A Comparison of Ammonia Inhibition on the Anaerobic Digestion of Organic Acids Between Digestate from Anaerobic Co- and Mono-Digestion Systems

By

Gloria Ruiz-Orozco

An Undergraduate Thesis Submitted to

Oregon State University

In partial fulfillment of
the requirements for the
degree of

Baccalaureate of Science in BioResource Research,
BioProducts and BioEnergy Option

November 13, 2020

APPROVED:

Tyler Radniecki, CBEE, College of Engineering	11/13/20
Ashley Berninghaus, Environmental Engineering, College of Engineering	11/13/20
Katharine G. Field, BRR Director	11/13/20

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.

Gloria Ruiz-Orozco	**11/13/20**

1. Introduction

Wastewater treatment plants have become a topic of interest in recent years due to their ability to produce renewably energy, a new demand for communities. The final stage in wastewater treatment, anaerobic digestion, has the potential to increase energy production, especially when combined with food waste. Anaerobic co-digestion, with the addition of fats, oils, and greases (FOG), produces more biogas than conventional anaerobic digestion, providing an added economic benefit for treatment plants[21]. Even so, the process is still risky because of a variety of operational parameters that are not yet fully understood. This research project focused on ammonia inhibition, which can occur due to the breakdown of protein rich FOG, resulting in the accumulation of ammonia in the anaerobic digester. The goal of this research was to test the inhibitory effects of varying levels of ammonia on FOG co-digestion.

Anaerobic digestion has been used for treatment of wastewater sludge for over 100 years[11,17]. In the United States, however, the uptake of anaerobic digestion has been slow in comparison to Europe[11,13,17]. The United States has around 1200 wastewater treatment plants producing biogas through anaerobic digesters. A little more than half of these elect not to use the gas for energy. Of those that use the gas for energy, 33% turn the energy into electricity for the plant, about 10% sell the electricity back to the grid and only 2% can inject the biogas into natural gas pipelines[17]. The plants that use the gas for energy may not be operating optimally and many still flare off some of the gas produced[17].

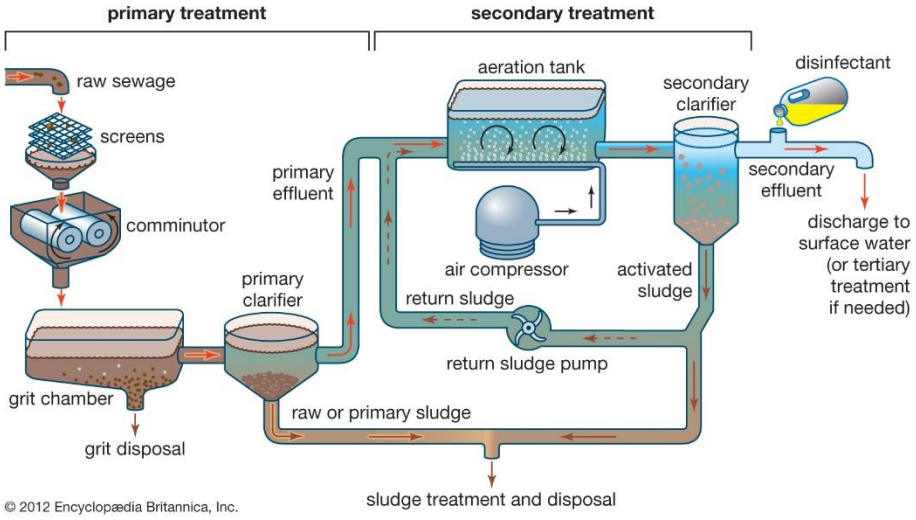

Fig. 1. *overview of wastewater treatment plant*[26]

The wastewater treatment process is not the same everywhere, but a general process diagram is shown above (Fig. 1). The wastewater flows in and the grit is removed early in the process before the primary clarifier. Then the solids settle in the primary clarifier and are left behind as primary sludge. This primary sludge is primarily composed of fecal matter. It can also be polluted with heavy metals and organic micro-pollutants. The primary clarifier effluent, containing non-settleable solids and dissolved contaminants, then flows into the aeration tank (Fig. 1). Air is bubbled through the wastewater in the aeration basin to promote the growth of microbes (*i.e.* activated sludge) that will remove the non-settleable and dissolved contaminants that remain (Fig. 1). These microbes settle out in the secondary clarifier and form waste activated sludge. The effluent from the secondary clarifier is disinfected and sent out to the environment[22,23]. Both primary and waste activated sludge are sent to the anaerobic digester for further treatment. The primary sludge and waste activated sludge in wastewater treatment are reduced in mass, pathogen content and odor during anaerobic digestion. The digested sludge is then sent out to another facility to be dewatered, dried, and disposed of.

Anaerobic digestion is a four-step process consisting of hydrolysis, acidogenesis, acetogenesis and methanogenesis (Fig. 2)[15]. Hydrolysis occurs when hydrolytic enzymes degrade complex polymer substrates into monomers; such as carbohydrates into sugars, proteins into amino acids and lipids to organic fatty acids [15,21]. The community that drives anaerobic digestion forward and turns these monomers into volatile fatty acids (VFAs), like propionate, butyrate, and acetate as well as carbon dioxide and hydrogen, is primarily made up of *Bacteria*, commonly called acidogens, which are grouped based on their specific function in the anaerobic digester[15,21]. In acetogenesis, the volatile fatty acids are further converted into acetate by another group of *Bacteria* called acetogens, which produce hydrogen and carbon dioxide as by-products[15]. Finally, through methanogenesis, methane is produced through two different pathways by *Archaea*. The first pathway is performed by acetoclastic methanogens, which convert acetate to methane. The second pathway is performed by hydrogenotrophic methanogens, which use hydrogen and carbon dioxide to produce methane.

