Correlating Sludge Constituents with Digester Foaming Risk Using Sludge Foam Potential and Rheology

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Abstract

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Foam potential and viscometer ramp tests (VRTs) were conducted for three municipal wastewater treatment plants to determine if these methods can relate mechanisms of foaming to physical and biological constituents in sludge. At all plants, digester volatile solids (VS) concentration correlated ($R^2 > 0.41$) with increases in plastic viscosity, a VRT parameter corresponding to foaming risk. Plastic viscosity also correlated with foam-causing bacteria *Gordonia* ($R^2 = 0.38$). Foam potential test values increased with *Microthrix parvicella* ($R^2 > 0.28$). For one plant, suspected foam-causing bacteria *Mycobacterium* negatively correlated with parameters representing foam risk. Microscopic filament counting correlated ($R^2 = 0.97$) with quantitative polymerase chain reaction (qPCR) for *Gordonia*, suggesting that the more accessible counting method can reliably quantify foam-causing bacteria. Foam potential tests and VRTs resulted in plant-specific correlations with foam-related constituents. Therefore, these tests may provide useful evidence when investigating causes of digester foam events.

Keywords: Anaerobic digestion foaming, *Gordonia*, *Microthrix parvicella*, Rheology

1. Introduction

Foaming issues in anaerobic digesters decrease the effective capacity of digesters, clog gas equipment, create safety hazards from overflowing sludge, and in extreme cases, cause collapse of digester covers (Chapman, 2011). Digester foaming consists of two types of foaming, herein defined as surface foaming and gas entrainment. Surface foaming involves large bubbles stabilized by surface active particles; gas entrainment occurs when smaller bubbles are obstructed in sludge by large particles or soluble surface active molecules (Pagilla, 2015). Gas entrainment is also governed by sludge rheology, which imparts friction on bubbles and impedes the escape of gas from sludge (Chapman, 2011). Regardless of type of foaming, digester foaming issues are common and causes are often left unidentified (Shroedel et al., 2011). To identify the causes of digester foaming events, suspected causes of foaming must correlate with measurable characteristics of sludge that assess foaming risk.

Limited experimental evidence supports proposed causes of digester foaming (Ganidi et al., 2009). However, some sludge constituents are commonly believed to contribute to digester foaming. Filamentous *Gordonia amarae* have been observed during digester foaming events and cause foaming because of their hydrophobic cells and release of biosurfactants from both viable and lysed cells (Hernandez and Jenkins, 1994; Iwahori et al., 2001; Pagilla, 2015; Petrovski et al., 2010; Subramanian et al., 2015). Another recognized group of foam-causing bacteria, *Microthrix parvicella* have been observed in full-scale digester foaming events; their hydrophobic, filamentous cells promote foaming by stabilizing gas bubbles (Westlund et al., 1998). Aside from *G. amarae* and *M. parvicella*, other bacteria are suggested to cause foam due to hydrophobic cells or release of biosurfactants (He et al.,

2017; Kougias et al., 2014); *Mycobacterium* also possess these characteristics (Petrovski et al., 2010). They are linked to foaming in secondary treatment but not to digester foaming (Maza-Marquez et al., 2016; Rosso et al., 2018). Relative to foaming in secondary treatment, the complexity of digester foaming complicates understanding of how bacteria contribute to foaming (Pagilla, 2015).

Additionally, high loading of volatile solids (VS) causes digester foaming, but there is no consensus on the physical mechanism that results in foaming. High loading of digesters has been reported to correlate with digester foaming theoretically due to increases in volatile fatty acids (VFAs) and gas production (Gerardi, 2006; Kanu et al., 2015; Massart et al., 2006). Alternatively, high organic loading can lead to the accumulation of hydrophobic or surface active solids, which promote foam formation and stability (Gerardi, 2006; Kanu et al., 2015; Pagilla, 2015).

Finally, detergents or man-made surface active agents (surfactants) cause foaming by lining the liquid-gas interface that forms foam (Vardar-Sukan, 1998). Anionic surfactants, particularly linear alkylbenzene sulfonates (LAS), are common in municipal wastewater due to their ubiquity in commercial products (Mungray and Kumar, 2009). There are no published cases of digester foaming caused by anionic surfactants, but their surface-active properties and ubiquity make them an important potential factor.

Quantifying the magnitude of foaming or the risk of foaming is required to investigate causes of digester foaming. Bench-scale methods assess foaming risk, independent of the conditions within digesters (e.g. pressure, temperature, etc.). The foam potential test has multiple iterations, but all provide an empirical quantification of foam production from dispersion of gas through digester sludge (Hernandez and Jenkins, 1994; J. Jiang et al.,

2016; Marneri et al., 2009; Ross and Ellis, 1992; Zábranská et al., 2002). Foam potential tests have been used for quantifying risk of surface foaming, particularly in secondary treatment, but there is doubt over the test's ability to quantify gas entrainment risk for digester foaming (Pagilla, 2015).

The viscometer ramp test (VRT) was developed to characterize the potential for gas entrainment (Bartek et al., 2017). The viscometer measures shear rate and stress data, which are fitted to rheological models to derive characteristic parameters like plastic viscosity, yield stress, consistency index, and flow behavior index. These parameters theoretically relate to gas entrainment by the relationship between apparent viscosity, mixing, and bubble rise velocity. High apparent viscosity results in slower bubble rise and more gas entrainment (Bartek et al., 2017; Chapman, 2011). Low mixing intensity present low shear conditions, yielding the highest apparent viscosity and stoppage of bubble rise (Bartek et al., 2017; Chapman, 2011). Both the VRT and foam potential test characterize digester foaming risk, which should increase with suspected causes like foam-causing bacteria, VS, and surfactants, independent of foaming occurring at full-scale conditions.

To investigate how sludge constituents add to the potential for digester foaming, foam potential tests and VRTs were regularly conducted for an 8-month period with digester sludge from three municipal wastewater treatment plants. It was hypothesized that if the bench-scale tests were useful in identifying the relative impact of constituents known to cause digester foaming, foam potential tests and VRTs would show significant correlations with increases in foam-related sludge constituents. Furthermore, observations from past digester foaming and foaming management at each treatment plant were used to reason why past events may have been caused or how they were resolved.

2. Methods and Materials

2.1. Plant process and digester description

King County operates three regional wastewater treatment plants, West Point treatment plant (WTP), South treatment plant (STP), and Brightwater treatment plant (BWTP), each with different operating conditions (Table 1).

Table 1. Highlighted features of King County regional wastewater treatment plants.

WTP	WTP	STP	BWTP
Design Average Wet Weather Flow	133 MGD	115 MGD	29 MGD
Secondary Treatment Features[a,b]	HPO	CAS with SVI control (with anaerobic basin)	MBR (with anoxic basin)
Sludge Thickening[c]	GBT	DAFT	GBT
Number of Primary Digesters[d]	5	4	3
Average Digester SRT, Days	28	25	30
Digester Mixing[e]	Gas mixing (draft tubes or diffusers)	Gas diffusers	Mechanical draft tube

[a] HPO = high purity oxygen; CAS = conventional activated sludge; SVI = sludge volume index (settleability); MBR = membrane bioreactor

[b] Secondary treatment is preceded with conventional primary clarifiers at all plants.

[c] GBT = gravity belt thickener; DAFT = dissolved air floatation thickener. Sludge is blended from primary and secondary treatment solids.

[d] WTP has only floating covers on primary digesters while STP only floating covers and BWTP only fixed covers; all are mesophilic digesters.

[e] Recirculation pumps contribute to mixing and heating at digesters at all plants.

2.1.1. West Point treatment plant experience with digester foaming

Within the 5 years preceding this study, WTP digesters experienced at least 2 significant digester foaming events, persisting multiple months and resulting in rapid decreases in pressure-based liquid level readings as sludge density decreased with more entrapped gas (Fig. 1.) while the level of the floating cover did not significantly change. During the sample collection of this study, digester foaming issues were mitigated by pumping sludge from top portions of digesters to a temporary storage basin; this mitigation method was similar to the common practice of surface wasting. Additionally, Foam-A-Tac 435

(Enterprise Specialty Products Inc., Laurens, SC) was dosed to digesters for to collapse foams and provide short-term relief from foam progression. The management of digester foaming at WTP prevented any excessive issues with digester foaming during this study.

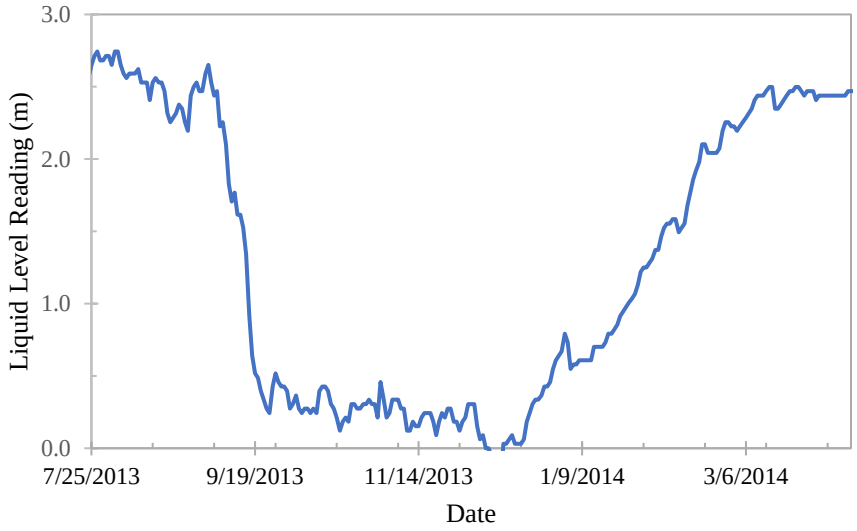

Fig. 1. West Point digester foaming event in 2013. Liquid level readings are shown for WTP Digester #3. Readings decreased from approximately 2.5 m down to less than 0 m over the course of a week. These erroneous readings are indicative of decreasing sludge density.

2.1.2. South treatment plant experience with digester foaming

Prior to this study, STP had foam events in 2015 and 2016, both occurring in late August and lasting between 1-2 weeks. In 2015, the foaming had caused the floating cover of one digester to be misaligned. These foam events occurred when secondary aeration and mean cell residence time (MCRT) were operated for nitrification-denitrification as opposed to optimizing settleability. Foaming issues were successfully mitigated with increased gas mixing and gas-sparging to encourage movement of entrained gas out of the sludge. Both

events were resolved once the MCRTs were reduced to prevent nitrification and optimize settleability. During this study, STP did not experience digester foaming issues.

2.1.3. Brightwater treatment plant experience with digester foaming

Since BWTP started its operation in 2011, there were two digester foaming events. These events were recognized by decreases in pressure-based liquid level readings and increasing quantity of sludge from the foaming digester to the sludge storage tank. In 2012, a planned power outrage resulted in rapid volume expansion (RVE) from the lack of mixing. In November of 2018, a second RVE event occurred from a lack of mixing caused by an update of the online process control system; this was the only digester foaming event that occurred during the study. BWTP digesters have not had foaming issues in the primary digesters when mixing has been present.

2.2. Digester sampling and analyses

2.2.1. Sample collection and handling

Regular sampling of digester sludge was conducted for each of King County's regional wastewater treatment plants. For WTP and STP, digester samples were collected every two weeks from June 2018 to January 2019, totaling 16 samples per plant. For BWTP, digester samples were collected every month from June 2018 to February 2019, totaling to 8 samples. All samples were grab samples collected the morning of each sample date and were transported in coolers with ice for no more than 2 hours. Samples were stored at 4 ^{o}C and held for no more than 24 hours before analysis, unless otherwise indicated.

