# Effect of Fat, Oil, and Grease (FOG) and Inoculum Source on Co-digestion in Batch Anaerobic Digesters

by Ceili T. Shannon

# A THESIS

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Honors Baccalaureate of Science in Environmental Engineering (Honors Scholar)

> Presented August 24, 2015 Commencement June 2016

## AN ABSTRACT OF THE THESIS OF

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Abstract approved: \_\_\_\_\_

Tyler Radniecki

Anaerobic co-digestion of fats, oils, and grease (FOG) for increased methane (CH4) production has been of increasing interest to municipal wastewater treatment plants (WWTP) due to the potential economic benefit of using the produced biogas for cogeneration of energy. FOG loading increase must be done carefully to mitigate the risk for anaerobic digester upset or failure. Batch anaerobic digesters, containing inoculum collected from either Gresham or Corvallis WWTPs, were used to test the effects of 7 FOG sources (bakery, cherry brine, mayonnaise, mixed FOG, vitamin, "Westside", and yogurt wastes) at various FOG loadings on batch anaerobic digester performance. There were three goals of this study: (1) compare the ability of different FOG sources to increase biogas production and to find the optimal loading rate for each FOG source, (2) compare the success of inoculum from a facility with a FOG addition program (Gresham) with one that had never received FOG additions (Corvallis), (3) find signals of anaerobic digester upset through high FOG loadings. FOG addition was able to increase batch anaerobic digester biogas and CH4 productions by up to 425 % and 333 %, respectively. Higher FOG loadings as compared to lower FOG loadings were associated with lower pH and biogas and CH<sub>4</sub> yields, and increased risk of failure.

Key Words: Fats, Oil, and Grease (FOG), Anaerobic Digestion, Co-digestion, Methanogenesis

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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# Introduction

Increasing interest for alternate sources of energy production has prompted some municipal wastewater treatment plants (WWTP) to add fats, oils, and grease (FOG) to their anaerobic digesters for increased biogas, and thereby methane (CH4), production [1, 2]. Biogas is a byproduct of the solids stabilization process, and consists of carbon dioxide (CO<sub>2</sub>), CH<sub>4</sub>, and other trace gases [3]. The additional volatile solids (VS) contained in the FOG have been shown to increase production of biogas in small-scale batch tests, benchtop continuous digesters, and industrial practice [2, 4, 5, 6, 7].

Anaerobic digestion is a biological process commonly used to treat high organic municipal wastewater sludge [8], including primary solids (PS) and waste activated sludge (WAS). Its chief purpose is to reduce sludge volume and stabilize the wasted solids by reducing biological oxygen demand (BOD) as the microbes consume the VS present in the solids. VS reduction of combined influent PS and WAS ranges from 36 to 52 %, while VS reduction of combined influent PS, WAS and FOG ranges from 24 to 72 %, as observed in mesophilic benchtop continuous anaerobic digesters with hydraulic retention times ranging from 10 to 20 days [3, 9, 10, 11, 12]. Stabilization of the sludge occurs through a three step, syntrophic process: hydrolysis of substrate, acidogenesis, and then methanogenesis (**Figure 1**).

Facultative aerobic and anaerobic bacteria use water to break down lipids, proteins, and carbohydrates in complex biosolids and organics, including FOG. The smaller fatty acids can then be consumed by acid-forming bacteria to produce organic acids, mostly acetate formed by acetogens [3]. Methanogens use the organic acids to generate energy, and create biogas as a byproduct. The portion of anaerobically digestible substrate available can be estimated by VS content, though a portion of the VS are considered difficult to degrade. Approximately 80% of incoming organics are turned into biogas in a properly functioning digester and 60 to 70 % of that biogas is made up of CH4 [3, 5, 11].



*Figure 1:* Anaerobic digestion occurs in a three-step process. Unlike aerobic processes, where the bacterial consortium work in parallel, anaerobic digestion relies on this process to be completed in series [3, 8].

Collecting the biogas from WWTP anaerobic digesters is important for employee and community safety because CH<sub>4</sub> can be explosive at concentrations as low as 4.0 to 16.0 % by volume in air [13]. Combusting the CH<sub>4</sub> has gained an added importance to reduce the effect of greenhouse gas emissions, as CH<sub>4</sub> is a 25 times more potent greenhouse gas than CO<sub>2</sub> [14]. Traditionally, produced biogas has been simply flared to mitigate explosion risk [8]. Other facilities harness the heat generated by the combusted CH<sub>4</sub> to maintain appropriate anaerobic digester temperatures, while some have begun to use cogeneration to augment their energy needs [3, 8].

Cogeneration can be cost-prohibitive, particularly for smaller facilities. Economic feasibility can be improved if production of biogas, or more specifically CH<sub>4</sub>, can be increased. Biogas production has been estimated using VS reduction, with typical values ranging between 0.75 to 1.12 m<sup>3</sup>/kg VS destroyed [3, 8]. FOG wastes are high in VS

content, ranging from 17 to 93 % by weight [10, 11]. Thus, the goal of FOG addition is to increase biogas, and CH<sub>4</sub> by introducing more VS.

The Gresham, OR, WWTP has had great success in their FOG additions and new cogeneration system, which now provides 92 % of their power needs and an income of about \$250,000 per year from transporters for FOG disposal [15]. However, adding FOG to an anaerobic digester does not necessarily directly translate to an increase in biogas production. Anaerobic digesters are at risk of upset, as defined by an observed reduction in pH, biogas or CH<sub>4</sub> production/yield, CH<sub>4</sub> to CO<sub>2</sub> ratio, and/or overall VS destruction [3, 6]. Digester upset can lead to digester failure, where all appreciable biological activity ceases.

A sudden change in operational conditions, including FOG loading or type, could cause digester upset or failure [3, 5, 6], which would be costly, require a lengthy clean-up, and for the Gresham WWTP result in the accumulation of 67,000 gallons per day of untreated sludge from the sewage and wastewater generated by 114,000 residents [16, 17]. Thus, FOG must be added at controlled rates in order to simultaneously promote digester success, meet effluent solids requirements, and improve CH<sub>4</sub> generation [3, 5, 6].

Gas generation from an anaerobic digester can fluctuate drastically depending on: FOG constituents, VS feed rate, and the biological activity and type of the microbes [8]. Different FOG sources may have varying success at promoting biogas production, as the presence of nutrients and other constituents differs. Microbes require nutrients for their biological processes, so the addition of a nutrient rich FOG source may provide beneficial resources for the microbial community [18]. However, high concentrations of certain nutrients or other constituents can prompt digester upset through inhibition or toxicity [3, 19]. Inhibition is typically reversible and caused by constituents that reduce the microbe's ability to degrade the substrate, whereas toxicity or inhibition include: ammonia, heavy metals, alternate electron acceptors, cyanide, long-chain fatty acids (LCFA), and high volatile solids loading [3, 21, 11, 19]. The presence of toxic or inhibitory constituents does not necessarily mean the digester will experience failure [8].

LCFA result from the breakdown of high lipid biosolids, like FOG and have been linked to anaerobic digester upset [6, 22, 21, 22], though there are studies conflict on whether these issues are due to toxicity [23, 3, 24] or inhibition [11, 25, 26, 21]. There are multiple proposals as to why LCFA prompt digester upset. One proposed reason is that they reduce the microbe's tolerance to free acid as the LCFA structural similarity allows them to dissolve the microbes cell wall [3]. Another source observed two possible scenarios, depending on the LCFA source. First was that the microbes were being encapsulated in a hydrophobic layer. The encapsulated microbes were unable to absorb the nutrients as quickly and experienced a lag phase in their growth. However, after the encapsulation was reduced, and the rate of CH<sub>4</sub> production increased [25]. An alternate source of LCFA was observed to precipitate out as small white masses floating on the surface of the anaerobic digester inoculum [25]. The FOG separation makes the present VS inaccessible to the microbes, and therefore the potential biogas and methane yields are unachievable.

High VS concentration (i.e. from FOG addition) can also prompt digester upset through product inhibition. A common result of high VS concentration is an increased acid production rate that methanogenic archaea cannot match, resulting in the accumulation of free acid and a pH drop outside the optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25]. Methanogenic archaea are very pH sensitive, and methanogenesis typically stops below a pH of 6.0, whereas acidogenic bacteria can function at lower pH [3]. Thus, acetogens continue to produce acid, and the methanogens continue to be inhibited [6], allowing even more free acid to accumulate.

In the present study, FOG types sourced from a variety of industrial processes were tested to explore the effects of different potential FOG constituents. Wastes were namely: vitamin, bakery, yogurt, mayonnaise, maraschino cherries, and an undisclosed "Westside" waste. FOG additions were normalized by their VS content and added at increasing loading rates to inoculum in batch reactors. Biogas and CH<sub>4</sub> volumes, and pH were measured throughout the 14 day experiments, and were used to explore optimal VS loading rates and inhibition characteristics of each individual FOG.

Anaerobic digester inoculum source was another variable considered. The microbial consortia present can greatly affect anaerobic digester success [27], and the microbial consortia within an anaerobic digester has been shown to shift in response to changes in operating conditions, sources of added FOG, and FOG loading rates [3, 28, 29]. A sudden change in VS loading can prompt digester failure [3, 5, 6], but a gradual increase in VS loadings has been linked to improved digester acclimation and reduced inhibition [5, 30], signifying that acclimated anaerobic digesters are able to accept higher toxic loadings if allowed time to adjust [3, 30] and have an increased tolerance for substrate loading increases if previously exposed [31, 26, 21]. Some of the changes undergone by an anaerobic digester's microbial consortia during acclimation may be associated with physiological changes as opposed to changes in the microbial types [26]. FOG tests were conducted on inoculum collected from two facilities: one that adds FOG, and one that does not. Gresham's anaerobic digesters had been receiving FOG for over a year, and therefore had an opportunity to acclimate to the influent FOG, whereas Corvallis' anaerobic digesters had not been receiving FOG additions. The inoculums' responses to FOG additions were tested to explore the effect of inoculum source on digester success.

