

Accepted Manuscript

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PII: S0960-8524(11)00414-7
DOI: [10.1016/j.biortech.2011.03.057](https://doi.org/10.1016/j.biortech.2011.03.057)
Reference: BITE 8290

To appear in: *Bioresource Technology*

Received Date: 13 January 2011
Revised Date: 16 March 2011
Accepted Date: 17 March 2011

Please cite this article as: Ganidi, N., Tyrrel, S., Cartmell, E., The effect of organic loading rate on foam initiation during mesophilic anaerobic digestion of municipal wastewater sludge, *Bioresource Technology* (2011), doi: [10.1016/j.biortech.2011.03.057](https://doi.org/10.1016/j.biortech.2011.03.057)

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1 **The effect of organic loading rate on foam initiation during**
2 **mesophilic anaerobic digestion of municipal wastewater sludge**

3
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8 **Abstract**

9 The impact of increasing organic load on anaerobic digestion foaming was studied at both
10 full and bench scale. Organic loadings of 1.25, 2.5 and 5 kg VS m⁻³ were applied to bench-
11 scale digesters. Foaming was monitored at a full scale digester operated in a comparable
12 organic loading range over 15 months. The bench scale batch studies identified 2.5 kg VS
13 m⁻³ as a critical threshold for foam initiation while 5 kg VS m⁻³ resulted in persistent
14 foaming. Investigation of a full scale foaming event corroborated the laboratory observation
15 that foaming may be initiated at a loading rate of ≥ 2.5 kg VS m⁻³. Experimental findings
16 on foam composition and differences in the quality characteristics between foaming and
17 non-foaming sludges indicated that foam initiation derived from the combined effect of the
18 liquid and gas phases inside a digester and that the solids/biomass ultimately stabilized
19 foaming.

20 **Keywords:** foaming; anaerobic digestion; organic loading; sludge, filaments.

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1

2 **1 Introduction**

3 Foaming during mesophilic anaerobic digestion (AD) has been encountered in sewage
4 treatment works (STWs) worldwide with significant impacts on process efficiency and
5 operational costs (Pagilla et al. 1997, Westlund et al. 1998, Barjenbruch et al. 2000, Barber
6 2005, Ganidi et al. 2009, Dalmau et al. 2010). A number of researchers have suggested that
7 shock (inconsistent) loadings or digester overloading, measured as kg volatile solids per m³
8 of digester per day (kg VS m⁻³ d⁻¹), can be a foaming cause (Pagilla et al. 1997,
9 Barjenbruch et al. 2000, Moen 2003, Barber 2005). It has been previously postulated that
10 this may result in partial sludge degradation and associated accumulation of surface active
11 substances, either directly found in the feed sludge such as lipids, proteins and detergents or
12 as intermediates such as biosurfactants and fatty acids (Barjenbruch et al. 2000, Moen
13 2003, Barber 2005). Shock loading, originating from a change in the composition of feed
14 sludge, could involve a sudden qualitative change (increase in solids, surface active agents,
15 organic content etc.) thus increasing the loading to the digester. Alternatively it could be the
16 result of an increase in the amount of surplus activated sludge (SAS) content in the feed
17 sludge (usually > 40% by volume) thus increasing the number of filamentous bacteria in a
18 digester. Filamentous bacteria have previously been identified as a cause of digester
19 foaming (Hernandez and Jenkins 1994, Westlund et al. 1998, Pagilla et al. 1997). However,
20 experimental evidence that overloading or shock loading results in foaming is lacking and
21 identification of a critical loading rate for foam initiation in conventional AD is unknown.

1 The relationship between the organic loading rate and AD foaming is investigated in this
2 paper. Bench-scale batch digesters were operated at different organic loading rates to
3 establish if a critical threshold for foam initiation and stabilization could be identified.
4 Additionally, data from an extended monitoring programme of a full scale digester was
5 used to provide complementary information.

6 **2 Materials and Methods**

7 *2.1 Description of the full scale sewage treatment works and sample collection*

8 A digester at a STWs in the UK (population equivalent (PE) approximately 230,000) was
9 studied over a 15 month period. The STWs consisted of an activated sludge plant (ASP)
10 and two primary mesophilic (35°C) gas-mixed anaerobic digesters (3700 m³ volume each).
11 The works received no industrial sludge imports. Retention times in the primary digesters
12 ranged between 12-14 days. Both digesters were fitted with sensors (Charis Technology
13 Ltd, Chatham, UK) set to raise an alarm when the foam level exceeded a set point of
14 approximately 50 cm.

15 Monthly grab samples of feed and digested sludge (10 L) were collected over a 15 month
16 period and when required prior to the batch laboratory experiments. Feed sludge was
17 collected from a pipe outlet after the feed blending tank and before digestion. Digested
18 sludge was collected from the overflow outlet at the top of the digester. The same digester
19 was sampled at all times. Samples were not collected when operational problems were
20 recorded on site that could have affected the digester's performance. The feed sludge
21 samples had an estimated ratio of primary to SAS sludge between 6:4. Samples for the full

1 scale digester analysis were stored in plastic containers in a cold room at 4 °C for no more
2 than 4 days. Samples for the batch experiments were placed in a water bath at 35 °C
3 immediately after collection to ensure viability of bacteria.

