

# Correlating sludge constituents with digester foaming risk using sludge foam potential and rheology

Kota Nishiguchi and Mari K. H. Winkler

## ABSTRACT

Foam potential and viscometer ramp tests (VRTs) were conducted for three municipal wastewater treatment plants to determine if these methods can relate to 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.

**Key words** | anaerobic digestion foaming, *Gordonia*, *Microthrix parvicella*, rheology

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## 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 & Jenkins 1994; Iwahori *et al.* 2001; Petrovski *et al.* 2010; Pagilla 2015; 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 (Kougias *et al.* 2014b; He *et al.* 2017); *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).

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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 (Massart *et al.* 2006; Kanu *et al.* 2015). Alternatively, high organic loading can lead to the accumulation of hydrophobic or surface active solids, which promote foam formation and stability (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 (Vardar-Sukan 1998). Anionic surfactants, particularly linear alkylbenzene sulfonates (LAS), are common in municipal wastewater due to their ubiquity in commercial products (Mungray & 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.

Often, the adverse effects of foaming begin before foaming events are noticed (Kougias *et al.* 2014a). Therefore, quantification of the magnitude and risk of foaming can help with the investigation and potential prevention of foaming events. 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 (Jiang *et al.* 2018). 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 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 (Chapman 2011; Bartek *et al.* 2017). Low mixing intensity present low shear conditions, yielding the highest apparent viscosity and stoppage of bubble rise (Chapman 2011; Bartek *et al.* 2017). 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 eight-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.

## METHODS AND MATERIALS

### 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 <sup>a,b</sup>	HPO	CAS with SVI control (with anaerobic basin)	MBR (with anoxic basin)
Sludge thickening <sup>c</sup>	GBT	DAFT	GBT
Number of primary digesters <sup>d</sup>	5	4	3
Average digester SRT, days	28	25	30
Digester mixing <sup>e</sup>	Gas mixing (draft tubes or diffusers)	Gas diffusers	Mechanical draft tube

<sup>a</sup>HPO = high purity oxygen; CAS = conventional activated sludge; SVI = sludge volume index (settleability); MBR = membrane bioreactor.

<sup>b</sup>Secondary treatment is preceded with conventional primary clarifiers at all plants.

<sup>c</sup>GBT = gravity belt thickener; DAFT = dissolved air floatation thickener. Sludge is blended from primary and secondary treatment solids.

<sup>d</sup>WTP and STP primary digesters use floating covers while BWTP only fixed covers; all are mesophilic digesters.

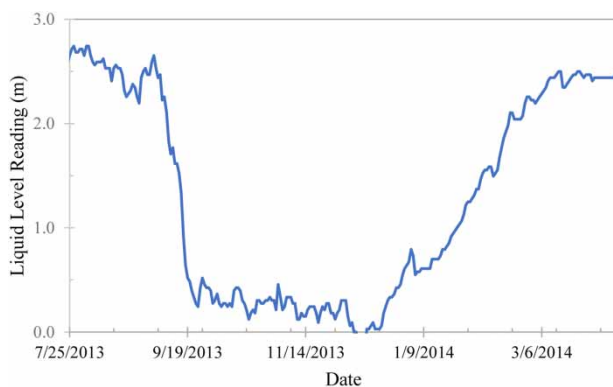
<sup>e</sup>Recirculation pumps contribute to mixing and heating at digesters at all plants.

### West point treatment plant experience with digester foaming

Within the five years preceding this study, WTP digesters experienced at least two 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 (Figure 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 like the common practice of surface wasting. Additionally, Foam-A-Tac 435 (Enterprise Specialty Products Inc., Laurens, SC, USA) 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.

### 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



**Figure 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.

settleability. During this study, STP did not experience digester foaming issues.

### 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 outage resulted in rapid volume expansion (RVE) from the lack of mixing. In November 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.

### Digester sampling and analyses

#### 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 eight 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 h. Samples were stored at 4°C and held for no more than 24 h before analysis, unless otherwise indicated.