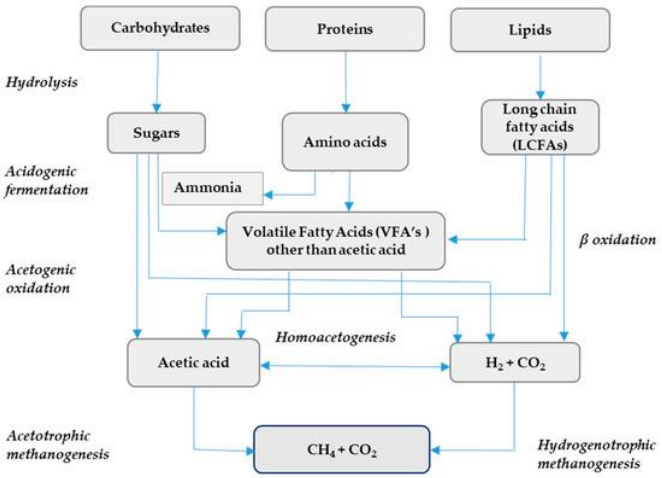

Fig. 2. *Anaerobic digestion pathway for biogas. Sourced from*[7]

The methane produced by wastewater treatment plants can be used to produce electricity or can be burned for process heat[4].

A way to increase the amount of biogas is through the addition of FOG for co-digestion[1]. FOG is helpful for wastewater treatment plants aiming to meet their electricity needs through on-site generation, because it increases biogas production by 30% or more, due to its high energy content[10]. FOG is a readily available organic waste, usually from the food service industry, that left alone is also an environmental pollutant[10].

Anaerobic co-digestion with FOG can potentially result in difficulties that would lead a plant to ultimately not implement the technique. One such difficulty is ammonia inhibition. Ammonia inhibition is a concern due to the protein content commonly found in FOG, which some studies estimate to be between 227-9209 mg/L[10]. While high concentrations of FOG within an anaerobic digester are desirable to increase biogas production, the hydrolysis of proteins within the FOG may produce ammonia at levels that are inhibitory to the anaerobic digestion process.

Ammonia is a necessary nutrient for bacterial growth but is also considered to be the largest inhibitor of anaerobic codigestion[21]. It is produced through the degradation of nitrogenous complexes, such as protein, and it is present as free ammonia (NH_3) and the ammonium ion (NH_4^+) in anaerobic digestate[21]. Both species are present in the anaerobic digestate to begin with but can accumulate further during anaerobic digestion[21]. Free ammonia is more toxic than the ammonium ion, primarily due to the smaller size of NH_3, which allows it to permeate a cell membrane easily, leading to an increase of pH due to the basic nature of NH_3[9,12]. The accumulation of NH_3 creates a hyperosmotic pressure surrounding the cell, because of the increase of pH. The cell tries to balance out the pH and pressure and in turn shrivels and dies[12].

Currently, a wide range of ammonia concentrations have been reported as inhibitory to anaerobic digestion. These can begin as low as 40 mg/L and get as high as 450 mg/L of free ammonia[5,19]. This range is partially due to the wide variety of substrates used for experiments, and partially due to relative novelty of the subject. Some ammonia levels, however, are not harmful to anaerobic digestion, and the exact levels that are inhibitory to anaerobic digestion of a specific substrate need to be further characterized.

Work done previously in this lab compared the sensitivity to ammonia inhibition between a digester performing anaerobic co-digestion and one performing anaerobic mono-digestion. In order to further understand the effect of ammonia inhibition on anaerobic co-digestion of FOG, this study aimed to better understand the effect of ammonia inhibition on the different steps of the anaerobic digestion process and the consumption of VFAs.

The experiments in this study were each conducted with one of three VFAs (Fig. 3.). The VFAs are all found naturally in the anaerobic digestion pathway and vary in length[28]. The sensitivity of anaerobic digestion specifically during the steps of acidogenesis, acetogenesis, and methanogenesis can have a large impact on biogas production, and as such be a large determinant of economic feasibility for a wastewater treatment plant[28].

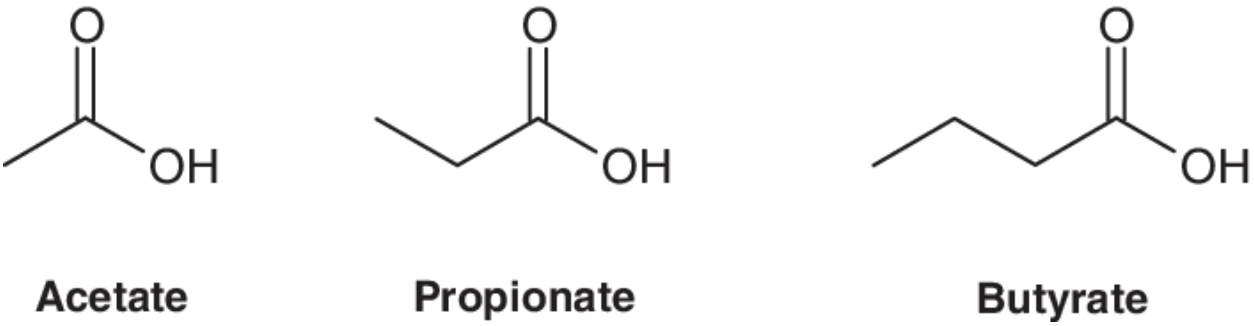

Fig. 3. *The structures of the VFA used in this study*

Another purpose of this study was to understand how the differences between the microbial community in an anaerobic digester performing co-digestion and one performing mono-digestion affect their sensitivity to ammonia inhibition. The digestate used in this study contained microbes from a FOG co-digestion system. The results of these experiments will be compared to a similar study performed on anaerobic mono-digestion, to ascertain the differences between the two.