4 **2.2 Bench scale batch anaerobic digestion rig operation**

5 Digestion took place in 12, 1 L, glass bottles (Fisher Scientific Ltd, Loughborough UK)
6 placed in a thermostatically-controlled water bath (Patterson Scientific Ltd, Luton, UK) at
7 35 °C. The digestion bottles were sealed with rubber bungs (Fisher Scientific Ltd,
8 Loughborough, UK) and gas was collected in glass collection columns (length: 110 cm,
9 diameter: 5 cm) by displacement of water containing hydrochloric acid (pH <4). Magnetic
10 stirrers (Patterson Scientific Ltd, Luton, UK) were placed underneath the water baths to
11 keep the sludge in suspension.

12 Three organic loading rates (OLR), 1.25, 2.5 and 5 kg VS m⁻³, plus a control containing
13 only digested seed sludge were tested in triplicate during 10 day batch anaerobic digestion.
14 Each batch experiment was repeated three times (Experiments 1, 2 and 3) to demonstrate
15 reproducibility of the results obtained. The loading rates selected were based on the full
16 scale digester loading rates and from information on recommended OLR found in the
17 literature for conventional, mesophilic, anaerobic digesters, ranging from 0.5 to 7.2 kg VS
18 m⁻³ d⁻¹ (Handbooks of UK Wastewater Practice 1996, Water Pollution Control Federation
19 1996, Brown 2002, Gerardi 2003, Harrison et al. 2004, Bolzonella et al. 2005, Braguglia et
20 al. 2007, Zupančič et al. 2008, Cartmell and Chinaglia 2009). The proportion of feed to
21 digested sludge varied for each batch experiment as it was based on the volatile solids
22 content of the feed sludge in order to achieve the required loading. The height of foam in

1 the bottles was measured daily and foam was subsequently destroyed daily by stirring. On
2 Day 3 of batch digestion, one digestion bottle per loading rate was removed from the water
3 bath for sludge and foam analyses. On Day 10, the digestion was stopped, when gas
4 production was $< 30 \text{ cm}^3 \text{ d}^{-1}$ and similarly sludge and foam samples were collected for
5 analysis from the remaining bottles.

6 **2.3 *Foaming test apparatus and methodology completed on the full scale digester*** 7 ***samples***

8 The foaming tests were carried out on 1 L feed and digested sludge and sludge-centrate
9 samples, collected from the full scale STWs as described in section 2.1. Sludge -centrate
10 samples were obtained by centrifuging either the feed (feed-centrate) or digested sludge
11 (digested-centrate) samples at 2000 g for 15 minutes and subsequently collecting the
12 supernatant. The foaming potential was determined by measuring the foam height after
13 aeration at $0.5 \text{ L minute}^{-1}$ for 10 minutes (NG et al. 1977, Morey et al. 1999, Desphande
14 and Barigou 2000, Desphande and Barigou 2001, Dedhia et al. 2004, Nakajima and
15 Mishima 2005). The apparatus comprised of a column (length: 1 m, diameter: 5.2 cm) with
16 a plastic, fine-bubble diffuser placed at the bottom. Foaming propensity and foam stability
17 were determined during the foaming tests. Foaming propensity was calculated based on the
18 amount of foam generated from a sample after aeration normalized over the solids content
19 of the sample in order to allow comparison of the foaming potential of different samples
20 (Equation 1). Foam stability was monitored indirectly by measuring the foam height 1 hour
21 after aeration ceased (Equation 2). All measurements were carried out in triplicate.

1 **2.4 Analytical methods**

2 Total solids (TS), volatile solids (VS) and alkalinity determination were carried out
3 according to the Standard Methods for the Examination of Water and Wastewater (APHA,
4 Greenberg et al. 1998). Solid-free sludge samples were obtained after centrifugation at
5 2000 g for 15 minutes and filtration (0.45 μm glass-fiber filter papers, Whatman,
6 Maidstone, UK) and were analyzed for alkalinity by titration of 0.02 M hydrochloric acid.
7 Total volatile fatty acids (tVFAs), including acetic, propionic, n- and iso- butyric, n- and
8 iso-valeric acid, were determined in sludge samples acidified with concentrated sulphuric
9 acid to stop microbial activity and stored at $-20\text{ }^{\circ}\text{C}$ until analysis. Filtration through 0.45
10 μm glass-fiber filter papers was carried out prior to analysis by high performance liquid
11 chromatography (HPLC) (Shimadzu VP Series, Shimadzu, UK) using a Bio-Rad
12 fermentation column (Bio-Rad, Hercules, CA, USA) for separation of VFAs. The column
13 temperature was set at $65\text{ }^{\circ}\text{C}$ and 1 mM sulphuric acid was used as mobile phase at 0.8 ml
14 minute^{-1} flow. Volatile fatty acid detection was performed by a UV detector at 208 nm
15 (Galanos et al. 1995, Sanford et al. 2002, Parawira et al. 2004). Soluble COD (SCOD) was
16 determined in samples after centrifugation at 2000 g for 15 minutes and filtration by a COD
17 kit (VWR, Lutterworth, UK). The absorbance was measured by a Spectroquant Nova 60
18 Spectrophotometer (VWR, Lutterworth, UK). Dissolved organic carbon (DOC) was
19 determined with a Shimadzu TOC – 5000A analyzer in samples after centrifugation and
20 filtration as described previously. Filamentous bacteria were identified according to
21 Eikelboom (2000). Gram stains (HD Supplies, Aylesbury, UK) with safranin as the counter
22 stain and Neisser stains (Fisher Scientific, UK) were used for staining of filaments in

1 sludge. Light microscopy (BHB, Olympus) was then used to identify the species according
2 to a scale from 1 to 5, 1 being the lowest and 5 being the highest, was used to describe the
3 abundance. Analysis of foam samples was carried out based on the methods as described
4 above.

