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Effect of thermal hydrolysis and ultrasounds pretreatments on foaming in anaerobic digesters

N. Alfaro [1], R. Cano, F. Fdz-Polanco *

University of Valladolid, Chemical Engineering and Environmental Technology Department, EII (sede Mergelina), 47011 Valladolid, Spain

HIGHLIGHTS

- Foaming is a common problem in anaerobic digesters at WWTP.
- Thermal hydrolysis at 170 °C mitigated foaming in continuous pilot scale reactors.
- Thermal hydrolysis and ultrasounds are efficient tools to prevent foaming.
- Filamentous bacteria abundance is drastically reduced after pretreatments.
- Foam potential and stability parameters do not predict anaerobic foaming.

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ABSTRACT

Foam appears regularly in anaerobic digesters producing operational and safety problems. In this research, based on the operational observation at semi-industrial pilot scale where sludge pretreatment mitigated foaming in anaerobic digesters, this study aimed at evaluating any potential relationship between foaming tools applied to activated sludge at lab-scale (foam potential, foam stability and *Microthrix parvicella* abundance) and the experimental behavior observed in pilot scale and full-scale anaerobic digesters. The potential of thermal hydrolysis and ultrasounds for reducing foaming capacity was also evaluated. Filamentous bacteria abundance was directly linked to foaming capacity in anaerobic processes. A maximum reduction of *M. parvicella* abundance (from 5 to 2) was reached using thermal hydrolysis with steam explosion at 170 °C and ultrasounds at 66.7 kWh/m^3, showing both good anti-foaming properties. On the other hand, foam potential and stability determinations showed a lack of consistency with the bacteria abundance results and experimental evidences.

1. Introduction

Foam appears regularly in the biological reactors and secondary clarifiers from wastewater treatment plants (WWTP). One of the main sources of foam formation is attributed to the presence of bacteria such as *Microthrix parvicella*, *Gordonia amarae*, *Type 0041*, *Rhodococcus*, *Dietzia*, *Mycobacterium*, *Skermania*, *Tsukamurella*, *Nocardia*, *Nostocoida*... (Iwahori et al., 2001). Among them, *M. parvicella* and *G. amarae* appear to be the main responsible of foam formation as a result of two mechanisms: filament hydrophobicity due to the high content of mycolic acid in their wall and the production of surfactant extracellular enzymes inducing stabilization of air bubbles, causing foam (Pagilla et al., 2002).

When activated sludge (WAS) with filamentous bacteria is anaerobically treated, foam is found as well in the digesters. Anaerobic foaming is caused by WAS filamentous bacteria, which could survive and even grow under anaerobic mesophilic conditions despite being obligate aerobes (Ganidi et al., 2009). In this context, Pagilla et al. (1997) found a direct relationship between excessive *Nocardia (G. Amarae)* levels in WAS and foaming events in the anaerobic digester. Likewise, Westlund et al. (1998b) stated that the prevention of foaming in the anaerobic digesters can be achieved by controlling the growth of *M. parvicella* in activated sludge. On the other hand, non-biological factors such as organic loading rate, mixing, and primary/activated sludge solids ratio also influence foaming in anaerobic digesters (Subramanian and Pagilla, 2014). The generation and accumulation of foam in anaerobic digesters causes a wide variety of operational problems such as clogging of pumps, fouling of gas collection pipes, blockage of gas mixing devices, a loss of effective digester volume, and a decrease

* Corresponding author. Tel.: +34 983423172.
E-mail addresses: natalia.alfaro@iq.uva.es (N. Alfaro), ffp@iq.uva.es (F. Fdz-Polanco).
[1] Tel.: +34 983423172.

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in both biogas production and volatile solids removal (Dalmau et al., 2010).