Finding alternate sources of energy is important for maintaining modern life. The Gresham WWTP used to be one of the largest consumers of energy from the city grid; now, they have the potential of producing energy in excess of their needs using their anaerobic digesters, if appropriate FOG loading rates can be achieved. The risk of upsetting their two-100,000 gallon anaerobic digesters through addition of FOG prompted Gresham WWTP to approach Oregon State University regarding optimal VS loading rates and the potential of increasing their CH4 production. This work will provide Gresham WWTP information regarding their current FOG loading rate efficiency. It will also be useful to all WWTP currently adding or considering adding FOG to their anaerobic digesters for increased CH4 production by analyzing the comparative success of different types of FOG.

# Methods

### Anaerobic Digester Inoculum Preparation

Anaerobic digester inoculum was collected from Oregon wastewater treatment facilities in Gresham and Corvallis. Several trips to both facilities for inoculum pick-up were made throughout the testing period. Gresham anaerobic digesters had been receiving FOG additions for a year, whereas Corvallis anaerobic digesters had not been receiving FOG. Inoculum was collected in 4 L jugs with a stir bar in the bottom and were sealed with a screw-on lid. The high rate of biogas production from fresh inoculum during transport from the WWTPs necessitated the pressure in the jug be periodically relieved en route to the lab.

In the lab, inoculum jugs were sealed using Parafilm and a rubber cork with two ports, one ending at the base of the jug (Port 1), the other ending in the headspace (Port 2). Silicone tubes were attached to the external end of each port and sealed with clamps to prevent oxygen infiltration. Inoculum was manually shaken to homogenize the mixture before being mixed with a stir plate. The tube ending in the inoculum jug headspace was fed into an inverted graduated cylinder filled with water to collect biogas (**Figure 2**).



**Figure 2**: Excess VS removal was completed by keeping the inoculum jug contents in anaerobic conditions using a rubber cork and tubing attached with two ports and allowing approximately two weeks to consume remaining substrate. A graduated cylinder was inverted and a stand kept the lip of the graduated cylinder from the base of a tub of water to allow gas from Port 2 to be collected for daily measurements.

The volume of biogas produced was measured daily via gas displacement for approximately two weeks, or until biogas production ceased. Excess VS removal was conducted to ensure that the measured inoculum VS concentration represented the bacterial population present and not the residual substrate from the WWTP. New inoculum was collected at a minimum of every few months to verify potency and mitigate error associated with microbial consortium die-off. Prepared inoculum was stored at 4 °C to reduce microbial activity during storage in between experiments.

## Anaerobic Digester Substrates

#### Sources

PS and WAS were collected once from the Gresham Wastewater Treatment plant and used throughout all experiments. FOG types were collected from various industrial processes, and provided by the producing facility or via Gresham WWTP. No special preparation of substrates was required. Substrates were stored in the cold room at approximately 4 °C.

The various FOG sources tested were:

- Bakery Waste
- Maraschino Cherry Brine
- Mayonnaise Waste
- Mixed FOG (from mixed collection tank at Gresham facility)
- Vitamin Waste
- Westside Manufacturing Waste
- Yogurt Waste

#### Characterization

All substrates (FOG, WAS and PS) were characterized for total solids (TS), volatile solids (VS), and pH density. Substrates were shaken to ensure a homogeneous mixture, then approximately 20 mL of substrate was aliquoted for characterization. Substrate pH was measured using a Fisher Scientific accumet benchtop pH/ion meter, model 25. TS and VS concentrations were measured as

described below. VS concentration for substrates was assumed representative of CH<sub>4</sub> production potential.

Three clean crucibles per prepared inoculum were heated in a combustion oven at 500 °C for 45 minutes to remove any present VS. Empty crucibles were allowed to cool to room temperature in a desiccator, then massed ( $M_{cru}$ ). Liquid samples of 5 mL ( $V_{samp}$ ) were placed into each crucible. The combined mass of crucible and wet sample ( $M_{cru,wet}$ ) was measured, allowing for the calculation of inoculum density ( $\rho_{inoc}$ ) (Equation 1).

$$\rho_{inoc} = (M_{cru,wet} - M_{cru}) / V_{samp}$$
(1)

The crucibles and samples were dried at 108 °C overnight. The dried samples were massed ( $M_{cru,dry}$ ), and used to calculate TS (**Equation** Error! Reference source not found.).

$$TS = (M_{cru,dry} - M_{cru}) / V_{samp}$$
(2)

The samples were then placed in a combustion oven for 45 minutes at 550 °C, allowed to cool in a desiccator for 20 min, and then massed ( $M_{cru,comb}$ ). The combusted mass was used to calculate VS (**Equation 2**).

$$VS = (M_{cru,comb} - M_{cru}) / V_{samp}$$
(2)

## Inoculum Characterization

The inoculum's VS and TS concentrations and pH were measured as described above. The inoculum jug was manually shaken to homogenize the mixture prior to sample removal. The anaerobic conditions in the inoculum jug were maintained by attaching a 120 mL syringe to Port 1 before removing the clamps from the rubber tubes that sealed the inoculum jug.

Approximately 120 mL of inoculum was extracted by the syringe then re-injected into the jug to verify homogenization. An inoculum sample of 20 mL was removed for

characterization. The VS concentration for prepared inoculum was assumed representative of the biomass present.

## Batch Anaerobic Digester Construction

Batch experiments were conducted using 155 mL bottles with rubber septa lids and contained PS, WAS, FOG, and WWTP anaerobic digester inoculum (**Figure 3**). Batch anaerobic digesters contained a specific ratio of constituents (Table 1).

This ratio was based off of volumetric flow information provided by Gresham, and the VS content of their mixed FOG used for co-digestion (complete calculations presented in **Appendix A**, Table 6 and Table 7). Inoculum, WAS, and PS were normalized by their volume, whereas FOG loading was normalized by VS concentration as different FOG sources have different VS content. WAS and PS used throughout the experiments was from a single collection from the Gresham WWTP.



*Figure 3: Batch Reactor anaerobic digesters contained potentially 4 constituents: Inoculum, WAS, PS, and FOG.* 

Constituent	Set-Up Ratio					
Constituent	Value					
Innoculum	92.7 mL					
WAS	4.2 mL					
PS	2.5 mL					
FOG	n (7.5) mg VS					
* Note: n is the desired multiple						
that accounts for changes in FOG						

**Table 1:** Anaerobic digesters were set-up based on a ratio of inoculum to substrates. Most of the anaerobic digester components were calculated based on their volume, but FOG sources were normalized by their VS content.

loading

Appropriate volumes of reactor substrates and inoculum were added to the bottle, as based on the constituent ratio for the desired FOG loading (see **Table 2** for average anaerobic digester constituents). The batch anaerobic digesters was sealed and purged with N<sub>2</sub> gas for 10 minutes to remove O<sub>2</sub>. The jug of inoculum was removed from the cold room, and manually shook to promote a homogeneous mixture. A 120 mL syringe was attached to Port 1. The sealing clamps were removed from Port 1, the desired volume of inoculum removed from the jug, and clips replaced. The periodic addition of N<sub>2</sub> gas to the inoculum jug was required as large volume removal of inoculum caused a caving of the jug sides.

**Table 2:** Anaerobic digesters were set up based on calculations specific to the desired FOG loading. An average of components for all conducted batch anaerobic digester tests at all FOG loadings using both Gresham and Corvallis inoculum is presented with 95 % confidence.

	Volume [mL]			VS [mg]		
	Average	Average 95 % Conf		Average	95 % Conf	
Inoculum	89.5	2.2		1369	82	
WAS	4.1	0.1		105	5	
PS	2.4	0.1		81	2	
FOG	4.6	2.4		287	106	
Total	100.7	1.4		1842	107	

The batch anaerobic digesters were quickly opened, and the inoculum was immediately injected before the anaerobic digesters were resealed and purged with N<sub>2</sub> gas for another

10 minutes. Bottle was opened, inoculum quickly injected inside, bottle reclosed, then purged with nitrogen for 10 minutes. Once all of the batch anaerobic digesters were constructed, an IM1 needle was attached to a lever-lock valve and a gas tight syringe was used to remove excess gas from the system. The needle was inserted into the batch anaerobic digesters which allowed the excess gas causing internal pressure to freely enter the syringe until atmospheric pressure was reached. The needle was removed and the batch anaerobic digesters were sealed with Parafilm.

### Batch Anaerobic Digester Operation

#### Incubation

The batch anaerobic digesters were stored inverted onto a shaker table and were shook at 100 rpm in the dark at 35 °C. The batch anaerobic digesters were incubated for 14 days to represent a typical anaerobic digester solids retention time [3, 9, 10, 11]. Biogas was generally measured daily, while pH was measured every 3 days. Time between biogas samplings were often adjusted if biogas production was particularly high or low.

#### Biogas Characterization

The produced biogas volume was measured using a IM1 needle attached to a valve and a gas-tight syringe, as described in previous section. Thin needles were used to prevent gas escape from septa. If gas production was greater than 20 mL, the valve was closed as the syringe reached near max volume, emptied and then repeated until all biogas was removed.

#### *CH*<sup>4</sup> and *CO*<sub>2</sub> *Measurements*

Between 4 and 20 mL of biogas, depending on volume biogas produced by reactor, was injected into a sealed 155 mL bottle containing 100 mL of 10 N NaOH. Contents were shaken to precipitate out  $CO_2$  as carbonate ( $CO_3^{2-}$ ). The volume of remaining gas, assumed primarily CH<sub>4</sub>, was measured using the gastight syringe as described above.

### pH Measurements

The pH of each reactor was sampled approximately every 3 days, and on day 14 when reactors were decommissioned. Each reactor was gently shaken, inverted, and 2 mL of reactor contents were removed using a 16 G1/2 " needle and 5 mL syringe. The sample was injected into a clean, 50 mL centrifuge tube. A calibrated pH probe was used to find the pH of each batch reactor sample.

#### Final VS Content Measurements

At the end of each experiment, selected batch anaerobic digesters were tested for total suspended volatile solids (TSVS). TSVS was measured to estimate remaining undigested substrate present in the batch anaerobic digesters for the calculation of the anaerobic digesters substrate utilization rate.