Ammonia inhibition in anaerobic digestion has been extensively studied in the past, but anaerobic co-digestion with FOG is still a relatively novel process. Thus, this study aimed to advance the field by characterizing the sensitivity of anaerobic co-digestion to ammonia inhibition and determine which reactions (*i.e.* acidogenesis, acetogenesis or methanogenesis) are the most sensitive to ammonia inhibition.

2. Methods and Materials

2.1 Batch Bottle Set Up

The anaerobic digestate was sourced from the Gresham wastewater facility in Gresham OR, and was used within 2 hours of collection. Gresham has added FOG, sourced from local restaurants, to their anaerobic digester since 2012[20,25].

The batch anaerobic digesters were set up as seen in Ana Aranda's thesis, in summary[2]:

Wheaton 155 mL glass bottles with tert-butyl rubber septa were inoculated with 100mL of anaerobic digestate, 5.5 mL of an NH_4Cl solution (500 mg/L, 1000 mg/L or 1500 mg/L), and 5.5 mL of a 120 g/L solution of each respective volatile fatty acid (VFA) tested (sodium acetate, sodium propionate, and sodium butyrate). The anaerobic digestate control did not have an NH_4Cl solution or VFA added to it to ensure the anaerobic digestate could produce gas on its own under non-inhibitory conditions. The VFA control did not have NH_4Cl solution added to it to show the effect the VFA alone had on the digestate. The bottles were sparged with N_2 gas for approximately five minutes to achieve an anaerobic environment. The batch anaerobic digesters were placed upside down in an incubated shaker at 37ºC and 120 rpm.

All batch reactors (Table 1) were run in quintuplets over the course of 14 days. The experiment was repeated twice, changing only the VFA, from sodium acetate to either sodium propionate or sodium butyrate.

Table 1

Summary of set-up conditions for each VFA experiment.

Conditions	Ammonia Concentration Added (mg/L)	Expected Initial VFA Concentration (g/L)
Anaerobic Digestate Control	0	0
Acetate Control	0	6
Propionate Control		
Butyrate Control		
Acetate + 500 mg/L ammonium	500	6
Propionate + 500 mg/L ammonium		

Butyrate + 500 mg/L ammonium		
Acetate + 1000 mg/L ammonium		
Propionate + 1000 mg/L ammonium	1000	6
Butyrate + 1000 mg/L ammonium		
Acetate + 1500 mg/L ammonium		
Propionate + 1500 mg/L ammonium	1500	6
Butyrate + 1500 mg/L ammonium		

2.2 Biogas Testing

The biogas was sampled on the 1st, 2nd, 3rd, 4th, 7th, 9th, and 14th day or when needed due to a build-up of gas pressure in the batch anaerobic digesters. On each sampling day, a 20 mL gas tight glass syringe fitted with a 20G x 1" BD Precision Glide sterile needle attached to a lever-lock valve was used to relieve the pressure of the batch anaerobic digester and to measure the volume of gas produced. A 2 mL crimp top vial with steel wool inside was used as an intermediate scrubbing step to reduce both water vapor and hydrogen sulfide prior to analyzing gas composition. The gas in the crimp top vial was sampled for methane, nitrogen and carbon dioxide content using an HP 5890 gas chromatograph thermal conductivity detector (GC-TCD) with argon carrier gas at a flow rate of 20 mL/min with a packed column (Supelco 15' x 1/8" SS support 60/80 Carboxen 1000). The method was isothermal at 220 ºC.

2.3 Anaerobic Digestate pH Measurements

On the 1st, 7th, and 14th day of the experiment, anaerobic digestate was extracted using a 3 mL syringe fitted with a 20G x 1" BD PrecisionGlide sterile needle. The pH was measured using a calibrated pH probe (Thermo Orion 9156BNWP). The pH measurements were conducted immediately to reduce possibility of equilibration with the air, and any possible pH changes that could result.

2.4 Dissolved Ammonium Assay

On the 1st, 7th, and 14th day of the experiment, 2 mL of anaerobic digestate was pipetted into 2 mL centrifuge tubes and centrifuged for 3 minutes at 14,000 rpm. Prior to further analysis, the supernatant was frozen at 20 ℃. A modified 2-phenylphenol method was used to measure the

dissolved ammonium concentrations in the clear supernatant. 25 µL of each diluted sample was combined with 175 µL of citrate reagent, 50 µL of 2-phenylphenol nitroprusside reagent, and 25 µL of buffered hypochlorite reagent in a 96-well plate. The plate was placed into a BioTek Synergy 2 microplate reader, incubated at 37 °C for 15 min, shaken for 30 s, and absorbance was read at 660nm.