Different methods for controlling foam in activated sludge systems are available (Martins et al., 2004), which could also be implemented in anaerobic digesters to prevent foaming. Of them, non-specific methods such as chlorination, ozonation or addition of hydrogen peroxide, water sprays or polymers involve the addition of external chemicals, do not entail a long term corrective action and present detrimental downstream effects in the WWTP; on the other hand, specific preventive methods by operational adjustments allow a permanent foam control. Ganidi et al. (2009) reviewed the main operational parameters that influence foaming in anaerobic digesters and the preventive measures against it: reactor mixing configuration (mechanical preferable), operation temperature (high temperatures reduce foaming), organic loading rate (low preferable), reactor shape (egg-shaped preferable as cylindrical) and surface active agents addition (no supportive experimental data found). There is a need for cost-effective technologies capable of reducing foaming in anaerobic digestion without compromising the performance of anaerobic digestion.

In this regard, different reviews (Neyens and Baeyens, 2003; Pérez-Elvira et al., 2006; Carrère et al., 2010; Carlsson et al., 2012) indicated that sludge pretreatments before digestion could mitigate or even suppress the risk of foaming in the digester while improving the anaerobic digestion process (enhancement in methane production, kinetics, or digestate hydrodynamics and dewaterability...). However, the anti-foaming potential of sludge pretreatments was rather speculative and no supportive data were indeed provided in the above mentioned reviews. Concerning experimental works that confirm these effects, scarce studies have been published in this area. One of the first reported studies in which a pretreatment was applied in order to prevent foaming showed that aerobic thermophilic pretreatment to mixed sludge was able to reduce pathogens and control *Nocardia* (Pagilla et al., 1996). Pagilla et al. (1998) applied chemical hydrolysis (chlorination) to prevent foaming in anaerobic digesters but obtained unsuccessful results since foaming capacity was increased. Pilli et al. (2011) claimed that filaments disruption takes place after just 2 min of sonication, while Sandino et al. (2005) reported foaming reduction with the application of ultrasounds to waste activated sludge in mesophilic digesters. On the other hand, Barjenbruch and Kopplow (2003) showed the superior performance of thermal hydrolysis (121 °C for 60 min) compared to mechanical or enzymatic pretreatments to prevent foaming. As well, the effect of *Cambi* thermal hydrolysis pretreatment in anaerobic foaming was studied, showing a positive influence on foaming mitigation (Marneri et al., 2003). On the other hand, Hoyle et al. (2006)and Marneri et al. (2003) established a systematic analysis of foaming prevention by the assessment of foaming tools: foam potential, foam stability and bacteria abundance.

Based on the operational observation at semi-industrial pilot scale where sludge pretreatment mitigated foaming in anaerobic digesters, this study aimed at evaluating any potential relationship between foaming tools applied to activated sludge at lab-scale and the experimental behavior observed in pilot scale and full-scale anaerobic digesters. As well, two pretreatment technologies (thermal hydrolysis and ultrasounds) at different pretreatment conditions will be tested to assess their effect on foam mitigation.

2. Methods

2.1. Sludge sampling

Waste activated sludge was sampled from the sludge recirculation line of the aerobic section in the WWTP of Valladolid (Spain) during episodes of foaming in the aeration basins, secondary clarifiers and anaerobic digesters (March–April 2013). All samples were immediately transported to the laboratory, characterized and subjected to the pretreatments below descried. The WAS, characterized according to Standard methods (APHA, 2005) contained a total solid content of 9.7 g/L (70% volatile solids) and total and soluble chemical oxygen demands of 11.3 g/L and 1.9 g/L, respectively.

2.2. Pretreatments

Thermal hydrolysis pretreatment was carried out in a pilot plant (Fdz-Polanco et al., 2008) in the WWTP of Valladolid. A thermal hydrolysis reactor containing 10 L of WAS was heated with direct steam injection in batch mode. The pilot plant was equipped with automatic valves and a data acquisition and control system that controlled the steam inlet (to maintain the desired operation temperature) and sludge outlet (steam explosion to the flash tank) once the reaction time had elapsed. This device operated at different temperatures (from 100 °C up to 200 °C), hydrolysis times and with or without steam explosion. In addition, a thermal pretreatment was performed at laboratory scale at lower temperatures (below 100 °C) in a simpler device without steam explosion. Thermal hydrolysis tests were performed in two different series corresponding to the two experimental devices. Laboratory scale trials were devised to study the influence of low temperature pretreatments at 50 and 90 °C at three different hydrolysis times (15, 30 and 60 min) with no steam explosion. Pilot scale plant trials evaluated the influence of higher temperatures (over 100 °C) applying or not steam explosion Three levels for hydrolysis time and temperature were selected for each operational variable according to typical values obtained from previous experience (Ferreira et al., 2014): hydrolysis times at 5, 15 and 30 min and temperatures at 120, 150 and 170 °C (Table 1).