The total suspended solids (TSS) from 60 mL mixed liquor ( $V_{MLSS}$ ) sample was pelleted via centrifugation at 1000 rpm for 20 minutes. The supernatant was decanted and its volume recorded ( $V_{sup}$ ). The separated SS were diluted with 15 mL ( $V_{dil}$ ) of deionized (DI) water. The VS of the TSS was measured as described above. The calculation of TSVS using masses was adjusted to account for water addition (**Equation 4**).

$$TSVS = (M_{cru,comb} - M_{cru}) / V_{samp} * (V_{dil} + V_{MLSS} - V_{sup}) / V_{sup}$$
(3)

#### Data Analysis

## *Loading Rate [g VS<sub>FOG</sub> / L inoculum]*

The mass of VS added via a FOG source was divided by the volume of inoculum used to co-digest the FOG loading. The set-up of anaerobic digesters often required slight variation in constituent volumes (see **Appendix A**). These variations in constituent volume were to account for the limited volume of the 155 mL batch anaerobic digesters. If inoculum volume was maintained throughout all tested FOG loadings, the total volume of batch anaerobic digesters at the highest loadings would be greater than 155 mL. The calculated FOG loading rate used

was to normalize loadings based on the constituent ratio used to set up each of the batch anaerobic digesters.

#### (Specific) Cumulative Biogas and CH<sub>4</sub> productions [L / g VS<sub>inoculum</sub>]

The cumulative biogas and CH<sub>4</sub> productions at 14.0 days was divided by the initial VS content of the anaerobic digester inoculum used during digester set-up to account for the potentially different bioactivities.

# (Specific) Biogas and CH<sub>4</sub> Yields [mL / g VS<sub>inoculum</sub>/g VS<sub>substrate</sub>]

The cumulative biogas and CH<sub>4</sub> production values were divided by the mass of VS added via PS, WAS, and FOG additions to the anaerobic digester.

#### $CH_4$ content, or $CH_4$ to Biogas Ratio [V/V]

During biogas testing, between 3 and 20 mL of biogas was injected into a 10 N NaOH solution and manually inverted to remove CO<sub>2</sub> from the biogas, and the volume of the remaining gas recorded. The volume of the remaining gas was divided by the injected gas volume to get a volume to volume ratio of CH<sub>4</sub> to biogas.

# Results

Anaerobic Digester Constituents Characterization

# Inoculum

The 2.5 year testing period was split into two parts, the first half focusing on Gresham inoculum, and the second focusing on Corvallis inoculum. Anaerobic digester inoculum was collected a total of 6 times from the Gresham WWTP and 4 times from the Corvallis WWTP. The density, TS content and VS content measured was measured for each inoculum collection. **Table 3** presents the average results from the characterization of the Gresham and Corvallis inoculums.

*Table 3:* Inoculum collected at different times had differing characteristics. The average density, TS, and VS are presented.

	pH	g/L					
Inoculum		Density		TS		VS	
		AVG	95% conf	AVG	95% conf	AVG	95% conf
Gresham	7.39	1142	163.9	28	3.6	17	2.4
Corvallis	7.2	1039	65.1	35	23.0	14	1.9

# Substrate

The focus of this study was to review how the different FOG sources would affect digester performance. PS, WAS, and all FOG sources used for co-digestion were analyzed for density, and TS and VS content (**Table 4**).

*Table 4:* Substrate characteristics are presented. Density, TS content, and VS content were measured in triplicate, so averages and 95 % confidence interval is presented.

	pН	[g/L]						
Substrate		Density		TS			VS	
		AVG	95% conf	AVG	95% conf	AVG	95% conf	
PS	5.14	953	14.9	38	3.4	34	2.1	
WAS	6.02	983	2.8	40	0.3	29	0.2	
Bakery		1004	6.7	172	1.5	170	1.5	
Cherry Brine	3.87	1044	16.2	110	1.9	75	1.5	
Mayonnaise		953	13.8	221	7.4	215	7.4	
Mixed FOG	4.88	991	24.7	13	0.7	12	0.7	
Vitamin	5.63	1046	0.0	209	0.1	189	0.2	
Westside		964	0.8	103	1.2	95	1.2	
Yogurt	5.83	966	8.6	93	1.6	85	1.5	

\* Note: TS = Total Soids; VS = Volatile Solids

WAS = Waste Activated Sludge; PS = Primary Solids

#### Bakery Waste

The highest loading tested, 1.6 g VS<sub>FOG</sub>/L inoculum, increased the cumulative biogas production by 69 % (13.9  $\pm$  0.03 L/g VS<sub>inoculum</sub>) (**Figure Figure 4a** and **b**), CH<sub>4</sub> production by 57 % (0.15  $\pm$  0.02 L/g VS<sub>inoculum</sub>) (*Figure 4b*), and decreased the CH<sub>4</sub> to biogas ratio by 7.4 % (0.73 V/V) (*Figure 4d*), as compared to control. The highest biogas and CH<sub>4</sub> yield (0.65  $\pm$  0.02 L/g VS<sub>inoc</sub>/g VS<sub>sub</sub> and 0.49  $\pm$  0.01 L/g VS<sub>inoc</sub>/g VS<sub>sub</sub>, respectively) were 11 % and 9.8 % higher than the control and observed from 0.81 g VS<sub>FOG</sub>/L inoculum loading (*Figure 4e* and **f**). CH<sub>4</sub> to biogas ratios ranged from 73 to 79 %, with a 79 % CH<sub>4</sub> content being observed in the control and the lowest tested loading (0.0 g VS<sub>FOG</sub>/L inoculum and 0.08 g VS<sub>FOG</sub>/L inoculum) (*Figure 4d*). Loading concentrations had a negligible effect on pH, though a slight initial pH drop was observed for all sets at 3.8 days (the first pH test after experimental set-up) but all quickly recovered (*Figure 4c*).

#### Cherry Brine

The highest loading tested, 1.6 g VS<sub>FOG</sub>/L inoculum, increased the cumulative biogas production by 49 % (0.17 ± 0.01 L / g VS<sub>inoculum</sub>) (*Figure* 5a and v), CH4 production by 30 % 0.12 ± 0.00 L / g VS<sub>inoculum</sub>) (*Figure* 5b), and decreased the CH4 to biogas ratio by 13 % (72 % CH4 V/V) (*Figure* 5d), as compared to control. The highest biogas and CH4 yields (0.56 ± 0.00 and 0.46 ± 0.01 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>, respectively) were observed in the control (0.0 g VS<sub>FOG</sub>/L inoculum loading) and all FOG loadings resulted lower biogas and CH4 yields as compared to the control (*Figure* 5f). Biogas and CH4 yields were not linearly correlated with cherry brine loading (*Figure* 5e). CH4 to biogas ratios ranged from 72 to 83 %, the highest observed recorded at 0.08 g VS<sub>FOG</sub>/L inoculum. There was a negligible effect on pH, though all sets had lower pH values on day 3, though all stayed above pH 6.5 (Figure 5c).

#### Mayonnaise Waste

Batch anaerobic digesters with small mayonnaise waste loadings showed increased cumulative gas production and yields for both biogas and CH<sub>4</sub>, as compared to the control (Figure 6b). A loading of 0.81 g VS<sub>FOG</sub>/L inoculum prompted the highest biogas production (Figure 6a), increasing cumulative biogas production by 47 % (0.22  $\pm$  0.02), CH<sub>4</sub> production by 35 % (0.16  $\pm$  0.03 L / g  $VS_{inoculum}$  (Figure 6b), and decreased the CH<sub>4</sub> to biogas ratio by 8 % (74 % CH<sub>4</sub>) (Figure 6d), as compared to the control. The highest biogas yield was also recorded for the 0.81 g VS<sub>FOG</sub>/L inoculum, at 7.6 % increase  $(0.79 \pm 0.06L/g$  $VS_{inoc}$  / g VS<sub>sub</sub>) (Figure 6e), and CH<sub>4</sub> yields were only reduced by 1 % (0.58 ± 0.09 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>) (Figure 6f). Gas production and yields were not linearly correlated to loading, and instead peaked at the  $0.81 \text{ g VS}_{FOG}/L$  inoculum and thereafter decreased as loading was increased (Figure 6f). The CH<sub>4</sub> to biogas ratios ranged from 65 to 80 % with the highest ratio observed at  $0.81 \text{ g VS}_{FOG}/L$ inoculum. Although the sets dropped slightly from initial pH, all recorded pH values did not deviate outside of the optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25], the final pH was inversely proportional to loading (Figure 6c).

## Mixed FOG

A loading of 0.86 g VS<sub>FOG</sub>/L inoculum prompted the highest cumulative biogas and CH<sub>4</sub> production with an increase of 72 % and 154 %, respectively (0.27  $\pm$ 0.02 and 0.15  $\pm$  0.02 L/g VS<sub>inoculum</sub>, respectively) \ (**Figure 7a** and **b**). Similarly, the biogas yield increased by 26 % (0.96  $\pm$  0.01 L/g VS<sub>inoc</sub>/g VS<sub>sub</sub>), while the CH<sub>4</sub> yield increased by 87 % (0.54  $\pm$  0.02 L/g VS<sub>inoc</sub>/g VS<sub>sub</sub>) (**Figure 7e** and **f**). The CH<sub>4</sub> to biogas ratio increased by 48 % (56 % CH<sub>4</sub> V/V) (**Figure 7d**). The CH<sub>4</sub> to biogas ratios ranged from 38 to 70 %, and the highest ratio was observed from the 2.42 g VS<sub>FOG</sub>/L inoculum loading (**Figure 7d**). The recorded pH did not deviate outside of optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25] for any of the batch anaerobic digesters (**Figure 7c**).