2.5 Calculations

2.5.1 Free Ammonia Calculation

Equation 1 was used to calculate the free ammonia from the total ammonium concentrations and pH values[14]. The pK_a used in these calculations was 9.3.

$$(10^{-pKa+pH})[NH_4^+] = [NH_3] \qquad \textbf{Equation 1}$$

2.5.2 Rate of Change Calculations

The rates of change for the Gresham experiments were calculated using the slope of the cumulative methane production for the volatile organic acid control, volatile organic acid and 500 mg/L of ammonia, volatile organic acid and 1000 mg/L of ammonia, and volatile organic acid and 1500 mg/L of ammonia. The results of the cumulative methane produced were graphed and the linear portion of the first few days was used for the slope. The rates of change for the Corvallis experiments were calculated the same way as above, except for the propionate experiment. For the propionate experiment, the lag period in the beginning of the study was considered, and the rate of change was found for day five through seven.

2.5.3 Significance Calculations

Separation significance was calculated using the student's t-Test in Excel with the Data Analysis tool, assuming unequal variances[29].

3. Results

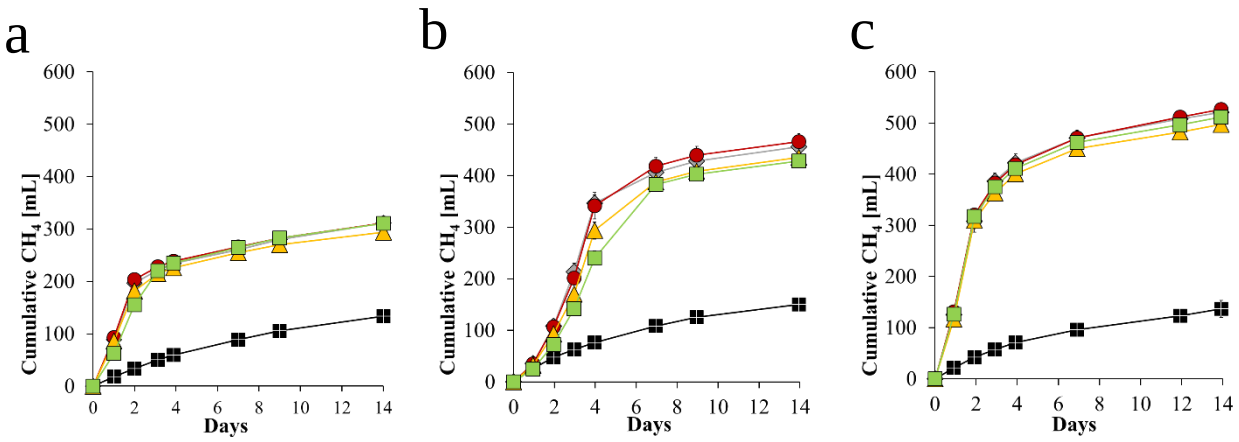

Fig. 4. *Cumulative methane produced for acetate (a), propionate(b) and butyrate (c) over the time of the experiment. The conditions consist of control with no volatile organic acid (■), volatile organic acid control (◆), volatile organic acid and 500 mg/L of ammonia (●), volatile organic acid and 1000 mg/L of ammonia (▲), and volatile organic acid and 1500 mg/L of ammonia (■). Error bars represent 95% confidence intervals.*

The acetate experiment produced the least amount of methane at 311 mL, and the amount of methane produced in the 1000 mg/L of ammonium condition was significantly different from the rest of the conditions (p-value 0.035) (Fig. 4a). The cumulative methane produced was higher in the propionate experiment and butyrate experiment, at 466 mL and 527 mL respectively (Fig. 4b and 4c). For the last 7 days of the propionate experiment, there were two groups of conditions that did not exhibit significant separation: the propionate control and the propionate and 500 mg/L of ammonium (p-value 0.37) and propionate with 1000mg/L of ammonium and 1500 mg/L of ammonium (p-value 0.24). If there were separation seen, this would mean there was a difference between these conditions. The propionate experiment exhibited significant separation between these two groups (p-value 0.01) (Fig. 4b). The butyrate experiment did not achieve significant separation, apart from butyrate and 500 mg/L of ammonium and 1000 mg/L of ammonium (p-value 0.02) (Fig. 4c).

