Technical University of Denmark

Final Project Report

ForskEL 2009-1-10255

Solutions for foaming problems in biogas plants

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Final report

1 Project details

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Project identification	ForskEL project no. 2009-1-10255
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Entity responsible for the project	Technical University of Denmark, Department of Environmental Engineering, Miljøvej, Building 113, DK-2800 Kgs. Lyngby. Telephone: +45 45251429, Email: iria@env.dtu.dk
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Executive summary

Foaming is a common and serious problem in many biogas plants, affecting negatively the overall digestion process and causing severe operational, economical, environmental drawbacks. At the Bioenergy Group of Denmark Technical University we have performed an extensive research on foaming in biogas plants fed with agro-industrial wastes, in the frame of the project ForskEL-10255 "Solutions for foaming problems in biogas plants". It was found that foaming occurrence is very common in Denmark as 15 out of 16 full-scale Danish biogas plants experienced foaming incidents either in the main biogas reactor or in the pre-storage tank, resulting to 20-50% biogas production loss. The two main causes of foaming were the chemical composition of the substrate and the organic loading rate of the digester. However, in one case study of Lemvig biogas plant, foaming was derived from a combined effect of feedstock composition (acidic whey) and mixing pattern. Concerning the feedstock composition, we concluded that protein rich substrates and lipid rich substrates can result in persistent foam. Nevertheless, the foaming tendency of proteins is greater (i.e. they cause foam even if present at lower concentrations than lipids), as amino acids have strong capability to stabilize foam. In manure-based biogas reactors, we identified an OLR of 3.5 gVS/L-reactor-day as "safe" threshold to prevent foaming. Above this threshold and depending on the influent feedstock stable foam is generated. However, it should be underlined that this OLR threshold cannot be a universal indicator for foaming, as foam can be affected by shape of digesters, mixing system etc. Microbial analysis revealed significant variations in the microbiology of biogas reactors before and after foam formation. A number of genera could be linked to foaming due to their properties. Finally, our analysis identified for the first time the presence of a species (operational taxonomic unit) whose abundance was increased in all reactors after foam formation;

this microorganism was found distantly similar to bacteria related to foam (Nocardia and Desulfotomaculum). As antifoaming strategies, we tested some commercial defoamers (as reference) and several chemical compounds to evaluate their efficiency on foam suppression. Natural oils (e.g. rapeseed oil) along with fatty acids (e.g. oleic acid) exhibited the best performance in foam suppression and did not result in process imbalance. From the overall results, this project delivered 10 ISI publications, 13 contributions in conference proceedings and 2 presentations to stakeholders.

2 Background

Anaerobic digestion (AD) foaming is the one of the most important practical problems which occasionally occurs in many full-scale biogas plants affecting negatively the overall digestion process. Foaming results in severe operational, economical and environmental problems for biogas plants. Concerning the operational problems, the entrapped solids in the foam may lead to blockage of the tubing or mixing systems and also fouling of the pumps. Moreover, in sludge digestion systems, foaming contributes to the creation of an inverse solids profile with higher solids concentrations at the top of the reactor, resulting in the formation of dead zones and thus reducing the digester active volume. It has also been reported that foaming incidents in centralized biogas plants resulted in methane production loss for shorter of longer periods. Significant economic losses that arise from foaming in biogas plants include extra labour work, additional maintenance and cleaning costs, increased oil consumption in combined heat and power systems (CHP) and finally electricity loss due to the reduced biogas that is produced. Finally, the adverse environmental impacts of foaming are owing to the overflowing of the pre-storage or digester tanks, and to methane emissions from the effluent, due to oversaturation of methane in the liquid phase.

In the cited literature there are a number of studies investigating potential causes of foaming during anaerobic digestion processes. However, the complicated mechanism and structure of foam along with the several compounds that are contained in the AD substrate make it difficult to correlate the foaming properties of a biomedia (i.e. foaming tendency and stability) to individual causes of foaming. The most significant parameters, which will be extensively presented in a following section of the current review, are the organic overload, feedstock composition, the presence of specific microorganisms and operational parameters. Nevertheless, most of the foaming studies in the cited literatures were associated with activated sludge systems and not with biogas reactors treating manure together with other organic agro-industrial wastes.

In common practice, foaming is rarely prevented but is detected once the negative effects of the foam have already influenced the process. The biogas plant operators usually monitor the problem by visual observation or when the security valve or alarm activates. Methods for foam suppression are classified into four main groups; mechanical, physical, chemical and biological methods. Mechanical and physical methods include the utilisation of thermal, electrical or mechanical applications. Chemical methods are based on the addition of antifoaming agents that disrupt the

foam bubbles. Biological methods targets on the limitation of the biological activities of foam promoting microorganisms.

From the above mentioned, it is therefore important to carry out a thorough study in order to deeply understand the foaming phenomenon in the manure digestion process, in order to find the proper operation strategies that could avoid foaming incident, as well as to find the cheap, fast, and effective method to resolve the foaming once it occurs.

3 Project results

3.1 Work packages

1) Collection of data / information

Data were collected by interviewing and conducting surveys to managers of 16 Danish full-scale biogas plants (i.e. 80% of the total centralized biogas plants in Denmark). Survey participants were provided with questionnaires involving questions concerning the occurrence, control and possible causes of anaerobic digestion foaming based on their individual experiences. Among the questions, the participants provided information concerning the operational parameters and the feedstock characteristics of the biogas plants. Additionally, one case study of foaming in Lemvig full-scale biogas plant was also investigated. Lemvig biogas plant's operation data during foaming period were analyzed and reactor samples were collected to determine the physical-chemical properties and microbial communities.

2) Experimental investigations to provoke foaming and strategies for prevention / recovery of foaming

Several experiments were conducted in order to identify the causes and solutions for foaming. Initially, the compounds commonly present in agro-industrial wastes were tested for their potentials to cause foaming in raw and digested manure (i.e. foaming tendency and foaming stability), as the survey reported foaming in both pre-storage tank and the main digester. By conducting lab-scale experiments, the major causes for foaming in continuously fed biogas reactors were identified and microbial analyses were performed to investigate potential association of foaming with the presence of specific microorganisms. The microbial analysis could reveal for the first time the presence of a species (operational taxonomic unit) whose abundance was increased in all manure reactors after foam formation; this microorganism was found distantly similar to bacteria related to foam. Finally, several potential antifoam compounds were tested both in batch and continuous reactor experiments to evaluate their suitability to be used as antifoaming agents in manure-based digester.

3) Evaluation and set-up of rules for the biogas plant operators

The results obtained were summarized in ***10 scientific manuscripts*** (two under submission), ***13abstracts in conference proceedings*** and ***2 presentations to stakeholders***. All the published experimental work for this project is can be found at section "5 Publication and dissemination" and are attached at section 6 (Appendixes) of the current manuscript.

3.2 Project organization

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All the staff members involved in the current project are affiliated in the Bioenergy Group of the Department of Environmental Engineering, Technical University of Denmark (DTU-ENV).The Bioenergy Group headed by Irini Angelidaki. The project was managed by Irini Angelidaki. Panagiotis Kougias and Kanokwan Boe were involved in the design and execution of project's research work (as Work Package leaders), distribution of project tasks, guidance and supervision of other researchers.

3.3 Work packages, milestones and time schedule

In order to address the objectives and the hypotheses of this project, the research project was divided in three major work packages (WP). The initial duration of the project was 3 years; however, due to the reduced man-power in some period (from maternity leave), and significant importance of the findings for the biogas plants, the project was extended for 2 more years. The project has been successfully fulfilled as it shown in the following section.

Table 1. Duration of project activities

Project Activities	Year 2009				Year 2010				Year 2011				Year 2012				Year 2013				Year 2014			
	1	2	3	4	1	1	1	2	3	4	2	3	4	2	3	4	1	2	3	4	1	2	3	4
1) Collection of Data (WP1)																								
2) Physical/Chemical effect of feed stocks on foaming (WP2)																								
2.1) Physical/Chemical effect of antifoaming compounds (WP2)																								
3) Reactor experiment: effect of feed stocks and operational parameters on foaming (WP2)																								
3.1) Reactor experiment: effect of antifoam on foam caused by organic overloading (WP2)																								
3.2) Reactor experiment: effect of antifoam on foam with high content of lipid and protein (WP2)																								
3.3) Batch experiment: Toxicity and degradibility test of antifoam compound in manure digester (WP2)																								
4) Microbial analysis in CSTR reactors (WP2)																								
5) Lemvig foaming case study (WP3)																								
6) Evaluation/Guidelines (WP3)																								

3.4 Objectives

The main objective of the project was to elucidate foaming problems in biogas reactors treating agro-industrial wastes, by identifying the causes of foam initiation and stabilization and by proposing solutions to solve foaming incidents. More specifically with this project, we were aiming to:

- Survey foaming causes and solutions applied in Danish centralized biogas plants and perform a case study concerning foaming incidents in full-scale biogas plant
- Investigate the foaming properties of specific compounds commonly present in agro-industrial wastes
- Identify the potential causes of foaming in manure-based biogas reactors
- Characterize the microbial community of biogas reactors suffering from foaming incidents
- Investigate antifoaming solutions using chemical compounds

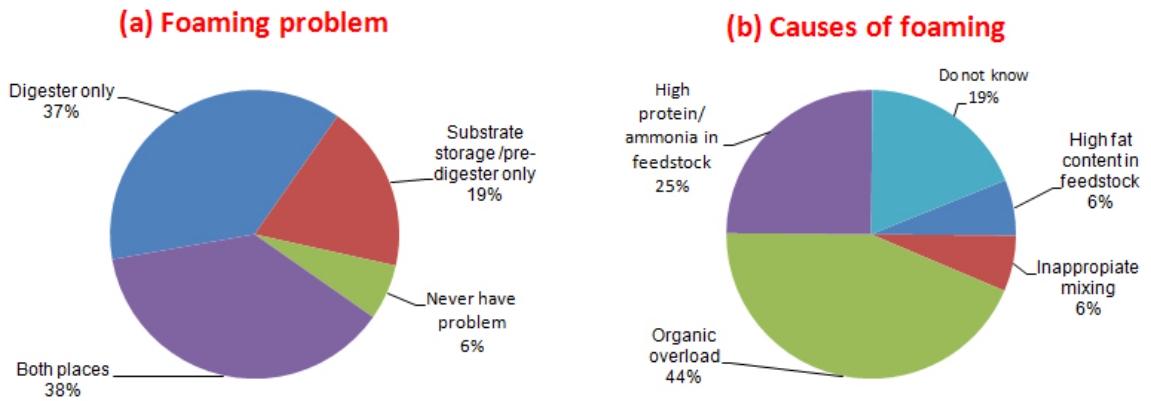
3.5 Results of the project

In this part the results from the project activities are briefly summarized. At the end of each activity description, we provide the relevant materials that include all the information and data,

along with a hyperlink that redirects to corresponding manuscript, abstract in conference proceedings, or presentation.

3.5.1 Survey on causes and solution of foaming in Danish full-scale biogas plants

In the beginning of the project we collected data and information from biogas plant operators concerning previous experiences on foaming. More specifically, to assess the prevalence of anaerobic digestion foaming in Demark, a survey in 16 Danish full-scale biogas plants was conducted. Survey participants were provided with questionnaires involving questions concerning the occurrence, control and possible causes of anaerobic digestion foaming based on their individual experience. Among the questions, the participants provided information concerning the operational parameters and the feedstock characteristics of the biogas plants.The results from the questionnaires showed that 15 biogas plants had experienced foaming problems (Fig. 1a). Foaming appeared mainly in the main digester and occasionally also in the substrate storage/pre-digester. Nevertheless, 38% of the plants observed that foaming occurred in both places. Foaming was an intermittent phenomenon that typically occurred up to three times per year in most of the plants, and the duration of the incidents lasted from one day to three weeks. The biogas plants estimated their biogas production loss during foaming period as 20-50%. Concerning the causes of foaming, most of the biogas plant reported that foaming problem was connected with organic overload (Fig. 1b). The second most dominant cause for foaming was the high protein content in the feedstock. High fat content in feedstock and inappropriate mixing were also reported as correlating with foaming in these biogas plants, although at a lower extend than protein. Type of mixing and mixing speed could also have influence on foaming. The most common solution that the plant operators applied to suppress foaming was the decrease of the digester's organic load (Fig. 1c). The second most applied approach to counteract foaming in the biogas plants was the addition of antifoaming agents. According to the survey results, the addition of antifoam compounds, such as foam absorbers, oils or lime, was a common approach accounted for 29% of the reported antifoam strategies. Other solutions involved adjustment of the stirring speed, increase of the flow rate, decrease of reactor temperature, and dilution of the reactor content.



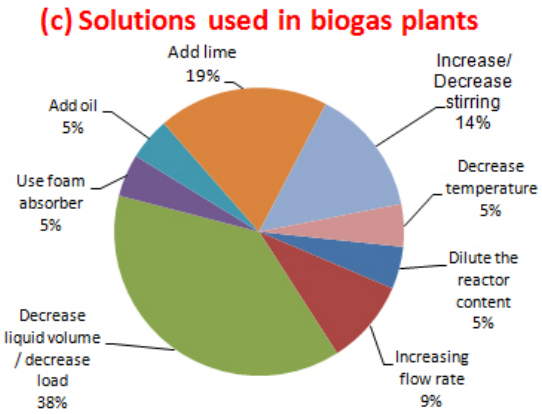


Fig. 1 Results from survey for the Danish full-scale biogas plants; (a) occurrence of foaming incidents, (b) potential causes of foaming, and (c) solutions applied to suppress foaming

All the information and results obtained from this activity were published in the ISI publication 4J, were included in the following conference proceedings 8C, 10C, 13C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes).

3.5.2 Case study concerning foaming incidents in full-scale biogas plant

During the project period, one Danish full-scale biogas plant, Lemvig biogas plant, was facing intensive foaming problem in one of the four reactors without any clear reasons (Fig. 2). Therefore, we investigated the main causes specifically for this foaming incident by analysing the chemical and microbial composition in the samples obtained from all the reactors of the biogas plant. The results from the sample analysis showed that the values of some parameters in the foaming reactor were significantly different compared to the non-foaming ones. More specifically, alkalinity and biosurfactants activity were found at higher levels. Additionally, by determining the foaming properties of the obtained samples we have found that the digestate from the foaming reactor presented higher tendency for foam formation and stronger foam stability. The microbial community of all reactors was characterised by PCR-DGGE analysis. The results showed that the bacterial composition in all reactors was not significantly different, although one genus of facultative anaerobic, endospore-forming bacteria, *Paenibacillus* was found in the foaming reactor. Moreover, we could not identified the presence of filamentous bacteria *Microthrix parvicella* and *Gordonia sp*., which according to literatures were the main cause of foaming in other AD systems (i.e. sludge digesters). Finally, we examined operational parameters of the biogas plant, such as the mixing pattern of all reactors (Fig. 3) and the feedstock composition. We concluded that the foaming incident at Lemvig biogas plant was caused by a combination of the chemical properties of the substrate (such as acidic substrate and substrate with high protein content) and the mixing pattern of the foaming reactor.

Fig. 2 Foam overflow from the reactor of Lemvig biogas plant

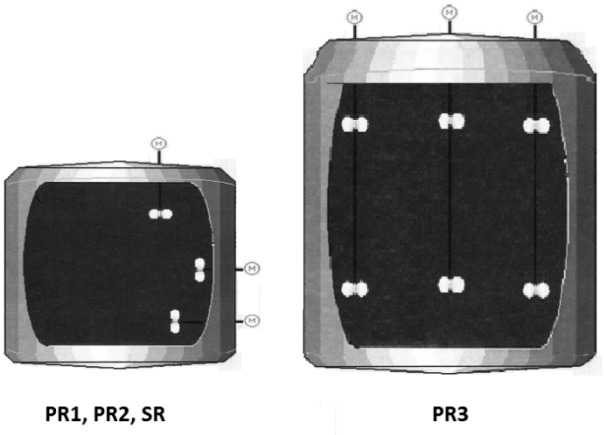


Fig. 3 Stirring pattern of biogas reactors at Lemvig biogas plant. PR3 is the foaming reactor.

All the information and results obtained from this activity were published in the ISI publication 4J, were included in the following conference proceedings 8C, 10C, 13C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendices).

3.5.3 Physicochemical tests to determine foaming properties of specific compounds

Using the data collected from the questionnaires and the gained knowledge from the case study in Lemvig biogas plants, we investigated the correlation of physicochemical properties of substrates and intermediate compounds, such as surface tension, surfactant property, and hydrophobicity, compared to the properties of foaming tendency and foam stability. For this reason, we selected 15 compounds that are commonly present in manure-based digestion systems and determined their foaming properties as single substrates and when mixed together (complex mixture). We have found that there was no consistent correlation between the foaming potential and the properties of hydrophobicity, Oil Displacement Area or surface tension of the solution. We concluded that the best way to determine the foaming property of manure mixture is to directly measure foaming tendency and foam stability (Fig. 4).Additionally, we found that the tendency of manure to generate foaming is decreasing when it became more diluted (Fig. 5). However, the change of manure concentration from 1 to 6%TS did not significantly affect the surface tension. Finally, among the tested compounds we found that the high content of lipids (Na-oleate) or proteins (gelatine) could significantly increase foaming potential of the solutions.

Fig. 4 Apparatus used for the determination of foaming tendency and stability

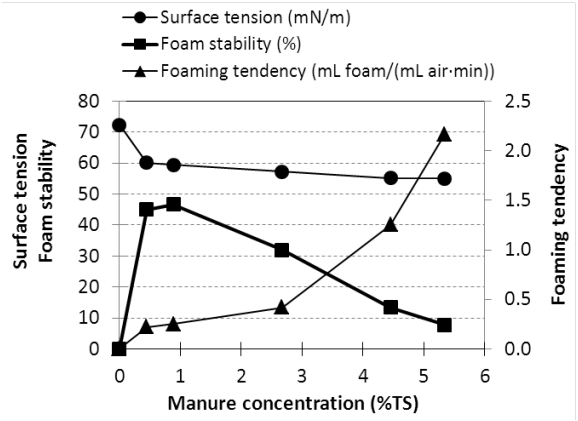


Fig. 5 Surface tension and foaming potential of manure at different concentrations

All the information and results obtained from this activity were published in the ISI publication 8J, were included in the following conference proceedings 12C and13C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes).

3.5.4 Causes of foaming in continuously fed biogas reactors

After the physiochemical tests could identify proteins and lipids as potential foam promoters, we conducted experiments in Continuously Stirred Tank Reactors (CSTR) in order to investigate the effect of these feedstock compositions and the effect of organic loading rate (OLR), on foaming. The experiment was carried out in 5 CSTR reactors (Fig. 6). The whole experiment was divided into four periods. During each period, the OLR and the concentration of gelatine, as a representative of proteins, or Na-Oleate, as a lipid representative compound, were increased in the feed explicitly, in order to distinguish between the effect of OLR, protein, and lipid, on foaming in the digesters. We found that the organic load was the main factor affecting foaming. Moreover, we have observed that protein-rich manure substrate initiated foaming at lower OLR than the lipid-rich manure substrate. Another significant finding was that foaming from gelatine had more stable volume compared to Na-Oleate. Additionally, we identified an OLR of 3.5 gVS/(L-reactor·day) as "safe" to avoid foaming incidents in manure based biogas reactors (Fig. 7). However, by increasing the OLR to 5.2 gVS/(L-reactor·day), high concentrations of Na-Oleate promoted foaming, while high concentrations of gelatine decreased foaming due to lower biogas production caused by the elevated ammonia concentration (i.e. ammonia inhibition). Finally, we demonstrated that foaming is rather related to increase of biogas production and not inhibition. If the foam was efficiently removed, foaming would not decrease methane production unless the process was inhibited by other reasons.

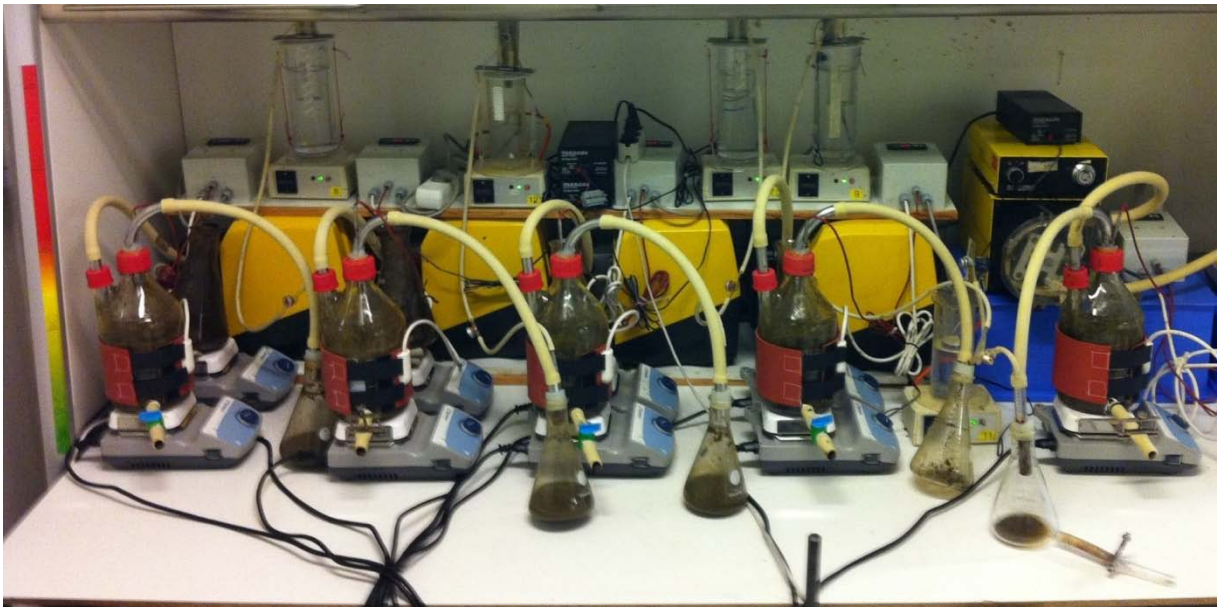


Fig. 6 Experimental setup of continuously fed biogas reactors

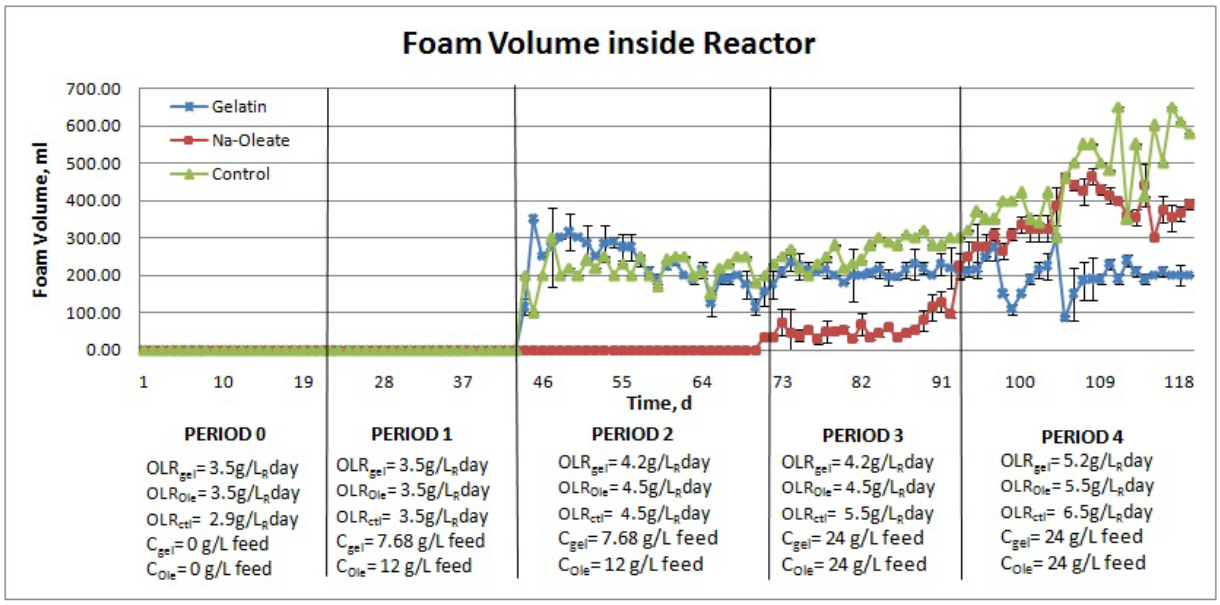


Fig. 7 Foam formation in the biogas reactors fed with manure supplemented with protein, or lipid, or carbohydrate

All the information and results obtained from this activity were published in the ISI publication 6J and 7J, were included in the following conference proceedings 9C, 11C and 12C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes).

3.5.5 Tests of antifoaming agents for manure digestion systems

Nowadays, many antifoaming agent solutions have emerged and abundance of commercial choices is available. Though, it is well known that an antifoaming agent may not be suitable for every

application and in worst case its addition in anaerobic digestion systems could deteriorate the whole process. Therefore, in this activity we investigated the antifoam efficiency of 14 non-commercial and commercial antifoaming agents in raw and digested cattle manure samples, using an aeration test method. Two aeration tests were performed (Fig. 8-9). In the first aeration test, the efficiency of each antifoam at different concentration levels was investigated. During the second aeration test, two different antifoam application methods (headspace and bottom injection) were evaluated. The current study was the first research that provided a qualitative and quantitative comparison of the specific substances concerning their ability to suppress foam in manure substrates. From the obtained results, it was found that natural oils, saturated and non-saturated fatty acids, tributylphosphate and the tested commercial antifoams were the most efficient compounds to suppress foam in both raw and digested cattle manure samples. Moreover, we have shown that the antifoams present stronger effect when applied in the bottom of the reactor. Thus, one strategy to obtain efficient antifoaming results is to ensure that the antifoam is fully dispersed in the solution.

All the information and results obtained from this activity were published in the ISI publication 5J and 9J, were included in the following conference proceedings 1C and 9C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes).

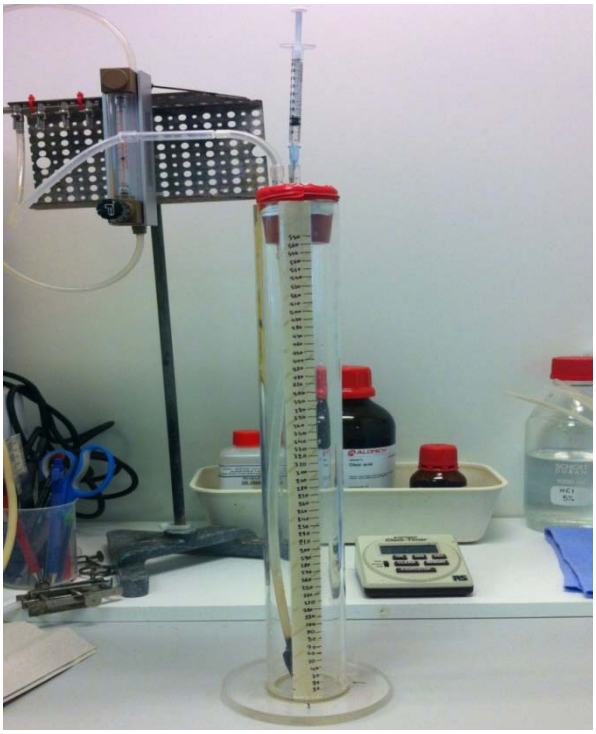


Fig. 8 Headspace injection method; the antifoam is sprinkled from the top of the aeration column.

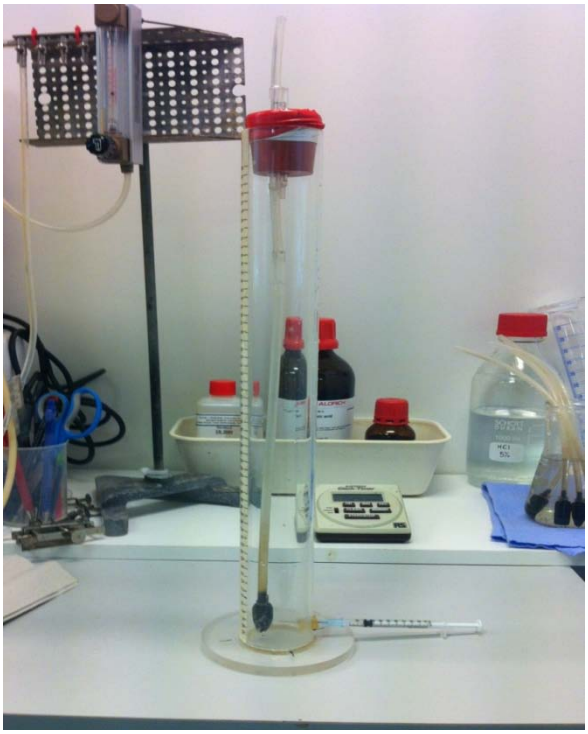


Fig. 9 Bottom injection method; the antifoam is directly added in the manure sample.

3.5.6 Foam suppression in reactors overloaded by carbohydrates

Based on the results from the physicochemical tests, we applied the most efficient antifoaming agents in lab scale CSTR reactors that were suffering from foaming incidents due to overload with carbohydrate-rich manure substrate (Fig 10).The aim of this study was to compare the foam reduction efficiencies and their influence on process performance under continuous operation of manure-based biogas reactors. Moreover, batch assays were carried out in order to determine the biodegradability of these antifoams, and to investigate their effect on the biomethanation of cattle manure. Each antifoam was tested at concentrations of 0.05, 0.1 and 0.5% v/v feed.The results from batch experiments showed that rapeseed oil, oleic acid and octanoic acid enhanced methane production when co-digested with cattle manure. These antifoams could also efficiently suppress foam under continuous reactor operation. We have concluded that rapeseed oil was most suitable as antifoam for manure digestion under organic overload, since it presented positive effect on the process at all applied dosages. Moreover, the price of rapeseed oil is lower compared to the prices of the other tested antifoams and this can contribute to a more economical solution for the full scale biogas plants. The optimal dosage of rapeseed oil will be dependent on the severity of foaming incident as the antifoaming efficiency of rapeseed oil increased with increased dosages. Finally, tributylphosphate strongly inhibited the biogas process and we characterize it as not suitable as antifoam in anaerobic digestion systems.

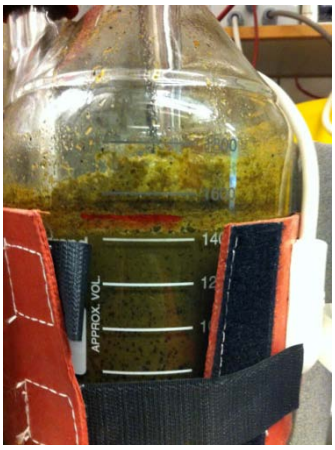


Fig. 10 Foam formation in CSTR overloaded with carbohydrate-rich manure substrate.

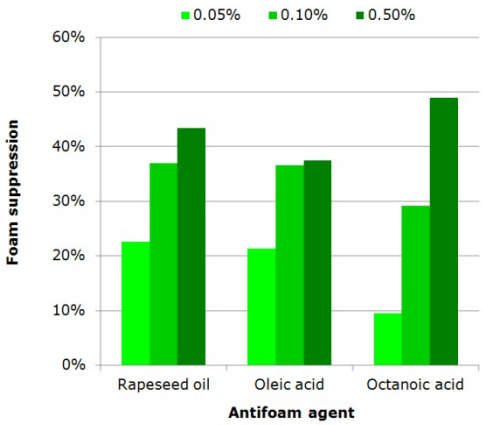


Fig. 11 Foam suppression efficiency of the tested antifoaming agents.

All the information and results obtained from this activity were published in the ISI publication 3J and 9J, were included in the following conference proceedings 1C and 9C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes) of the current

3.5.7 Foam suppression in reactors overloaded by lipids or proteins

In this study we further investigated the two antifoams that had shown the strongest antifoaming potential in the previous activity, which were rapeseed oil and oleic acid. In this activity we

performed continuous reactor experiments in order to investigate the defoaming efficiency of these compounds in reactors that were fed with protein- or lipid-rich manure substrate. The aim was to identify which of these antifoams and in which applied dosage is more suitable for foam suppression in cases that lipid or protein is the main cause of foaming. The influence of the antifoams on the biomethanation process was investigated both in batch assays and under continuous operation of manure-based biogas reactors. From batch experiments we found that rapeseed oil enhanced methane production both in protein- and lipid-rich substrates at all tested dosages. In contrary, the addition of oleic acid presented a negative influence on the biomethanation process at higher concentration. However, at low concentrations of oleic acid a possible synergistic effect was observed, as the methane yield from co-digestion was found to be higher than digestion of individual substrates. The results from the continuous reactor experiments investigating the defoaming efficiency of rapeseed oil and oleic acid are presented in Tables 2 and 3. It was found that rapeseed oil could efficiently suppress foaming by 40-52% in the reactor that was fed with proteins and by 46-51% in the reactor that was fed with lipids. Finally, oleic acid managed to suppress foaming by 30-49% in the reactor that was fed with proteins and by 40-56% in the reactor that was fed with lipids.

Table 2. Defoaming efficiency in biogas reactors fed with protein rich manure substrate.

	Rapeseed Oil		Oleic Acid	
	0.1% v/v_{feed}	0.5% v/v_{feed}	0.1% v/v_{feed}	0.5% v/v_{feed}
Methane yield ($mLCH_4/gVS$)	215±31	233±28	162±16	179±25
Defoaming efficiency (%)	39.98	52.26	30.44	48.92

Table 3. Defoaming efficiency in biogas reactors fed with lipid rich manure substrate.

	Rapeseed Oil		Oleic Acid	
	0.1% v/v_{feed}	0.5% v/v_{feed}	0.1% v/v_{feed}	0.5% v/v_{feed}
Methane yield (mLCH4/gVS)	301±43	344±24	311±42	354±16
Defoaming efficiency (%)	45.96	51.11	40.52	56.64

All the information and results obtained from this activity were published in the ISI publication 9J and 10J, were included in the following conference proceeding1C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes).

3.5.8 Microbial analysis of foaming reactors

In the present activity, we elucidated the possible correlation between foaming and the presence of specific microorganisms in manure-based biogas reactors (Fig. 10). The microbial ecology of continuous fed digesters overloaded with proteins, lipids and carbohydrates before and after foaming incidents was characterized using 16S rRNA gene sequencing. Moreover, the microbial diversity in the liquid and foaming layer was assessed. We have found that the microbial ecology in biogas reactors before and after foam formation varies significantly. Microorganisms that were reported in the cited literatures to contribute in foaming in sludge digesters or wastewater treatment plants were not found. However, we have found a number of genera that could be

linked to foaming as they produce biosurfactants, or contain mycolic acid in their cell wall, or have ability to decrease the surface tension of the culture media (Fig. 11). Finally, our analysis identified for the first time the presence of a species (operational taxonomic unit) whose abundance was increased in all reactors after foam formation; this microorganism was found distantly similar to bacteria related to foam (*Nocardia* and *Desulfotomaculum*). Additionally, in the current case it seemed that barley, which is commonly present in manure at high concentrations, contributed to the stabilisation of foam structure and was not the actual cause that initiates foam. More specifically, by visual inspection, particles from barley were observed to be transferred to the liquid surface due to the biogas bubbles, and accumulated in the foaming layer; thus contributing in foam stabilization. It is worth to highlight that this was the first study reporting the changes in the microbial population of biogas reactors that were fed with agro-industrial wastes, before and after foaming incidents.

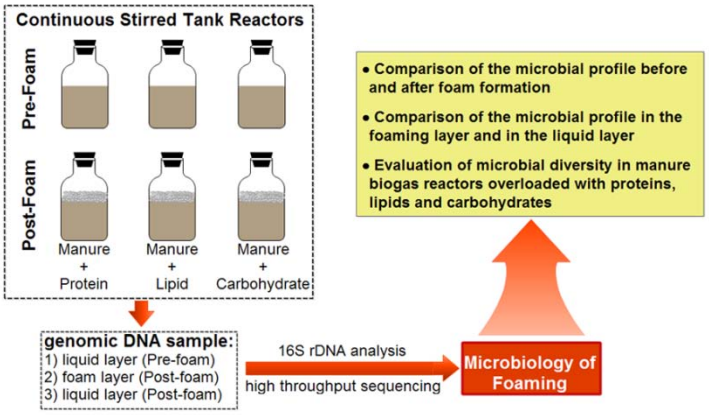


Fig. 10 Experimental setup along with the objectives of the research activity.

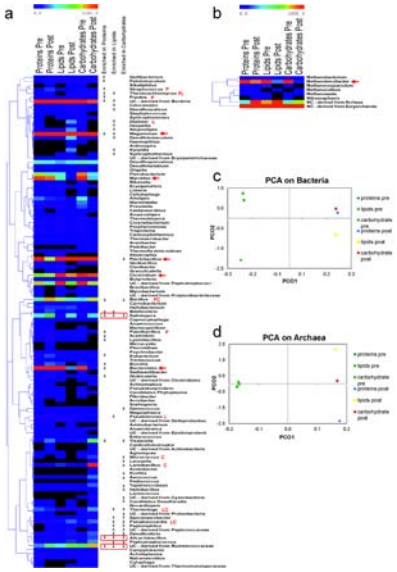


Fig. 11 Hierarchical clustering and heatmap showing abundance and composition of microbial community in foaming reactors.

All the information and results obtained from this activity were published in the ISI publication 1J and 2J, were included in the following conference proceedings 2C, 3C, 4C, 5C, 6C and 7C and presentations 1P and 2P. The corresponding list of disseminations activities can be found in section "5 Publication and dissemination" and are attached at section 6 (Appendixes) of the

3.6 Technical results achieved

The major technical results obtained from this project are:

1. No consistent correlation was found between the foaming potential and the physiochemical properties such as hydrophobicity, ODA or surface tension of the manure mixture substrates.

2. The proper way to determine foaming properties of the manure solution was the direct measurement of foaming tendency and foam stability. In this project, an improved method for measuring foam tendency and foam stability had been established.
3. Based on physicochemical test, Na-oleate and acetic acid showed the tendency to increase foaming formation. Additionally, gelatine and NH_4^+ had moderate effect on increasing foaming tendency, and gelatine had very strong effect on increasing foam stability.
4. Lipids and proteins showed the highest potential to create foam in the manure digester. Due to their strong ability to stabilize foam, high organic content of Na-oleate or gelatine was considered as main potential foaming problem.
5. The organic loading rate of a reactor is a major factor that affects foam formation.
6. Foam formation caused by overloading increases the stability of the foam and can lower the methane yield in case the feeding substrate is only cattle manure.
7. From aeration test, natural oils, fatty acids, and tributylphosphate acid could effectively decrease foaming in the digested manure.
8. From the aeration test, the foam suppression efficiency increased when increasing antifoam dosage. Though, for some specific compounds (e.g. rapeseed oil or commercial antifoam agents), the maximum efficiency was achieved up to a critical concentration (0.1% v/v), and further increase of the antifoam dosage did not have any further effect on foam suppression.
9. From the continuous experiments, it was found that tributylphosphate inhibited methanogenesis, despite the fact that this compound presented remarkable antifoam activity during aeration tests.
10. Rapeseed oil and oleic acid were able to suppress the foam in manure based biogas reactors up to a certain percentage (approximately 56%) without causing any problems to the whole anaerobic process.
11. It was found that in overloaded biogas reactors, at antifoam concentrations of 0.05% and 0.1% v/v_{feed}, rapeseed oil was the most efficient agent, as the foam in the reactors was reduced by 22.6% and 37.0%, respectively. In contrary, Octanoic acid had highest antifoaming efficiency at a concentration of 0.5% v/v_{feed}, which could suppress foam by 48.94%. Rapeseed oil at 0.5% v/v_{feed} presented also good antifoam efficiency of 43.33%.
12. In reactors fed with protein-rich substrate, the defoaming efficiency of rapeseed oil and oleic acid was 40-52% and 30-49%, respectively.
13. In reactors fed with lipid-rich substrate, the defoaming efficiency of rapeseed oil and oleic acid was 46-51% and 40-56%, respectively.
14. The addition of antifoaming agents together with the influent feedstock (bottom injection) was found to be more efficient compared to the sprinkling addition (headspace injection). This was due to the fact that the antifoam agents were better dispersed when they were added directly into the medium. However, by headspace injection the foam was destroyed in shorter time.
15. A number of genera that were found in biogas reactors could be linked to foaming as they produce biosurfactants (*Lactobacillus, Bacillus, Pseudomonas, Thermotoga*), others contain mycolic acid in their cell wall (*Thermoactinomyces, Pseudonocardia*) or have ability to

decrease the surface tension of the culture media (*Micrococcus, Streptococcus*). *Frankia, Dialister* and *Paenibacillus* are known to be correlated to foaming phenomenon but their mechanism is still unclear.

16. The microbial ecology in the liquid phase of a foaming reactor is significantly different from the foaming phase.
17. Cattle manure contains large amounts of barley fibres, which can contribute significantly on foam stabilisation in the biogas reactor.

4 Project conclusion and perspective

4.1 Conclusions

Anaerobic digestion (AD) foaming is the one of the most important practical problems which occasionally occurs in many full-scale biogas plants, affecting negatively the overall digestion process. More specifically, foaming is an intermittent phenomenon that typically occurred up to three times per year in most of the Danish biogas plants, and the duration of the incidents lasted from one day to three weeks, resulting in 20-50% biogas production loss. From physicochemical and continuous experiments, it was concluded that the main causes of foaming in manure-based biogas reactors were the organic overload and the feedstock composition. Especially, substrates that are rich in proteins or lipids tend to generate foam in the reactor. In contrary with the sludge digestion systems, filamentous foam promoting bacteria (e.g. *Microthrix parvicella*, *Nocardia sp.*) were not found in the reactor during foaming, and therefore they cannot be considered as cause for foaming in manure reactors. However, our analysis identified for the first time the presence of a species (operational taxonomic unit) whose abundance was increased in all reactors after foam formation; this microorganism was found distantly similar to bacteria related to foam (*Nocardia* and *Desulfotomaculum*). The utilisation of natural oils or fatty acids as antifoaming agents was concluded to be reliable and efficient methods to suppress foaming. The optimal dosage of these antifoams will be dependent on the severity of foaming incident, as the antifoaming efficiency of these antifoams increased with increased dosages in most cases. From several experiments, we concluded that rapeseed oil is recommended to be used in manure based biogas reactors to achieve rapid and efficient foam destruction.

4.2 Perspective

The current project investigated the main causes and solutions for foaming problems in manure biogas reactors. The major future challenges from the outcomes of this project are; (a) the deciphering of the foaming mechanism and (b) the deep investigation of the defoaming action and properties of chemical solutions. These will significantly contribute in optimizing antifoaming strategies and therefore improve their efficiency of foam suppression in the biogas reactors. Concerning the foaming mechanism, it is yet disputed whether specific parameters are associated with foam initiation or foam stabilization. These parameters include operating conditions of reactors (e.g. temperature, HRT), presence or relative abundance of specific microorganisms and

compounds that are commonly present in manure-based biogas reactors (e.g. barley). Sampling from full-scale biogas plants that are suffering from foaming incidents is absolutely necessary in order to decipher the foam mechanism. Regarding the defoaming action and properties of chemical solutions, the challenge targets to identify and produce antifoaming agents that are efficient, cost-effective and specialised for the manure-based biogas system. Nowadays there are a number of different commercial antifoaming agents available. However, their chemical composition is not given by the manufacturers and the purchase price for them is still very high. Additionally, their effectiveness is not ensured as most of them have not been tested under real AD applications. The selection of non-proper antifoam agents can lead to deterioration of the methanogenic process and as a consequence will affect severely the biogas plant economy. Therefore, once the effectiveness of antifoaming agents is evaluated in laboratory scale reactors, the next step is the full scale implementation if biogas plants in order to evaluate their applicability under real conditions. The development of antifoaming scenarios targeting specific causes of foaming (i.e. organic overload, feedstock composition and microbial composition) will contribute in deciphering the antifoaming mechanism and can be attractive not only for the biogas plant operators but also for Danish consulting companies and chemical industries.

5 Publication and dissemination

The publications and the dissemination efforts for the current project are:

5.1 Manuscripts published

1J De Francisci, D., Kougias, P.G., Treu, L., Campanaro, S., Angelidaki, I., 2015.**Microbial diversity and dynamicity of biogas reactors due to radical changes of feedstock composition**. Bioresource Technology. 176, 56-64.

2J Kougias, P.G., De Francisci, D., Treu, L., Campanaro, S., Angelidaki, I., 2014.**Microbial analysis in biogas reactors suffering by foaming incidents**. Bioresource Technology. 167, 24-32.

3J Kougias, P.G., Boe, K., Tsapekos, P., Angelidaki, I., 2014. **Foam suppression in overloaded manure-based biogas reactors using antifoaming agents**. Bioresource Technology. 153, 198-205.

4J Kougias, P.G., Boe, K., O-Thong, S., Kristensen, L.A., Angelidaki, I., 2014. **Anaerobic digestion foaming in full-scale biogas plants: a survey on causes and solutions**. Water Science and Technology. 69, 889-895.

5J Kougias, P. G., Tsapekos, P., Boe, K., Angelidaki, I., 2013. **Antifoaming effect of chemical compounds in manure biogas reactors**.Water Research. 47, 6280-6288.

6J Kougias, P. G., Boe, K., Angelidaki, I., 2013. **Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors**. Bioresource Technology. 144, 1-7.

7J Angelidaki I and Kougias P.G., 2013. **Når biogasanlægget skummer over**. Journal FiB - Forskning i Bioenergi, (46), pp 21

8J Boe, K., Kougias, P., Pacheco, F., O-Thong, S., Angelidaki, I., 2012.**Effect of substrates and intermediate compounds on foaming in manure digestion systems**. Water Science & Technology. 66, 2146-2154.

5.2 Manuscript in submission

9J Kougias, P.G., Boe, K., Angelidaki, I., 2015. **Solutions for foaming problems in biogas reactors using natural oil or fatty acid defoamers**. Submitted to journal "Energy and Fuels"

10J Kougias, P.G., Boe, K., Sif Einarsdottir, E., Angelidaki, I., 2015. **Foam suppression in biogas reactors fed with protein and lipid rich substrates**. Submitted to journal "Bioresource Technology".

5.3 Conference proceedings

1C Kougias, P.G., Boe, K., Angelidaki, I., 2014. **Antifoaming effect of rapeseed oil and oleic acid in biogas reactors**.Biogas Science 2014 International conference on anaerobic digestion, October 26-30, 2014, Vienna, Austria

2C Kougias, P.G., De Francisci, D., Treu, L., Campanaro, S., Angelidaki, I., 2014.**Metagenomic analysis of foaming in biogas reactors**.Biogas Science 2014 International conference on anaerobic digestion, October 26-30, 2014, Vienna, Austria

3C De Francisci, D., Kougias, P.G., Treu, L., Campanaro, S., Angelidaki, I., 2014.**Changes in the microbial profile of biogas reactors due to variations in the feedstock composition**.Biogas Science 2014 International conference on anaerobic digestion, October 26-30, 2014, Vienna, Austria

4C De Francisci, D., Kougias, P.G., Treu, L., Campanaro, S., Angelidaki, I., 2014.**Microbial diversity and dynamicity of biogas reactors fed with different substrates**. 2nd International Conference on Biogas Microbiology-ICBM, June 10-12, 2014, Uppsala, Sweden

5C Kougias, P.G., De Francisci, D., Treu, L., Campanaro, S., Angelidaki, I., 2014.**Comparative microbial analysis before and after foaming incidents in biogas reactors**.2nd International Conference on Biogas Microbiology-ICBM, June 10-12, 2014, Uppsala, Sweden

6C Campanaro, S., Treu, L., Kougias, P.G., De Francisci, D., Angelidaki, I., 2014.**Comparative analysis of the microbial diversity in liquid and foaming layer in biogas reactors**.2nd International Conference on Biogas Microbiology-ICBM, June 10-12, 2014, Uppsala, Sweden

7C Treu, L., Campanaro, S., De Francisci, D., Kougias, P.G., Angelidaki, I., 2014.C **A novel bioinformatic strategy to characterise microbial communities in biogas reactors**.2nd International Conference on Biogas Microbiology-ICBM, June 10-12, 2014, Uppsala, Sweden

8C Kougias, P.G., Boe, K., O-Thong, S., Kristensen, L.A., Angelidaki, I., 2013. **Anaerobic digestion foaming in Danish full-scale biogas plants: a survey on causes and solutions**, 13th World Congress on Anaerobic Digestion. 25-28 June 2013, Santiago de Compostela, Spain

9C Kougias, P.G., Tsapekos, P., Angelidaki, I., 2014.**Foaming in manure based digesters: Effect of overloading and foam suppression using antifoam agents**. XXXV CIOSTA & CIGR V Conference 2013, 3-5 July 2013, Billund, Denmark

10C Kougias, P.G., Boe, K., O-Thong, S, Angelidaki, I., 2012.**Influence of microbial composition on foam formation in a manure-based digester**, Symposium of The Danish Microbiological Society, Copenhagen, Denmark

11C Kougias, P.G., Boe, K., Angelidaki, I., 2012. **Foaming in manure based digesters-Causes and solutions**, Nordic Biogas Conference. 23-25 of April 2012, Copenhagen, Denmark.

12C Boe, K., Kougias, P., Pacheco, F., O-Thong, S., Angelidaki, I., 2011.**Effect of substrates and intermediate compounds on foaming in manure digestion**

systems. International Symposium on Anaerobic Digestion of Solid Waste and Energy Crops, Vienna, Austria, 28/8-1/9/2011

13C Pacheco, F., O-Thong, S., Boe, K., Angelidaki, I., 2011.**Systematic investigation of the effect of feedstock composition, and filamentous bacteria cells on foaming in manure anaerobic digestion systems**.12th World Congress on Anaerobic Digestion. 31 October-4 November 2011, Guadalajara, Mexico

5.4 Presentations to stakeholders

1P Kougias, P.G., Boe, K., Angelidaki, I., 2014. **Skumdannelse: 1.Kortlægning af skumningsproblemer på biogasanlæg, 2.Udvikling af strategier for at undgå skumning.**Rådnetank II - et fortsætter kursus, 8-9 December,Silkeborg, Denmark

2P Kougias, P.G., Boe, K., Angelidaki, I., 2013. **Skumdannelse: 1.Kortlægning af skumningsproblemer på biogasanlæg, 2.Udvikling af strategier for at undgå skumning.** Rådnetank II - et fortsætter kursus, 3-4 October, Silkeborg, Denmark

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Finally, we would like to express our thanks to all the researchers and students that participated in the project activities; Dr. SompongO-Thong, Dr. Davide De Francisci, Dr. Laura Treu, Dr. Stefano Campanaro, Panagiotis Tsapekos, Elma Sif Einarsdottir and Francisco Pacheco López.

6. Appendix A (manuscripts-poster-presentation)

Appendix A.1

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Microbial diversity and dynamicity of biogas reactors due to radical changes of feedstock composition

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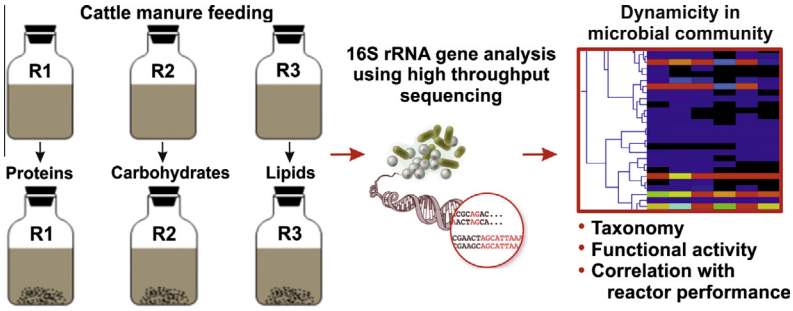
HIGHLIGHTS

- 16S rRNA analyses on biogas reactors prior and after substrate change.
- Comparison of microbial dynamicity in response to change of feedstock composition.
- Reactors process performance was correlated with the microbiological shifts.
- The metabolic pathways and the dynamicity of specific genera were correlated.

GRAPHICAL ABSTRACT



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ABSTRACT

The anaerobic digestion process is often inhibited by alteration of substrates and/or organic overload. This study aimed to elucidate changes of microbial ecology in biogas reactors upon radical changes of substrates and to determine their importance to process imbalance. For this reason, continuously fed reactors were disturbed with pulses of proteins, lipids and carbohydrates and the microbial ecology of the reactors were characterized by 16S rRNA gene sequencing before and after the imposed changes. The microbial composition of the three reactors, initially similar, diverged greatly after substrate change. The greatest increase in diversity was observed in the reactor supplemented with carbohydrates and the microbial community became dominated by lactobacilli, while the lowest corresponded to the reactor overfed with proteins, where only *Desulfotomaculum* showed significant increase. The overall results suggest that feed composition has a decisive impact on the microbial composition of the reactors, and thereby on their performance.

1. Introduction

Anaerobic digestion is a complex process, widespread in anaerobic environments, in which organic matter is degraded by different types of microorganisms to form biogas. Examples of such environments are freshwater sediments, wetlands and the digestive tracts of animals. Anaerobic digestion is also a widely applied method for treating different types of wastes (agricultural, industrial and domestic), not only for the concomitant energy production, but also for the transformation of the organic residues into fertilizer to be used in agricultural purposes.