The ultrasound homogenizer converts electrical energy in mechanical vibrations (ultrasounds), which are transmitted to the sample by a sonotrode to produce cavitation. Test samples were sonicated in a UP400S Hielscher ultrasound equipment (Germany) with a nominal power of 400 W and 24 kHz frequency. The sonication time and power level (up to 200 W, which was the maximum attainable power) could be varied and controlled. Four ultrasounds tests series of batch experiments with WAS were carried out varying sonication time and power. Sonication time was manually controlled for each batch at two different levels (1 and 5 min) and power was set at two different levels for each sonication time (200 and 100 W). Unfortunately, for the highest power and time, the device broke and the final test could not be completed. Table 2 compiles the experimental design.

Table 1
Experimental setup for thermal hydrolysis tests.

Thermal hydrolysis		
Temperature (°C)	Time (min)	Steam explosion (Yes/No)
50/90	15	No
	30	
	60	
120/150/170	5	No
	15	
	30	
120/150/170	5	
	15	Yes
	30	

Table 2
Experimental setup for ultrasounds tests.

Ultrasounds		
Power (W)	Time (min)	Energy input (kWh/m^3)
100	1	6.7
	5	33.3
200	1	13.3
	5*	66.7

* Not completed.

2.3. Foam potential and foam stability

The influence of the pretreatment on the foaming of WAS was assessed in terms of foam potential (FP) and foam stability (FS). These two parameters were determined following the method established by Ho and Jenkins (1991) using Alka-Seltzer tablets to produce foam. An adaptation of the methodology proposed by Hoyle et al. (2006) was followed in the experiments. Foam potential tests were performed by introducing 250 mL of sludge in a 1000 mL graduated glass cylinder and adding 2 tablets of Alka-Seltzer® (Bayer, Spain) in consecutive small portions. When Alka-Seltzer contacted the sample, effervescence took place and foam was generated. The volume of generated foam (in mL) represented the foam potential. Then, foam stability was measured as the foam half-life (min), defined as the time needed to dissipate half of the volume of foam generated in the foaming potential test.

Foam potential and foam stability were analyzed in triplicate before and after each pretreatment assay in the same day in which the pretreatment was performed. Final foam potential and final foam stability of each sample were the average of three measures. Analysis of variance (ANOVA) was performed to evaluate results significance with a degree of confidence of 95% ($\alpha = 0.05$) and to assess the influence of different factors on the studied parameters.

2.4. Microscopy

For the determination of the foam-generating filamentous bacteria species and their abundance in the raw, thermally hydrolyzed and sonicated samples, Gram-stained and Neisser-stained were applied prior to microscopic observation. Each sample was stained in triplicate and observed three times per slide at random locations. The microscope used was a LEICA, DM4000 B model. Stains and identification cards (Jiménez-Sánchez, 1998) were used to identify the filamentous bacteria species existing in the samples.

Eikelboom criterion was used to determine the abundance of identified filamentous bacteria (Eikelboom, 2006). This criterion consisted of the observation of the stained-sample under the microscope followed by the determination of its abundance (from 1 to 5). The final abundance of each sample was the average of the three abundance counts performed.