2.2.2. Foam potential and viscometer ramp tests

Foam potential measurements were collected in accordance with an established protocol (Pagilla, 2015). Samples of digester sludge were tested in 1 L graduated cylinders with fine pore diffusers. Sludge was filled to the 200 mL mark and the height was recorded. The sludge was then aerated at 1.5 L/min for 30 min, and the maximum foam height and settled foam height (1 min after the end of aeration) were recorded. Unstable foam index (Eq. 1) and stable foam index (Eq. 2) characterize the potential for foam initiation and the creation of persistent foam, respectively. The suggested use of these indices is to conduct testing over an extended period to establish a relative scale of foam severity; the provided example (shown in Appendix A) predicts severe foaming from an unstable foam index greater than 3 and stable foam index greater than 0.5 (Pagilla, 2015). In this study, stable fractions of foam (Eq. 3) were also calculated to quantify the relative stability of foams.

$$Unstable\ Foam\ Index = \frac{Maximum\ Foam\ Height - Initial\ Height}{Initial\ Height} \quad (1)$$

$$Stable\ Foam\ Index = \frac{Settled\ Foam\ Height - Initial\ Height}{Initial\ Height} \quad (2)$$

$$Stable\ Fraction\ of\ Foam = \frac{Stable\ Foam\ Index}{Unstable\ Foam\ Index} \quad (3)$$

VRTs were done with a Brookfield DV2T viscometer in conjunction with the RheocalcT software (Brookfield Middleboro, MA). Samples of digester sludge were measured with the LV3C spindle. Samples were warmed by water bath to ± 1°C of 35°C and kept at that temperature through the duration of the test. The viscometer and software were used to record values of shear stress (τ) in Pa with increasing shear rate (γ) in s^{-1} ; the collected data were linearized and regressed to the Casson and Ostwald (power) models. These two

models were chosen because they have been shown to provide the best fits for anaerobic digester sludge (Civelekoglu and Kalkan, 2010). The Casson model (Eq. 4) gave plastic viscosity (η_p) in mPa-s and yield stress (τ_o) in Pa while the Ostwald model (Eq. 5) gave consistency index (k) in mPa-s^n and flow behavior index (n), which is unitless. Yield stress and consistency index characterize conditions at low shear, representing the potential for stoppage of bubbles when there is no mixing (visualization of shear profile characteristics in Appendix B). Plastic viscosity and flow behavior index predominantly characterize the apparent viscosity when shear is present. Increasing trends in these parameters represent increased friction on rising bubbles (shear profile characteristics shown in Appendix B).

$$\sqrt{\tau} = \sqrt{\eta_p \gamma} + \sqrt{\tau_o} \quad (4)$$

$$\tau = k\gamma^n \quad (5)$$

2.2.3. DNA extraction and qPCR

Samples for DNA extraction were pelleted and stored at -80^{o}C. DNA was extracted from the pelleted mass of each sample with DNeasy PowerBiofilm Kits (Qiagen Germantown, MD). The extracted samples were analyzed with qPCR to quantify foam-causing bacteria. Well known foam-causing bacteria were targeted with primers for *M. parvicella* and *Gordonia* (for *G. amarae*). Additionally, *Mycobacterium* primers were used to investigate their contribution to digester foaming. Total bacteria were also targeted to normalize quantities of bacteria between samples and primer sets. Primers (list of primers and references in Appendix C) and standards were produced by Eurofins Genomics (Louisville, KY) as custom oligonucleotides. FastStart Essential DNA Green Master kit (Roche Branchburg, NJ) was used for PCR-grade water and master mix. Volumes and

concentrations used for each qPCR reaction are summarized in Table C.2 (Appendix C). The qPCR reactions were conducted using a Lightcycler 96 Gallery (Roche Branchburg, NJ). The parameters for the reactions for each primer set are summarized in Table C.3, Appendix C. Standards were prepared as a serial dilution from 10^8 copies/µL down to 10 copies/µL for each primer set.

2.2.4 Suspended solids concentration and anionic surfactants

Measurements of total suspended solids (TSS) were done in accordance with EPA Method 160.2 (US Environmental Protection Agency, 1971a); To reduce filtration time, two filters were used per sample and sample volumes were adjusted such that 2 mL of sample was diluted with wash water in the filtration apparatus, ensuring that the solids were evenly distributed across the filter. Measurements of volatile suspended solids (VSS) were done in accordance with EPA Method 160.4 (US Environmental Protection Agency, 1971b). Volatile fractions of solids were calculated to be VSS as a fraction of TSS.

Anionic surfactants were measured using the Hach 8028 kit and Hach DR/4000 spectrophotometer (Hach, Loveland, CO). Sludge samples were diluted (1000x) to fit the method's measurable concentration range. Hach concentrations were reported as mg/L of LAS, though the method detects other anionic surfactants in addition to LAS.

2.2.5. Special sample collection and 16S rRNA gene sequence analysis

16S sequencing data were compiled by MR DNA lab (Shallowater, TX, USA) with Illumina MiSeq and were processed with uSearch (Edgar, 2010). A single set of samples were sent for sequencing from WTP, STP, and BWTP, collected on the week of June 18th, 2018. Additionally, five sets of samples were collected from the foam layer of a digester at

WTP, digester contents that flowed onto the digester cover, for 16S sequencing data to compare the foam layer to bulk sludge. Lastly, samples were collected after the startup of a WTP digester, reseeded with biosolids from STP, for foam potential testing, 16S sequencing, and qPCR analysis.

2.3 Gram staining and counting filamentous foam-causing bacteria

An alternative method of quantifying foam-causing bacteria was tested. Surface foam from WTP secondary treatment, known to contain filamentous foam-causing bacteria, was collected and mixed into samples of WTP digester sludge under anaerobic conditions for 12 hours to simulate the behavior of foam-causing bacteria after entering a digester. The resulting samples were analyzed by both qPCR and a modified filament counting method (Gray, 2004). Samples diluted (10x) and 30 μL were spread over 18 mm^2, prepared in duplicates. The dried area was gram stained (Sigma Aldrich St. Louis, MO) and observed under 1000x magnification for 20 randomly selected fields per slide. For each field at 1000x, gram-positive *Gordonia* with branching filaments were identified and the number of intersections, between filaments and the vertical centerline of the field, were recorded. Calculations for the total number of intersections was adapted from a previously developed counting technique (Gray, 2004) to determine intersections per milligram of VSS.

2.4 Correlation Analysis

Correlations found in this study were computed using Tableau software (Seattle, WA). Values for coefficient of determination (R^2) were used to quantify the linear correlations between two datasets. Values of R^2 closer to 1 indicated a stronger linear correlation; positive and negative correlations were recorded. Like other studies investigating foam-

causing bacteria in full-scale treatment plants, an R^2 of 0.250 ($|r| = 0.500$) was used as a threshold value for an acceptable correlation (X. T. Jiang et al., 2016; Miłobędzka and Muszyński, 2015). Probability values (*p*-values) were also calculated. Stronger correlations were expected to have *p*-values less than 0.05.

Correlation analysis was separated by plant. It is suggested that every plant has a different prediction of foam severity from foam potential testing (Pagilla, 2015). This plant specificity was assumed to also apply to VRT measurements. Separating correlations by plant allows isolation of relationships between physical measurements and sludge constituents that are the most relevant to each plant.

3. Results and discussion

3.1. Mycobacterium investigated by 16S sequencing analysis and qPCR

Sequencing and qPCR analysis were conducted to compare the microbial populations of WTP, STP, and BWTP, to identify relevant foam-causing bacteria at WTP, and to study the development of foam potential during a restart of a WTP digester. The comparison of relative abundances from sequences between plants revealed that the genus *Mycobacterium* was abundant at WTP (4.6%) and BWTP (2.9%) but not at STP (0.1%). *Mycobacterium* have not yet been reported to cause digester foaming, but their high abundances corresponded to the treatment plants with the highest digester foam potential (WTP and BWTP), suggesting they may be an overlooked group of foam-causing bacteria. Also, this study investigated their abundance with sequencing in the foam layer at WTP, which revealed that *Mycobacterium* and *Gordonia* were both approximately 4 times more abundant in the digester foam layer than in bulk sludge. These trends were confirmed with

qPCR analysis, showing that *Mycobacterium* and *Gordonia* were 2.6 and 4.5 times more abundant in the foam layer, respectively. Both *Mycobacterium* and *Gordonia* have hydrophobic cell surfaces and cell walls that contain mycolic acid, characteristics thought to induce foaming in secondary treatment processes (Davenport et al., 2008). The shared characteristics between *Mycobacterium* and *Gordonia* may explain their high presence in the digester foam layer at WTP, similar to observations in secondary foam in other research (Winkler et al., 2016). For the digester startup at WTP, biosolids from non-foaming STP digesters were used to seed the restarting WTP digester. In first months of the startup, foam potential indices increased, as did the *Mycobacterium* and *Gordonia* concentrations in the digester sludge. It was assumed at this point of the study that both unstable and stable foam indices could effectively show foaming risk related to foam-causing bacteria. Thus, the concurrence of increasing foam potential with increasing *Mycobacterium* and *Gordonia* validated the interest in monitoring their relationship to digester foaming (Fig. 2).

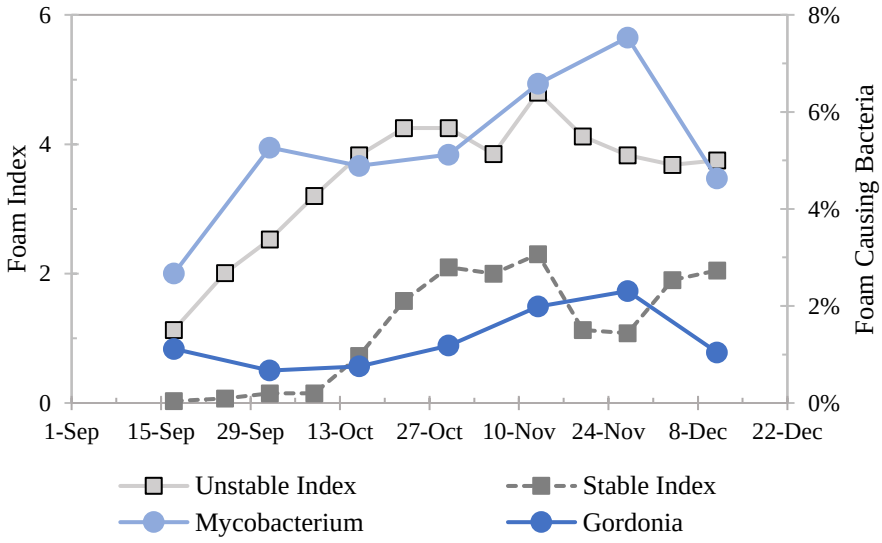

Fig. 2. Increasing foam potential and foam-causing bacteria concentrations observed during startup of WTP Digester 4. Trends with foam potential indices coincide with *Mycobacterium* and *Gordonia* concentrations measured by qPCR.

3.3. West Point Treatment Plant digester foaming evaluation

Foam potential tests and VRTs were conducted to evaluate operator-friendly tests to quantify risk of digester foaming at WTP, as a batch experiment showed positive relationships between these tests and *Gordonia* sourced from WTP (experiment results shown in Appendix D). VRTs showed notable correlations with suspected causes of foaming (Table 2.A; correlations illustrated graphically in Appendix E). For instance, *Gordonia* concentrations showed positive correlations with plastic viscosities ($R^2 = 0.384$, *p*-value < 0.05). This rheological trend represents increased sludge viscosity and slower bubble rise, suggesting that *Gordonia* promote gas entrainment in WTP digesters. Sludge viscosity might have increased as a result of slow degradation of their cells, mycolic acids released upon cell lysis, and biosurfactants travelling with cells (Hernandez and Jenkins, 1994; Pagilla, 2015; Petrovski et al., 2010). *Gordonia* are difficult to avoid for WTP digesters due to the foam trapping and *Gordonia* growth that occur upstream in the HPO system, resulting in high *Gordonia* concentrations in the digester feed. However, once *Gordonia* enters the digesters, their effects on digester foaming can effectively be reduced by removing sludge from the top portion of digesters (surface wasting), where *Gordonia* concentrations are highest.

Additionally, solids concentration, particularly volatile solids, in WTP digester sludge correlated positively with plastic viscosity ($R^2 = 0.522$, *p*-value < 0.05). The relationship between volatile solids concentrations and plastic viscosity suggests that increased volatile solids lead to viscous sludge that may impede the rise of gas. Other studies reported that solids destruction and hydrolysis lowers viscosity (Battistoni et al., 1990; Brar et al., 2005) and that anaerobically digested sludges decrease in viscosity during digestion (Monteiro,

1997). To reduce the impact of volatile solids, organic loading rates can be reduced or digester SRT can be increased to ensure that solids do not accumulate due to the slow rate of solids destruction.

