Profiles. Retrieved from https://americanbiogascouncil.org/resources/state-profiles/

[9] Chen, H., Wang, W., Xue, L., Chen, C., Liu, G., & Zhang, R. (2016). Effects of Ammonia on Anaerobic Digestion of Food Waste: Process Performance and Microbial Community. Energy & Fuels, 30(7), 5749–5757. https://doi.org/10.1021/acs.energyfuels.6b00715

[10] McArdle, C. (2017, May 11). The illustrated step-by-step guide to anaerobic digestion. Retrieved May 26, 2019, from The Opus Energy Blog website: http://www.opusenergyblog.com/illustrated-step-step-guide-anaerobic-digestion/

[11] Biomass to Biogas. (n.d.). Retrieved May 26, 2019, from E Instruments | e-inst.com website: https://www.e-inst.com/training/biomass-to-biogas/

[12] Jhansi, S., and Mishra, S. (2013). Wastewater Treatment and Reuse: Sustainability Options. Consilience: The Journal of Sustainable Development 10: 1 – 15.

[13] Bailey R.S. (2007). Anaerobic digestion of restaurant grease wastewater to improve methane gas production and electrical power generation potential. Proceedings of the 80th Annual Technical Exhibition and Conference of the Water Environment Federation: 6793-6805.

[14] Salama, E.-S., Saha, S., Kurade, M. B., Dev, S., Chang, S. W., & Jeon, B.-H. (2019). Recent trends in anaerobic co-digestion: Fat, oil, and grease (FOG) for enhanced biomethanation. Progress in Energy and Combustion Science, 70, 22–42. https://doi.org/10.1016/j.pecs.2018.08.002

[15] Liou, J. S.-C., & Madsen, E. L. (2008). Microbial Ecological Processes: Aerobic/Anaerobic. In S. E. Jorgensen & B. D. Fath (Eds.), Encyclopedia of Ecology (pp. 2348–2357). https://doi.org/10.1016/B978-008045405-4.00254-8