5 **2.5 Statistical methods**

6 Descriptive statistics were carried out for all data involving the calculation of mean values,
7 standard deviations and standard errors. Further statistical analysis of the data involved
8 examination of the normality of the data and subsequently one-way analysis of variance
9 (ANOVA) using Statistica.

10

11 **3 Results and Discussion**

12 **3.1 Investigation of the effect of organic loading on foaming at bench scale**

13 Persistent, recurring foaming was recorded from the highest loading tested (5 kg VS m^{-3}),
14 in all three repeated bench scale batch experiments (Experiments 1-3) with foam volumes
15 varying from 1.1 % to 18.9 % of the total volume of the digestion bottle. The 2.5 kg VS m^{-3}
16 loading did not consistently produce foam during the digestion period with foam volumes
17 varying from 0% to 3.8% of the total volume of the digestion bottle during all three
18 experiments. The daily foam production is illustrated in Figure 1. It is important to
19 highlight that foam was destroyed daily by stirring after each recording and reappeared
20 within a day. No foaming was recorded in either the control or the $1.25 \text{ kg VS m}^{-3}$ loading
21 digestion bottles during batch digestion any of the three experiments.

1 Additional information on gas and methane production, solids reduction, tVFAs and
2 alkalinity for the three experiments showed that the digestion process was not inhibited at
3 any of the loading rates tested. The highest cumulative gas production was recorded from
4 the 5 kg VS m⁻³ loading (1445 – 1959 mL biogas) with cumulative gas production from the
5 1.25 and 2.5 kg VS m⁻³ loading at 91 – 495 mL and 402 – 996 mL biogas, compared to a
6 range of 131 – 399 mL in the control. The 5 kg VS m⁻³ loading consistently produced a
7 higher methane content (52 – 67 %) than the 1.25 (35 % – 56 %) and 2.5 kg VS m⁻³ (38 –
8 58 %) treatments during all three experiments. The tVFAs in digested sludge at the end of
9 batch digestion did not exceed 28 mg L⁻¹ in any of the treatments. Alkalinity values from
10 all three loadings ranged from 2950 to 3325 mg L⁻¹. There was a statistically significant
11 difference for both SCOD and DOC values (SCOD P=0.004, DOC P=0.002, a=95 %) in
12 digested sludge at the end of batch digestion (Day 10) with values in sludge from the 5 kg
13 VS m⁻³ loading significantly higher than those from the 1.25 kg VS m⁻³ loading. Yet, the
14 same was not observed between the 1.25 and 2.5 kg VS m⁻³ loading. The TS content in the
15 foam samples (5.7 – 7.5 %) (Table 1) was significantly higher than that in sludge (2.2 – 4.5
16 %). On the contrary, VS did not vary considerably between sludge (49 – 77 %) and foam
17 (51 – 66 %).

18 The tVFAs values in the foam varied from 1 to 65 mg L⁻¹ during the batch digestion period
19 of all three experiments. The individual VFAs observed in the foam and sludge were acetic,
20 n-butyric and iso-valeric acids whilst no iso-butyric and n-valeric acids were present in
21 either sludge or foam during the experiments. This indicated that there was no preferential
22 partitioning of individual VFAs into the foam. Dissolved organics concentrations in foam

1 ranged from 5 to 493 mg L⁻¹ on Days 3 and 10 during the three experiments and generally
2 increased on Day 10 compared to Day 3.

3 Three filament species were identified in sludge and foam samples in this work, *Microthrix*,
4 *N.limicola I* and *N.limicola III*, as shown in Table 2. The filament abundance was overall
5 moderate as it did not exceed a filament index (FI) of 3. After the 10-day digestion period
6 *N.limicola I* was no longer present in sludge or foam during experiment 1 but *Microthrix*
7 and *N.limicola III* were still present in sludge and foam samples.

8 **3.2 Investigation of a foaming event at full scale**

9 The studied full scale digester had no foaming issues for over a year prior to
10 commencement of the work presented here. During the monitoring period a foaming event
11 was recorded in the digester between two sampling occasions (26.03.08 and 8.04.08). The
12 foam disappeared after 3-4 days and no antifoam was dosed during the event due to the
13 nature and duration of foaming.

14
15 The OLR of the full scale digesters varied from 1.44 to 2.84 kg VS m⁻³ d⁻¹ during the 15-
16 month monitoring period with an average value of 2.25 kg VS m⁻³ d⁻¹ (SD: 0.43 kg VS m⁻³
17 d⁻¹). The foaming incident followed loadings of 2.81 and 2.68 kg VS m⁻³ d⁻¹ on 14.03.08
18 and 26.03.08, respectively, as seen in Figure 2. The full-scale digester achieved 37 % TS
19 reduction and 42 % VS reduction only on 5.11.07 (Figure 3) with all the other values being
20 below that range which was attributed to the effect of dilute feed. The VS reduction ranged
21 from 11 % to 42 % during the monitoring period with an average value of 28 % (±SD:8%).
22 All tVFAs values in the digester ranged from 0 to 83 mg L⁻¹. In addition, all alkalinity