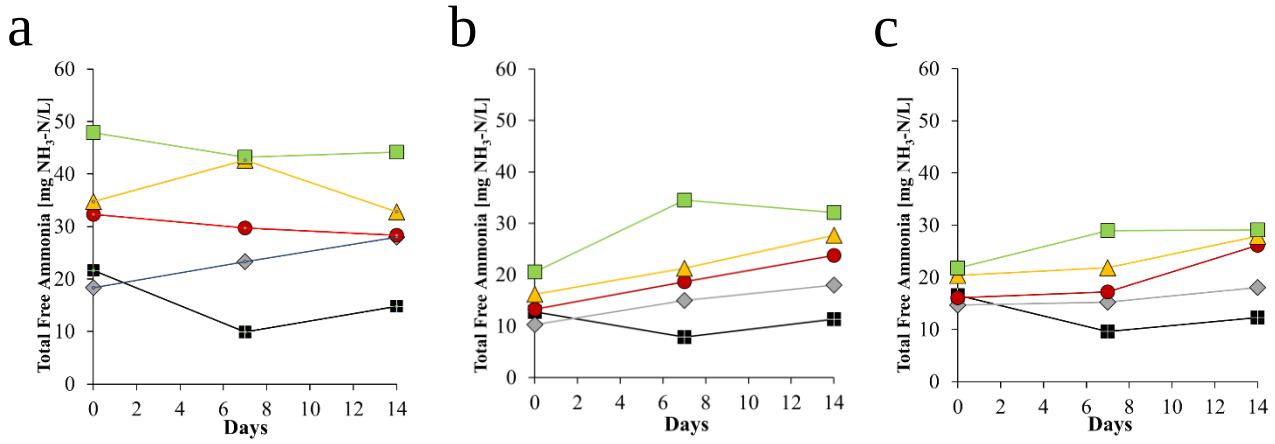

Fig. 5. *Total free ammonia for Gresham experiments on acetate (a), propionate (b) and butyrate (c) over the time of the experiment. The conditions consist of control with no volatile organic acid (■), volatile organic acid control (◆), volatile organic acid and 500 mg/L of ammonia (●), volatile organic acid and 1000 mg/L of ammonia (▲), and volatile organic acid and 1500 mg/L of ammonia (■). Error bars represent 95% confidence intervals.*

The acetate experiment had the highest amount of free ammonia, at 47.86 mg NH_3-N/L, for the 1500 mg/L of ammonium condition (Fig. 5a). The propionate and butyrate experiments had similar levels of free ammonia, propionate having the higher of the two at 34.49 mg NH3-N/L (Fig. 5b and 5c).

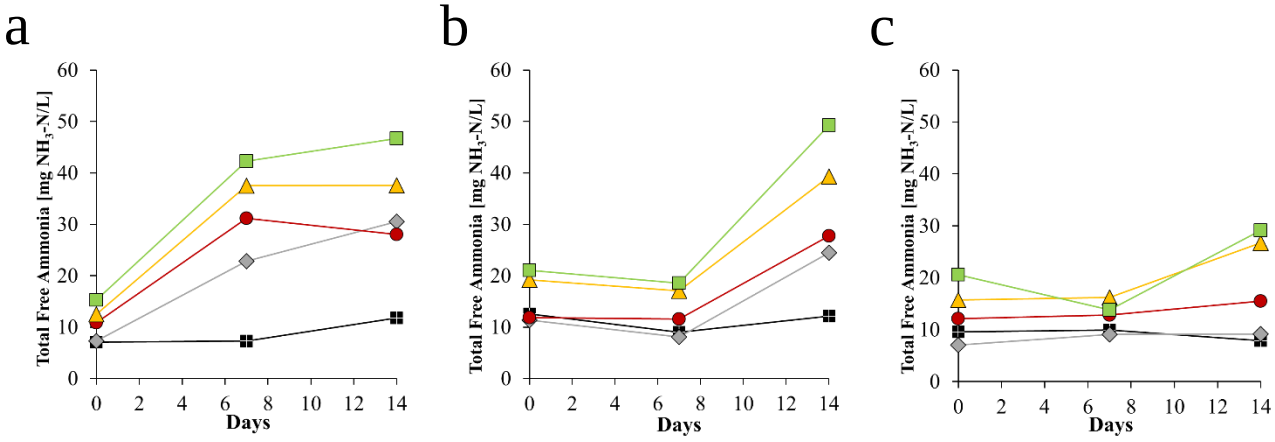

Fig. 6. *Total free ammonia for Corvallis experiments on acetate (a), propionate(b) and butyrate (c) over the time of the experiment[2]. The conditions consist of control with no volatile organic acid (■), volatile organic acid control (◆), volatile organic acid and 500 mg/L of ammonia (●), volatile organic acid and 1000 mg/L of ammonia (▲), and volatile organic acid and 1500 mg/L of ammonia (■). Error bars represent 95% confidence intervals.*

The total free ammonia for the Corvallis acetate experiment was considerably lower at the beginning of the experiment and then higher for the end of the experiment, peaking at 46.68 mg NH_3-N/L from 15.26 mg NH_3-N/L (Fig. 6a). For the propionate condition, the experiment started out with comparable results to the Gresham condition, but ended up being much higher for the 1500 mg/L of ammonium condition, at 49.24 mg NH_3-N/L (Fig. 6b and 5b). The levels of free ammonia for the Corvallis butyrate experiment were like its Gresham counterpart, but were in general slightly lower (Fig. 6c and 5c).

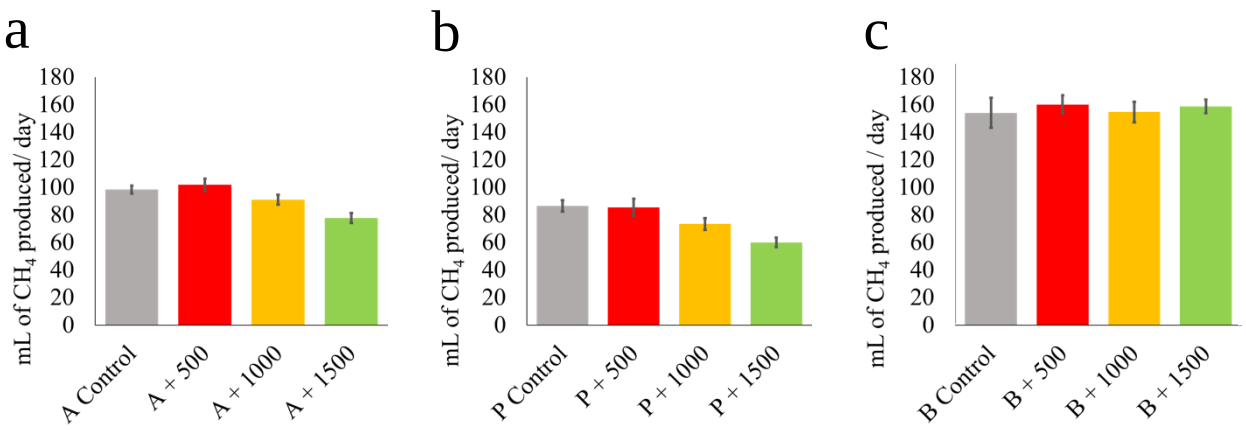

Fig. 7. *Starting rate of change for acetate (a), propionate (b), and butyrate (c) for the Gresham experiments. Error bars represent 95% confidence intervals.*