2.5. Thermal hydrolysis plant and continuous digesters

WAS was concentrated to 12–14% TS using a commercial centrifuge and then pretreated in a Continuous Thermal Hydrolysis (CTH) industrial prototype operating at 30 min hydraulic retention time (HRT), 170 °C and 7.6 bar, followed by steam explosion to atmospheric pressure (Fdz-Polanco et al., 2008). The main elements of the CTH prototype were a preheater receiving steam from the flash, a reactor with direct steam injection at 10 bar and a flash tank at atmospheric pressure where steam explosion took place. Then, three identical 200 L digesters (A, B and C) were operated at 35 °C to treat mixed sludge from the WWTP of Valladolid as described in Souza et al. (2013). Digesters A and B were fed with hydrolyzed activated sludge and fresh primary sludge (50% VS w/w), while digester C was kept as a control fed with fresh mixed sludge (50% VS w/w). Digesters A and C were operated at a HRT of 20 days, while digester B was operated at a HRT of 10 days. After a month of operation, the feed of reactor B was modified by replacing the hydrolyzed activated sludge by untreated fresh sludge.

3. Results and discussion

3.1. Continuous digesters foaming episode

In February 2013, filamentous bulking was detected in the aerobic biological process from the WWTP of Valladolid. Few days later, uncontrolled foaming came into view in the anaerobic full-scale digesters, causing severe problems of maintenance and operation. Similarly, anaerobic reactor C fed with fresh sludge, which mimicked the performance of full-scale digesters, also experienced severe foaming until completely clogging of the biogas line. However, reactors A and B, fed with hydrolyzed WAS and fresh primary sludge, did not experience foaming at any time. When reactor B feed was changed by untreated fresh sludge, four days later, persistent foam formation was detected in B. It has to be mentioned as well that the organic load of pilot reactors was about 2 kgVS/m^{3}d, which was below the critical organic load of 2.5 kgVS/m^{3}d established by Ganidi et al. (2011) as a threshold for foam initiation.

As indicated by Ganidi et al. (2009), these experimental findings showed a clear and consistent correlation between aerobic filamentous bulking and foam formation in the anaerobic digesters. Furthermore, there was a clear experimental evidence of the effect of the thermal hydrolysis pretreatment of activated sludge on preventing foaming in anaerobic digesters, as suggested by Neyens and Baeyens (2003), Pérez-Elvira et al. (2006), Carrère et al. (2010), and Carlsson et al. (2012). Our results were in agreement with those reported by Marneri et al. (2003), who observed a prevention in foaming in lab-scale digester fed with thermally hydrolyzed sludge.

During this foaming episode, WAS was also sampled from WWTP to perform further lab-scale trials to deeper study this phenomenon and find some explanation to this behavior.

3.2. Foam potential and stability tests

The average foam potential (FP) obtained in control samples varied depending on the period in which WAS was sampled. The average FP from control samples was 204.6 ± 26.3 mL, which showed a considerable standard deviation; however, considering each control sample, the triplicates showed much lower deviations (average standard deviation: 2.9 mL). Then, each set of assays was separately studied for each pretreatment technique. Results of average FP, expressed in mL, obtained in all the assays for lab-scale thermal treatment, thermal hydrolysis and ultrasounds pretreatments are shown in Tables 3–5, respectively.

According to the results shown in Table 3, it could be stated that FP from samples treated at 50 °C was similar than FP from control

Table 3
Results of average foam potentials (in mL) for thermal lab-scale tests.

	Temperature (°C)	Time (min)		
		15	30	60
Control	–		198.3 ± 2.4	
Thermal lab-scale	50	220.0 ± 4.1	248.3 ± 2.4	170.0 ± 0.0
	90	111.7 ± 2.4	150.0 ± 0.0	111.7 ± 2.4

Table 4
Results of average foam potentials (in mL) for thermal hydrolysis pilot plant tests (SE = Steam Explosion).

	Temperature (°C)	Time (min)					
		With SE			Without SE		
		5	15	30	5	15	30
Control	–		248.3 ± 2.4			193.3 ± 4.7	
Thermal hydrolysis	120	481.7 ± 2.4	590.0 ± 0.0	480.0 ± 0.0	380.0 ± 0.0	340.0 ± 0.0	340.0 ± 0.0
	150	588.3 ± 2.4	380.0 ± 0.0	451.7 ± 2.4	251.7 ± 2.4	250.0 ± 0.0	141.7 ± 2.4
	170	351.7 ± 2.4	261.7 ± 2.4	310.0 ± 0.0	221.7 ± 2.4	200.0 ± 0.0	221.7 ± 2.4

Table 5
Results of average foam potentials (in mL) for and ultrasounds tests.