To control digester foaming at WTP, surface wasting is recommended to address *Gordonia* and reduction of volatile solids is suggested to reduce gas entrainment. For foaming from unknown causes, it is recommended to observe trends from both foam potential tests and VRTs. The foam potential test was developed to quantify surface foaming risk and has uncertain applicability for gas entrainment (Pagilla, 2015) while VRT provides the rheological parameters primarily related to gas entrainment risk (Bartek et al., 2017). Given that the foam potential and VRT parameters did not correlate at WTP (Table 3.A), trends from both tests are required to monitor the risk of digester foaming by both surface foaming and gas entrainment. From VRT results, increasing trends in sludge viscosity or yield stress requires increased mixing to remove entrapped gas. From the foam potential test, increasing foam potential requires reduced gas mixing to avoid surface foam production, as gas mixing may provide the mixing energy to remove entrained gas but still worsen digester foaming (Chapman, 2011; Pagilla, 2015).

Table 2.
Correlation (R^2) between foam evaluation methods and sludge constituents of digester foaming at **A.** WTP, **B.** STP, and **C.** BWTP. Under the "Range" column, each cell represents the range of values measured in the units specified in parenthesis for each sludge constituent.

A.	**WTP**	UFI	SFI	SF	PV	YS	CI	FB	Range[a]
Gordonia (%)		-0.040	0.109	0.197	0.384	0.003	0.002	0.237	0.2 - 7.1
Mycobacterium (%)		-0.016	0.051	0.083	0.151	0.071	0.088	0.010	3.8 - 15.1
M. Parvicella (%)		-0.136	-0.027	0.000	0.046	0.177	0.202	-0.020	0.0 - 0.1
TSS (mg/L)		-0.246	-0.196	0.010	0.346	0.004	0.001	0.256	24500 - 33050
VSS (mg/L)		-0.008	0.002	0.013	0.522	0.032	0.029	0.230	16750 - 23822
Volatile Fraction (mg/mg)		-0.043	0.002	0.008	0.445	0.109	0.173	0.031	0.67 - 0.76
Anionic Surfactants (mg/L)[b]		-0.153	0.010	0.080	-0.044	0.000	-0.001	-0.029	22- 44
B.	**STP**	UFI	SFI	SF	PV	YS	CI	FB	Range[a]
Gordonia (%)		0.075	0.034	0.035	0.001	-0.112	-0.279	0.408	0.1 - 0.7
Mycobacterium (%)		0.020	0.669	0.652	0.488	-0.005	-0.006	0.725	0.2 - 1.0
M. Parvicella (%)		0.002	0.276	0.280	0.098	0.005	0.000	0.203	0.0 - 9.8
TSS (mg/L)		0.008	0.660	0.744	0.460	0.010	0.012	0.500	24550 - 32400
VSS (mg/L)		0.017	0.679	0.759	0.461	0.002	0.004	0.482	18100 - 24950
Volatile Fraction (mg/mg)		0.085	0.289	0.303	0.159	-0.047	-0.023	0.131	0.74 - 0.79
Anionic Surfactants (mg/L)[b]		-0.017	-0.068	0.033	0.002	-0.270	-0.270	0.125	30 - 59
C.	**BWTP**	UFI	SFI	SF	PV	YS	CI	FB	Range[a]
Gordonia (%)		-0.139	0.010	0.134	0.082	0.006	0.006	0.014	0.1 - 0.4
Mycobacterium (%)		0.055	-0.356	-0.593	-0.642	-0.020	-0.033	-0.039	3.1 - 9.7
M. Parvicella (%)		0.619	0.339	0.093	0.091	-0.230	-0.227	0.258	0.0 - 12.6
TSS (mg/L)		0.082	0.123	0.405	0.357	0.006	0.012	0.026	23050 - 30700
VSS (mg/L)		0.043	0.101	0.290	0.409	-0.017	-0.008	0.103	16433 - 26350
Volatile Fraction (mg/mg)		0.004	0.032	0.023	0.324	-0.326	-0.268	0.361	0.71 - 0.86
Anionic Surfactants (mg/L)[b]		0.159	0.046	0.045	-0.032	0.589	0.539	-0.378	36 - 55

[a] Range of values (minimum - maximum) measured during the study (see Appendix F).

[b] An outlier from each plant was filtered out of analyses for correlations (see Fig. F.3., Appendix F).

Key

Positive correlation ($R^2 = 1$)
Lowest correlation ($R^2 = 0$)
Negative correlation ($R^2 = 1$)

UFI - Unstable Foam Index
SFI - Stable Foam Index
SF - Stable Fraction of Foam

PV - Plastic Viscosity
YS - Yield Stress
CI - Consistency Index
FB - Flow Behavior Index

Table 3.
Correlation between foam potential parameters and calculated rheological parameters at **A.** WTP, **B.** STP, and **C.** BWTP

A.	**WTP**	Plastic Viscosity	Yield Stress	Consistency Index	Flow Behavior Index
Unstable Foam Index		-0.045	-0.003	-0.010	-0.002
Stable Foam Index		0.008	-0.002	-0.013	0.052
Stable Fraction		0.043	-0.002	-0.008	0.085
B.	**STP**	Plastic Viscosity	Yield Stress	Consistency Index	Flow Behavior Index
Unstable Foam Index		0.001	-0.453	-0.420	0.063
Stable Foam Index		0.507	0.000	0.006	0.444
Stable Fraction		0.477	0.079	0.091	0.398
C.	**BWTP**	Plastic Viscosity	Yield Stress	Consistency Index	Flow Behavior Index
Unstable Foam Index		0.036	-0.350	-0.380	0.434
Stable Foam Index		0.583	0.022	0.025	0.063
Stable Fraction		0.216	0.419	0.468	-0.152

Key

Positive correlation ($R^2 = 1$)
Lowest correlation ($R^2 = 0$)
Negative Correlation ($R^2 = 1$)

3.4. South Treatment Plant Digester Foaming Evaluation

Foam causing bacteria concentrations correlated with foam evaluation parameters (Table 2.B). However, only *M. parvicella* were present in high concentrations and showed weak correlations ($R^2 = 0.276$, p-value < 0.05) with foam potential parameters, suggesting that the bacteria are not the primary contributors to digester foaming at STP (correlations shown graphically in Appendix E; distribution of measured concentrations in Appendix F). However, past digester foaming at STP coincided with conditions in secondary treatment that favored the enumeration of *M. parvicella*, when secondary mean cell residence times (MCRTs) were raised to accommodate nitrification (MCRT = 7-9 days); foaming decreased when secondary MCRTs were lowered (MCRT = 3.5-4.5 days) and nitrification was diminished. Under nitrification conditions, nitrate in the return activated sludge resulted in the anaerobic selector zone (ahead of the aeration zone) to become anoxic. Growth of *M. parvicella* has been claimed to be favored at long SRTs (>12 days), low temperatures, low dissolved oxygen (DO) conditions, and uptake of long chain fatty acid (LCFA) in anoxic

selectors (Jenkins et al., 2003; Nielsen et al., 2002). Though *M. parvicella* was not the primary contributor to foam risk at STP during this study, past foaming experience at STP showed that less favorable conditions for *M. parvicella* (reduction of nitrification) reduced digester foaming issues at full-scale. The exact cause of *M. parvicella* enumeration cannot be determined with the information available in this study, but increased MCRT, low DO from increased oxygen demand from nitrification, and the presence of an anoxic selector could have favored their growth.

Furthermore, foam potential tests showed that volatile solids concentrations increased stable foam indices ($R^2 = 0.679$, p-value < 0.05) and stable fractions of foam ($R^2 = 0.759$, p-value < 0.05), suggesting that high volatile solids contribute to foam stability. Shown on Table 2.B., high volatile solids concentrations also correlated to increased plastic viscosities ($R^2 = 0.461$, p-value < 0.05) and flow behavior indices ($R^2 = 0.482$, p-value < 0.05), which relate to slow bubble rise. Thus, the risk of digester foaming by stable foam production and gas entrainment may be minimized by decreasing organic loading rates or increasing digester SRT, as both methods reduce the organic solid concentrations of digester sludge.

To control digester foaming, increased gas mixing can be applied to remove entrained gas. To ensure that gas mixing at STP will not result in both surface foaming and gas entrainment, trends in foam potential and VRT parameters should be observed. Results in this study show that foam potential was relatively low (range of values measure shown in Appendix F), meaning there is low risk of surface foam production at STP. In past digester foam events, increased mixing removed entrained gas without producing problematic surface foams. Thus, low foam potential may justify increased gas mixing to resolve gas entrainment issues.

3.5. Brightwater Treatment Plant digester foaming evaluation

Counter to expectations, *Mycobacterium* concentrations at BWTP negatively correlated to test parameters associated with foam stability (R^2 = 0.593, *p*-value < 0.05) and gas entrainment (R^2 = 0.642, *p*-value < 0.05; correlation graph shown in Appendix E), suggesting they reduce the likelihood of surface foaming and gas entrainment. Conversely, *M. parvicella* correlated with foam potential parameters that relate to foam production (R^2 = 0.619, *p*-value < 0.05), which is in line with literature (Westlund et al., 1998) and the results from STP. Notably, because BWTP operates with a membrane bioreactor system, the system is designed for an average MCRT of 10 days. Additionally, the secondary treatment system is designed with an anoxic basin. Given that *M. parvicella* have been observed to grow in secondary systems with high MCRTs (>12 days) and anoxic basins (Jenkins et al., 2003; Nielsen et al., 2002), the secondary treatment system at BWTP may have provided favorable conditions for *M. parvicella* growth, particularly during periods when the MCRT exceeded the design average. Though the specific cause of *M. parvicella* growth was not determined in this study, *M. parvicella* were shown to relate to increased risk of digester foaming at BWTP.

Similarly, volatile solids concentrations at BWTP correlated to stable fractions of foam (R^2 = 0.290, *p*-value = 0.17) and plastic viscosities (R^2 = 0.409, *p*-value = 0.09). The positive relationships with these foam evaluation parameters showed that volatile solids contributed to foam stability and increased viscosity, corresponding to persistent foams and slow bubble rise, respectively. Unique to BWTP, volatile fractions negatively correlated with yield stress (R^2 = 0.326, *p*-value = 0.14) and consistency index (R^2 = 0.268, *p*-value =0.19), theoretically corresponding to decreased trapping of bubbles (Bartek et al., 2017; Chapman,

2011). Therefore, volatile solids at BWTP contribute to gas entrainment by slowing the rise of gas out of the sludge when mixing is present, rather than complete entrapment under unmixed conditions. Like WTP and STP, gas entrainment risk may be reduced at BWTP by decreasing organic loading rates or increasing digester SRT.

Lastly, anionic surfactants positively correlated with yield stress ($R^2 = 0.589$, *p*-value = 0.39) and consistency index ($R^2 = 0.539$, *p*-value = 0.37). The mechanisms relating anionic surfactants to sludge rheology are not clear. But it is known that increases in yield stress and consistency index correspond to bubble entrapment, suggesting anionic surfactants contribute to digester foaming by stopping bubble movement in digester sludge.

Notably, the correlations discussed for BWTP have weaker statistical support as evidenced by high *p*-values for solids concentrations and anionic surfactants (other *p*-values listed in Appendix G). Sampling and analysis for BWTP were half as frequent as analysis at WTP and STP, which reduced the number of data points to support correlations. Thus, increased frequency of analysis could have improved the understanding of connections between sludge constituents and measurements of foam risk at BWTP.

Regardless of the constituents that promote digester foaming, experiences with foaming events at BWTP have demonstrated that applying mechanical mixing to the digesters controls the occurrence of foaming. As a proactive measure, rheological parameters from VRTs should be monitored ahead of changes to mixing conditions to ensure that reduced mixing does not coincide with high gas entrainment risk.

3.6. Practical Application of Foam Evaluation Methods

The aim of this study was to investigate how foam evaluation methods may be used to project foaming events and how the methods relate to sludge constituents known to cause digester foaming. For the application of foam potential test and VRTs, it is important to consider that both tests were only capable of characterizing the risk of digester foaming, as opposed to the occurrence of foam in full-scale digesters. Evidently, the foam evaluation parameters (unstable foam index, stable foam index, plastic viscosity, etc.) fluctuated though periods when digesters did not experience foaming issues. Mentioned previously, authors suggest long term testing to accurately relate foam potential testing to severity of foaming (Pagilla, 2015). This recommendation aligns with the results in this study, as the average stable foam indices for the three treatment plants (0.93 at WTP, 0.53 at STP, and 2.41 at BWTP) correspond to predictions of "severe foaming" (example severity scale in Appendix A) despite the absence of severe foaming at the treatment plants. The discrepancy exemplifies how bench-scale predictions of digester foaming are relative to each plant. Thus, foam evaluation methods should be used to evaluate the relative potential for foaming at each plant and not to quantify the magnitude of foaming at full-scale.