1 values (average of 3860 mg L^{-1} , SD: 868 mg L^{-1}) were within the recommended range
2 ($2000\text{-}5000 \text{ mg L}^{-1}$) (Handbooks of UK Wastewater Practice 1996) apart from one
3 occasion (8.04.08) right after the foaming incident with alkalinity in digested sludge
4 observed at 5780 mg L^{-1} . The DOC concentrations were variable with average values of
5 1175 mg L^{-1} (SD: 319 mg L^{-1}) and 282 mg L^{-1} (SD: 87 mg L^{-1}) for feed and digested sludge,
6 respectively, over the monitoring period. The DOC in digested sludge samples increased in
7 the period between 14.03.08 and 8.04.08 compared to values obtained in previous sampling
8 occasions and the increase in DOC coincided with the foaming event with the maximum
9 DOC value (440 mg L^{-1}) recorded just after the foaming incident.

10 Feed sludge samples and feed-centrate samples showed a variable foaming propensity
11 during the monitoring period (Table 3). The high foaming propensity values of feed and
12 feed-centrate recorded on 9.07.07, 14.03.08 and 26.03.08 did not have an impact on
13 digested sludge foaming propensity. It was noticed, however, that the two highest foaming
14 propensity values were found in the feed-centrate samples before the foaming incident was
15 recorded at the full scale but not in the whole sludge sample. Digested sludge foaming
16 propensity was consistently much lower than digested-centrate foaming propensity during
17 the monitoring period. Foam stability was observed on two occasions in the feed sludge
18 sample only (9.07.07, $11.76 \pm 1.55 \text{ cm}^3 \text{ foam mL}^{-1} \text{ air min}^{-1}$, 5.11.07, $0.27 \pm 0.03 \text{ cm}^3 \text{ foam}$
19 $\text{mL}^{-1} \text{ air min}^{-1}$). However, this had no impact on the digested sludge foam stability and was
20 not linked with foaming observed on the full scale digester.

21 Five filament species were found in the full scale digester, including *N.limicola I* and *III*,
22 *Microthrix*, 0041, 0581 (Figure 4). The overall abundance did not exceed a filament index

1 of 3. In detail, the species 0041 and 0581 had the highest abundance during the monitoring
2 period with FI between 1- 3 and 1-2.5, respectively. *Microthrix* abundance did not exceed a
3 FI of 1.5 during the monitoring period. Before the foaming event was recorded at full scale,
4 the abundance of 0041 species increased from 2 to 3 on the filament index scale (26.03.08).
5 Yet, digested sludge contained only one other species, *N.limicola III*, at low abundance
6 (FI<1) just before the foaming event.

7 **3.3 Organic loading as a foaming cause**

8 This work attempted to identify a critical organic loading threshold for foam initiation and
9 stabilization during conventional mesophilic AD. It is appreciated that each digester could
10 in practice operate with its own critical threshold (Dalmau et al. 2010). However, the bench
11 scale batch anaerobic digestion experiments on sludge obtained from a non-foaming full
12 scale digester identified the 2.5 kg VS m⁻³ loading as critical for foam initiation with foam
13 lasting between 1 and 4 days and the 5 kg VS m⁻³ loading resulting in persistent foaming.
14 The bench scale findings also demonstrated that sludge obtained from a non-foaming full
15 scale digester has the potential to foam under critical conditions (increased organic
16 loadings) at bench scale.

17
18 Overloading/shock loading of digesters can result in partial sludge degradation and
19 associated accumulation of surface active substances, either directly found in the feed
20 sludge such as lipids, proteins and detergents or as intermediates such as biosurfactants and
21 fatty acids (VFAs) often leading to digester instability and subsequently foaming (Ross and
22 Ellis 1992, Pagilla et al. 1997, Westlund et al. 1998, Barjenbruch et al. 2000, Moen 2003,

1 Barber 2005). Standard monitoring and analysis during the bench scale batch experiments
2 carried out in this work (gas and methane production, solids reduction, tVFAs and
3 alkalinity) showed no clear evidence of partial sludge degradation and therefore unstable
4 digestion due to higher organic loadings. Gas and methane production increased with
5 increasing organic loading with the 5 kg VS m⁻³ loading consistently producing more
6 methane (52 – 67 %) than the 1.25 (35 % – 56 %) and 2.5 kg VS m⁻³ (38 – 58 %)
7 treatments. Total VFAs in digested sludge at the end of batch digestion did not exceed 28
8 mg L⁻¹ from all loadings and all three batch experiments (upper recommended limit for
9 AD:300 mg L⁻¹) and alkalinity values ranged from 2950 to 3325 mg L⁻¹. Further
10 determination of SCOD and DOC in digested sludges obtained at the end of the batch
11 experiments showed a statistically significant difference only in sludges between the 1.25
12 (non-foaming) and 5 kg VS m⁻³ d⁻¹ (foaming) loading. Data from the long term monitoring
13 of the performance of the full scale digester were in accordance with the bench scale
14 findings supporting the theory that foaming can occur when no signs of inhibition or
15 imbalance are observed. An increase in DOC (440 mg L⁻¹, average: 282 mg L⁻¹) and
16 alkalinity values (5780 mg L⁻¹, average: 3860 mg L⁻¹) followed the foaming incident but
17 there was no VFAs accumulation in the digester or other clear sign of digestion inhibition.
18 The average organic loading to the digester during the monitoring period was 2.25±0.43 kg
19 VS m⁻³ d⁻¹ with foaming following loadings of 2.81 and 2.68 kg VS m⁻³ d⁻¹, verifying the
20 bench scale findings that foaming may be initiated at loading rates ≥2.5 kg VS m⁻³. In
21 addition, these findings indicated that typical monitoring of AD involving gas and methane
22 production, solids reduction and tVFAs is not adequate to identify differences between
23 foaming and non-foaming digesting sludge.