Nowadays, the biogas plants proliferate and extra biomass is continuously needed to meet the surplus needs and new substrates are tested for their potential as feedstocks. Specifically in Denmark, most of the centralized biogas plants are co-digesting manure (at an amount of 70%) and other organic residues mainly derived from food industries (at an amount of 30%). Mixed feedstocks are usually

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very complex and can contain several compounds that could either result in a successful combination for the biomethanation or in contrary could inhibit the AD process. The reasons for such instability are generally connected to the characteristics and complexities of the microbial communities responsible for the process, which are still poorly understood. These communities are commonly divided into three distinct groups of microorganisms: primary fermenting bacteria, anaerobic oxidizing bacteria and methanogenic archaea (Angelidaki et al., 2011). The primary fermenting bacteria hydrolyze polymers (such as polysaccharides, proteins and lipids) to monomers (such as sugars, amino acids and long-chain fatty acids). This first step is characterized by the action of specific hydrolytic enzymes (amylases, proteases and lipases) produced and secreted by this group of microorganisms. The monomers produced in this step are then further reduced to alcohols (methanol, ethanol), short-chain fatty acids and organic acids (formic, acetic, propionic, butyric and pentanoic), hydrogen and carbon dioxide. The oxidizing bacteria oxidize these reduced products to acetate, hydrogen, formate and CO_2 (Angelidaki et al., 2011). At this point aceticlastic methanogens are responsible for the methanogenic degradation of acetate whereas the hydrogenotrophic methanogens directly convert hydrogen and CO_2 to methane. The balance within these distinct microbial groups is pivotal to the quality and yield of the methane produced and, again, is directly connected to its overall stability of the process (Demirel and Yenigün, 2002). Unfortunately, this balance is very fragile, as these groups are significantly different in respect to growth rate, physiology and nutritional needs. Several substances are known to cause unbalance and/or inhibition to anaerobic digestion systems, due to their negative effect on the bacterial growth of specific microorganisms and/or specific shifts in the microbial communities which are characterized by the accumulation of organic acids and a decrease of methane yield (Chen et al., 2008). Up to now it is still unclear how the utilization of specific substrates as reactor feeding corresponds to different microbial communities in anaerobic digestion systems. This information would be fundamental for the development of strategies for improving the performance of biogas plants that utilize agro-industrial wastes as feedstock. In order to address this issue, in the present study the microbial ecology of the reactors was screened before and after substrate change to gain a deeper understanding on how the microbial ecology of the biogas reactors responds to radical variations of substrate.

2. Methods

2.1. Waste characteristics and preparation of the feedstock

The raw cattle manure (CM) used as the main feedstock (of the reactors) derived from Hashøj biogas plant, Denmark. Upon arrival, the manure was shredded and sieved (5 mm) to separate large particles which could block tubing during reactor feed, and was stored at −20 °C. The frozen manure was thawed at 4 °C for 2–3 days before use. The manure had a pH of 8.09 ± 0.01, total solids (TS) and volatile solids (VS) content of 74.5 ± 0.1 and 59.4 ± 0.4 g/L, respectively. The total Kjeldahl nitrogen (TKN) and ammonium nitrogen (NH_4^+) were 3.30 ± 0.17 and 2.11 ± 0.14 g/L, respectively. Finally, the concentration of the volatile fatty acids (VFAs) in the raw manure was 11.11 ± 0.98 g/L.

2.2. Experimental setup and operation

The experiment was carried out in three continuous stirred tank reactors (CSTR) with a total and working volume of 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer and was equipped with a thermal jacket in order to maintain the operating temperature steady at 54 ± 1 °C. Throughout the experiment the hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The influent feedstock was automatically provided twice per day using peristaltic pumps. Biogas production was recorded using an automated displacement gas metering system with 100 ml cycle. At start-up all the reactors were inoculated with thermophilic inoculum obtained from Snertinge biogas plant, Denmark. The experiment was divided in two periods; (a) during the first period all the reactors, denoted as R1, R2 and R3, were fed exclusively with CM and therefore they were considered as triplicates, (b) during the second period the influent feedstock of each reactor was radically changed by adding to the CM either gelatine (9 g/L-feed), as a representative of proteins (R1), or sodium oleate (Na-Oleate, 12 g/L-feed), as a representative of lipids (R2) or glucose (40 g/L-feed), as a representative of carbohydrates (R3). The amount of each compound added to cattle manure was chosen according to our previous study (Kougias et al., 2013), so as to ensure distinct process performance variations due to the altered feed with the specific organic compound, without causing process failure due to overloading. The concentration of each compound added in the cattle manure feedstock and the organic loading rate of the reactors are presented in Table A1. Samples were taken from each reactor prior to the feedstock change and after reaching the new steady state conditions (biogas production variation less than 10% for more than 6 consecutive days) with the new substrates. DNA was extracted from each sample and the corresponding microbial composition was determined via analyses of 16S rRNA amplicons.

2.3. Analytical methods

Total solids (TS), volatile solids (VS), total nitrogen (TKN), total ammonia and pH were determined according to APHA standard methods for the examination of water and wastewater (APHA, 2005). The methane and the CO_2 content in biogas were determined using a gas chromatograph (Mikrolab, Aarhus A/S, Denmark), equipped with a thermal conductivity detector (TCD). Volatile fatty acids (VFAs) analysis was performed using a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) as described by Kougias et al. (2013). During the whole experiment the biogas production was recorded on daily basis, while the methane content in biogas, pH and the concentration of the VFA were measured twice or three times per week. All the determinations were performed in triplicate.

2.4. Sampling and DNA extraction

Samples (100 ml) from each reactor were collected and processed as described in Kougias et al. (2014a). Total microbial DNA from samples was isolated and purified using the PowerSoil® DNA Isolation Kit (MO BIO laboratories, Inc.) according to the protocol provided by the manufacturer. The hypervariable V3 region of bacterial and archaeal 16S rRNA genes was amplified using the same procedure reported in Kougias et al. (2014a) (Appendix A). The concentration of each amplicon was determined by using both NanoDrop (Thermo Scientific) and Qbit fluorometer (Life Technologies) and equivalent amounts of each amplicon were pooled in a single tube. The obtained amplified product was purified with the Agencourt AMPure kit (Beckman Coulter, Inc.) and the final DNA elution was performed with 40 µl of water. Identical amount of each amplicon was pooled and sent to DTU Multi-Assay Core (DMAC) for library preparation (according to the standard protocol Ion Xpress Plus gDNA and Amplicon Library Preparation, Life Technologies) and Ion Torrent PGM sequencing (316 chip, Ion Sequencing 200 kit, Life Technologies).

2.5. Analysis of the 16S rRNA gene sequences

Sequences in FASTQ format were converted to FASTA format using the FASTX-Toolkit. Raw sequence data were submitted to the NCBI sequence read archive database (SRA) with accession numbers reported in Table 1. Terminal parts of sequences having quality lower than 13 were trimmed and sequences shorter than 50 bp after trimming were removed. Low quality sequences (minimum quality threshold of 13 on more than 10% of the sequence) were removed. The sequences in FASTQ format were uploaded to the database server MG-RAST and were de-replicated (Gomez-Alvarez et al., 2009). The sequences with the low quality sequences were identified and discarded using a modified dynamic trim (Cox et al., 2010). Sequences with a score lower than 15 and with a quality lower than 5 were removed. Detection of chimeras was performed using the UCHIME algorithm (http://drive5.com/usearch/manual/uchime_algo.html) of USEARCH software but their presence was found negligible.

The abundance of each genus, the Principal Coordinate Analysis (PCoA) and the Alpha diversity indices were calculated with the MG-RAST toolkit using the "best hit classification" method. Alpha diversity estimates mean Operational Taxonomic Unit (OTU) diversity at local scale (Whittaker, 1972). It was calculated for all samples using MG-RAST software. The alpha diversity is based on the taxonomic annotations for the predicted rRNA genes and is a single number that summarizes the distribution of OTU-level annotations in a dataset. PCoA was performed with MG-RAST software to assess community composition inferred using 16S rRNA annotation. The data has been normalized to values between 0 and 1, the graph was drawn using Bray Curtis distance.

The annotation sources considered were SSU, M5RNA, RDP and Greengenes databases using MG-RAST software with two different thresholds to compare results with medium and high specificity. The maximum e-value of 1e-5, a minimum alignment length of 50 bp for databases and minimum % identity cutoffs of 50% and 97%, respectively, were chosen. The two thresholds resulted in no differences in archaea identification in term of number of genera (384) and species (575). Regarding bacterial classification the results were slightly more abundant with the lower threshold than with higher one both at genus (5577 vs 5553) and species level (11,589 vs 11,721). For this reason we proceeded with all the other analysis using the higher threshold because of the higher reliability of the results.

In order to perform a direct comparison between the samples, the relative abundance of each genus was calculated. To simplify the discussion we classified genus according to their relative abundance as predominant (>5%), abundant ($\geqslant$1%), medium (0.1–0.99%), rare (0.01–0.09%) and very rare (<0.01%). Very rare microorganisms were not considered in the discussion and those with less than 10 reads in all samples were discarded. Relative abundance values were used to build heatmaps using the MeV 4.8.1 software (Saeed et al., 2003). The hierarchical clusterings were performed using average linkage method and with Pearson correlation as distance metric. Log_2 values have been calculated to better highlight changes in microorganism abundance from period 1 to period 2. As major changes in the microbial community we discuss those higher than a threshold of twofold change in relative abundance.

2.6. Bioinformatics strategy: classification at species level and KEGG analysis

Reliable microorganism identification at species level was obtained using a strategy based on BLAST similarity search and classification with a self-written perl script (Campanaro et al., 2014). It was created to obtain a more strict control on the 16S rRNA classification at the species level, with estimation of its reliability. Briefly, the software performs local BLAST similarity search and evaluates the results with high stringency (95% up to 100%), returning all the possible candidate species with unique or multiple matches. In this process, different categories of reliability are generated: some can lead to univocal species identification even in the same genus, while others give multiple matches with the same probability each. Species assigned as unique matches with high probability (100% and 99.7%) were considered reliable and therefore used for discussion (Table A3), while others having multiple matches were excluded.

Moreover, specific perl scripts were developed to perform comparisons among different genera according to the corresponding COG and KEGG composition. A database with COG and KEGG annotations was generated, using the IMG 4 Data Management system (http://img.jgi.doe.gov), including all the microbial genomes available at the Integrated Microbial Genomes and Metagenomes (IMG). Both the complete and draft microbial genomes were considered (453 archaea and 15,484 bacteria), removing those having less than 500 genes, which were considered "incomplete genomes". Species belonging to the same genus were grouped using the annotation downloaded from the same website. The script takes as input a list of genera, for each genus it recovers all the microbial species and for each species it calculates the number of genes belonging to each COG and KEGG category. At this point, for each genus, the average number of genes belonging to each COG class and each KEGG pathway was calculated and this allowed a comparison of COG and KEGG gene content among different genera.

Table 1
Sequencing results summary and alpha diversity.

	NCBI SRA ID	Filtered reads	Assigned reads	% Reads assigned	Alpha diversity	
Archaea						
R1	SRP033444	98,724	41,269	42	3.20	Period 1
R2	SRP033444	125,214	58,482	47	2.78	
R3	SRP033444	106,126	46,686	44	2.84	
R1	SRP033447	117,616	47,727	41	3.01	Period 2
R2	SRP033448	107,381	53,992	50	1.74	
R3	SRP033449	116,389	60,184	52	1.69	
Bacteria						
R1	SRP033444	231,018	58,779	25	54.60	Period 1
R2	SRP033444	269,983	80,568	30	42.48	
R3	SRP033444	258,121	74,729	29	48.24	
R1	SRP033447	250,721	70,865	28	53.34	Period 2
R2	SRP033448	224,213	66,144	30	30.42	
R3	SRP033449	197,692	54,637	28	66.82	

For these analyses, only the genera increasing more than fourfolds in period 2 were considered.

3. Results and discussion

Up to now it has been disputed whether and at which extent the presence of specific microorganisms in anaerobic digestion systems is correlated with the utilization of specific substrates as reactor feeding. Furthermore, specifically for biogas plants using agro-industrial wastes as feedstock, the potential correlation of specific microorganisms and substrates is not yet elucidated. In order to address this issue, we established the response of microbial ecology composition upon changes of the feed substrate to the reactors. We focused mainly on the genera whose relative abundance was highly diverse (i.e. increase or decrease by at least 2-folds).

3.1. Microbial diversity and dynamicity of biogas reactors in response to radical change of feedstock composition

The experimental work was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3, operating under thermophilic conditions. The whole experiment was divided in two periods; during the first experimental period all the reactors were fed with CM, while in the second period the feed was CM supplemented with proteins (R1), lipids (R2) and carbohydrates (R3). Samples were taken from each reactor at two times. The first was just prior to the feedstock change (period 1) and the second one after the reactors had reached steady state with the new feedstock (the mixed substrates) (period 2).

All the sequencing results are summarized in Table 1. Regarding bacteria, it was possible to perform taxonomic assignment of less than half of the total sequenced reads (on average 67,620 per sample). This is partly due to the stringent criteria used for taxonomic classification. Among the total, 16% of reads matched to unclassified microorganisms at genus level. This was due to the lack in databases of 16S rRNA sequences belonging to microorganisms not yet discovered nor isolated and the presence in the same databases of sequences belonging to many unknown and uncharacterized microorganisms.

For archaea the reads assigned were half of the total sequenced (on average 51,390 per sample), but 90% of them matched to unclassified microorganisms. This tremendous lack of information in archaeal databases is a major obstacle for the comprehension and understanding of anaerobic digestion systems and needs urgent solution.

Principal coordinate analysis (PCoA, Fig. 1) together with alpha diversity index (Table 1) enable to assess the community composition inferred using 16S rRNA annotation at OTU level. PCo1 and PCo2 explained 42% and 23% of the total bacterial community variations and 45% and 26%, respectively, for archaea (Fig. 1). It was seen, as expected, that samples of the three CSTR taken in period 1 clustered closely (especially for archaea), while samples taken in period 2 diverged greatly. This shift in period 2 is clearly due to the microbial adaptation to the different organic substrates added to the different reactors. Alpha diversity values indicate a much higher community complexity for bacteria compared to archaea, which is in agreement with previous findings (Jaenicke et al., 2011; Wirth et al., 2012). This suggests that the archaeal community in biogas reactors represents a small fraction of the whole microbiome. It is also noteworthy that all these values generally decreased in period 2, indicating a reduction in the community complexity, except for bacteria in R3 (Table 1). This behavior of the community complexity in the three reactors could be explained by the nature of the specific substrates added. For R1 and R2 the complexity was reduced, as both proteins and lipids are known to be potential inhibitors for many bacteria and archaea, due to the toxicity offered by the resulting ammonia and Long Chain Fatty Acids (LCFA) (Fotidis et al., 2014a; Kougias et al., 2014b; Palatsi et al., 2010). Only selected groups of microorganisms are surviving in such environments, due to the toxicity pressure. These tolerant microorganisms were subsequently enriched and thereby reduction of the microbial complexity was observed. On the other hand, glucose is a substrate which supports growth of a wide spectrum of bacteria. This resulted in increased microbial complexity in R3.

In particular, according to the PCoA and Alpha index results (Fig. 1, Table 1), it is clear the bacterial communities in R1 and R2 are reduced in variability, respectively slightly and strongly, while in R3 are increased. Finally, the highest bacterial variability was observed in R3 during period 2. Moreover a tremendous increment of many *Lactobacillus* species in this reactor was observed, as described in detail in Section 3.3.

Regarding archaea, R2 and R3 strongly reduced their diversity from period 1 to period 2, which instead increased slightly for R1 (Fig. 1, Table 1). This indicates that the archaeal population is strongly correlated and influenced differently by the feedstock composition of biogas reactors. It was observed that there was a direct correlation between the bacterial and archaeal dynamics in R2, while an inverse correlation was found in R1 and R3. Unfortunately, a possible explanation for this behavior could not be conceived. As reported in a recent study, a putative metabolic context in order to understand the changes in the diversity of methanogenic archaea accompanying the acclimation, especially to protein substrates, is yet difficult to be established (Ács et al., 2013).

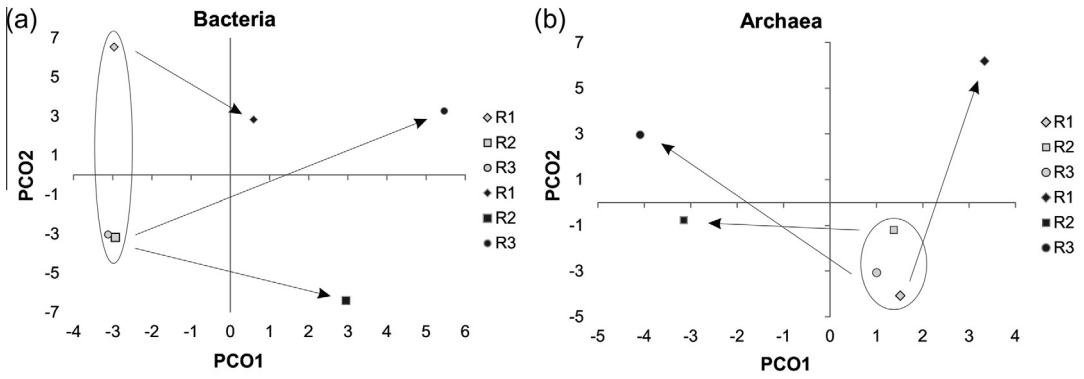

Fig. 1. PCoA analyses describe bacterial (a) and achaeal (b) composition and variability. Results corresponding to period 1 are grouped by a circle; arrows indicate the shifts occurred in period 2, which correspond to communities' dynamicity.

3.2. General consideration at phylum and genus level

Our results confirm that the predominant microbes contributing to the decomposition of CM in biogas reactors include members of Firmicutes and Bacteroidetes, which is in accordance with previous reports (Riviere et al., 2009; Sundberg et al., 2013). These two phyla are common to all substrate changes with a very similar relative abundance in all the three reactors (67% mean value). As a general trend, bacteria that are found to increase in relative abundance mainly belong to Firmicutes, Actinobacteria, Proteobacteria and Cyanobacteria. It was previously proven that microorganisms often found in anaerobic digestion systems belong to these phyla (Wirth et al., 2012). The results of the present study confirm predominance and adaptation of these phyla as they are found to increase their dominance at all the conditions tested. Microorganisms mainly belonging to Bacteroidetes, Spirochaetes and Synergistetes are those that as a general trend decrease in relative abundance after substrate change, suggesting that these phyla are not adapted to counteract and survive these conditions.

Excluding the unclassified microorganisms, the common predominant genera in all three reactors were *Megamonas* (Firmicutes) and *Bacteroides* (Bacteroidetes), while *Flectobacillus* (Bacteroidetes), *Clostridium* (Firmicutes), *Holdemania* (Firmicutes) and *Myroides* (Bacteroidetes) were the predominant genera of the microbial community in two out of the three CSTRs. All the identified genera changed to some extent their relative abundance at period 2. The results corresponding to the predominant genera are summarized in Fig. 2 and in Table A2.

Regarding the archaeal communities the only predominant genus identified was *Methanobrevibacter*. This methanogen is found to decrease in relative abundance in all the CSTRs. The data showed the presence of another specific archaeon, still unclassified, belonging to Euryarchaeota that is predominant and common to the reactors, both in period 1 and 2. Unfortunately, due to the lack of information regarding archaea in databases it was not possible to provide any comment on the predominance of this microorganism.

Finally, *Methanoculleus* was found to increase in all three reactors. In R1 it reached a medium level of relative abundance (from 0.03% to 0.15%, 5-fold) while it remained at lower abundance in R2 and R3. Among the *Methanoculleus* genus *M. bourgensis*, *M. thermophilus*, *M. marisnigri*, *M. palmolei*, *M. chikugoensis* and *M. receptaculi* were found to increase. A possible explanation for the general increase of the *Methanoculleus* sp. could be provided by the KEGG analysis performed on the archaea population. As it can be seen in Fig. 3a *Methanoculleus* sp. has higher gene content compared to the other genera in specific pathways, some directly involved in the biomethanation process. As a consequence, these features could give advantages for the survival of *Methanoculleus* sp. in different growing environments. This hypothesis has been already validated in a previous study for the pathways of reductive citric cycle and glycolysis (Anderson et al., 2009). More specifically, it was proven that *Methanoculleus* species and some closely related genera pose the ability to use ethanol and a variety of secondary alcohols as electron donors for methanogenesis. Additionally, the higher increase in the relative abundance of this genus specifically in the reactor fed with proteins could be explained as it was previously shown that *Methanoculleus* has a relative high tolerance to ammonia (Fotidis et al., 2014b). Our results are also in agreement with a recent study in which the impact of protein, lipid and cellulose containing mixed substrates on microbial communities was investigated. The qPCR results from this study revealed that to the gelatin substrate corresponded the highest abundance of *Methanoculleus* sp. compared to all the other tested substrates (Wagner et al., 2013).

3.3. Microbial dynamicity in biogas reactors overloaded with proteins

The microbial analysis of the reactor where proteins were supplemented to the feed (R1) did not reveal a great response in the bacterial microbial community, especially when compared with the other two reactors. However, interesting shifts in relative abundance of archaea were observed.

The methane yield during period 1 reached on average 142 ml CH_4/g VS. Methane yield decreased after day 30 to approximately 91 ml CH_4/g VS, followed by a sharp increase in the VFA levels and decrease in the pH (Fig. 4). The main compound that contributed in the increase of VFA was acetate, while the propionate concentration remained stable.

While such a profound impact was observed in the reactor performance after introduction of proteins in the feed, the metagenomic analysis revealed no relevant bacterial shift, especially in

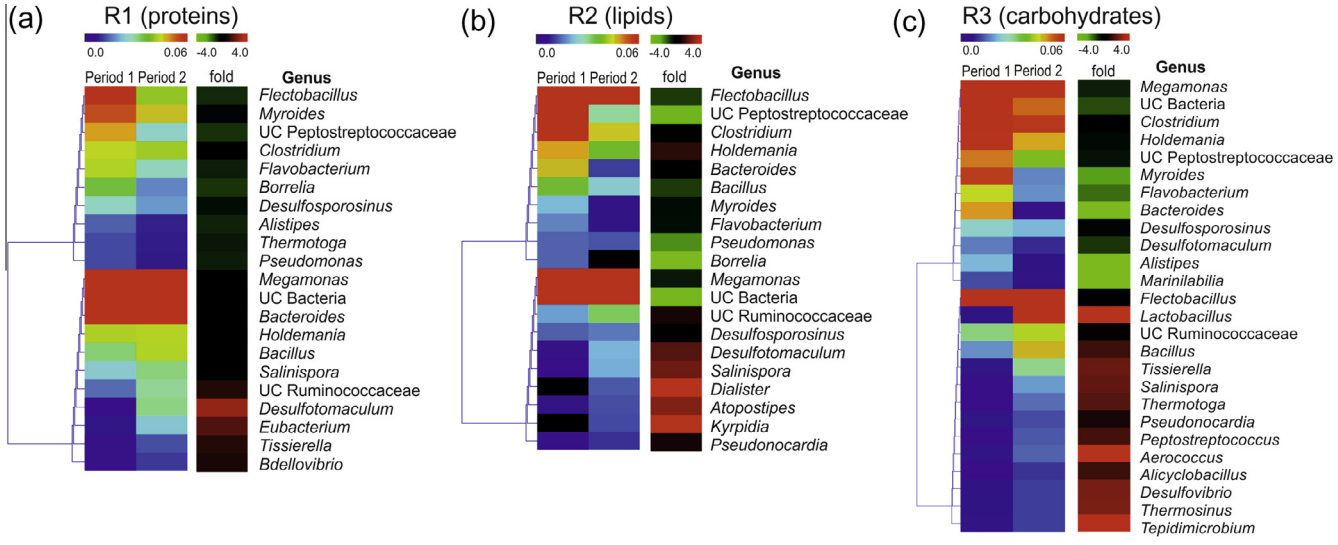

Fig. 2. Hierarchical clustering of the results obtained on bacterial communities of R1 (a), R2 (b) and R3 (c). Only abundant genera were considered. Relative abundance identified for each sample was represented as a heat map (left part of each panel) and colored to evidence increased (red) and decreased (green) genera (right part of each panel). Correspondence between colors and relative abundance/fold change is reported in the scales at the top of each panel. Acronym "UC" indicates unclassified bacteria.

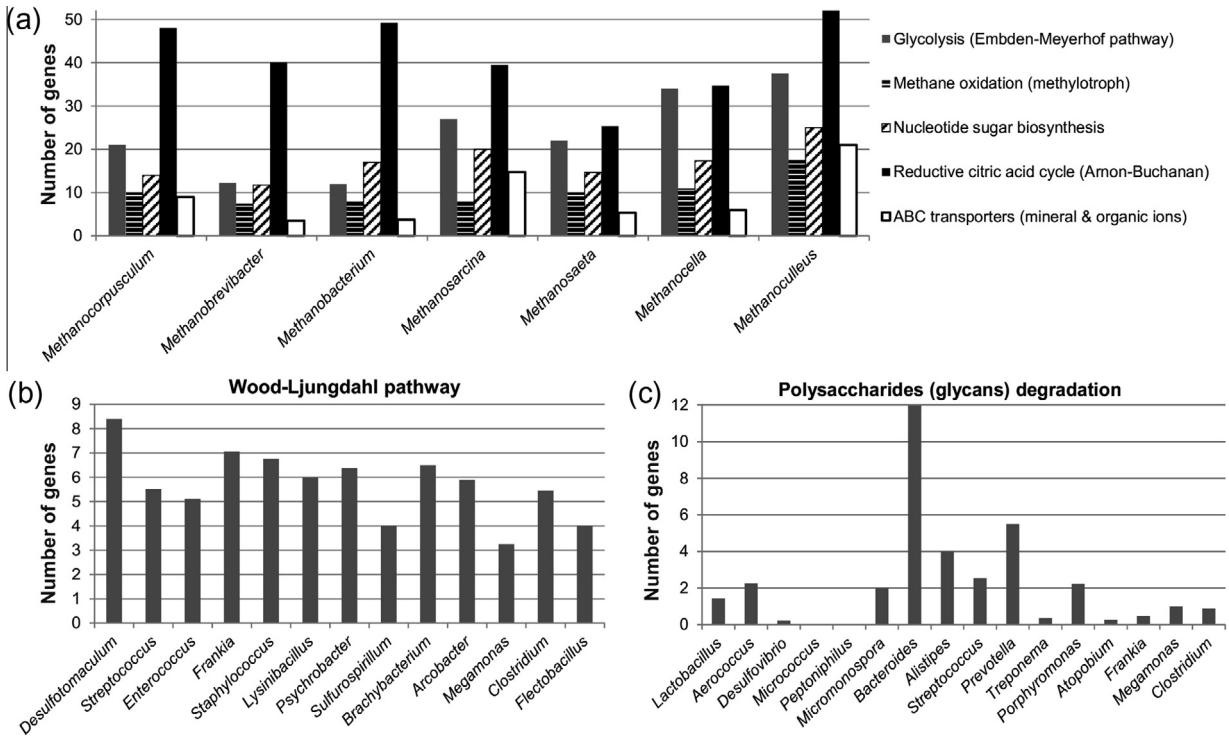

Fig. 3. Results of the KEGG analysis performed on all the microorganisms identified; (a) analysis on archaea genera identified in this study. The *y* axis refers to the number of genes present in the genera and belonging to the pathways reported in the legend, (b) results performed on bacterial genera specific for R1 considering the Wood–Ljungdahl pathway genes, (c) analysis performed on bacterial genera specific for R3 considering the genes involved in polysaccharides degradation.

comparison to R2 and R3. The only exceptions were *Desulfotomaculum* (increases from 0.4% to 3.1%, ~7-fold, probably connected with the producton of H_2S from protein degradation) and *Eubacterium* (increases from 0.7% to 2.3%, ~3-fold). Among these two genera the most abundant species identified were *D. alcoholivorax*, *D. geothermicum* and *D. thermobenzoicum*. The third one is probably the most relevant as it is known to be able to perform propionate oxidation (Plugge et al., 2002).

A rational to explain this lack of dynamicity can derive from the monitoring of the biogas reactor process. The methane yield during the steady state of period 2 was on average 87 ml CH_4/g VS presenting a significant decrease (37.7%) compared with the period that the reactor was fed only with CM. This inhibition could be explained by the increase of ammonia concentration in the reactor, due to the degradation of gelatine which negatively affected the methanogenesis (Kougias et al., 2013). Ammonia accumulation

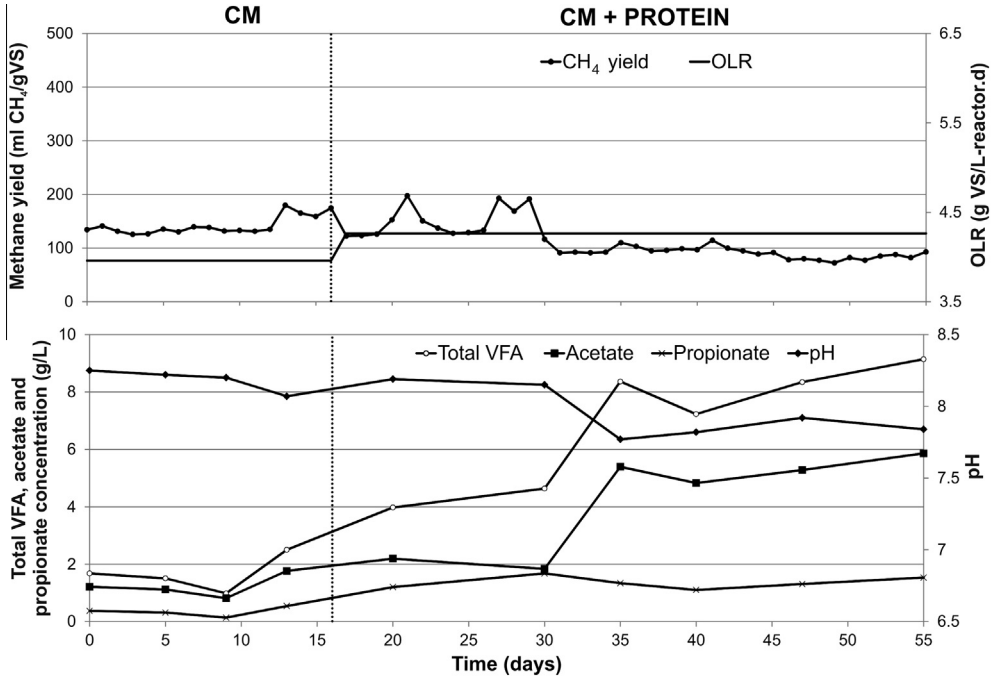

Fig. 4. Process performance of reactor (R1) fed with CM and gelatin (protein).

has led to inhibition of methanogens, except the ones tolerant to ammonia, such as *Methanoculleus*, which consequently increased in relative abundance. Ammonia inhibits specifically aceticlastic methanogenesis, while hydrogenotrophic methanogenesis is more resistent to ammonia and can still proceed. Acetate can still be utilized for methane production, through acetate oxidation, when hydrogenotrophic methanogens act synergistically with syntrophic acetate-oxidizing (SAO) bacteria. In this set of reactions, the methyl and carboxyl groups of acetate are first oxidized to CO_2 with concomitant production of H_2. This process is endergonic and therefore unfavorable but can proceed when hydrogen is subtracted from the system by hydrogenotrophic methanogens, with a total energetic balance of −30 kJ (Stams et al., 2006). As the energy gain from this reaction is relatively low, these syntrophs are expected to grow slowly, with the overall microbial community requiring a longer time to adapt to the ammonia-rich environment.

In order to obtain more insights on the effect of ammonia (derived from protein degradation) on the microbial community, a KEGG analysis was performed using the nine genes belonging to the Wood–Ljungdahl pathway, among which the most important is carbon monoxide dehydrogenase/acetyl-CoA synthase (Fig. 3b). The results clearly show that *Desulfotomaculum*, the genus presenting the highest increase (~7-fold) at period 2, contains the complete pathway, suggesting that the change of substrate is indeed having a direct, although slow effect on the microbial composition.

Probably a better understanding of the dynamic changes taking place in reactors undergoing the same variation in substrate could be achieved via a metatranscriptomic and/or metaproteomic study.

An interesting result is an unclassified predominant archaeon that increases 3 fold (from 2.7% to 8.6% of relative abundance) only in this reactor. Other archaea (but with a relative abundance just below the threshold) found to increase more than 2-folds are *Methanosaeta*, *Nitrososphaera* and *Methanocella*, belonging respectively to Methanosarcinales, Nitrososphaerales and Methanocellales.

3.4. Microbial dynamicity in biogas reactors overloaded with lipids

A strong divergence in the profile of the microbial community of R2 was observed between period 1 and 2. This dynamicity corresponds to a reduction in the community variability which is probably due to the specialization of bacteria to the lipid substrate.

The methane yield during the period in which the reactor was fed only with CM reached on average 154 ml CH_4/g VS (Fig. 5). Approximately 4 days before the radical change of the influent feedstock a minor upset in the methane yield was observed, which was probably due to a fouling of the pump that provided inconsistent volume of feedstock to the reactor. After the addition of Na-Oleate, the methane yield started to increase till day 30, when a dramatic decrease was observed together with an accumulation of VFA and a pH decrease to approximately 7.6. Similarly to the protein treatment, the main compound that contributed in the increase of VFA was acetate, while the increase of propionate resulted to be negligible. The methane yield during the period in which the reactor was fed with CM supplemented with lipids decreased to an average value of 81 ml CH_4/g VS. The loss in the methane production could be due to accumulation of LCFA which is a known bacterial inhibitor (Kougias et al., 2014b; Palatsi et al., 2010).

The predominant genera in period 2 are *Megamonas, Flectobacillus* and *Clostridium*, the first increasing in relative abundance from 14.5% to 31.7% (~2-fold), the other two decreasing (Table A2). The only identified species belonging to the genus *Megamonas* is *M. hypermegale*, increasing from 0.1% to 0.2% (~2-fold). This species, previously classified as *Bacteroidetes* (Morotomi et al., 2007), can ferment glycerol and inositol and possesses key enzymes of the pentose phosphate pathway.

A general trend after lipid addition was the decrease of the most abundant genera, partially compensated by the massive increase of *Dialister* (increasing from 0.01% to 1.4%, more than 100-fold) and *Kyrpidia* (not detectable in period 1 and 1.27% in period 2). This increase is even more striking when considering that both genera decrease in R1 and R3. Among the species identified only *D. propionicifaciens* and *D. succinatiphilus* have been sequenced and characterized. They have been both observed to produce succinic acid (Morotomi et al., 2008), which suggests a specific ability for these microbes to metabolize lipids. *Kyrpidia* is a novel genus, almost uncharacterized. One of the few representative species, *K. tusciae*, has been reported to grow heterotrophically only on short chain

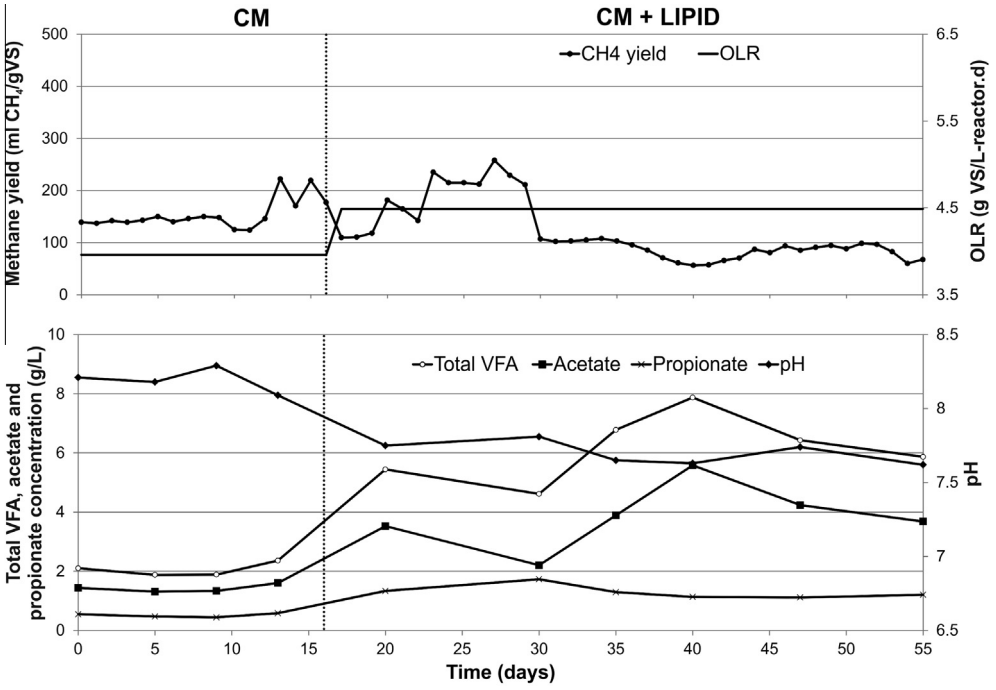

Fig. 5. Process performance of reactor (R2) fed with CM and Na-Oleate (lipid).

fatty acids, amino acids and alcohols, while sugars are not metabolized (Klenk et al., 2011).

A genus that is well known to be involved in lipid metabolism and it is reported to increase after lipid addition in AD is *Syntrophomonas* (Sousa et al., 2007a,b). Our results are partially in agreement as it was found to slightly increase (from 0.15% to 0.20% of relative abundance); at species level *S. sapovorans* was identified.

In the archaeal community a strong decrease in relative abundance was observed for the majority of the identified genera. This finding was in accordance with the lower methane production registered and could justify the decrease in the methane yield by 47.2% compared with the methane yield during the period that the reactor was fed only with CM. In particular *Methanocorpusculum*, belonging to the order Methanomicrobiales, accounts for 0.9% of the community in period 1, while it was nearly undetectable in period 2. The only genus that slightly increased (1.6-fold) was *Methanoculleus*, while the relative abundance of *Methanocella* and the predominant unclassified archaea remained essentially constant.

3.5. Microbial dynamicity in biogas reactors overloaded with carbohydrates

The profile of the microbial community in R3, where carbohydrates were added to the feed in period 2, presented the highest dynamicity. A profound transformation in the community was observed, with a dramatic increase in the variability of the microbial population.

The reported variation in the microbial community was reflected in the reactor performance after substrate change (Fig. 6). The methane yield during the period that the reactor was fed only with CM reached on average 158 ml CH_4/g VS. After the feedstock change, the methane yield was slightly inhibited for a short period (days 17–22) and then slightly increased reaching 180 ml CH_4/g VS, corresponding to an increase of approximately 13.7%. However, according to the stoichiometric conversion of glucose to methane and carbon dioxide, if all the glucose added to the CM was fully converted to methane, the corresponding methane yield would be 271 ml CH_4/g VS, i.e. approximately 34% higher compared to the observed. A rational behind the reactor's process performance can be found in the presence of specific genera. After the addition of glucose in the reactor, the VFA profile changed with the main contributor to be propionic acid (Fig. 6). It is well known that propionate accumulates in cases of process imbalance, and is more persistent compared to other accumulated intermediates (Angelidaki et al., 2006). It was previously reported that propionate/acetate ratio over 1.25 may lead to biomethanation process failure (Wang et al., 2012). In the present study, it was found that after day 35 till the end of the experimental period the propionate/acetate ratio was on average 1.25. A possible explanation regarding the accumulation of propionic acid could be that a considerable portion of the glucose was metabolized to lactic acid, and subsequently the lactic acid was further transformed into propionate. It was previously found that the conversion of lactic acid to propionic acid can be performed by some *Clostridium* and *Megasphaera* species (Prabhu et al., 2012; Tracy et al., 2012). Indeed, in our study, it was found that after the addition of glucose lactobacilli became the most dominant genera of the community (Table A2), the concentration of propionic acid was significantly increased, and finally *Megasphaera elsdenii*, which is one of the species responsible for the conversion of lactic acid to propionic acid, increased its relative abundance (~9-fold).

Regarding the whole bacterial community, the highest increase in relative abundance was observed for *Tissierella* (~4-fold), *Aerococcus*, (~26-fold), *Desulfovibrio* (~5-fold), *Tepidimicrobium* (~15-fold) and mainly for *Lactobacillus* (more than 60-fold). By comparing the predominant genera in all the tested substrates, *Lactobacillus* is the one that presents the highest increase in relative abundance. A more detailed analysis at species level revealed that a large variety of lactobacilli increased: *L. amylophilus, L. camelliae, L. concavus, L. coryniformis, L. dextrinicus, L. diolivorans, L. manihotivorans and L. sharpeae*. In parallel several genera decrease as a result of glucose addition: *Bacteroides, Alistipes, Marinilabilia* and *Prevotella*. These genera are among the most studied in

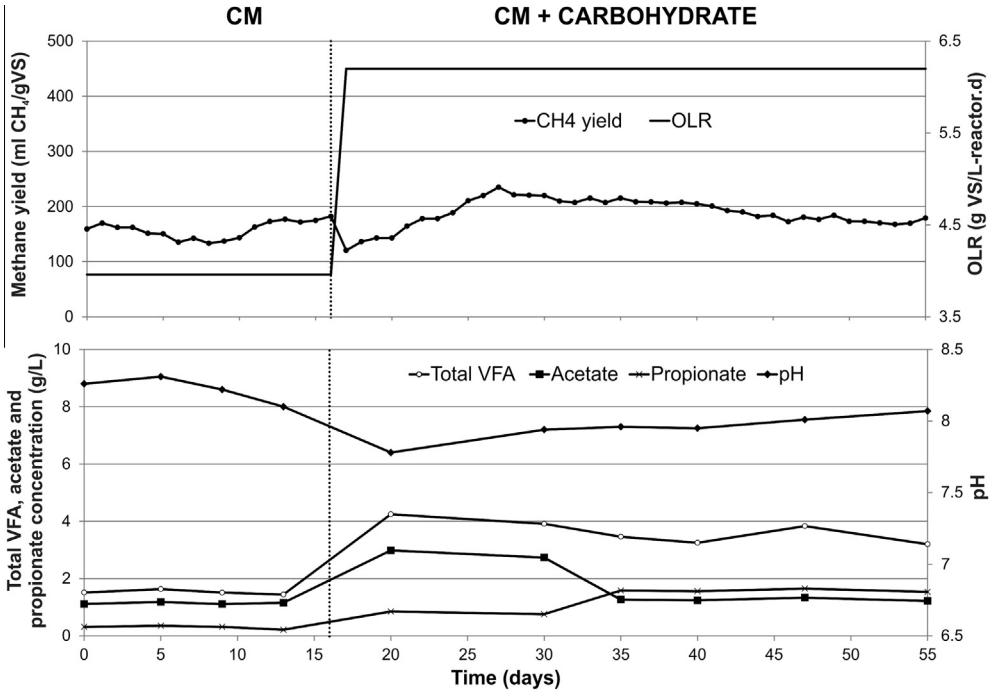

Fig. 6. Process performance of reactor (R3) fed with CM and glucose (carbohydrate).

anaerobic digestion systems, and they all belong to Bacteroidales. It is reported in literature that lactobacilli are specialized in monosaccharide utilization and are therefore capable of outcompeting other genera for growing on this substrate, while members belonging to Bacteroidales are specialized in polysaccharides degradation compared to the direct utilization of monosaccharides (Comstock, 2009). The dataset relative to R3 is in perfect accordance with these findings. This is also supported by a KEGG analysis focused on ten genes involved in polysaccharide degradation, like glycoside hydrolase and polysaccharide lyase. The abovementioned genera all contain a higher number of those genes (especially *Bacteroides*) compared to the others, confirming the presence of similar metabolic characteristics in different species of the same genus (Fig. 3c). These distinctive features determine the success of some species outcompeting those less adapted to the substrate modification. This result also evidences the presence of a dynamic microbial community composed by competing species that are likely to exhibit a complementary distribution with a small number of genera becoming dominant under a specific environmental setting (Freilich et al., 2010).

4. Conclusions

This work determined the effects of radical changes of feedstock composition on the microbial community of biogas reactors using high throughput 16S rRNA gene sequencing. The microbial community in all reactors changed dramatically into a new consortium depending on the substrate overload. The dynamicity in the bacterial population affected the degradation process and this was characteristically depicted in the concentration and profile of VFA. The microbial dynamicity was also reflected as a common trend in all the three substrates, resulting in a decrease of total methanogens abundance. The latter was also verified by the corresponding decrease of methane yield.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.10.126.

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Microbial analysis in biogas reactors suffering by foaming incidents

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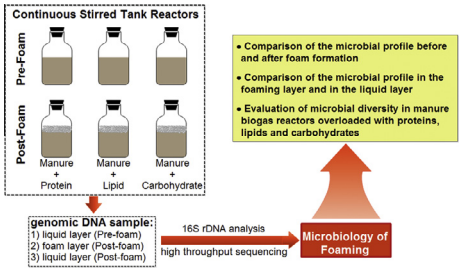
HIGHLIGHTS

- 16S rDNA analyses were performed on biogas reactors prior and after foam incidents.
- Genera known to be involved in foaming increased in abundance after foam formation.
- A microbe similar to known foaming bacteria increased in all reactors after foaming.
- Some species vary their abundance in foaming layer compared to the liquid one.

GRAPHICAL ABSTRACT



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ABSTRACT

Foam formation can lead to total failure of digestion process in biogas plants. In the present study, possible correlation between foaming and the presence of specific microorganisms in biogas reactors was elucidated. The microbial ecology of continuous fed digesters overloaded with proteins, lipids and carbohydrates before and after foaming incidents was characterized using 16S rRNA gene sequencing. Moreover, the microbial diversity between the liquid and foaming layer was assessed. A number of genera that are known to produce biosurfactants, contain mycolic acid in their cell wall, or decrease the surface tension of the media, increased their relative abundance after foam formation. Finally, a microorganism similar to widely known foaming bacteria (*Nocardia* and *Desulfotomaculum*) was found to increase its relative abundance in all reactors once foam was observed, regardless of the used substrate. These findings suggest that foaming and specific microorganisms might have direct association which requires to be further investigated.

1. Introduction

Foaming has been characterized as one of the most significant drawbacks in biogas plants as it affects severely the overall anaerobic digestion process. Foam is a gas–liquid dispersion having a gas content of more than 95% (Varley et al., 2004) and is accumulated on the surface of the liquid phase as a stable viscous layer. It has been recorded that the foam is typically created in the pre-storage feeding tank and/or in the main reactor. The negative impacts of foaming in biogas plants include severe operational problems, such as blockage of the tubing systems and pump failure, caused by the entrapped solids in the foam. Ganidi et al. (2009) reported that in activated sludge systems, foaming can create an inverse solids profile with higher solids concentrations at the top of a digester, leading to the formation of dead zones and thus reducing the digester active volume. A recent survey reported that 15 out of 16 full-scale biogas plants in Denmark have faced foaming problems in the digester and/or in the pre-storage feeding tank, resulting in 30–50% biogas production loss (Kougias et al., 2014a). Another drawback of foaming is the negative economic consequences for the biogas plant owners, due to income losses

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as a result of the reduced biogas production, costs for extra labor work and additional maintenance costs (Barber, 2005). Finally, adverse environmental impacts arise from foaming, caused by the overflowing of the pre-storage or digester tanks.

In the literature, a number of potential causes of foaming have been recorded. Boe et al. (2012) reported that the feedstock composition and especially high lipid and/or protein content are strongly correlated with foam formation. In recent studies, organic overloading of a digester was identified as the main cause of foaming in manure-based biogas reactors (Kougias et al., 2013, 2014b). Other operational parameters, such as temperature, digester shape and type of the mixing devices have been suggested as parameters that can induce foam formation (Barber, 2005).

Furthermore, a number of reports in the literature associate foaming with the presence of specific microorganisms. Kragelund et al. (2010) reported that high abundance of filamentous bacteria, and most frequently members of the *Actinobacteria*, branched filamentous *Mycolata* (formerly denoted Nocardioforms) and *Microthrix parvicella* are correlated with foaming. Moreover, Ganidi et al. (2011) who investigated a foaming event in a full scale digester reported the presence of several filamentous microorganisms and identified five species in sludge samples, including *Nostocoida limicola I* and *III*, *Microthrix*, 0041, 0581. Finally, Guo and Zhang (2012), who performed a metagenomic analysis focused on bulking and foaming bacteria (BFB) in activated sludge, found that the most abundant and frequent BFB are *N. limicola I* and *II*, *Mycobacterium fortuitum*, Type 1863, and *M. parvicella*.

Foaming is a complex phenomenon and yet it is not clear whether the presence or relative abundance of filamentous bacteria can surely result in foam initiation. Petrovski et al. (2011) examined 65 *Mycolata* species isolated from foams in order to explain their role in activated sludge foam formation. However, not all of them managed to form stable foams, and with some isolates, the bases for their foaming behavior was characterized as uncertain. Heard et al. (2008) investigated three strains of the filamentous bacteria *Gordonia amarae*, isolated from wastewater treatment plants, in order to determine their effect on foam formation and stabilization. Their study concluded that the presence of *G. amarae* was not directly linked with foam formation but increased the foam persistence and stability in the samples.

Foaming has mainly been studied in connection to wastewater treatment plants and activated sludge systems, where aerobic or anoxic conditions are mainly used. Even in case of anaerobic reactors the feeding substrate consists of a biological floc composed of microorganisms. However, in biogas reactors treating agricultural residues and industrial wastewaters, anaerobic conditions are present, and consequently the microorganisms involved in these processes are different than in wastewater plants and activated sludge systems. To the best of our knowledge a possible correlation between foaming and the presence of specific microorganisms in biogas plants has not yet been reported. Moreover, the aforementioned studies focus on microbial analyses performed only after foaming occurrence; thus comparisons of the microbial ecology prior and after foaming were never performed. In this study we aimed to elucidate a potential association of specific microorganisms with foaming incidents in manure-based biogas reactors overloaded with different feedstock composition (i.e. proteins, lipids and carbohydrates). The identification of the microorganisms populating the reactors was performed before and after foam formation in order to obtain a better and deeper insight of the microbial changes. Finally, another aim was to compare the microbial communities of the liquid layer versus the foaming layer of the reactors, in order to determine potential differences on the microbial diversity. This was done to investigate whether the specific microorganisms involved in foaming are accumulated on the surface of the reactor, contributing to foam formation and therefore their abundance would be higher in the foaming layer.

2. Methods

2.1. Waste characteristics and preparation of the feedstock

The raw cattle manure used in the experiment was obtained from Hashøj biogas plant, Denmark. After arrival, the manure was shredded and sieved (5 mm) to separate large particles and stored at −20 °C. The frozen manure was thawed at 4 °C for 2–3 days before use. The characteristics of manure are presented in Table 1.

2.2. Experimental setup and operation

The experiment was carried out in three CSTR reactors having a total and a working volume of 2 and 1.5 L, respectively. The reactors were continuously stirred using magnetic stirrers. Moreover, the reactors were equipped with thermal jackets in order to maintain the operating temperature steady at 54 ± 1 °C. Each reactor was fed with a different substrate which was found to have an influence on foam formation in our previous study (Kougias et al., 2013). More specifically, it was found that foam is generated by increasing the organic loading rate of manure-based biogas reactors above 4.2 gVS/(L-reactor·day) by adding proteins, lipids or carbohydrates. Thus, in the present study the influent manure was supplemented with gelatine as a representative of proteins (R1), Na-Oleate as a representative of lipids (R2) and glucose as a representative of carbohydrates (R3) in order to ensure that foam would be formed in the reactors. The hydraulic retention time (HRT) of all reactors was kept constant and was equal to 15 days. The whole experiment was divided into two periods. During the first period, the reactors were fed only with cattle manure. Once steady state conditions were reached, liquid sample from all reactors was obtained for DNA extraction and metagenomic analysis. After sampling, the feedstock composition of each reactor was changed by the addition of gelatine or Na-Oleate or glucose (second experimental period). As a consequence, foam formation was observed in all reactors approximately after one HRT period. Once the daily volume of the formed foam was steady, samples directly from the liquid phase of the reactors and also from the formed foam were taken for DNA extraction.

2.3. Analytical methods

Total solids (TS), volatile solids (VS), pH, total nitrogen (TKN) and total ammonia were measured according to APHA standard methods for the examination of water and wastewater (APHA, 2005). Volatile fatty acids (VFAs) were measured using a gas chromatograph (Shimadzu GC-2010AF, Kyoto, Japan), (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) and a FFAP fused-silica capillary column, 30 m × 0.53 mm I.D., film thickness 1.0 μm, using nitrogen as a carrier gas (Kougias et al., 2013). All the determinations were performed in triplicate.

Table 1
Cattle manure characteristics.

Parameter	Unit	Values
pH	–	8.09 ± 0.01
Total solids (TS)	g/L	74.5 ± 0.1
Volatile solids (VS)	g/L	59.4 ± 0.4
Total Kjeldahl nitrogen (TKN)	g-N/L	3.30 ± 0.17
Ammonium nitrogen (NH_4^+)	g-N/L	2.11 ± 0.14
Total volatile fatty acids (VFA)	g/L	11.11 ± 0.98

2.4. Sampling and DNA extraction

Samples (100 mL) from each reactor were collected and 2 mL were transferred to 2.0 mL microcentrifuge tubes (Eppendorf). The samples were subsequently centrifuged at 13,400 rpm for 10 min. The resulting supernatant was discarded and from each pellet 0.25 g of solid fraction was used for further analyses. Total microbial DNA from manure reactor samples was isolated and purified using the PowerSoil® DNA Isolation Kit (MO BIO laboratories, Inc.) according to the protocol provided by the manufacturer. The hypervariable V3 region of bacterial and archaeal 16S rRNA genes was amplified using the same procedure reported in Luo et al. (2013) (Appendix A) in order to obtain amplicons of ~100 bp that are better suited to the construction of the libraries for the Ion Proton™ System. A low number of PCR cycles should allow maintaining proportionality between the species abundance present in the starting sample. Moreover, it is well known that measurements performed in the PCR plateau are not necessarily directly related to the starting material (Freeman et al., 1999). Therefore, in the present study, the amplification was performed with a low number of cycles (i.e. 26 cycles) in order to maintain a better proportion between the initial DNA copies and the final obtained sequences.