	Power (W)	Time (min)	
		1	5
Control	–	178.3 ± 2.4	
Ultrasounds	100	331.7 ± 2.4	380.0 ± 0.0
	200	320.0 ± 0.0	–

samples. Therefore, FP was not affected by a thermal treatment at low temperature. However, when raising the temperature level to 90 °C, a decrease of FP was clearly observed (ANOVA showed that temperature was a critical factor, with $F = 34.7$ versus $F_{critic} = 18.5$). This study confirmed that temperature was a key parameter to reduce foaming in anaerobic digesters, as previously reported by Ganidi et al. (2009). However, the effect of pretreatment time on FP was not significant ($F = 5.2$ came down below F_{critic}).

On the contrary, when thermal hydrolysis pretreatment was applied at higher temperatures (results in Table 4), FP values increased compared to the control values, resulting in worse anti-foaming properties. However, the highest temperatures (170 °C) prevented foaming at most, leading to the lowest FP. Similarly, the hydrolysis time was not a decisive variable to reduce FP. In fact, the ANOVA test did not show a significant influence of both variables on FP. On the other hand, steam explosion in the pretreatment negatively influenced FP, since lower FPs were obtained in the samples without steam explosion (and lower relative increases respect to the control sample).

When WAS samples were sonicated (Table 5), an increase of FP was produced regardless of the power and sonication time.

The average foam stability (FS) obtained in control samples were as well very dependent on the sampling period. The average FS from these samples was 11.07 ± 2.14 min, which showed again a considerable standard deviation. However, considering each control sample, triplicates standard deviations were below 0.1 min. Then, each set of assays was separately studied for each pretreatment technique. Results of average FS obtained in all assays, expressed in minutes, are shown in Tables 6–8 for lab-scale thermal treatment, thermal hydrolysis and ultrasounds pretreatments, respectively.

FS after thermal pretreatment at lab-scale (Table 6) was strongly affected by temperature, very similar to the trend exhibited by FP. In this particular case, both temperature and time had a significant influence, although temperature influence was much higher ($F = 65{,}535$ versus $F_{critic} = 18.5$). The highest temperature (90 °C) almost halved control sample FS.

Table 7 shows the results of thermal hydrolysis pretreatment from the pilot plant. Although it is difficult to find a clear pattern of the pretreatment effect on FS, the highest FS reductions were reached at the highest hydrolysis time (30 min), which under some operational conditions were below the FS value of the control sample, especially when no steam explosion occurred. Temperature and time in this case did not show any significant influence (F values dropped down below F_{critic}). The fact that FS obtained in thermal hydrolysis without steam explosion was lower than the one with steam explosion could be certainly attributed to the higher FP obtained for samples subjected to thermal hydrolysis with steam explosion in previous test.

Concerning ultrasounds (Table 8), the lowest power input (100 W) mediated a decrease on FS which did not occur at highest power. The results here obtained suggested that there was no influence of sonication time on FS.

The results here achieved concerning FP and FS indicated that WAS presented a higher foaming capacity after both thermal hydrolysis and ultrasounds pretreatment. This finding contradicted experimental studies (Westlund et al., 1998a; Marneri et al., 2003) where WAS FP was reduced after heating at low temperature (70 °C) or thermal hydrolysis, respectively. The obtained increases of FP and FS after pretreatments were in agreement to those obtained by Pagilla et al. (1998), who obtained higher FP and FS following WAS pretreatment by chlorination. Hence, the cell fragments resulting from the breakdown of the sludge flocs during pretreatment were still hydrophobic and were more exposed to the bulk liquid. The pretreatments might have also helped to release anti-foam-stabilizing materials from cells. Thus, it could be stated that the pretreated WAS was still able to produce foam but its behavior in the subsequent anaerobic digester was a priori unknown. Interestingly, these results contradicted the findings obtained from the continuous digesters, where foaming was suppressed with thermal hydrolysis at 170 °C and 30 min with steam explosion. This could be explained by the fact that the cell fragments responsible of foaming in WAS were mixed and degraded inside the anaerobic digester, leading to a reduction of the pretreated WAS foaming capacity (considering that the digester is perfectly mixed (CSTR), thus the average composition in the digester is not anymore the feed composition, but the effluent's one).