### 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 (Equation (1)) and stable foam index (Equation (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 (Equation (3)) were also calculated to quantify the relative stability of foams.

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

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

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

VRTs were done with a Brookfield DV2T viscometer in conjunction with the RheocalcT software (Brookfield, Middleboro, MA, USA). Samples of digester sludge were measured with the LV3C spindle. Samples were warmed by water bath to  $\pm 1$  °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 ( $\tau$ ) in Pa with increasing shear rate ( $\gamma$ ) in  $s^{-1}$  (example data shown in Appendix B); 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 & Kalkan 2010). The Casson model (Equation (4)) gave plastic viscosity ( $\eta_p$ ) in Pa-s and yield stress ( $\tau_o$ ) in Pa while the Ostwald model (Equation (5)) gave consistency index ( $k$ ) in Pa-s<sup>n</sup> 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. 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.

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

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

## DNA extraction and qPCR

Samples for DNA extraction were pelleted and stored at  $-80$  °C. DNA was extracted from the pelleted mass of each sample with DNeasy PowerBiofilm Kits (Qiagen, Germantown, MD). The extracted samples were analyzed with quantitative polymerase chain reaction (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 (listed in the Table 2) and standards were produced by Eurofins Genomics (Louisville, KY) as custom oligonucleotides. FastStart Essential DNA Green Master kit (Roche Branchburg, NJ, USA) was used for PCR-grade water and master mix. Volumes and concentrations used for each qPCR reaction are summarized in Table C.1 (Appendix C). The qPCR reactions were conducted using a Lightcycler 96 Gallery (Roche Branchburg, NJ, USA). The parameters for the reactions for each primer set are summarized in Table C.2, Appendix C. Standards were prepared as a serial dilution from  $10^8$  copies/ $\mu$ L down to 10 copies/ $\mu$ L for each primer set.

**Table 2** | Primers used for qPCR analysis

Target bacterium	Primer name	Sequence (5'-3')	Expected amplicon length	Reference
<i>Gordonia</i> (genus)	G268F	AGGCGGGTCTCTGGGTAGTA	411	a.
	G1096R	ATAACCCGCTGGCAATACAG		
<i>M. Parvicella</i>	S-S-M.par-0828-S-21	GGTGTGGGGAGAACTCAACTC	210	b.
	S-S-M.par-1018-A-17	GACCCCGAAGGACACCG		
<i>Mycobacterium</i> (genus)	AFB genus FWD-06	CCGCAAGGCTAAAACCTCAA	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 & Young (2005).

b. Kaetzke et al. (2005).

c. Richardson et al. (2009).

d. Rupf et al. (1999).

### 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 8,028 kit and Hach DR/4000 spectrophotometer (Hach, Loveland, CO, USA). Sludge samples were diluted (1,000x) 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.

### Special sample collection and 16S rRNA gene sequence analysis

16S sequencing data were compiled by MR DNA laboratory (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.

### 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 h 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  $\mu$ L were spread over 18 mm<sup>2</sup>, prepared in duplicates. The dried area was Gram stained (Sigma Aldrich, St. Louis, MO, USA) and observed under 1,000x

magnification for 20 randomly selected fields per slide. For each field at 1,000x, 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.

### Correlation analysis

Correlations found in this study were computed using Tableau software (Seattle, WA, USA). Values for coefficient of determination ( $R^2$ ) were used to quantify the linear correlations between two datasets. 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 (Miłobędzka & Muszyński 2015; Jiang *et al.* 2016). 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). Separating correlations by plant allows isolation of relationships between physical measurements and sludge constituents that are the most relevant to each plant.