#### Vitamin Waste

The highest vitamin waste loading of 13.0 g VS<sub>FOG</sub>/L inoculum increased cumulative biogas production by 291 % (0.65  $\pm$  0.11 L / g VS<sub>inoculum</sub>) (Figure 8a), CH<sub>4</sub> production by 254 % (0.41  $\pm$  0.12 L / g VS<sub>inoculum</sub>) (Figure 8b), and decreased CH<sub>4</sub> to biogas ratio by 9 % (64 % CH<sub>4</sub> V/V) (Figure 8d), as compared to control. The biogas and CH<sub>4</sub> yields were also decreased by 41 % and 45 %, respectively (0.46  $\pm$  0.01 and 0.29  $\pm$  0.02 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>, respectively) (Figure 8b). The highest biogas and CH<sub>4</sub> yields (0.65  $\pm$  0.11 and 0.46  $\pm$  0.07 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>, respectively) were observed from the controls with 0.0 g VS<sub>FOG</sub>/L inoculum. The CH<sub>4</sub> to biogas ratios ranged from 64 to 74 %, the highest ratio observed ratio from lowest loading (0.08 g VS<sub>FOG</sub>/L inoculum), and lowest observed ratio from highest loading (13.0 g VS<sub>FOG</sub>/L inoculum) (Figure 8e and f). Vitamin waste loading had a negligible effect on pH range observed (Figure 8c).

#### Westside Waste

The highest Westside waste loading of  $1.54 \text{ g VS}_{FOG}/L$  inoculum increased cumulative biogas production by 72 % ( $0.21 \pm 0.01 \text{ L} / \text{g VS}_{\text{inoculum}}$ ), and CH<sub>4</sub> production by 71 % ( $0.15 \pm 0.01 \text{ L} / \text{g VS}_{\text{inoculum}}$ ) (Figure 9a and b). This high load had a CH<sub>4</sub> to biogas ratio (73 % CH<sub>4</sub> V/V) and biogas and CH<sub>4</sub> yields ( $0.44 \pm 0.02 \text{ L} / \text{g VS}_{\text{inoc}} / \text{g VS}_{\text{sub}}$ , respectively) comparable to the control (Figure 9d-f). Westside waste loading had a negligible effect on pH range observed, all sets staying near neutral (Figure 9c). Biogas yields fell by 2.1 %, while CH<sub>4</sub> yields fell by 4.8 % range. The CH<sub>4</sub> to biogas ratios ranged from 73 to 76 %.

#### Yogurt Waste

The highest yogurt waste loading tested, 1.61 g VS<sub>FOG</sub>/L inoculum, increased the cumulative biogas by 40 % production  $(0.19 \pm 0.01 \text{ L}/\text{ g VS}_{inoculum})$  (Figure 10a and b), CH<sub>4</sub> production by 40 %  $(0.15 \pm 0.00 \text{ L}/\text{ g VS}_{inoculum})$  (Figure 10b), and increased the CH<sub>4</sub> to biogas ratio by 1 % (78 % CH<sub>4</sub> V/V) (Figure 10d), as compared to control. Biogas and CH<sub>4</sub> yields were also decreased by 20 % and 19 %, respectively  $(0.53 \pm 0.02 \text{ and } 0.42 \pm 0.01 \text{ L}/\text{ g VS}_{inoc}/\text{ g VS}_{sub})$  (Figure 10e and f). Gas yields were inversely correlated to loading with the highest biogas

and CH<sub>4</sub> yields ( $0.57 \pm 0.11$  and  $0.38 \pm 0.06$ ) being observed from the controls ( $0.0 \text{ g VS}_{FOG}/L$  inoculum). CH<sub>4</sub> to biogas ratios ranged from 77 to 78 %, and were not correlated to yogurt waste loading (**Figure 10d**). Yogurt waste loading in the tested range had a negligible effect on pH (**Figure 10c**).



*Figure 4:* Bakery waste in Gresham inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 5:* Cherry Brine in Gresham inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 6:* Mayonnaise waste in Gresham inoculum results, including (*a*) cumulative biogas; (*b*) cumulative biogas percent difference from control; (*c*) pH; (*d*)  $CH_4$  to biogas ratio; (*e*) biogas and  $CH_4$  yields; and (*f*) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



**Figure 7:** Mixed FOG in Gresham inoculum results, including (a) cumulative biogas (note y-axis bounds); (b) cumulative biogas percent difference from control; (c) pH; (d) CH<sub>4</sub> to biogas ratio; (e) biogas and CH<sub>4</sub> yields (note y-axis); and (f) biogas and CH<sub>4</sub> yields percent difference from control. Error bars represent 95 % confidence.



**Figure 8:** Vitamin waste in Gresham inoculum results, including (a) cumulative biogas (note y-axis bounds); (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



**Figure 9:** Westside waste in Gresham inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 10:* Yogurt waste in Gresham inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.
### Bakery Waste

The highest loading at 18.2 g VS<sub>FOG</sub>/L inoculum resulted in a cumulative biogas production increase of 72 % ( $0.20 \pm 0.02 \text{ L}/\text{g} \text{VS}_{\text{inoculum}}$ ) (Figure 11a and b), a cumulative CH<sub>4</sub> production increase of 22 % ( $0.09 \pm 0.00 \text{ L}/\text{g} \text{VS}_{\text{inoculum}}$ ) (Figure 11b), and a 28 % reduction in CH<sub>4</sub> to biogas ratio (44 % CH<sub>4</sub> V/V) (Figure 11d) as compared to the control. This loading was less successful than 13.1 g VS<sub>FOG</sub>/L inoculum loading, which increased cumulative biogas production by 225 % ( $0.38 \pm 0.04 \text{ L}/\text{g} \text{VS}_{\text{inoculum}}$ ), CH<sub>4</sub> production by 152 % ( $0.18 \pm 0.04 \text{ L}/\text{g} \text{VS}_{\text{inoculum}}$ ), and decreased CH<sub>4</sub> to biogas ratio by 23 %, ( $0.48\% \text{ CH}_4 \text{ V/V}$ ) as compared to the control.

The highest biogas and CH<sub>4</sub> yields ( $0.72 \pm 0.06$  and  $0.45 \pm 0.12$  L/g VS<sub>inoc</sub>/g VS<sub>sub</sub>) were observed from the control, with 0.0 g VS<sub>FOG</sub>/L inoculum loading, and were inversely correlated to loading (**Figure 11e** and **f**). The CH<sub>4</sub> to biogas ratios ranged from 44 to 62 %. The batch anaerobic digesters containing the two highest FOG loadings had recorded pH values (5.9 and 5.0) that were lower than the optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25] (**Figure 11c**), the CH<sub>4</sub> to biogas ratios (48 and 44 % CH<sub>4</sub> V/V) fell below reported typical range of 60 to 70 % CH<sub>4</sub> by volume [3], and the biogas production rate decreased or stopped during the 14 day testing period. The batch anaerobic digester loaded at 13.1 g VS<sub>FOG</sub>/L inoculum began to recover, returning to a pH above 6.5 after 11.83 days. All batch anaerobic digesters tested had an initial pH drop, recorded at 5.13 days (first test after set-up), though control tended to stay closest to neutral throughout experiment.

## Cherry Brine

The highest cherry brine loading, with 1.8 g VS<sub>FOG</sub>/L inoculum, increased the cumulative biogas production by 74 % ( $0.19 \pm 0.00 \text{ L} / \text{g VS}_{\text{inoculum}}$ ) (Figure 12a and b), CH<sub>4</sub> production by 51 % ( $0.13 \pm 0.00 \text{ L} / \text{g VS}_{\text{inoculum}}$ ) (Figure 12b), and decreased the CH<sub>4</sub> to biogas ratio by 13 % (66 % CH<sub>4</sub> V/V) (Figure 12d), as

compared to the control. The highest biogas and CH<sub>4</sub> yields ( $0.64 \pm 0.08$  and  $0.50 \pm 0.13 \text{ L}/\text{g VS}_{inoc}/\text{g VS}_{sub}$ , respectively) were observed from the control anaerobic digesters with 0.0 g VS<sub>FOG</sub>/L inoculum loading (**Figure 12e** and **f**). CH<sub>4</sub> to biogas ratios ranged from 66 to 76 % with the highest being observed from 0.0 g VS<sub>FOG</sub>/L inoculum, and were inversely correlated to loading (**Figure 12d**). Cherry brine loading had a negligible effect on pH range observed, though the pH for all anaerobic digesters dropped slightly from the initial pH and quickly recovered (**Figure 12c**).

#### Mayonnaise Waste

The highest cumulative biogas and CH<sub>4</sub> productions (0.17  $\pm$  0.01 and 0.15  $\pm$  0.06 L / g VS<sub>inoculum</sub>, respectively) from mayonnaise waste were observed at the 1.61 g VS<sub>FOG</sub>/L inoculum loading, which resulted in a 139 % biogas production increase and a 174 % CH<sub>4</sub> production increase (**Figure 12a** and **b**). The CH<sub>4</sub> to biogas ratio increased by 15 % (88 % CH<sub>4</sub> V/V) as compared to control (**Figure 12d**). The highest biogas and CH<sub>4</sub> yields (0.48  $\pm$  0.03 and 0.43  $\pm$  0.19 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>) were observed from the batch anaerobic digesters loaded with 1.61 g VS<sub>FOG</sub>/L inoculum (**Figure 12e** and **f**). The biogas and CH<sub>4</sub> yields were not linearly correlated to mayonnaise waste loading, peaking at a mayonnaise waste loading of 1.61 g VS<sub>FOG</sub>/L inoculum. The CH<sub>4</sub> to biogas ratios ranged from 24 to 88 % with the highest being observed from the control with 0.0 g VS<sub>FOG</sub>/L inoculum, and was inversely correlated to mayonnaise waste loading. Anaerobic digesters at higher loading prompted the pH to deviate outside of the optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25] (**Figure 12c**).

### Mixed FOG

The lowest mixed FOG loading of 0.86 g VS<sub>FOG</sub>/L inoculum increased cumulative biogas production by 55 % (0.16  $\pm$  0.02 L / g VS<sub>inoculum</sub>) (**Figure 14a** and **b**), CH<sub>4</sub> production by 113 % (0.11  $\pm$  0.01 L / g VS<sub>inoculum</sub>) (**Figure 14b**), and increased the CH<sub>4</sub> to biogas ratio by 38 % (65 % CH<sub>4</sub> V/V) (**Figure 14d**), as compared to control. The highest observed biogas and CH<sub>4</sub> yields (0.58  $\pm$  0.03 and 0.38  $\pm$  0.01 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>) resulted from batch anaerobic digesters at the lowest loading (0.86 g VS<sub>FOG</sub>/L inoculum), at 14 % and 57 % higher than the control, respectively (**Figure 14e** and **f**). Biogas and CH<sub>4</sub> yields were inversely correlated to loading. The CH<sub>4</sub> to biogas ratios ranged from 48 to 76 % with the highest observed ratio resulting from 0.0 g VS<sub>FOG</sub>/L inoculum, and were inversely related to loading. Mixed FOG loading had a negligible effect on pH, though all sets dropped slightly from initial pH and quickly recovered (**Figure 14c**).