Foam evaluation parameters can function as surrogates to predict foam but cannot identify causes without additional evidence of correlation specific to each treatment plant. For example, *M. parvicella* correlated with foam potential parameters at STP and BWTP while *Gordonia* correlated with plastic viscosity at WTP. Confirmation of the presence of these foam-causing bacteria (e.g. with gram staining or qPCR) combined with trends from the foam-evaluation methods provide necessary evidence to identify foam-causing bacteria as the cause of digester foaming issues at full-scale. Similarly, trends in solids concentrations

should be observed in parallel with trends in increasing viscosity to inform decisions on adjusting digester feeding to reduce the risk of digester foaming by gas entrainment.

The foam potential tests and VRTs should be applied in making decisions on mixing as mitigation strategy for digester foaming. Results from VRTs show trends of increasing risk of gas entrainment, which can theoretically resolve with increased mixing. However, digesters with gas mixing, in particular, are more prone to foaming by surface foaming (Pagilla et al., 1997). To ensure that mixing does not worsen foaming, it is recommended to monitor the risk of surface foaming with regular foam potential testing such that decisions to increase gas mixing do not coincide with high risk of surface foaming.

3.7 Alternative quantification of foam-causing bacteria at wastewater treatment plants

Simple and accurate quantification of foam-causing bacteria can supplement foam evaluation methods to identify foam-causing bacteria as the cause of digester foaming in full-scale digesters. The filament counting method was tested for this application (example microscopic images shown in Appendix H). Results from the counting method were well correlated to qPCR analysis (Fig. 3), suggesting that filament counting is a suitable method for quantifying the foam-causing bacteria in digester sludge. Furthermore, filament counting requires less equipment, training, and time than qPCR analysis, making the method more accessible than qPCR analysis. As a note, it has been theorized that partial degradation of *G. amarae* in anaerobic digesters results in weak retention of gram stains (Hernandez et al., 1994), which can complicate identification and counting. Nonetheless, filament counting was shown to be an accessible method of quantifying *G. amarae* in digester sludge, which may provide context to foam evaluation methods.

An important consideration with the quantification of foam-causing bacteria is that *G. amarae* and *M. parvicella* are widely studied and are conveniently identifiable by their filamentous structure and retention of gram-stains. However, these two bacteria may not be solely responsible for microbially mediated foaming, and other lesser-known foam-causing bacteria may contribute to foaming (Appendix I shows correlations using the combined concentrations of *Gordonia*, *M. parvicella*, and *Mycobacterium*). Furthermore, contributions to foam risk may not be exclusively proportional to the quantity of foam-causing bacteria and may be related to the activity of foam-causing bacteria, as exemplified with *Mycobacterium* in other research (Maza-Marquez et al., 2016). In this case, identification and quantification alone would not provide enough information to quantify the contribution of foam-causing bacteria. For this reason, qPCR used in this study and the proposed alternative (filament counting method) are limited in their ability to quantify biological constituents that promote foaming.

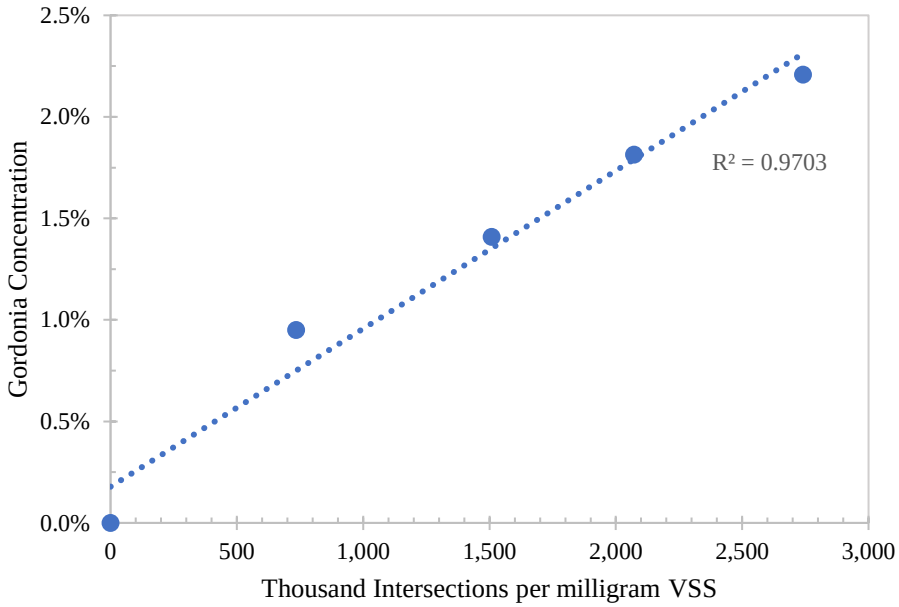

Fig. 3. Correlation between qPCR and Filament Counting. Concentrations measured by filament counting (intersections per mg VSS) correlate well with concentrations of *Gordonia* (%) measured by qPCR.

4. Conclusion

Foam potential tests and VRTs had different trends for each plant but offered a quick risk assessment for surface foaming and gas entrainment, respectively. Therefore, it is recommended to use foam evaluation methods, but only with other supporting evidence, to identify the cause and mechanism of foaming. Specifically, this work showed that a) increased *Gordonia* concentrations at WTP related to increase risk of gas entrainment, b) at STP and BWTP, *M. parvicella* positively correlated with surface foaming risk, and c) in all treatment plants, increased volatile solids concentrations corresponded to more viscous sludge and higher risk of gas entrainment.

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Appendices

Appendix A: Example foam severity scale provided by Pagilla (2015).

The following foam severity scale (Table A.1.) was provided as an example scale to use to interpret foam potential testing results (Pagilla, 2015). The author of this severity scale suggests long term use of foam potential testing to develop severity scales for individual treatment plants that can help predict foaming events.

Table A.1 Example severity scale for interpretation of foam potential test results

Foam Rating	Stable Foam Index	Unstable Foam Index
Non-Foaming	0 - 0.1	0 - 1.0
Mild Foaming	0.1 - 0.3	1.0 - 2.0
Foaming	0.3 - 0.5	2.0 - 3.0
Severe Foaming	> 0.5	> 3

Appendix B: Shear Profile Characteristics

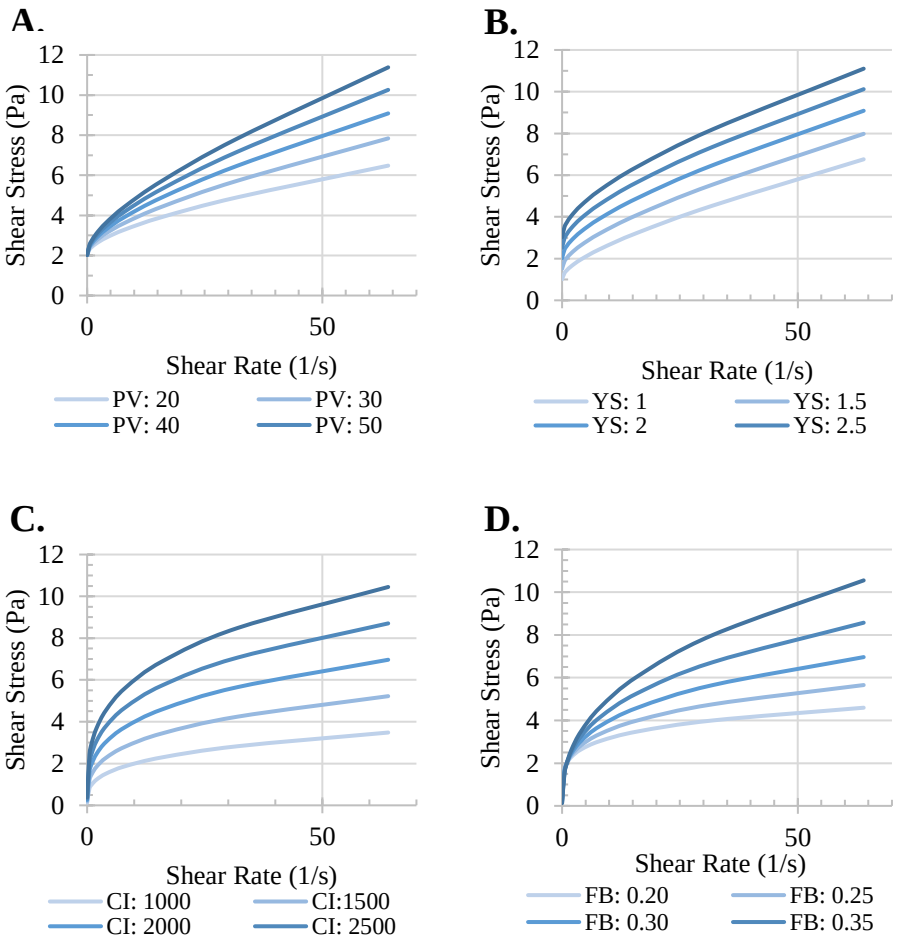

Fig. B.1. Shear profiles for different **A.** plastic viscosities, **B.** yield stresses, **C.** consistency indices, and **D.** flow behavior indices.

Table B.1.
Interpretation of shear profiles from Fig.B.1. with respect to gas entrainment. High apparent viscosities at low shear rates relate to full entrapment of bubbles. High apparent viscosities at high shear rates relate to slow bubble rise.

		Trend in Apparent Viscosity	
	Increasing Trend in Parameter	Low Shear (Bubble Entrapment)	High Shear (Slow Bubble Rise)
A.	Plastic Viscosity	↑	↑
B.	Yield Stress	↑↑	↑
C.	Consistency Index	↑↑	↑
D.	Flow Behavior	↓	↑↑

Appendix C: qPCR Reaction Parameters

Table C.1.
Primers used for qPCR analysis

Target Bacterium	Primer Name	Sequence (5'-3')	Expected Amplicon Length	Reference
Gordonia (genus)	G268F	AGGCGGGTCTCTGGGTAGTA	411	a.
	G1096R	ATAACCCGCTGGCAATACAG		
M. Parvicella	S-S-M.par-0828-S-21	GGTGTGGGGAGAACTCAACTC	210	b.
	S-S-M.par-1018-A-17	GACCCCGAAGGACACCG		
Mycobacterium (genus)	AFB genus FWD-06	CCGCAAGGCTAAAACTCAAA	149	c.
	AFB genus REV-01	TGCACACAGGCCACAAGGGA		
Total bacteria	S-D-Bact-0509-S-17	ACTACGTGCCAGCAGCC	297	d.
	S-D-Bact-0784-A-22	GGACTACCAGGGTATCTAATCC		

a. (Shen and Young, 2005)
b. (Kaetzke et al., 2005)
c. (Richardson et al., 2009)
d. (Rupf et al., 1999)

Table C.2.
Reaction volumes and concentrations for qPCR

	Volume per Reaction (µL)	Concentration
PCR Grade H_2O[a]	3	-
Mastermix[a]	5	-
Forward Primer	0.5	10 mM
Reverse Primer	0.5	10 mM
Sample DNA	1	1 ng/µL

[a] Provided in FastStart Essential DNA Green Master Kit

Table C.3.
Lightcycler parameters for qPCR analysis

Program	*Gordonia*	*M. Parvicella*	*Mycobacterium*	Total Bacteria
Pre-Incubation	600 sec. at 95°C	600 sec. at 95°C	600 sec. at 95°C	600 sec. at 95°C
3-Step Amplification	No. of Cycles: 30	No. of Cycles: 35	No. of Cycles: 35	No. of Cycles: 35
	10 sec. at 95°C	10 sec. at 95°C	10 sec. at 95°C	10 sec. at 95°C
	10 sec. at 60°C	10 sec. at 60°C	10 sec. at 60°C	10 sec. at 60°C
	50 sec. at 72°C	30 sec. at 72°C	16 sec. at 72°C	30 sec. at 72°C
Acquisition (Reading)	3 sec. at 81°C	3 sec. at 81°C	3 sec. at 81°C	3 sec. at 81°C
Melting	10 sec. at 95°C	10 sec. at 95°C	10 sec. at 95°C	10 sec. at 95°C
	60 sec. at 65°C	60 sec. at 65°C	60 sec. at 65°C	60 sec. at 65°C
	1 sec. at 97°C	1 sec. at 97°C	1 sec. at 97°C	1 sec. at 97°C

Appendix D: Foam evaluation methods with batch test samples inoculated with *Gordonia*

D.1. Methodology

A sludge sample from a non-foaming digester at STP was collected, according sample handling and storage methods in Section 2.2.1. Foam from secondary treatment, known to contain *Gordonia amarae*, was collected from the surface of the mixed liquor channel at WTP. The secondary foam was centrifuged to remove gaseous volume and was added to STP digester sludge in varying concentrations. These samples were mixed under anaerobic conditions at 37.8^{o}C for 12 hours to ensure thorough integration of *Gordonia* into sludge samples. Foam potential tests and VRTs were conducted after the mixing period. Concentrations of *Gordonia* were determined by qPCR.