## RESULTS AND DISCUSSION

### 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, sequencing in the foam layer at WTP revealed that *Mycobacterium* and *Gordonia* were both approximately four times more abundant in the digester

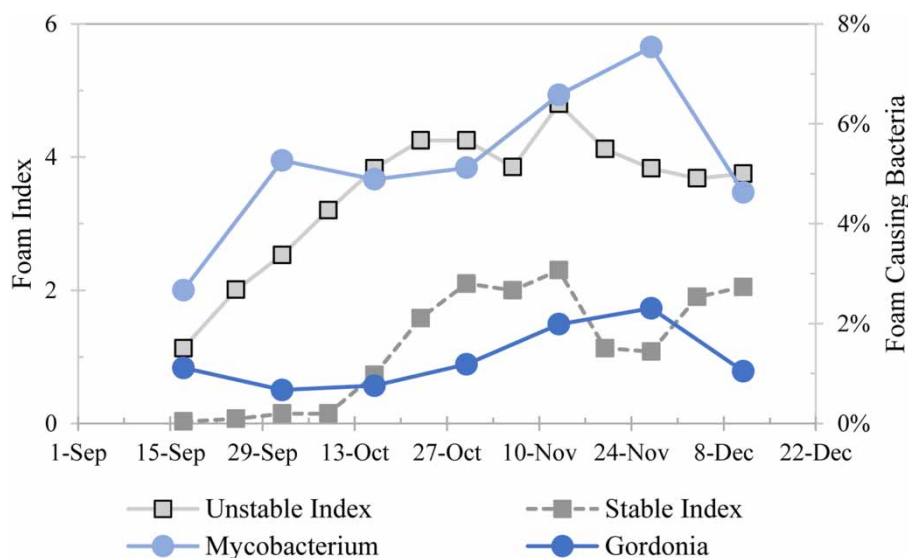
foam layer than in bulk sludge. Trends were confirmed with qPCR analysis, showing that *Mycobacterium* and *Gordonia* were 2.6 and 4.5 times more abundant in the foam layer, respectively. Both *Mycobacterium* and *Gordonia* have hydrophobic cell surfaces and cell walls that contain mycolic acid, characteristics 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 the 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 (Figure 2).

### 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 3A). 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 & Jenkins 1994; Petrovski *et al.* 2010; Pagilla 2015). *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  $< 0.05$ ), which concurs with apparent viscosity measurements by Cheng & Li (2015). 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) and that anaerobically digested sludges decrease in viscosity during digestion (Monteiro 1997). To reduce the



**Figure 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.

**Table 3** | Correlation ( $R^2$ ) between foam evaluation methods and sludge constituents of digester foaming at A. WTP, B. STP, and C. BWTP



(a)	WTP	UFI	SFI	SF	PV	YS	CI	FB	Range <sup>a</sup>
	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) <sup>b</sup>	-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 <sup>a</sup>
	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) <sup>b</sup>	-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 <sup>a</sup>
	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) <sup>b</sup>	0.159	0.046	0.045	-0.032	0.589	0.539	-0.378	36 - 55

Under the "Range" column, each cell represents the range of values measured in the units specified in parenthesis for each sludge constituent

<sup>a</sup>Range of values (minimum – maximum) measured during the study (see Appendix E).

<sup>b</sup>An outlier from each plant was filtered out of analyses for correlations (see Figure E.3., Appendix E).

Key

	Positive correlation ( $R^2 = 1$ )	UFI – Unstable Foam Index	PV – Plastic Viscosity
	Lowest correlation ( $R^2 = 0$ )	SFI – Stable Foam Index	YS – Yield Stress
	Negative correlation ( $R^2 = 1$ )	SF – Stable Fraction of Foam	CI – Consistency Index
			FB – Flow Behavior Index

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 4A), 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 4** | 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 ( $R^2 = 1$ )

### South treatment plant digester foaming evaluation

Foam causing bacteria concentrations correlated with foam evaluation parameters (Table 3B). 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 (distribution of measured concentrations in Appendix E). 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 (Nielsen *et al.* 2002; Jenkins *et al.* 2003). Although *M. parvicella* were not the primary contributors 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 3B, 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 E), 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.



## 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  $< 0.05$ ), 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 (Nielsen *et al.* 2002; Jenkins *et al.* 2003), 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. Although the specific cause of *M. parvicella* growth was not determined in this study, *M. parvicella* was 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 (Chapman 2011; Bartek *et al.* 2017). 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 F). 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.