#### Vitamin Waste

The vitamin waste loading of 13.1 g VSFoG/L inoculum increased the cumulative biogas production by 426 % ( $0.63 \pm 0.01 \text{ L}/\text{g} \text{ VS}_{\text{inoculum}}$ ) (Figure 15a and b), CH<sub>4</sub> production by 33 % ( $0.32 \pm 0.05 \text{ L}/\text{g} \text{ VS}_{\text{inoculum}}$ ) (Figure 15b), and decreased the CH<sub>4</sub> to biogas ratio by 24 % ( $50 \% \text{ CH}_4 \text{ V/V}$ ) (Figure 15d), as compared to control. The highest observed biogas and CH<sub>4</sub> yields ( $0.72 \pm 0.06$  and  $0.45 \pm 0.12 \text{ L}/\text{g} \text{ VS}_{\text{inoc}}/\text{g} \text{ VS}_{\text{sub}}$ , respectively) occurred from the control with 0.0 g VSFOG/L inoculum (Figure 15e and f). Biogas and CH<sub>4</sub> yields were inversely correlated to vitamin waste loading. The CH<sub>4</sub> to biogas ratios ranged from 47 to 61 %, with the highest observed resulting from the control, and were inversely correlated to vitamin waste loading. The highest vitamin waste loading tested prompted the pH to drop below 5, well outside of the optimal range of 6.8 to 7.2 [3, 8, 19, 22, 24, 25] (Figure 15c).

#### Westside Waste

The highest two Westside waste loadings, of 13.1 and 18.2 g VS<sub>FOG</sub>/L inoculum, were not statistically different from each other in regards to biogas production (**Figure 16a** and **b**), with both averaging about 200 % higher ( $0.36 \pm 0.06$  and  $0.36 \pm 0.02$  L/g VS<sub>inoculum</sub>) than control. A loading of 13.1 g VS<sub>FOG</sub>/L inoculum prompted a 273 % increase in CH<sub>4</sub> production ( $0.27 \pm 0.09$  L/g VS<sub>inoculum</sub>), while an 18.2 g VS<sub>FOG</sub>/L inoculum prompted only a 175 % increase ( $0.20 \pm 0.01$  L/g VS<sub>inoculum</sub>) (**Figure 16b**). Batch anaerobic digesters loaded with 13.1 g VS<sub>FOG</sub>/L inoculum had an observed CH<sub>4</sub> to biogas ratio increase of 32 % (75 % CH<sub>4</sub> V/V), as compared to control (**Figure 16d**). The highest biogas and CH<sub>4</sub> yields ( $0.46 \pm 0.03$  and  $0.37 \pm 0.05$  L/g VS<sub>inoc</sub>/g VS<sub>sub</sub>, respectively) were

observed at the 1.61 g VS<sub>FOG</sub>/L inoculum loading, increasing by 10.6 % and 14.2 % respectively as compared to the control (**Figure 16e** and **f**). The biogas and CH<sub>4</sub> yields were inversely correlated to Westside waste loading. The CH<sub>4</sub> to biogas ratios ranged from 56 to 77 %, inversely related to Westside waste loading. Westside waste loading had a negligible effect on pH, though all sets dropped slightly from initial pH then quickly recovered (**Figure 16c**).

### Yogurt Waste

The lowest yogurt waste loading tested, 1.61 g VS<sub>FOG</sub>/L inoculum, had the highest biogas and CH<sub>4</sub> productions (0.11 ± 0.03 and 0.09 ± 0.02 L / g VS<sub>inoculum</sub>, respectively), at 51 and 73 % higher than control (**Figure 17a** and **b**), while the CH<sub>4</sub> to biogas ratio increased by 15 % (89 % CH<sub>4</sub> V/V) (**Figure 17d**), as compared to the control. The highest biogas and CH<sub>4</sub> yields (0.57 ± 0.11 and 0.38 ± 0.06 L / g VS<sub>inoc</sub> / g VS<sub>sub</sub>, respectively) were observed from the control with 0.0 g VS<sub>FOG</sub> /L inoculum loading (**Figure 17e** and **f**). The biogas and CH<sub>4</sub> yields were inversely correlated to loading. The CH<sub>4</sub> to biogas ratios ranged from 57 to 89 %, the lowest CH<sub>4</sub> content resulting from the highest loading rate (18.2 g VS<sub>FOG</sub>/L inoculum). Yogurt waste oading had negligible effect on pH, though all sets dropped slightly from initial pH and quickly recovered (**Figure 17c**).



*Figure 11:* Bakery waste in Corvallis inoculum results, including (*a*) cumulative biogas; (*b*) cumulative biogas percent difference from control; (*c*) pH; (*d*) CH<sub>4</sub> to biogas ratio; (*e*) biogas and CH<sub>4</sub> yields; and (*f*) biogas and CH<sub>4</sub> yields percent difference from control. Error bars represent 95 % confidence.



*Figure 12:* Cherry waste in Corvallis inoculum results, including (*a*) cumulative biogas; (*b*) cumulative biogas percent difference from control; (*c*) pH; (*d*)  $CH_4$  to biogas ratio; (*e*) biogas and  $CH_4$  yields; and (*f*) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 13:* Mayonnaise waste in Corvallis inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 14:* Mixed FOG in Corvallis inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 15:* Vitamin waste in Corvallis inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 16:* Westside waste in Corvallis inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.



*Figure 17:* Yogurt waste in Corvallis inoculum results, including (a) cumulative biogas; (b) cumulative biogas percent difference from control; (c) pH; (d)  $CH_4$  to biogas ratio; (e) biogas and  $CH_4$  yields; and (f) biogas and  $CH_4$  yields percent difference from control. Error bars represent 95 % confidence.

# Discussion

## Gresham Inoculum, Low FOG Loadings

Comparison of the effect of FOG addition to batch anaerobic digesters containing Gresham inoculum for co-digestion resulted in the FOG source's apparent division into two groups. Specifically, mayonnaise waste was closely correlated to mixed FOG, while the other FOG sources (bakery, cherry brine, vitamin, Westside, and yogurt wastes) were similar.

Both mixed FOG and mayonnaise waste had a peak in cumulative biogas production (**Figure 18c**), biogas yield (**Figure 19c**), cumulative CH<sub>4</sub> production (**Figure 20c**), and CH<sub>4</sub> yield (**Figure 21c**) at loading 0.81 or 0.86 VS<sub>FOG</sub>/L inoculum (mixed FOG and mayonnaise waste, respectively). After the peak, there was a negative trend as FOG loading increased.

Mixed FOG's peak in cumulative biogas production and biogas yield at 0.86 g VSFOG /L was 22 % higher than mayonnaise waste's peak in cumulative biogas production and biogas yield at 0.81 g VSFOG/L for cumulative biogas production and biogas yield (*Figure* 18c and *Figure* 20c). The mixed FOG loading at this peak was 6.2 % higher than the mayonnaise waste loading. Thus, the higher mixed FOG loading may in part, but not entirely, account for the greater biogas production and yield success observed for mixed FOG. Mixed FOG also had a 42 % higher cumulative and 60 % higher biogas yield at loading 2.42 g VS<sub>FOG</sub>/L, and a 60% higher cumulative biogas production and 57% higher biogas yield at loading 3.23 g VS FOG/ L as compared to mayonnaise waste at the same loadings (*Figure 18c* and *Figure 20c*). This suggests that the Gresham inoculum may have had been better able to handle mixed FOG compared to the mayonnaise waste due to adaptation of the initial inoculum that has occurred. Previous exposure to a FOG source has been linked to improved anaerobic digester tolerance to substrate loading increases [31, 26, 21]. While the mayonnaise waste behaved similarly to mixed FOG, the mayonnaise waste was a new substrate that the batch anaerobic digesters had not been previously acclimated to and thus had less success since Gresham started to feed their

anaerobic digesters mixed FOG. Another possibility is a possible difference in LCFA content between the two FOG sources, though LCFA content measurements were outside the scope of this study. High LCFA content has been linked to reduced biogas in batch anaerobic digesters [22, 23], which would help explain the strong negative trend as FOG loading increased after the peak.

The other tested FOG sources, namely bakery waste, cherry brine, vitamin waste, Westside waste, and yogurt waste, did not have the same peak, and instead had more linear trends in biogas production and yield, CH<sub>4</sub>, production and yield, and methane content. Gresham batch anaerobic digesters loaded with these five FOG sources had a positive increase in cumulative biogas production (**Figure 18a** and **b**) and cumulative CH<sub>4</sub> production (**Figure 20a** and **b**) as loading increased. Biogas yield (**Figure 19a** and **b**) and CH<sub>4</sub> yield (**Figure 21a** and **b**) remained near constant or decreased slightly as loading increased. The CH<sub>4</sub> to biogas ratio for these 5 FOG sources also remained between 73 % and 79 % CH<sub>4</sub> by volume at all FOG loadings tested in Gresham inoculum (**Figure 22a** and **b**).

These 5 FOG sources were closely correlated, but could be loosely separated into two sub-groups: bakery waste with Westside waste, and yogurt waste; and cherry brine with vitamin waste. Bakery waste, Westside waste, and yogurt waste consistently closely entwined, whereas cherry and vitamin waste values fell below bakery, Westside, and yogurt waste values in biogas yield, cumulative CH<sub>4</sub> production, and CH<sub>4</sub> yield at 0.81 g VS FOG/L. Without further testing of FOG constituents, a definite reason why they cluster cannot be determined. It is perhaps due in part to a lower LCFA content, though LCFA content was not measured. Alternatively, perhaps FOG loadings were low enough for all of these substrates that minimal inhibition or toxicity occurred.