D.2. Results

Both unstable and stable foam indices linearly increased with increasing *Gordonia* as shown on Fig. C.1.A. Additionally, plastic viscosity and yield stress increased with *Gordonia* concentrations (Fig. C.1.B). Under the controlled conditions of this experiment, foam potential tests and VRTs showed increasing potential for digester foaming for samples containing higher concentrations of *Gordonia*.

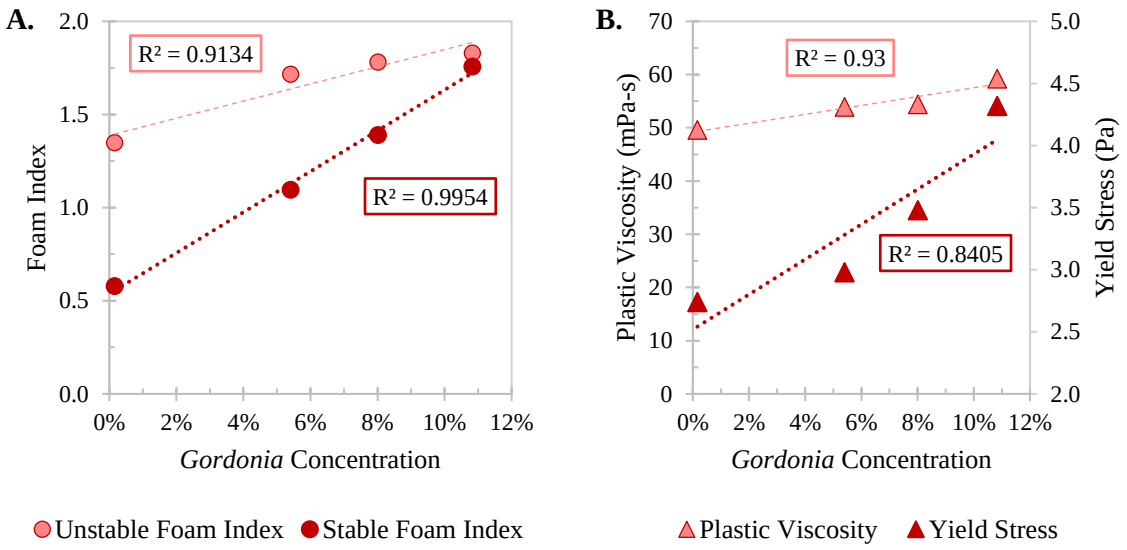

Fig. D.1. Increasing **A.** foam potential parameters and **B.** VRT parameters for sludges with increasing added concentrations of *Gordonia*.

Appendix E: Selected correlations between physical measurements and sludge constituents

The following graphs contain correlations from foam potential tests and VRTs. As a note, the linear regressions provided in each graph were computed through Excel (Microsoft, Redmond, WA), which differ from calculations for coefficients of determination (R^2) that were done with Tableau (Seattle, WA).

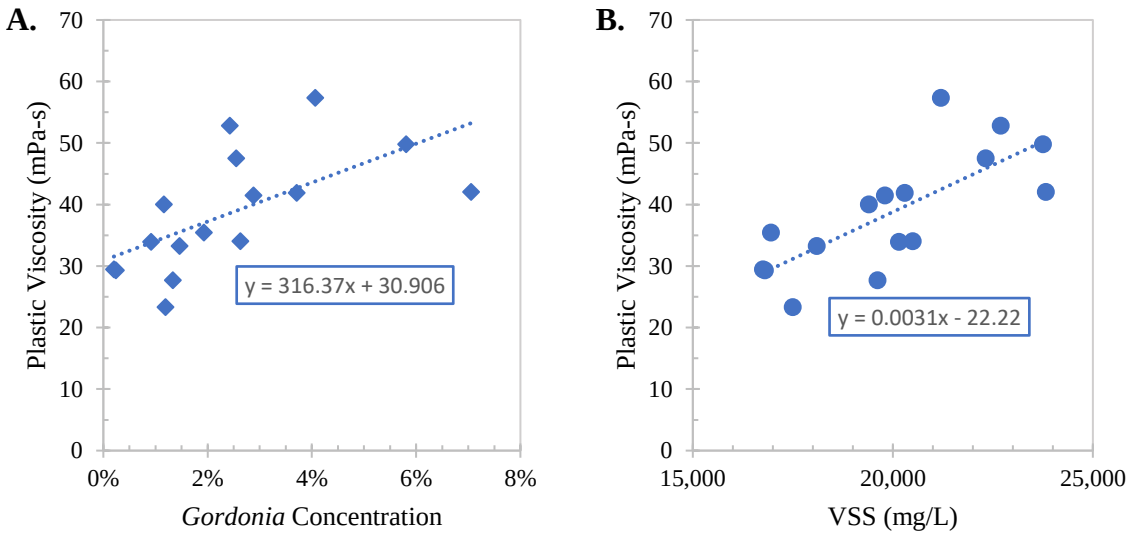

Fig. E.1. Correlations from digester sludge at WTP between **A.** plastic viscosity and *Gordonia* (R^2 = 0.384) in addition to **B.** plastic viscosity and VSS (R^2 = 0.522).

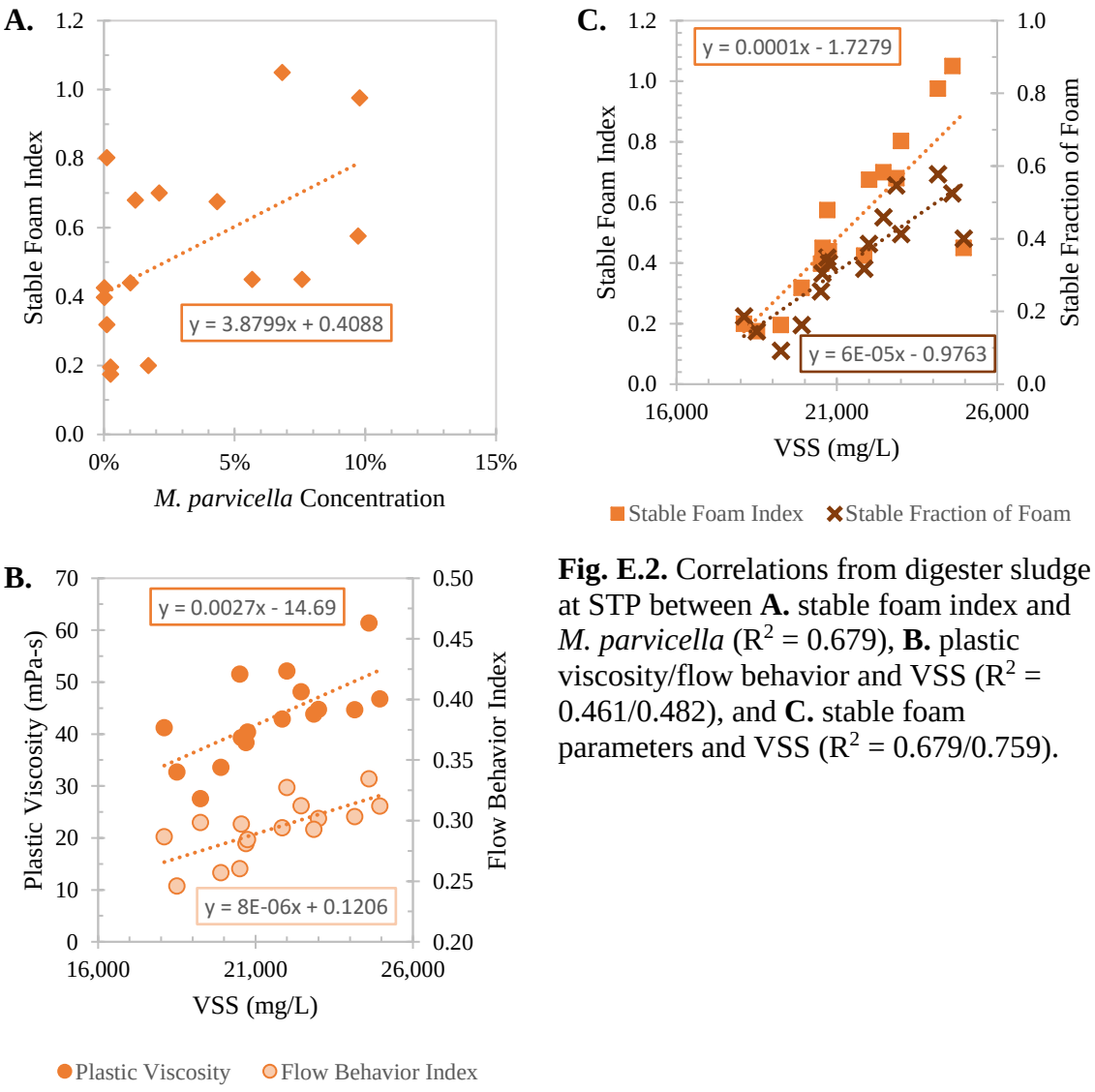

Fig. E.2. Correlations from digester sludge at STP between **A.** stable foam index and *M. parvicella* (R^2 = 0.679), **B.** plastic viscosity/flow behavior and VSS (R^2 = 0.461/0.482), and **C.** stable foam parameters and VSS (R^2 = 0.679/0.759).

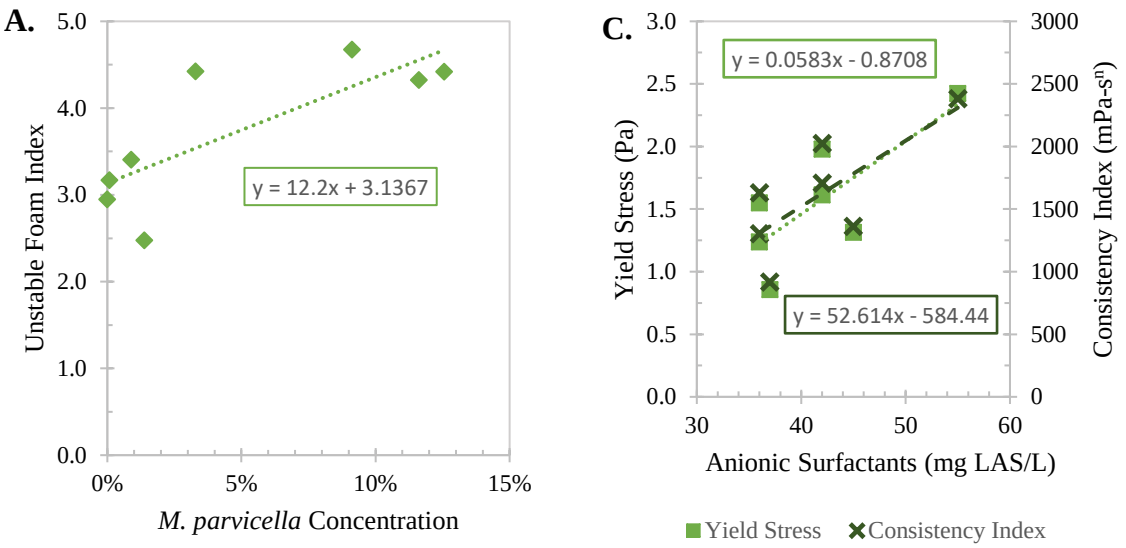

Fig. E.3. Correlations from digester sludge at BWTP between **A.** unstable foam index and *M. parvicella* (R^2 = 0.619), **B.** plastic viscosity and VSS (R^2 = 0.409), and **C.** yield stress/consistency index and anionic surfactants (R^2 = 0.589/0.539).