## 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.

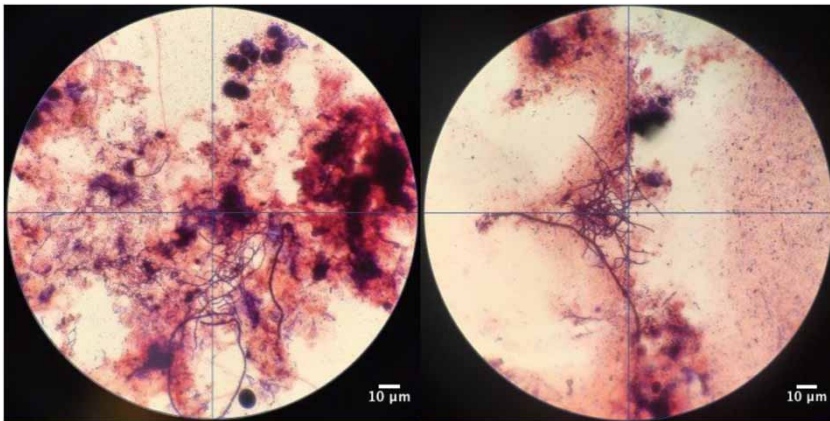
The foam potential tests and VRTs should be applied in making decisions on mixing as mitigation strategy for digester foaming. Results from VRTs show trends of increasing risk of gas entrainment, which can theoretically resolve with increased mixing. However, digesters with gas mixing, in particular, 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.

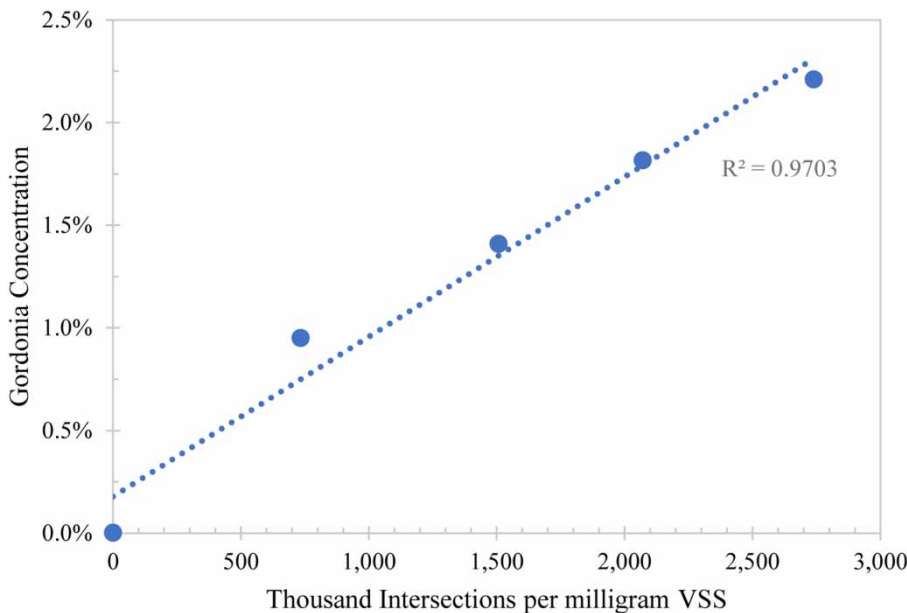
#### Alternative quantification of foam-causing bacteria at wastewater treatment plants

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 (examples shown in Figure 3).

Results from the counting method were well correlated to qPCR analysis (Figure 4), suggesting that filament



**Figure 3** | 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.



**Figure 4** | Correlation between qPCR and filament counting. Concentrations measured by filament counting (intersections per mg VSS) correlate well with concentrations of *Gordonia* (%) measured by qPCR.

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. 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.

## 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 increased 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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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this paper is available online at <https://dx.doi.org/10.2166/wst.2020.180>.

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