1 3.4 Foams as 3-phase systems

2 To understand the key components of foam and the mechanisms of foam generation in AD,
3 the theory of Davenport and Curtis (2002), examining foam as a 3-phase system with gas-
4 liquid-solid interactions, has been taken into consideration.

5 3.4.1 Gas phase

6 The gas phase is always present in AD in the form of biogas production with digester gas
7 mixing systems having an increased gas content. Gas production alone could not have
8 resulted in foaming as the presence of surface active agents is necessary for foam initiation
9 (Vardar-Sukan 1998, Glaser et al. 2007). However, the higher gas production of the 5 kg
10 VS m⁻³ loading compared to the 2.5 kg VS m⁻³ loading could have contributed to foaming
11 as increased gas rates can increase foam formation as demonstrated by Varley et al. (2004).

13 3.4.2 Liquid phase

14 Research has shown that the onset of foaming in liquids is due to the presence of
15 surfactants and biosurfactants, abundant in wastewater and sludge. They have both
16 hydrophobic and hydrophilic properties and tend to accumulate at air – liquid interfaces
17 increasing surface activity. When gas is introduced into solution, a thin liquid film is
18 formed around the gas bubbles as they reach the air – liquid interface preventing them from
19 bursting (Hug 2006, Ganidi 2009). The foaming propensity tests carried out in feed and
20 digested sludge and their centrate samples collected from the full scale digester showed that
21 surfactants were consistently present in all samples examined with digested – centrate
22 demonstrating the highest foaming propensity. The latter did not seem to be affected by the

1 feed and feed-centrate foaming propensity according to the experimental findings (Table 3)
2 indicating that the surfactants in the digested-centrate responsible for foaming under
3 aeration were produced during anaerobic digestion. Additionally, the liquid phase of the
4 full scale digester contained significant amounts of surfactants during the monitoring
5 period, yet, the foaming potential was decreased in the presence of solids as foaming
6 propensity of the digested sludge samples was considerably lower than that of the centrates
7 in all cases (average foaming propensity of digested sludge: $0.4 \text{ mm g}^{-1} \text{ TS}$, average
8 foaming propensity of centrate: $31.7 \text{ mm g}^{-1} \text{ TS}$), potentially due to interactions between
9 solid particles and the surfactants. The literature suggests that in complex surfactant
10 systems, such as sludge, which contain a number of surfactants, the foaming potential could
11 be enhanced or reduced depending on the surfactant – surfactant and particle - surfactant
12 interactions (Glaser et al. 2007, Eisner et al. 2007). Additionally, increases in temperature
13 in liquids containing surfactants result in increased surface activity (lower surface tension)
14 and enhanced foaming potential (Barber 2005). Therefore, the behavior of the surfactants in
15 the full scale digester and their impact on foam initiation is dependent on the effect of
16 mesophilic temperatures and surfactant – surfactant, particle - surfactant interactions. In
17 order to gain a better understanding of foam creation and stabilization, the liquid phase of
18 foams generated at bench scale was analyzed for DOC and tVFAs. The amount of tVFAs
19 found in foam on both Day 3 and Day 10 of batch digestion was relatively low ($1 - 65 \text{ mg}$
20 L^{-1}) and concentrations found in the corresponding sludge samples were $0 - 266 \text{ mg L}^{-1}$ on
21 both Day 3 and Day 10 with no consistent evidence of VFAs partitioning in the foam.
22 Thus, it was considered that VFAs were potentially not an essential component of AD
23 foams. However, hydrophobic compounds present in the DOC foam concentration (5 to

1 493 mg L⁻¹ on Day 3 and Day 10) could have contributed to the generation and perhaps
2 stabilization of foaming by accumulating at air interfaces and preventing air bubbles from
3 bursting.

4 3.4.3 Solid phase

5 Recent studies have shown that wastewater foam stabilization is mainly due to the
6 filamentous *Gordonia* and *M.parvicella* but there is evidence suggesting that non
7 filamentous mycolic-acid containing microorganisms, of which specific species have not
8 yet been identified, also act as stabilizing agents (Hug 2006, Heard et al. 2008). Five
9 filamentous species were found in sludge in the full scale digester, of which no reference
10 has been found in the literature for the four species, *N.limicola I* and *III*, 0041 and 0581 in
11 relation to foaming. Investigation of the contribution of *Microthrix* to foam stabilization
12 during bench scale batch digestion in this work showed insignificant partitioning to foam as
13 the filament abundance was either the same or higher by only one unit of the filament index
14 in foam compared to sludge. Consequently, foam stabilization due to the presence of
15 filamentous bacteria was not clearly seen during this work. It is possible that the high solids
16 content in the foam could have been attributed to the presence of mycolic acid containing
17 microorganisms, which in addition to the filamentous bacteria stabilized the foam at bench
18 scale.