Table 6
Results of average foam stabilities (in minutes) for thermal lab-scale tests.

	Temperature (°C)	Time (min)		
		15	30	60
Control	–		7.99 ± 0.07	
Thermal lab-scale	50	7.09 ± 0.08	6.09 ± 0.11	7.06 ± 0.0.3
	90	4.14 ± 0.04	3.04 ± 0.03	4.13 ± 0.06

3.3. Filamentous bacteria abundance

The dominant species of filamentous bacteria was identified as *M. parvicella*. This microorganism was responsible of foaming production and found in high abundance in the control WAS samples surrounding the flocs like a skein-shaped.

To carry out the full identification of the species, Gram-stained samples and Neisser-stained samples were also observed using microscopy. *M. parvicella* is Gram-positive and Neisser-negative (with Neisser-positive granules) and therefore it was observed under the microscope as purple-blue and yellow–brown,

Table 7
Results of average foam stabilities (in minutes) for thermal hydrolysis pilot plant tests (SE = Steam Explosion).

	Temperature (°C)	Time (min)					
		With SE			Without SE		
		5	15	30	5	15	30
Control	–		11.03 ± 0.08			11.18 ± 0.10	
Thermal hydrolysis	120	16.03 ± 0.03	14.06 ± 0.05	13.04 ± 0.02	16.09 ± 0.06	11.08 ± 0.07	8.07 ± 0.08
	150	16.04 ± 0.02	13.04 ± 0.03	12.03 ± 0.04	13.07 ± 0.05	10.03 ± 0.03	8.12 ± 0.08
	170	18.07 ± 0.05	12.14 ± 0.10	8.04 ± 0.07	11.11 ± 0.08	11.06 ± 0.06	10.08 ± 0.06

Table 8
Results of average foam stabilities (in minutes) for ultrasounds tests.

	Power (W)	Time (min)	
		1	5
Control	–	15.08 ± 0.07	
Ultrasounds	100	11.04 ± 0.04	11.23 ± 0.06
	200	15.07 ± 0.06	–

respectively. Gram and Neisser staining of control samples by microscopy observation showed that filamentous bacteria were the same as the microorganism that were determined at first with identification cards.

When WAS samples were thermally hydrolyzed (with or without steam explosion) and sonicated, a disruption of the filaments was produced. These fragmented filaments were released into the inter-flocular liquid, leading to different abundance values of *M. parvicella*.

The average filamentous bacteria abundance obtained in the control samples was 5 (*abundant*) in every case. The results of average filamentous bacteria abundance obtained in all the assays are enclosed in Table 9. It was noteworthy that an abundance value could be set for the ultrasound test at 200 W and 5 min, although it was just partially sonicated until the device broke (the sample could be observed under the microscope although FP and FS tests could not be performed because of the absence of enough sample volume).

Filamentous bacteria abundance was unaffected by the thermal treatment at laboratory scale at 50 and 90 °C (Table 9). It remained with constant at 5, with filamentous bacteria showing a density over 20 units/floc. However, filamentous bacteria abundance was greatly affected by thermal hydrolysis pretreatment, especially when steam explosion took place. A maximum reduction of abundance (from 5 to 2) occurred at 170 °C for 15 and 30 min with steam explosion, which were the most aggressive conditions. Both variables (temperature and hydrolysis time) showed a significant influence on the filamentous bacteria abundance reduction, especially the former ($F = 28$ versus a $F_{critic} = 6.9$ applying ANOVA). The same behavior was observed when WAS samples were thermally hydrolyzed without steam explosion: a minimum abundance of 3 was reached at 170 °C for 15 and 30 min. However, the effect of steam explosion on reducing filaments abundance was evident: lower abundance values were reached when steam explosion occurred regardless of the thermal hydrolysis operational conditions tested (Table 9).