Starting rate of change was highest for the butyrate experiment. There was no significant difference seen in the rate of change for any of the conditions for the butyrate experiment (Fig. 7c). In the acetate experiment the acetate control condition and the condition with 500 mg/L of ammonium added were the only two conditions that had no significant separation (p-value 0.25) (Fig. 7a). Likewise, the propionate control condition and the propionate and 500 mg/L of ammonium added were the only two conditions that had no significant separation (p-value 0.77) (Fig. 7b).

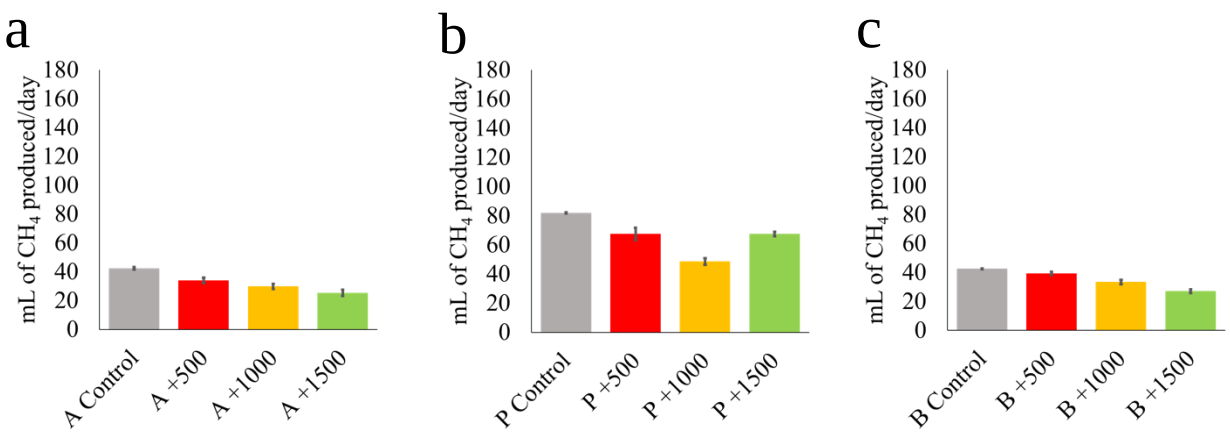

Fig. 8. *Starting rate of change for acetate (a), propionate (b), and butyrate (c) for the Corvallis experiments[2]. Error bars represent 95% confidence intervals.*

The acetate experiment had significant separation between all the conditions (Fig. 8a). The propionate and 500 mg/L of ammonium added, and the propionate and 1500 mg/L added conditions were the only ones of the propionate experiment that do not have significant separation (p-value 1) (Fig. 8b). The butyrate experiment had had significant separation between all conditions (Fig. 8c).

4. Discussion

On the second day of the acetate experiment, the acetate condition with 1500 mg/L of ammonium was significantly separated from all other conditions (p-values 1.43E-05, 2.65E-03, 1.36E-04, 7.90E-05) for cumulative methane. On the last day of the experiment, the cumulative methane did not achieve significant separation between the conditions, other than the condition with 1000 mg/L of ammonium added (p-values 0.01, 0.03, 5.7×10^{-3}) (Fig. 4a). The condition with 1000 mg/L of added ammonium had the lowest biogas produced, which could indicate the other conditions were not as affected by the ammonium added to it (Fig. 4a).

The free ammonia levels for acetate with 1000 mg/L of added ammonium reached 42.67 mg NH_3-N/L after seven days and at the end of the experiment, the same condition had a free ammonia level of 32.76 mg NH_3-N/L (Fig. 5a). This was not the highest level of free ammonia. The free ammonia levels of the acetate experiment reached 44.2 mg NH_3-N/L for the 1500 mg NH_4-N/L condition and produced about the same amount of gas as the acetate control, which had 28 mg NH_3-N/L (p-value 0.87) (Figures 4a and 5a).

Other studies found that there is 80% inhibition with 40 mg of free ammonia [19,24]. Although we did reach 40 mg of added free ammonia, those levels showed 0.24 ± 0.01% inhibition. The study cited calculated inhibition based on methanogenic activity, whereas our study measured inhibition on relative methane biogas produced[24]. Additionally, other studies have found inhibition to be at a much higher threshold[9]. It is possible that our experiments only measured the beginning stages of inhibition, and the actual inhibitory levels for added ammonium are higher than 1500 mg NH_4-N/L.

When referencing the starting rate of change for acetate, there is significant difference seen in all conditions except between the acetate control and acetate and 500 mg/L of ammonium added (p-value 0.25) (Fig. 7a). Additionally, the inhibition for the 1500 mg/L condition in the first two days was 21 ±1.1 % inhibition. However, the amount of methane produced in the end of the experiment was very similar between conditions, implying that the ammonia affected the beginning stages of the experiment the most.