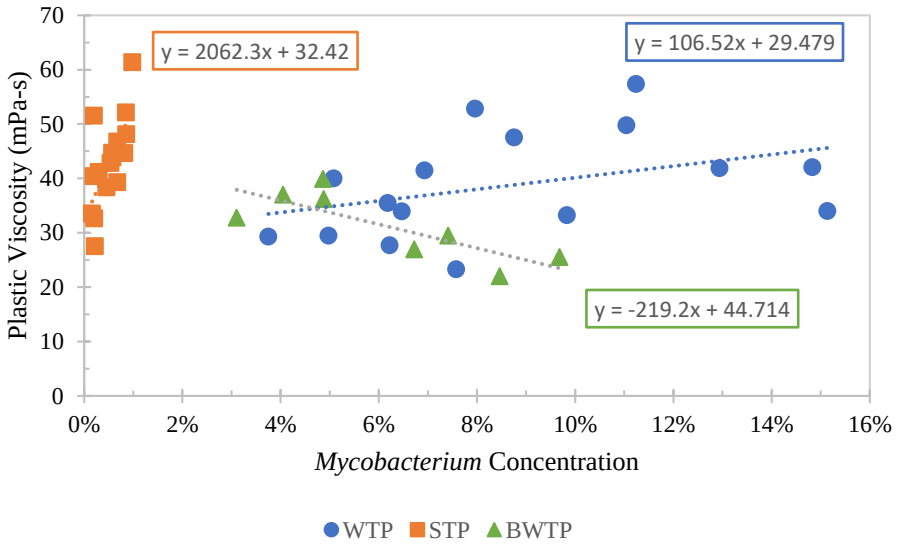

Fig. E.4. Correlation between *Mycobacterium* and plastic viscosity at all three treatment plants. Correlations WTP were positive at WTP (R^2 = 0.151), positive at STP (R^2 = 0.488), and negative at BWTP (R^2 = 0.642).

Appendix F: Box plots of measurements from each treatment plant

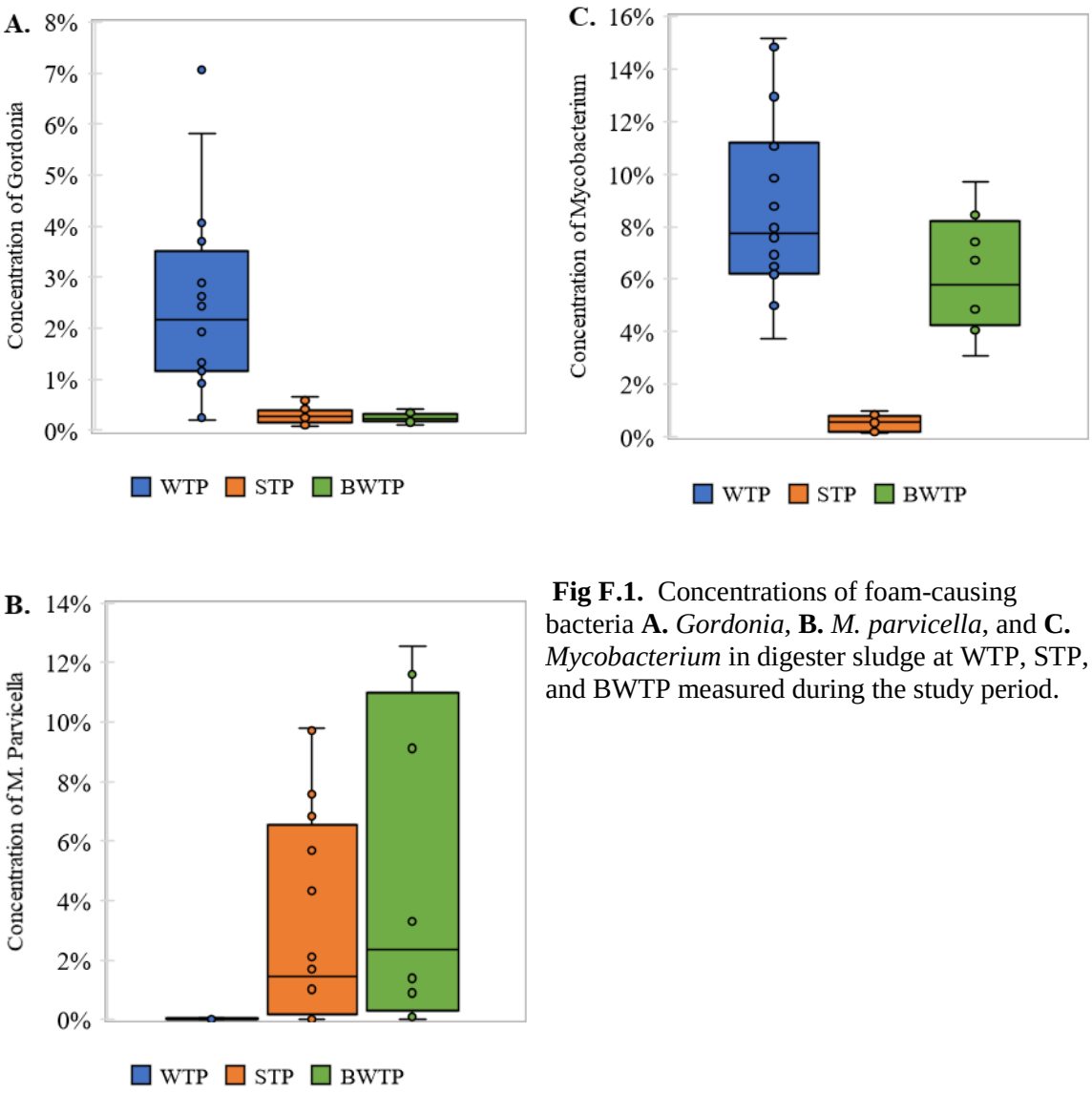

Fig F.1. Concentrations of foam-causing bacteria **A.** *Gordonia*, **B.** *M. parvicella*, and **C.** *Mycobacterium* in digester sludge at WTP, STP, and BWTP measured during the study period.

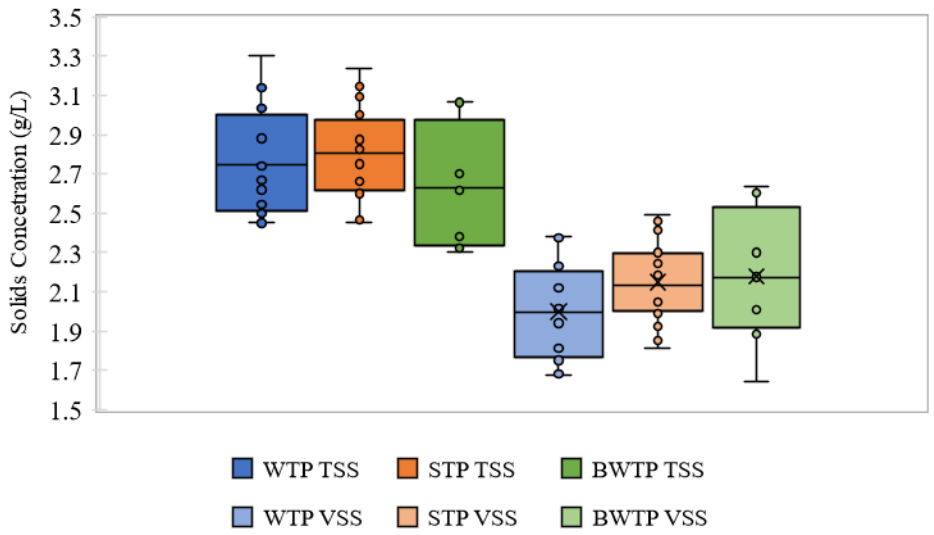

Fig F.2. Total and volatile suspended solids of digester sludge measured at WTP, STP, and BWTP during the study period.

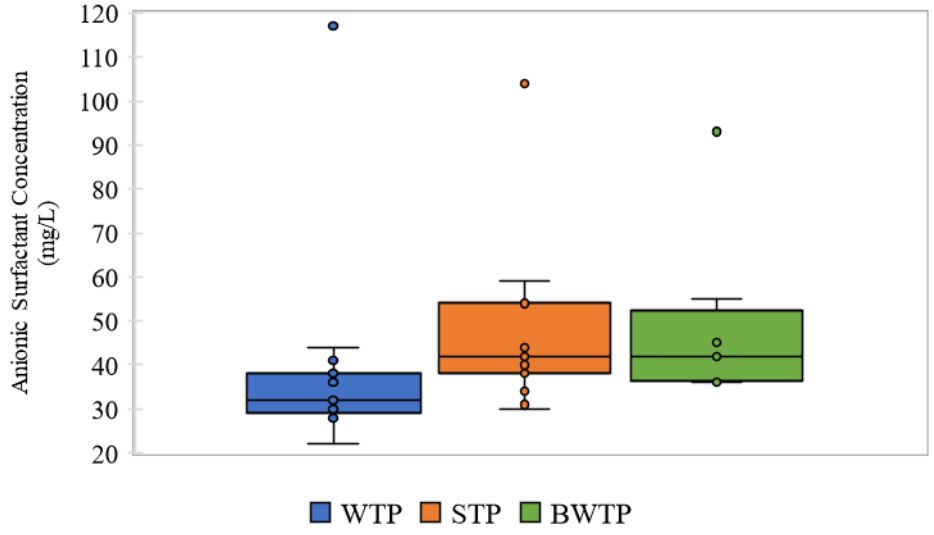

Fig F.3. Anionic surfactants in digester sludge measured at WTP, STP, and BWTP during the study period. Three outliers, one from each plant, were removed from correlation calculations presented in the Sec. 3.

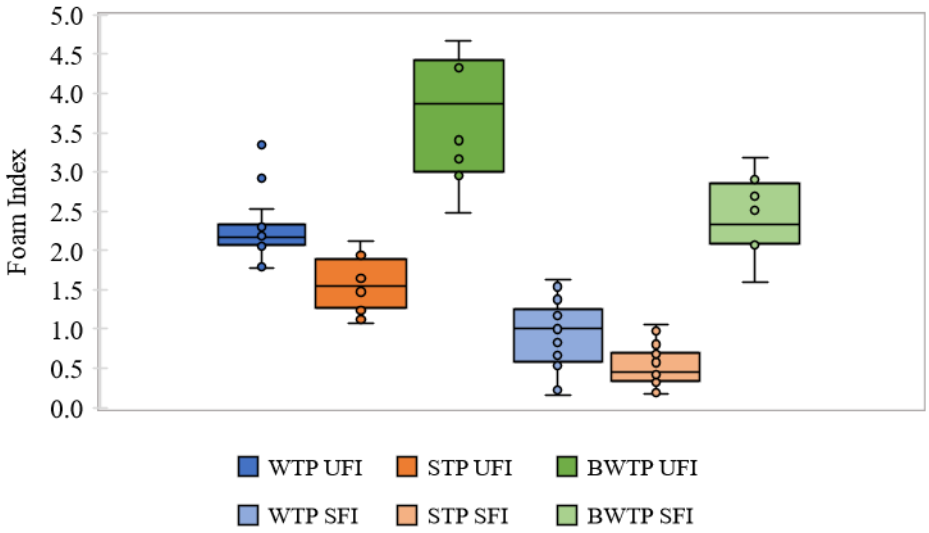

Fig F.4. Unstable and stable foam indices of digester sludge measured at WTP, STP, and BWTP during the study period.