Concerning results of sonicated samples (Table 9), the same abundance result was obtained at 200 W and 5 min than in thermally hydrolyzed samples at 170 °C for 15/30 min. This abundance value of 2 entailed that filamentous bacteria were *common* but not present in all flocs. Again, the highest reduction was obtained for the harsher pretreatment conditions (full power and longer time), reaching the same bacteria reduction than thermal pretreatment.

Full concordance between abundance results and experimental evidence from continuous digesters was thus observed, reaching a minimum abundance of 2 for thermal hydrolysis at 170 °C and 30 min with steam explosion. In fact, Westlund et al. (1998a) found a direct relationship at Bromma WWTP between *M. parvicella* abundance and foaming episodes in anaerobic digesters. Likewise, Pagilla et al. (1997) found a correlation between the excessive *Nocardia* levels and foam accumulation in anaerobic digesters. In addition, the low presence of filaments obtained after thermal hydrolysis at 170 °C was directly linked to the lack of foaming properties in the anaerobic process. In this context, Westlund et al. (1996) established a limit of filament abundance at 3 to prevent foaming associated with *M. parvicella* in activated sludge. Hence, based on the assumption that *M. parvicella* abundance below 3 in WAS do not support foaming during anaerobic digestion, it could be stated that both ultrasounds (at 66.7 kWh/m^3) and thermal hydrolysis (at 170 °C with steam explosion) pretreatments were efficient techniques to prevent foaming in continuous anaerobic digesters.

3.4. Final considerations: energy consumption and results correspondence

Finally, it has to be said that there was no correlation between FP and FS results and bacteria abundance results. While the last

Table 9
Results of average filamentous bacteria abundance counts according to Eikelboom criterion (SE = Steam Explosion).

Assay	Time (min)	Thermal lab-scale			Thermal hydrolysis						Ultrasounds	
					With SE			Without SE				
		15	30	60	5	15	30	5	15	30	1	5
Control	–						5					
Temperature (°C)	50	5	5	5								
	90	5	5	5								
	120				5	4	4	5	5	5		
	150				4	4	3	5	4	4		
	170				3	2	2	4	3	3		
Power (W)	100										4	3
	200										4	2

results concerning filamentous bacteria abundance revealed that the lowest filaments abundance were mediated by WAS pretreatments at the most aggressive conditions, FP and FS tests did not show the beneficial influence of pretreatments and in some cases showed a negative influence of pretreatments on foaming reduction. However, based on the present results, observational evidences in continuous digesters (pilot reactors A and B) and findings by other authors, it could be concluded that thermal hydrolysis at 170 °C with steam explosion ensured foaming destruction in the anaerobic digester by pretreating WAS. Therefore, FP and FS tests to pretreated WAS samples using Alka-Seltzer did not appear to be adequate to measure and predict foaming capacity of WAS in anaerobic digesters, despite being efficient tools in conventional activated sludge systems.

Concerning energy consumption by pretreatments, it is important to highlight that thermal hydrolysis is able to provide energy self-sufficient processes (Pérez-Elvira et al., 2008), but ultrasounds technology usually presents higher energy consumptions. Specific energy inputs of 66.7 kWh/m^3 were used in this study, which negatively compared with the critical energy feasibility limit for ultrasound test of 2 kWh/m^3 (10 g TS/L sludge) established by Cano et al. (2014). Energy considerations could suppose an important aspect in view of full-scale application of the studied pretreatments.

4. Conclusions

Thermal hydrolysis and ultrasound pretreatments to activated sludge were effective in order to prevent foaming in anaerobic digesters. Filamentous bacteria abundance (*M. parvicella)* was drastically reduced (from 5 to 2) by thermal hydrolysis with steam explosion at 170 °C and 15 min and by ultrasounds at 66.7 kWh/m^3. Foam potential and stability were useful and simple tools to measure foaming capacity but showed a lack of consistency with the bacteria abundance results and experimental evidences and therefore were not convenient to predict anaerobic digestion foaming. On the other hand, filamentous bacteria abundance was directly related with anaerobic foaming capacity.

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