The DNA purity was determined using NanoDrop (Thermo Scientific), the quantification has been performed with the Qubit fluorimeter (Life Technologies) and the absence of primers and primer dimers was evaluated using 2100 Bioanalyzer (Agilent DNA 1000 Kit). Identical amount of each amplicon was pooled and sent to DTU Multi-Assay Core (DMAC) for library preparation (according to the standard protocol Ion Xpress Plus gDNA and Amplicon Library Preparation, Life Technologies) and Ion Torrent PGM sequencing (316 chip together with the Ion Sequencing 200 kit, Life Technologies).

2.5. Analysis of the 16S rRNA gene sequences

The sequences obtained from the Ion Proton™ for each sample were ranging from 136876 (bacteria, sample obtained from the foam phase of the reactor overloaded with carbohydrates) to 375994 (bacteria, sample obtained from the liquid phase of the reactor overloaded with proteins). Sequences in FASTQ format were converted to FASTA format using the FASTX-Toolkit. Raw sequence data were submitted to the NCBI sequence read archive database (SRA) with accession numbers SRP033444 (pre-foaming), SRP033447 (liquid layer post-foaming, bioreactor overloaded with proteins), SRP033448 (liquid layer post-foaming, bioreactor overloaded with lipids), SRP033449 (liquid layer post-foaming bioreactor overloaded with carbohydrates), SRP033453 (foam layer, bioreactor overloaded with proteins), SRP033454 (foam layer, bioreactor overloaded with lipids), and SRP033455 (foam layer, bioreactor overloaded with carbohydrates). Only high quality sequences have been used in the analyses and trimming strategy is described in detail in Appendix A. The abundance of each genus, the Principal Component Analysis (PCA) and the rarefaction curves were calculated with the MG-RAST toolkit using the "best hit classification" method (Fig. A.1), considering the annotation sources "RDP", "SSU" and "Greengenes", with maximum *e*-value cutoff of $1e^{-5}$, a minimum % identity cutoff of 97% and a minimum alignment length cutoff of 50 bp.

Rarefaction plots are included in Appendix section showing the curve of annotated species richness for each sample. Each curve is a plot of the total number of distinct species annotations as a function of the number of sequences analyzed and provides an indication of diversity, separately for bacteria and archaea. Moreover, PCA is a representation of multiple samples and their taxonomic annotations and shows the differences between the microbial communities of different samples before and after foaming occurrence (Fig. 1). Detection of chimeras was performed but their presence was found negligible (Appendix A).

Statistical analysis was carried out using STAMP software in order to identify significantly abundance differences for each genus among the samples (Parks and Beiko, 2010). The fact that the comparison was performed between two conditions (i.e. before and after foam formation) without biological replicates was overcome by applying statistical hypothesis Fisher's exact test (Rivals et al., 2007). Fisher's exact test with $n \times 2$ contingency tables (where n was the total number of genera in the samples) was used to determine the statistical differences among the tested samples since in some cases the abundance was less than five. Additionally, multiple comparisons were assessed using "Bonferroni correction" method setting the p-value to 0.01.

In order to perform a direct comparison between the samples, the relative abundance of each genus was used. For this reason, the number of sequences of each genus was normalized taking into consideration the total number of sequences of each sample after the filtering process.

The file with the number of sequences in tabular format, normalized considering the sample with the lower number of sequences, was used to build a heatmap representative of the abundance of each genus in the four samples analyzed using the MeV 4.8.1 software (Saeed et al., 2003). The clustering was performed using Pearson's correlation to highlight genera having similar behavior. The color scale in MeV software has been settled in order to make clearly visible both changes in low abundance and super-abundant genus (lower limit 0 sequences, midpoint 200 sequences, and upper limit 3000 sequences).

Sequences classified by MG-RAST as belonging to genus *Salinispora* were extracted and analyzed separately. Their number was variable ranging from 354 in sample SRP033444 to 2199 in sample SRP033447. Sequences of SRP033447 sample were clustered at three different levels of similarity with CD-HIT software. The representative sequence of the cluster was used as reference for clustering all other samples, with the same procedure. A blast search was performed on "Greengenes" and RDP databases using the representative sequence obtained after clustering.

3. Results and discussion

Up to now it has been disputed whether foaming in anaerobic digestion systems is correlated with the presence or relative abundance of specific microorganisms and also whether the microorganisms act as foam initiators or foam stabilizers. This is mainly due to the complexity of the foam structure and to the high diverse microbial population in environmental applications in which foaming is presented. Furthermore, specifically for biogas plants that are utilizing agro-industrial wastes as feedstock, the potential correlation of specific microorganisms and foaming is not yet defined. In order to address this issue, in the present study the microbial ecology of the reactors was screened before and after foaming incidents focusing on the genera whose relative abundance were increasing significantly after foaming (Fig. A.2–4 provide the whole set of results for the genera that significantly changed their abundance). To obtain a comprehensive characterization of the complex microbial communities of the reactors, a deep sequencing of the 16S rRNA genes of bacteria and archaea producing a number of reads much higher compared to previous studies was performed. This allowed the identification of even the less abundant components of the microbial community. Furthermore, in previous studies a range of different threshold and different methods were used to identify species involved in foaming; sometimes the use of very low thresholds can lead to

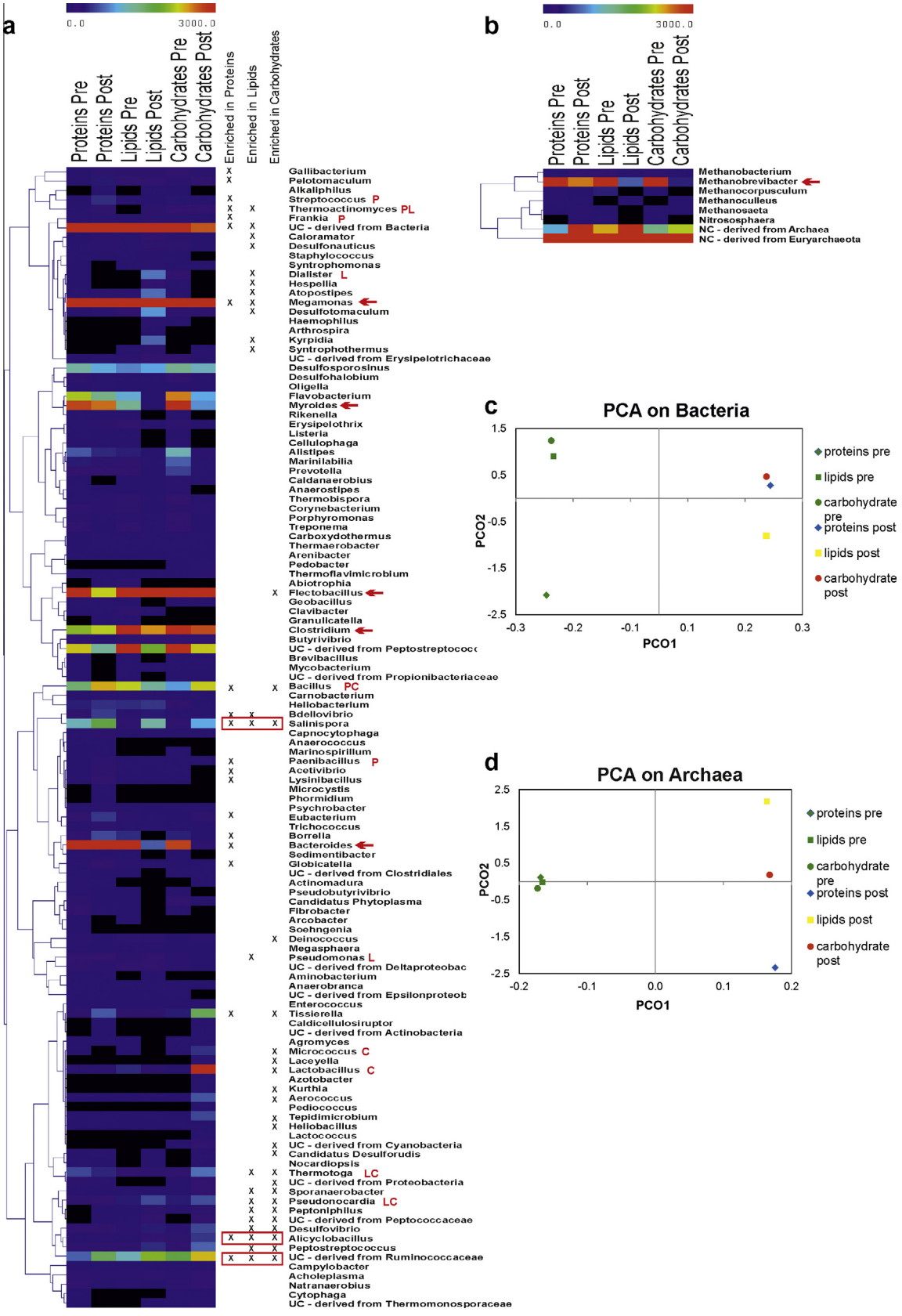

Fig. 1. Hierarchical clustering of the results obtained from the analysis of the foaming reactors before and after foaming incidents for bacteria (a) and for archaea (b). Number of normalized sequences identified from each sample was represented as a heat map. Correspondence between colors and number of sequences is reported in the scale at the top-left of the figure. Genera not identified in a specific reactor are reported in black, and those with increasingly higher abundance are reported in blue, green, yellow, orange and red. In the right part of the figure the genera significantly increased after the foaming incident in the reactors fed with proteins (P), lipids (L) and carbohydrates (C) are marked with a "x" symbol. The red arrows illustrate the most abundant genera. Principal Component Analysis depicts the bacteria (c) and archaea (d) communities composition, separately. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

incorrect assignment of sequences at the species and genus level. For this reason, the accuracy of the results was assured by setting a very stringent threshold for the identification of the microorganisms. Finally, as this is the first research trying to correlate foaming incidents in such digestion systems, a direct comparison of our results with already cited literature was not possible. So, in order to associate these microorganisms with foam formation, the current analysis was also based on knowledge from other studies related to different scientific fields (i.e. food science, water research, veterinary medicine etc.).

3.1. Global overview of the microbial ecology of all the manure-based reactors

A global representation of the abundance determined for the genera identified using 16S rRNA gene sequencing is reported in Fig. 1, where the number of sequences is represented as a heat map. Only genera whose abundance were higher than 0.025% (with respect to the total number of sequences) and were present in at least one reactor were considered in subsequent analyses and are reported in figure. This reduced the possible effect of sequencing artifacts without excluding 'rare' taxa, defined as those accounting for less than 1% of total frequency. As expected there was a very low number of genera highly represented in the microbial community (depicted in green, yellow, orange and red and indicated by a red arrow) like *Megamonas*, *Myroides*, *Bacteroides*, *Flectobacillus*, *Clostridium*. At the contrary the "rare" and "low abundance" biosphere (depicted in blue and azure) is represented by a high number of different genera. Bacteria and archaea were tested separately. In regards to the archaeal community, *Methanobrevibacter* was the most abundant genus identified. However, despite the high abundance and the increased presence of some archaeal genera after foaming incidents, it was not possible to correlate any of the identified genera with this phenomenon, and therefore archaea are not further discussed (Fig. A.2–4). Finally, Fig. 1 highlights the high number of genera whose relative abundance changed after foaming in at least one of the reactors examined.

Another set of analyses were performed in the liquid layer versus the foaming layer of the reactors suffering by foaming incidents. It has been previously described that in activated sludge systems the filamentous microorganisms are attached to biogas bubbles and transferred to the air/liquid interface of the reactor. As a consequence their accumulation on the surface along with their biosurfactants production, lead to an increase of the surface activity (i.e. decrease the surface tension of the substrate) and therefore contribute to foam formation (Ganidi et al., 2009; Heard et al., 2008). Based on these concepts we hypothesized that specific microorganisms involved in foam formation and stabilization could be concentrated in the "foaming layer". The results of these analyses revealed that there are some species that vary their relative abundance in this layer compared to the liquid one (Fig. A.5–7). A major finding was related to genus *Salinispora* which was the only common high abundant microorganism identified at a genus level in all reactors (Fig. 3–5). The number of sequences assigned to this genus using MG-RAST software was found to significantly increase after foaming occurrence by approximately 62%, 276% and 188% in the reactors overloaded with proteins, lipids and carbohydrates, respectively. This observation, along with the fact that the taxonomic classification software can exhibit biases toward certain taxonomic groups and could have a lower phylogenetic resolution using some 16S rRNA gene variable regions (Qunfeng and Claudia, 2012) led to the performance of an additional analysis. All sequences classified as *Salinispora* in our samples were clustered at 97% and the representative sequence obtained was manually classified using blast. It was found that the two best-hits with approximately 92% and 91% similarity were with some well-known foam related genera, which are *Nocardia* and *Desulfotomaculum*, respectively. After excluding the possibility that this was a chimeric sequence, and since the similarity was nearly identical with these genera belonging to different phyla, it could be drawn that this microorganism (Operational Taxonomic Unit, OTU) cannot be assigned to any existing taxonomic group. Lower similarity (87%) with *Salinispora arenicola* and *Salinispora tropica* suggested a possible reason for the incorrect taxonomic assignment obtained using MG-RAST. Moreover, by using the same clustering method it was confirmed that this microorganism significantly increases its abundance after foaming in all reactors and particularly in the foam layer (Fig. 2).

3.2. Microbial ecology in foaming reactors overloaded with protein-rich substrate

In the reactors overloaded by proteins a number of genera increased their abundance after foam formation (Fig. 3). According to the literature the genera that have a correlation with foaming were *Bacillus*, *Streptococcus*, *Thermoactinomyces*, *Frankia* and *Paenibacillus*. The population of *Bacillus* was increased by 69% after foaming incident. It has been documented that severe foaming was caused during the production of biopesticides using *Bacillus thuringiensis* with sewage sludge as a feedstock (Vidyarthi et al., 2000). Moreover, *Bacillus subtilis* produce a cyclic lipopeptide surfactin that possess both excellent foaming properties and high ability in foam stabilization (Razafindralambo et al., 1998). A significant increase was found for *Streptococcus*, whose population after foaming was found to be 1134% higher than before foaming incident. Furukawa et al. (2010) reported that the population of *Streptococcus* sp. was markedly higher in the stable yellowish foam that was formed in the coastal waters and sand as a result of fecal pollution. This study concluded that these species were mainly concentrated on the water surface into the foam bubbles and the dissolved organic compounds presented surface activity and decrease the surface tension. It has been previously proven that also in anaerobic digestion foaming is associated with the decrease of the surface tension of the substrate (Boe et al., 2012). This could explain the role of *Streptococcus* on foam formation in our specific case.

Concerning *Thermoactinomyces*, the relative abundance of this genus increased by 347%. It is acknowledged that this thermophilic endospore-forming genus belongs to *Actinomyces*. *Actinomyces* have been greatly associated with foaming as they are extremely hydrophobic due to the presence of mycolic acid on their cell walls (Ganidi et al., 2009). *Frankia* sp. also increased remarkably after the appearance of foaming, by approximately 1114%. An analysis performed on stable viscous foam obtained from activated sludge aeration basin surfaces revealed the probable presence of these microorganisms (93% similarity) (Wagner and Cloete, 2002). Finally, the genus *Paenibacillus*, which has been identified as a cause for non-filamentous sludge bulking (Oerther and Iyer, 2003), showed a significant increase (331%).

3.3. Microbial ecology in foaming reactors overloaded with lipid-rich substrate

Fig. 4 illustrates the genera whose population had significantly changed after foam formation once the reactors were overloaded with high lipid substrate. The genera among these that were found in literature to be correlated to foaming were *Dialister*, *Pseudonocardia*, *Thermoactinomyces*, *Pseudomonas* and *Thermotoga*. *Dialister* was almost absent before foaming (on average 0.003% of the total reads), but its abundance increased remarkably after foaming (on average 0.41% of the total reads). Ohnishi et al. (2010) correlated foaming with this genus during the investigation of biohydrogen

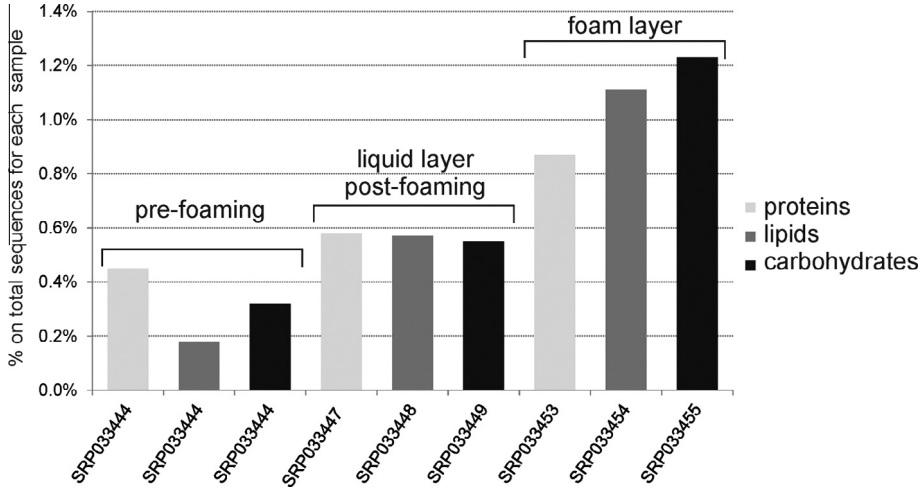

Fig. 2. Abundance of the unassigned OTU similar to foam related genera in the nine samples analyzed. Percentage on vertical axes was calculated considering the total number of sequences for each sample. Light gray, dark gray and black colors correspond to the reactors overloaded with proteins, lipids and carbohydrates, respectively. The horizontal axis labels are the SRA codes of each sample.

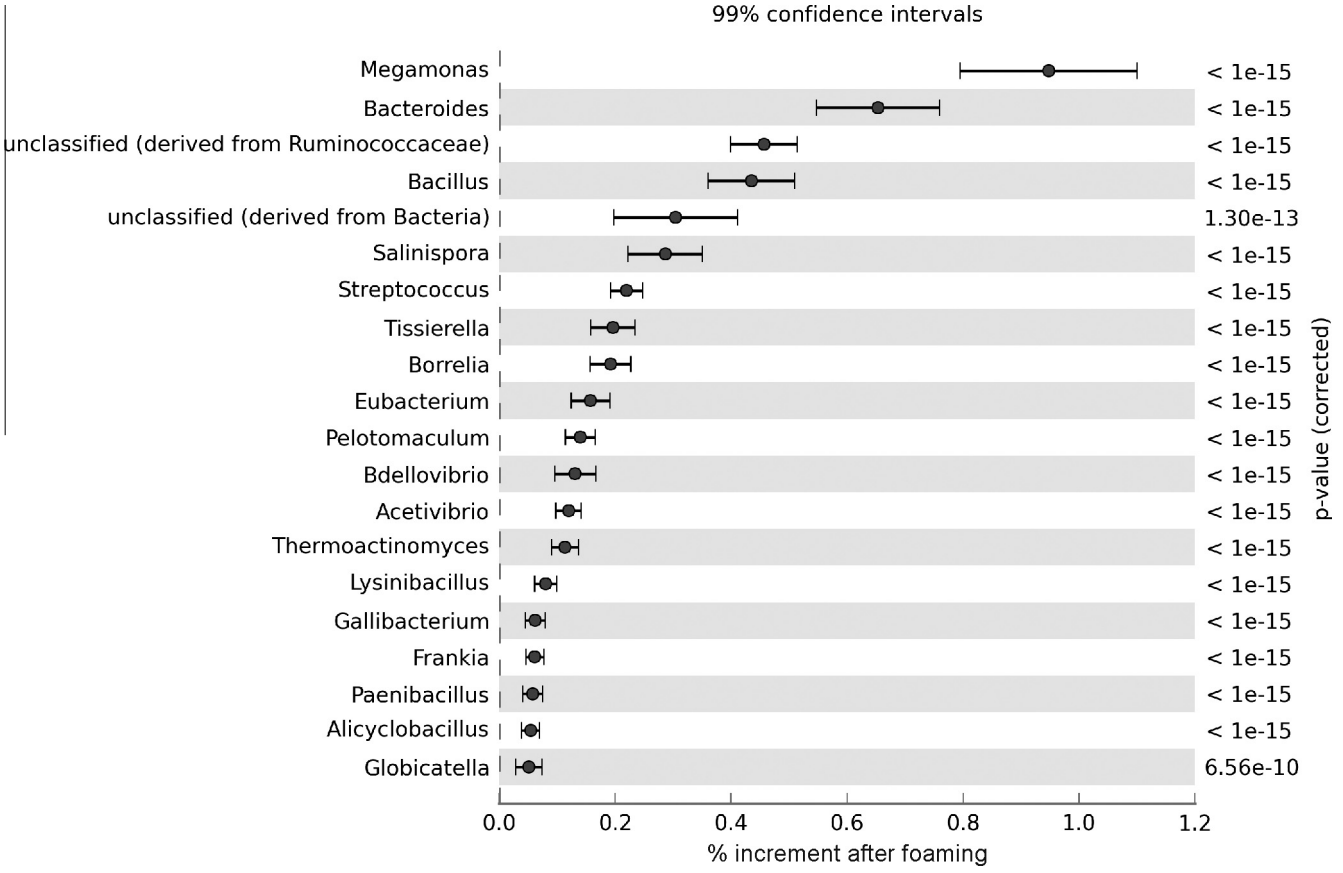

Fig. 3. The difference between the proportions represents the increment in the number of reads (reported as %) after foaming. It was calculated as in terms of comparison among samples obtained by the reactor overloaded with protein-rich substrate before and after foaming formation. The difference between the proportions was calculated as in terms of the total normalized reads while the ratio between the number of reads before and after foaming is reported in the text. Only bacterial genera that increase their abundance after foaming are reported.

production through dark fermentation of garbage slurry using different inocula. They reported significant foaming after 24-h in the case when leaf-litter cattle-waste compost was used as inoculum, while foaming was not remarkable when soil, kitchen waste or garbage compost was the used inoculum. The DGGE analysis revealed the abundance of *Dialister* in the used microflora. *Pseudonocardia* sp. presented an increase of 36%. This genus belongs to the *Mycolata* morphotype that is characterized by the presence of mycolic acid in their cell walls and, as mentioned already for *Thermoactinomyces*, that was probably the cause of foam. *Thermoactinomyces* was found again in the current condition (reactor overloaded with lipid-rich substrate) but in this case its correlation was even more striking, as it was totally absent before foaming appearance. *Pseudomonas* and *Thermotoga* increased their abundance after foam

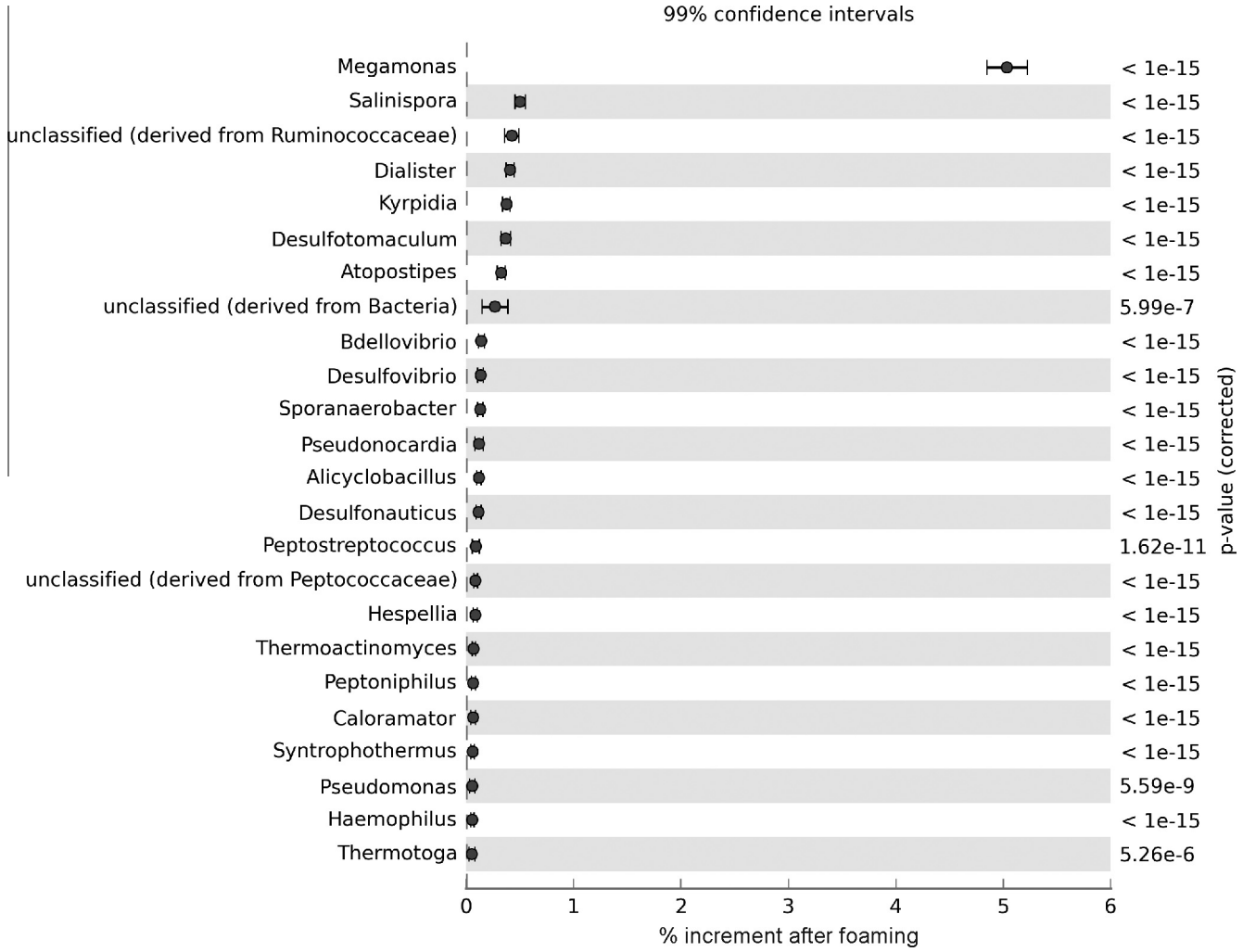

Fig. 4. The difference between the proportions represents the increment in the number of reads (reported as %) after foaming. It was calculated as in terms of comparison among samples obtained by the reactor overloaded with lipid-rich substrate before and after foaming formation. The difference between the proportions was calculated as in terms of the total normalized reads, while the ratio between the number of reads before and after foaming is reported in the text. Only bacterial genera that increase their abundance after foaming are reported.

formation by 51% and 29%, respectively. Both these genera are known to produce biosurfactants (Salleh et al., 2011; Törnvall and Hatti-Kaul, 2007). It has been previously stated that the biosurfactants are amphiphilic compounds, such as hydroxylated and cross-linked fatty acids, glycolipids, proteins, lipoproteins, phospholipids and polysaccharide–lipid complexes, which are produced during the metabolic activity of specific microorganisms (Ganidi et al., 2009). Heard et al. (2008) reported that foaming in activated sludge digesters was linked with the presence of biosurfactants which contribute in foam partitioning and prevent foam destruction. Moreover, in a recent study it was observed that the increased organic loading in manure-based biogas reactors fed with lipids and facing foaming incidents could have stimulated microbial production of biosurfactants (Kougias et al., 2013).

3.4. Microbial ecology in foaming reactors overloaded with carbohydrate-rich substrate

Fig. 5 illustrates the genera whose population had significantly increased after foam formation once the reactors were overloaded with high carbohydrate substrate. The genera that were correlated with foaming were *Lactobacillus*, *Bacillus*, *Thermotoga*, *Micrococcus*, and *Pseudonocardia*. The high level of *Lactobacillus* (this genus increased in abundance on average from 0.052% to 3.4% of the total reads) was clearly consequence due to the radical change of the substrate and the addition of carbohydrates. It is well known that the lactic acid bacteria (LAB) as lactobacilli and pediococci, exhibit enormous capacity to degrade different carbohydrates (Pithva et al., 2012). Concerning foaming, several studies have identified lactobacilli as a foaming promoter. Lactobacilli have been used to produce single cell protein (Kam et al., 2012) and therefore its presence in biogas reactors can lead to the creation of foam due to the high possibility of cell leaking of proteins as a consequence of intense and vigorous agitation. Moreover, the main product of lactic fermentation is lactic acid. Lactic acid from biotechnological processes (based on the fermentation of sugars such as glucose by bacteria) is widely used in the food industry as foaming agent (Moldes et al., 2001). Moreover, it has also been proven that during the fermentation process driven by *Lactobacillus pentosus*, biosurfactants with foaming properties are produced (Vecino et al., 2013). Extracellular water-soluble biosurfactants from *Micrococcus* (which increased in this case by 1838%) have been also identified as a foaming agent and this genus also utilizes sugars as substrate which lowers the surface tension of the culture filtrates to the extent of 57.5 ± 0.3 mN m^{-1} in the case of glucose (Das et al., 1998). Raw manure tends to produce foam at a surface tension of

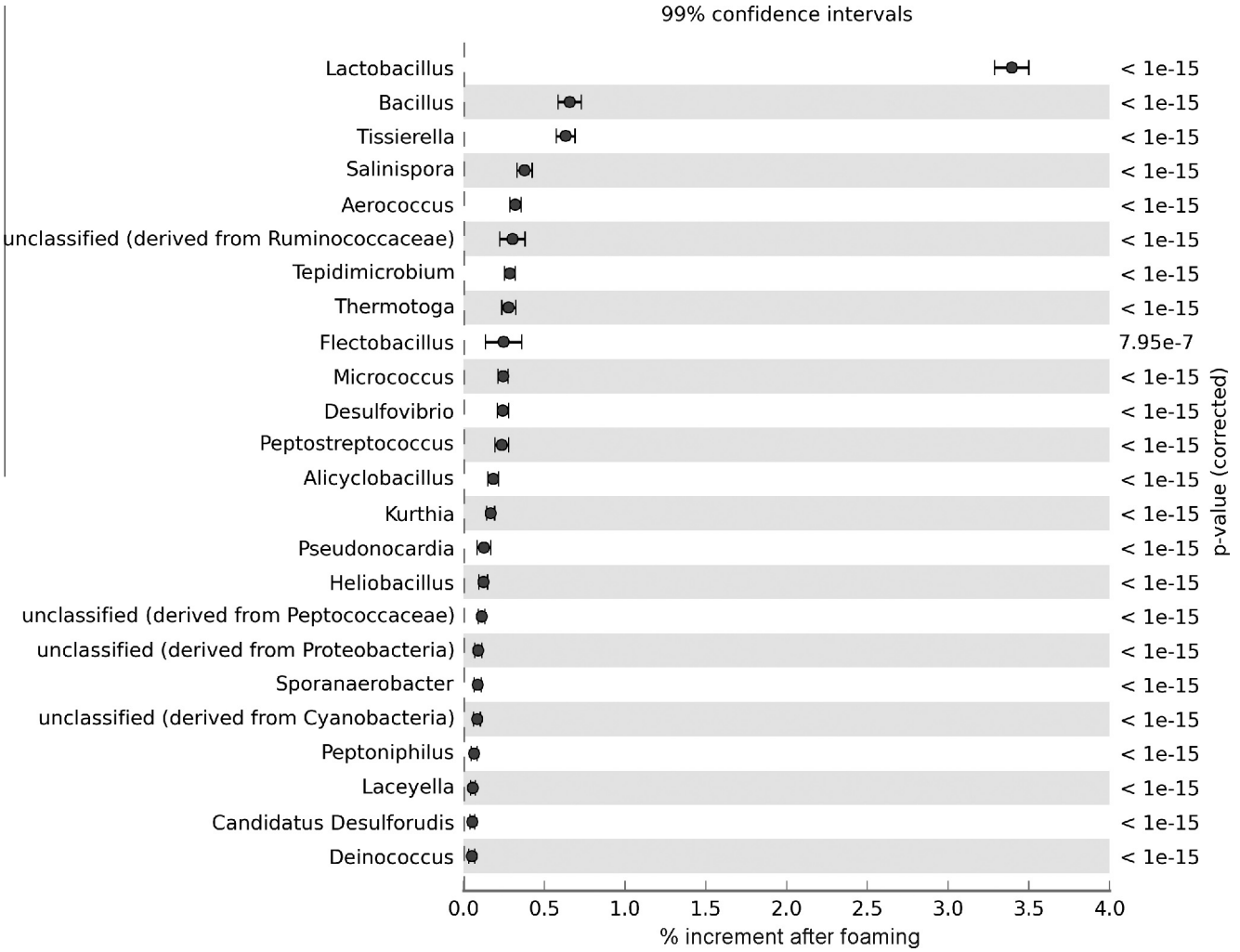

Fig. 5. The difference between the proportions represents the increment in the number of reads (reported as %) after foaming. It was calculated as in terms of comparison among samples obtained by the reactor overloaded with carbohydrate-rich substrate before and after foaming formation. The difference between the proportions was calculated as in terms of the total normalized reads while the ratio between the number of reads before and after foaming is reported in the text. Only bacterial genera that increase their abundance after foaming are reported.

60.11 mN m^{-1} (Boe et al., 2012); therefore the presence of *Micrococcus* could have attributed to a further decrease of surface tension leading to a corresponding increase of foaming tendency.

Bacillus increases its relative abundance after foaming by 78%, *Thermotoga* by 150% and *Pseudonocardia* by 26%. These three genera have already been discussed in this study in relation to their correlation with foaming.

3.5. General considerations and observations from the study

In the present study, the reactors were fed with manure containing high amounts of barley. This component of the manure is also a fundamental ingredient in the production of beer and it has been proved to contribute to the formation and stabilization of the beer foam due to the activity of one of its proteins (Brey et al., 2003). It cannot be excluded that the presence of barley in the studied reactors could play a part in foaming even if it was obviously present in the reactors before foaming incidents. So, in the present case it seemed that barley contributed in the preservation of foam structure and was not the actual cause of foam. More specifically, by visual inspection particles from barley were observed to be transferred on the liquid surface due to the biogas bubbles and accumulated in the foaming layer; thus contributing in foam stabilization.

Another interesting observation is that some of the microorganisms previously identified as cause of foaming had a very low relative abundance even after the foaming incident (e.g. *Paenibacillus*). However, they were included in the discussion because this phenomenon could be the result of the cumulative contribution of different genera rather than the individual action of a specific one (i.e. not only dominant species but also non-dominant ones might significantly contribute to foaming generation).

It is important to report that no archaea was found to be associated with foaming incidents, even if some archaea were found to increase their abundance in correspondence to foam formation.

Finally, as stated previously, this is first study reporting the changes in the microbial population of biogas reactors that were fed with agro-industrial wastes, before and after foaming incidents. Therefore, these initial results are essential for deciphering the foaming phenomenon in biogas plants and particularly important as a stimulus for further research and investigation of the role and contribution of specific microorganisms during foaming. The ability to define an abundance profile of different microbes involved in foaming could lead in the future to predict and prevent foam formation, once community shifts in a specific direction are

detected in time. It should be highlighted that the stringent threshold which was set for the identification of the microbial ecology assured the maximum possible accuracy of the analysis, even if in the cited literature the corresponding threshold was remarkably lower. As a consequence, microorganisms that were reported in the cited literature to contribute generally in foaming were not found. Nevertheless, in case this threshold is decreased at levels of 90% or below (as in other studies found in the cited literature that report association of microorganisms and foaming) these relative to foaming microorganisms were present (e.g. *Nocardia* and *Desulfotomaculum*).

4. Conclusions

Significant variations in the microbiology of biogas reactors were recorded before and after foam formation. A number of genera could be linked to foaming as they produce biosurfactants, or contain mycolic acid in their cell wall or decrease the surface tension of the media. Finally, our analysis identified for the first time the presence of a species (operational taxonomic unit) whose abundance was increased in all reactors after foam formation; this microorganism was found distantly similar to bacteria related to foam (*Nocardia* and *Desulfotomaculum*). The results from the present study are pivotal for deciphering foaming problems in biogas plants.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2014.05.080.

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Foam suppression in overloaded manure-based biogas reactors using antifoaming agents

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HIGHLIGHTS

- Rapeseed oil, oleic and octanoic efficiently suppressed foaming in biogas reactors.
- Rapeseed oil was the most suitable antifoam for overloaded reactors.
- Rapeseed oil showed synergistic effect on methane yield when digested with manure.
- Tributylphosphate inhibited severely the anaerobic digestion process.

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ABSTRACT

Foam control is an imperative need in biogas plants, as foaming is a major operational problem. In the present study, the effect of oils (rapeseed oil, oleic acid, and octanoic acid) and tributylphosphate on foam reduction and process performance in batch and continuous manure-based biogas reactors was investigated. The compounds were tested in dosages of 0.05%, 0.1% and 0.5% v/v_{feed}. The results showed that rapeseed oil was most efficient to suppress foam at the dosage of 0.05% and 0.1% v/v_{feed}, while octanoic acid was most efficient to suppress foam at dosage of 0.5% $v/v_{feed.}$ Moreover, the addition of rapeseed oil also increased methane yield. In contrast, tributylphosphate, which was very efficient antifoam, was found to be inhibitory to the biogas process.

1. Introduction

Foaming is a serious problem, occasionally occurring in full-scale biogas plants, negatively affecting both the environment and the economy of biogas plants. Foaming often results in the formation of an inverse solids profile with higher solids concentrations at the top of a reactor, leading to the formation of dead zones and consequently reducing the reactor active volume (Ganidi et al., 2009). The direct consequences of foaming are severe operational problems, such as blockage of mixing devices and collapse of pumps. The adverse environmental impacts from foaming are related to overflowing from the pre-storage or digester tanks and increased emissions of methane through the effluent, due to oversaturation of methane in the liquid phase (Kougias et al., 2013a). The economy of the biogas plants is negatively affected by income losses as a result of the reduced biogas production, costs for extra labour and cleaning work and additional maintenance costs (Barber, 2005; Barjenbruch et al., 2000). It has been previously reported that foaming in full-scale manure digestion systems led to reduced biogas production for shorter or longer periods (Nielsen and Angelidaki, 2008). Similarly, our recent survey results showed that 15 out of 16 full-scale biogas plants in Denmark have faced foaming problems in the digester and/or in the pre-storage feeding tank, resulting in 30–50% biogas production loss (Kougias et al., 2013b).

In the cited literature, there are several studies identifying feedstock composition, organic loading rate and the presence of specific microorganisms as potential causes of foaming (Dalmau et al., 2010; Kougias et al., 2013a). However, organic overload was reported to be the major cause of foaming in most of the Danish full-scale biogas plant (Kougias et al., 2013b). It is well known that the digester overloading can result in partial degradation of organic matter and accumulation of surfactants or biosurfactants (Ganidi et al., 2011). The excessive concentration of these substances can potentially contribute to foaming phenomena. Specifically, in manure-based biogas reactors, a recent study has proven that organic overloading was the main factor contributing to foaming and

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identified that an organic loading rate (OLR) of 3.5 g VS/(L-reactor·day) was the critical threshold for foam initiation (Kougias et al., 2013a).

An efficient method to prevent or suppress foaming incidents is of a great importance in order to avoid the difficulties during the process control and equipment handling (Riera et al., 2006). There are several techniques to prevent or suppress foaming including chemical, mechanical, physical and biological methods (Vardar-Sukan, 1988; Riera et al., 2006). The most common solution for foaming in bioprocesses is the chemical method using antifoaming agents or defoamers. Antifoaming agents are substances that are applied prior to foam formation in order to prevent foaming, while defoamers are compounds applied after foam formation in order to suppress it (Denkov, 2004; Miller, 2008). However, antifoaming agents and defoamers are fundamentally the same chemicals despite their different classification depending on their mode of application (Pelton, 2002). Consequently, in common practice, antifoaming agents and defoamers are terminological identical describing those compounds that are used for antifoaming and generally are referred as antifoams.

Antifoams are strong surface-active substances that can decrease the surface tension of the solvent (Joshi et al., 2009; Xu et al., 2010). A typical antifoam consists of oil or hydrophobic solid particles or a mixture of both (Delvigne et al., 2009). Specifically, it has been previously reported that nonpolar oils (mineral and silicone oils) and polar oils (fatty alcohols and acids, alkylamines, alkylamides, tributylphosphate) have been successfully used to reduce foaming (Denkov, 2004).

So far, the exact mechanism of the antifoams is not well understood (Barber, 2005). Nevertheless, the general function of the antifoams is that they disrupt the stability of the liquid films in the foam, increasing the rate of the liquid drainage and thus enhance foam destruction (Winterburn and Martin, 2012).

Nowadays, there is an abundance of available commercial antifoams (Junker, 2007). However, it is well known that the foam suppression efficiency of a specific antifoam is highly dependent on the applied process and may not be suitable for every application (Winterburn and Martin, 2012). Moreover, the applied dosage and the mode of addition can also affect the foam suppression efficiency. It has been previously reported that each antifoam has a specific concentration that presents its optimum antifoaming effect, below which is less effective, while above the optimum concentration may act as foam stabilizer (Karakashev and Grozdanova, 2012). According to the antifoam product specifications from manufacturers, a typical dosage of commercial antifoaming agents suggested for bioprocesses is 0.1% v/v (Kougias et al., 2013c).

The immediate effect of different antifoams had been investigated in our previous study based on physiochemical tests (Kougias et al., 2013c). In this study, we further investigated the four antifoams that had shown strong antifoaming potential in our previous study, which were rapeseed oil, oleic acid, tributylphosphate and octanoic acid. The aim of this study was to compare the foam reduction efficiencies and their influence on process performance under continuous operation of manure-based biogas reactors. Moreover, batch assays were carried out in order to determine the biodegradability of these antifoams, and to investigate their effect on the biomethanation of cattle manure.

2. Methods

2.1. Waste characteristics and preparation of the feedstock

The raw cattle manure used in the experiment was obtained from Hashøj biogas plant, Denmark. After arrival, the manure was shredded and sieved (5 mm) to separate large particles and stored at −20 °C. The frozen manure was thawed at 4 °C for 2–3 days before use. The manure had a pH of 7.43 ± 0.01, total solids (TS) and volatile solids (VS) content of 61.6 ± 0.7 and 47.5 ± 0.6 g/L, respectively. The total Kjeldahl Nitrogen (TKN) and ammonium Nitrogen NH_4^+ were 3.30 ± 0.17 and 2.11 ± 0.14 g-N/L. The concentration of total Volatile fatty acids (VFA) in raw manure was 5.54 ± 0.1 g/L.

2.2. Tested antifoams

The four antifoams tested in this study included oleic acid (90%, Sigma–Aldrich), octanoic acid ($\geqslant$98%, Sigma–Aldrich), rapeseed oil, and tributylphosphate. The rapeseed oil used was normal edible oil bought from supermarket, which had low content of erucic acid (less than 2%). The edible rapeseed oil contained mainly oleic acid (51–70%), linoleic acid (15–30%), alpha-linoleic acid (5–14%), and palmitic acid (2.5–7%) (Codex Alimentarius, 1999).

2.3. Effect of the antifoams on cattle manure biomethanation

In order to investigate the effect of the antifoams on the methane production of cattle manure, batch assays were performed to determine methane potential of cattle manure. Pure antifoams at three different concentrations mixed with cattle manure were inoculated with digested manure. The methane potential was determined according to the guidelines of the biochemical methane potential (BMP) protocol (Angelidaki et al., 2009). The inoculum was obtained from a thermophilic full-scale biogas reactor, co-digesting cattle manure and industrial wastes. All tests were performed in triplicate. The test bottles used for BMP of pure cattle manure and pure antifoams had total and working volume of 547 and 300 mL, respectively. For the BMP of pure antifoam, in each bottle 100 mL inoculum, 200 mL water, and approximately 0.3 mL antifoam, were added which corresponded to the initial organic loading of 1 gVS/L. For the BMP of pure cattle manure, each bottle was added with 100 mL inoculum, 200 mL water, and 18 mL cattle manure, which corresponded to the initial organic loading of 6.43 gVS/L. The test bottles used for BMP of cattle manure supplemented with antifoam had total and working volume of 320 and 100 mL, respectively. In each bottle 80 mL inoculum, 18 mL cattle manure, and 2 mL of a mixture of water and antifoam were added. Each antifoam was tested at concentrations of 0.05%, 0.1% and 0.5% v/v. After inoculation, all bottles were flushed with nitrogen gas, sealed with rubber stoppers and aluminium screw caps, and incubated at 54 ± 1 °C. The methane content in the headspace was regularly measured until the methane production has stopped.

2.4. Continuous reactor experiment

The selected four antifoams were also tested for their antifoaming efficiencies and effects on biomethanation under continuous reactor operation. The experiment was carried out in five continuous stirred tank reactors (CSTR). Each reactor had total and working volume of 2 and 1.5 L, respectively. The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The reactors were continuously stirred using a magnetic stirrer and during the experiment the reactors temperature was maintained at 54 ± 1 °C using thermal jackets. The substrate was automatically provided twice per day using peristaltic pumps with timers. Biogas production was measured by an automated displacement gas metering system with 100 mL cycle (Angelidaki et al., 1992) The production of biogas and the volume of foam in the reactors were recorded daily, while methane content in biogas and VFA concentration were measured once or twice per week. Initially, all reactors were inoculated with thermophilic inoculum, and fed with

raw cattle manure until steady state conditions were achieved. Subsequently, a stepwise increase of the OLR was performed by adding glucose to the substrate, until the OLR of the reactors reached 6.5 g VS/(L-reactor·day). This OLR was chosen based on the results from our previous investigation (Kougias et al., 2013a), where the OLR of 6 g VS/(L-reactor·day) resulted in an extensive and persistent foaming. Once the daily foam volume production in the reactors was steady, a certain concentration of rapeseed oil (R1), oleic acid (R2), octanoic acid (R3) and tributylphosphate (R4) was added to the feedstock bottle of each corresponding reactor. The fifth reactor (R5) was kept as a control and no antifoam was added. The antifoam addition was done in three periods (period I, II, and III), with the antifoams dosages of 0.05%, 0.1%, and 0.5% v/v_{feed}, respectively. During each period and once the reactor had a stable daily foam production, the foam reduction efficiency was calculated, and the addition of the antifoam was stopped until the foam production inside the reactor reached back to its initial level (i.e. before adding antifoam). The period that lasted from the stoppage of the antifoam addition till the time which the foam volume recovered back to its initial state was also recorded as foam recovery period in order to compare the duration of the relapse effect of antifoams.

2.5. Foaming potential methodology and calculation

The daily foam production inside the reactor was determined by measuring the average foam height and multiplying with the surface area of the reactor. After measurement, the reactor was rapidly stirred or manually shaken until the foam totally broke down. The volume of foam production was recorded daily. The foam reduction efficiency of the antifoams was calculated by the following equation:

$$\text{Foam reduction efficiency}(\%) = (1 - (\text{Foam volume after antifoam addition} / \text{Foam volume before antifoam addition})) * 100.$$

2.6. Analytical methods

Total solids (TS), volatile solids (VS), pH, total nitrogen (TKN) and total ammonia were determined according to APHA standard methods for the examination of water and wastewater (2005). For the batch assays, the methane content in biogas was measured using a gas-chromatograph (Shimadzu GC-8A, Tokyo-Japan) equipped with a glass column (2 m, 5 mm OD, 2.6 mm ID) packed with Porapak Q 80/100 mesh (Supelco, Bellefonte, PA, USA) and with a flame ionization detector (FID). During the continuous reactor experiment, the methane and the CO_2 content in biogas were measured using a gas chromatograph (Mikrolab, Aarhus A/S, Denmark), equipped with a thermal conductivity detector (TCD). Volatile fatty acids (VFAs) analysis was performed in a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) as described by Kougias et al. (2013a). All the determinations were performed in triplicate.

2.7. Statistical analysis

Data analysis was conducted using "Graphpad Prism" version 5 software (Graphpad Software, Inc., San Diego, California). Descriptive statistics were performed for all variables, mean values and standard deviations were calculated. Normality test and subsequently one-way analysis of variance (ANOVA) was carried out in order to compare and define significant differences ($p < 0.05$) among the methane yields during the continuous operation of the reactors.

3. Results and discussion

3.1. Methane potential of pure antifoams and cattle manure

The methane potential of the antifoams and the cattle manure used, obtained from the BMP assays are summarised in Table 1. The theoretical methane potential of the cattle manure used was 509 mL-CH_4/gVS-added, calculated from the manure composition according to the method described by Angelidaki et al. (2009). Moreover, the theoretical methane potentials of antifoams were calculated by using stoichiometric conversion of the compounds to methane and carbon dioxide, assuming full degradation of each substrate (Table 1). From the BMP assays, the maximum methane production from pure cattle manure reached after 17 days and was 399 mL-CH_4/gVS-added, which corresponded to 78% of the theoretical value. The incomplete conversion of cattle manure to biogas was probably due to the high content of recalcitrant organic materials such as straws (Fang et al., 2011). For rapeseed oil, oleic and octanoic acid, the maximum methane production was achieved after 25 days. The methane production was relatively slow and a lag phase was observed in all bottles (data not shown). This was due to the slow degradation of long chain fatty acids (LCFA). The degradation of LCFA through a β-oxidation pathway has been previously reported as the rate-limiting step during the anaerobic digestion process (Lalman and Bagley, 2002). Moreover, the degradation of the by-products of LCFA, such as palmitic and myristic from the degradation of linoleic and oleic acid, could also inhibit the biogas process (Lalman and Bagley, 2001). The methane potential obtained from BMP assays of rapeseed oil, oleic and octanoic acid were 704, 837 and 623 mL-CH_4/gVS-added, respectively, corresponding to 70%, 83% and 73% of the theoretical yields. In contrast, no methane production was observed from tributylphosphate, revealing a strong inhibition of the biogas process. This corresponded to the report that tributylphosphate is a bacteriostatic compound which is very persistent in the natural environment (Berne et al., 2005).

3.2. Effect of antifoams on anaerobic biomethanation of cattle manure

Possible synergistic or inhibitory effects of antifoams on biomethanation of cattle manure were investigated by comparing the methane yields of pure antifoams and pure cattle manure, with the methane yields from the cattle manure supplemented with antifoams at different concentrations. The results showed that rapeseed oil enhanced the methane yield of the mixed substrate (i.e. antifoams and cattle manure) at all concentrations, while oleic acid and octanoic acid had positive effect up to concentration of 0.1% v/v (Fig. 1a). The higher methane yields of the mixed substrates were mainly due to the higher methane potential of antifoams compared to pure cattle manure (Table 1). The methane yields of manure added with rapeseed oil at concentrations of 0.05%, 0.1% and 0.5% v/v were 498 ± 3, 530 ± 6 and 655 ± 8 mL-CH_4/gVS-added, corresponding to 89%, 91% and 92% of the theoretical yield, respectively. The methane potential of this mixture calculated by

Table 1
Methane yield of cattle manure and antifoams from batch experiments.

Substrate	Initial Load gVS/L	Methane yield mL CH_4/gVS added	Theoretical methane yield at 55 °C mL CH_4/gVS added
Cattle manure	6.43	399 ± 18	509
Rapeseed oil	1	704 ± 13	1007
Oleic acid	1	837 ± 0.3	1013
Octanoic acid	1	623 ± 1.2	856
Tributylphosphate	1	No methane	705

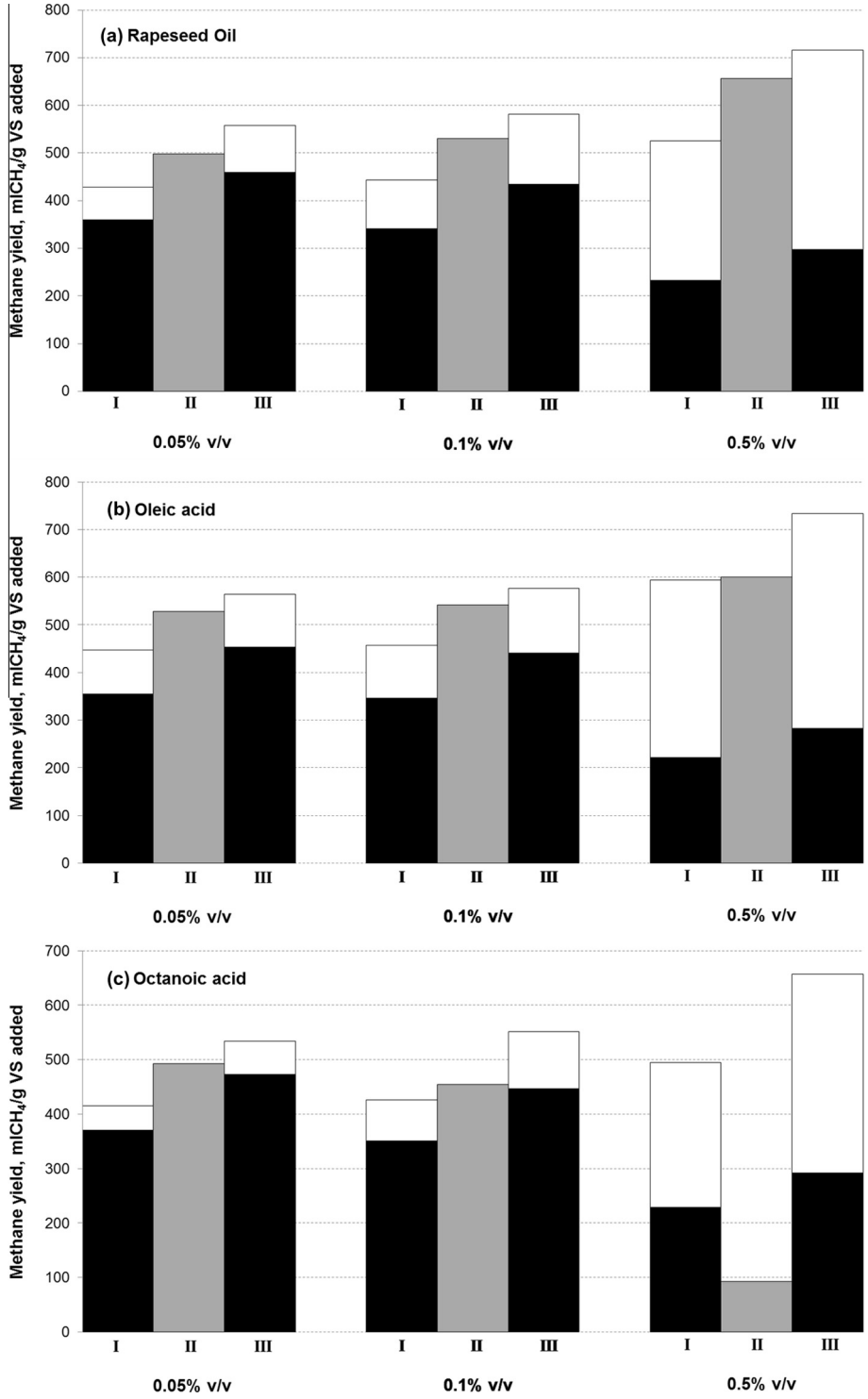

Fig. 1. Methane yield of cattle manure (black column), antifoams (white column), and mixture of cattle manure with 0.05%, 0.1% and 0.5% v/v antifoams (grey column); (I) from BMP assays of single substrates, (II) from BMP assays of mixed substrates, and (III) from theoretical yield.

summation of the BMP results of pure cattle manure and pure rapeseed oil at concentration of 0.05%, 0.1% and 0.5% v/v, were 429, 443 and 526 mL-CH_4/gVS-added, respectively, which were 16–25% lower than the BMP results from the mixed substrate. The higher degradation efficiency of the mixed substrate indicated that there was a synergic effect when co-digesting cattle manure and rapeseed oil up to concentration of 0.5% v/v. The synergistic effect was probably due to an active microbial activity supported by the readily biodegradable organics in the cow manure. The microbial activity would contribute to higher hydrolytic capacity for the LCFA degradation (O-Thong et al., 2012).