No significant correlation based on TS content, VS content, or density was found to explain correlation of FOG source's effect on anaerobic digestion, except that mixed FOG and mayonnaise waste were at opposite ends of the TS and VS concentrations. Of all FOG sources, mixed FOG had the lowest TS and VS concentrations ( $13 \pm 0.7$  and  $12 \pm 0.7$  g / L, 83 and 85 % lower, respectively, than the next lowest FOG source's

concentrations) while mayonnaise waste had the highest VS and TS concentrations (221  $\pm$  7.4 and 215  $\pm$  7.4 g / L, 14 and 6 % higher, respectively, than the next highest FOG source's concentrations). This could signify that the tendency of a FOG source to prompt batch anaerobic digesters to peak at a relatively low loading could be independent from VS and TS concentrations. However, it could also signify that FOG sources at the extremely low or high TS and VS contents are likely to peak at low FOG loadings.

Direct comparison of co-digestion results from low FOG loading experiments in batch anaerobic digesters containing Gresham inoculum revealed some general trends regarding the effect of FOG additions on batch anaerobic digester success.

- All FOG sources were able to increase cumulative biogas production and cumulative CH<sub>4</sub> production for at least one trial when used for co-digestion (Appendix B, Figure 34 and Figure 36).
- The highest loadings tested resulted in biogas and CH<sub>4</sub> yields that were either close to or significantly lower than control yields (**Appendix** B, **Figure 35** and **Figure 37**). This signifies that the CH<sub>4</sub> production potential based on the FOG loading is not being fully met. Higher FOGloadings can lead to digester upset and incomplete VS destruction [3] potentially due to the inhibition of the microbial community prompted by the increased VS concentrations.
- All batch anaerobic digesters at FOG loadings tested remained above 60 % CH<sub>4</sub> by volume (the low bound of the typical methane content range [3, 5, 11]) excluding the mixed FOG experiments (**Appendix B**, **Figure 38**). However, the mixed FOG experiment's low CH<sub>4</sub> content was not attributed to FOG loading because the experimental control, which received no additional FOG, had a CH<sub>4</sub> content of only 48 % by volume.



*Figure 18:* The seven FOG sources were tested at various loadings (g VS<sub>FOG</sub>/L inoculum) in batch anaerobic digesters seeded with Gresham inoculum, and their cumulative biogas production data are presented. Strong similarities in co-digestion results for specific FOG sources were observed, and were grouped by (a) bakery waste, Westside waste, and yogurt waste; (b) cherry brine and vitamin waste; and (c) mayonnaise waste and mixed FOG. Confidence intervals included are for 95 %.



**Figure 19:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Gresham inoculum, and their biogas yield data are presented. Strong similarities in co-digestion results for specific FOG sources were observed, and were grouped by (a) bakery waste, Westside waste, and yogurt waste; (b) cherry brine and vitamin waste; and (c) mayonnaise waste and mixed FOG. Confidence intervals included are for 95 %.



**Figure 20:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Gresham inoculum, and their cumulative CH<sub>4</sub> production data are presented. Strong similarities in co-digestion results for specific FOG sources were observed, and were grouped by (**a**) bakery waste, Westside waste, and yogurt waste; (**b**) cherry brine and vitamin waste; and (**c**) mayonnaise waste and mixed FOG. Confidence intervals included are for 95 %.



**Figure 21:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Gresham inoculum, and their CH<sub>4</sub> yield data are presented. Strong similarities in co-digestion results for specific FOG sources were observed, and were grouped by (a) bakery waste, Westside waste, and yogurt waste; (b) cherry brine and vitamin waste; and (c) mayonnaise waste and mixed FOG. Confidence intervals included are for 95 %.



**Figure 22:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Gresham inoculum, and their CH<sub>4</sub> to biogas data are presented. Strong similarities in co-digestion results for specific FOG sources were observed, and were grouped by (a) bakery waste, Westside waste, and yogurt waste; (b) cherry brine and vitamin waste; and (c) mayonnaise waste and mixed FOG.

## Corvallis Inoculum

Comparison of the effect of FOG addition to batch anaerobic digesters containing Corvallis inoculum for co-digestion resulted in the FOG source's apparent division into two groups. Specifically, mayonnaise waste was closely correlated to mixed FOG, and can be differentiated from the other FOG sources (bakery waste, vitamin waste, Westside waste, and yogurt waste). In an attempt to prompt batch anaerobic digester upset, FOG sources were co-digested at very high loadings in Corvallis inoculum (up to 23 times higher than most of the Gresham loadings). Only select FOG sources were tested at lower loadings equivalent to the majority of FOG loadings tested in Gresham inoculum.

When considering all tested loadings, it is apparent that mayonnaise waste and mixed FOG behaved similarly, as both had a peak in cumulative biogas production (**Figure 23a**), biogas yield (**Figure 24a**), cumulative CH<sub>4</sub> production (**Figure 25a**), and CH<sub>4</sub> yield (**Figure 26a**) at loading 0.81 to 0.86 g VS<sub>FOG</sub>/L inoculum. After the peak, there was a negative trend as FOG loading increased. This strong negative trend could be attributed to the FOG source contents.

The high loadings of mayonnaise waste had visible white balls in the batch anaerobic digesters. Pereira et al. suggest that these white masses are the separation of LCFA from the anaerobic digester contents [25]. Although mixed FOG did not display the same tendency to separate out white masses, a high concentration of LCFA could still be present and affecting the anaerobic digester microbes' cellular structure or nutrient uptake [25]. A high LCFA content would potentially explain the strong negative trend as FOG loading increased after the peak, as high LCFA content has been linked to reduced biogas in batch anaerobic digesters [22, 23].

Mayonnaise (**Figure 23b**, **Figure 24b**, **Figure 25b**, *Figure 26b*, and **Figure 27b**), Westside and yogurt wastes (**Figure 23c**, **Figure 24c**, **Figure 25c**, **Figure 26c**, and **Figure 27c**) were tested at 1.61 g VS<sub>FOG</sub>/L inoculum, but there was no differentiating results as all batch anaerobic digesters responded similarly to this loading. Mixed FOG was not tested at 1.61 g VS<sub>FOG</sub>/L inoculum, while vitamin and bakery wastes were not tested at 0.81 or 1.61 g VS<sub>FOG</sub>/L inoculum Corvallis inoculum.

Based only on the controls and highest FOG loadings tested on Corvallis inoculum (13.07 to 20.8 g VS<sub>FOG</sub>/L inoculum), biogas yields (**Figure 24b** and **c**) and CH4 yields (**Figure 26b** and **c**) decreased as loading increased for all FOG sources. All FOG sources, excluding mayonnaise, also showed a negative trend in CH4 to biogas ratio (**Figure 27b** and **c**). Bakery, vitamin, and yogurt wastes had higher cumulative biogas and CH4 production at a FOG loading of 13.1 *g* VS<sub>FOG</sub>/L inoculum than at a FOG loading of 18.2 g VS<sub>FOG</sub>/L inoculum (**Figure 24c, Figure 23c** and **Figure 25c**), Westside's biogas and CH4 values were not statistically different, whereas mayonnaise waste and mixed FOG had higher cumulative biogas production and CH4 at loading 18.2 to 20.8 g VS<sub>FOG</sub>/L inoculum (**Figure 23b, Figure 24b**, and **Figure 25b**). This suggests that higher loadings led to decreased VS destruction and anaerobic digester upset, likely due to inhibition prompted by VS overload.

Direct comparison of co-digestion results from low FOG loading experiments in batch anaerobic digesters containing Corvallis inoculum revealed some general trends regarding the effect of FOG additions on batch anaerobic digester success.

- All FOG sources were able to increase cumulative biogas production and cumulative *CH*<sup>4</sup> production for at least one trial when used for co-digestion (Appendix C, Figure 39 and Figure 41).
- The highest loadings tested resulted in biogas and CH<sub>4</sub> yields that were significantly lower than control yields (Appendix C, Figure 40 and Figure 41). This signifies that the CH<sub>4</sub> production potential based on the FOG loading is not being fully met. High loadings can lead to digester upset and incomplete VS destruction [3].
- All batch anaerobic digesters whose CH<sub>4</sub> to biogas ratio went below 50 % CH<sub>4</sub> by volume stopped producing biogas before the conclusion of the 14 day test, excluding one mayonnaise waste experiment loaded at 13.07 g VS<sub>FOG</sub>/L inoculum FOG loadings whose CH<sub>4</sub> content dropped to 24 % by volume (Appendix C, Figure 42). This CH<sub>4</sub> content is the lowest observed throughout the entire study and seems unreasonable. A biogas ratio below 60 % CH<sub>4</sub> by volume (the low bound of the typical methane content range [3, 5, 11]) and low

biogas production are signs of anaerobic digester failure [3, 6]. This may have occurred due to inhibition associated with the overloading of VS prompting upset of the batch anaerobic digesters.



**Figure 23:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Corvallis inoculum, and their cumulative biogas production data are presented. Strong similarities in co-digestion results for specific FOG sources were observed and grouped accordingly. Review of lower loadings for (a) Mayonnaise waste and mixed FOG revealed a peak at 0.81 to 0.86 g  $VS_{FOG}/L$  inoculum. Higher FOG loadings are present for (b) mayonnaise and mixed wastes, and (c) bakery, vitamin, Westside, and yogurt wastes. Confidence intervals of 95 % are included.



**Figure 24:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Corvallis inoculum, and their biogas yield data are presented. Strong similarities in co-digestion results for specific FOG sources were observed and grouped accordingly. Review of lower loadings for (a) Mayonnaise waste and mixed FOG revealed a peak at 0.81 to 0.86 g  $VS_{FOG}/L$  inoculum. Higher FOG loadings are present for (b) mayonnaise and mixed wastes, and (c) bakery, vitamin, Westside, and yogurt wastes. Confidence intervals of 95 % are included.



**Figure 25:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Corvallis inoculum, and their cumulative CH<sub>4</sub> production data are presented. Strong similarities in co-digestion results for specific FOG sources were observed and grouped accordingly. Review of lower loadings for (**a**) Mayonnaise waste and mixed FOG revealed a peak at 0.81 to 0.86 g  $VS_{FOG}/L$  inoculum. Higher FOG loadings are present for (**b**) mayonnaise and mixed wastes, and (**c**) bakery, vitamin, Westside, and yogurt wastes. Confidence intervals of 95 % are included.