The propionate experiment had the most signs of inhibition; there was significant separation between all conditions except between the propionate control and the propionate and 500 mg/L of added ammonium (p-value 0.3) and between the 1000 and 1500 mg/L of added ammonium concentration (p-value 0.38) (Fig. 4c). Propionate had the highest percent inhibition at the end of the experiment compared to the other two experiments. For the same 1500 mg/L condition, propionate had 6.1 ± 0.22% inhibition while acetate and butyrate had 0.24 ± 0.01% and 1.79 ±0.07% inhibition respectively.

The propionate experiment also produced the least amount of methane cumulatively, which could be because when accumulated, propionate is considered the least energetically favorable of the three VFAs[8]. The starting rate of change showed that propionate and 1500 mg/L of ammonium had significantly lower rate of change than propionate and 1000 mg/L (p-value 1.25E-3) at 60 mL/day and 73.39 mL/day respectively. Additionally, the 1000 mg/L condition was significantly lower than the 500 mg/L condition (p-value 0.02) (Fig. 7b). This shows separation from ammonia that was not seen in the other experiments, as the two conditions with highest amount of added ammonium were significantly separated from the rest of the conditions throughout the experiment.

In Ana Aranda's thesis[2], the results measured efficiency through overall biogas production. The conditions of her experiment were the same as this experiment and the VFA used for each experiment were also the same. However, the digestate used came from Corvallis OR, which does not use FOG in their wastewater treatment plant.

Although the added ammonium was the same for both experiments, the free ammonia concentrations varied. The Corvallis acetate experiment started with less free ammonia than the Gresham acetate experiment and ended with very similar levels of free ammonia (Fig. 7a and Fig. 8a). The 1500 mg NH_4-N/L condition reached 46.7 mg NH_3-N/L, while the same condition in the Gresham experiment ended at 44.2 mg NH_3-N/L[2].

The larger amount of gas produced could be due to the presence of residual FOG in the digestate from Gresham. In previous studies, residual FOG has been shown to have an impact on digestate methane production due to adaption from the microbial communities[27]. This may explain why Gresham performed relatively better than the Corvallis experiments, as the residual FOG may have been a selective pressure for *Bacteria* and *Archaea* that are less sensitive to ammonia inhibition.

The limitations of this study include the range of added ammonium concentration. Given other studies have measured ammonia inhibition starting at 500 mg and up to 1100 mg NH_3-N/L, the range in future studies could include more conditions with higher amounts of free ammonia[5,19]. Because adding free ammonia is done through adding ammonium, and is only an estimation, the expected range should aim to be higher than these values. The experiments would be the same other than the range of ammonium added, which would now include 7 conditions including the control and VFA control. The conditions with ammonium added would be 2000 mg/L, 2500 mg/L, 5000 mg/L, 7500 mg/L and 10000 mg/L of ammonium added. These levels of added ammonium, given an average of 1200 mg/L of ammonium in the digestate and a 7.2 pH, would potentially translate to free ammonia levels ranging from 10 mg/L to 89 mg/L NH_3-N/L. Although this range is lower than some literature values, it could provide more insight to when significant inhibition occurs.

Overall, the FOG co-digestion was more resilient to ammonia inhibition than digestate that did not use FOG co-digestion. The digestate was found to be largely uninhibited to levels of free

ammonia inhibition up to 50 mg NH_3-N/L, potentially due to the acclimation of the microbial community to the FOG added into the system at Gresham.

5. Appendix A: Supplementary Data

Additional Figures.pdf

6. Sources

1. Appels, L., Baeyens, J., Degrève, J., Dewil, R., 2008. Principles and potential of the anaerobic digestion of waste-activated sludge. Progress in Energy and Combustion Science 34, 755–781. https://doi.org/10.1016/j.pecs.2008.06.002
2. Index Catalog // ScholarsArchive@OSU [WWW Document], n.d. URL https://ir.library.oregonstate.edu/catalog?f%5Bcreator_sim%5D%5B%5D=Aranda%2C+Ana+A.&locale=en (accessed 11.13.20).
3. Astals, S., Peces, M., Batstone, D.J., Jensen, P.D., Tait, S., 2018. Characterising and modelling free ammonia and ammonium inhibition in anaerobic systems. Water Research 143, 127–135. https://doi.org/10.1016/j.watres.2018.06.021
4. Awe, O.W., Zhao, Y., Nzihou, A., Minh, D.P., Lyczko, N., 2018. Anaerobic co-digestion of food waste and FOG with sewage sludge – realising its potential in Ireland. International Journal of Environmental Studies 75, 496–517. https://doi.org/10.1080/00207233.2017.1380335
5. Dai, X., Duan, N., Dong, B., Dai, L., 2013. High-solids anaerobic co-digestion of sewage sludge and food waste in comparison with mono digestions: Stability and performance. Waste Management 33, 308–316. https://doi.org/10.1016/j.wasman.2012.10.018
6. Duan, N., Dong, B., Wu, B., Dai, X., 2012. High-solid anaerobic digestion of sewage sludge under mesophilic conditions: Feasibility study. Bioresource Technology 104, 150–156. https://doi.org/10.1016/j.biortech.2011.10.090
7. Li, C., Champagne, P., Anderson, B.C., 2015. Enhanced biogas production from anaerobic co-digestion of municipal wastewater treatment sludge and fat, oil and grease (FOG) by a modified two-stage thermophilic digester system with selected thermo-chemical pre-treatment. Renewable Energy 83, 474–482. https://doi.org/10.1016/j.renene.2015.04.055