However, oleic and octanoic acid presented a positive impact on the methane yield of the manure mixtures only up to a concentration of 0.1% v/v (Fig. 1b and c). The methane yields of manure supplemented with oleic acid at concentration of 0.05% and 0.1% v/v were 528 ± 4 and 541 ± 6 mL-CH_4/gVS-added, respectively, which corresponded to 94% of theoretical methane yield and 18% higher than the summation of BMP from single compounds.

However, the mixture of manure with 0.5% v/v oleic acid resulted in a methane yield of 600 ± 9 mL-CH_4/gVS-added, corresponding to 82% of theoretical yield and only 1% higher than the summation of the BMP from single compounds. The mixtures of manure and octanoic acid at concentrations of 0.05% and 0.1% v/v resulted in the methane yield of 492 ± 6 and 454 ± 7 mL-CH_4/gVS-added, corresponding to 92% and 82% of the theoretical values and were 19% and 7% higher than the summation of BMP from single compounds. Nevertheless, the addition of 0.5% v/v octanoic acid inhibited significantly the biomethanation process. The achieved methane yield was only 92 ± 4 mL-CH_4/gVS-added, which corresponded to 14% of the theoretical value and 81% lower than the summation of the BMP from single compounds. It could be explained that the mixture of manure with 0.5% v/v octanoic acid corresponded to a concentration of 33 mM octanoic acid, which was more inhibitory compared to the concentration of 6.9 mM in the BMP assays of pure octanoic acid. Another study also reported that octanoic acid at a concentration of 10 mM resulted in 50% inhibition of aceticlastic methanogens in batch tests using sludge as substrate (Koster and Cramer, 1987).

Finally, the results from the BMP assays of manure added with tributylphosphate revealed a total inhibition of the process since no methane was produced at all concentrations of tributylphosphate.

3.3. Effect of antifoams under continuous reactor operations

The results from reactor experiments are shown in Figs. 2 and 3. Fig. 2a shows that rapeseed oil addition resulted in higher methane yield compared to the control reactor. The reactor added with rapeseed oil had average methane yield of 306 ± 17, 282 ± 10 and 363 ± 14 mL CH_4/gVS-added at the dosages of 0.05%, 0.1% and 0.5% v/v_{feed}, respectively, while the control reactor had methane yield of 273 ± 12 mL CH_4/gVS-added. According to the statistical analysis after the addition of 0.05% and 0.5% v/v_{feed} rapeseed oil the increase in the methane yield was statistically significant. Moreover, the antifoam efficiency of rapeseed oil increased at higher concentrations, as the antifoam efficiencies of rapeseed oil were 21%, 38% and 43% at the end of phase I, II and III, respectively.

As mentioned in the Section 2, each period of antifoams addition was followed by a foam recovery period where no antifoams were applied. The foam recovery time was 12 days after the stoppage of 0.05%, 0.1% v/v_{feed} rapeseed oil addition. This indicates that the dosages of 0.05% and 0.1% v/v_{feed} resulted in the same relapse effect inside the reactor. After the end of phase III, the reactor was operated for 7 days and it did not manage to recover to its initial foam levels.

Fig. 2b shows that oleic acid addition also resulted in higher methane yield compared to the control reactor and this increase

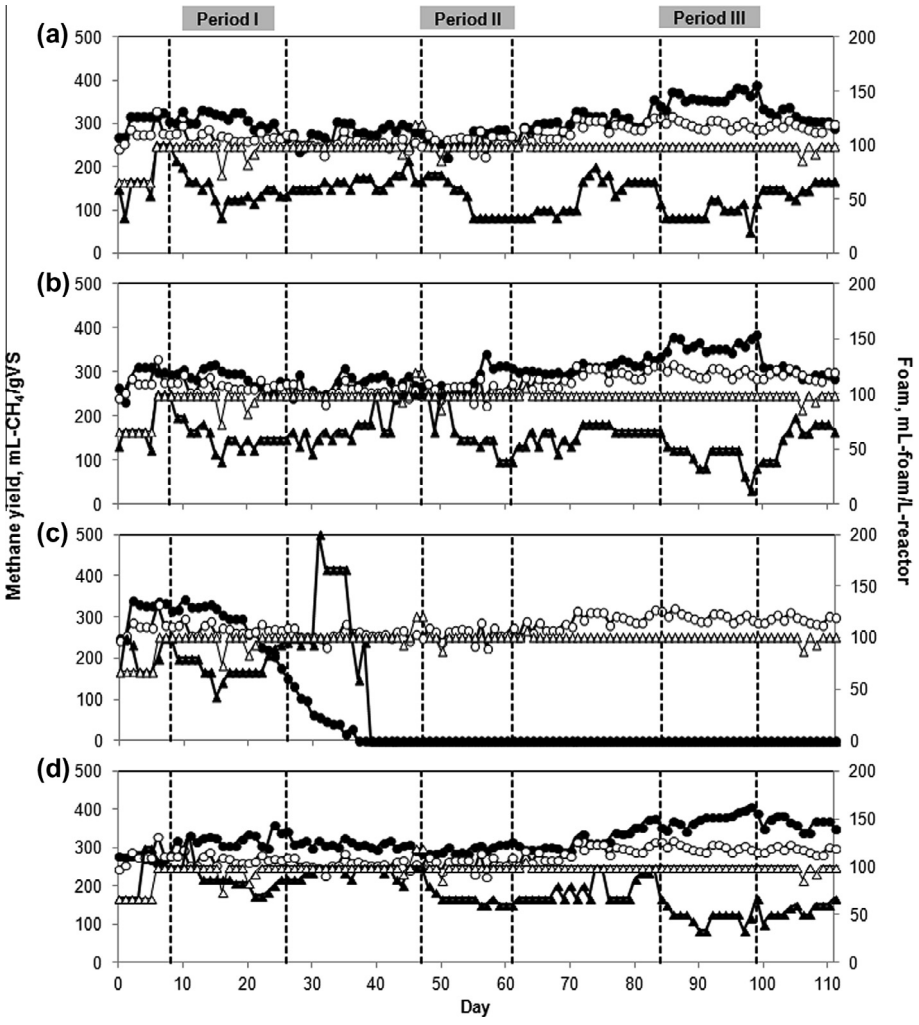

Fig. 2. Methane yield and foam formation in the control reactor (○ and △, respectively), compared with the reactors treated with rapeseed oil (a), oleic acid (b), tributylphosphate (c) and octanoic acid (d). Periods (I), (II) and (III) corresponded to antifoam dosages of 0.05%, 0.1% and 0.5% v/v_{feed}, respectively. Symbols ● and ▲ represent methane yield and foam formation in the treated reactors, respectively.

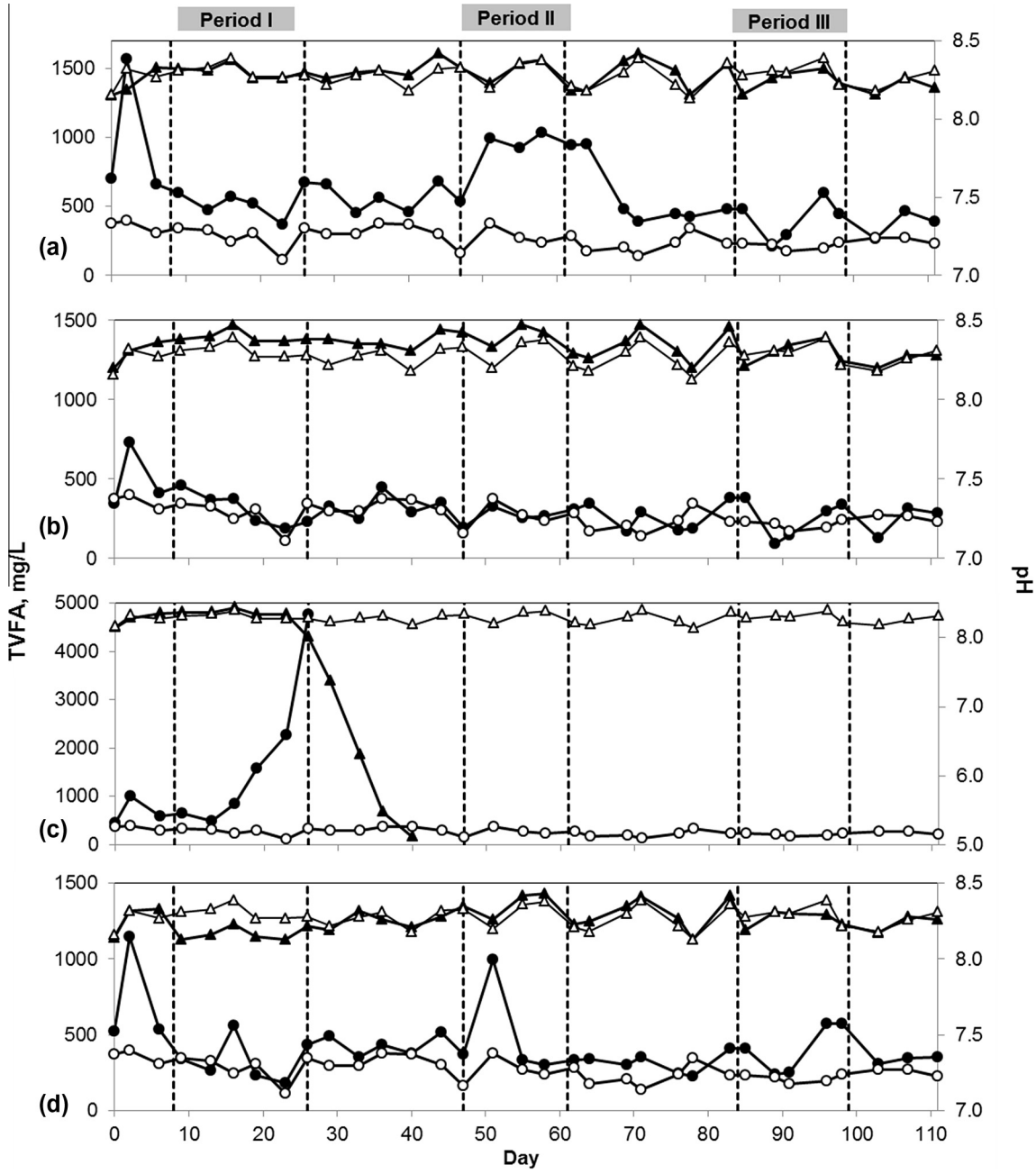

Fig. 3. Total volatile fatty acids (TVFA) and pH in the control reactor (○ and △, respectively), compared with the reactors treated with rapeseed oil (a), oleic acid (b), Tributylphosphate (c) and Octanoic acid (d). Period I, II and III corresponded to antifoam dosages of 0.05%, 0.1% and 0.5% v/v_{feed}, respectively. Symbols ● and ▲ represent TVFA and pH in the treated reactors, respectively.

was statistically significant at concentrations over 0.1% v/v_{feed}. The reactor fed with manure supplemented with oleic acid had average methane yield of 296 ± 10, 284 ± 18 and 360 ± 13 mL CH_4/gVS-added at the dosages of 0.05%, 0.1% and 0.5% v/v_{feed}, respectively. The antifoam efficiency of oleic acid was slightly lower than rapeseed oil at all dosages. However, they had the same tendency of higher antifoam efficiency at higher dosages, as the antifoam efficiencies of oleic acid were 18%, 36% and 38% at the end of phase I, II and III, respectively.

The foam recovery period was 12, 12, and 7 days after stop adding 0.05%, 0.1% and 0.5% v/v_{feed} oleic acid, respectively. It was found that the relapse effect of oleic acid at concentrations of 0.05% and 0.1% v/v_{feed} was achieved at the same time period, similarly to the rapeseed oil. One explanation could be that the addition of higher concentration of LCFA antifoams stimulated faster degradation of LCFA, thus the antifoam effect ceased when the antifoams were removed by degradation. It has also been previously reported that the conversion of LCFA became faster and more effective along the successive pulses as the biomass become adapted (Neves et al., 2009). The fast degradation of 0.5% v/v_{feed} antifoams was confirmed by the higher methane yield and no VFA accumulation during the period III (Figs. 2a and b, 3a and b).

Fig. 2d shows that octanoic acid addition also resulted in higher methane yield compared to the control reactor. The reactor fed with manure supplemented with octanoic acid had average methane yield of 286 ± 9, 297 ± 8 and 377 ± 18 mL CH_4/gVS-added at the dosages of 0.05%, 0.1% and 0.5% v/v_{feed}, respectively. These were higher than the methane yield from both reactors with rapeseed

oil and oleic acid at the dosages of 0.1% and 0.5% v/v_{feed}. The results from the statistical analysis showed that the methane yields in the reactor with octanoic acid were significantly higher than the corresponding ones of rapeseed oil at concentrations of 0.1% and 0.5% v/v_{feed}, and significantly increased compared to the reactor with oleic acid at concentration of 0.5% v/v_{feed}. One reason could be due to the shorter chain of octanoic acid which resulted in faster degradation. The antifoam efficiency of octanoic acid was much lower than rapeseed oil and oleic acid at the dosages of 0.05% and 0.1% v/v_{feed}, but higher at dosage of 0.5% v/v_{feed}. The antifoam efficiencies of octanoic acid were 9%, 30% and 49% at the end of phase I, II and III, respectively. This showed that the antifoam efficiency of octanoic acid was significantly increased at higher dosages.

The foam recovery period was 3 and 12 days after stopping the addition of 0.05% and 0.1% v/v_{feed} octanoic acid, respectively. It should be highlighted that foam recovery was much faster when low dosages of octanoic acid were applied. This could be due to its faster degradability compared to rapeseed oil and oleic acid. However, octanoic acid extended foam recovery period of the reactor as its concentration was increased, indicating that the degradation capacity for octanoic acid was not stimulated with higher dosage. Similarly with the previous antifoam agents, the reactor was not recovered within 7 days after stopping the defoamer addition at a concentration of 0.5% v/v_{feed}.

Fig. 3 presents the pH and VFA levels of the reactor during the experiment. In all reactors except tributylphosphate, the pH values were similar and within the range of 8.0–8.5, which was within the optimum range for methanogenesis (between 5.5 and 8.5) (Angelidaki et al., 2011). This indicates that rapeseed oil, oleic acid and octanoic acid did not have significant effect on the pH of the reactors. One explanation could be due to the low water solubility of fatty acids which decreased as the amount of carbon increased. Thus, these fatty acids as in original form (i.e. prior degradation) would have minimal effect on pH of the reactors. However, the degradation products of fatty acids, such as acetic acid, might affect the reactors pH if they are present at high concentration. The concentration of VFA has also been suggested as a biogas process indicator since an increase in VFA concentration is a result of process imbalance (Boe et al., 2007). The determination of the VFA showed that total VFA concentrations in all reactors, except tributylphosphate, were low despite of a small increase in VFA during the addition of 0.1% v/v_{feed} rapeseed oil. This indicates that the process was stable during the whole experimental period, showing that the addition of fatty acids antifoams at these dosages did not cause process imbalance.

Tributylphosphate was the antifoam that showed the highest antifoaming potential in the physiochemical tests (Kougias et al., 2013c). However, in contrary to the fatty acids antifoams, tributylphosphate strongly inhibited the biogas process. From Fig. 2c and Fig. 3c, methane production decreased and VFA accumulated along with the addition of 0.05% v/v_{feed} tributylphosphate, indicating the inhibition of methanogenesis. The methane yield decreased to 176 mL CH_4/gVS-added and the VFA concentration increased up to 4.8 g/L at the end of period I. The average antifoam efficiency of 0.05% v/v_{feed} tributylphosphate was 13%, which was lower than rapeseed oil and oleic acid. The pH dropped from 8.2 to 5 and the process did not recover after the stoppage of tributylphosphate addition. For that reason, further increase of tributylphosphate concentration was not investigated. Thus, it was concluded that this antifoam was not suitable for anaerobic digestion application.

In general, the effect of antifoam on biogas process is also depending on the application method. In the present study the antifoams were added in the feed bottles, which is the common method generally applied in full scale biogas plants. Thus, the analyses of all the data comparing the four tested antifoams provided information about their efficiency on foam destruction that can be directly utilized in real practice from the biogas plant operators. To sum up the results from both the batch tests and continuous mode experiment, it was shown that the antifoam which was most suitable for anaerobic digestion of cattle manure under organic overload was rapeseed oil, since it presented positive effect on the process at all dosages. Moreover, the price of rapeseed oil is lower compared with the prices of the other tested antifoams and this can contribute in a more economic feasible solution in order to suppress foaming incidents.

4. Conclusions

The results from batch experiments showed that rapeseed oil, oleic and octanoic acid enhanced methane production when co-digested with cattle manure. These antifoams could also efficiently suppress foam under continuous reactor operation. It was concluded that rapeseed oil was most suitable as antifoam for manure digestion under organic overload. The optimal dosage of rapeseed oil will be dependent on the severity of foaming incident as the antifoaming efficiency of rapeseed oil increased with increased dosages. Finally, tributylphosphate strongly inhibited the biogas process and was not suitable as antifoam in anaerobic digestion.

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Anaerobic digestion foaming in full-scale biogas plants: a survey on causes and solutions

P. G. Kougias, K. Boe, S. O-Thong, L. A. Kristensen and I. Angelidaki

ABSTRACT

Anaerobic digestion foaming is a common operation problem in biogas plants with negative impacts on the biogas plants economy and environment. A survey of 16 Danish full-scale biogas plants on foaming problems revealed that most of them had experienced foaming in their processes up to three times per year. Foaming incidents often lasted from one day to three weeks, causing 20–50% biogas production loss. One foaming case at Lemvig biogas plant has been investigated and the results indicated that the combination of feedstock composition and mixing pattern of the reactor was the main cause of foaming in this case. Moreover, no difference in bacterial communities between the foaming and non-foaming reactors was observed, showing that filamentous bacteria were not the main reason for foaming in this case.

Key words | anaerobic digestion, causes, full-scale biogas plants, foaming, solutions

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INTRODUCTION

Anaerobic digestion foaming is a serious drawback that is occasionally recorded in many full-scale biogas plants. Foam is a gas-liquid dispersion, containing more than 90% gas and is typically created in the main biogas reactor or in the pre-storage tank as a viscous deep-brown coloured layer (Oerther *et al.* 2001; Varley *et al.* 2004).

It is well documented that foam formation is causing upsets in the digestion process. Specifically, foaming results in operational problems, caused by the entrapped solids that are present in the foam, resulting in blockage of mixing devices and collapse of pumps (Dalmau *et al.* 2010). Inadequate or bad mixing leads to an inverse solids profile in the digesters, forming dead zones and thus reducing the active volume of the reactor. As a consequence biogas production is reduced for shorter or longer periods (Nielsen & Angelidaki 2008). Furthermore, an increase in the operational expenditures are linked to foaming, due to income losses, manpower overtime and maintenance costs (Barjenbruch *et al.* 2000; Barber 2005). Foaming can also lead to negative environmental impacts caused by the overflowing of the pre-storage or digester tanks.

In the cited literature there are a number of studies aiming to identify the potential causes of foaming. Surface active agents, such as surfactants or biosurfactants generated by the metabolic processes were found to lower the surface tension of the substrate and enhance foaming potential (Barber 2005). Ross & Ellis (1992) identified organic overloading and subsequently the accumulation of acetic acid as the cause of foam formation in wastewater sludge digesters. Moreover, Boe *et al.* (2012) compared the effect of several substrates and intermediate compounds on foaming in a manure digestion system and reported that high content of lipids or proteins could promote foaming. Filamentous microorganisms and especially *Gordonia* species and *Microthrix parvicella* are known to be the major cause of foaming in sludge digesters as they are attached to the gas bubbles and accumulated on the surface of the reactor (Heard *et al.* 2008). Dalmau *et al.* (2010) have developed a knowledge-based model to predict the risk of foaming in activated sludge systems, by selecting as inputs the organic loading rate, the variation in organic loading rate and the presence of filamentous microorganisms. Operating factors, such as inadequate mixing, temperature fluctuations have been also suggested as foaming causes (Barjenbruch *et al.* 2000; Barber 2005).

As it can be easily understood, an effective strategy to prevent and/or suppress foaming is imperative in order to avoid the unfavourable effects of foaming incidents. However, the identification of foam causes cannot always

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assure the implementation of a precise and successful antifoam strategy. This is due to the facts that foam has a complex structure and there are a lot of applications that foaming incidents occur, which might require different solutions for precise and efficient foam suppression. Nowadays, there is an abundance of commercial products that can be used as antifoaming agents (Junker 2007). Though, it is well known that an antifoaming agent may not be suitable for every application (Routledge & Bill 2012). Commonly, these products contain oil or dispersed hydrophobic solid particles or a mixture of both (Marinova & Denkov 2001) and are classified as antifoams or defoamers. Antifoams include compounds that are added to a solution prior to foam formation in order to prevent excessive foaming, while defoamers include agents that are added once the foam is formed and are able to break it down in short time (Miller 2008). However, in common practice the terminology of antifoams and defoamers is identical referring to compounds that can eliminate foaming (Kougias *et al.* 2013a).

In environmental applications, foaming has been traditionally associated with activated sludge systems (Barber 2005). Thus, there is lack of experimental data and scientific information concerning foaming in manure-based digesters. More specifically, the analysis of foaming incidents and their correlation with operational parameters of full-scale biogas plants has never been studied. The aim of the present research was to survey foaming incidents in Danish full-scale biogas plants and to establish knowledge for disclosing potential causes of foaming, in order to achieve a stable operation in Danish biogas plants.

MATERIALS AND METHODS

To assess the prevalence of anaerobic digestion foaming in Demark, a survey in 16 Danish full-scale biogas plants was conducted (i.e. 80% of the total centralised biogas plants in Denmark). Survey participants were provided with questionnaires involving questions concerning the occurrence, control and possible causes of anaerobic digestion foaming based on their individual experience. Among the questions, the participants provided information concerning the operational parameters and the feedstock characteristics of the biogas plants.

During the project period, on case study of foaming in full-scale biogas plant was also investigated. In order to identify the cause of foaming incidents at Lemvig biogas plant, the plant operation data during foaming period were analysed. Moreover, reactor samples were collected for analysis of physical–chemical properties and microbial communities.

Analysis techniques

Analysis of pH, total solids, volatile solids and alkalinity was according to *Standard Methods for the Examination of Water and Wastewater* (APHA 2005). The biosurfactant activity of the samples was determined by the oil displacement test as described by Morikawa *et al.* (2000). In a Petri dish (15 cm diameter), 10 μL of Murban crude oil were dropped on the surface of 50 mL distilled water, and subsequently a thin oil film was immediately formed. Thereafter, 20 μL of sample was placed onto the centre of the oil film and a clear circle inside the oil film was formed. The diameter of this circle was measured after the period of 30 s. Oil displacement area (ODA) was calculated as the area (cm^2) of the clear circle.

The foaming potential of solution was determined by the aeration method modified from Bikerman method described by Boe *et al.* (2012). The apparatus comprised of an Imhoff settling cone with a ceramic diffuser placed at the bottom. Through the diffuser, air was introduced into the system to promote foaming. The air pressure was controlled with a pressure gauge and set at 1 bar. The air flow rate was set at 60 mL/min and was controlled and measured with a flow-meter. A 50 mL sample was aerated in the settling cone for 10 min. The foam height in the settling cone was measured twice; once just after the 10 min aeration period and another one after 1h. The foaming potential was defined using two parameters: foaming tendency and foam stability. The foaming tendency (mL-foam/(mL-air · min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). The foam stability was determined as percentage of foam remaining in the settling cone at 1 h after aeration compared to the volume of foam right after aeration. After each aeration, the ceramic diffuser was replaced or cleaned with distilled water containing 5% of HCl. The determination of the foaming potential was performed in triplicate.

The dynamic population of Bacteria and Archaea in samples derived from all full-scale reactors of Lemvig biogas plant were analysed. Genomic DNA extraction, polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE), and sequencing were made as described in (Zhao *et al.* 2009).

RESULTS AND DISCUSSION

Survey of anaerobic digestion foaming at full-scale biogas plants in Denmark

A survey of full-scale biogas plants in Denmark revealed that foaming is a widespread problem and the causes and consequences of foaming control are not fully understood. The results from the questionnaires showed that 15 biogas plants had experienced foaming problems (Figure 1(a)). Foaming appeared mainly in the main digester and occasionally also in the substrate storage/pre-digester. Nevertheless, 38% of the plants observed that foaming occurred in both places. Foaming was a periodic problem that typically occurred up to three times per year in most of the plants, and the duration of the incidents lasted from one day to three weeks. The duration of the incidents classifies the foam occurring in biogas reactors as metastable. Metastable foams can persist indefinitely if they are absolutely protected from disturbing influences, and thus the use of chemical antifoaming agents is needed in order to destabilise them (Vardar-Sukan 1998). The biogas plants estimated their biogas production loss during foaming period as 20–50%. However, some plants experienced up to 90% of biogas production loss, and some extreme conditions could lead to total process failure. This can be explained by the observation that different parameters can lead in different severity and type of foaming. Additionally it has been previously reported that in some cases in which more than one parameter was involved in foam formation, there was a synergistic effect on foam promotion (Kougias *et al*. 2013b).

Most of the biogas plant reported that the foaming problem was connected with organic overload (Figure 1(b)). This is in agreement with another study that the digester overloading can result in partial degradation of organic

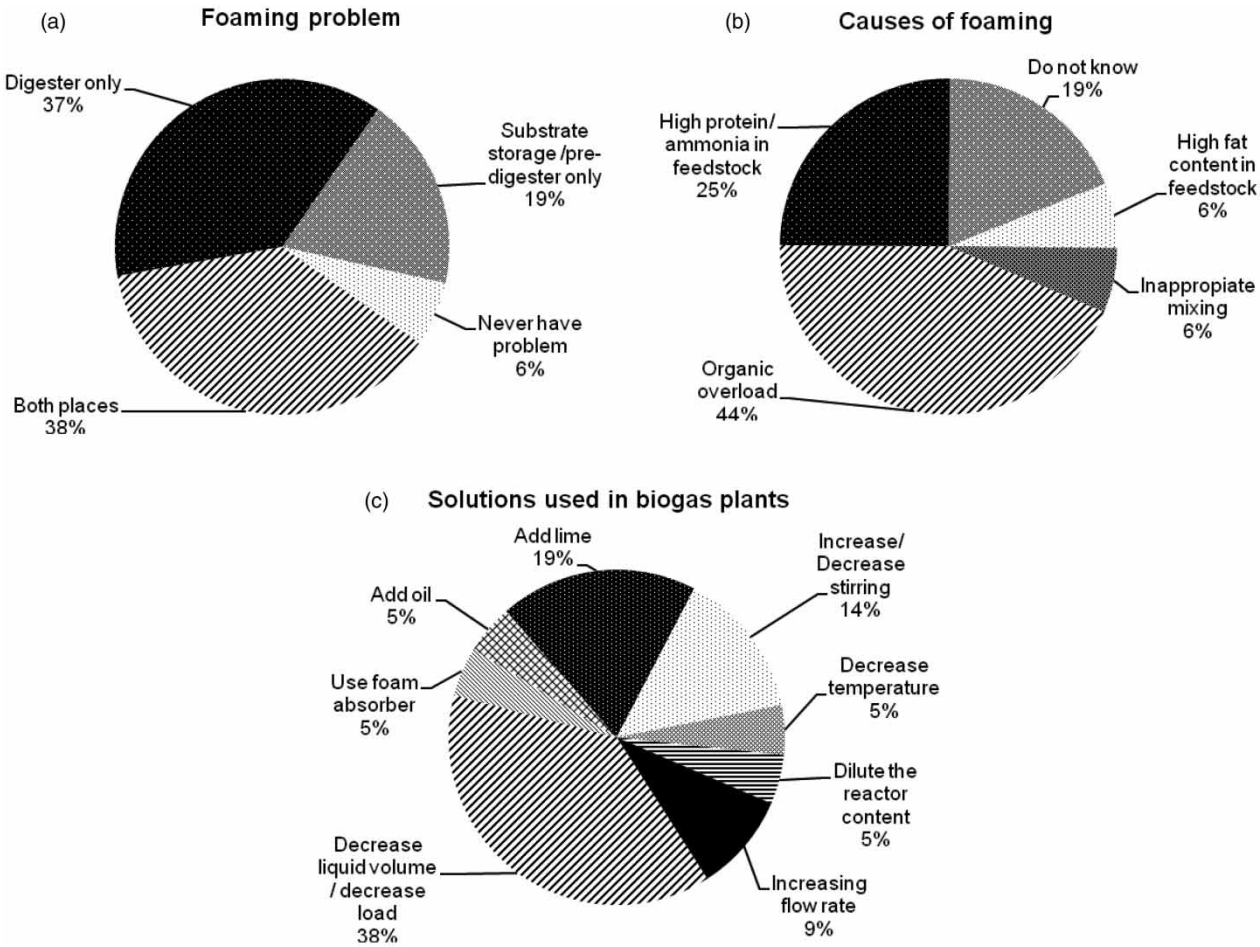

Figure 1 | Results from survey for the Danish full-scale biogas plants: (a) occurrence of foaming incidents, (b) potential causes of foaming, and (c) solutions applied to suppress foaming.

matter and accumulation of surfactants or biosurfactants (Ganidi *et al*. 2011). The excessive concentration of these substances can potentially contribute to foaming phenomena. The second most dominant cause for foaming, indicated by the 25% of the biogas plants, was the high protein content in the feedstock. The mechanism of foam formation by proteins compounds is based on the synthesis of ionisable structures with both hydrophobic and hydrophilic available ends. Specifically, protein foams are formed by a protein film surrounding a gas bubble creating a structure that holds bubbles in place (Foegeding *et al*. 2006). Our previous study has also shown that the protein compounds such as gelatine had a strong effect on increasing the foaming tendency of manure under the physiochemical tests (Boe *et al*. 2012). High fat content in feedstock and inappropriate mixing were also reported as correlating with foaming in these biogas plants, although at a lower extent than protein. Our previous physiochemical tests had shown that the derivatives of lipids such as sodium oleate also had the ability to promote foam in manure (Boe *et al*. 2012). It is also well known that protein and lipid are the main causes of foaming in the high-rate anaerobic digesters (Lettinga & Hulshoff Pol 1991). Type of mixing and mixing speed could also have influence on foaming. Pagilla *et al*. (1997) compared two full-scale anaerobic sludge digesters treating primary sludge and thickened waste-activated sludge, and concluded that more foam was accumulated in the gas-mixed digester than in the mechanically-mixed digester. Brown & Sale (2002) reported that foaming incidents in a high rate digestion plant were attributed also by vigorous mixing with non-confined gas. In general, inappropriate mixing could result in formation of dead zones inside the reactor, resulting in partial degradation of organic matter, accumulation of surface active compounds and consequently potential foaming risks. Finally, according to the survey, it should be highlighted that one-fifth of the biogas plants could not suggest any potential causes that promote foaming in their processes.

Unfortunately, foaming is often detected after the adverse impacts of the foam effect have already been initiated. The plant operators normally detected the problem by visual observation or when the security valve or alarm activates. The most common solution that the plant operators applied to suppress foaming was the decrease of the digester's organic load (Figure 1(c)). This was in good agreement with the results from our previous reactor experiments, which concluded that organic overload was the main cause of foaming in manure digesters (Kougias *et al*. 2013b). Thus, the decrease of the organic load could minimise foam formation. The second most applied approach to counteract foaming in the biogas plants was the addition of antifoaming agents. As stated previously, the metastable foams could be suppressed by the use of chemical antifoaming agents. According to the survey results, the addition of antifoam compounds, such as foam absorbers, oils or lime, was a common approach accounted for 29% of the reported antifoam strategies. Other solutions involved adjustment of the stirring speed, increase of the flow rate, decrease of reactor temperature, and dilution of the reactor content.

Lemvig biogas plant case study

During the project period, one Danish full-scale biogas plant, Lemvig, was facing intensive foaming problem in one of the four reactors without any clear reasons. The biogas plant has four continuously stirred tank reactor (CSTR) biogas reactors, and only one of the reactors was facing foaming problems while the three other reactors had no foaming incidents. The biogas plant applied serial-CSTR configuration (Boe & Angelidaki 2009) with three primary reactors, denoted as PR1, PR2 and PR3, followed by a second-stage reactor, denoted as SR (Figure 2). Reactor PR1, PR2 and PR3 were running in parallel under the same HRT of 23 days, and fed with the same substrate which was manures (cattle manure, pig manure and chicken manure), and industrial wastes at a ratio of 75 and 25%, respectively. Reactor SR was fed with effluent from the primary reactors. All four reactors were operated at thermophilic temperature (52 °C). The working volume of reactor PR1, PR2, and SR were equal to 2,400 m^3, while reactor PR3 was 7,100 m^3. Stirring speed of PR1, PR2 and SR was 2,400 m^3, while the stirring speed of PR3 was 16 rpm. Excessive foaming incidents occurred only in PR3, with the maximum foam formation approx. 1,065 m^3 foam/day.

For the case study, the operational parameters, the feedstock composition, and the samples from all reactors were investigated, in order to define the potential causes of foam formation. The results from the sample analysis showed that the values of some specific parameters in PR3 were significantly different from other reactors (Table 1). Alkalinity and biosurfactant activity were found at higher levels in PR3. It was previously reported that an increase in alkalinity leads to a decrease of surface tension, rendering the substrate more surface active and more prone to foam (Nges & Liu 2010). Moreover, the findings of the present study are in accordance with Ganidi *et al*. (2011) who reported a high level of alkalinity in a full-scale sludge digester with foaming incidents.

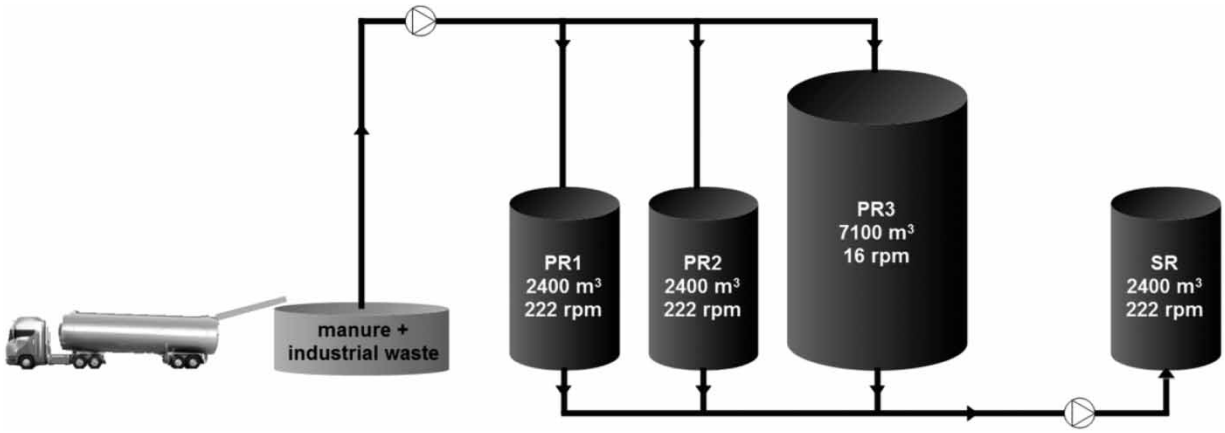

Figure 2 | Lemvig biogas plant reactors scheme.

Table 1 | Characteristics of the feedstock composition and samples obtained by the reactors

	Feedstock		Reactors			
Parameters	**Manure**	**Industrial waste**	**PR1**	**PR2**	**PR3**	**SR**
pH	6.5	4.3	8.13	8.11	8.21	8.15
Alkalinity (g/L as $CaCO_3$)	6.2	0	5.2	4.5	8.1	7.5
Biosurfactant activity (mm^2)	12.5	3.1	5	8.2	9.1	7.3
Total VFA (g/L)	8.9	24.6	0.02	0.02	0.14	0.09
Foaming tendency (mL foam/mL-air.min)	50–100	20	25–50	10–90	100–200	100–150
Foam stability (mL)	0	0	0	0	30	30

The determination of the foaming properties revealed a high tendency for foam formation and strong foam stability in the samples from PR3. Specifically, the maximum foaming tendency for PR1, PR2, PR3 and SR were found to be 50, 90, 200 and 150 mL foam/mL-air.min. Foaming stability was recorded only in PR3 and SR. As it can be easily understood, the characteristics of the samples derived from SR were deeply influenced by the corresponding ones of the PR3. However, the receiving of PR3 effluent did not cause foam promotion in the SR.

Since all primary reactors were fed with the same OLR and feedstock composition, the other possible factors that might have influences on the foaming incident could be the microbial community established in these reactors or the mechanical construction of the reactors. However, results from microbial analysis showed that the bacterial composition in all reactors were not significantly different, although one genus of facultative anaerobic, endospore-forming bacteria, *Paenibacillus*, that was present in the industrial wastes feedstock, was identified only in PR3. To the best of our knowledge, there is no available reference indicating that this bacterial genus could potential enhance foaming. Moreover, the well-known foam formation bacteria, *Gordonia* sp., was also investigated. It was previously reported that *Gordonia* sp. filamentous bacteria were the main cause of foaming in two full-scale anaerobic sludge digesters (Pagilla *et al*. 1997). However, no *Gordonia* sp. was detected in the tested samples obtained from all reactors, indicating that *Gordonia* sp. was not the cause of foaming in this case.

The reactor PR3 was significantly different from other reactors in regarding to the reactor size, mixing pattern, and mixing speed. Reactor PR3 had large reactor size with continuously slow agitation (16 rpm), while other reactors had smaller size with intermittently, fast, agitation (222 rpm) (Figure 3). It was possible that the high mixing speed was capable of providing fast gas-liquid transfer, and thus better removal of gas bubbles from the liquid mixtures. In contrary, the mixing device in PR3 had low-mixing speed and only created turbulences at the impeller tips, which did

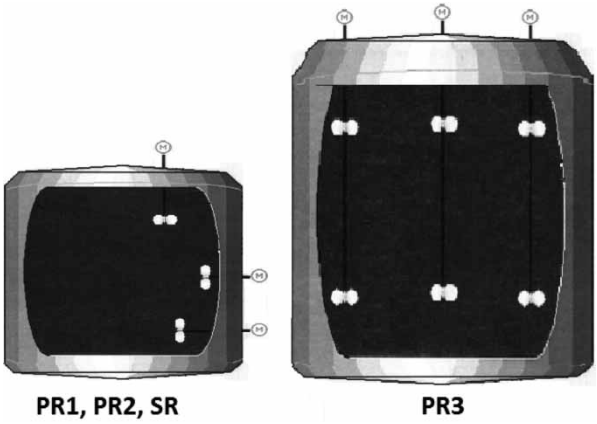

Figure 3 | Stirring pattern of biogas reactors at Lemvig biogas plant.

not manage to break down the foam once it was formed. As a consequence, excess foam was accumulated in the digester.

One important observation during the foaming incident was that the foam formation was significantly increased around 5–10 min right after the feed with the acidic feed mixture of industrial wastes (containing acidic whey). This could be due to the inadequate gas-liquid transfer which resulted in super-saturation of carbon dioxide in the reactor PR3 liquid mixtures. As a consequence of the acidic feed entering the reactor, the pH in the reactor was reduced, resulting in change of the carbonate balance and thereby in high amounts of CO_2 release. Another interesting observation in this case was that the foaming incident gradually disappeared from PR3 after the biogas plant totally omitted chicken manure from their feedstock. During the foaming period, the biogas plant received a large amount of chicken manure in their feedstock. Chicken manure has a high protein content which was known to promote foam according to our results from laboratory-scale experiment on the effect of substrate composition on foaming in a manure digester (Kougias *et al.* 2013b). Nevertheless, from the microbial analysis results and the holistic monitoring of the digester operational parameters, it was concluded that the foaming incident at Lemvig biogas plant was caused by a combination of the chemical properties of the substrate (such as acidic substrate and substrate with high protein content) and the mixing pattern of the reactor, rather than to the microbial ecology inside the reactors.

CONCLUSIONS

Foaming has been recorded in the majority of the full-scale biogas plants in Denmark, causing 20–50% biogas production loss. The biogas plant operators indicate the organic overload and the high protein and ammonia concentration in the feedstock as the most dominant factors for foaming. Unfortunately, foaming is rarely prevented but once it is detected the decrease of the organic load or the liquid volume are the most common solutions to suppress the foaming incident. A case study research at Lemvig biogas plant indicated that the feedstock composition combined with mixing pattern of the reactor has to be taken into serious consideration in order to avoid foaming incidents.

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Antifoaming effect of chemical compounds in manure biogas reactors

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ABSTRACT

A precise and efficient antifoaming control strategy in bioprocesses is a challenging task as foaming is a very complex phenomenon. Nevertheless, foam control is necessary, as foam is a major operational problem in biogas reactors. In the present study, the effect of 14 chemical compounds on foam reduction was evaluated at concentration of 0.05%, 0.1% and 0.5% v/v_{sample}, in raw and digested manure. Moreover, two antifoam injection methods were compared for foam reduction efficiency. Natural oils (rapeseed and sunflower oil), fatty acids (oleic, octanoic and derivative of natural fatty acids), siloxanes (polydimethylsiloxane) and ester (tributylphosphate) were found to be the most efficient compounds to suppress foam. The efficiency of antifoamers was dependant on their physicochemical properties and greatly correlated to their chemical characteristics for dissolving foam. The antifoamers were more efficient in reducing foam when added directly into the liquid phase rather than added in the headspace of the reactor.

1. Introduction

Foaming is often appearing in biogas reactors and is one of the most significant problems in biogas plants, negatively affecting the anaerobic digestion (AD) process. Generally, foam is gas–liquid dispersion with gas content more than 90%, which is accumulated on the surface of the liquid phase as a stable viscous, and in manure digesters appears as dark brown-colored layer (Oerther et al., 2001; Varley et al., 2004). Several studies reported that the most dominant factors contributing to foaming are the operational parameters of the digester (i.e. organic overload, temperature fluctuation, inadequate mixing), the feedstock composition and the presence of specific microorganisms (Ganidi et al., 2009; Dalmau et al., 2010; Boe et al., 2012). Once the major causes for foaming have been identified, a successful method to control or counteract foaming would be possible. Methods for foam prevention and suppression are classified into four large groups; mechanical, physical, biological and chemical methods (Vardar-Sukan, 1998). Mechanical and physical methods are based on the utilization of thermal, electrical or mechanical applications. Biological methods aim to prevent or suppress foam by limiting biological activities of foam promoting microorganisms, for example, by decreasing organic loading rate, remove feedstock components that favors the growth of foam promoting microorganisms, or by adding phages. Chemical methods refer to the addition of antifoaming agents in the digesters. Nevertheless, the complexity of the foam structure makes it difficult to apply a precise and efficient antifoam strategy.

A possible solution to prevent or suppress foaming incidents is the use of antifoaming agents also called defoamers. Antifoaming agents are strongly surface-active chemical compounds capable to decrease the surface tension of the

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solvent (Joshi et al., 2009; Xu et al., 2010). Moreover, Lacasse and Baumann (2004) reported that the antifoaming agents replace the foam producing substances in the air/liquid boundary and therefore their surface tension should be lower compared with the corresponding one of the foam producing compounds. Yet, the mechanism of foam suppression using antifoaming agents is not clearly understood (Barber, 2005). However, a general approach is that the antifoaming agents disrupt the stability of the liquid films in the foam by increasing the rate of the liquid drainage and subsequently the foam bubbles are penetrated (Winterburn and Martin, 2012).

There is a wide variety of different products that can be used as antifoaming agents with dissimilar compositions and properties, but usually common antifoams contain an oil or dispersed hydrophobic solid particles or a mixture of both (Marinova and Denkov, 2001). Antifoams and defoamers are fundamentally the same chemicals despite their different classification depending on their mode of action (Pelton, 2002). Antifoams include compounds that are added to a solution prior to foam formation in order to prevent excessive foaming (Denkov, 2004), while defoamers include agents that are added once the foam is formed and are able to break it down in short time (Miller, 2008). However, the terminology of antifoaming agents or antifoams and defoamers is identical in common practice, describing these compounds that are used to counteract foaming, either before or after the foam is created.

Nowadays, many antifoaming agent solutions have emerged and abundance of commercial choices is available (Junker, 2007). Though, it is well known that an antifoaming agent may not be suitable for every application (Routledge and Bill, 2012).

As a consequence, a number of different compounds should be examined to define which is the most appropriate for a certain treatment.

The ideal antifoaming agent for manure digesters should have cheap price, give fast response, suppress foam efficiently at low dosage, and not be inhibitory to the biogas process. It should also be biodegradable and should not have negative impact on the environment since the digester effluent is normally applied as fertilizer on farmland. In order to achieve efficient foam suppression, the antifoaming agent should fulfill the ideal criteria above. Moreover, some additional factors that can influence the biogas process should also be identified, such as method of application (i.e. optimal dosage, point of application), temperature and pH effect on foam suppression efficiency, and possible reaction with other compounds present in the digesters.

The efficiency of an antifoaming agent is based on its chemical composition, type of foam (i.e. metastable or unstable) and the chemical composition of the substrate/medium in the reactor (Vardar-Sukan, 1998). In general, the evaluation of the efficiency of an antifoaming agent depends on the physicochemical parameters that determine the properties of foam films (Exerowa and Kruglyakov, 1998).

Parameters such as antifoaming agent dosage, costs of the antifoaming agent, and influence on the biogas process, are essential for evaluation of the economic viability of the antifoaming strategies. It has been previously reported that each agent has a specific concentration where it has its optimum antifoaming effect, below which is less effective, while above the optimum concentration may act as foam stabilizer (Karakashev and Grozdanova, 2012). Denkov (2004) reported that the typical antifoam concentration range of emulsions is in the range of 0.1–1 wt%, while the concentration range of the active compounds in the emulsions is equal to 0.01–0.1 wt %. Yet, according to the specifications of manufacturers a typical dosage of commercial antifoaming agents suitable for bioprocesses is 0.1% v/v.

In full scale biogas plants the defoamers are added in the pre-storage tank, so as to be diluted with the substrate, creating a homogenous mixture. This means that the defoamers are directly mixed into the reactor content and are not dispersed by spraying onto the formed foam. Most of the antifoaming agents used in AD processes are commercial and often the specific composition of these chemical solutions is not provided by the suppliers. In the cited literature, scientific information and experimental data concerning the efficiency of chemical compounds on AD foam prevention and suppression are very limited.

The aim of this work was to investigate the antifoam efficiency of 14 non-commercial and commercial antifoaming agents in manure samples, using an aeration test method. Two aeration tests were performed. In the first aeration test, the effect of concentration level of the antifoaming agents was investigated. During the second aeration test, two different antifoam application methods (headspace and bottom injection) were evaluated. The current study is the first research that provides a qualitative and quantitative comparison of the specific substances concerning their ability to suppress foam in manure substrates.

2. Materials and methods

2.1. *Manure characterisation*

The raw cattle manure used in the experiment was obtained from Hashøj biogas plant, Denmark. After arrival to the laboratory, the manure was shredded and sieved to separate large particles and stored at −20 °C. The frozen manure was thawed at 4 °C for 2–3 days before use. The digested cattle manure was derived from laboratory reactors facing foaming problems due to overloading (Kougias et al., 2013). The reactors (working volume 1.5 L, total volume 2.0 L) were operated at thermophilic conditions with a Hydraulic Retention Time (HRT) of 15 days. The reactors were fed with a mixture of raw cattle manure and glucose. Specifically, in raw manure was added sufficient quantity of glucose, so as the final mixture to obtain a total solids content of 11% and consequently the Organic Loading Rate (OLR) of the reactors was 6.2 g VS/L-reactor day. The characteristics of raw and digested manure are presented in Table 1.

2.2. *Antifoaming agents*

The 14 antifoaming agents used were among the categories of Long Chain Fatty Acids (LCFA), natural oils, esters, commercial antifoams, salts and alcohols. Two mono-unsaturated and two saturated LCFA were selected; erucic acid (~90%,

Table 1 – Cattle manure characteristics.

Parameter	Unit	Raw cattle manure	Digested cattle manure
		Values	
pH	–	7.43 ± 0.01	8.25 ± 0.01
Total solids (TS)	g/L	61.6 ± 0.7	69.6 ± 0.2
Volatile solids (VS)	g/L	47.5 ± 0.6	48.7 ± 0.2
Total Kjeldahl Nitrogen (TKN)	g/L	3.30 ± 0.17	2.76 ± 0.05
Ammonium Nitrogen ($N-NH_4^+$)	g/L	2.11 ± 0.14	1.27 ± 0.03
Total Volatile fatty acids (VFA)	mg/L	5535 ± 431.6	2114.4 ± 133
Acetate	mg/L	3151.1 ± 353.4	733.7 ± 30.3
Propionate	mg/L	1288.3 ± 65.7	858.8 ± 56.4
Iso-butyrate	mg/L	138.6 ± 2.0	147.2 ± 11.5
Butyrate	mg/L	608.6 ± 10.4	149.6 ± 11.5
Iso-valerate	mg/L	191.2 ± 0.4	169.2 ± 15.2
Valerate	mg/L	126.8 ± 0.0	26.2 ± 6.4
n-Hexanoate	mg/L	30.6 ± 0.3	29.7 ± 1.7

Sigma–Aldrich), oleic acid (90%, Sigma–Aldrich), stearic acid (95%, Sigma–Aldrich) and octanoic acid (≥98%, Sigma–Aldrich). The category of natural oils involved rapeseed and sunflower oil that were obtained commercially. Two different esters were examined in the experiments; Sorbitan monostearate, denoted as Span 60 (Sigma–Aldrich) which is an ester of sorbitan and stearic acid and tributylphosphate (≥99%, Sigma–Aldrich) which is an ester of orthophosphoric acid with n-butanol. Two commercial antifoams were tested; Struktol SB 2113 Dimethylpolysiloxane, denoted as PDMS, which an emulsion of silicone and Struktol SB 2080, which is derivative of natural fatty acids. From the category of salts, three compounds were selected as antifoams: polyaluminium chloride (Akzo Nobel), calcium chloride dihydrate (≥99%, Sigma–Aldrich) and magnesium chloride hexahydrate (~99%, Sigma–Aldrich). As a representative of alcohols, ethanol (99.99%, Kemetyl) was selected. The non-commercial compounds tested in the present study were reported in the cited literature to possess direct or indirect antifoam properties (Tsuge et al., 1984; Vardar-Sukan, 1988; Jain et al., 1989; Westlund et al., 1998; Marinova and Denkov, 2001; Zhang et al., 2003; Barber, 2005; Ran et al., 2011). However, none of these substances was tested in manure digestion systems. The examined concentrations of each antifoaming agent were three; 0.05%, 0.1% and 0.5% v/v_{sample}.