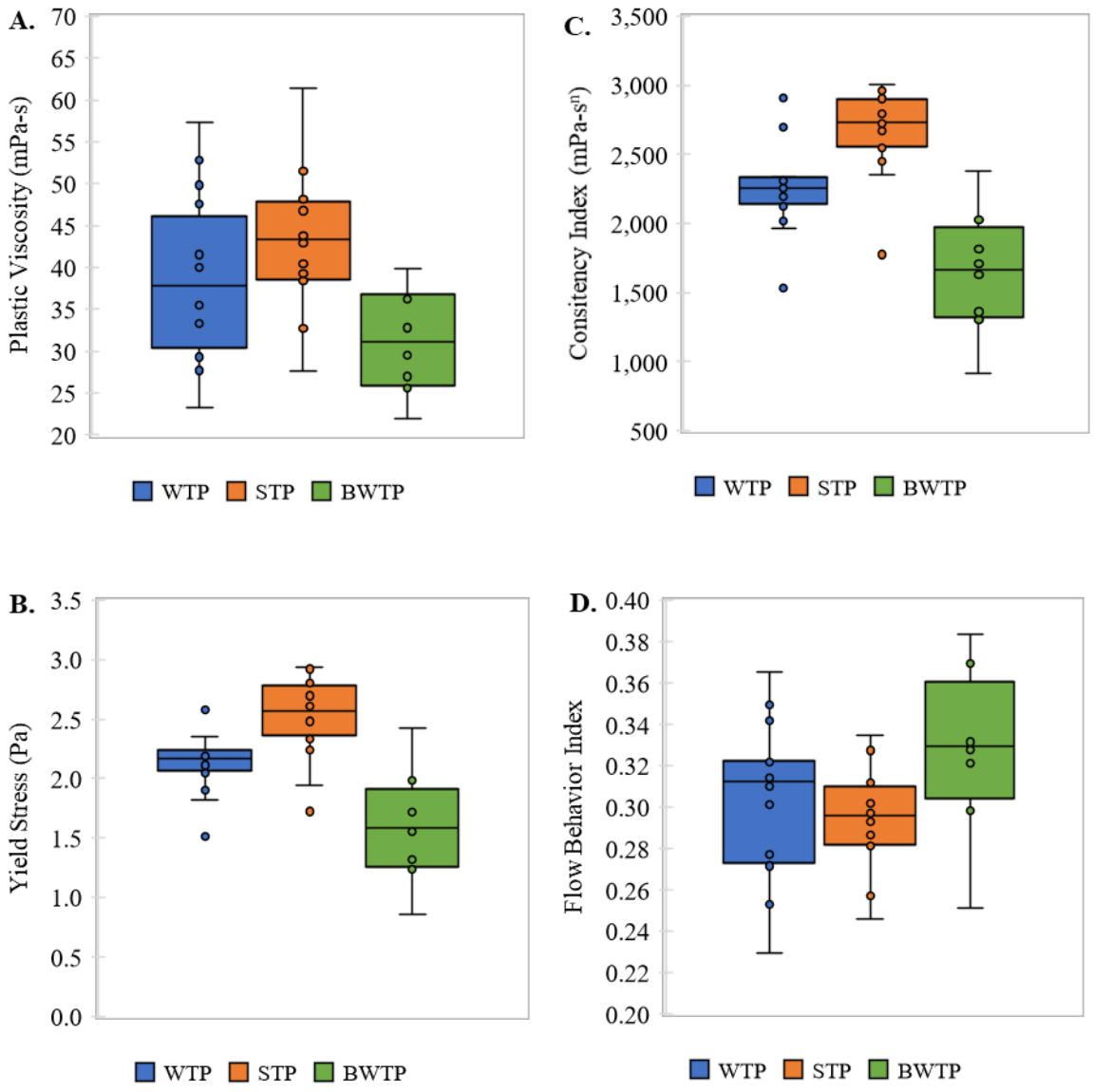

Fig F.5. Viscometer ramp test parameters **A.** plastic viscosity, **B.** yield stress, **C.** consistency index, and **D.** flow behavior index of digester sludge at WTP, STP, and BWTP measured during the study period.

Appendix G: *p*-values for correlations found between foam potential tests/VRTs and sludge constituents.

Table G.1.

p-values for correlations foam potential test/VRT parameters and sludge constituents.

A.	**WTP**	UFI	SFI	SF	PV	YS	CI	FB
Gordonia (%)		0.474	0.228	0.097	**0.014**	0.850	0.874	0.070
Mycobacterium (%)		0.653	0.417	0.297	0.152	0.336	0.284	0.724
M. Parvicella (%)		0.177	0.561	0.989	0.443	0.118	0.093	0.617
TSS (mg/L)		0.060	0.847	0.432	**0.021**	0.816	0.936	0.054
VSS (mg/L)		0.098	0.905	0.321	**0.002**	0.524	0.541	0.071
Volatile Fraction (mg/mg)		0.729	0.436	0.341	**0.007**	0.230	0.123	0.530
Anionic Surfactants (mg/L)*		0.479	0.572	0.752	0.451	0.107	**0.035**	**0.011**
B.	**STP**	UFI	SFI	SF	PV	YS	CI	FB
Gordonia (%)		0.306	0.495	0.485	0.925	0.206	**0.035**	**0.008**
Mycobacterium (%)		0.600	**0.000**	**0.000**	**0.003**	0.801	0.781	**0.001**
M. Parvicella (%)		0.873	**0.037**	**0.035**	0.239	0.939	0.939	0.080
TSS (mg/L)		0.748	**0.000**	**0.000**	**0.004**	0.688	0.688	**0.002**
VSS (mg/L)		0.627	**0.000**	**0.000**	**0.004**	0.807	0.807	**0.003**
Volatile Fraction (mg/mg)		0.274	**0.032**	**0.027**	0.126	0.572	0.572	0.169
Anionic Surfactants (mg/L)*		0.856	0.349	0.244	0.252	0.445	0.445	0.139
C.	**BWTP**	UFI	SFI	SF	PV	YS	CI	FB
Gordonia (%)		0.363	0.813	0.373	0.492	0.854	0.854	0.779
Mycobacterium (%)		0.577	0.118	**0.025**	**0.017**	0.739	0.668	0.638
M. Parvicella (%)		**0.021**	0.130	0.464	0.468	0.229	0.232	0.199
TSS (mg/L)		0.492	0.394	0.090	0.118	0.850	0.794	0.700
VSS (mg/L)		0.624	0.442	0.168	0.088	0.762	0.837	0.439
Volatile Fraction (mg/mg)		0.880	0.671	0.720	0.141	0.139	0.189	0.115
Anionic Surfactants (mg/L)*		0.327	0.767	0.578	0.697	0.389	0.374	0.247

*An outlier from each plant was filtered out of analyses for correlations (see Fig. C.3., Appendix C).

Key

p value < 0.05

UFI - Unstable Foam Index
SFI - Stable Foam Index
SF - Stable Fraction of Foam
PV - Plastic Viscosity
YS - Yield Stress
CI - Consistency Index
FB - Flow Behavior Index

Appendix H: Example images from filament counting

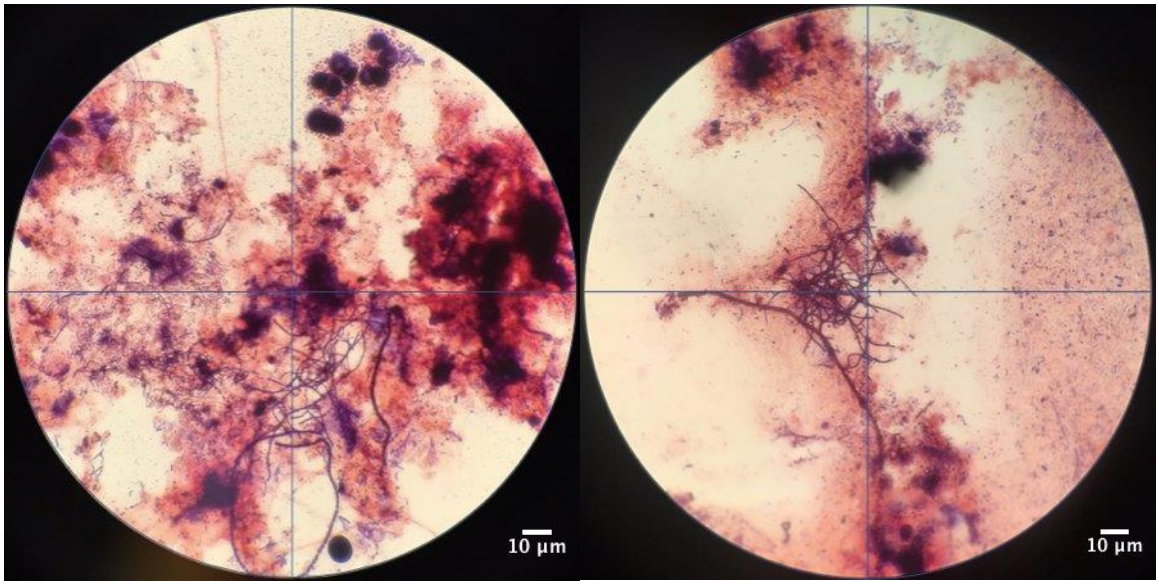

Fig. G.1. Example images used to count filaments for WTP digester sludge. The vertical line across each field was used to count the number of intersections with foam-causing bacteria; horizontal lines were used to align the vertical lines.

Appendix I: Correlation between total foam-causing bacteria and foam potential test/VRT parameters.

Foam potential test and VRT parameters were compared with the combined concentrations of *Gordonia*, *Mycobacterium*, and *M. parvicella*. This approach was applied to all the data from the three treatment plants and separately applied to each treatment plant (Table I.1). From all treatment plants, the strongest correlation was between unstable foam index and combined foam-causing bacteria ($R^2 = 0.254$). This correlation was likely influenced by the correlation between *M. parvicella* and unstable foam index at BWTP (Table 2.C). Shown on Table I.1, the correlations at each plant varied from those of the entire data pool. Because of the discrepancies in strength and direction (positive/negative) of correlations, it is clear that the relationships between foam-causing bacteria and foam potential test/VRT parameters were specific to each plant.

Table I.1.
Correlations between foam potential test/VRT parameters and combined total of *Gordonia, M. parvicella,* and *Mycobacterium*.

Combined Foam-Causing Bacteria[a]	UFI	SFI	SF	PV	YS	CI	FB
All Three Plants	0.254	0.230	0.154	0.001	-0.162	-0.165	0.177
WTP	-0.023	0.088	0.143	0.248	0.036	0.048	0.075
STP	0.004	0.319	0.323	0.121	0.002	0.000	0.268
BWTP	0.752	0.103	-0.382	-0.001	-0.282	-0.297	0.176

[a] Sum of concentrations (%) of *Gordonia, M. parvicella,* and *Mycobacterium.*

Key

Positive correlation ($R^2 = 1$)
Lowest correlation ($R^2 = 0$)
Negative correlation ($R^2 = 1$)

UFI - Unstable Foam Index
SFI - Stable Foam Index
SF - Stable Fraction of Foam

PV - Plastic Viscosity
YS - Yield Stress
CI - Consistency Index
FB - Flow Behavior Index

Supplementary Material: Investigation of Sulfate and Sulfide

SA.1 Effects of sulfate-reducing bacterial activity on physical properties of sludge

Sulfate reducing bacteria (SRB), specifically *Desulfotomaculum*, was suggested to be foam-causing bacteria based on 16s sequencing of digester foam (Kougias et al., 2014). The mechanisms by which SRB may contribute to digester foam were not established. A possible explanation is that SRB produce biosurfactants that promote foaming, as SRB in wastewater are known to secrete extra-polymeric substances (EPS) (Hao et al., 2013). Alternatively, production of hydrogen sulfide may result in cell toxicity (Gerardi, 2003; Parkin et al., 1990). The resulting cell lysis may alter the physical properties of digester sludge to promote foaming. Without other studies to substantiate the original reporting of foam related to SRBs, it is unclear if or how SRBs contribute to digester foaming.

To investigate the role of SRB in digester foaming, operational data from two municipal digester foaming events were analyzed for SRB activity. Additionally, batch tests with digester sludge were conducted, adding sulfate and sulfide. It was hypothesized that if SRB growth promoted digester foaming, then addition of sulfate to digester sludge would increase foam potential. Similarly, if H_2S toxicity led to foaming, then addition of sulfide would increase foam potential of digester sludge.

SA.2. Sulfate Addition and Sulfide Addition Experiment Methodology

For the sulfate-addition experiment, digester sludge collected from WTP was aliquoted into 200 mL samples. To encourage sulfate reducing bacteria (SRB) growth, duplicate samples received 2882 mg/L of sulfate from Na_2SO_4 and 2880 mg/L of COD from either magnesium lactate or a combination of sodium acetate and sodium propionate; control

samples did not receive sulfate. Samples were incubated anaerobically for 10 days at 37.8^{o}C and were measured for foam potential; filtered supernatants were analyzed for lactate, acetate and propionate (Thermo Scientific™ Dionex™ ICS-3000, Waltham, MA) in addition to sulfate (Thermo Scientific™ Gallery™ Plus, Waltham, MA). Pressure changes were monitored *in situ* with Oxitop systems (WTW, Giessen, Germany) to confirm microbial activity.