**Figure 26:** The seven FOG sources were tested at various loadings (g  $VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Corvallis inoculum, and their  $CH_4$  yield data are presented. Strong similarities in co-digestion results for specific FOG sources were observed and grouped accordingly. Review of lower loadings for (a) Mayonnaise waste and mixed FOG revealed a peak at 0.81 to 0.86 g  $VS_{FOG}/L$  inoculum. Higher FOG loadings are present for (b) mayonnaise and mixed wastes, and (c) bakery, vitamin, Westside, and yogurt wastes. Confidence intervals of 95 % are included.



**Figure 27:** The seven FOG sources were tested at various loadings ( $g VS_{FOG}/L$  inoculum) in batch anaerobic digesters seeded with Corvallis inoculum, and their  $CH_4$  to biogas data are presented. Strong similarities in co-digestion results for specific FOG sources were observed and grouped accordingly. Lower loadings of (**a**) Mayonnaise waste were reviewed. Higher FOG loadings were tested for (**b**) mayonnaise and mixed wastes, and (**c**) bakery, vitamin, Westside, and yogurt wastes. Confidence intervals of 95 % are not included.

# Gresham and Corvallis, Cross-Over Loadings

Gresham experiments were conducted first at conservative loadings, which were found not to prompt digester upset. During the second phase of testing using Corvallis inoculum, higher loadings were tested to attempt inducing shock load conditions. However, select lower loadings tested previously in Gresham inoculum were repeated in Corvallis inoculum to allow for direct comparison of inoculum co-digestion performance in batch anaerobic digesters. The question being explored was whether Gresham inoculum due to previous exposure to FOG loadings would surpass Corvallis inoculum performance. Cherry brine and mayonnaise waste were tested at two cross over loadings, while mixed FOG, yogurt, and vitamin were each tested at a single cross-over loading.

Co-digestion results of cherry brine was comparable in Gresham and Corvallis inoculum seeded batch anaerobic digesters (**Figure 28**). In comparing the Gresham to Corvallis inoculums for cherry waste, close correlation between Gresham and Corvallis inoculum results for cumulative biogas production and CH<sub>4</sub> values (**Figure 28a** and **c**), Corvallis inoculum had higher values for biogas and CH<sub>4</sub> yield (**Figure 28b** and **d**), while Gresham inoculum had a higher CH<sub>4</sub> to biogas ratio (**Figure 28e**).

Mayonnaise waste also performed similarly in both Gresham and Corvallis inoculums. However, at the highest cross-over loading tested, the Corvallis inoculated batch anaerobic digesters values for all parameters (cumulative biogas production. Biogas yield, cumulative CH<sub>4</sub> production, CH<sub>4</sub> yield, and CH<sub>4</sub> to biogas ratio) were higher than Gresham batch anaerobic digester at the same loading (**Figure 29**). Co-digestion of mayonnaise waste in both inoculums resulted in a peak at the 0.081 g VS <sub>FOG</sub>/L inoculum loading, as observed in the results for cumulative biogas production and biogas yield (**Figure 29a** and **b**). The peak at the 0.081 g VS <sub>FOG</sub>/L inoculum loading for Gresham waste was also evident in the cumulative CH<sub>4</sub> production and CH<sub>4</sub> yield results, but large variance in the Corvallis data at the higher cross-over loadings a possible peak indiscernible (**Figure 29c** and **d**). The CH<sub>4</sub> content is the largest difference between the two inoculums when co-digesting mayonnaise waste, as the Gresham inoculum had a negative trend as loading increased, whereas Corvallis had a positive trend (**Figure 29e**). This trend may be due to neither anaerobic digester inoculums being previously acclimated to mayonnaise waste loading. With neither inoculum source acclimated, both were more or less at the same disadvantage.

Comparing cross-over loadings for other FOG sources also showed Gresham did not generally outperform Corvallis. Often, Corvallis inoculum performance was comprarable to Gresham inoculum, or Corvallis outperformed Gresham inoculum, as evidenced by the co-digestion of cherry brine, mayonnaise, and vitamin waste (**Figure 30**, **Figure 31**, **Figure 32**) However, mixed FOG and yogurt wastes in Gresham inoculated batch anaerobic digesters had significantly higher values for cumulative biogas productin (**Figure 30**a and **Figure 31**a), biogas yield (**Figure 30b** and **Figure 31b**), cumulative CH<sub>4</sub> production (**Figure 30c** and **Figure 31c**), and CH<sub>4</sub> yield (**Figure 30d** and **Figure 31d**), though both had lower CH<sub>4</sub> to biogas ratios (**Figure 30***e* and **Figure 31e**).

The significantly higher success yogurt waste and mixed waste co-digested in Gresham inoculated anaeorbic digesters as opposed to Corvallis batch anaerobic digesters is likely due to previous acclimation to the FOG source. Mixed FOG was a sample of the FOG slurry used by the Gresham WWTP for co-digestion in their onsite anaerobic digesters, and a portion of that mixed FOG is believed to be a diary waste. Dairy waste and yogurt are very similar in TS content, VS content, and density (**Table 5**), so perhaps they would behave similarly. The Gresham inoculum had the benefit of previous exposure, and was already acclimated the mixed FOG source, and possibly the yogurt waste, whereas Corvallis was newly exposed. Anaerobic digesters acclimated to a waste tend to be more tolerant to the waste [31, 26, 21].

**Table 5:** Dairy versus yogurt waste characterization to evaluate the possibility that Gresham's higher success at higher yogurt waste loadings may be due to previous acclimation to a similar waste.

	pН	g/L					
Substrate		Density		TS		VS	
		AVG	95% conf	AVG	95% conf	AVG	95% conf
Dairy	5.83	960.6	4.00	87.0	2.68	77.8	2.26
Yogurt		966	8.6	93	1.6	85	1.5

**Figure 31** and **Figure 32** show no appreciable trend related to FOG source composition, including density, TS, and VS, is observed for these data when comparing batch anaerobic digesters seeded with Gresham versus Corvallis inoculum, related to the limited number of substrates tested at cross-over loadings.



**Figure 28:** Comparing results Corvallis (•) versus Gresham ( $\Delta$ ) inoculums with cherry brine for co-digestion verified strong correlation between the two inoculums at the loadings tested for (**a**) biogas production, (**b**) biogas yield, (**c**) CH<sub>4</sub> production, (**d**) CH<sub>4</sub> yield, (**e**) and CH<sub>4</sub> to biogas ratio. Batch anaerobic digesters were loaded between 0.00 and 1.84 g VS<sub>FOG</sub>/L inoculum for Corvallis inoculum, and 0.00 to 1.61 g VS<sub>FOG</sub>/L inoculum for Gresham inoculum.



**Figure 29:** Comparing results Corvallis (•) versus Gresham ( $\Delta$ ) inoculums with mayonnaise production waste for co-digestion showed Corvallis inoculum exceeded success of Gresham inoculum at the loadings tested for (**a**) biogas production, (**b**) biogas yield, (**c**) CH<sub>4</sub> production, (**d**) CH<sub>4</sub> yield, (**e**) and CH<sub>4</sub> to biogas ratio. However, the two inoculums behaved similarly. Batch anaerobic digesters were loaded between 0.0 and 1.61 g VS<sub>FOG</sub>/L inoculum.



CH<sub>4</sub> to Biogas Ratio



**Figure 30:** FOG sources were tested at the loading  $0.84 \pm 0.04$  g VS<sub>FOG</sub>/L inoculum in batch anaerobic digesters seeded with Corvallis ( ) or Gresham () inoculums. The control bar is an average of controls for all represented experiments. Gresham inoculum did not consistently do better than Corvallis when comparing (a) biogas production, (b) biogas yield, (c) CH<sub>4</sub> production, (d) CH<sub>4</sub> yield, (e) and CH<sub>4</sub> to biogas ratio. Corvallis and Gresham inoculated batch anaerobic digesters performed similarly, except for the mixed FOG digesters where Gresham performed better. Substrates are organized by increasing density from left to right.



**Figure 31:** FOG sources were tested at the loading  $1.64 \pm 0.08$  g VS FOG/L inoculum in batch anaerobic digesters seeded with Corvallis ( $\blacksquare$ ) or Gresham ( $\blacksquare$ ) inoculums. The control bar is an average of controls for all represented experiments. Gresham inoculum did not consistently do better than Corvallis when comparing (**a**) biogas production, (**b**) biogas yield, (**c**) CH<sub>4</sub> production, (**d**) CH<sub>4</sub> yield, (**e**) and CH<sub>4</sub> to biogas ratio. Corvallis inoculated batch anaerobic digesters performed similarly or better, except for the yogurt waste digesters where Gresham performed better. Substrates are organized by increasing density from left to right.













**Figure 32:** FOG sources were tested at the loading  $13.02 \pm 0.11$  g VS FOG/L inoculum in batch anaerobic digesters seeded with Corvallis ( ) or Gresham () inoculums. The control bar is an average of controls for all represented experiments. Gresham and Corvallis inoculum performed similarly in regards to (a) cumulative biogas production, (b) biogas yield, (c) cumulative CH4 production, (d) CH<sub>4</sub> yield, (e) and CH<sub>4</sub> to biogas ratio. Substrates are organized by increasing density from left to right.

# Combined Gresham and Corvallis Trends

### Maximum Observed Biogas/CH<sub>4</sub> Production and Yield

All FOG sources were able to increase cumulative biogas and CH<sub>4</sub> production and cumulative CH<sub>4</sub> production for at least one trial in both inoculum types when used for co-digestion, but not all FOG types were able to increase biogas or CH<sub>4</sub> yields. FOG addition was able to increase biogas production by 291 % in Gresham inoculum and 425 % in Corvallis inoculum (both from the addition of vitamin waste, at FOG loadings 13.0 and 13.1 g VS<sub>FOG</sub> / L inoculum, respectively). The same vitamin waste loading resulted in the highest cumulative CH<sub>4</sub> production, at 254 % in Gresham inoculum and 333 % in Corvallis inoculum. The maximum biogas yield in Gresham inoculum was 26.3 % and in Corvallis inoculum was 15.76 % (0.86 g VS FOG/L inoculum from mixed FOG, and 1.61 g VS FOG/L inoculum from mayonnaise waste). CH<sub>4</sub> yield was increased by a maximum of 86.6 % and 56.8 % by the 0.86 g VS FOG/L inoculum loading of mixed FOG in Gresham and Corvallis inoculum, respectively.