8. Li, J., Ban, Q., Zhang, L., Jha, A.K., 2012. Syntrophic Propionate Degradation in Anaerobic Digestion: A Review. Int. J. Agric. Biol. 14, 9.
9. Liu, Y., Ngo, H.H., Guo, W., Peng, L., Wang, D., Ni, B., 2019. The roles of free ammonia (FA) in biological wastewater treatment processes: A review. Environment International 123, 10–19. https://doi.org/10.1016/j.envint.2018.11.039
10. Long, J.H., Aziz, T.N., Reyes, F.L. de los, Ducoste, J.J., 2012. Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. Process Safety and Environmental Protection, Special Issue on Energy from Waste 90, 231–245. https://doi.org/10.1016/j.psep.2011.10.001
11. Lora Grando, R., de Souza Antune, A.M., da Fonseca, F.V., Sánchez, A., Barrena, R., Font, X., 2017. Technology overview of biogas production in anaerobic digestion plants: A European evaluation of research and development. Renewable and Sustainable Energy Reviews 80, 44–53. https://doi.org/10.1016/j.rser.2017.05.079
12. Luther, A.K., 2015. Ammonia toxicity in bacteria and its implications for treatment of and resource recovery from highly nitrogenous organic wastes. Rutgers University - Graduate School - New Brunswick. https://doi.org/10.7282/T3668G53
13. McCarty, P.L., Smith, D.P., 1986. Anaerobic wastewater treatment. Environ. Sci. Technol. 20, 1200–1206. https://doi.org/10.1021/es00154a002
14. Niu, Q., Qiao, W., Qiang, H., Hojo, T., Li, Y.-Y., 2013. Mesophilic methane fermentation of chicken manure at a wide range of ammonia concentration: Stability, inhibition and recovery. Bioresource Technology 137, 358–367. https://doi.org/10.1016/j.biortech.2013.03.080
15. Rabii, A., Aldin, S., Dahman, Y., Elbeshbishy, E., 2019. A review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. energies 12, 1106. https://doi.org/10.3390/en12061106
16. Song, Y.-C., Kwon, S.-J., Woo, J.-H., 2004. Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic- and thermophilic digestion of sewage sludge. Water Research 38, 1653–1662. https://doi.org/10.1016/j.watres.2003.12.019
17. US EPA, R. 03, 2016. Types of Anaerobic Digesters [WWW Document]. US EPA. URL https://www.epa.gov/anaerobic-digestion/types-anaerobic-digesters (accessed 5.15.20).

18. Wang, H., Zhang, Y., Angelidaki, I., 2016. Ammonia inhibition on hydrogen enriched anaerobic digestion of manure under mesophilic and thermophilic conditions. Water Research 105, 314–319. https://doi.org/10.1016/j.watres.2016.09.006
19. Wang, Y.-T., Pai, P.-C., Latchaw, J.L., 1989. Effects of Preozonation on Anaerobic Biodegradability of O-Cresol. Journal of Environmental Engineering 115, 336–347. https://doi.org/10.1061/(ASCE)0733-9372(1989)115:2(336)
20. Wastewater Treatment Plant | City of Gresham [WWW Document], n.d. URL https://greshamoregon.gov/Wastewater-Treatment-Plant/ (accessed 4.14.20).
21. Yenigün, O., Demirel, B., 2013. Ammonia inhibition in anaerobic digestion: A review. Process Biochemistry 48, 901–911. https://doi.org/10.1016/j.procbio.2013.04.012
22. "Primary vs. Secondary Sludge." *ClearCove*, 24 Jan. 2014, http://www.clearcovesystems.com/primary-vs-secondary-sludge/.
23. "The Difference Between Primary and Secondary Wastewater Treatment." *Worm Farm Waste Systems*, 19 Feb. 2020, https://www.wormfarm.com.au/the-difference-between-primary-and-secondary-wastewater-treatment/.
24. Belmonte, M., Hsieh, C.-F., Figueroa, C., Campos, J.L., Vidal, G., 2011. Effect of free ammonia nitrogen on the methanogenic activity of swine wastewater. Electronic Journal of Biotechnology 14, 2–2.
25. Hottman, S., Oregonian, T., 2012. Gresham's wastewater treatment plant leading way in power production, alternative energies [WWW Document]. oregonlive. URL https://www.oregonlive.com/gresham/2012/07/greshams_wastewater_treatment.html (accessed 9.2.20).
26. Wastewater treatment - Primary treatment | Britannica [WWW Document], n.d. URL https://www.britannica.com/technology/wastewater-treatment/Primary-treatment (accessed 9.2.20).
27. Amha, Y.M., Sinha, P., Lagman, J., Gregori, M., Smith, A.L., 2017. Elucidating microbial community adaptation to anaerobic co-digestion of fats, oils, and grease and food waste. Water Research 123, 277–289. https://doi.org/10.1016/j.watres.2017.06.065
28. Wang, Q., Kuninobu, M., Ogawa, H.I., Kato, Y., 1999. Degradation of volatile fatty acids in highly efficient anaerobic digestion. Biomass and Bioenergy 16, 407–416. https://doi.org/10.1016/S0961-9534(99)00016-1

29. Excel Formula for t Test | Educational Research Basics by Del Siegle [WWW Document], n.d. URL https://researchbasics.education.uconn.edu/excel-formula-for-t-test/ (accessed 10.29.20).