The sulfide-addition experiment required 100 mL per sample, aliquoted from sludge collected at WTP. To cause sulfide inhibition and toxicity, duplicate samples received 500 mg/L of sulfide from Na_2S, based on previously reported sulfide inhibition (Karhadkar et al., 1987), with 7% HCl added to account for the alkalinity supplied by Na_2S; these samples also received 50 mg/L acetate from sodium acetate. Control samples only received 50 mg/L of acetate; negative control samples received no solutions. All samples were anaerobically incubated at 37.8^{o}C for 10 days. At the start of the 10th day, all samples, except for the negative controls, received 25 mg/L of acetate to stimulate gas production. Pressure increase 1-hour after addition of acetate was recorded with Oxitop systems. The rate of pressure increase was calculated from data points collected 40 to 120 minutes after addition of acetate. Finally, samples were analyzed for foam potential, pH, and TSS/VSS.

SA. 3. Results and Discussion for Investigation of Sulfide and Foaming at WTP

SA.3.1. Increase in H_2S gas during past WTP foaming

Analysis of past operational data revealed that H_2S gas concentrations increased at the start of foam events at WTP in both 2013 and 2015 (Fig. 4). The coincidence of H_2S increases and digester foaming suggested that the production of H_2S contributed to digester foaming.

This prompted an investigation of whether growth of SRB or toxicity from H_2S affect foaming in digester sludge.

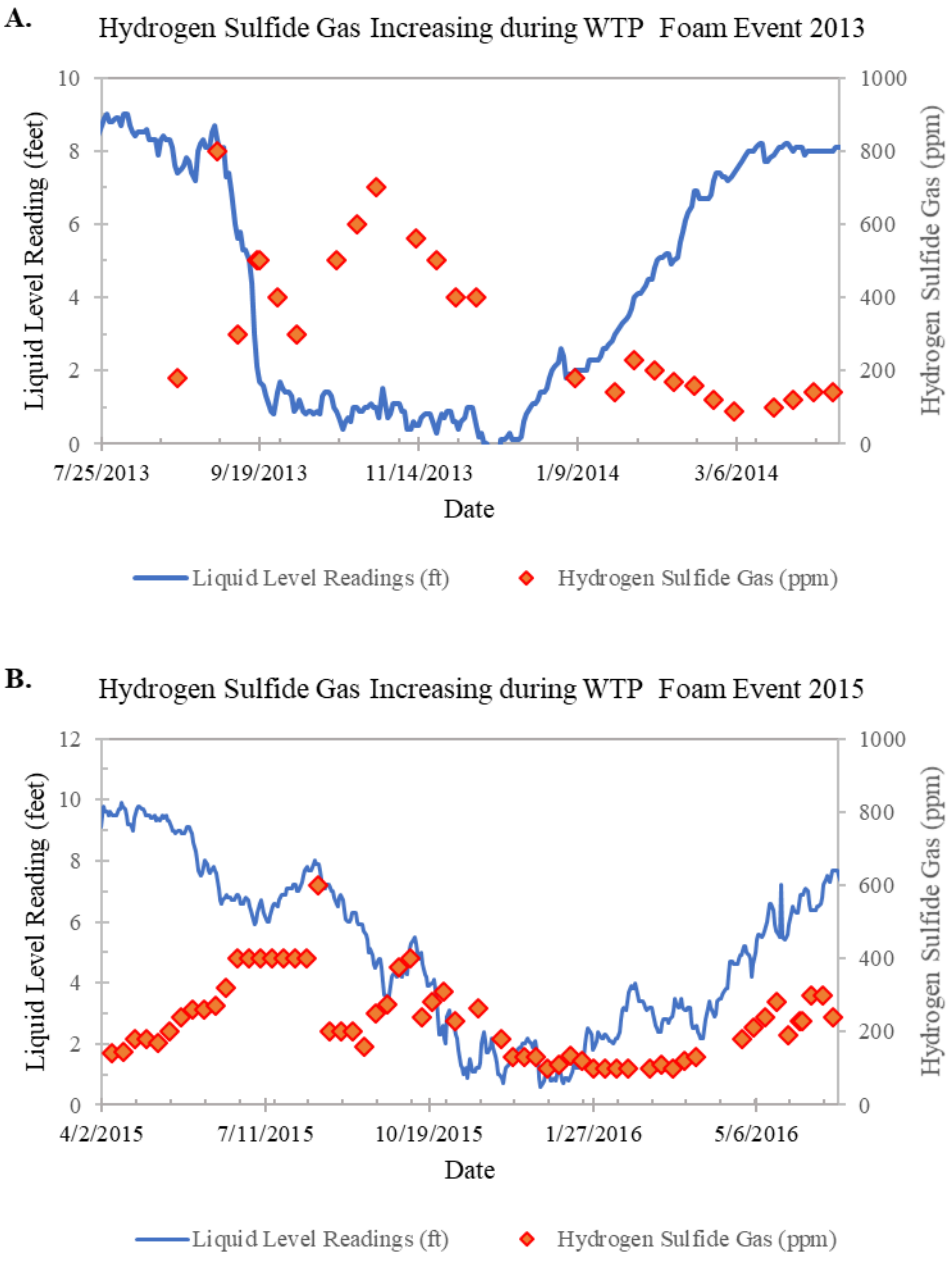

Fig.4. Hydrogen sulfide gas concentrations increased at the start of WTP digester foam events in **A.** 2013 and **B.** 2015.

SA.3.2. Batch testing to induce foaming by addition of sulfate and sulfide

Batch testing of WTP digester sludge, aimed at inducing foaming through SRB growth, showed that foam potential did not increase under conditions favorable to SRB (Table 4). In samples added with sulfate, less than 300 mg/L of sulfate was consumed over 10 days of incubation (Table 4). The resulting sludge had similar foam potential to sludges that did not receive sulfate (Table 4), suggesting that SRB activity and H_2S production had no effect on the foam potential of WTP sludge.

Table 4. Summary of results after 10-day incubation during sulfate addition experiment.

Sample	COD Removed [a]	Sulfate Consumed [b]	Unstable Foam Index	Stable Foam I
Acetate/Propionate	99.93% (+/- 0.00%)	-	4.35 (+/- 0.55)	0.73 (+/- 0.0
Acetate/Propionate + Sulfate	99.93% (+/- 0.00%)	5.4% (+/- 1.5%)	4.08 (+/- 0.33)	0.63 (+/- 0.0
Lactate	99.95% (+/- 0.00%)	-	3.68 (+/- 0.02)	0.43 (+/- 0.0
Lactate + Sulfate	99.94% (+/- 0.00%)	9.2% (+/- 0.5%)	3.75 (+/- 0.05)	0.45 (+/- 0.1

Notes:

[a] 2880 mg/L COD added by either acetate/propionate or lactate at start of incubation

[b] 2882 mg/L Sulfate added at start of incubation

Sulfide toxicity was induced in batch experiments that were inoculated with WTP digester sludge to determine if H_2S promotes foaming independent of SRB activity. Pressure data confirmed that sludge receiving only acetate had higher pressure increase and rate of pressure increase than negative control samples (Fig. 5.A, Fig. 5.B). Samples exposed to sulfide during incubation had about 45% less pressure increase and 59% lower rate of pressure increase than sludges only receiving acetate (Fig. 5.A, Fig. 5.B), showing that exposure to sulfide had diminished gas production. As expected, digester sludge exposed to sulfide had experienced inhibition. The toxicity from H_2S did not lead to higher foam potential, as the foam potential of all samples were similar (Fig. 5.C). To summarize, the exposure to sulfide led to toxic effects on WTP digester sludge but did not lead to increased foam potential.

In conclusion, observed increases in H_2S during past WTP foam events likely did not contribute to digester foaming. Batch testing of WTP digester sludge showed that the addition of sulfate neither lead to significant SRB activity nor increased foaming potential. Furthermore, sulfide addition led to inhibition of gas production but did not affect foam potential. Thus, increased H_2S was most likely a tangential trend that occurred during past WTP foam events and was not the cause of foaming.

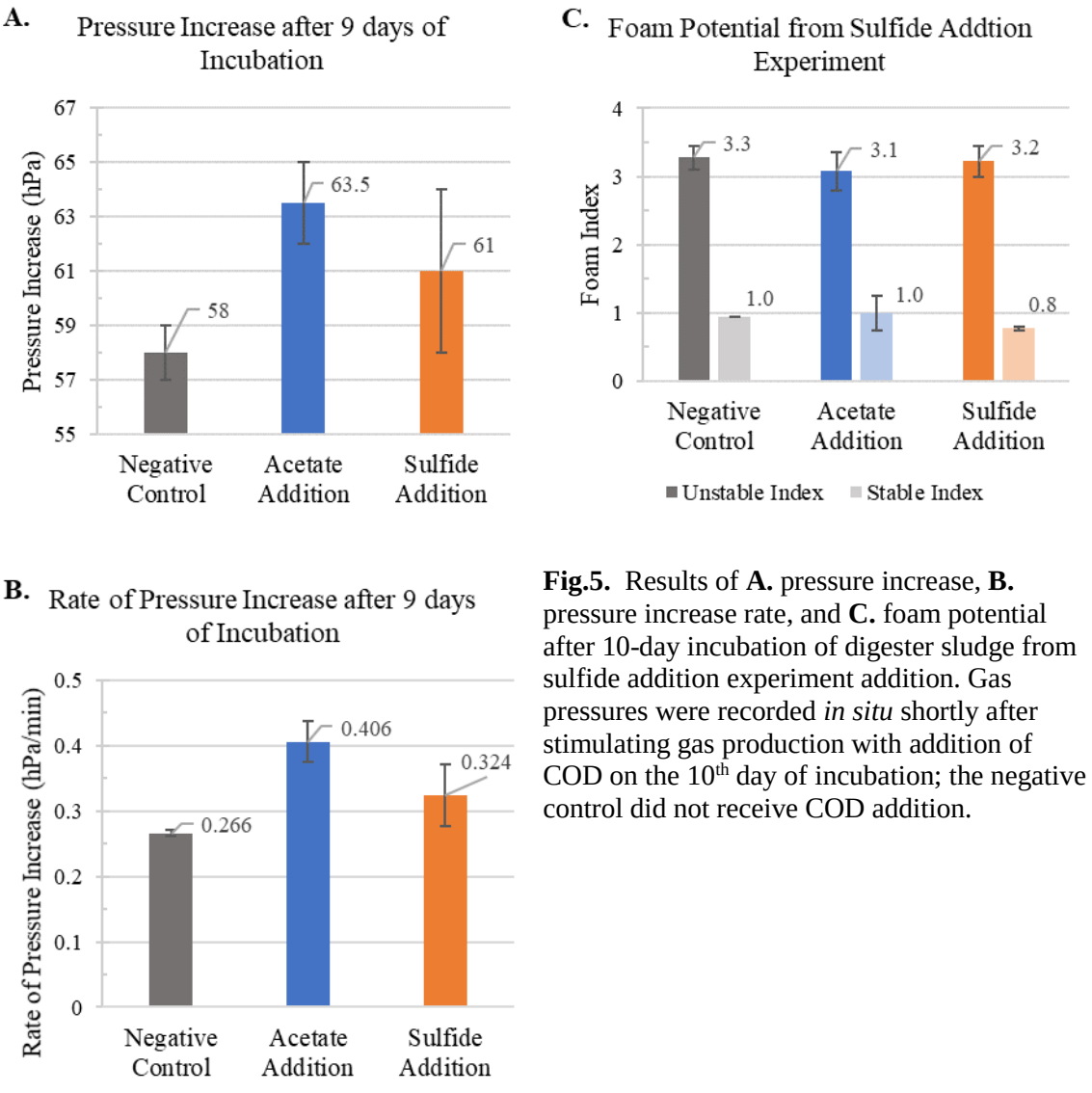

Fig.5. Results of **A.** pressure increase, **B.** pressure increase rate, and **C.** foam potential after 10-day incubation of digester sludge from sulfide addition experiment addition. Gas pressures were recorded *in situ* shortly after stimulating gas production with addition of COD on the 10th day of incubation; the negative control did not receive COD addition.