2.3. *Foaming potential methodology and calculation*

The foaming potential of a sample was determined by the aeration method as described by Boe et al. (2012). The apparatus was consisted of an acrylic cylinder (inside diameter 4.5 cm, height 40 cm) with a ceramic air diffuser (diameter 1.5 cm, length 2.5 cm) placed at the bottom of the cylinder (Fig. 1). The cylinder was closed with a rubber stopper during aeration. The rubber stopper had two openings; one was used for air supply to the diffuser and another one was used for the headspace injection of the antifoam and for air outlet. Additionally, the cylinder had a small opening in the bottom that was sealed with a rubber stopper to avoid any leakage. This opening was used in order to inject the antifoams directly in the liquid phase of the sample (bottom injection). A 100 mL sample was added in the cylinder and was aerated twice with an air flow rate of 120 mL/min for 10 min each time. In the beginning the sample consisted only of manure and after the first aeration period the volume of the formed foam was recorded. Then, without stopping the air supply, the antifoaming agent was injected in the column and subsequently the mixed sample that contained manure and antifoam was aerated for another 10 min. Just after the second aeration, the volume of foam in the column was measured again.

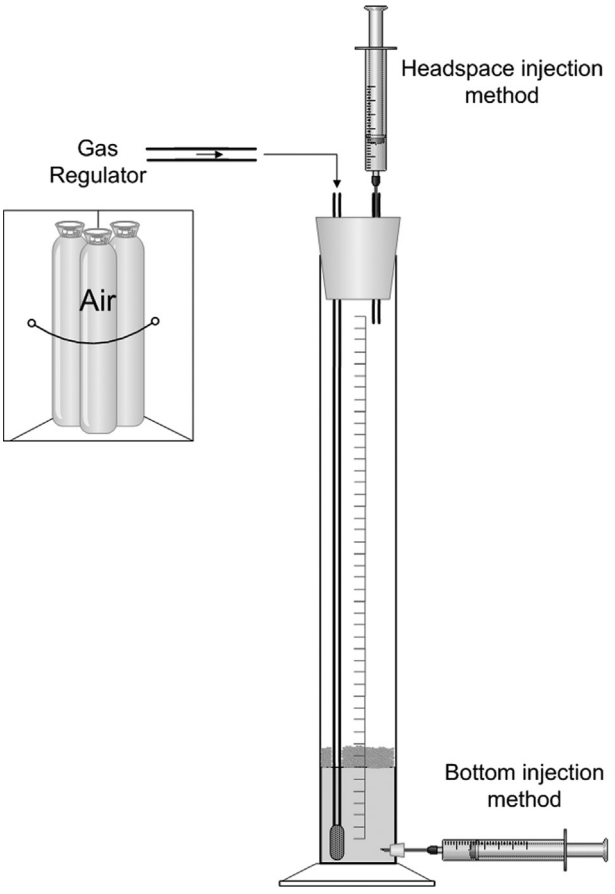

Fig. 1 – Foaming apparatus used for the aeration method.

Thereafter, the air supply was stopped and the sample was kept in the column in order to estimate the foam stability by measuring the remaining foam after 1 h. The foaming potential was defined using three parameters, namely as foaming tendency (Eq. (1)), foam stability (Eq. (2)) and foam reduction efficiency (Eq. (3)).

$$\text{Foaming Tendency} = \text{Volume of foam right after aeration (mL)/air flow rate (mL/min)} \quad (1)$$

$$\text{Foam Stability, \%} = (\text{Foam volume remaining in the cylinder at 1 h after aeration (mL)/Foam volume right after aeration (mL)} * 100 \quad (2)$$

$$\text{Foam Reduction Efficiency, \%} = (1 - \text{Foaming tendency of sample with antifoaming agent/Foaming tendency of sample without antifoaming agent}) * 100 \quad (3)$$

All the determinations were performed in triplicate.

2.4. Aeration tests

Two aeration tests were conducted. In the first test, aeration was applied to raw and digested manure samples and foam tendency and stability were determined. Subsequently, antifoaming agent was added on the formed foam, and the foam properties were determined again. In that test 14 antifoaming agents were evaluated for their efficiency to suppress the formed foam. Five of the antifoaming agents that exhibited the highest efficiency from this aeration test were chosen for further investigation in a second aeration test. In the second test, the sample was aerated for 10 min in order to create foam. After 10 min of aeration, antifoaming agents were quickly injected to the sample while continuing aeration for another 10 min. After the aeration stopped, the volume of foam left in the column was then recorded. The antifoaming agents were injected to the sample from two different points; either by applying directly on top of the foam surface (headspace injection), or by injecting into the liquid phase at the bottom of the sample (bottom injection)."

The sample used in the second aeration test was a mixture of raw cattle manure and glucose.

2.5. Statistical analysis

Data analysis was conducted using "Statistical Package for the Social Science SPSS for Windows" version 20. Descriptive statistics were performed for all variables and mean values, standard deviations and standard errors were calculated. Normality test and subsequently one-way analysis of variance (ANOVA) was carried out in order to compare the quantitative variables between the different groups.

3. Results and discussion

3.1. Effect of chemical compounds on foam reduction in raw and digested cattle manure

During the first aeration test, the differences in the foaming properties of raw and digested manure with and without the addition of antifoaming agents were determined in order to evaluate the foam reduction efficiency of 14 commercial and non-commercial compounds. The average foaming tendency of raw and digested cattle manure were 2.65 ± 0.81 mL-foam/mL-air min and 2.97 ± 0.82 mL-foam/mL-air min, respectively. No foaming stability was recorded, as after ending the air supply, the foam collapsed in less than 1 min. These results are in accordance with a previous study reporting that an increase in manure concentration at 6% TS raises the foaming tendency value up to more than 2 mL-foam/mL-air min, while decreasing the foaming stability of the medium (Boe et al., 2012).

Fig. 2 depicts the foam reduction efficiency of the antifoaming agents in raw cattle manure samples. The results showed that the foam reduction efficiency was influenced by the concentration of the agents that were injected in the substrate. Specifically, it was observed that an increase in the antifoam dose improved the foam reduction efficiency in almost all tested antifoaming agents. Thus, at antifoam concentration equal to 0.05% v/v_{sample} (Fig. 2A) and 0.1% v/v_{sample} (Fig. 2B), only Struktol SB 2080 and tributylphosphate managed to suppress the foam totally, while by increasing the antifoam concentration to 0.5% v/v_{sample} (Fig. 2C) PDMS, Struktol SB 2080, rapeseed oil, sunflower oil and tributylphosphate succeeded to fully break down the formed foam.

Statistical analysis of the data revealed that the efficiency of the antifoaming agents varied significantly among the same added concentration and as a consequence it was possible to classify them into groups according to their foam reduction efficiency. The different groups are marked with letters a-e in Fig. 2 and the statistical significance to distinguish between the groups of applied antifoaming agents within a certain concentration was set at $p < 0.05$. At the lowest antifoam concentration, 0.05% v/v_{sample} (Fig. 2A), the group of most efficient antifoaming agents included the commercial antifoams, the natural oils, tributylphosphate and octanoic acid. These compounds managed to suppress the foam in a range of 81–100%. Yet, the efficiency of sunflower oil did not vary significantly with the corresponding one of oleic acid that achieved a foam suppression of 67%. In contrary, the least efficient antifoaming agent was ethanol that presented adverse impact on foam suppression, by promoting foam formation by approximately 30%. The ability of ethanol to act as foam promoter, when its concentration in the substrate is very low, is of a great importance, because ethanol is a well known intermediate in anaerobic digestion process (Chen et al., 2008). Nevertheless, in the present study it was found that ethanol can only enhance foam formation when diluted in raw manure (Fig. 2A), while when added in digested manure it behaves as antifoam with a limited efficiency (Fig. 3). This indicates that the amounts of ethanol that are normally present in a biogas reactor will probably not

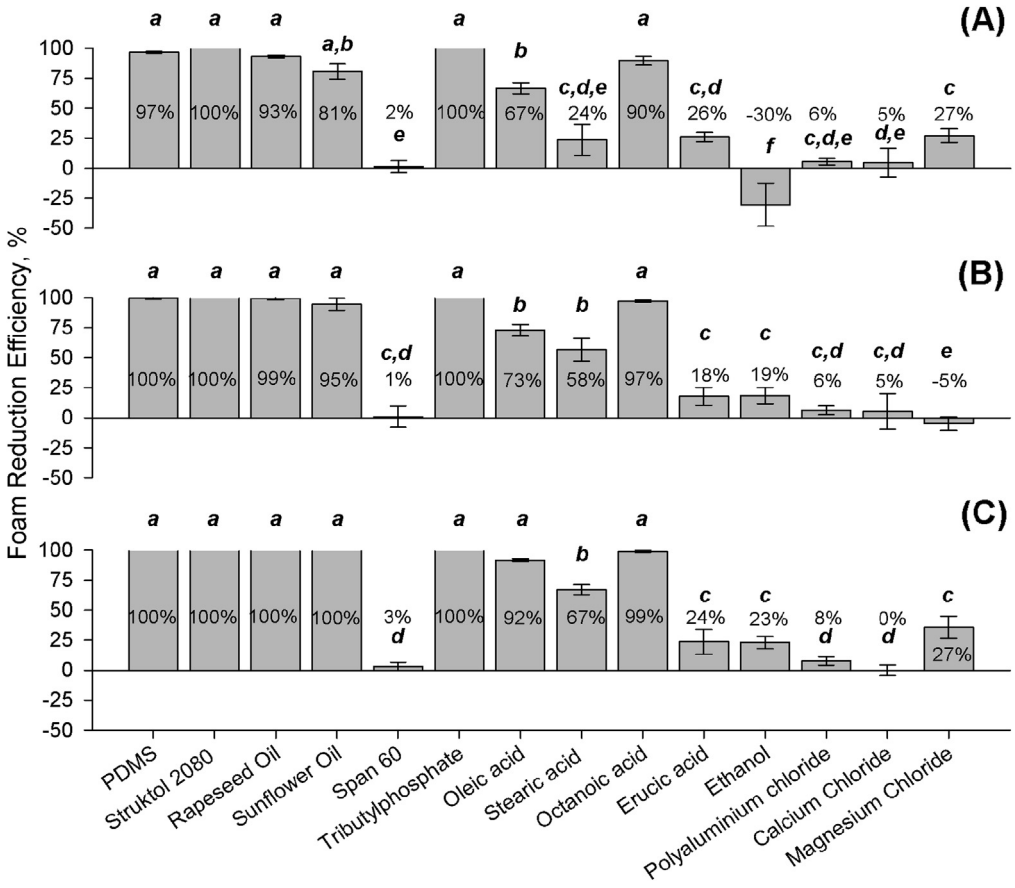

Fig. 2 – Foam reduction efficiency of antifoaming agents in raw cattle manure; antifoam concentration (A) 0.05% v/v_{sample}, (B) 0.1% v/v_{sample} and (C) 0.5% v/v_{sample}. Different letters above the bars signify distinct statistical groups ($p < 0.05$) between the applied antifoaming agents within a certain concentration. The letter above a bar designates the group that the corresponding antifoaming agent belongs in, according to insignificant statistical difference in efficiency (ANOVA).

cause any foaming problem. The selective ability of ethanol to act as foam promoter or as foam suppressor could be possibly explained due to the different protein profile of raw and digested cattle manure. It has been previously reported that ethanol in low concentrations can increase the foaming stability of aqueous protein solutions, while in higher amounts may lead to foam instability together with the aggregation of proteins in bulk solutions associated with denaturation (Ekemen et al., 2011). However, the specific antifoaming mechanism of short chain alcohols, like ethanol, has not been thoroughly investigated.

By increasing the antifoam concentration to 0.1% v/v_{sample} in the substrate (Fig. 2B), the commercial antifoams, the natural oils, tributylphosphate and octanoic acid still remained in the group of the most efficient antifoaming agents. The foam reduction efficiency of these agents varied significantly compared to all the other tested compounds, as they managed to break down the formed foam by 95–100%. In contrast to the previous applied antifoam dosage, ethanol did not enhance foam formation, but magnesium chloride slightly promoted foaming. This is in accordance with a previous study where magnesium slightly increased the foaming tendency, and thus promoted foaming, when added as a single compound in raw manure (Boe et al., 2012). According to the same research, magnesium has the potential to decrease foaming tendency in the presence of other compounds like LCFA, because magnesium tends to form insoluble salts which could precipitate surfactants and thus reduce foaming. However, Zhang et al. (2004) reported that magnesium could present defoaming effect in the absence of oils, although this effect could not be explained.

At the highest added antifoam concentration, 0.5% v/v_{sample} (Fig. 2C), oleic acid was classified in the group of the most efficient antifoaming agents along with the best ones found during the treatments in which 0.05% and 0.1% v/v_{sample} antifoam were applied. The foam reduction efficiency of this group was in the range of 92–100%.

It should be highlighted that oleic acid managed to increase its foam reduction efficiency from approximately 67–73% and finally to 92%, with increasing dosing from 0.05%, to 0.1% and to 0.5% v/v_{sample} oleic acid, respectively. It is well known that oleic acid, as all LCFAs, has an amphiphilic structure and thus in acidic form is poorly soluble in aqueous solutions, while in the form of salts it is relatively hydrophilic (Haridas et al., 2005; Alves et al., 2009). In that way, when oleic acid was added in raw manure samples showed a lipophilic behavior,

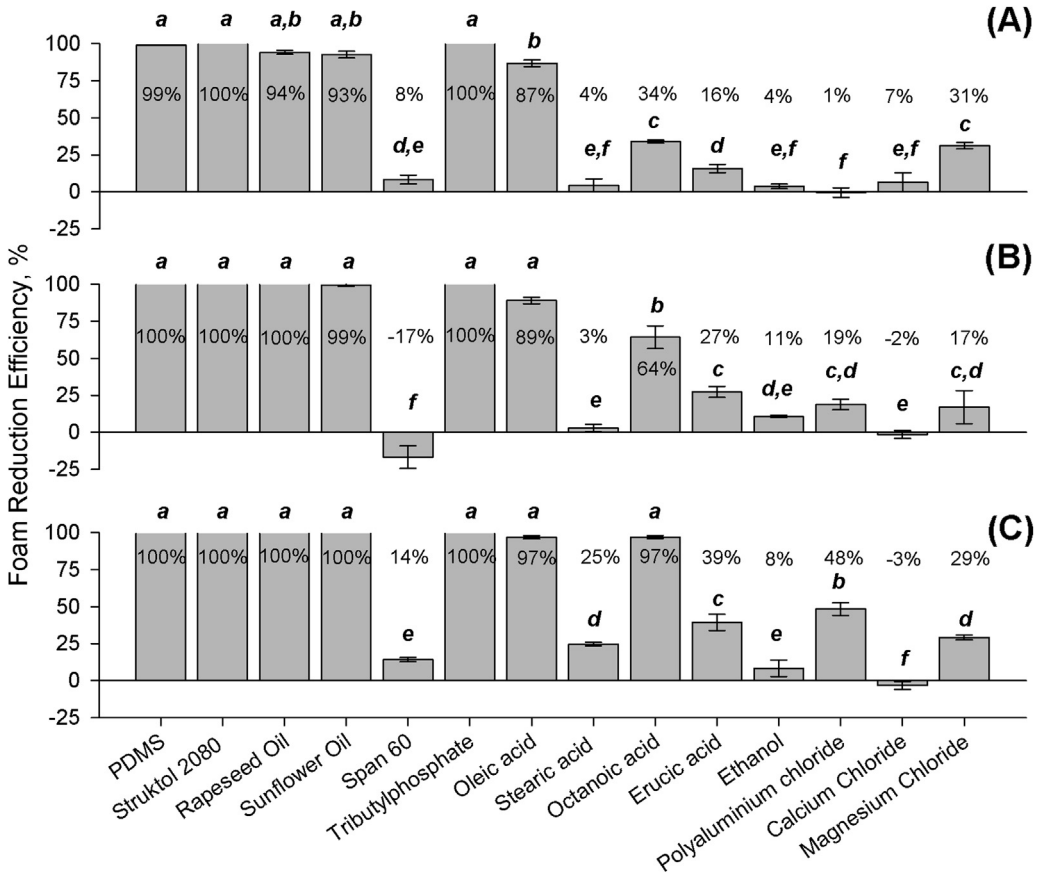

Fig. 3 – Foam reduction efficiency of antifoaming agents in digested cattle manure; antifoam concentration (A) 0.05% v/v_{sample}, (B) 0.1% v/v_{sample} and (C) 0.5% v/v_{sample}. Different letters above the bars signify distinct statistical groups ($p < 0.05$) between the applied antifoaming agents within a certain concentration. The letter above a bar designates the group that the corresponding antifoaming agent belongs in, according to insignificant statistical difference in efficiency (ANOVA).

not allowing foam bubbles to be formed on the liquid surface of the substrate.

As described previously, the digested cattle manure obtained from the liquid phase of the reactors that was having foaming incidents during the sampling time caused by organic overloading. By visual inspection it could be observed that the foam generated after the aeration of the digested cattle manure without the addition of antifoaming agents was the same as the one created inside the reactors under operation. This indicates that the applied aeration method could simulate the foam formation that actually occurred inside the reactors. Fig. 3 illustrates the foam reduction efficiency of the tested compounds on digested cattle manure samples. The ability of the antifoaming agents to improve their influence on foam break down as their concentration in the substrate increased was also shown in digested manure.

Based on the statistical analysis, the commercial antifoaming agents, the natural oils and tributylphosphate were the most efficient antifoaming agents, managing to suppress the foam by approximately 93–100%, when their concentration in the substrate was 0.05% v/v_{sample} (Fig. 3A). On the other hand, the least efficient compound was polyaluminium chloride. It was shown that the addition of 0.05% v/v_{sample} of polyaluminium chloride was not sufficient enough to reduce the formed foam. However, its efficiency was considerably improved by injecting higher amounts of this compound in the substrate (Fig. 3B and C). Westlund et al. (1998) reported that polyaluminium salt at concentrations of 3–6 g Al kg^{-1} TSS (total suspended solids) exhibited good results on foam suppression. The foam reduction efficiency of natural oils did not vary significantly with the corresponding one of oleic acid (87%). It should be highlighted that at every applied antifoam concentration the impact of oleic acid on foam reduction was higher in digested manure, compared with raw manure samples. The same trend was observed also for erucic acid, indicating that the improved ability on foam reduction in digested manure was correlated to the unsaturated LCFA. Nevertheless, an explanation for this correlation is not yet understood, due to the fact that manure contains several compounds that could potentially be linked with foaming.

Oleic acid was classified along with the commercial antifoams, natural oils and tributylphosphate in the group of the most efficient antifoam agents at a concentration of 0.1% v/v_{sample} (Fig. 3B). The effectiveness of this group was in a range of 89–100%. Moreover, it was observed that Span 60 enhanced foam formation by approximately 17%. Span 60 did not

present reliable antifoam activity, as its efficiency was in general very low and not stable, considering that in different added amounts acted either as foam suppressor or foam promoter. Probably, Span 60 could not act as a successful antifoaming agent when added as a single compound in the substrate. Marinova and Denkov (2001) reported that Span 60 exhibited strong effect on the antifoam activity in both anionic and nonionic surfactant media, when added in antifoams containing silica, due to the achievement of a synergistic effect between the compounds.

Finally, at the highest added antifoam concentration, 0.5% v/v_{sample} (Fig. 3C), octanoic acid was classified in the group of the most efficient antifoaming agent together with the best ones found during the antifoam applications of 0.05% and 0.1% v/v_{sample}. In general, octanoic acid presented a stronger antifoam behavior in raw cattle manure and was able to suppress the foam totally in digested manure samples only in the highest antifoam dosage. In the present study it was proven that the foam reduction efficiency of the saturated LCFA was higher in raw manure, as the same behavior was also observed in case of stearic acid. As discussed previously, manure is a complex substrate and a possible elucidation of this property of the saturated LCFA is not clear yet.

To sum up the results from the first aeration test, the commercial antifoaming agents, the natural oils (i.e. rapeseed and sunflower oil), oleic acid, octanoic acid and tributylphosphate presented the highest foam reduction efficiency among the 14 tested compounds, both in raw and digested cattle manure samples. The results from the current aeration test are in accordance with other researches that have reported significant high efficiency of antifoaming agents with identical or similar chemical composition with the commercial antifoaming agents and tributylphosphate used in the present study (Tsuge et al., 1984; Marinova and Denkov, 2001; Barber, 2005). However, the high foam reduction efficiency of natural oils was remarkable, and is in contrast from what has been previously reported from Vardar-Sukan (1998). Vardar-Sukan (1998) has reported that the efficiency of these compounds is limited because they have high viscosity, are easily metabolized and are poorly dispersed in the medium. Moreover, in the present study it was shown that oleic acid could successfully reduce the formed foam when added as a single compound in the substrate. In the cited literature, oleic acid exhibited good defoaming action when mixed with other compounds (e.g. mixtures of oleic acid and triolein) (Zhang et al., 2003). However, the defoaming action of oleic acid was not tested in the absence of these substances. To the best of our knowledge there are not any other experimental information and studies to compare the effect of octanoic acid found in the present test.

The evaluation of the ability of the antifoaming agents to break down the foam was based mainly on physicochemical properties and reactions with the substrate. The present test of the effectiveness of certain antifoaming agents is a common type of foam-related test (Höfer et al., 2000). However, before applying these antifoaming agents in a full scale biogas plant it is very crucial to test them in a continuous digestion system, so as to investigate their long term effect on the bioprocess and also their influence on the microbial ecology of the reactors. Specifically, a combined analysis on foam reduction efficiency and biodegradability of different compounds is an important issue for future investigations. The antifoaming agents should be added in a manure biogas reactor until obtaining steady state conditions and their effect on the biomethanation process should be studied. Moreover, it should be examined whether the addition of antifoaming agents create unfavorable or toxic conditions for the growth of the specific microorganisms that are necessary for the achievement of methanogenesis. These will help in order to avoid a possible deterioration and unstable performance of the biogas reactor. Another important issue that will have great impact on foam prevention and foam suppression is the understanding of the mechanisms of foam formation and foam destruction in manure-based matrices. This will lead to the more efficient strategies to solve foaming problem in manure digesters.

3.2. *Evaluation of the application method of the antifoaming agent*

Five selected antifoaming agents exhibiting the highest foam reduction efficiency in the first aeration test were investigated by applying them in the substrate by two different methods. The antifoaming agents used were rapeseed oil, oleic acid, octanoic acid, tributylphosphate and Struktol SB 2080. Each antifoaming agent was examined again in three different concentrations, 0.05%, 0.1% and 0.5% v/v_{sample}.

Fig. 4 illustrates the foam reduction efficiency of the applied antifoaming agents by injecting them in the headspace and from the bottom of the column. Tributylphosphate and Struktol SB 2080 managed to break down the foam totally, even at the lowest added antifoam concentration, in both injection methods. This indicates that these compounds are capable on changing the interfacial properties of a liquid, resulting in extensive foam suppression. Their strong defoaming behavior seemed to be independent on the applied injection method and the amount of antifoam added in the substrate. However, it was observed that the effect of tributylphosphate on foam reduction was quicker than the effect of Struktol SB 2080. This was probably due to their different mechanism on foam destruction owing to the chemical composition of each compound. Tributylphosphate is a solvent and has a lower surface tension (0.029 N/m) (Andrukh et al., 2011) compared with the surface tension of cattle manure (0.052–0.059 N/m) (Boe et al., 2012). Vardar-Sukan (1998) has reported that when the surface tension of the antifoaming droplet is lower than the corresponding one of the foaming film, the antifoaming agent is rapidly spreading on the surface of the film, leading the liquid of the media to be squeezed and as a result the film gets thinner and collapses. In contrary, the chemical composition of Struktol SB 2080 is derivative on natural fatty acids having hydrophobic behavior. Vardar-Sukan (1998) has reported that when a hydrophobic droplet contacts the foaming film, the liquid of the media flows away from the droplet leading the foam to collapse. Furthermore, it was observed in both antifoaming agents that in the case of bottom injection the needed time for total foam collapse was longer compared with the headspace injection (data not shown).

Rapeseed oil, oleic acid and octanoic acid were more efficient to reduce the formed foam when bottom injection

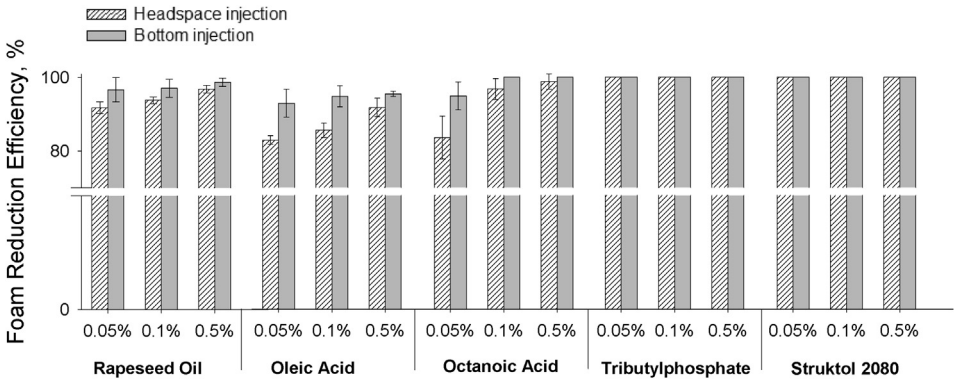

Fig. 4 – Foam reduction efficiency of antifoaming agents during headspace and bottom injection methods.

method was applied compared with the headspace injection. This could be explained due to the fact that the antifoaming agents were dispersed more widely when added directly into the liquid phase of the medium and as a consequence their physicochemical effect on changing the foaming properties was increased. This is in accordance with Jha et al. (2000) who reported that the antifoam efficiency of an agent is highly depending on its spreading ability in the medium. Moreover, it should be highlighted that these compounds presented instant antifoam activity during headspace injection, resulting in an immediate destruction of the foam, up to a limit. This was due to the instantaneous interaction between the compounds and the foam bubbles. Denkov (2004) previously described the ability of oils and of mixtures of oils together with hydrophobic particles, to rupture foam films quickly by a bridging-stretching mechanism. According to this mechanism, once the antifoam globule enters the foam film it creates an oil bridge followed by capillary pressures at the oil–water and air–water interfaces. Subsequently, the bridge stretches and as a consequence the foam film is ruptured. In addition, it was also observed that during both injection methods and as the concentration of the added compounds was increased, the needed time for foam destruction was shorter (data not shown).

Furthermore, the efficiency of rapeseed oil, oleic acid and octanoic acid to reduce foam was increased as the total solids concentration increased. As described previously, the substrate used during this test, was raw cattle manure with glucose, and was more concentrated compared to the raw manure used during the first aeration test. It has been previously been reported that manure with high TS content, was able to produce thicker foams than diluted manure (Zhang and Zhu, 2005). This was also found in the present investigation, since the foaming tendency of raw cattle manure with glucose was found 11% higher than the corresponding one without glucose addition. Based on this finding and according to the Eq. (3), for a certain foaming tendency of a substrate with the addition of antifoaming agent (equal numerator of the equation), the foam reduction efficiency of raw manure with glucose would be higher (higher denominator of the equation), compared to raw manure (lower denominator of the equation). However, in the present study, it was observed that the increased foam reduction efficiency was mainly owing to a further decrease in the foaming tendency of the mixed substrate after adding the antifoaming agents. More specifically, the foaming tendency of the mixed substrate after the addition of oleic acid at concentrations of 0.05%, 0.1% and 0.5% was 48%, 27% and 22% lower, respectively. The decrease of the foaming tendency was even greater in case of octanoic acid as its addition at concentrations of 0.05%, 0.1% and 0.5% resulted in lowering the foaming tendency of the mixed substrate by 81%, 95% and 80% respectively, compared with the foaming tendency of raw manure.

Antifoam injection from the bottom of the reactor, showed stronger foam suppression effect compared with the headspace injection method. This shows that the common practice of the full scale biogas plants to add the antifoaming agents in the pre-storage tank is a good practice for antifoam application. Nevertheless, in cases where the foam is created rapidly and its immediate suppression is imperative, it is recommended to apply the headspace injection method by spraying the antifoaming agents onto the formed foam.

4. Conclusions

By determining the defoaming efficiency of 14 commercial and noncommercial substances, it was found that rapeseed oil, sunflower oil, oleic acid, octanoic acid, tributylphosphate and the commercial antifoams were the most efficient compounds to suppress foam in raw and digested cattle manure samples. Moreover, the antifoams showed stronger effect when applied in the bottom of the reactor. These findings can contribute in the development of antifoam strategies in manure based anaerobic digesters.

Acknowledgments

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Når biogasanlægget skummer over

Skum i biogasanlæg kan være et både økonomisk og miljømæssig problem for mange anlæg. Skum betyder faldende gasproduktion, overfyldte lagertanke og udslip af metangas. Høj organisk belastning er den vigtigste årsag til skum i reaktoren, men sammensætningen af biomassen har også en vis indflydelse.

Foto: DTU Miljø

Forsøg med skumdannelse i laboratorieanlæg.

Af Irini Angelidaki og Panagiotis Kougias

Mange biogasanlæg oplever i perioder, at der dannes skum i toppen af reaktoren eller i for- eller efterlagertanke. Derved falder gasproduktionen, dels fordi det aktive volumen i reaktortanken reduceres, dels fordi skummet kan have en negativ indflydelse på den biologiske proces.

Indtørret skum kan endvidere forårsage alvorlige driftsproblemer i form af blokerede gasmålere og ødelagte pumper. Skumning er således både en økonomisk belastning for mange biogasanlæg, men det er også et miljømæssigt problem, fordi skummet kan give anledning til overfyldte lagertanke og udslip af metangas.

Årsager til skumning

På DTU Miljø har vi med støtte fra ForskEL-programmet undersøgt, hvad de primære årsager er til, at der dannes skum i biogasanlæg. Er det biomassens sammensætning, den organiske belastning af reaktoren eller en kombination af begge dele?

Ved trinvist at øge den organiske belastning og koncentrationen af fedt, proteiner og kulhydrater i laboratorieanlæg har vi gennem en periode på tre måneder kunnet følge årsagerne til skumdannelse.

Resultaterne viser, at den organiske belastning er den vigtigste årsag til, at der dannes skum i reaktoren, mens sammensætningen af biomassen i kombination med den organiske belastning kan forårsage skumning.

Mere specifikt kan proteiner indlede skumdannelsen ved en lavere organisk belastning end fedtstoffer. Desuden er mængden af skum, forårsaget af proteiner, forholdsvis stabil og bliver ikke forøget ved en yderligere forøgelse af den organiske belastning eller ved en forøgelse af proteinindholdet i biomassen.

Projektet afsluttes i efteråret 2014.

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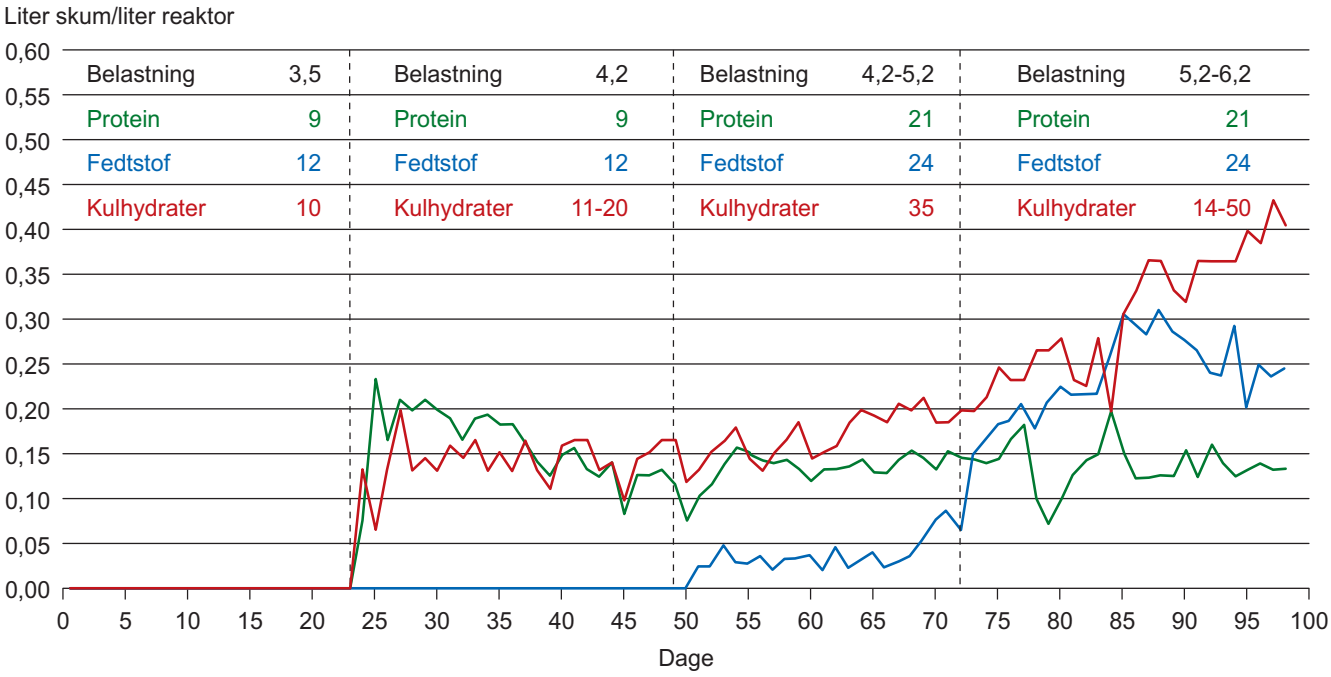

Skumdannelse i laboratorieanlæg med varierende belastning og forskellige typer biomasse.

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Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors

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HIGHLIGHTS

- Organic loading rate was the main factor affecting foaming.
- An organic loading rate of 3.5 gVS/(L-reactor·day) was safe to avoid foaming.
- Proteins initiated foaming at lower organic loading rate than lipids.
- Foaming from gelatine had more stable volume compared to Na-Oleate.
- Foaming is rather related to increase of biogas production and not inhibition.

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ABSTRACT

Foaming is one of the major problems that occasionally occur in biogas plants, affecting negatively the overall digestion process. In the present study, the effect of organic loading rate (OLR) and feedstock composition on foaming was elucidated in continuous reactor experiments. By stepwise increasing the OLR and the concentration of proteins or lipids in the substrate, foaming in biogas reactors was investigated. No foam formation was observed at the OLR of 3.5 g volatile solids/(L-reactor·day). Organic loading was the main factor affecting foam formation in manure digester, while the organic composition, such as content of proteins or lipids were factors that in combination with the organic loading were triggering foaming. More specifically, gelatine could initiate foam formation at a lower OLR than sodium oleate. Moreover, the volume of foam produced by gelatine was relatively stable and was not increased when further increasing either OLR or gelatine concentration in the feed.

1. Introduction

Foaming is a serious problem in many biogas plants, since it affects negatively the overall digestion process. The foam is typically formed in the main biogas reactor or in the pre-storage tank. Entrapped solids in the foam cause severe operational problems, such as blockage of gas meters, and collapse of pumps. In sludge digesters, foaming leads to inverse solids profile with higher solids concentrations at the top of a digester, resulting in the formation of dead zones and thus reducing the digester active volume (Ganidi et al., 2009). It has also been observed in full-scale manure digestion that foaming incidents lead to reduction of biogas production for shorter or longer periods (Nielsen and Angelidaki, 2008). Foaming incidents result in negative economic consequences for the biogas plant, due to income losses as a result of the reduced biogas production, costs for extra labour work and additional maintenance costs (Barber, 2005; Barjenbruch et al., 2000). Moreover, foaming presents adverse environmental impacts owing to the overflowing of the pre-storage or digester tanks and increased losses of methane through the effluent, due to oversaturation of methane in the liquid phase.

In the literature, a number of possible causes for foam formation in sewage sludge digesters have been suggested. Ross and Ellis (1992) who investigated the correlation between the sludge loading and foaming in the digester suggested that the overloading and the accumulation of acetic acid resulted in reduction of gas production and stable foam formation. Foaming in sludge digesters sometimes links to foaming incident in the activated sludge process where filamentous bacteria could be the main reason, as they produce biosurfactants and contribute in foam partitioning which prevents foam destruction (Heard et al., 2008). Dalmau et al. (2010) have developed a knowledge-based model to predict the risk of foaming in sludge digesters, by selecting organic loading rate, variation in organic loading rate, and presence of filamentous microorganisms, as inputs. Moreover, Barjenbruch et al. (2000) and Barber (2005) also reported that inadequate mixing, temperature

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fluctuations, hydrophobic substances and extracellular polymeric materials could increase foam formation in the sludge digestion tanks. Among all the above mentioned causes, organic loading rate (OLR) and substrate composition have crucial effect on foaming in most cases. Organic overloading of a digester may lead to accumulation of intermediate degradation compounds that promote or stabilize foam. Thus, substances promoting foaming in biogas reactors can either be surface active agents that are present in the complex feed mixtures, such as fats, oils, greases and polymers (Barber, 2005; Massart et al., 2006) or intermediate degradation compounds, such as volatile fatty acids (VFAs) (Westlund et al., 1998). Murto et al. (2004) investigated co-digestion of sewage sludge with pig-manure and reported that no foam was observed in a reactor operating with an OLR of 3.1 gVS/(L-reactor d), while another reactor with higher OLR (3.7 gVS/(L-reactor d)) had unstable performance and foaming incidents. Nevertheless, another study reported foaming incidents at lower organic loading (Ganidi et al., 2011). In this investigation the critical organic loading for foam formation in 1 L batch reactors digesting sewage sludge was 2.5 gVS/L-reactor, while at organic loading of 5 gVS/L-reactor stable foam was observed.

In anaerobic co-digestion systems, the feeding substrates contain a variety of different compounds. Among these compounds, proteins and lipids have been classified as foaming agents. From a previous investigation using physicochemical tests we have concluded that high content of sodium oleate (Na-Oleate), as representative of lipids, or the high content of gelatine, as representative of proteins, in manure mixtures, had strong positive correlation to foaming (Boe et al., 2012). The foaming effect from proteins is based on the synthesis of ionisable structures with both hydrophobic and hydrophilic available ends. Specifically, protein foams are formed by a protein film surrounding a gas bubble creating a structure that holds bubbles in place (Foegeding et al., 2006). The foaming effect from lipids is mainly caused by the fatty acids where the carboxylic end is free. Both long-chain fatty acids (LCFA) and short-chain fatty acid such as acetic acid have structures with free polic ends (carboxylic ends), which could exhibit surfactant properties (Boe et al., 2012). The identification of the foaming causes in a co-digestion system is difficult, since several compounds with potential foam promoting properties, are present. In particular, there has never been a thorough investigation of foaming problems in manure based digesters, although the aforementioned problem is often appearing in full-scale plants. This study is the continuation of the work following our preliminary investigation of the effect of substrate compound on foaming by physicochemical tests (Boe et al., 2012). The aim of this study was to investigate the effect of proteins and lipids, which had shown strong foaming potential in our previous study, and the effect of organic loading rate (OLR), on foaming in continuous stirred tank reactors (CSTR) reactors, in order to identify the correlation between OLR, substrate composition, foam formation and process performance.

2. Methods

2.1. Waste characteristics and preparation of the feedstock

Cattle manure obtained from Snertinge biogas plant, Denmark, was used in the experiment. After arrival, the manure was shredded and sieved (5 mm) to separate large particles and stored at −20 °C. The frozen manure was thawed at 4 °C for 2–3 days before use. The characteristics of manure are presented in Table 1.

2.2. Experimental set up and operation

The experiment was carried out in five CSTR reactors. The total and the working volume of each reactor were 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54 ± 1 °C using thermal jackets. The five CSTR reactors represented two duplicate reactors for the test of protein (R1&R2), lipid (R3&R4) and one carbohydrate reactor (R5), respectively. The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The OLR of all reactors was increased by adding different organic compounds to the manure substrate as shown in Table 2. In reactors R1&R2, the influent manure was supplemented with gelatine as a representative of proteins, while in R3&R4 it was supplemented with Na-Oleate, as a representative of lipids. Reactor R5 was used for investigating the effect of OLR with addition of glucose. The whole experiment was divided into four periods. During each period, the OLR and gelatine or Na-Oleate concentration in the feed were increased explicitly, in order to distinguish between the effect of OLR, protein, and lipid, on foaming in the digesters. The reactors were automatically fed twice a day using peristaltic pumps, and biogas production was measured by an automated displacement gas metering system with 100 mL cycle (Angelidaki et al., 1992). Biogas production and foam formation were recorded daily, while methane content in biogas, volatile fatty acids concentration, and foaming potential in the liquid sample were measured once or twice per week.

2.3. Foaming potential methodology and calculation

The foaming potential of the solutions was determined by the aeration method as described by Boe et al. (2012). The apparatus was consisted of an Imhoff settling cone and a ceramic air diffuser placed at the bottom of the cone. A 50 mL sample, derived from each reactor, was aerated for 10 min in the settling cone with an air flow rate of 60 mL/min. The foam height in the settling cone was measured right after the aeration stopped and again 1 h later. The foaming potential was defined using three parameters: foaming tendency, foam stability and foam volume inside the reactor. The foaming tendency (mL-foam/(mL-air·min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). The foam stability was determined as percentage of foam remaining in the settling cone at 1 h after aeration compared to the volume of foam right after aeration. The determinations of foaming tendency and stability were carried out in triplicate. The foam formation inside the reactors was recorded daily. The volume of foam produced was determined by the measured average foam height multiplied with the surface area of the reactor. After each measurement, the reactor was rapidly stirred or shaken until all foam disappeared. Hence, the daily amount of foam that was formed inside the reactors was measured.

Table 1
Cattle manure characteristics.

Parameter	Unit	Values
pH	–	7.05 ± 0.01
Total solids (TS)	g/L	55.1 ± 0.80
Volatile solids (VS)	g/L	43.3 ± 0.79
Total kjeldahl nitrogen (TKN)	g-N/L	3.74 ± 0.06
Ammonium nitrogen (NH_4^+)	g-N/L	1.93 ± 0.02
Total volatile fatty acids (VFA)	mg/L	5447 ± 507
Acetate	mg/L	3729 ± 425
Propionate	mg/L	980 ± 68
Iso-butyrate	mg/L	122 ± 4
Butyrate	mg/L	379 ± 15
Iso-valerate	mg/L	173 ± 2
Valerate	mg/L	57 ± 2
n-hexanoate	mg/L	7 ± 0

Table 2
Experimental plan.

Period	Days	Reactor	OLR (gVS/L-reactor·d)	Added organic compounds (g/L-feed)*		
				Gelatine	Na-Oleate	Glucose
I	1–23	R1&2	3.5	9	–	–
		R3&4	3.5	–	12	–
		R5	3.5	–	–	10
II	24–49	R1&2	4.2	9	–	11
		R3&4	4.2	–	12	10
		R5	4.2	–	–	20
III	50–72	R1&2	4.2	21	–	–
		R3&4	4.2	–	24	–
		R5	5.2	–	–	35
IV	73–99	R1&2	5.2	21	–	14
		R3&4	5.2	–	24	16
		R5	6.2	–	–	50

* VS content of gelatine, Na-oleate and glucose were 98%, 81% and 99%, respectively.

2.4. Analytical methods

Total solids (TS) were determined by drying the samples at 103–105 °C to constant weight according to APHA standard methods for the examination of water and wastewater (2005). There after the dried samples were ignited at 550 °C to constant weight in order to determine the volatile solids (VS) (APHA (American Public Health Association), 2005). pH measurements were performed by a digital PHM210 pH meter that was connected to the Gel pH electrode (pHC3105–8, Radiometer analytical). Total nitrogen (TKN) and total ammonia were measured according to APHA standard methods for the examination of water and wastewater (2005). The methane and CO_2 content in biogas were determined with a gas-chromatograph (Shimadzu GC-8A, Tokyo-Japan) equipped with a glass column (2 m, 5 mm OD, 2.6 mm ID) packed with Porapak Q 80/100 mesh (Supelco, Bellefonte, PA, USA) and with a flame ionization detector (FID). Volatile fatty acids (VFAs) analysis was prepared by adding 0.1 mL of 34% H_3PO_4 to 1.5 mL sample in a 2 mL Eppendorf tube and centrifuged at 13,000 rpm for 10 min. The supernatant (1 mL) was transferred into the GC vial prepared with 100 μL internal standard (4-methyl-valeric acid) for analysis in a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) and a FFAP fused-silica capillary column, 30 m × 0.53 mm I.D., film thickness 1.0 μm, using nitrogen as a carrier gas. The oven temperature was initially set at 50 °C for 3.5 min. and then increased 25 °C/min to 130 °C followed by 10 °C/min to 210 °C, and kept at final temperature for 10 min. The injection port and detector temperatures were 150 °C and 230 °C, respectively. All the determinations were performed in triplicate.

3. Results and discussion

3.1. Effect of organic loading rate on foaming

The effect of OLR, as the main operational parameter, on foaming and reactor performance was investigated with and without the addition of protein or lipid. During the start-up period, all reactors were fed with raw cattle manure only, corresponding to the OLR of 2.89 gVS/(L-reactor·day), and no foam was observed. The effect of OLR with addition of excess glucose to the influent manure, in reactor R5 was investigated and the results are shown in Fig. 1.

A clear tendency of increased organic loading and increased methane production rate and foam formation can be seen in Fig. 1. Foaming inside this reactor was first observed at the OLR of 4.2 gVS/(L-reactor·day), and more volume of foam was produced as the reactor was more overloaded. Specifically, at the OLR of 4.2, 5.2 and 6.2 gVS/(L-reactor·day), the corresponding foam that was created inside the reactor was 0.15, 0.18 and 0.37 L/L-reactor, respectively. This could be explained by the combination of the increased biogas production rate which increased foam bubbling, and inadequate degradation of surface active agents during the high loading rates. Moreover, increased organic loading could have stimulated microbial production of biosurfactants. Additionally, Ganidi et al. (2009) reported that digester overloading can result in partial degradation of organic matter and accumulation of biosurfactants. The exceeded concentration of these substances can potentially contribute to foaming phenomena. To our knowledge, no previous investigation concerning correlation of OLR and foaming in manure based digesters, has been published, so it was not possible to compare our findings with cited literature. Nevertheless, a recent report investigating foaming in sludge batch digesters concluded that an organic loading of 2.5 gVS/L-reactor was the critical threshold for foam initiation while 5 gVS/L-reactor resulted in persistent foaming (Ganidi et al., 2011).

Contrary to the increase in foaming with OLR, the methane yield remained almost constant during the whole experiment (Fig. 1). The methane yield was 231, 271, 241 and 242 mL CH_4/gVS for the corresponding OLR of 3.5, 4.2, 5.2 and 6.2 gVS/(L-reactor·day), respectively. If we assume that all glucose added in the feed was fully degraded in the reactor, the theoretical methane yield at OLR of 3.5, 4.2, 5.2 and 6.2 gVS/(L-reactor·day) would be 217, 241, 267 and 284 mL CH_4/gVS, respectively. This indicates that the increase of OLR lead to lower degradation efficiency. The difference between actual and theoretical methane yields was up to 15% at OLR of 6.2 gVS/(L-reactor·day). However, the average VFA concentration at OLR of 3.5 and 6.2 gVS/(L-reactor·day) were 1.0 and 1.9 g/L, respectively. The increased VFA concentration of 0.9 g/L corresponded to the loss methane production of 22 mL

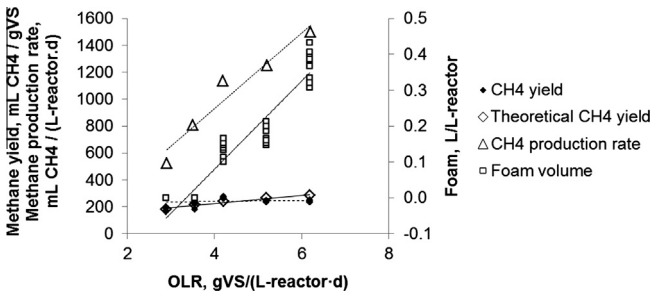

Fig. 1. Effect of OLR on methane yield, methane production rate, and foam formation without addition of protein or lipid. The OLR was increased by adding glucose.

CH_4/(L-reactor·d), which was much lower than the total methane loss of 261 mL CH_4/(L-reactor·d). Thus, it would be expected that large fractions of slowly degraded organic compounds in the manure came out from the reactor undigested. It is also worth mentioning that during the daily foam-removal procedure by fast stirring, significant amount of biogas was released into gas phase and the biogas production rate was clearly increased. This observation could explain the decrease of biogas production in the digester with foaming, indicating that the decrease was due to oversaturation of the liquid phase with biogas. Thus, a larger portion of methane was leaving the digester with the liquid phase, rather with the gas phase as biogas. The lower methane production during foaming periods, could have been mistaken as process inhibition, but was probably rather due to lack of equilibrium between gas and liquid phase and loss of methane with the effluent. It has also been previously reported that the foam covering the liquid surface prevents efficient gas–liquid transfer, thus decreases the biogas production (Ganidi et al., 2009). If the foam was not daily removed in our experiment, the methane production would have been lower. Moreover, it should be highlighted that during the experimental periods when the OLR was equal to 5.2 gVS/(L-reactor·day) or higher, the foam was very stable and thick. During these periods the foam could not be destroyed by stirring; could only be destroyed by vigorously manual shaking of the reactor.

3.2. Effect of organic loading rate under addition of protein and lipid

Fig. 2, illustrates the correlation between the OLR, methane yield and foam formation at specific concentrations of protein or lipid in the feed. During the first experimental period at OLR 3.5 g VS/(L-reactor·day) (Fig. 2a and c), no foam appeared although protein and lipids were added to the feed (gelatine 9 g/L-feed and Na-Oleate 12 g/L-feed, respectively), indicating that OLR was the main factor affecting foam formation, and low OLR was enough to prevent foaming regardless of protein or lipids addition. At this OLR, the average methane yield from the Na-Oleate reactor was higher than from the gelatine reactor (approx. 296 and 167 mL-CH_4/gVS, respectively). This was because Na-Oleate is more reduced compound compared to gelatine, resulting in higher specific methane potential (Boe and Angelidaki, 2009). From Fig. 2a and c, when increasing the OLR of both reactors from 3.5 to 4.2 gVS/(L-reactor·d), foaming was observed in the gelatine reactor, but not in the Na-Oleate reactor although gelatine and Na-Oleate had similar concentrations of 9 and 12 g/L-feed, respectively. This demonstrates that gelatine had stronger ability to initiate foam formation at this OLR than Na-Oleate. At gelatine concentration of 9 g/L-feed (Fig. 2a), the increase of OLR by adding glucose 11 g/L-feed corresponded to the theoretical extra methane production of 271 mL CH_4/(L-reactor·d). However, the actual increase of methane production was 413 mL CH_4/(L-reactor·d). This suggested that the degradation efficiency of the whole substrate was improved despite of foam formation, probably due to the higher C/N ratio helped counteracting the effect of ammonia toxicity (Hartmann et al., 2002). Moreover, this could be specific for our experimental set up, in which foam was mechanically destroyed every day, thus, all biogas entrapped in the foam was efficiently released. In contrary, the increase OLR by adding glucose in Na-Oleate reactor did not increased methane yield (Fig. 2c), suggesting that the process became more inhibited, as increasing the easily degradable fraction in the substrate should have improved the overall methane yield.

At high concentration of gelatine and Na-Oleate (21 and 24 g/L-feed, respectively), foaming was observed in both reactors at the OLR of 4.2 gVS/(L-reactor·d) (Fig. 2b and d). From this stage, further increasing OLR to 5.2 gVS/(L-reactor·d) led to increased volume of foam in the Na-Oleate reactor, but not in the gelatine reactor. At Na-Oleate concentration of 24 g/L-feed (Fig. 2d), the increase of OLR by adding glucose 16 g/L-feed corresponded to the theoretical extra methane production of 394 mL CH_4/(L-reactor·d). However, the actual increase of methane production was 1175 mL CH_4/(L-reactor·d). The additional methane production might be contributed by the improved Na-Oleate degradation at period IV compared to period III. It is worth to highlight that, the Na-Oleate concentration of 24 g/L-feed at OLR 5.2 gVS/(L-reactor·d) was tested at the end of the experiment (period IV). Increased methane yield was observed, which was probably due to microbial adaptation after long-term addition of Na-Oleate (period I–III). Microbial community shifts as a consequence of the exposure to LCFA pulses has previously been reported (Baserba et al. 2012). After adaptation, the reactor can manage to recover and thus increase the methane yield. This was confirmed by the results shown in Fig. 3c and d; during the foaming period, no VFA accumulation

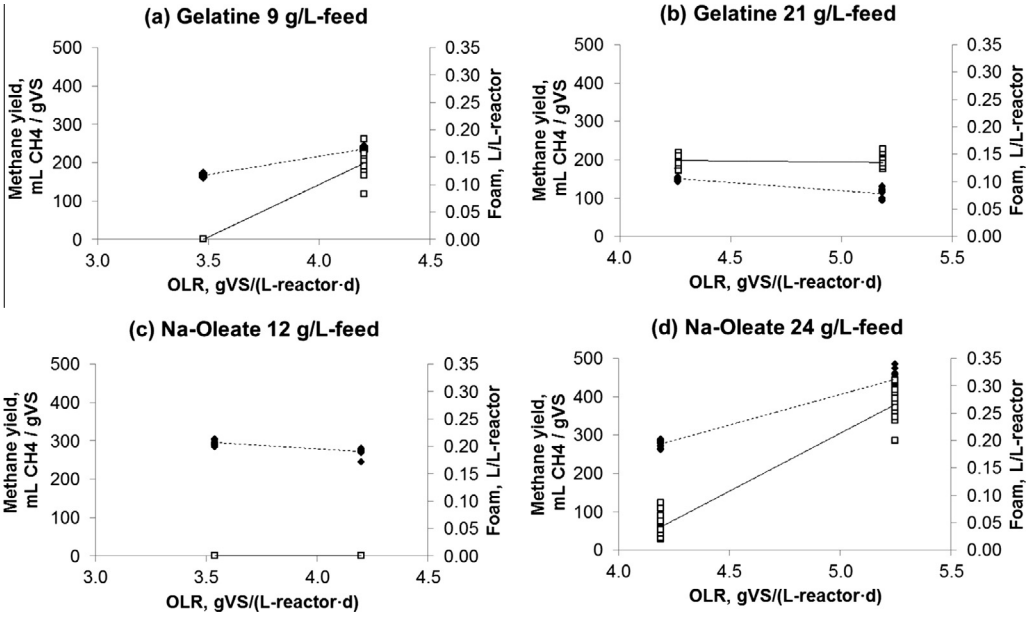

Fig. 2. Effect of OLR on methane yield (♦) and foam formation (□) with the addition of gelatine or Na-Oleate, or glucose.

was observed, and the methane yield was also increasing. Moreover, the daily disruption of the foam was enough to maintain high methane production in the reactor. In contrary, although the same amount of foam was observed in the gelatine reactor when increasing the OLR from 4.2 to 5.2 gVS/(L-reactor·d), the methane yield from this reactor was decreased, suggesting that the increased gelatine concentration had adverse effects on the microorganisms. This could be seen from the Fig. 3a and b, where the methane yield was decreasing while the VFA and ammonia concentration in the reactor increased.