19 Overall, foam initiation derives from the liquid phase when the gas phase is present and the
20 solid phase can enhance and stabilize foaming. Subsequently, it can be concluded that
21 although increased organic loading has been identified here as a foaming cause, it is
22 believed that the effect of increased organic loading on the digestion process and

1 specifically on the liquid fraction of sludge (measured here as increased DOC) initiated
2 foaming.

3 **4 Conclusions**

- 4 • An organic loading of 2.5 kg VS m⁻³ was identified as a critical organic loading
5 threshold for foam initiation for sludge obtained from a non-foaming full scale
6 digester while 5 kg VS m⁻³ resulted in persistent foaming.
- 7 • Survival of filamentous microorganisms during AD was evident at full scale and
8 five species were identified (*Microthrix*, *N.limicola I & III*, 0041, 0581) with an
9 overall abundance not exceeding a filament index of 3.
- 10 • *Microthrix*, *N.limicola I* and *III* were present in sludge and foam samples during
11 bench scale batch digestion. However, their contribution to foaming at bench scale
12 was considered insignificant.

13 Acknowledgements

14 The authors gratefully acknowledge funding for this work from Anglian Water,
15 Northumbrian Water, Severn Trent Water, Thames Water, United Utilities and Yorkshire
16 Water.
17

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FIGURE LEGENDS

Figure 1: Daily foam production as a percentage of the volume of the digestion bottle (\pm SD) during batch anaerobic digestion of Experiments 1, 2 and 3.

Figure 2: Volatile solids loading ($\text{kg VS m}^{-3} \text{d}^{-1} \pm$ SD) of the full scale digester

Figure 3: Total and volatile solids reduction ($\% \pm$ SD) for the full scale digester

Figure 4: Filamentous bacteria abundance in digested sludge of the full scale digester

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$$\text{foaming propensity} = \frac{\text{mm of foam after aeration}}{\text{gram total solids}}$$

Equation 1: Foaming propensity in mm g⁻¹

$$\text{foam stability} = \frac{\text{foam volume after 1hr settling, cm}^3}{\text{air flow rate, ml.min}^{-1}}$$

Equation 2: Foam stability in cm³ ml⁻¹ min⁻¹

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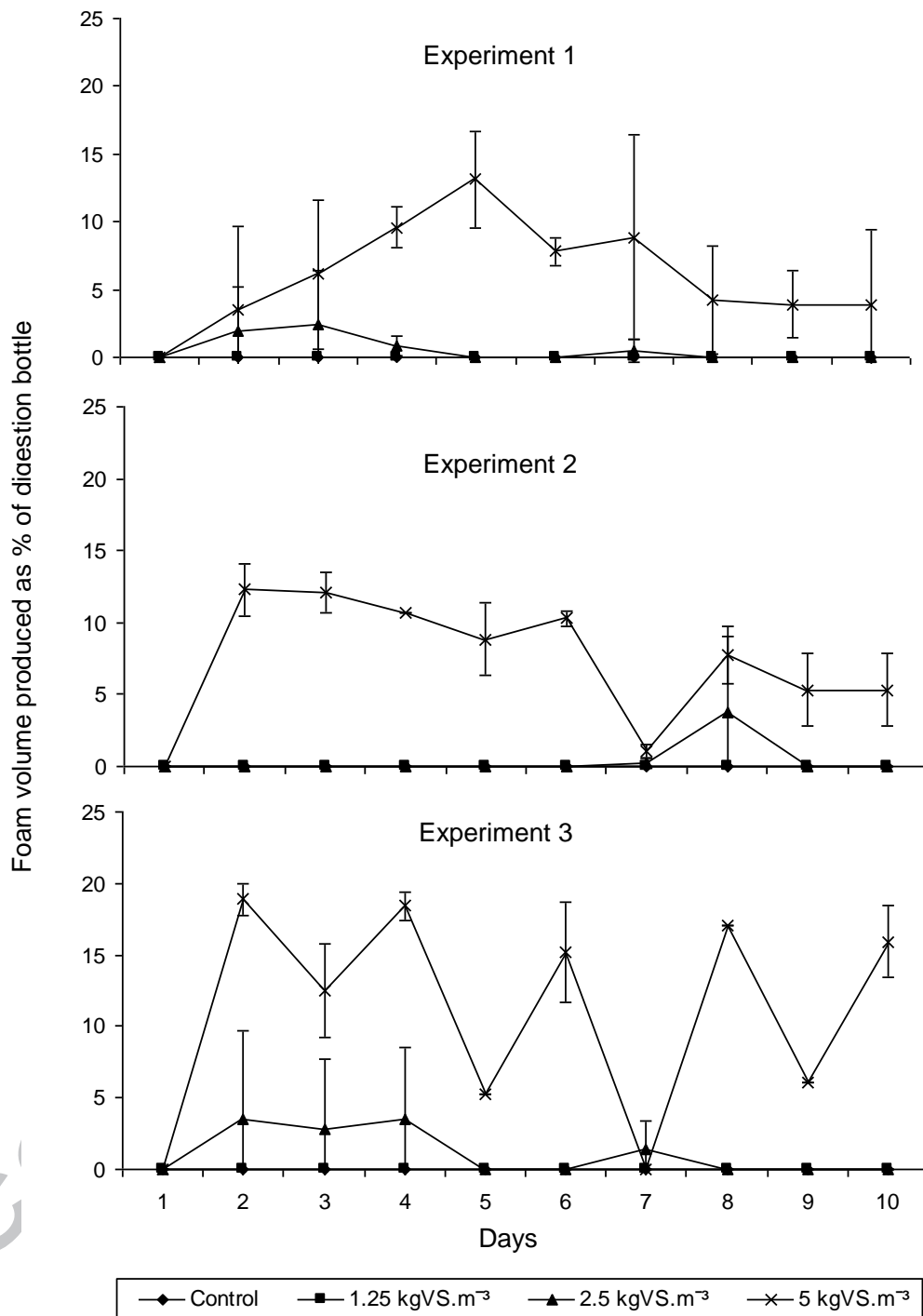