Vitamin, mayonnaise, and mixed FOG are recurring wastes that prompted the highest increases. Vitamin waste prompted the highest cumulative production increases, while the maximum yields were observed at the mayonnaise waste and mixed FOG peaks. Testing the nutrient content of the FOG sources was outside the scope of this study, but vitamin waste may have been particularly successful due to a high nutrient content that was a beneficial resource to the microbes.

### Lowest Recorded pH

pH data was collected throughout each experiment, and the lowest recorded pH for each set at each loading was recorded (**Figure 33a**). The lowest recorded pH was found to be inversely proportional to FOG loading. CH<sub>4</sub> to biogas ratio was found to increase as the lowest recorded pH increased (**Figure 33b**). This trend between the CH<sub>4</sub> to biogas ratio and the lowest recorded pH was strongest for the Corvallis inoculum at the highest loadings tested.
Both trends are consistent with other sources, as high FOG loadings has been attributed to a pH drop due to increased acidogen activity. The associated pH drop prompts methanogenesis inhibition, explaining the low CH<sub>4</sub> content as pH decreases. [3, 8, 19, 22, 24, 25] The association between higher loadings leading to increased biogas production, but a lower CH<sub>4</sub> content has been reported by Wan et al [12]. This trend is also more apparent when lower pH values are reached, showing that near neutral pH has less of an effect on CH<sub>4</sub> content.

Combined Gresham inoculum data and Corvallis inoculum data showed that batch anaerobic digesters whose pH: fell below 5 failed; was between 5.5 and 5.8 recovered; and was above 5.9 was not prone to failure. The importance of maintaining pH for anaerobic digester performance has often been reported. However, batch anaerobic digester success at a pH as low as 5.9 as was observed in this study is outside the reported optimal range for methanogens of 6.8 to 7.2 [3, 8, 19, 22, 24, 25].



Lowest Observed pH versus Loading Rate [g VS<sub>FOG</sub>/ L inoculum]

*Figure 33:* Correlations were observed between (*a*) lowest observed pH and loading rate ( $g VS_{FOG}/L$  inoculum, trendline for all data, and (*b*) the CH<sub>4</sub> to biogas ratio and the lowest observed pH, trendline for Corvallis data only.

#### Conclusions

All FOG sources were able to increase cumulative biogas and CH<sub>4</sub> production and cumulative CH<sub>4</sub> production for at least one trial in both inoculum types when used for codigestion, but not all FOG types were able to increase biogas or CH<sub>4</sub> yields. FOG addition was able to increase batch anaerobic digester biogas production by 291 % (vitamin, 13.0 g VS<sub>FOG</sub>/L inoculum) in Gresham and 425 % (vitamin, 13.0 g VS<sub>FOG</sub>/L inoculum) in Gresham and 425 % (vitamin, 13.0 g VS<sub>FOG</sub>/L inoculum) in Gresham and 425 % (vitamin, 13.0 g VS<sub>FOG</sub>/L inoculum) in Gresham and 15.8 % (mayonnaise, 15.8 g VS<sub>FOG</sub>/L inoculum) in Corvallis; CH<sub>4</sub> production by 254 % (Westside, 13.1 g VS<sub>FOG</sub>/L inoculum) in Gresham and 333 % (vitamin, 13.1 g VS<sub>FOG</sub>/L inoculum) in Corvallis; CH<sub>4</sub> yield by 86.6 % (mixed FOG, 0.86 g VS<sub>FOG</sub>/L inoculum) in Corvallis. Higher loadings had significantly lower biogas and CH<sub>4</sub> yields, suggesting incomplete VS destruction and unmet CH<sub>4</sub> production potential.

Limited correlation by FOG characteristics (density, TS, and VS) were observed, but mayonnaise and mixed FOG wastes were two FOG sources who were strongly correlated in cumulative gas production and biogas yields in both Gresham and Corvallis seeded anaerobic digesters. Both had a peaks at the 0.86 g VS FOG/L inoculum FOG loading, followed by an inverse relationship with FOG loading. Visible white masses were seen in the batch anaerobic digesters with the highest mayonnaise waste loading, which may suggest the presence of high LCFA concentrations, which could explain the negative trend at higher loading rates [25].

Comparison of Gresham batch anaerobic digesters to Corvallis batch anaerobic digesters suggests Gresham inoculum was acclimated to mixed FOG and yogurt waste by Gresham WWTP established FOG addition program, whereas Corvallis was not previously acclimated. At the tested cross-over loadings, Gresham inoculum had a more successful response to the yogurt and mixed FOG loadings, based on cumulative gas production and gas yields [3, 6], as compared to the Corvallis inoculum. In general, Gresham did not out-perform Corvallis waste which suggests that inoculum acclimation is specific to waste used, not just to VS content.

Batch anaerobic digester failure signs were similar between Corvallis and Gresham inoculums, and revealed by the trends between lowest observed pH with loading and CH<sub>4</sub> content. Lowest pH observed was inversely correlated to FOG loading rate. Furthermore, the lowest observed pH was correlated to batch anaerobic digester success. Batch anaerobic digesters whose pH: fell below 5 failed; was between 5.5 and 5.8 recovered; and was above 5.9 was not prone to failure. Lower pH also was associated with decreased CH<sub>4</sub> content, and digester failure occurred when CH<sub>4</sub> content fell below 50 %.

Further areas of research potential are present, particularly in better analyzing the correlations between FOG characteristics and anaerobic digester success. The present study suggests a possible correlation with density, but limited data prevented further analysis. Literature also suggests the carbon to nitrogen ratio, COD, and present nutrients would be parameters for analysis [18, 32]. These FOG characteristics may help explain the strong correlation between mayonnaise waste and mixed FOG.

Overall, FOG additions to batch anaerobic digesters using inoculums from two different sources can increase biogas and CH<sub>4</sub> cumulative productions and yields. Higher loadings were associated with a significantly lower pH and increased risk of failure as compared to lower loadings. Acclimation of anaerobic digester inoculum did affect anaerobic digester success at increased FOG loadings. The limitations associated with scale-up of batch experiments should be noted [33]. However, this study suggests that Gresham can significantly increase loading their loading by as much as 10 to 20 times their current loading rate (equivalent to a 0.81 to 1.61 g VSFOG/L) with limited risk of failure if the digester is allowed to acclimate to the introduced waste.

## Appendix A

	Gresham's Volumetric Flow Rates	Benchtop Reactor Volumetric flow Rates		Batch Reactor Loadings Unadjusted	
	Provided by J.	To make 100	Use benchtop values for volumetric ratio		
	Maag via e-mail,	[mL/d] reactor	multiply FOG Loading by a factor		
	[GPD]	[mL/d]	[mL]	[mg VS]	
WAS Loading	42000	4.2	4.2	84.4	
PS Loading	25000	2.5	2.5	78.5	
FOG Loading	6100	0.61	n * (0.61)	n * (7.5)	
Innoc. Loading	926900	92.7	92.7	VS Inoculum,i	
Total	100000	100.0	V <sub>TOTAL,i</sub>	VS <sub>TOTAL,i</sub>	

**Table 6:** Batch anaerobic digester set-up relied on volumetric flow rates as provided by Gresham WWTP [16], as seen in **Equations 5** through 9. Small FOG loadings did not significantly affect the calculated volume.

Note: WAS = Waste Activated Sludge; PS = Primary Solids; FOG = Fats, Oils and Grease; n = desired multiple to account for FOG loading adjustments

**Table 7:** Higher FOG loadings required batch anaerobic digester set-up calculations to be adjusted for volume. Batch reactor contents volume and VS concentration ratios from the calculations unadjusted for volume were maintained after adjustment, as seen in **Equations 10** through 18.

	Gresham WWTP Anaerobic Digester	Benchtop CSTR Anaerobic Digester		Batch Anaerobic Digester		
	[GPD]	[mL/d]	[mL]	[mg VS]		
WAS	42000	4.2	4.2	$M_{WAS,VS,i} = (4.2 \text{ mL}) VS_{WAS}$	[5]	
PS	25000	2.5	2.5	$M_{PS,VS,i} = (2.5 \text{ mL}) VS_{PS}$	[6]	
FOG	6100	0.61	n (0.61)	$M_{FOG,VS,i} = n (0.61 \text{ mL}) VS_{mixed}$	[7]	
Inocculum	926900	92.7	92.7	M <sub>inoc,VS,i</sub> = (92.7 mL) VS <sub>Inoculum</sub>	[8]	
Total	100000	100.0	V <sub>TOTAL, i</sub>	$M_{TOTAL,VS,i} = \Sigma M_{x,vs,i}$	[9]	

Note: CSTR = Continuously Stirred Tank Reactor; WAS = Waste Activated Sludge; PS = Primary Solids; FOG = Fats, Oils and Grease; n = desired multiple to account for FOG loading adjustments

### **Appendix B**



*Figure 34:* Gresham inoculated batch anaerobic digester biogas production data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 35: Gresham inoculated batch anaerobic digester biogas yield data for all tested substrates is presented to review how FOG source and loading affect digester success.* 



*Figure 36:* Gresham inoculated batch anaerobic digester  $CH_4$  production data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 37:* Gresham inoculated batch anaerobic digester  $CH_4$  yield data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 38:* Gresham inoculated batch anaerobic digester  $CH_4$  to biogas ratios for all tested substrates is presented to review how FOG source and loading affect digester success.

# Appendix C



*Figure 39:* Corvallis inoculated batch anaerobic digester biogas production data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 40:* Corvallis inoculated batch anaerobic digester biogas yield data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 41:* Corvallis inoculated batch anaerobic digester CH<sub>4</sub> production data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 42:* Corvallis inoculated batch anaerobic digester  $CH_4$  yield data for all tested substrates is presented to review how FOG source and loading affect digester success.



*Figure 43:* Corvallis inoculated batch anaerobic digester  $CH_4$  to biogas ratios for all tested substrates is presented to review how FOG source and loading affect digester success.

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