The increase of foam formation in the Na-Oleate reactor when increasing OLR could be explained by the higher biogas production.

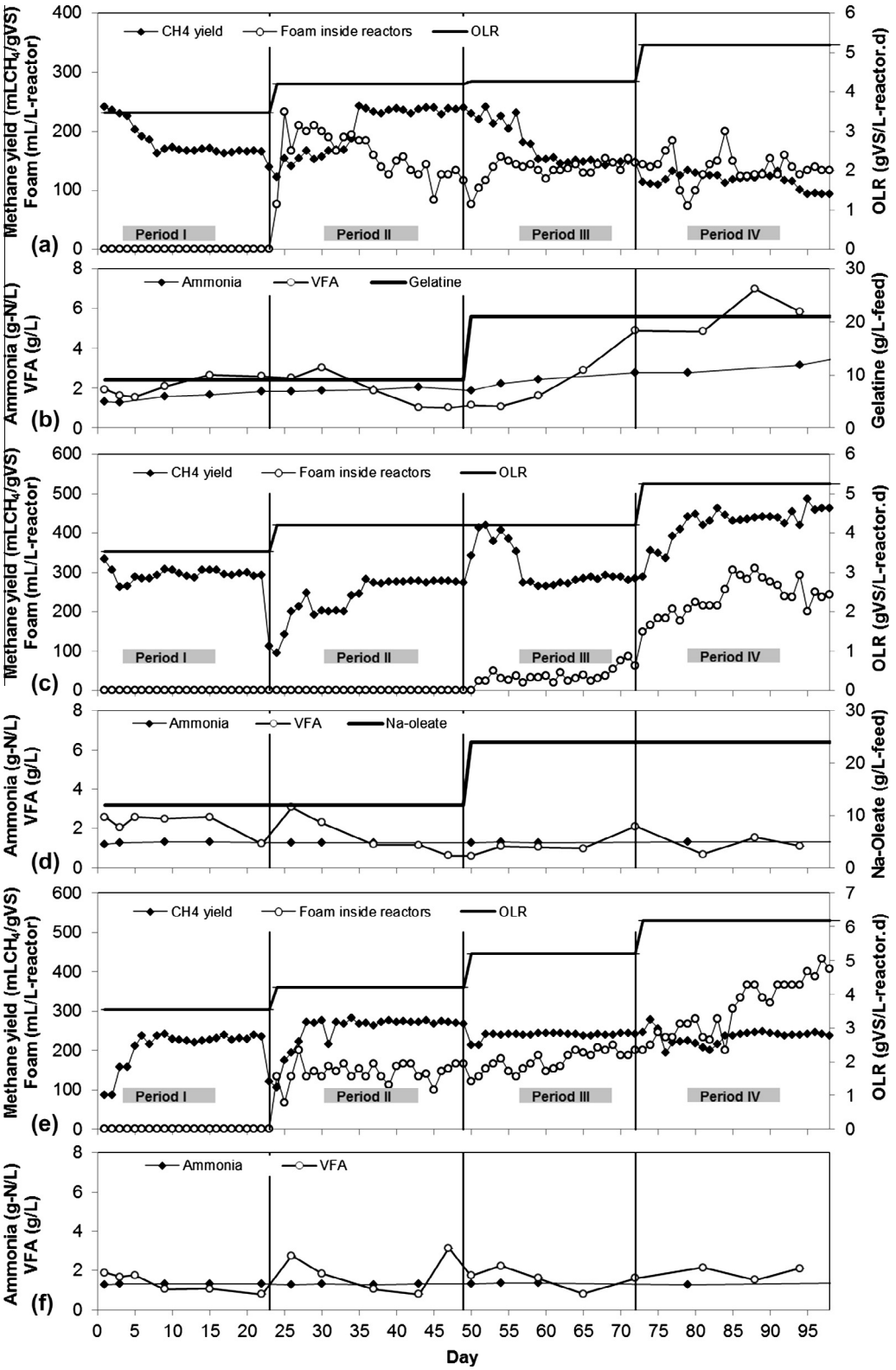

Fig. 3. Process performance of reactor R1&R2 (a, b), R3&R4 (c, d), and R5 (e, f) during all periods of experiment.

It has to be noted that the foam formed in the gelatine reactor and Na-Oleate reactor also looked different. By visual inspection it could be observed that the foam created in the gelatine reactor was tight and compact, while the foam created in the Na-Oleate reactor was light bubbles with colourful reflections. The light foam caused by Na-Oleate was easy to expand by the increased biogas production. In contrast, the foam caused by gelatine was relatively stable in volume due to its compactness. Moreover, gelatine degradation provides ammonia, which could cause process inhibition and thereby counteract foam formation, as the reduced biogas production, would generate less gas bubbles to promote foam.

3.3. Effect of protein and lipid compounds on foaming

Fig. 4a–f depicts the correlation between the concentration of proteins and lipids in the feedstock, the methane yield and the amount of foam that was formed inside the reactors. At OLR of 3.5 VS/(L-reactor·day) (Fig. 4a and b), no foam formation was observed in the reactors with and without addition of gelatine and Na-Oleate. At OLR of 4.2 VS/(L-reactor·day) (Fig. 4c and d), Na-oleate concentration of 12 g/L-feed resulted in antifoaming effect (Fig. 4d). However, the antifoaming effect of Na-Oleate decreased when the Na-Oleate concentration increased to 24 g/L-feed. At this OLR, addition of gelatine did not increase the amount of foam formation. However, the reactor with gelatine (Fig. 4c at gelatine concentration 9 g/L-feed, R1&R2, period II) had lower methane yield than the reactor with glucose (Fig. 4c, at gelatine concentration 0 g/L-feed, R5, period II). This could be due to the degradation of gelatine increased ammonia concentration in the reactor which negatively affected the microorganisms. Both ammonia and VFA level in gelatine reactor were higher than in the control reactor (added glucose only) at the same OLR of 4.2 gVS/(L-reactor d), indicating that reactor added with gelatine was more stressed than the reactor added with glucose. From this stage, further increase of the gelatine concentration in the feed resulted in further decrease of methane yield, indicating that the process was even more inhibited (Fig. 3a and b). High VFA concentration indicates imbalanced microbiological activity and enhances foaming problems (Resch et al., 2011). The accumulation of the intermediate compounds (VFA) was an indication of an imbalanced inhibited process. Additionally, increasing the concentration of Na-Oleate in the feed should result in higher methane yield due to the higher theoretical methane potential of Na-Oleate compared to glucose (939 and 395 mL CH_4/gVS at STP for Na-Oleate and glucose, respectively). The addition of Na-Oleate at 12 g/L-feed corresponded theoretically to the extra methane production of 365 mL CH_4/(L-reactor d). However, from Fig. 4d, the methane yield did not increase with the increase of Na-Oleate, indicating process inhibition.

At OLR of 5.2 VS/L-reactor·day (Fig. 4e and f), foam formation was observed in all reactors both with and without addition of gelatine and Na-Oleate. At this OLR, the increase gelatine concentration decreased methane yield due to ammonia toxicity, while the increase Na-Oleate concentration increased methane yield as a results of long-term adaptation to LCFA as discussed above. The change in foam volume at this OLR was also similar to the previous section, where the increased gelatine concentration decreased foam volume due to the decreased biogas production caused by ammonia inhibition, and increased Na-Oleate increased biogas

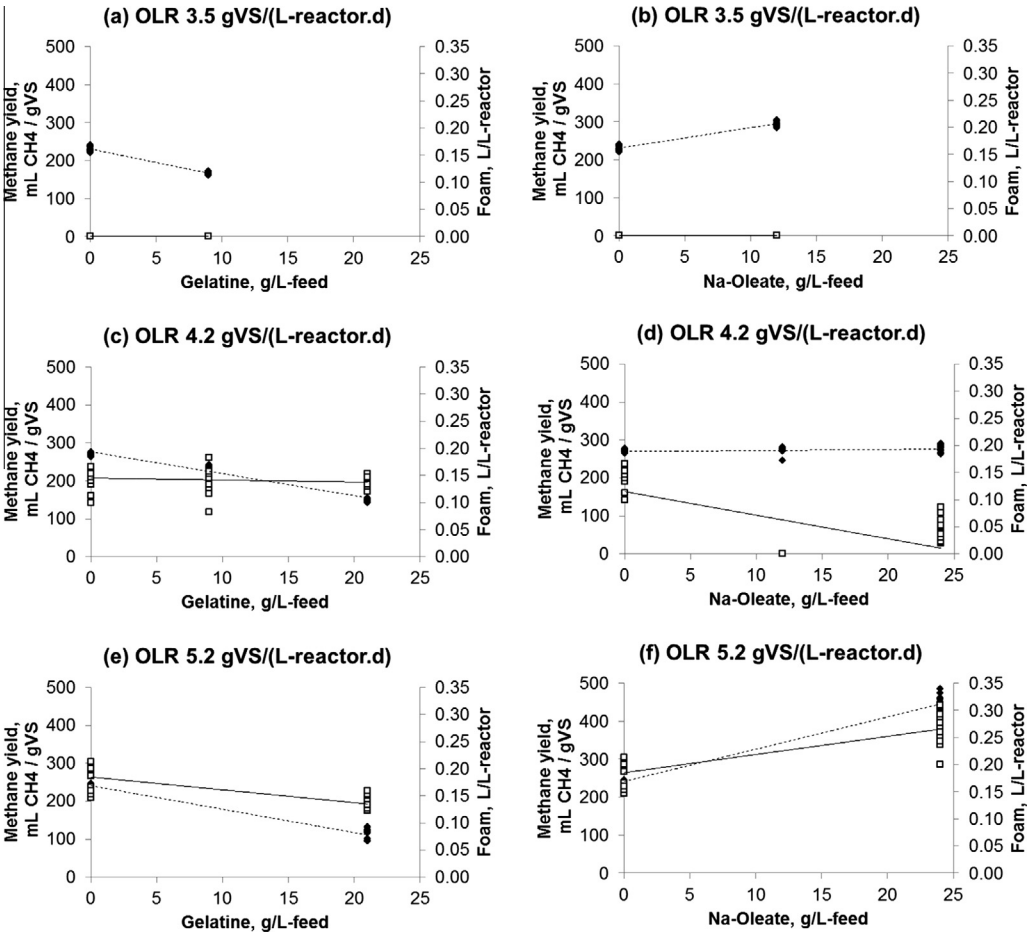

Fig. 4. Effect of protein (a, c, e) and lipid (b, d, f) on methane yield (♦) and foam formation (□) Na-Oleate at OLR of 3.5 (a, b), 4.2 (c, d), and 5.2 (e, f) gVS/(L-reactor·day).

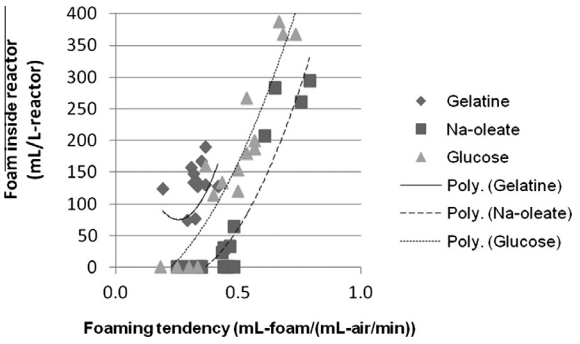

Fig. 5. Correlation of foaming tendency and the volume of foam produced inside the reactors.

production and thus increased foaming. Moreover, Na-Oleate is known to have ability to promote foam (Beneventi et al., 2001). Hejnfelt and Angelidaki (2009) investigated the anaerobic digestion of slaughterhouse by-products and observed foam formation in batch and CSTR experiments when the initial concentration of lipids reached 5 g/kg. Unfortunately, only limited information concerning the critical threshold of lipid concentration for initiation of foaming in anaerobic digesters is existing in the literature (Ganidi et al., 2009).

3.4. The use of foaming tendency as an indicator of foaming inside the reactor

In our pervious study (Boe et al., 2012), we investigated different physiochemical parameters that could promote foaming in manure samples. The parameters that were used to indicate foaming in the physiochemical test were foaming tendency and foam stability. In this study, we also investigated the potential of using foaming tendency or foam stability as foaming indicator inside the reactor at continuous operation. During the foaming period, liquid samples were taken from the reactor for measurement of foaming tendency and foam stability as described in the section Materials and Methods. The correlation of foaming tendency and the volume of foam produced inside the reactors is shown in Fig. 5. The foaming tendency showed relatively good correlation with the volume of foam produced inside the reactor with glucose and Na-Oleate addition. However, the correlation was not clear for the gelatine reactor since the foam volume inside the gelatine reactor did not change much during the experiment. From these results, it could be suggested that the foaming tendency can be used as indicator to predict foaming potential of the sample, although it would be best to observe foaming directly inside the reactor. Additionally, the foam stability of was zero in all tests (results not shown), indicating that this parameter was not sensitive enough to predict foaming in the manure digester.

4. Conclusions

Organic load was the main factor affecting foaming. Gelatine initiated foaming at lower OLR than Na-Oleate. Foaming from gelatine had more stable volume compared to Na-Oleate. No foaming occurred at OLR 3.5 gVS/(L-reactor·day). At OLR 4.2 gVS/(L-reactor·day), low concentration of Na-Oleate showed antifoaming effect. At OLR 5.2 gVS/(L-reactor·day), increased Na-Oleate promoted foaming, while increased gelatine decreased foaming, due to lower biogas production caused by the elevated ammonia concentration. Foaming is rather related to increase of biogas production and not inhibition. If the foam was efficiently removed, foaming would not decrease methane production unless the process was inhibited by other reasons.

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Effect of substrates and intermediate compounds on foaming in manure digestion systems

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ABSTRACT

Manure contains several compounds that can potentially cause foaming during anaerobic digestion. Understanding the effect of substrates and intermediate compounds on foaming tendency and stability could facilitate strategies for foaming prevention and recovery of the process. In this study, the effect of physicochemical properties of substrates and intermediate compounds on liquid properties such as surface tension, surfactant property, and hydrophobicity were investigated and compared with the effect on foaming tendency and foam stability. The results showed that there was no consistent correlation between foaming potential and hydrophobicity, oil displacement area (ODA) or surface tension of the tested solutions, and the best way to determine the foaming property of the solution was to directly measure foaming tendency and foam stability. Na-oleate and acetic acid showed the highest potential to create foam in a manure digester. Moreover, high organic loading of lipids and protein, and high concentrations of acetic and butyric acids also showed a strong tendency to create foaming during anaerobic digestion. Due to their great ability to stabilize foam, high organic loadings of Na-oleate or gelatine were considered to be the main potential foaming problem.

Key words | anaerobic digestion, foaming tendency, intermediate compounds, manure, substrates

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INTRODUCTION

Anaerobic digestion (AD) has gained increased attention in recent years, which has led to the development of many biogas plants worldwide. Many biogas plants, especially in Denmark, are co-digestion plants, where different types of organic industrial wastes are digested together with manure. In these plants, foaming typically occurs in the main reactor or in the pre-storage tank. Foaming often results in operational problems, such as blockage of mixing devices, and collapse of pumps, due to entrapped solids in the foam (Ganidi *et al.* 2009). The bad mixing leads to an inverse solids profile in the digesters with higher solids concentrations at the top of a digester, resulting in the formation of dead zones and thus reducing the digester active volume. As a result, biogas production is decreased for shorter or longer periods (Nielsen & Angelidaki 2008). Serious economic consequences are linked to foaming, due to income losses, extra labour need, and maintenance costs (Barjenbruch *et al.* 2000; Barber 2005). Furthermore, foaming often causes environmental problems due to overflowing of pre-storage or digester tanks.

Pure water cannot have foam unless a surface active agent is present as an impurity. Surface active agents, such as surfactants or bio-surfactants, generated under metabolic processes, decrease the surface tension of the liquid and thus enhance foaming potential (Barber 2005). Other factors for foam formation include a gaseous phase and the presence of hydrophobic material. Foam in anaerobic digestion systems consists of three phases, which are gas bubbles, liquid (wastewater or soluble microbial products) and solid particles (microorganisms or suspended solids). Foam formation and stabilization require at least the presence of gas bubbles and solid particles in the bulk liquid (Dynarowicz & Paluch 1989). During the last 10 years, many studies have focused on foaming problems in sludge digesters from wastewater treatment and several causes for foaming have been suggested. Ross & Ellis (1992) suggested that organic overloading and the accumulation of acetic acid were the cause of foaming in sludge digesters. Pagilla *et al.* (1997) suggested that *Gordonia* filamentous bacteria were the cause of foaming, where *Gordonia* was identified in

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two full-scale sludge digesters. Other factors such as inadequate mixing, temperature fluctuation, shock load, extracellular polymeric substances (EPS) and hydrophobic substances have also been suggested as foaming causes in sludge digesters (Barjenbruch *et al*. 2000; Barber 2005).

The results from the above mentioned studies are, however, contradictory and the information provided is either site-specific or the supporting experimental information is limited. So far, there has never been a thorough investigation of a foaming problem in a manure-based digester, which is the main anaerobic digestion technology used in Denmark. Foaming is one of the major problems that occasionally occurs and leads to production loss in the Danish full-scale biogas plants. There is a need for investigation of the foaming causes in this system in order to find the method to avoid as well as to resolve the problem. This work aims to identify the potential causes of foaming in manure digesters. The specific compounds commonly present in a manure digester are investigated for their effects on liquid properties and foaming potential in manure.

MATERIALS AND METHODS

The cow manure used in the experiment was obtained from Vegger biogas plant, Denmark. After arrival, the manure was shredded and sieved to separate large particles and stored at −20 °C until further use. Freezing is a common and suitable technique for manure storage as it preserves the original physical and chemical characteristics of the sample (Van Kessel *et al*. 1999; Kaparaju & Rintala 2005; Pognani *et al*. 2012). The characteristics of manure are presented in Table 1.

Table 1 | Cow manure characteristics

Parameter	Unit	Value
pH	–	6.98 ± 0.07
Total solids (TS)	g/L	89 ± 1
Volatile solids (VS)	g/L	71 ± 1
Ammonium (N-NH_4^+)	g/L	3.4 ± 0.4
Total volatile fatty acids (VFA)	g/L	9.1 ± 0.1
Acetic acid	g/L	5.8 ± 0.4
Butyric acid	g/L	0.78 ± 0.02
Propionic acid	g/L	1.84 ± 0.07
Iso-butyric acid	mg/L	222 ± 1
Valeric acid	mg/L	101 ± 4
Iso-valeric acid	mg/L	283 ± 3

Physicochemical effect of substrates and intermediate compounds on liquid properties and foaming potential

The effect of individual substrates and intermediate compounds on liquid properties and foaming potential was investigated in both water and manure. The liquid properties investigated were surface tension, surfactant property and hydrophobicity. The foaming potential was measured as foaming tendency and foam stability. For the preparation of this test, the frozen manure was thawed and diluted to 5% in water to obtain a total solid (TS) content of 0.45%. This solution was kept at 4 °C until use for a maximum of 6 days. Fifteen compounds were chosen, which are proteins (peptone, gelatine), carbohydrates (cellulose, starch, sucrose), lipids (fish oil, sodium oleate, glycerol, sunflower oil), cations (Ca^{2+}, Na^+ and Mg^{2+}), volatile fatty acids (acetic acid, butyric acid) and ammonia. The concentration of each compound in a 50 mL solution was chosen in the range of 0.2–6 g/L, which is in accordance with its typical concentration that is commonly found in manure digesters.

The effect of the sample matrix was also investigated in manure, by comparing the surface tension and foaming potential of manure at different concentrations corresponding to the total solid (TS) content of 0.45, 0.89, 2.67, 4.45 and 5.34%.

Physicochemical effect of substrates and intermediate compounds in a complex mixture

In a co-digestion system, several substrates and intermediate compounds are present in a digester at the same time, which can affect foaming in the digester differently. To investigate the effect of each compound in a complex mixture, a fractional factorial design of experiments was carried out, where all the compounds involved were tested at different concentrations. Assuming that the interaction between each compound is insignificant, the design matrix could be reduced to 16 combinations of 15 compounds in manure as shown in Table 2. From each test combination, the liquid properties and foaming potential were measured. The liquid properties measured were surface tension, surfactant property and hydrophobicity. The foaming potential was determined as foaming tendency. The results from each test combination were then analysed using Design-Expert® software (Stat-Ease Inc., USA) to determine the influence of each compound on liquid properties and foaming potential in manure.

Table 2 | Concentrations (g/L) of compounds involved in each test combination

Combinations:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Gelatine	–	–	0.8	–	–	–	0.6	0.6	0.7	0.6	–	–	0.6	0.7	0.6	–
Peptone	1.1	–	1.2	–	1.1	–	1.3	1.2	–	–	1.0	–	–	1.2	–	1.4
Fish oil	–	1.4	–	–	1.5	1.3	1.3	–	1.4	1.3	–	–	–	1.6	–	1.1
Sunflower oil	1.1	–	1.2	–	–	1.2	–	–	–	1.1	–	1.2	1.1	1.2	–	1.1
Glycerol	1.6	1.1	–	–	–	1.2	1.8	–	–	–	1.3	–	1.6	1.2	1.0	–
Na-oleate	–	–	2.0	–	2.0	2.0	–	–	2.0	–	2.0	2.0	–	2.0	2.0	–
Starch	2.8	2.8	–	–	3.0	–	–	2.9	–	2.9	–	3.0	–	3.0	2.9	–
Sucrose	–	3.0	–	–	–	–	–	2.9	3.3	–	3.1	2.9	3.0	3.0	–	2.8
Cellulose	2.8	–	–	3.0	3.0	3.0	–	2.9	2.9	–	–	–	2.9	3.1	–	–
Acetic acid	–	3.0	3.0	3.0	–	3.0	3.0	3.0	–	–	–	3.0	–	3.0	–	–
Butyric acid	3.0	–	–	3.0	–	–	3.0	–	3.0	3.0	3.0	3.0	–	3.0	–	–
NH_4^+	–	8.3	7.7	7.6	7.0	–	–	–	–	9.0	8.1	–	7.2	7.1	–	–
Ca^{2+}	–	–	–	11.2	9.8	–	10.5	–	–	–	–	10.7	10.9	11.5	10.4	10.2
Mg^{2+}	3.0	30.4	29.0	30.0	–	–	–	–	30.0	–	–	–	–	29.0	29.0	30.0
Na_+	–	–	–	12.0	–	11.0	–	9.0	–	10.0	11.0	–	–	10.0	10.0	11.0

Analytical methods

Analysis of pH, TS and volatile solids (VS) was according to Standard Methods for the Examination of Water and Wastewater (1998). All analyses were carried out in triplicate. Volatile fatty acids (VFA) were determined by gas chromatograph (GC Shimadzu) with flame ionisation detector (FID). Methane content in biogas was determined using a gas chromatograph (GC Shimadzu) equipped with a Porapak 60/80 molecular sieve column and a flame ionisation detector (FID).

Surface tension measurement was carried out by the Wilhelmy plate method as described by Glinski *et al.* (2000) and Elmitwalli *et al.* (2001).

The surfactant property of the solution was measured using the oil displacement test. By placing 10 μL Murban crude oil on the surface of 50 mL distilled water in a Petri dish (15 cm diameter), a thin oil film formed immediately. Thereafter, 20 μL of sample was dropped onto the centre of the oil film. A clear circle inside the oil film was formed, and the diameter was measured after 30 s. The area (cm^2) of the clear circle was calculated as the oil displacement area (ODA) (Morikawa *et al.* 2000).

The hydrophobicity was determined by mixing 2 mL of diesel oil with the same amount of sample for 2 min and letting it stand for 24 h. The emulsification activity was then calculated as the percentage of the height of emulsified layer divided by the total height of the liquid column (Thampayak *et al.* 2008).

The foaming potential of the solution was determined by an aeration method modified from the Bikerman method described by Beneventi *et al.* (2001). The apparatus was comprised of an Imhoff settling cone with a diffuser placed at the bottom. A 50 mL sample was aerated in the settling cone with the air flow rate of 60 mL/min. for 10 minutes. The foam height in the settling cone was measured right after aeration was stopped and again at 1 hour later. The foaming potential was defined using two parameters: foaming tendency and foam stability. *The foaming tendency* (mL-foam/(mL-air·min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). *The foam stability* was determined as percentage of foam remaining in the settling cone at 1 h after aeration compared with the volume of foam right after aeration.

RESULTS AND DISCUSSION

Physicochemical effect of substrates and intermediate compounds on liquid properties and foaming potential

The liquid properties, which are surface tension, ODA and hydrophobicity, were measured in this experiment for establishing a possible correlation between these parameters to foam formation in our tested solutions, in order to find appropriate foaming indicators. Morikawa *et al.* (2000) introduced ODA measurement as an indicator of surfactant activity, based on the fact that the surface pressure of the

surfactant displaces the oil. From our test results, the ODA value of pure water and manure were zero. Figure 1(a) shows that fish oil and Na-oleate significantly increased ODA in water up to 1.52 and 0.48 cm^2, respectively, while the rest of the compounds did not show a significant effect. In manure, only Na-oleate significantly increased ODA up to 0.7 cm^2.

Concerning hydrophobicity, it has been reported that the increased chain length of the hydrophobic part of the surfactants can increase foam formation (Beneventi *et al.* 2001). From our results, the hydrophobicity of pure water and manure were 0% and 50%, respectively. From Figure 1 (b), all tested compounds increased hydrophobicity of water to a level similar to manure. On the other hand, their effects were different in manure. The strongest hydrophobic behaviour was observed from sucrose, NH_4^+ and Ca^{2+}, as they have increased the hydrophobicity value by 5.87, 4.85 and 3.58% respectively. In contrast, Na-oleate and cellulose reduced the hydrophobicity of the manure by 3.04 and 2.67%, respectively. The effect of Na-oleate on hydrophobicity could be explained as being due to the presence of the free carboxylic end, which is not so hydrophobic compared with neutral oils where the carboxylic end is not available. This contradicts the generalisation that lipids are characterised as hydrophobic organic molecules (Ganidi *et al.* 2009). Overall, the effects of compounds on hydrophobicity of manure did not demonstrate consistent correlation with the effects on the ODA.

Surfactants decrease surface tension and cause foam in the solution (Mulligan 2005). The surface tension of pure water and manure (0.45%TS) in this test was found to be 72.34 and 60.11 mN/m, respectively. A similar result has been demonstrated by Vardar-Sukan (1998), who reported the surface tension of pure water as 72 mN/m at 20 °C. Figure 1(c) shows that most of the tested compounds, except cations, decreased surface tension of both water and manure. The effects from proteins and lipids were stronger than carbohydrates. This has also been stated by many researchers, who reported the property of proteins and lipids to behave as surface active compounds capable of decreasing the surface tension of their solvent and to enhance foaming potential (Vardar-Sukan 1998; Barber 2005; Rouimi *et al.* 2005; Foegeding *et al.* 2006, Glaser *et al.* 2007; Junker 2007).

Na-oleate could also be considered as a surfactant, as its effect followed most of the surfactant behaviour reported in literature where a clear correlation between the ability to decrease surface tension and the increase of ODA in the solution was observed. This correlation did not fit with other lipids in this experiment, which are neutral oils, i.e. do not have a free polar end (carboxylic end) and therefore do not exhibit surfactant properties. Cations, including NH_4^+, clearly increased surface tension of the solution, but had no consistent relationship to the change of both ODA and hydrophobicity of the solution. The increase in surface tension from cations did not correspond to the change of foaming tendency either. The effect of cations on foaming tendency could be explained in that NH_4^+ and Na^+ were resulting in soluble salts which slightly increased foaming tendency in the solutions, while Ca^{2+} and Mg^{2+} tended to form insoluble salts which could precipitate the surfactants e.g. LCFA, and therefore decreased foaming tendency. As foaming tendency and foam stability are the most direct measurement of foaming property of the solution, and had been widely used for determination of foaming property of the solution (Deshpande & Barigou 2001; Ganidi *et al.* 2011), the effect of compounds based on foaming tendency and foam stability would be the most reliable indicator. Moreover, although the liquid properties such as surface tension, ODA, and hydrophobicity had been reported to correlate with foaming potential caused by surfactants, and a similar trend was also observed for Na-oleate in this experiment, the correlation could not generally be applied to other substrates and intermediate compounds tested in this experiment.

Foaming potential was determined by two parameters; foaming tendency and foam stability. From the test results, Na-oleate strongly increased foaming tendency in both water and manure, while other compounds had a relatively low effect. This was due to its surfactant properties (Na-oleate is a soap). In contrast, acetic acid significantly increased foaming tendency in manure but had no effect in water. Ganidi *et al.* (2009) have also reported that accumulation of acetic acid was one of the possible causes of foaming in a sludge digester. Although gelatine and NH_4^+ had relatively low foaming tendencies, the results from measurement of foam stability (Figure 1(d & e)) showed that they could potentially cause a foaming problem in the manure digester due to their ability to create foam stability. Na-oleate also created foam stability in both water and manure, which emphasised its' strong potential to cause a foaming problem in a manure digester.

From this study, the effect of compounds on foaming potential could not be correlated to the changes of surface tension, ODA, or hydrophobicity in manure. It has also been previously reported that no consistent relationship was detected between the foaming abilities and the hydrophobicity of mycolata bacteria from activated sludge

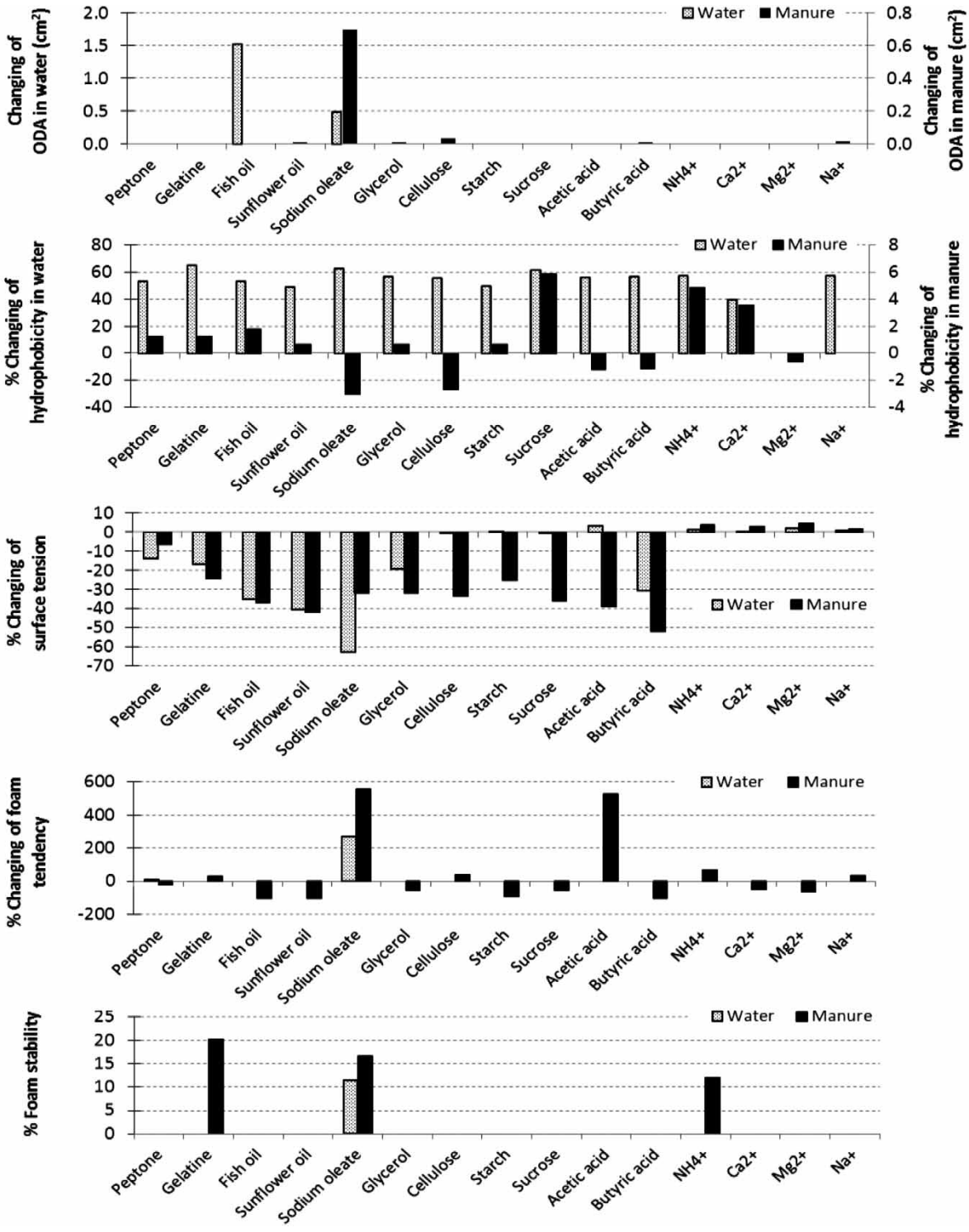

Figure 1 | Effect of feedstock compounds on solution properties and foaming in water and manure.

(Stratton *et al.* 2002). Other hypotheses have also been reported. Blackall & Marshall (1989) demonstrated that the antifoam cations that adsorb to negative charges on the bacterial cell surface via positive charges on their ends, thus coating the cell, could reduce foam formation in activated sludge. The study results from Stratton *et al.* (2002)

also supported that the mechanisms of these bindings are surface charge-related. Comparing to our study, the strong cations such as Ca^{2+} and Mg^{2+} with heavy molecules tended to decrease foaming tendency. This was due to the decreased availability of surfactant compounds due to the bindings of the polar end. In contrast, cations such as NH_4^+ and Na^+ did not show this ability. It was also probably due to the fact that Na^+ and NH_4^+ can form sodium and ammonium soaps with long-chain fatty acids, i.e. compounds that ionise in water to a compound with both hydrophobic and hydrophilic ends, which could act as a surfactant and cause foaming (Palatsi *et al.* 2009). Another example of charge effect on foaming is the foaming from protein. Foaming from protein is well known and widely applied in food industries. Their foaming effect is probably again based on the formation of ionisable compounds with both hydrophobic and hydrophilic available ends. Protein foams are formed by a protein film surrounding a gas bubble creating a structure that holds bubbles in place (Foegeding *et al.* 2006). Proteins consist of different proportions of amino acids and some of them are charged in the solution. At pH 7, basic amino acids such as arginine and lysine carry a net positive charge, while acidic amino acids such as aspartic and glutamic acids have a negative charge. The ratio of these amino acids determines the net charge of a protein. A combination of basic and acidic proteins creates very stable foams due to their opposite charges. However, acidic proteins are much more common in nature. It was also known from food industries that fats could destroy the protein foams by displacing the proteins that form the air bubble surface, causing the bubble to collapse (Hart 1989). The same tendency was observed in this study where gelatine strongly increased foam stability, while lipids such as fish oil, sunflower oil and butyric acid tended to decrease foaming.

Effect of sample matrix on foaming in manure

The quantity, composition, and consistency of cattle manure influence the selection and the design of manure-handling facilities. In its strictest definition, animal manure refers only to feces and urine. However, bedding, feed wastage, rain, soil, milk-house wastes or wash, and more are mixed with the feces and urine on many farms. Manure is a complex substrate and showed considerable potential to create foaming. The test results showed that cattle manure had a surface tension around 52–59 mN/m at a normal feed concentration of 3–6% TS. The low surface tension of manure compared with water (72 mN/m) could possibly be due to the presence of natural and synthetic surfactants, oils, greases, proteins, lipids and polymers. However, the increase of manure concentration from 1 to 6% TS did not significantly affect the surface tension. In contrast, the increase of manure concentration corresponded to an increase in foaming tendency, up to more than 2 mL-foam/(mL-air.min) at 6% TS. However, the foaming stability was lowered at high TS concentration (Figure 2). Again, it was obvious in these results that surface tension was a poor foaming indicator in complex slurries such as manure.

Physicochemical effect of substrates and intermediate compounds in a complex mixture

Figure 3(a) shows the change of ODA, hydrophobicity, surface tension and foaming tendency in manure to which had been added combinations of different compounds. From statistical analysis of this data, the factor of influence of each compound on liquid properties and foaming tendency was then calculated and plotted in Figure 3(b).

Similar to the results from testing of a single compound, in a complex mixture most of the tested compounds decreased the surface tension of the manure mixture, except starch and cations. According to literature, carbohydrates and inorganic salts are not surface active substances as they are able to increase surface tension of water when dissolved. Matubayasi & Nishiyama (2006) and Matubayasi & Yoshikawa (2007) showed that aqueous solutions of sodium inorganic salts, glucose, sucrose, nitrate anions, sulphates salts and different ammonium salts, respectively, were not surface active agents as in all cases

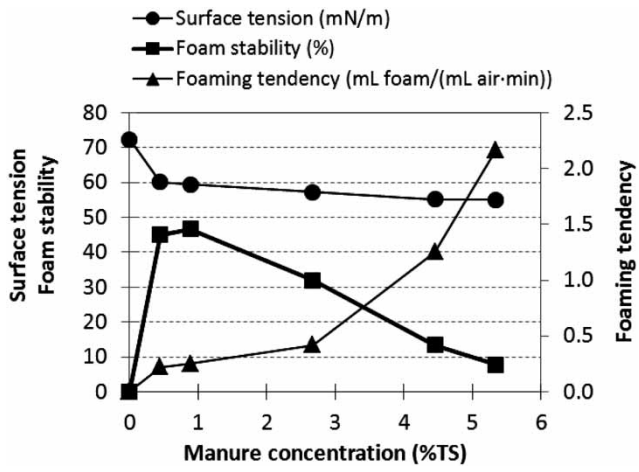

Figure 2 | Surface tension and foaming potential of manure at different concentrations.

surface tension within the concentration ranges studied was higher than the pure water surface tension value of approximately 72 mN/m. From Figure 3(a), similar to the results of a single compound, the combined effect of multi-compound mixtures on surface tension did not show a consistent relationship with the change of ODA and hydrophobicity either.

From the overview of the results in Figure 3(b), some of the compounds showed similar effects on foaming tendency of manure both when testing as a single compound and when testing in a complex mixture. In both tests, gelatine, Na-oleate, cellulose, acetic acid and NH_4^+ increased foaming tendency in the manure, while fish oil, glycerol, butyric acid and Ca^{2+} decreased foaming tendency. However, some compounds showed opposite effects on foaming tendency when tested in a complex mixture compared with when testing as a single compound, which were sunflower oil, sucrose, Mg^{2+} and Na^+. Moreover, some compounds showed a stronger effect on increasing foaming tendency when tested in a complex mixture, for example, gelatine and peptone. Both gelatine and peptone increased foaming tendency most strongly in this test, which could be characterized as strong foaming agents in a complex mixture. Similar results have been reported in several studies (Rouimi *et al.* 2005; Foegeding *et al.* 2006, Glaser *et al.* 2007). It was also noticed that Na-oleate was the only compound which showed a consistent relationship between liquid properties and foaming tendency, similarly to when testing as a single compound.

CONCLUSION

There was no consistent correlation between the effect on foaming potential of the substrate and the effect on hydrophobicity, ODA or surface tension of the solution. The best way to determine foaming property of the solution was to directly measure foaming tendency and foam stability. Na-oleate and acetic acid showed a tendency to increase foaming in all tests, both for physiochemical effect and during anaerobic digestion. However, acetic acid did not create stable foam. In contrast, gelatine and NH_4^+ had a moderate effect on increasing foaming tendency, but had a very strong effect on increasing foam stability. Lipids tended to decrease foaming tendency according to physiochemical tests, however, they could also increase foaming during anaerobic digestion due to their high organic load. Na-oleate was the strongest foam forming

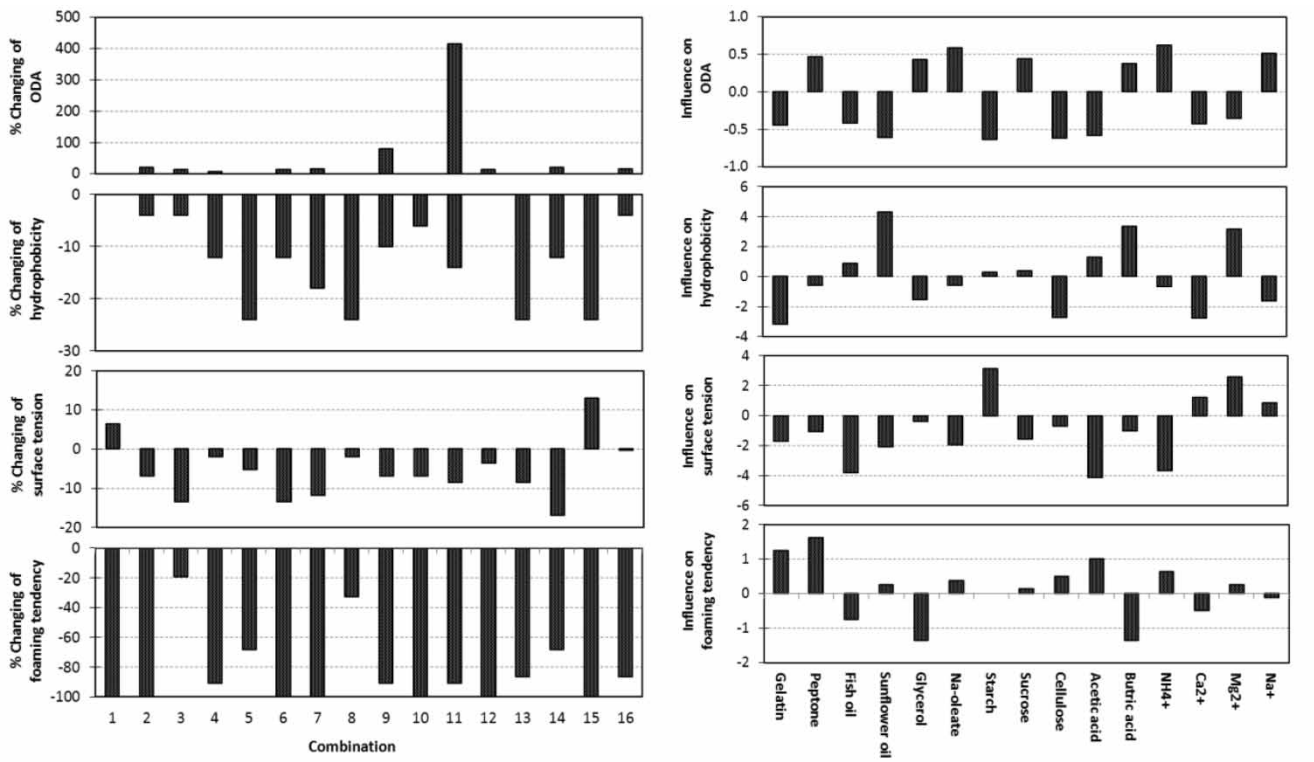

Figure 3 | Effect of substrates and intermediate compounds in a complex mixture; (a) change of liquid properties and foaming potential in a manure solution with different combinations of compounds, (b) statistical analysis for the influence of compounds on liquid properties and foaming in the manure mixture.

agent due to its surfactant property and its high organic load. Ca^{2+} and Mg^{2+} tended to decrease foam tendency in the solution. In conclusion, Na-oleate and acetic acid showed the highest potential to create foam in the manure digester. Due to their strong ability to stabilize foam, a high organic content of Na-oleate or gelatine was considered as the main potential foaming problem.

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Foam suppression in biogas reactors fed with protein and lipid rich substrates

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Abstract

Foaming is a major operational problem in biogas plants. In manure co-digestion system, the feedstock often contains potential foam promoting substances such as protein and lipid. The efficiency of antifoams on biogas process could also be influenced by the feedstock and chemical composition inside the reactor. This study aimed to investigate the effect of rapeseed oil and oleic acid on foam reduction and process performance when foaming was caused by the addition of protein or lipid, under batch and continuous reactor operation. The antifoams were tested in dosages of 0.05, 0.1 and 0.5% v/v_{feed}. The results from continuous reactor operation showed that both rapeseed oil and oleic acid were efficient to suppress foam both at the dosage of 0.1 and 0.5% v/v_{feed}. However, rapeseed oil showed better synergy effect on the biogas production, especially in the case that reactor was overloaded with protein.

Keywords

Biogas; antifoams; proteins; lipids; foaming

1. Introduction

Nowadays, more and more biogas plant operators raise the imperative necessity in finding efficient and cost effective antifoaming solutions for the biogas plants in order to avoid the dramatic consequences of foaming incidents. Foaming problem occurs intermittently in the biogas plants, lasting from one to three weeks, resulting commonly in 30–50% biogas production loss and typically occurs up to three times per year

(Kougias et al., 2014a). The causes of foaming in biogas reactors have been previously investigated, identifying the feedstock composition, the organic overload, and the presence of specific microorganisms as the main parameters that promote foam (Dalmau et al., 2010; Kougias et al., 2014c; Moeller et al., 2014). The lifetime duration of foaming incidents classifies the foam in full-scale biogas plants as metastable. Metastable foams can persist indefinitely if they are absolutely protected from disturbance influences (Vardar-Sukan, 1998). Therefore, it is clearly understood that an efficient antifoaming action should be applied in order to destabilise the formed foam.

Foam suppression methods are divided into four main groups; mechanical, physical, biological and chemical methods. The most commonly applied solution in biotechnological processes is the chemical method by using antifoams. Antifoams are defined as surface active chemical substances that, when dispersed in the foaming media, will destroy the foam by causing bubble coalescence (Junker, 2007). Several chemical antifoams are available in the market with varying degrees of foam destruction effectiveness (Moeller et al., 2012a).

The efficiency of antifoam is depending on many parameters and it is well known that one antifoam may not be suitable for every application (Routledge and Bill, 2012). One important parameter defining the suitability of the chemicals to be used as antifoam especially for biological process is the toxicity of the antifoam chemicals. A clear example is the use of tributylphosphate (TBP). TBP is polar oil that showed excellent antifoam efficiency in various processes (Privitera et al., 2014). However, when TBP was applied in anaerobic digestion systems for biogas production, fatal inhibition of methanogenesis was recorded both in batch and continuous feeding reactors (Kougias et al., 2014b). This indicates that the suitable chemical composition of the antifoams is

important in order to avoid negative impacts and deterioration of the process. However, the specific chemical composition of the antifoams is usually not provided by the suppliers, and thus, several compounds should be tested in order to select the most efficient one for each specific application. Another important parameter that should be taken into consideration for the economy of the biogas plants is the cost of antifoams application, which depending on both the price of the chemicals and the required dosage. It was estimated that the cost of antifoams application to suppress foaming incidents in a biogas plant was 500-600€ per foaming event (Moeller et al., 2012b). The cost and also the efficiency of antifoam are directly linked with the applied dosage. It has been previously reported that each antifoam has a specific concentration that presents its maximum antifoaming effect, below which is less effective, while above the optimum concentration it may instead act as foam stabilizer (Karakashev and Grozdanova, 2012). Nevertheless, according to the antifoam product specifications from manufacturers, an indicative typical dosage of commercial antifoams suggested for bioprocesses is 0.1% v/v (Kougias et al., 2013b).

The characteristics of the ideal antifoam specifically for biogas plants that are treating manure and other agro-industrial wastes have been previously described by Kougias et al., (2013b). In summary, the antifoam should have a low cost, suppress foam quickly and efficiently at low application dosage, be biodegradable and not inhibitory for the biogas process. Finally, it should not have negative impact on the environment since the digester effluent is normally applied as fertilizer on farmland.

In practice, antifoams are applied in two different ways in full-scale biogas plants; either by mixing the antifoam with the substrate in the pre-storage tank before feeding, or by spraying the antifoam directly on top of the liquid/foam surface inside the

reactors.

The effect of four different antifoams on foam suppression and process performance in the overloaded reactors had been investigated in our previous study (Kougias et al., 2014b). In this study we further investigated the effect of two antifoams on foam suppression and process performance in the reactors fed with lipid- or protein-riched substrates. The antifoams chosen for this study were rapeseed oil and oleic acid, which had shown the strongest antifoaming potential in the overloaded reactors. The aim of this study was to evaluate the suitability of these antifoams for suppressing foam that was caused by lipid or protein. The effect of the antifoams on the biomethanation process was investigated both in batch assays and in continuous reactor operations.

2. Materials and methods

2.1 Feedstock characteristics and preparation

The inoculum used in the experiment was digested manure obtained from a thermophilic anaerobic reactor of Snertinge biogas plant, Denmark. The feedstock was dairy cattle manure supplemented with lipids or proteins. Upon arrival, the manure was shredded and sieved (5 mm) to separate large particles and stored at -20^{o}C. The frozen manure was thawed at 4^{o}C for 2-3 days before use. The characteristics of the raw cattle manure are presented in Table 1. The raw cattle manure was added with 12 g/L Na-Oleate (≥99%, Sigma-Aldrich), or 9g/L gelatin (Fluka Chemika), to be used as a representative of the lipid- or protein-riches substrates, respectively. The concentrations of Na-Oleate and gelatine used in this study were based on our previous study, in order to ensure persistent formation of foam (Kougias et al., 2013a).

Table 1. Cattle manure characteristics

Parameter	Unit	Values
pH	-	7.3±0.04
Total solids (TS)	g/L	61.6±0.4
Volatile solids (VS)	g/L	48.1±0.4
Total Kjeldahl Nitrogen (TKN)	g-N/L	2.87±0.18
Ammonium Nitrogen (NH_4^+)	g-N/L	1.74±0.13
Total Volatile fatty acids (VFA)	g/L	7.77±0.53
Acetate	g/L	5.44±0.4
Propionate	g/L	1.39±0.09
Iso-butyrate	g/L	0.12±0.01
Butyrate	g/L	0.55±0.02
Iso-valerate	g/L	0.18±0.01
Valerate	g/L	0.06±0.00
n-hexanoate	g/L	0.02±0.00

2.2 Antifoams used in the experiment

Rapeseed oil and oleic acid (90%, Sigma-Aldrich) were used as antifoam in the current study. The rapeseed oil used was normal edible oil bought from supermarket, which had low content of erucic acid (less than 2%). The edible rapeseed oil contained mainly oleic acid (51-70%), linoleic acid (15-30%), alpha-linoleic acid (5-14%), and palmitic acid (2.5-7%) (Codex Alimentarius, 1999).

2.3 Biodegradability of antifoams and effect on biomethanation

The effect of antifoams on the methane potential of the feedstock was examined in batch assays. The methane yield was determined according to the guidelines of the biochemical methane potential (BMP) protocol (Angelidaki et al., 2009) .Two batch assays were conducted in triplicate bottles with a total and a working volume of 337 mL and 100 mL, respectively. The first batch assay was to determine the methane potential of cattle manure supplemented with Na-Oleate or gelatine, and the methane potential of antifoams as single substrate. In this assay, each bottle was filled with 80 mL inoculum, and 20 mL of water mixed with a corresponding amount of substrate, so that the initial organic load was equal to 1 gVS/L. The second batch assay aimed to investigate the effect of the antifoams on the biomethanation of cattle manure mixed with either Na-Oleate or gelatine as mixed substrate. Therefore, each bottle contained 80 mL inoculum, 6.66 mL manure mixed substrate, 13 mL water, and different concentrations of antifoams. Three antifoam concentrations were tested, which were 0.05% v/v_{feed}, 0.1% v/v_{feed}, and 0.5% v/v_{feed}. In both batch assays, after inoculation, all bottles were flushed with nitrogen gas to ensure anaerobic conditions, closed with rubber stoppers and aluminium screw caps, and incubated at 54 ± 1°C. The methane content in the headspace was regularly measured until the methane production has stopped.

2.4 Continuous reactor experiment

The foam reduction efficiency of rapeseed oil and oleic acid along with their effects on the biogas process were investigated under continuous reactor operation. Four Continuous Stirred Tank Reactors (CSTR) were used in the experiment, denoted as R1, R2, R3 and R4. Each reactor had a total and a working volume of 2 L and 1.5 L,

respectively, and operated at a constant hydraulic retention time (HRT) of 15 days. The reactors were continuously stirred using magnetic stirrers. Additionally, the reactors were equipped with silicone thermal jackets in order to maintain the operating temperature at 54±1^{o}C. Substrate was automatically fed to the reactors twice a day using peristaltic pumps controlled with timers. An automated displacement gas metering system with 100 mL cycle was connected to each reactor recording the biogas production (Angelidaki et al., 1992). During the start-up period, all the reactors were inoculated with thermophilic inoculum and were fed with cattle manure only. After reaching steady state conditions the influent feedstock was changed. R1 and R2 were fed with cattle manure supplemented with 9g/L gelatine, while R3 and R4 were fed with cattle manure supplemented with 12g/L Na-Oleate. Once the daily foam formation in the reactors was steady, rapeseed oil was added to the bottles of reactor R1 and R3, while oleic acid was added to the bottles of reactor R2 and R4. The experiment was divided into two periods corresponding to the different antifoam dosages (i.e. 0.1% v/v$_{feed}$, and 0.5% v/v$_{feed}$). Each period was followed by a recovery phase during which no antifoam was added in order to regenerate the foam formation. The duration of the recovery phases was recorded in order to evaluate the tolerance of reactors to the regeneration of foaming after stop adding antifoam. For the process monitoring, the biogas productions and the volume of the formed foam in the reactors were recorded daily, while methane content in biogas and VFA analysis of the liquid samples were performed once or twice per week.