Figure 1: Daily foam production as a percentage of the volume of the digestion bottle (\pm SD) during batch anaerobic digestion of Experiments 1, 2 and 3.

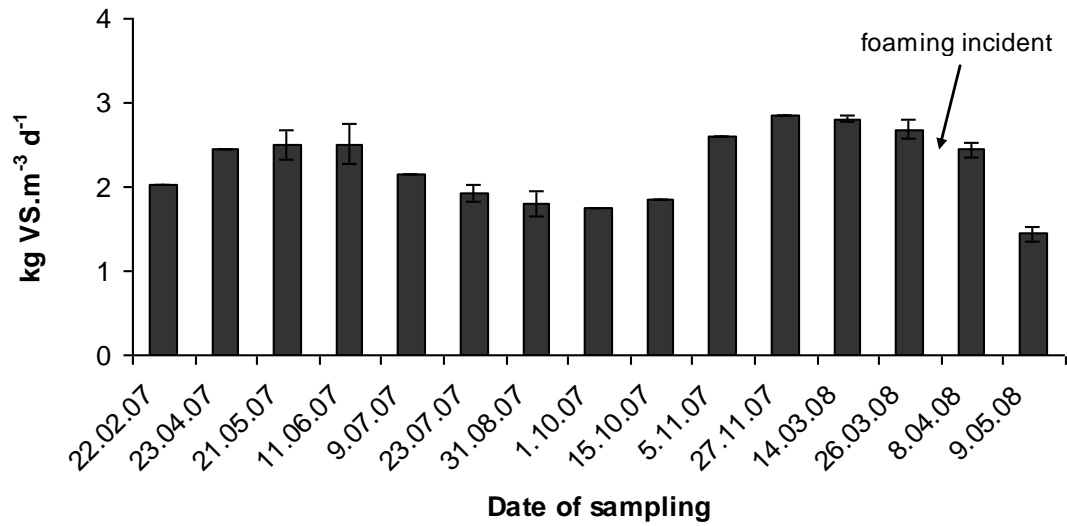


Figure 2: Volatile solids loading (kg VS m⁻³ d⁻¹ ±SD) of the full scale digester

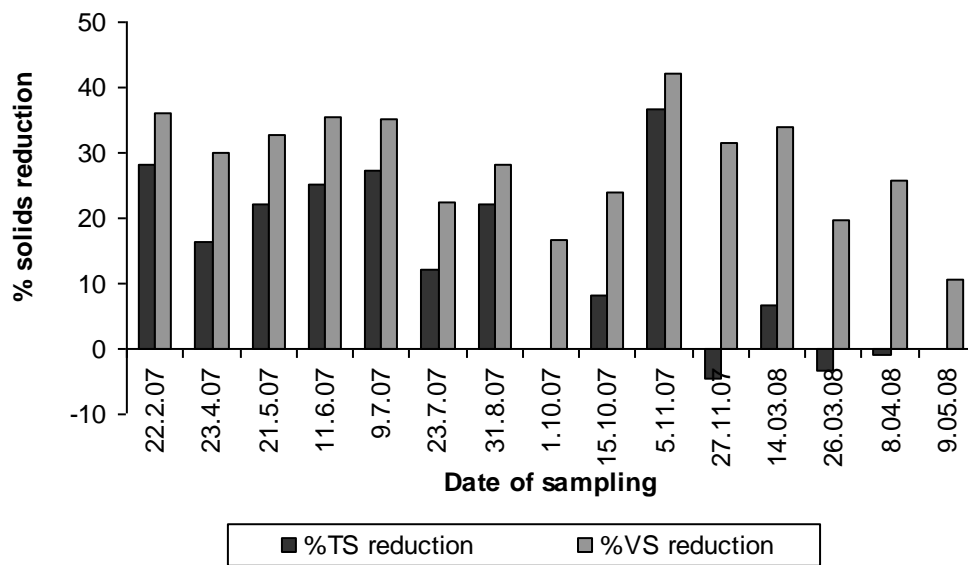


Figure 3: Total and volatile solids reduction (% \pm SD) for the full scale digester