2.3 Foaming potential methodology and calculation

The daily volume of foam formation inside the reactors was calculated from the foam

height above the liquid surface times with the surface area of the liquid. After each foam measurement, the stirring speed of the reactor was increased rapidly or the reactors were manually shaken in order to fully remove the foam. The ability of antifoams to suppress foaming was compared by the foam reduction efficiency, which was calculated using the following equation:

Foam reduction efficiency, (%) = (1-(Foam volume after antifoam addition/Foam volume before antifoam addition))*100

2.4 Analytical methods

Total solids (TS), volatile solids (VS), pH, total Kjeldahl nitrogen (TKN) and total ammonia were determined according to APHA standard methods for the examination of water and wastewater (2005). For the batch assays, the methane content in the test bottles was analysed using a gas-chromatograph (Shimadzu GC-14A, Tokyo-Japan) equipped with a molecular sieve column (2 m, 5 mm OD, 2.6 mm ID) packed with Porapak Q 80/100 mesh (Supelco, Bellefonte, PA, USA), and with a flame ionization detector (FID). Nitrogen was used as a carrier gas with a flow pressure of 2.0 kg/cm^2. The oven temperature was set to 110^oC, while the temperature of the detector and the injection port was 160^oC. Quantitification was based on standard gas mixtures with 100%, 60%, 40% and 5% methane in carbon dioxide, using 0.2 mL sampling volume. For the continuous reactor experiment, the methane and the carbon dioxide content in biogas were measured using a gas chromatograph (Mikrolab, Aarhus A/S, Denmark), equipped with a thermal conductivity detector (TCD) and packed columns (front column: 1.1 m x $^1/_{16}$ molsieve 137 + 0.7 m x $^1/_4$ Lithium Sord K8, back column 10 FF x $^1/_8$ SS, 60/80 molsieve 5A). The injection port, detector and oven temperature was set at

196 50°C. Hydrogen was used as carrier gas with a flow rate of 40 mL/min. Quantitification
197 was based on standard gas mixture containing 40% methane, 30% carbon dioxide and
198 30% nitrogen, using 0.5 mL sampling volume. Volatile fatty acids (VFA) analysis was
199 prepared by adding 0.1 mL of 34% H_3PO_4 to 1.5 mL sample in a 2 mL Eppendorf tube
200 and centrifuged at 13,000 rpm for 10 min. The supernatant (1 mL) was transferred into
201 the GC vial and added with 100 μL internal standard (4-methyl-valeric acid) before
202 analysis. VFA was analysed using a gas chromatograph (Shimadzu GC-2010, Kyoto,
203 Japan), equipped with a flame ionization detector (FID) and a FFAP fused-silica
204 capillary column (30 m × 0.53 mm I.D., film thickness 1.0 μm), using nitrogen as a
205 carrier gas. The oven temperature was initially set at 50 °C for 3.5 min. and then
206 increasing 25 °C/min until 130 °C, followed by 10 °C/min until 210 °C, and kept at
207 final temperature for 10 min. The injection port and detector temperatures were set at
208 150 °C and 230 °C, respectively. All the determinations were performed in triplicate.
209
210 ## 3. Results and Discussion
211 ### 3.1 Methane potential of single antifoam, and manure mixed with gelatine or
212 Na-Oleate
213 The biochemical methane potentials of single antifoam, and the cattle manure mixed
214 with gelatine or Na-Oleate as single substrate, are presented in Table 2. The theoretical
215 methane potentials were calculated based on the chemical composition of manure
216 (Angelidaki et al., 2009) and using the stoichiometric conversion of the antifoams to
217 methane and carbon dioxide, assuming full degradation of each substrate. It was
218 recorded that the achieved methane yield of the cattle manure supplemented with
219 gelatine was 324 mL CH_4/gVS-added, corresponding to 68% of the theoretical yield.

Similarly, the methane yield of manure containing Na-Oleate reached 67% of the theoretical, corresponding to a methane yield of 380 mL CH_4/gVS-added. The incomplete degradation of the organic matters could be due to the relatively high content of recalcitrant materials (i.e. large fraction of straws and fibers in cow manure), or due to the slow degradability and inhibition effect of Long Chain Fatty Acids (LCFA) in the case of Na-Oleate. The latter was also verified by the process monitoring, as it was clearly observed that the batch bottles containing cattle manure mixed with Na-Oleate presented a lag phase of approximately 14 days before the initiation of methane production. It has been previously reported that β-oxidation pathway, responsible for the degradation of LCFA, can become a rate-limiting step during the anaerobic digestion process because of the slow growth of the LCFA consuming microorganisms and the requirement of low hydrogen partial pressure (Nordell et al., 2013). Concerning the degradation of antifoams as pure substrates, the methane yield of rapeseed oil and oleic acid was 704 and 837 mL CH_4/gVS-added, respectively, corresponding to 70% and 83% of the theoretical yields. In this case the incomplete degradation both antifoams could also be due to the inhibition effect of the LCFA as described above. Moreover, as previously mentioned, rapeseed oil contains not only oleic acid, but also high content of linoleic acid. It has also been previously reported that linoleic acid was more inhibitory than oleic acid (Templer et al., 2006). Thus this could be the reason for the lower methane yield of rapeseed oil compared to pure oleic acid.

Table 2. Biochemical and theoretical methane potential of the used substrates

Substrate	Initial load (gVS/L)	Methane yield	Theoretical

		from batch assays (mL/gVSadded)	methane yield at STP conditions (mL/gVSadded)
Cattle manure+ Gelatine*	1	324±7	473
Cattle manure+ Na-Oleate*	1	380±10	568
Rapeseed oil	1	704±13	1100
Oleic acid	1	837±0.3	1106

*The concentration of gelatine and Na-Oleate added in manure were 9 g/L and 12g/L, respectively, in terms of TS, corresponding to 8.91 g/L and 9.72 g/L, respectively, in terms of VS.

3.2 Effect of antifoams on biomethanation of cattle manure mixed with gelatine or Na-Oleate

The influence of antifoams on biomethanation of cattle manure mixed with gelatine or Na-Oleate was examined in batch assays. Possible synergy or inhibition during the process was determined by comparing the achieved methane yields from the co-digestion of antifoam and manure mixture (cattle manure mixed with gelatine or Na-Oleate), with the expected methane yield calculated by adding the methane yield of single antifoam, to the methane yield of cattle manure mixture. The aggregated results from the batch assay are illustrated in Fig. 1 and 2.

Concerning the effect of rapeseed oil on the biomethanation process of cattle manure mixtures, it was found that the methane productions in both cases (gelatine and Na-

Oleate) were increased (Fig 1a and b). More specifically, in the case of gelatine, the methane yields of manure mixture added with rapeseed oil at concentrations of 0.05%, 0.1% and 0.5% v/v were 421 ± 24, 466 ± 33 and 674 ± 23 mL-CH_4/gVS-added, corresponding to 77%, 78% and 82% of the theoretical yield, respectively. The expected methane yield of these substrates, calculated by summation of the BMP from cattle manure mixture and from pure rapeseed oil at concentration of 0.05%, 0.1% and 0.5% v/v, were 370, 399 and 534 mL-CH_4/gVS-added, respectively, which were 14–26% lower than the BMP results from the mixed substrate. Similarly, in the case of Na-Oleate, the methane yields of manure mixture added with rapeseed oil at concentrations of 0.05%, 0.1% and 0.5% v/v were 419 ± 20, 504 ± 3 and 633 ± 17 mL-CH_4/gVS-added, corresponding to 66%, 75% and 74% of the theoretical yield, respectively. The expected methane yield of these substrates calculated by summation of the BMP from single compounds at concentration of rapeseed oil equal to 0.05%, 0.1% and 0.5% v/v, were 419, 444 and 559 mL-CH_4/gVS-added, respectively. In this case, it was found that the addition of 0.05% v/v rapeseed oil resulted in equal values of achieved and expected methane yield. However, higher concentrations of rapeseed oil exhibited a synergistic effect, leading to an increment of the achieved methane yield by 13% compared to the expected one. A possible explanation for the recorded synergistic effects is related to the less efficient degradation of rapeseed oil when the oil is used as single substrate (e.g. due to inhibition from LCFA), and to the contribution of the easily biodegradable organic matter of cattle manure, when rapeseed oil is used as co-substrate. This means that the expected methane yield, based on the individual BMPs of pure substrates, was underestimated because of the low biomethanation efficiency of rapeseed oil as single substrate. In contrary, during the co-digestion of manure mixture and rapeseed oil, the

easily degradable organic fraction of manure could have stimulated the activity of the microbial community, which, in turn, resulted in higher hydrolytic capacity for the LCFA degradation. Once the degradation of LCFA was stimulated and the effect of LCFA inhibition was minimised, the methane yield from co-digestion could be increased due to high energy content in the lipids. The positive influence of rapeseed oil on biogas yield during co-digestion strategies in manure based reactors was previously shown also in other studies (Zhou et al., 2011).

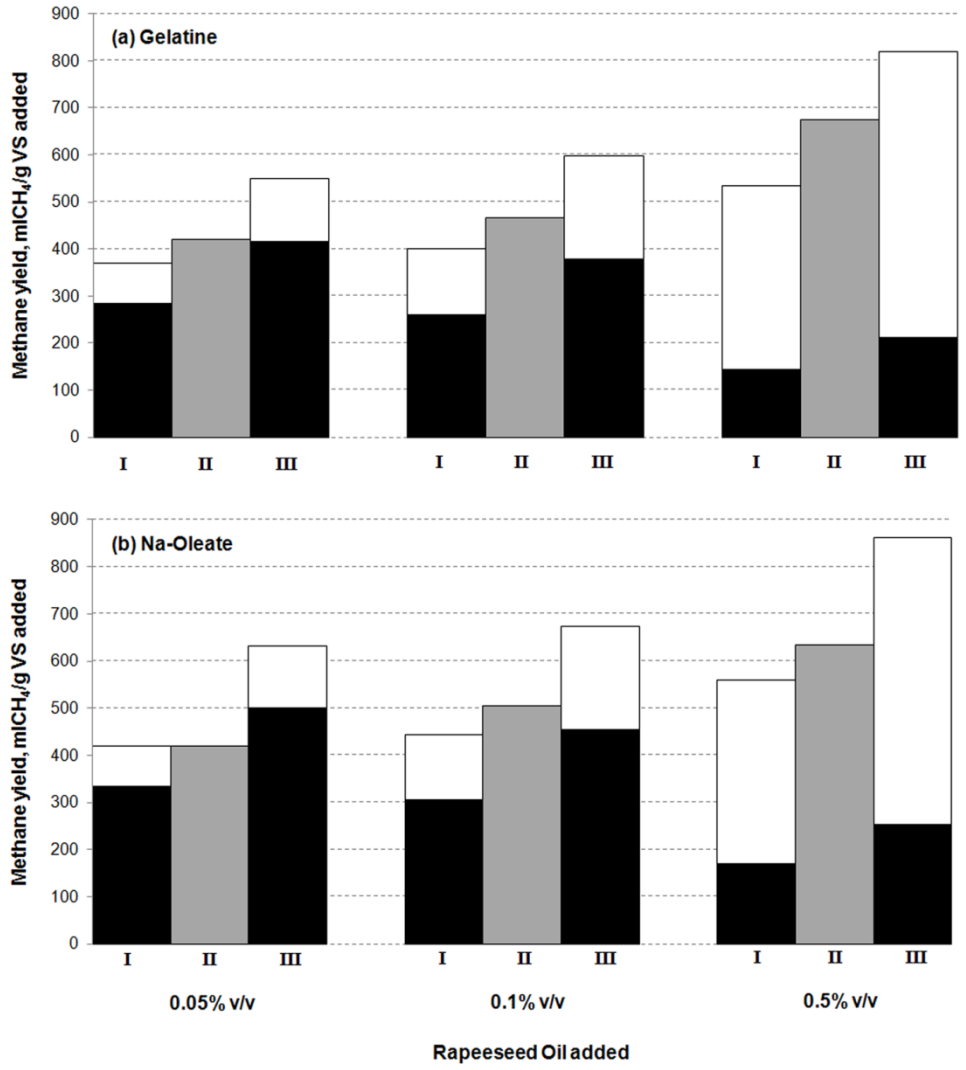
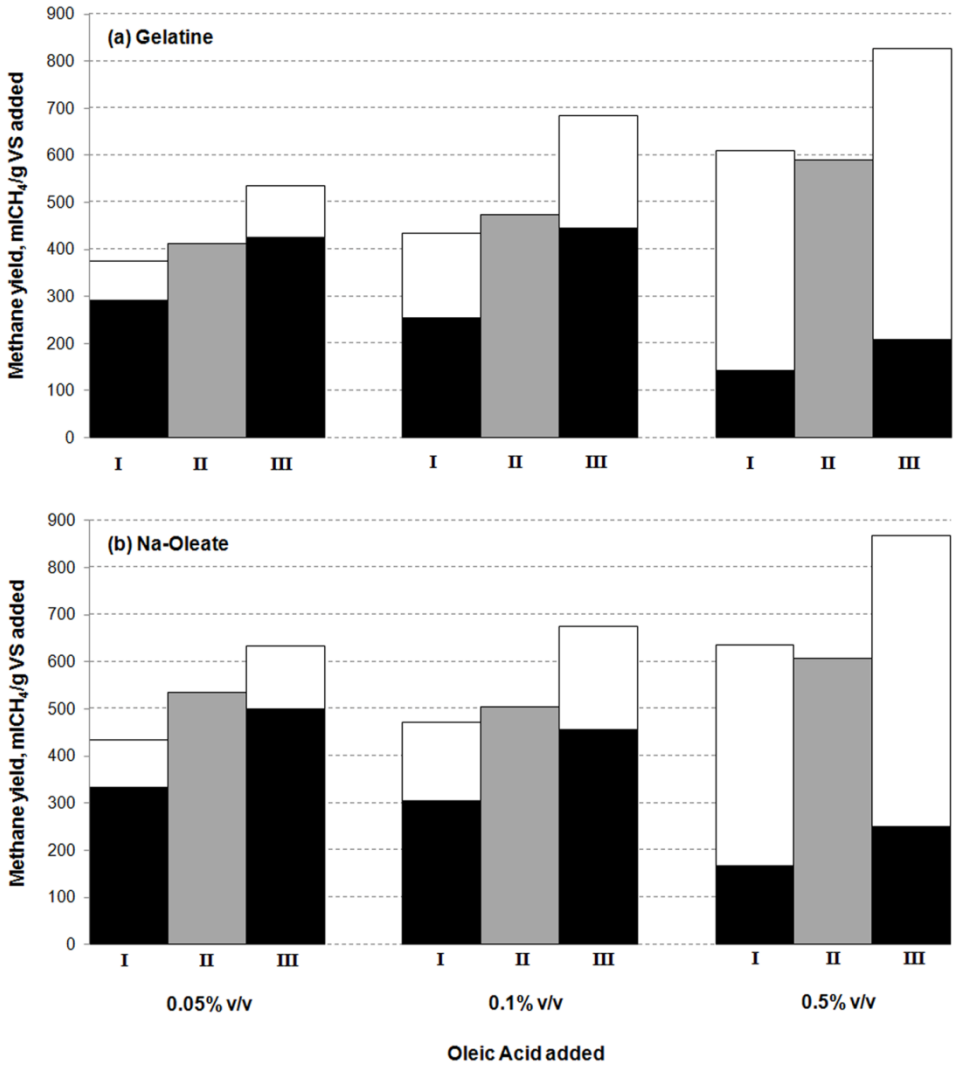


Fig. 1. Methane yield of cattle manure mixtures (black column), rapeseed oil (white

column) and cattle manure mixtures added with 0.05%, 0.1% and 0.5% v/v rapeseed oil (grey column) in case of manure co-digestion with gelatine (a) and Na-Oleate (b); (I) from BMP assays of single substrates, (II) from BMP assays of mixed substrates, and (III) from theoretical calculations.

In contrast to the results of rapeseed oil, the influent of oleic acid on biomethanation of cattle manure (Fig. 2) was found to be critically dependent on the added concentration of oleic acid. The synergy effect was only observed at dosages of 0.05% and 0.1% v/v in both cases (gelatine and Na-Oleate). By increasing the concentration of oleic acid to 0.5% v/v the methanogenesis was inhibited. These results was in agreement with our previous study in which the positive impact of oleic acid in batch reactors fed with carbohydrate rich manure mixture was presented only up to concentration of 0.1% v/v; over this concentration the beneficial contribution of oleic acid was neglected (Kougias et al., 2014b). It is well known that unsaturated LCFA, such as oleic acid, have inhibitory effect in anaerobic systems (Cuetos et al., 2010). Lalman and Bagley (2001) reported that oleic acid, even at low concentrations, inhibit aceticlastic methanogenesis and may cause a slight toxicity on hydrogenotrophic methanogenesis. In the reactor fed with gelatine, the methane yields of manure mixture added with oleic acid oil at concentrations of 0.05% and 0.1% were 413±32 and 473±58 mL-CH_4/gVS-added, corresponding to 77% and 70% of the theoretical yield, and were 10 and 8 % higher than the expected methane yield based on the summation of the individual BMPs, respectively. The achieved methane yield in the batch bottles with 0.5% v/v oleic acid reached 589±39 mL-CH_4/gVS-added (71% of the theoretical yield) which was 3.5% lower than the expected yield of these substrates. Additionally, in the Na-oleate

311 treatment, the methane yields of manure mixture added with oleic acid oil at
312 concentrations of 0.05%, 0.1% were 536±22 and 505±23 mL-CH_4/gVS-added,
313 corresponding to 85% and 75% of the theoretical values, and were found to be 23% and
314 7% higher than the expected methane yields. Finally, by increasing the concentration of
315 oleic acid to 0.5% v/v, the inhibition resulted in lowering the achieved methane yield by
316 4.5% compared to the expected one, producing 607±13 mL-CH_4/gVS-added (70% of
317 the theoretical methane yield).



318

319 **Fig. 2.** Methane yield of cattle manure mixtures (black column), oleic acid (white

column) and cattle manure mixtures added with 0.05%, 0.1% and 0.5% v/v oleic acid (grey column) in cases of manure co-digestion with gelatine (a) and Na-Oleate (b); (I) from BMP assays of single substrates, (II) from BMP assays of mixed substrates, and (III) from theoretical calculations

3.3 Effect of antifoams under continuous reactor operations

The continuous reactor experiment was conducted in order to study the antifoaming efficiency of rapeseed oil and oleic acid, added at different concentrations, in biogas reactors suffering from foaming incidents caused by proteins or lipids. During the experimental period that the antifoams were added at a concentration of 0.05% v/v_{feed}, operational problems occurred (i.e. overheat of reactor and blockage of tubing system) and it was necessary to re-inoculate the reactors. Therefore, the results from this period are excluded from the report.

Fig. 3 and Fig.4 illustrate the process performance of the reactors under continuous operation. In the beginning of the experiment, all reactors were fed with cattle manure only. On day 15, gelatine was added as co-substrate for the reactor R1 and R2 and Na-Oleate was added as co-substrate for the reactors R3 and R4. The start-up period lasted until all reactors produced persistent and stable foam daily. The methane yields of R1 and R2 during this period were similar on the average of 201 mL-CH_4/gVS-added, corresponding to 43% of the theoretical value, and the methane yields of R3 and R4 were on average 287 mL-CH_4/gVS-added, corresponding to 51% of the theoretical value. The low methane yields in all reactors could be directly linked with the process imbalances due to the overloaded with protein or lipid. At the end of start-up period, the daily volume of foam in R1 and R2 was on average 116 mL foam/L-reactor (Fig. 3a and b), while R3 and R4 had a lower foam volume of 92 mL foam/L-reactor (Fig. 3c and d).

The higher foam volume generated by protein compared to liquid could be due to the fact that protein and its degradation products (amino acids) have strong capability as foam stabiliser (Boe et al., 2012).

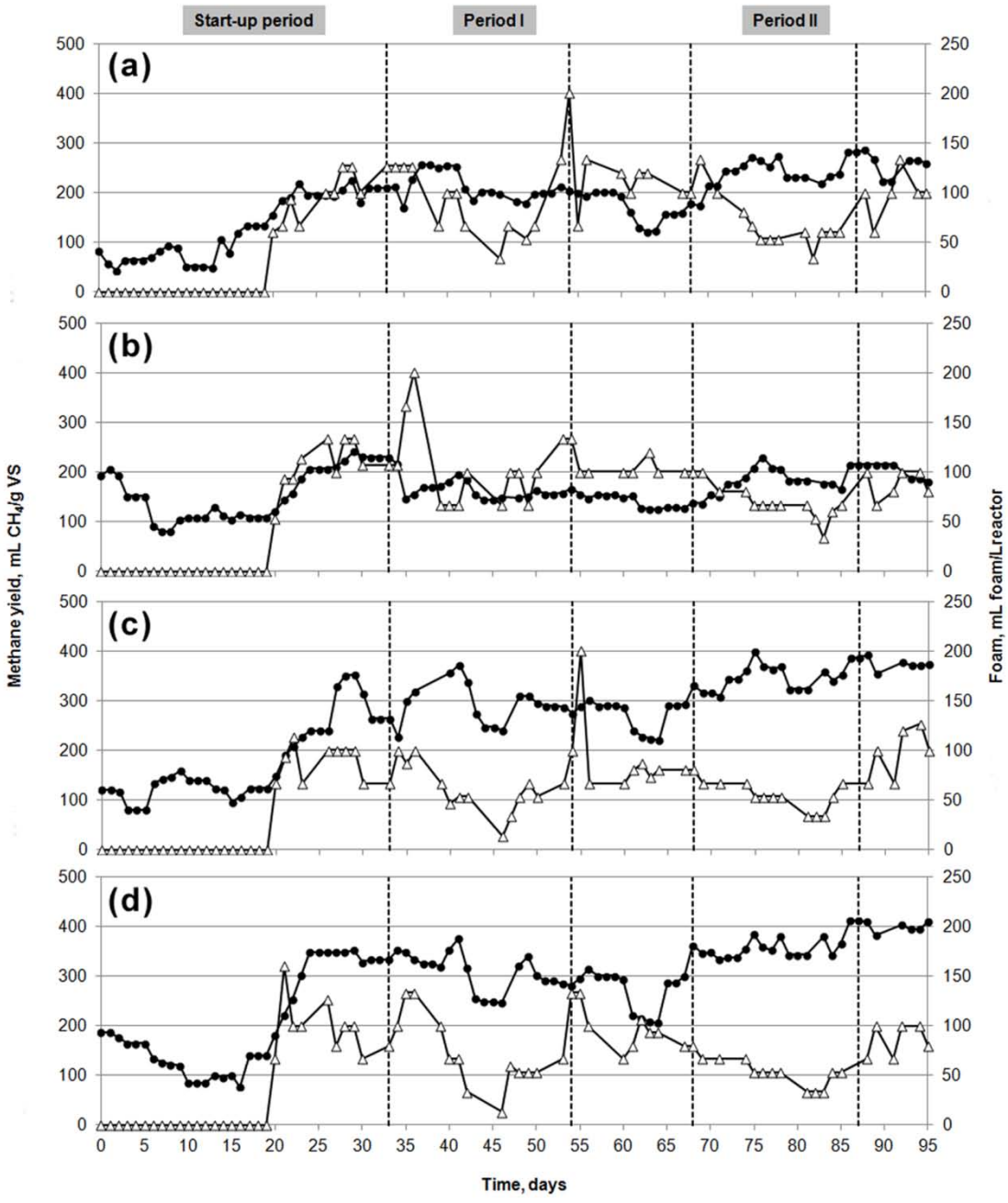


Fig. 3. Methane yield (●) and daily volume of foam production (△) in reactor R1 (a), R2 (b), R3 (c) and R4 (d)

Following the start-up period, rapeseed oil or oleic acid at a concentration of 0.1% v/v_{feed} were added to the feedstock of all reactors (Period I). The antifoams were applied until the achievement of stable foam suppression in the reactors. It was found that antifoam efficiency of rapeseed oil was higher compared to the oleic acid at the same dosage. More specifically, rapeseed oil managed to suppress foaming by 40% in the protein reactor (R1) and by 46% in the lipid reactor (R3) (Fig. 3a and c). In contrary, the foam suppression efficiency of oleic acid was 30% in R2 and 40% in R4 (Fig. 3b and d). Nevertheless, the initial effect of both antifoams on minimising the foam in the reactors occurred simultaneously within in a period of 7 days after their addition in the influent feedstock. Another interesting finding was that the addition of the oleic acid slightly inhibited the methane production. In the protein case, the methane yield of R2 stabilized at the average of 162 mL-CH_4/gVS-added, reaching only 34% of the theoretical value. The methane yield of R2 was 20% lower compared to the period that no oleic acid was added. In contrary, in the lipid case, the methane yield of R4 was in average of 311 mL-CH_4/gVS-added (52% of the theoretical yield), which was 8% higher compared to the period that no oleic acid was added. As discussed previously, oleic acid, even at low concentrations, might inhibit methanogenesis. However, the lower inhibition in the lipid reactor could also be due to the fact that the microbial community populating in R4 was more acclimatised to LCFA, as the reactor had been fed with supplemental amounts of Na-Oleate. Baserba et al., (2012) reported that continuous oleate pulses changed the microbial community of a reactor into a new consortium which was more specialised for LCFA degradation.

During period II, the foam suppression efficiency of both antifoams was higher as their added concentration was increased to 0.5% v/v_{feed}. This is in agreement with a

375 previous study reporting that the antifoaming dosage of 0.5% v/v_{feed} had higher
376 efficiency than the dosage of 0.1 v/v_{feed} (Kougias et al., 2013b). More specifically,
377 rapessed oil could suppress foaming by 52% in R1 (i.e. 13% more than in period I) and
378 by 51% in R3 (i.e. 13% more than in period I). Similarly, oleic acid could minimise the
379 foam by 49% in R2 (i.e. 18% more than in period I) and by 56% in R4 (i.e. 16% more
380 than in period I). During this period, the methane yields of R1 and R3 were in average
381 of 233 and 344 mL-CH4/gVS-added, respectively, which were 16% higher than the
382 corresponding reactors during the period that no rapeseed oil was added. This showed
383 the increase in synergy effect of rapeseed oil significantly in both protein and lipid
384 reactors at higher dosage. In case of oleic acid, the methane yield of R2 reached the
385 average of 179 mL-CH4/gVS-added, which was slightly increased compared to the
386 dosage of 0.1% v/v_{feed}, but still 11% lower than the period that no oleic acid was added.
387 The increased concentration of oleic acid did not significantly improve its synergy
388 effect in reactor operation. The effect of oleic acid as antifoam in the reactor fed with
389 Na-Oleate showed similar trend as in the previous period. The average methane yield of
390 R4 was approximately 354 mL-CH4/gVS-added, which was increased compared to the
391 dosage of 0.1% v/v_{feed}, corresponding to 23% higher than the period that no oleic acid
392 was added. The significant increase of methane yield in R4 confirmed the adaptation of
393 microbial community to the degradation of LCFA as the experiment continued.
394 Concerning the volatile fatty acids (VFA) and pH, the VFA increased mainly during
395 the start-up period and during the early stage of antifoam addition. The increase of VFA
396 in R1 when adding rapeseed oil at dosage 0.1% v/v_{feed} but not 0.5 v/v_{feed} indicated that
397 the system was not previously adapted to rapeseed oil but has undergone adaptation
398 relatively fast, as seen from the decreased in VFA afterward. In contrast, the reactor R2

399 did not successfully adapt to oleic acid, as the VFA concentration remained relatively
400 high and significantly increased when increasing the oleic acid dose to 0.5% v/v_{feed}.
401 Interestingly, the addition of both rapeseed oil and oleic acid as antifoam at both
402 concentrations did not disturb the stability of the reactors that has been fed with Na-
403 Oleate (R3 and R4). As discussed above, the microorganisms in R3 and R4 would have
404 been adapt to the LCFA after the long start-up period. In all reactors, the pH was
405 relatively stable in a range from 7.5 to 8.3, mainly due to the strong buffering capacity
406 in cow manure.

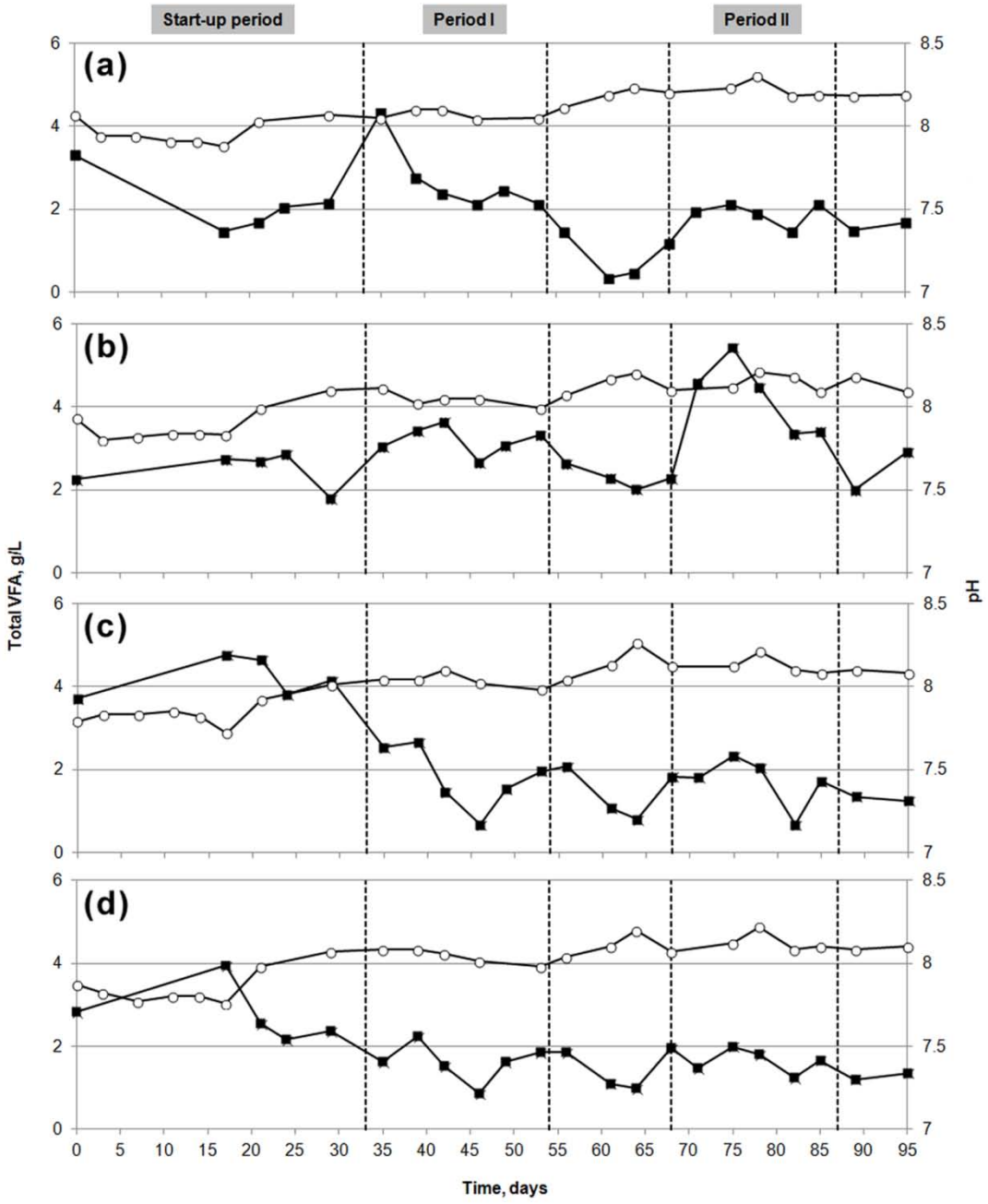


Fig. 4. VFA concentration (■) and pH (○) in reactor R1 (a), R2 (b), R3 (c) and R4 (d)

Each period where antifoam was added in the influent feedstock was followed by a foam recovery period where no antifoam was added. This was done in order to investigate cabablility of antifoams in preventing the regeneration of foaming. Surprisingly, it was recorded that shortly after the discontinuation of antifoams

(approximately 2 days) the foam was regenerated at almost equal quantities as in the initial stated (i.e. before any addition of antifoam). This could be due to the fact that the degradation process of antifoams occurred instantaneously, as their dose was significantly lower compared to the total working volume of the reactor; thus their concentration is rapidly decreasing resulting in total loss of antifoaming effect, unless raw antifoam is added in the reactor.

The contradictory behaviour of the oleate to act as foam promoter when added as fatty acid salt (Na-oleate), and act as antifoam when added in the form of fatty acid (oleic acid) is noteworthy. This can be explained by the chemical structure of these compounds based on their carboxylic ends. Fatty acids, like oleic acid, are hydrophobic substances with low solubility in water. Therefore, due to the hydrophobic behaviour they accumulate on the foaming surface and rapture the foaming film by bridging mechanisms (Denkov et al., 2014). In contrary, the salts of fatty acids are amphiphilic substances possessing both hydrophobic and hydrophilic ends (Scrimgeour, 2005). The hydrophobic end moves the substances towards the air phase, while the hydrophilic end tends to move them towards the liquid phase (Ganidi et al., 2009). As a consequence, the formation of such monolayers at liquid/air or liquid/surface interfaces promote the generation of foam. Additionally, due to its high solubility in water, Na-oleate cannot be maintained in the aqueous solutions as solid particles, in contrary with Ca-oleate which is insoluble (FAO, 1988). Zhang et al., (2003) reported that when oil is present in the medium (as oleic acid or rapeseed oil in this study) the solid particles of insoluble fatty acid salts can be attached to the oil drop surfaces and act as antifoams. Thus, it could be expected that the soluble Na-Oleate will act as foam promoter, while Ca-Oleate or free oleic acid will act as antifoams.

In summary, although oleic acid gave higher methane yield than rapeseeds oil when applied as single substrate, rapeseeds oil showed better synergy effect especially when added as antifoam in the reactor overloaded with gelatin. High concentration of oleic acid inhibited co-digested process in both cases of gelatin and Na-Oleate from the batch assays. The higher methane yield when applied oleic acid as antifoam in the reactor overloaded with Na-Oleate was not due to indigenous synergy effect, but rather due to the acclamation of microorganisms' capability for degrading LCFA.

4. Conclusions

The results from batch assays showed that rapeseed oil could increase methane yield when co-digested with cattle manure mixed with gelatin or Na-Oleate at all tested doses, while oleic acid showed inhibitory effect at the dose of 0.5% v/v. Both antifoams could efficiently suppress foam cause by gelatin or Na-Oleate under continuous reactor operation. It was concluded that rapeseed oil was more suitable as antifoam in both cases since antifoam showed stronger synergy effect. The efficiency of rapeseed oil as antifoam increased with increased dosages.

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Solutions for foaming problems in biogas reactors using natural oils or fatty acids as defoamers

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Solutions for foaming problems in biogas reactors using natural oils or fatty acids as defoamers

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ABSTRACT. Foaming is one of the most common and important problems in biogas plants leading to severe operational, economical and environmental drawbacks. As addition of easily degradable co-substrates for boosting the biogas production can suddenly raise foaming problem, the full-scale biogas plants face an increasing necessity in finding efficient and cost effective antifoaming solutions in order to avoid the dramatic consequences of foaming incidents. The most common solution to suppress foaming is the use of chemical defoamers. The present work is a mini review summarizing the aggregated results from our previous extensive research along with some unpublished data on defoaming by rapeseed oil and oleic acid in manure based biogas reactors. It was found that both compounds exhibited remarkable defoaming efficiency ranging from 30-57% in biogas reactors suffering from foaming problems promoted by addition of protein, lipid or carbohydrate co-substrates. However, in all examined cases the defoaming efficiency of rapeseed oil was greater than oleic acid and therefore this compound is recommended to be used in biogas reactors in order to solve foaming problems.

INTRODUCTION

Foam formation is a disturbance in full scale biogas plants, which can deteriorate the whole anaerobic digestion process. The severe consequences of foaming can lead to main operational problems, such as reactor overflow, fouling of mixing devices, and blockage of tubing systems. These affect negatively to the biogas plant economy, due to biogas production loss and increased maintenance cost. Foaming incidents are very common. Recent studies reported that 12 out of 15 biogas plants in Germany[1] and 15 out of 16 full-scale biogas plants in Denmark experienced foaming either in the main biogas reactor or in the pre-storage tank.[2]

The main causes of foam formation in biogas plants treating manure and organic industrial wastes have been previously identified. The chemical composition of the influent feedstock is highly correlated with foam formation.[3] The manure digester fed with co-substrates that are rich in lipid, protein, or general overload with easily degradable matter such as carbohydrates, are prone to generate foam.[4] However, the tendency of proteins to create foam seems to be higher compared to lipid or carbohydrate, due to the foam stabilizing ability of many amino acids. The latter is of a great importance, as in real practice the biogas plants are commonly co-digesting different types of organic wastes depending on their seasonal availability, and consequently the chemical composition of the influent feedstock is varying during the year. In most of the cases, the radical change of the influent feedstock is the reasoning for foaming and explains why foaming is an intermittent phenomenon. Another major cause of foaming is the organic overloading of the reactor. The direct correlation between organic overload and foam formation was proven experimentally in many studies[5-6] but yet there is not a universal critical threshold of organic loading rate (OLR) above which foam is generated. It was previously suggested that the partial degradation of the organic matter result in accumulation of hydrophobic substances, and thus stimulate foaming.[7]

Additionally, it was found that by applying the same high OLR in a biogas reactor, the thermophilic digestion is more resistant to foaming compared to the mesophilic one.[8] The presence of some specific microorganisms in biogas reactors might also cause foaming. Nevertheless, in contrary with the sludge digestion systems, where the presence of the filamentous foaming and bulking bacteria can be a direct cause of foam[9], the manure based co-digestion system could experience foaming without the presence of these filamentous bacteria. Kougias et al.[4] reported that indeed the microbial community in the manure digesters was significantly changed after foaming incidents; however the well-known foaming bacteria commonly found in sludge digesters (e.g. *Nocardia*, *Microthrix parvicella*) was not found in these manure digesters. The report identified for the first time the presence of a species (operational taxonomic unit), distantly similar to bacteria related to foam (*Nocardia* and *Desulfotomaculum*), whose abundance was increased after foam formation. Finally, other physical parameters such as the reactor's shape or the mixing intensity and patterns may also contribute to foam formation.[10]

As mentioned above, foaming is a complex phenomenon driven by many parameters. However, one general common basis for foam formation is the simultaneous co-existence of gas, liquid phase and also presence of amphiphilic compounds such as detergents or microbially produced bioemulgators. Moreover, particulate matter contributes to formation and stabilisation of foam. Nevertheless, the prediction of foaming incidents, which would as a consequence permit the biogas plant operators to take action for its prevention, is rather difficult. Foam detection in full-scale biogas plants is achieved by the use of normal level sensors or specialised foam sensors that are installed inside the reactors. The foam sensors can be either contact devices (e.g. capacitance or conductivity sensors) or contactless devices based on ultrasound, photo-detection or detection of head pressure variations.[11] Once foam is

detected, antifoaming strategies must be applied as soon as possible before excessive volume of foam is generated.

The most common strategy to suppress foaming in biogas plants is the chemical method using defoamers. Defoamers are commonly added to the reactor by sprinkling from top of the reactor over the foam layer, having as a major aim to induce rapid foam collapse.[12] Typically, a defoamer is composed by oils or hydrophobic solid particles, or a mixture of both.[13] Nowadays, a wide variety of defoamers has emerged and many commercial products are available.[14] However, despite the abundance of commercial defoamers, it is well known that an antifoaming agent may not be suitable for every application.[15] Additionally, the detailed chemical composition of the defoaming solutions is usually not provided by the manufacturers. Therefore, the determination of a proper defoaming product along with its applied dosage in biogas plants is yet an empirical procedure.

The purpose of the work was to aggregate the main results of our previous investigations in a single manuscript, so as to provide more comprehensive knowledge on defoaming strategies for biogas plants. Therefore, the present article is a mini review and summarises the results from different experiments along with some unpublished data investigating the defoaming efficiency of rapeseed oil and oleic acid in manure-based biogas reactors. In all studied cases, the main cause of foam formation was the addition of different types of easily degradable organic co-substrates. The study includes foaming assays and lab-scale continuous reactors experiments. The findings of the present study can give information for counteracting foaming in manure reactors that are co-digested with protein, lipid, or carbohydrate rich substrates.

EXPERIMENTAL SECTION

Preparation and characteristics of the influent feedstock

Dairy cattle manure was the main substrate in all experiments. Upon arrival, the manure was sieved using a plastic net (2 mm opening) to remove large particles and prevent clogging of reactor tubes and pumps. The sieved manure was stored at −20 °C and thawed at 4 °C for 2–3 days before use. The chemical composition of the manure used is presented in Table 1. In different reactor experiments, the raw manure was mixed with protein (9g/L gelatine, Fluka Chemika), lipid (12g/L Na-Oleate, ≥99%, Sigma-Aldrich) or carbohydrate (50g/L glucose, ≥99%, Sigma-Aldrich) before addition to the feed bottle. In the foaming assays, both raw and digested manure were used. The digested manure was collected from the effluent bottles of laboratory reactors facing foaming problems, as described by Kougias et al.[5]

Defoamers

Edible rapeseed oil with low content of erucic acid (less than 2%) and oleic acid (90%, Sigma-Aldrich) were tested as defoamers. The edible rapeseed oil contained mainly oleic acid (51–70%), linoleic acid (15–30%), alpha-linoleic acid (5–14%), and palmitic acid (2.5–7%).[16] Both defoamers were tested at concentration of 0.1% and 0.5% v/v sample.

Determination of the foaming properties

The foaming properties of the samples were determined using a foaming assay based on aeration, as previously described by Kougias et al.[17] All the measurements were performed in triplicate. The foaming properties were compared using two parameters, defined as foaming tendency and foam reduction efficiency.[17]

Continuous reactor experiments

The experiment was carried out in six continuous stirred tank reactors (CSTR). The total and the working volume of each reactor was 2 and 1.5 L, respectively. Each reactor was equipped with a magnetic stirrer to ensure homogenous mixing and a thermal jacket to maintain the temperature steady at 54 ± 1 °C. The hydraulic retention time (HRT) of all reactors was kept

constant at 15 days. The reactors were fed twice per day using a peristaltic pump. The influent feedstock was dairy cattle manure supplemented with 9 g/L gelatine as a representative of proteins, 12 g/L Na-Oleate as a representative of lipids and 50 g/L glucose as a representative of carbohydrates in order to ensure generation of foam.[5] The experiment was divided in three experimental periods. Once steady volume of foam was produced in the reactors (period I), a certain concentration of defoamer (i.e. rapeseed oil or oleic acid) was added to the feedstock bottle of each corresponding reactor. The examined concentrations of the defoamers were 0.1% v/v feed (period II) and 0.5%v/v feed (period III). By the end of periods II and III, the foam reduction efficiency of the defoamers was determined and subsequently the addition of the defoamer was stopped until the foam productivity inside the reactor return to the initial level (i.e. before adding defoamer). The foam height inside the reactor was measured daily, and the foam volume was then calculated based on the working volume of the reactor. After foam volume measurement, the stirring speed of the magnetic stirrers was increased or the reactors were manually shaken in order to destroy the foam layer completely. The defoaming efficiency of rapeseed oil and oleic acid was determined using the following equation:[18]

Defoaming efficiency, (%) = (1-(Foam volume after defoamer addition/Foam volume prior to defoamer addition))*100

Analytical methods

Total solids (TS), volatile solids (VS), pH, total nitrogen (TKN) and total ammonia were determined according to APHA standard methods for the examination of water and wastewater.[19] The chemical composition of biogas was determined using a gas chromatograph (Mikrolab, Aarhus A/S, Denmark), equipped with a thermal conductivity detector (TCD) and packed columns for compound separation (front column: 1.1 m x $^{1}/_{16}$

molsieve 137 + 0.7 m x $^{1}/_{4}$ Lithium Sord K8, back column 10 FF x $^{1}/_{8}$ SS, 60/80 molsieve 5A). The concentration of the Volatile Fatty Acids (VFA) was measured using a gas chromatograph (Shimadzu GC-2010, Kyoto, Japan), equipped with a flame ionization detector (FID) and a FFAP fused-silica capillary column, as described by Kougias et al.[5] All the determinations were performed in triplicate.

RESULTS AND DISCUSSION

Defoaming efficiency of rapeseed oil and oleic acid in raw and digested manure samples

The results from the foaming assay showed that both rapeseed oil and oleic acid presented high defoaming efficiency and could adequately suppress the foam in raw and digested cattle manure samples (Figure 1).

Rapeseed oil could totally suppress foaming in raw manure, and in digested manure supplemented with proteins or carbohydrates (Figure 1a, b and d). Additionally, its defoaming efficiency on digested manure supplemented with lipids was 87% and 93% (Figure 1c), at concentrations of 0.1% v/v_{sample} and 0.5% v/v_{sample}, respectively. The mechanisms beyond foam destruction by the addition of oils have been previously investigated and can be summarised as bridging–stretching, bridging–dewetting, and several mechanisms related to oil spreading.[20] In the bridging–stretching mechanism, the foam film surfaces are bridged by the oil globule with non-balanced capillary pressures at the oil−water and air−water interfaces; afterwards, the bridge stretches in radial direction until it ruptures in the bridge center, resulting in foam destruction.[12] The bridging–dewetting mechanism implies that once an oil bridge is formed between the two surfaces of the foam film, this bridge is "dewetted" from the aqueous phase, due to the hydrophobic surface of the oily globule.[12] However, in our case, the exact mechanism of foam destruction using rapeseed oil it was not clear.

Oleic acid could suppress the foam by 87-96% in raw manure samples (Figure 1a) and by 60-88%, 72-86% and 85-96% in digested cattle manures rich in proteins (Figure 1b), lipids (Figure 1c) and carbohydrates (Figure 1d), respectively. In the cited literatures, oleic acid exhibited good defoaming action when mixed with other compounds (e.g. mixtures of oleic acid and triolein).[21] The results from our study showed that oleic acid presented high defoaming efficiency even when oleic acid was applied alone, and not as a mixture with other substances. By comparing the two tested defoamers, oleic acid exhibited lower defoaming efficiency compared to the rapeseed oil. This difference could be due to the chemical composition of the defoamers, as rapeseed oil not only consists of monounsaturated fatty acids (like oleic acid), but also contains saturated (i.e. palmitic acid) and polyunsaturated (i.e. linoleic and alpha-linoleic) acids. Therefore, it could be possible that a combination of different fatty acids enhanced the ability of rapeseed oil to destabilise the foam. The results from the current study are contradicting a previous argument stating that the efficiency of natural oils to suppress foam is limited as they disperse poorly, have high viscosities and are metabolised.[11] Nevertheless, the current findings verify the hypothesis of the same research indicating that the effectiveness of a natural oil in foam suppression varies greatly with the type of medium,[11] and from our results the defoaming ability of rapeseed oil was remarkable in manure-based samples.

The defoaming efficiency of a chemical compound is highly associated with its applied dosage in the foaming medium. The obtained results showed that the defoaming efficiency increased with increasing concentrations from 0.1% v/v_{sample} to 0.5% v/v_{sample} both for rapeseed oil and oleic acid. It has also been previously reported that each defoamer has a specific concentration that presents its optimum defoaming effect, below which is less effective, while above the optimum concentration it may instead act as foam stabilizer.[22]

Defoaming efficiency of rapeseed oil and oleic acid in biogas reactors

The evaluation of the defoaming efficiency of rapeseed oil and oleic acid along with their effect on process performance and stability was performed under continuous biogas reactor operation. The results from the continuous experiments are summarised in Table 2, 3 and 4.

Concerning the reactors that were fed with cattle manure and proteins, the data in Table 2 were previously extensively presented by Kougias et al.[23] Protein was the cause for foaming in two reactors resulting in a persistent daily foam formation of 116±14 mLfoam/$L_{reactor}$. The addition of 0.1% v/v_{feed} of defoamers resulted in the foam reduction by approximately 40% in the case of rapeseed oil and 30% in the case of oleic acid. By increasing the defoamer's dosage to 0.5% v/v_{feed}, the defoaming efficiency of both compounds was almost equal in the range of 49-52% foam reduction (Table 2).

Regarding the process performance, it was found that oleic acid slightly inhibited the biomethanation process. An increase in the total VFA concentration was observed, and the methane yield was slightly lower than the period that no defoamer was added to the feed. It was previously reported that the long-chain fatty acids resulting from lipid degradation could affect the overall metabolism by increasing or decreasing microbial growth, product formation and substrate utilization.[11] More specifically, long chain fatty acids such as oleic acid are known to be potential inhibitor for many bacteria and archaea in anaerobic digestion process and only selected groups of microorganisms are surviving in such environments, due to the toxicity pressure.[24-25] In contrary, rapeseed oil did not present any negative effect on the process performance. It was found that the addition of rapeseed oil not only decreased the foaming problem but also resulted in increased methane yield as the compound was degraded. It is well known that the component responsible for oil inhibition is the long chain fatty acids (LCFA), while neutral oils are not inhibiting the microbial growth.[26] Fat and oils are first hydrolysed to LCFA and glycerol. Thereafter, the LCFA are degraded by beta-oxidation successively to shorter chained organic acids and finally to acetate. It is important

that when oils are added in a biogas reactor, that an active culture responsible of fast degradation of the released LCFA is present, in order to avoid accumulation of high concentrations of LCFA which might cause inhibition of the anaerobic process. Nevertheless, it should be also mentioned that in case of addition of oils and LCFA as defoamer, the needed concentrations are relatively small, and serious inhibition is not expected.

Concerning the reactors that were fed with cattle manure and lipids, the data in Table 3 were previously extensively presented by Kougias et al.[23] The reactors that were fed with lipid rich manure based substrate had a total daily foam formation of 92±17 mLfoam/$L_{reactor}$. The methane yield in the period that no defoamer was added was on average 286±18 mLCH_4/gVS. At added concentration of 0.1% v/v_{feed}, rapeseed oil could decrease the formed foam by approximately 46%, while the defoaming efficiency of oleic acid was 40%. At higher dosage, oleic acid presented a slightly higher defoaming efficiency, equal to 57%, while rapeseed oil had defoaming efficiency of 51% (Table 4).

Regarding the reactor operation, it was observed that the addition of both defoamers affected positively the overall process performance, increasing the methane yield and maintaining the VFA concentrations at lower levels compared to the period that no defoamer was added. This could be explained by the fact that the reactors were gradually acclimatised to lipid degradation. It was previously shown that the microbial community of a biogas reactor changed in response to continuous oleate addition, resulting in a new consortium specialized for LCFA degradation.[27]

A noteworthy observation from this study was that oleate presented foam promoting behaviour when added as fatty acid salt (Na-oleate) in the influent feedstock, while it presented defoaming properties when added in the form of free fatty acid (oleic acid). This is due to the chemical structure of this compound. Oleic acid in the free fatty acid form is hydrophobic substance with low solubility in aqueous solution. Due to its hydrophobic

behaviour, the oil globule of oleic acid that entries the foaming film surface could destabilise the foam and cause film rapture by bridging mechanisms.[20] In contrary, oleic acid salt such as Na-Oleate is amphiphilic compound,[28] which could form monolayers at liquid/air surface promoting the generation of foam.

Concerning the reactors that were fed with cattle manure and carbohydrates, the data in Table 4 were previously extensively presented by Kougias et al.[18] The recorded daily volume of the generated foam prior to the defoaming addition was 96±8 mLfoam/Lreactor. The addition of both defoamers at concentration of 0.1% v/v_{feed} presented equal defoaming efficiency of 36-37% (Table 4). By increasing the dosage of the defoamers to 0.5% v/v_{feed}, the defoaming efficiency of rapeseed oil increased to 43%, while the defoaming efficiency of oleic acid remained almost the same (37.5%). No process imbalance was observed as the methane yields increased and the total concentration of the total VFA remained at very low levels during the whole experiment. It could be noticed that the addition of oleic acid as defoamer did not cause imbalance in the reactors with carbohydrate, but caused inhibition in the reactors with protein. This could be due to the stress of the methanogens in the reactor with protein due to high concentration of ammonia resulting from protein degradation. Thus, the methanogens in protein reactor were more susceptible to other potential inhibitor.

CONCLUSIONS

The present work summarised all the results from previous experiments regarding the evaluation of defoaming efficiency of rapeseed oil and oleic acid both in raw and digested manure samples. Additionally, results from continuous experiments were presented aiming to investigate the effect of these defoamers on process performance and stability of biogas rectors. It was found that both compounds were capable to suppress foaming, exhibiting remarkable defoaming efficiency ranging from 30-57% in biogas reactors that were suffering foaming problems due to protein, lipid, or carbohydrate. Moreover, it was shown that the

defoaming efficiency of both compounds increased when increasing their applied dosage. However, in all examined cases the defoaming efficiency of rapeseed oil was greater than oleic acid and therefore this compound is recommended to be used in manure-based biogas reactors in order to solve the foaming problems. These results could be directly applied as one of the defoaming strategies in full-scale biogas plants treating agro-industrial wastes.