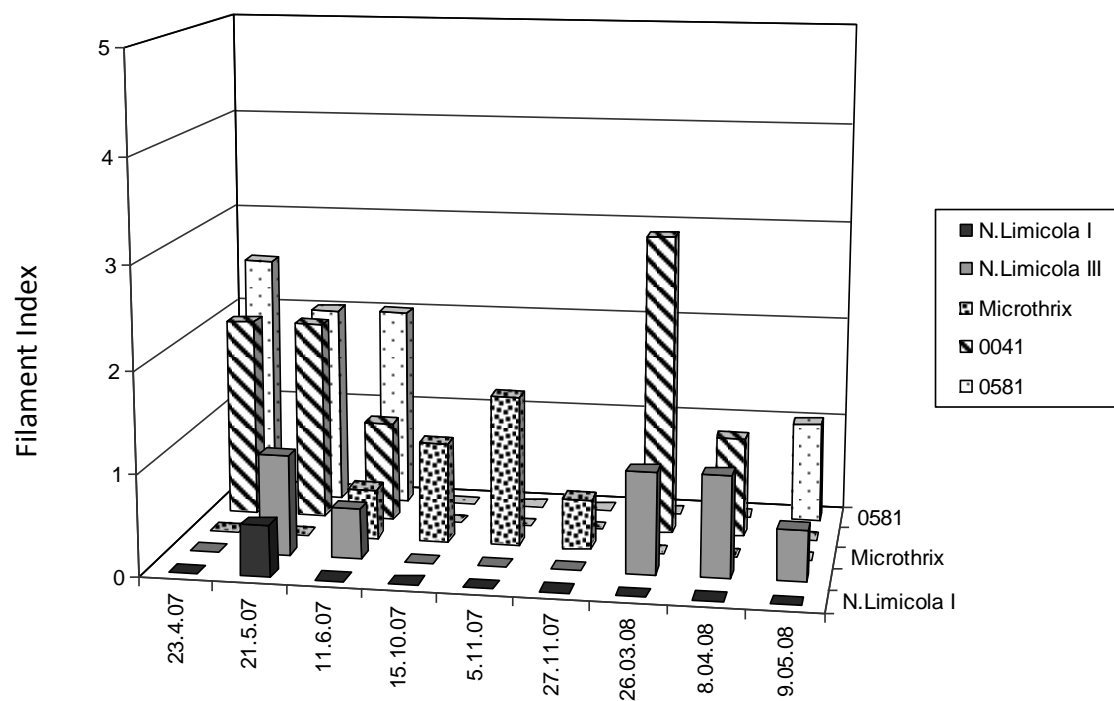


Figure 4: Filamentous bacteria abundance in digested sludge of the full scale digester

Table 1: Quality characteristics of foam samples from the 5 kg VS m⁻³ loading

	<i>Experiment 1</i>	<i>Experiment 2</i>	<i>Experiment 3</i>
TS (%)			
Day 3	5.7 ^a	7.4±0.3	6.3±0.4
Day 10	6.0 ^a	7.0±0.0	7.5±0.3
VS (%)			
Day 3	65.7 ^a	61.5±0.4	59.3±3.5
Day 10	66.2 ^a	63.4±0.1	51.4±1.4
tVFAs (mg L⁻¹)			
Day 3	6.0 ^a	3.1±4.5	29.0±18.4
Day 10	64.6 ^a	1.3±2.2	16.7±0.6
DOC (mg L⁻¹)			
Day 3	182.0±0.1	5.0 ^a	328.0±23.9
Day 10	324.0±12.8	273.0 ^a	493.0±15.7

(^a): Single analysis due to sample volume limitations

± : Standard deviation

Table 2: Filament index in sludge and foam samples during bench scale batch digestion

	<i>Microthrix</i>		<i>N.limicola I</i>		<i>N.limicola III</i>		
	Day 3	Day 10	Day 3	Day 10	Day 3	Day 10	
Experiment 1	non foaming sludge (<i>control</i>)	1	0.5	-	-	-	-
	foaming sludge (5 kg VS.m ⁻³ <i>loading</i>)	1	1	1	-	1	0.5
	foam (5 kg VS.m ⁻³ <i>loading</i>)	3	2	1	-	-	1
	non foaming sludge (<i>control</i>)	1.5	1	-	-	-	-
Experiment 2	foaming sludge (5 kg VS.m ⁻³ <i>loading</i>)	1	1	-	-	0.5	0.5
	foam (5 kg VS.m ⁻³ <i>loading</i>)	2	1	-	-	-	-
	non foaming sludge (<i>control</i>)	0.5	0.5	-	-	-	-
	foaming sludge (5 kg VS.m ⁻³ <i>loading</i>)	0.5	-	-	-	-	-
Experiment 3	foam (5 kg VS.m ⁻³ <i>loading</i>)	0.5	0.5	-	-	-	-
	foam (5 kg VS.m ⁻³ <i>loading</i>)	0.5	0.5	-	-	-	-

Table 3: Foaming propensity (mm of foam per gram TS) of feed and digested sludge samples and their centrates obtained from the full scale digester over the 15 month monitoring period

Date	Feed	Digested	Feed-centrate	Digested-centrate
9.07.07	21.7	0.1	1.7	39.3
1.10.07	1.1	0.4	2.0	8.3
15.10.07	2.7	0.4	0.0	63.5
5.11.07	3.6	0.6	0.0	43.3
27.11.07	1.6	0.2	5.5	45.0
14.03.08	1.3	0.0	34.6	8.7
26.03.08	0.2	0.2	45.7	21.3
8.04.08 ^a	0.8	0.5	3.6	12.6
9.05.08	1.1	0.9	17.5	43.7
mean	3.8	0.4	12.3	31.7
minimum	0.2	0.0	0.0	8.3
maximum	21.7	0.9	45.7	63.5
SD	6.8	0.3	16.9	19.6

^a: Sampling followed the foaming incident