FIGURES

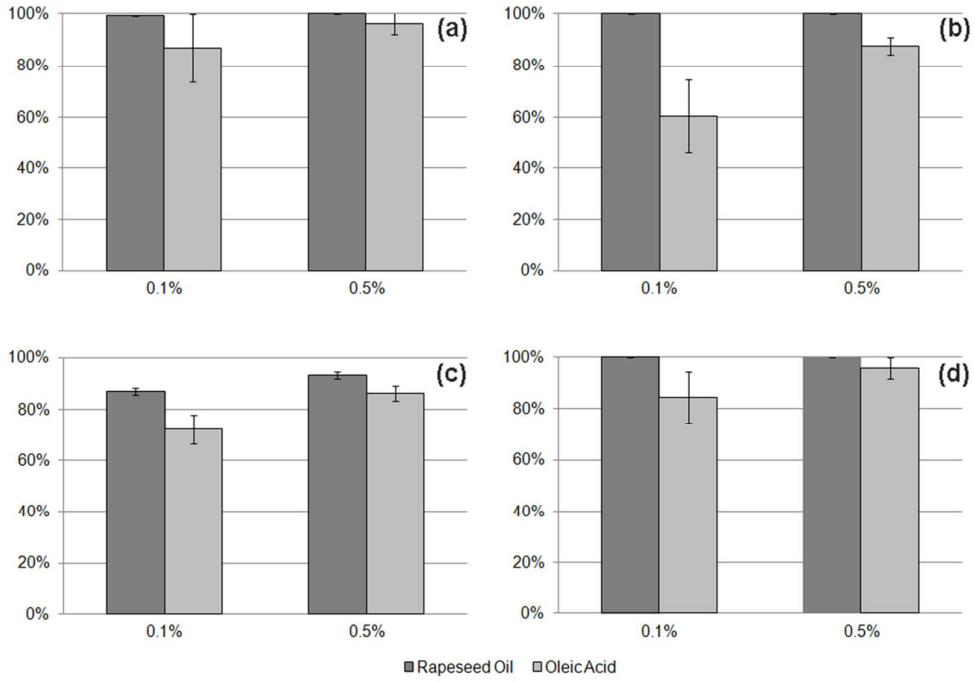

Figure 1. Defoaming efficiency (%) of rapeseed oil and oleic acid in (a) raw cattle manure, (b) digested cattle manure supplemented with proteins, (c) digested cattle manure supplemented with lipids, (d) digested cattle manure supplemented with carbohydrates. Both defoamers were tested at concentrations of 0.1% v/v_{sample} and 0.5% v/v_{sample}.

TABLES.

Table 1. Chemical composition of raw cattle manures used in the experiments

Parameter	Unit	Cattle manure 1*	Cattle manure 2**
pH	-	7.4±0.01	7.3±0.04
Total solids (TS)	g/L	61.6±0.7	61.6±0.4
Volatile solids (VS)	g/L	47.5±0.6	48.1±0.4
Total Kjeldahl Nitrogen (TKN)	g-N/L	3.30±0.17	2.87±0.18
Ammonium Nitrogen (NH_4^+)	g-N/L	2.11±0.14	1.74±0.13
Total Volatile fatty acids (VFA)	g/L	5.53±0.43	7.77±0.53
Acetate	g/L	3.15±0.35	5.44±0.4
Propionate	g/L	1.28±0.65	1.39±0.09
Iso-butyrate	g/L	0.13±0.02	0.12±0.01
Butyrate	g/L	0.60±0.01	0.55±0.02
Iso-valerate	g/L	0.19±0.04	0.18±0.01
Valerate	g/L	0.12±0.0	0.06±0.00
n-hexanoate	g/L	0.03±0.0	0.02±0.00

* Raw cattle manure 1 was used in the physicochemical tests. Also, the manure was supplemented with carbohydrate and used in the continuous mode experiment.

** Raw cattle manure 2 was supplemented with protein or lipid and used in the continuous mode experiment

Table 2. Results from the continuous operation of reactors fed with cattle manure supplemented with protein

		Rapeseed Oil		Oleic Acid	
Parameter	No defoamer	0.1% v/v_{feed}	0.5% v/v_{feed}	0.1% v/v_{feed}	0.5% v/v_{feed}
pH	7.98±0.11	8.07±0.03	8.22±0.05	8.06±0.04	8.14±0.05
Methane yield ($mLCH_4/gVS$)	201±27	215±31	233±28	162±16	179±25
% CH_4 in biogas	67.41±0.91	68.00±2.48	67.83±1.38	66.07±1.03	66.72±1.67
Total VFA (g/L)	2.18±0.53	2.43±0.26	1.75±0.37	3.17±0.38	3.95±0.66
Defoaming efficiency (%)	-	39.98	52.26	30.44	48.92

Table 3. Results from the continuous operation of reactors fed with cattle manure supplemented with lipid

		Rapeseed Oil		Oleic Acid	
Parameter	No defoamer	0.1% v/v_{feed}	0.5% v/v_{feed}	0.1% v/v_{feed}	0.5% v/v_{feed}
pH	7.92±0.14	8.05±0.03	8.13±0.05	8.06±0.03	8.12±0.06
Methane yield ($mLCH_4/gVS$)	286±18	301±43	344±24	311±42	354±16
% CH_4 in biogas	68.18±1.67	69.08±0.98	69.59±1.33	68.59±0.68	69.99±1.30
Total VFA (g/L)	2.76±0.80	1.58±0.82	1.73±0.56	1.62±0.50	1.64±0.29
Defoaming efficiency (%)	-	45.96	51.11	40.52	56.64

Table 4. Results from the continuous operation of reactors fed with cattle manure supplemented with carbohydrate.

		Rapeseed Oil		Oleic Acid	
Parameter	No defoamer	0.1% v/v_{feed}	0.5% v/v_{feed}	0.1% v/v_{feed}	0.5% v/v_{feed}
pH	8.35±0.06	8.32±0.07	8.28±0.05	8.41±0.06	8.33±0.08
Methane yield ($mLCH_4/gVS$)	288±14	282±10	366±11	303±28	363±12
% CH_4 in biogas	59.00±1.41	59.65±2.19	65.06±1.33	57.98±1.76	65.42±0.02
Total VFA (g/L)	0.44±0.14	0.98±0.06	0.44±0.15	0.26±0.05	0.26±0.10
Defoaming efficiency (%)	-	37.78	43.33	36.36	37.50

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. All authors contributed equally.

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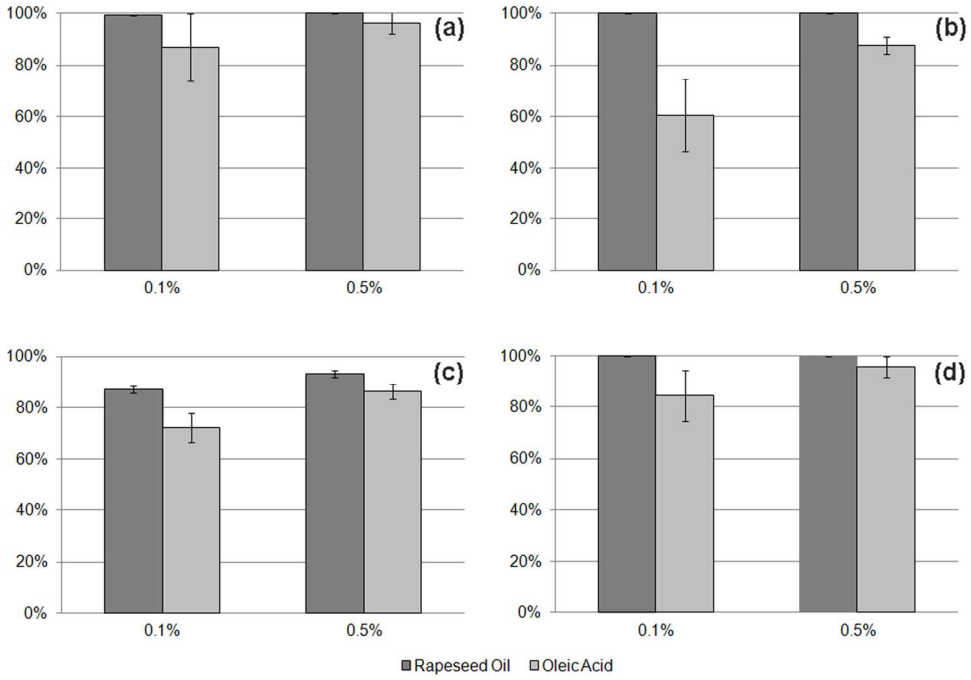

Figure 1. Defoaming efficiency (%) of rapeseed oil and oleic acid in (a) raw cattle manure, (b) digested cattle manure supplemented with proteins, (c) digested cattle manure supplemented with lipids, (d) digested cattle manure supplemented with carbohydrates. Both defoamers were tested at concentrations of 0.1% v/vsample and 0.5% v/vsample.
169x120mm (300 x 300 DPI)

Antifoaming effect of rapeseed oil and oleic acid in biogas reactors

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Foaming is one of the major problems that occasionally occur in the biogas plants, affecting negatively the overall digestion process. Foam typically occurs in the main biogas reactor or in the pre-storage tank and results in adverse operational, economical and environmental impacts. So far, the foaming problem in manure based digesters, which is the main anaerobic digestion process applied in Denmark, has not been thoroughly investigated. Several studies reported that the most dominant factors contributing to foaming are the operational parameters of the digester (i.e. organic overload, temperature fluctuation, inadequate mixing), the feedstock composition and the presence of specific microorganisms. Methods for foam prevention and suppression are classified into four large groups; mechanical, physical, biological and chemical methods. Nevertheless, the complexity of the foam structure makes it difficult to apply a precise and efficient antifoam strategy. The aim of the present study was to evaluate the foam reduction efficiency and to investigate the influence on process performance of rapeseed oil and oleic acid in continuous mode manure-based biogas reactors, which were suffering by foaming incidents.
The experiment was carried out in six continuous stirred tank reactors (CSTR). The total and the working volume of each reactor was 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54 ± 1 °C using thermal jackets. Each reactor was fed with a different mixed substrate, which was found to have an influence on foam formation in our previous study. Thus, the influent manure was supplemented with gelatine as a representative of proteins, Na-Oleate as a representative of lipids and glucose as a representative of carbohydrates. The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. Once the daily foam volume production in the reactors was steady, a certain concentration of rapeseed oil or oleic acid was added to the feedstock bottle of each corresponding reactor in order to evaluate their antifoaming potential. The antifoam addition was done in three periods (period I, II, and III), with the antifoams (rapeseed oil and oleate) dosages of 0.05%, 0.1%, and 0.5% v/v-feed, respectively. During each period and once the reactor had a stable daily foam production, the foam reduction efficiency was calculated, and the addition of the antifoam was stopped until the foam production inside the reactor reached back to its initial level (i.e. before adding antifoam). The results obtained by the present study revealed both compounds were suitable as antifoam for manure digestion. Moreover, rapeseed oil was more efficient in foam suppression in the reactor fed with carbohydrates. The optimal dosage of antifoam agent should be dependent on the severity of foaming incident as the foam reduction efficiency of both compounds increased as their concentration in the reactor was higher.

Simulation of the flexible biogas production from agricultural substrates and residues

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Due to more than 7500 running plants the biogas technology is a significant part of the renewable energy sources in Germany today. In general biogas plants have been constructed and designed to produce a stable and constant energy output. With the changing conditions within the energy sector in Germany biogas plants have to meet new requirements, especially the flexible supply of electricity to compensate for the divergence between energy demand and energy supply by uncontrolled sources like wind and solar power.

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Effect of mechanical pre-treatment methods on the anaerobic digestibility and structure change of meadow grass for biogas production

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Nowadays, biogas plants have been proliferated and there is an imperative need for additional feedstock in order to meet the surplus needs. Lignocellulosic residues are increasingly used as a substrate in full scale biogas plants and consequently grass from marginal lands can be an excellent source for extra biomass to be used for bioenergy production. More specifically, meadow grass is abundant in global agricultural area and therefore this substrate could be utilised for biogas production. However, the utilisation of biomass from these low input cultivated areas is economically feasible only from the highest yielding plots and the plots with close proximity to biogas plants. Moreover, an efficient pretreatment method is needed in order to increase the access of microorganisms to the degradable ingredients.
The present study aimed to examine different mechanical pre-treatment methods for meadow grass and to define whether the pre-treated meadow grass pose the desired characteristics (e.g. size, disruption of the heterogeneous structure of lignocellulose) for efficient biogas production. Moreover, in the present work we investigated potential correlation between the results derived from the standard protocol of biochemical methane potential in comparison with other alternative physicochemical methods (i.e. conductivity test, soluble chemical oxygen demand (SCOD)) in order to define the most accurate and less time-consuming process to evaluate the substrate's degradability efficiency.

Thus, in order to investigate the impact of different mechanical pre-treatments on the biodegradability of ensiled meadow grass, methane potential batch assays were performed. The methane potential was determined according to the guidelines of the biochemical methane potential (BMP)

protocol. All tests were performed in triplicate and the operating temperature was 54 ± 1 °C. Moreover, scanning electron microscopy (SEM) allowed the evaluation of the plant tissues deconstruction after each pre-treatment configuration.

The results obtained from the present study revealed that all the pre-treatment configurations enhanced the digestibility of the meadow grass. However, there was a statistical significant difference of the level of the degradation efficiency among the different configurations. The most efficient pre-treatment method resulted in more than 30% increase of the methane yield compared with the untreated ensiled meadow grass. The increased efficiency was also verified from the SEM observations, as distinct structural variations were observed. Finally, multiple linear regression models predicted the BMP with r2 equal to 56.9 and 42.2 by the results obtained from the conductivity and SCOD test, respectively.

Reed as an alternative biomass source for biogas production

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The strong growth in the global energy demand, coupled with the limitation of the fossil fuel resources, has resulted in an increased interest in the production of bioenergy from biomass. Biogas production from lignocellulosic materials can help increase the share of renewable energies. However, most of the biomass used for the biogas production are standing in competition with food and feed production. This leads to the search for other feedstocks, which does not compete with croplands. Common reed (Phragmites australis) is one of the most widely spread plants in the world, frequent in marshes and the littoral zone of lakes, rivers and estuaries. In some places, this grass can be considered as a pest, which contributes to clogging and overgrowth of water systems, decreasing the use of areas for recreational purposes. Due to its fast-growing properties and high biomass yields, it is recognized as a promising biomass source for renewable energy.
Common reed cannot be used in biogas plants without a pretreatment step, due to the recalcitrant nature of lignocelluloses. One of the most efficient pretreatment methods for lignocellulosic biomass is steam explosion. This consists of heating the biomass at high temperatures, followed by a mechanical disruption of the biomass fibers caused by a rapid pressure drop, thereby resulting in an increase of the biogas yields. The aims of this study were to determine the specific methane yields of steam-exploded reed as well as to identify how the different pretreatment conditions influence its physico-chemical characteristics. For this purpose, reed was pretreated with a steam explosion unit, using different time and temperature combinations and its effects on the material digestibility were analyzed in batch experiments according to VDI 4630. For every pretreatment, scanning electron microscopy (SEM) pictures and detailed chemical analyses of the substrates were carried out for a better understanding of the effect that the pretreatment severity has on the degradation process.

Results showed the great effects steam explosion has on reed, among which stand out the disruption and defibration of its structure, the decrease in the hemicellulose contents as well as the improvements on the specific methane yields up to 88 % comparing to the untreated sample. Thus, steam-exploded reed has a promising potential as source for methane production in anaerobic digestion systems. The utilization of this feedstock in biogas-based biorefinery concepts can help reduce current dependence on fossil energy sources at the same time that decreases land use competition between fuel and food production. Further attempts in optimizing the process should be made.

lulose rich biomass. The fungal biomass will be quantified using Real-Time PCR targeting the 18S rRNA gene of anaerobic fungi. Final results will be presented at the conference.

New Enzymes for the Degradation of Polyesters Under Anaerobic Conditions

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Aliphatic-aromatic polyesters like PBAT (poly(butylene adipate-co-butylene terephthalate)) are produced in industrial scale and are frequently used in bio waste bags or in food packaging. These polyesters can accumulate in biogas plants since the (municipal) bio waste is an interesting carbon source for biogas production. Different studies have proven the biodegradability of PBAT under aerobic conditions. However, only little information exists on PBAT degradation in anaerobic environments.
Here, quantification of degradation products and imaging methods (CLSM, SEM) clearly demonstrated anaerobic hydrolysis of PBAT and PBAT model substrates in biogas sludge. However, the detected hydrolysis rates are still too low for efficient PBAT degradation in industrial biogas plants. For this reason, enzymes from different anaerobic organisms (Clostridium species) were analyzed concerning their ability to hydrolyze PBAT. A selection of hydrolases from various Clostridium species were successfully heterologously expressed in E.coli BL21-Gold(DE3). The esterase activity assay with standard substrate 4-nitrophenyl acetate revealed high activities of up to 700 U/mg (vmax). The crystal structure of one esterase from C. botulinum was solved and revealed that the enzyme contains a metal ion that lies deep beneath the protein surface.
Degradation experiments were performed on a broad spectrum of PBAT model substrates with the aim to get a deeper insight into the reaction mechanisms of these esterases. HPLC/MS quantification of the hydrolysis products clearly proved that esterases from C. hathewayi and C. botulinum are able to hydrolyze PBAT and the model substrates. The esterase activity was detected under mild conditions as well as in biogas sludge.

Metagenomic analysis of foaming in biogas reactors

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Foaming is one of the major problems that occasionally occurring in biogas plants affecting negatively the overall anaerobic digestion (AD) process. According to a recent survey, 15 out of 16 full-scale biogas plants, which were surveyed in Denmark, faced foaming incidents in the main reactor and/or in the pre-storage feeding tank, resulting in 30-50% biogas production loss. The main causes of foaming in biogas plants fed with agricultural and industrial wastes are organic overloading, feed-

stock composition and operational parameters. However, the contribution of specific microorganisms on foam generation in this AD system has not been previously investigated. The aim of the present study was to define potential correlation between specific microorganisms and foaming in manure based biogas reactors overloaded by different feedstock composition (i.e. proteins, lipids and carbohydrates).

The experiment was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3. The total and the working volume of each reactor was 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54 ± 1 °C using thermal jackets. Each reactor was fed with a different mixed substrate, which was found to have an influence on foam formation in our previous study. Thus, the influent manure was supplemented with gelatine as a representative of proteins (R1), Na-Oleate as a representative of lipids (R2) and glucose as a representative of carbohydrates (R3). The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The whole experiment was divided into two periods. During the first period, the reactors were fed only with cattle manure. Once steady state conditions were reached, liquid sample from all reactors was obtained for DNA extraction and metagenomic analysis. After sampling, the feedstock composition of each reactor was changed by the addition of gelatine or Na-Oleate or glucose (second experimental period). As a consequence, foam formation was observed in all reactors approximately after one HRT period. Once the daily volume of the formed foam was steady, samples were taken again for DNA extraction and metagenomic analysis. Data derived from the 16S rDNA sequencing were compared in order to determine differences in the microbial consortium of the reactors prior and after foaming.

The results obtained from the present study revealed that significant variations in the microbiology of the manure-based biogas reactors were observed after foam formation. A number of genera could be linked to foaming as they produce biosurfactants (Lactobacillus, Bacillus, Pseudomonas, Thermotoga), others contain mycolic acid in their cell wall (Thermoactinomyces, Pseudonocardia) or decrease the surface tension of the media (Micrococcus, Streptococcus). Frankia, Dialister and Paenibacillus are known to be correlated to this phenomenon but their mechanism is still unclear. The results from the present study reinforce the idea that a microbial abundance threshold is critical for foam formation.

Process disturbances and their indicator microbs

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The anaerobic digestion of organic material to biogas is a highly sensitive process: small variations in the process chain such as a temperature increase or the overproduction of volatile fatty acids (VFA) in combination with insufficient buffer capacity can lead to a strong decrease in the biogas yield and hence dramatic economic loss, especially in the case of agricultural biogas plants (BGP). As the biogas production is mediated by a complex, metabolically interconnected microbial consortium, there is an urgent need to better harness the high levels of microbial community organization and functionality. Microbiological indicators as valuation standard for the stability, efficiency and adaptability of the occurring community or as an indicator of disturbances can only be established if it is possible to identify process-relevant indicator organisms in a positive and in a negative sense.

Since targeted disturbances in the operation of agricultural BGPs cannot be induced for economic reasons, unpredictable random process variations or failures are of significant value. Therefore, we carried out a comprehensive analysis of eight mesophilic agricultural BGPs with special emphasis on process-unfavorable events by screening the prevalent procedural-chemical parameters. For an inventory of the complex and dynamic microbial communities we used the community profiling

rearrangements of the biogas-producing community upon protein feeding and specific differences due to the individual protein substrates were recognized
The results amply reveal that these high-energy-content waste products can be converted to biogas, a renewable energy carrier with flexible uses that can replace fossil natural gas in its applications. Process control, with appropriate acclimation of the microbial community to the unusual substrate, is necessary. Metagenomic analysis of the microbial community by next-generation sequencing allows a precise determination of the alterations in the community composition in the course of the process.

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Changes in the microbial profile of biogas reactors due to variations in the feedstock composition

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Anaerobic digestion (AD) is a widely applied methodology for treating different types of wastes, which can be extremely complex and contain several compounds that could inhibit the AD process. Aim of this study is to gain a deeper understanding on how the microbial ecology of biogas reactors responds to radical changes of substrate.
The experimental work was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3, operating under thermophilic conditions (54±1oC). The hydraulic retention time (HRT) of the reactors was kept to 15 days. During the start-up period all the reactors were inoculated with thermophilic inoculum obtained from Hashøj biogas plant, Denmark. The whole experiment was divided in two periods; during the first experimental period all the reactors were fed with cattle manure, while in the second period the substrate contained cattle manure supplemented with proteins (R1), lipids (R2) and carbohydrates (R3). Samples were taken from each reactor prior to the feedstock change and after a time period of one HRT during which the reactors were fed with the corresponding mixed substrate. DNA was extracted from each sample and the corresponding microbial composition was determined via analyses of 16S amplicons.
Before the substrate change, the microbial composition of the three reactors was found to be extremely similar. The most abundant within the identified genera (relative abundance >1%) were Methanobrevibacter, Megamonas, Flectobacillus, Bacteroides, Clostridium, Myroides, Flavobacterium and Bacillus. After the substrate modification the profile of the microbial communities substantially changed within the relatively short period of time corresponding to one HRT.
In the reactor supplemented with carbohydrates, the relative abundance of microorganisms belonging to the genera Methanobravibacter and Bacteroides decrease 12 and 9 folds, respectively. Myroides and Flavobacterium also decreased their relative abundance. These decrements are compensated by the enormous increase (69 folds) of Lactobacillus. In the reactor supplemented with lipids we again observed an increment of Methanobrevibacter and Bacteroides (6 and 4 folds, respectively) together with Myroides (14 folds) Flectobacillus (2 folds) and Bacillus (decrease of 2 folds). Megamonas is the only genus that increased its relative abundance upon the addition of lipids (increase of 2 folds). In contrast with the aforementioned reactors, the one supplemented with proteins did not undergo substantial changes in its microbial composition. Methanobrevibacter is the only genus whose relative abundance varies significantly (decrease of 2 folds).

This simple analysis underlines the dynamicity of microbial communities populating biogas reactors showing that by changing the composition of the main substrate the profile of the microbial community undergoes a profound transformation and is significantly altered already after a relatively short period of time corresponding to one HRT.

Algae biomass as biogas substrate: laboratory fermentations and metagenomic studies

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Microalga biomass offers a promising alternative to the commonly used maize silage in biogas technologies. It can be cultivated on areas not suitable for other agricultural activities, algae do not compete with food production and may yield more biomass per unit land than any terrestrial plant. Alga biomass utilization can be part of a complex biorefinery strategy to supply high value chemicals, e.g. carotenes, enzymes, or other energy carriers such as hydrogen before the spent biomass is used for biogas generation.
There are essentially two approaches for mass cultivation of microalgae. In open ponds pure cultures are difficult to maintain, a mixture of algae and other microbes is formed. More sophisticated closed photobioreactors yield biomass of high microbiological purity but production cost may be disproportionate.

Two substrates were tested for their biogas potential. A mixture of Chlamydomonas sp. and Scenedesmus sp. and syntrophic bacterial partners (mostly representatives of the genus Rhizobia and Burkholderia belonging to the phylum Proteobecteria), called MIX, and an almost 100% pure Scenedesmus obliquus culture, cultivated in a tubular photobioreactor, were studied in laboratory CSTR biogas reactors. Reactors fed with maize silage and a 50-50% mixture of maize silage and MIX (on organic dry substance, oDS, basis) were included in the experimental set-ups. In addition to temperature control at 37°C, the pH and redox potential in the reactors and volumetric gas production were continuously monitored, the FOS/TAC, ammonium ion concentration, total organic carbon, total organic nitrogen, gas composition were measured regularly and the carbon/nitrogen (C/N) ratio of the input material was determined. The composition of the microbial community was established at regular intervals through metagenomic analysis of databases obtained by next generation DNA sequencing.

The MIX had a very low C/N ratio (5.3). The unfavorable C/N might be the reason why the biogas yield was lower than that of the maize silage (C/N=45). Replacing half of the MIX with maize silage improved the situation and the biogas yield was about the same as with maize silage alone. It is noteworthy, that the CH_4 content of the gas from MIX was significantly higher than in the case of maize silage. Taken together about 25% less CH_4 was produced from the MIX relative to maize silage. In the reactors containing MIX the Bacteria domain was quickly dominated by the Proteobacteria carried in with the substrate.
The C/N content of the S. obliquus culture was higher (C/N=9) although still below the optimum 20-30 value. As in the case of MIX co-fermentation with maize silage gave the best CH_4 yield. Biogas continued to evolve from the algae after discontinuing the daily substrate input, which indicated that the decomposition of the algal cell wall required longer time than that of the maize silage. In the Bacteria domain the Bacteroidetes phylum was most abundant.

The composition of the Archea domain was not altered by the addition of the new substrates.
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Anaerobic co-digestion of wastewater microalgae together with swine manure for biogas production

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Abstract Co-digestion of manure with several organic wastes is a common practice applied in Danish centralised biogas plants. The methane yield from manure is relatively low, thus, co-digestion of manure with organic substrates with a high content of lipids, proteins or carbohydrates, becomes necessary for the biogas plant's economy. Biogas plants currently utilise around 7% of manure and a large part of the organic wastes from the Danish industries. With the increased number of biogas plants in the near future, the organic wastes from industries will not be enough to support the strong need of co-substrates that give high methane potential in order to make the biogas plant economically viable. Thus, the new un-conventional types of co-substrate will also be utilised in the future. The biomasses that have recently gained increasing interest in Denmark are cellulosic residues such as grass, straws, coast seaweed, etc. The aim of this study was to investigate the possibility of co-digesting wastewater microalgae with swine manure that contain high nitrogen concentration in order to improve the C/N ratio and thus achieve better process performance. The efficiency of different mixing ratios of microalgae and manure in terms of methane production was evaluated in batch assays. Once the optimum mixing ratio was defined, the process was monitored in continuous reactor experiment. The experiment is currently ongoing and definite results are anticipated by the conference time.

2nd International Conference on Algal Biorefinery: A potential source of food, feed, biochemicals, biofuels and biofertilizers, August 27-29, 2014, Kgs. Lyngby, Denmark
ORAL PRESENTATION (Session 7. ALGAL APPLICATIONS)

An integrated process of biogas upgrading and microalgae production for co-digestion in biogas reactor

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Abstract The production of biogas during the anaerobic digestion process of organic residues is widely known as an emerging alternative energy technology. Therefore, biogas is envisioned as a key element in achieving 20% renewable energy by the year 2020, as set by the European Union. Additionally, in Denmark, the government proposed a surplus target of exploiting 50% of the manure produced in country for renewable energy production, which would be essentially fulfilled by extensive expansion of biogas facilities. Biogas mainly consists of CH_4 (40– 75%) and CO_2 (25–60%). The upgrade of biogas to methane content of more than 90% would increase its heating value and therefore it would be feasible to use biogas in the infrastructure of natural gas pipelines. The aim of this work was to investigate the possibility of coupling a biogas upgrading process to the cultivation of microalgae. The scrubbing system consisted of a gas-liquid separator containing an alkaline solution. Effluent from the scrubber (carbonate solution) was subsequently used as medium for microalgae cultivation such that dissolved inorganic carbon could be used instead of supplying gaseous carbon dioxide. The upgrading efficiency of the scrubber was tested by using different concentrations of the alkaline solution. Moreover, the growth of different microalgae species under various operational parameters (i.e. pH, carbonate concentration, dilutions of biogas reactor digestate as nutrient source) was screened in microplates and evaluated. Once the optimal growing conditions/ microalgae species was defined, continuous cultivation in photobioreactors was performed in order to monitor the process. The experiment is currently ongoing and definite results are anticipated by the conference time.

2nd International Conference on Algal Biorefinery: A potential source of food, feed, biochemicals, biofuels and biofertilizers, August 27-29, 2014, Kgs. Lyngby, Denmark
ORAL PRESENTATION (Session 5B ALGAE FOR WASTEWATER TREATMENT: Domestic wastewater studies)

5B.1

Microbial diversity and dynamicity of biogas reactors fed with different substrates

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Anaerobic digestion (AD) is widely applied method for treating different types of wastes. Nowadays, the biogas plants have proliferated and therefore extra biomass is needed in order to meet the surplus needs. In Denmark, most of the biogas plants are co-digesting manure (at an amount of 70%) and other organic residues mainly derived from food industries (at an amount of 30%) (Angelidaki and Ellegaard, 2003). As a consequence, the mixed feedstock is very complex and contains several compounds that could either result in a successful combination for the biomethanation or in contrary could inhibit the AD process. Aim of this study is to gain a deeper understanding on how the microbial ecology of the biogas reactors, mainly constituted by Archaea and Bacteria, responds to radical changes of substrate.

The experimental work was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3, operating under thermophilic conditions (54±1^{o}C). The hydraulic retention time (HRT) of the reactors was kept to 15 days. During the start-up period all the reactors were inoculated with thermophilic inoculum obtained from Hashøj biogas plant, Denmark. The whole experiment was divided in two periods; during the first experimental period all the reactors were fed with cattle manure, while in the second period the substrate contained cattle manure supplemented with proteins (R1), lipids (R2) and carbohydrates (R3). The amount of the different compounds added to the manure was based according to preliminary experiment in order to maintain the level of organic loading rate (OLR) stable (Kougias et al., 2013). Samples were taken from each reactor prior to the feedstock change and after a time period of one HRT during which the reactors were fed with the corresponding mixed substrate. DNA was extracted from each sample and the corresponding microbial composition was determined via analyses of 16S amplicons. During the experiment the daily biogas production, the composition of methane in biogas, pH and the concentration of volatile fatty acids (VFA) were recorded.

Before the substrate change, the microbial composition of the three reactors was found to be extremely similar. The most abundant within the identified genera (excluding < 1% relative abundance) were found to be *Methanobrevibacter*, *Megamonas*, *Flectobacillus*, *Bacteroides*, *Clostridium*, *Myroides*, *Flavobacterium* and *Bacillus*.

After the substrate composition was modified, taking in consideration only the genera whose relative abundance reached at least 1% before or after the change, the profile of the microbial communities substantially changed within a relatively short period of time, corresponding to one HRT.

In the reactor supplemented with carbohydrates, the relative abundance of microorganisms belonging to the genera *Methanobravibacter* and *Bacteroides* show the highest decrease (12 and 9 folds, respectively), *Myroides* 3 folds and *Flavobacterium* 2 folds. These decrements are compensated by the enormous increase (69 folds) of *Lactobacillus* and the more moderate of *Bacillus* (2 folds). In the reactor supplemented with lipids *Methanobrevibacter* and *Bacteroides* are again found to decrease of several folds (6 and 4, respectively) together with *Myroides* (14 folds) *Flectobacillus* (2 folds) and *Bacillus* (decrease of 2 folds). *Megamonas* is the only genus that increased its relative abundance upon the addition of lipids (increase of 2 folds). In contrast with the aforementioned reactors, the one supplemented with proteins did not undergo substantial changes in

its microbial composition. *Methanobrevibacter* is the only genus whose relative abundance varies significantly (decrease of 2 folds).
This simple analysis underlines the dynamicity of microbial communities populating biogas reactors showing that by changing the composition of the main substrate the profile of the microbial community undergoes a profound transformation and is significantly altered already after a relatively short period of time corresponding to one HRT.

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Conference topic: - Microbial inhibition and adaptation to perturbations <u>ORAL PRESENTATION</u>

Comparative microbial analysis before and after foaming incidents in biogas reactors

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Foaming is one of the major problems that occasionally occurring in biogas plants affecting negatively the overall anaerobic digestion (AD) process. According to a recent survey, 15 out of 16 full-scale biogas plants, which were surveyed in Denmark, faced foaming incidents in the main reactor and/or in the pre-storage feeding tank, resulting in 30-50% biogas production loss (Kougias et al., 2014). In activated sludge systems and in wastewater treatment plants the major causes of foaming are organic overload, the presence of surface active agents, operational parameters (e.g. digester's shape, mixing system etc) and filamentous microorganisms (e.g. *Gordonia species, Microthrix parvicella*) (Ganidi et al., 2009). However, the contribution of specific microorganisms on foam generation in biogas reactors fed with agro-industrial wastes has not been previously investigated. The aim of the present study was to elucidate the microbiology of biogas reactors fed with different substrates prior and after foaming incidents.
The experiment was carried out in three continuous stirred tank reactors (CSTR) denoted as R1, R2 and R3. The total and the working volume of each reactor was 2 and 1.5 L, respectively. Each reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54 ± 1 °C using thermal jackets. Each reactor was fed with a different mixed substrate, which was found to have an influence on foam formation in our previous study (Kougias et al., 2013). The hydraulic retention time (HRT) of all reactors was kept constant at 15 days. The whole experiment was divided into two periods. During the first period, the reactors were fed only with cattle manure. Once steady state conditions were reached, liquid sample from all reactors was obtained for DNA extraction and metagenomic analysis. After sampling, the feedstock composition of each reactor was changed by the addition of gelatine or Na-Oleate or glucose (second experimental period). As a consequence, foam formation was observed in all reactors approximately after one HRT period. Once the daily volume of the formed foam was steady, samples were taken again for DNA extraction and metagenomic analysis.
Results from the present study revealed significant variations in the microbiology of the manure-based biogas reactors after foam initiation. A number of genera could be linked to foaming as they produce biosurfactants (*Lactobacillus, Bacillus, Pseudomonas, Thermotoga*), others contain mycolic acid in their cell wall (*Thermoactinomyces, Pseudonocardia*) or decrease the surface tension of the media (*Micrococcus, Streptococcus*). *Frankia, Dialister* and *Paenibacillus* are known to be correlated to this phenomenon but their mechanism is still unclear. Finally, microorganisms that have a widely known association with foaming were identified when the identification threshold for the microorganisms was decreased to similar levels reported in the cited literature; however, the latter, due to its high importance, needs to be further investigated

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P6

Comparative analysis of the microbial diversity in liquid and foaming layer in biogas reactors

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Foaming incidents have been recorded in many biogas plants causing severe operational, economical and environmental problems (Kougias et al., 2014). However, the foaming phenomenon in biogas reactors fed with agro-industrial wastes has not been extensively investigated, especially with respect to the microbial composition of the digesters (Moeller et al., 2012). In the cited literature, it has been reported that specific microorganisms, which are mainly filamentous (e.g. *Gordonia species, Microthrix parvicella*), are attached to biogas bubbles and transferred to the air/liquid interface of sludge reactors or wastewater treatment works (Ganidi et al., 2009). Once these microorganisms accumulate on the liquid surface, they initiate biosurfactants production due to their metabolic activity, leading to the decrease of the surface tension and thus generate foaming. The aim of the present study was to investigate the microbial diversity in the liquid versus the foaming layer in manure-based biogas reactors suffering by foaming incidents in order to elucidate potential role and contribution of the microorganisms in foam promotion.
The experimental work was carried out in three thermophilic continuous stirred tank reactors (CSTR) fed with manure and supplemental amounts of lipids, proteins and carbohydrates. Once foaming was formed in the reactors, samples from the liquid and foaming layer were obtained and screened using 16S rDNA sequencing.
The results of these analyses revealed that there are indeed some species that significantly vary their relative abundance in the foaming layer compared to the liquid one (e.g. *Methanoculleus sp., Dialister sp.*). However, based on the cited literature and to the best of our knowledge there was not a direct correlation of these species with foaming. Further investigation is needed in order to define the properties of these species on foam generation. Finally, it was observed that particles of barley plant (that was contained in the raw manure as ingredient of animal nutrition) were accumulated in the foaming layer. It has been previously documented that barley contributes in stabilization of beer foam due to the activity of one of its proteins (Brey et al., 2003). For that reason, it could be hypothesised that the existence of barley particles in our reactors could contribute in foaming although their presence was also prior to the foaming incidents; their effect on foam formation or stabilization might be enhanced in correlation with other parameters (i.e. presence of specific microorganisms).

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Conference topic: - Microbial inhibition and adaptation to perturbations <u>POSTER PRESENTATION</u>

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A novel bioinformatic strategy to characterise microbial communities in biogas reactors

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Sequences encoding ribosomal Bacterial and Archeal genes are very similar among species of the same genus, in fact, in some cases, similarity is 99% or higher among the 1500 bp that compose the 16S rDNA. For this reason today it is still a challenge to gain the species level characterisation using 16S hypervariable regions, especially when working with the not high quality very short reads characteristics of next generation sequencers (Mande S.S. et al., 2012). Previous works analysed the microbial community composition in biogas reactors via 16S rDNA sequencing (Luo, G. et al., 2013; Werner, J.J. et al., 2011). For this reason we developed a bioinformatics strategy in order to create a tool to review the generated dataset and to obtain a more strict control on the bacterial composition at the species level, with estimation of its reliability. The program perform local similarity search and evaluate the results with high stringency (95 up to 100%) and returns all the possible candidate species with unique or multiple matches for each genus. In the process of species identification, different categories of reliability can be generated: certain can lead to univocal species identification even in the same genus, while others give multiple matches with the same probability. The software was used to analyse samples taken during the digestion process in three independent biogas reactors continuously fed with raw cattle manure. Among the most represented (>1%) considering the relative abundance of the community *Clostridium* resulted to be the most complex genus to elucidate. Some species in this genus, *Clostridium ultunense* and *Clostridium irregular,* have been assigned with high probability (100% and 99.7% of unique matches) while other 11 have only few unique matches (0.1 to 10%). *Bacteroides*, *Acetobacterium* and *Pseudomonas* genera had difficulties in the assignment, gathering medium-low probability as well (1 to 50%). On the contrary several other genera were assigned with high probability and no multiple matches. Some of them including only one species uniquely identified, some other including more than one (i.e. *Dialister succinatiphilus* with 37% and *Dialister propionicifaciens* with 63%, *Tissierella praeacuta with* 26% and *Tissierella creatinophila* with 74%, *Proteiniphilum acetatigenes* with 100%, *Halothermothrix orenii* with 100%, *Thermoflavimicrobium dichotomicum* with 100%). Furthermore comparative analyses with MG-RAST (Meyer F. et al., 2008) results have been performed to test our strategy. We also found that our method can be used to understand which hypervariable region of 16S rDNA is more efficient in the identification at the species level in different genera. Our conclusion is that the identification at the species level remains a challenge of major interest but it can be done reliably for specific genera. In fact we uniquely identified the species of up to 67% of the most abundant genera and we obtained a less reliable identification for the remaining 33%.

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Conference topic: Advanced methods for microbiological analysis <u>POSTER PRESENTATION</u>

Anaerobic digestion foaming in Danish full-scale biogas plants: a survey on causes and solutions

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Abstract
A survey on foaming in biogas reactors in Denmark revealed that foaming incidents have been documented in most of the full-scale biogas plants. Foaming was recorded up to three times per year and lasted from one day to three weeks, causing 20-50% biogas production loss. A case study research on foaming at Lemvig biogas plant indicated that the feedstock composition and the mixing speed of the reactor has to be taken into serious consideration in order to avoid foaming incidents.

Keywords
Anaerobic digestion; foaming; causes; solutions; full-scale biogas plants

INTRODUCTION

Anaerobic digestion foaming is a serious drawback that is occasionally recorded in many full-scale biogas plants. Foam is a gas-liquid dispersion with gas content of more than 90% and is typically created in the main reactor or in the pre-storage tank as a viscous deep-brown coloured layer (Oerther et al., 2001, Varley et al., 2004).

It is well documented that foam formation is causing upsets in the digestion process. Specifically, foaming results in operational problems, such as blockage of mixing devices and collapse of pumps caused by the entrapped solids in the foam (Dalmau et al., 2010). Bad mixing leads to inverse solids profile in the digesters, forming dead zones and thus reducing the active volume of the reactor. As a consequence biogas production is reduced for shorter or longer periods (Nielsen and Angelidaki 2008). Increase in the operational expenditures is linked to foaming, due to income losses, manpower overtime and maintenance costs (Barjenbruch et al., 2000; Barber 2005).

In the cited literature there are a number of studies aiming to identify the potential causes of foaming. Surface active agents, such as surfactants or biosurfactants generated by the metabolic processes were found to lower the surface tension of the substrate and enhance foaming potential (Barber, 2005). Moreover, high organic content of proteins or lipids has been reported that induce foaming phenomena (Boe et al., 2012). Filamentous microorganisms and especially *Gordonia* species and *Microthrix parvicella* are known to be the major cause of foaming in sludge digesters as they are attached to the gas bubbles and accumulated on the surface of the reactor (Heard et al., 2008). Operating factors, such as inadequate mixing, temperature fluctuations have been also suggested as foaming causes (Barjenbruch et al., 2000; Barber 2005).

Foaming has been traditionally associated with activated sludge systems (Barber 2005). Thus, there is lack of experimental data and scientific information concerning foaming in manure based digesters. The aim of the present research is to survey foaming incidents in Danish full-scale biogas plants and to establish knowledge for disclosing potential causes of foaming, in order to achieve a stable operation in Danish biogas plants.

MATERIALS AND METHODS

To assess the prevalence of anaerobic digestion foaming in Demark, a survey in 16 Danish full-scale biogas plants was conducted. Survey participants were provided with questionnaires involving

questions about the occurrence, control and possible causes of anaerobic digestion foaming based on their individual experience. Among the questions, the participants provided information concerning the operational parameters and the feedstock characteristics of the biogas plants. Moreover, in order to investigate the foaming incidents that occurred in Lemvig biogas plant, samples were taken for further analysis.

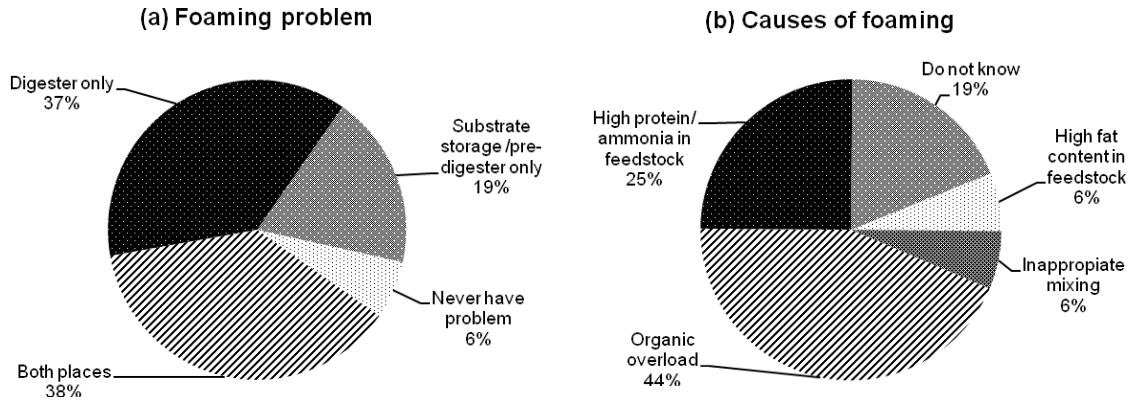
RESULTS AND DISCUSSION

Survey of anaerobic digestion foaming at full-scale biogas plants in Denmark

A survey of full-scale biogas plants in Denmark revealed that foaming is a widespread problem and the causes and consequences of foaming control are not fully understood. The results from the questionnaires showed that 15 biogas plants had experienced foaming problems (Fig. 1a). Foaming appeared mainly in the main digester and occasionally in the substrate storage/pre-digester. Nevertheless in 38% of the cases, foam appeared in both places. Foaming was periodic problem that typically occurred up to three times per year in most of the plants and the duration of the incidents lasted from one day to three weeks. The biogas production loss due to foaming was estimated to be 20-50%. However, in some cases it could cause up to 90% production loss and under extreme conditions lead to total process failure.

Most of the biogas plant operators believed that foaming problem was connected with organic overload (Fig. 1b). It is well known that the digester overloading can result in partial degradation of organic matter and accumulation of surfactants or biosurfactants (Ganidi et al., 2011). The excessive concentration of these substances can potentially contribute to foaming phenomena. The second most dominant cause for foaming, indicated by the 25% of the biogas plant operators, was the high protein and ammonia concentration in the feedstock composition. The foaming effect from proteins is based on the synthesis of ionisable structures with both hydrophobic and hydrophilic available ends. Specifically, protein foams are formed by a protein film surrounding a gas bubble creating a structure that holds bubbles in place (Foegeding et al., 2006). High fat content in feedstock and inappropriate mixing was also linked with foaming but at a lower extend. Finally, it should be highlighted that one fifth of the respondents was totally unaware for the causes that promote foaming.

Unfortunately, foaming is normally detected as soon as the adverse impacts of the foam effect have already been initiated. The plant operators become aware of the problem by visual observation or when the security valve or alarm activates. The most common solution to suppress foaming was the decrease of the digester's organic load (Fig. 1c). Other solutions involved adjustment of the stirring speed, the increase of the flow rate and more rarely the addition of compounds (i.e. foam absorbers, oils or lime), the temperature decrease or the dilution of the reactor content.



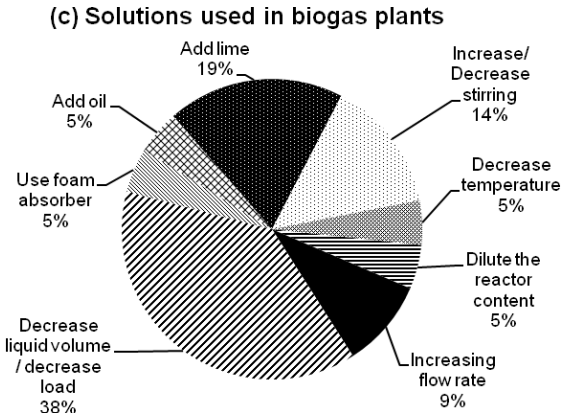


Figure 1. Survey for the (a) occurrence of foaming incidents, (b) causes of foaming and (c) solutions used to suppress foaming in Danish full-scale biogas plants.

Lemvig biogas plant case study

As a case study we investigated the operational parameters and the feedstock composition of one manure-based digester of Lemvig biogas plant in order to define the potential causes of foam formation. The biogas plant has four biogas reactors, and only one of the reactors was regularly facing foaming problems while the three other reactors, were not experiencing foaming incidents. Lemvig biogas plant has three primary reactors, denoted as PR1, PR2 and PR3, fed with the same substrate which was cattle manure and industrial waste at a ratio of 70% and 30% respectively (Fig. 2). Another reactor, namely as SR, was a second stage reactor fed with effluent from the primary reactors. The working volume and the stirring speed of PR1, PR2 and SR was 2400 m^3 and 200 rpm respectively while the working volume and the stirring speed of PR3 was 7100 m^3 and 16 rpm respectively. Excessive foaming incidents were recorded only in PR3 where the maximum foam formation was approximately 1065 tons foam/day.

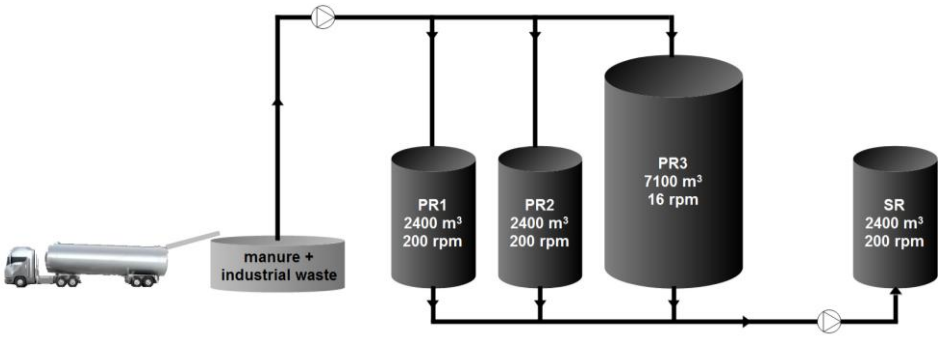


Figure 2. Lemvig biogas plant reactors.

The results from the sample analysis have shown that the values of specific parameters in PR3 presented significant differences compared with the corresponding values of the non-foaming reactors (Table 1). Alkalinity and biosurfactants activity were found at higher levels in PR3. It was previously reported that an increase in alkalinity leads to a decrease of surface tension, rendering the substrate more surface active and more prone to foam (Nges and Liu 2010). Furthermore, the determination of the foaming properties in PR3 revealed increased tendency for foam formation and stronger ability in foam stabilisation. The results from microbial analysis showed that the bacterial composition in all reactors was not significantly different indicating that foaming was not caused by differences in bacterial composition. In contrary, the quantitative and qualitative analysis of Archaea revealed significant differences concerning their composition in PR3 relative to the non-foaming reactors.

Moreover, one important observation was that the acidic feed mixture (containing acidic whey)

was directly pumped to the reactors. As a consequence of the acidic feed entering the reactor, the pH in the reactor was reduced, resulting in change of the carbonate balance and thereby in high amounts of CO_2 release. The low mixing speed in PR3 did not manage to break down the foam once it was formed and as a consequence excess foam was accumulated at the top of the digester.

Table 1. Characteristics of the feedstock composition and samples obtained by the reactors

Parameters	Feedstock		Reactors			
	Manure	Industrial waste	PR1	PR2	PR3	SR
pH	6.5	4.3	8.13	8.11	8.21	8.15
Alkalinity (g/L as $CaCO_3$)	6.2	0	5.2	4.5	8.1	7.5
Biosurfactant activity (mm^2)	12.5	3.1	5	8.2	9.1	7.3
Foaming tendency (ml foam/ml-air.min)	50-100	20	25-50	10-90	100-200	100-150
Foam stability (ml)	0	0	0	0	30	30

CONCLUSIONS

Foaming has been recorded in the majority of the full-scale biogas plants in Denmark, causing 20-50% biogas production loss. The biogas plant operators indicated the organic overload and the high protein and ammonia concentration in the feedstock as the most dominant factors for foaming. Unfortunately, foaming was rarely prevented but once it was detected the decrease of the organic load or the liquid volume were the most common solutions to suppress the foaming incident. A case study research at Lemvig biogas plant indicated that the feedstock composition, especially acidic waste feed, in combination with mixing of the reactor has to be taken into serious consideration in order to avoid foaming incidents.

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Foaming in manure based digesters: Effect of overloading and foam suppression using antifoam agents

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ABSTRACT

Anaerobic digestion foaming is one of the major problems that occasionally occur in full-scale biogas plants, affecting negatively the overall digestion process. The purpose of the present study was to investigate the effect of organic loading rate on foam formation and also to evaluate the antifoam efficiency of different chemical compounds on foam suppression. In order to investigate the effect of organic overloading on foam formation, a stepwise increase of the organic loading rate was performed by the addition of glucose in the feeding substrate. The investigation of possible solutions to counteract foam formation was achieved through the evaluation of the antifoam efficiency of five commercial and non-commercial chemical compounds. The results obtained from the above experiments showed that the organic loading rate had a significant impact on foam formation. Finally, it was observed the use of specific chemical antifoaming agents could minimise foam formation in manure samples However, the efficiency of the antifoaming agents varied significantly, revealing that their chemical composition affected differently the foam destruction mechanism.

Keywords: Anaerobic digestion, Foaming, Antifoaming agents, Organic overloading, Biogas

1. INTRODUCTION

Anaerobic digestion (AD) foaming is one of the major problems that occasionally occur in full-scale biogas plants, affecting negatively the overall digestion process. The foam is typically created either in the main biogas reactor or/and in the pre-storage tank and the entrapped solids in the foam cause severe operational problems, such as blockage of mixing devices and collapse of pumps (Ganidi et al., 2009). Furthermore, the foaming problem is linked with economic consequences for biogas plants, due to income losses derived from the reduced biogas production, extra labour work and additional maintenance costs (Barber, 2005). Moreover, foaming presents adverse environmental impacts owing to the overflowing of the pre-storage or digester tanks (Boe et al., 2012).

P. G. Kougias, P. Tsapekos, K. Boe and I. Angelidaki. “Foaming in manure based digesters: Effect of overloading and foam suppression using antifoam agents”. International Commission of Agricultural and Biological Engineers, Section V. CIOSTA XXXV Conference “From Effective to Intelligent Agriculture and Forestry”, Billund, Denmark, 3-5 July 2013.

A number of potential causes for foaming can be found in the cited literature. Boe et al., (2012) compared the effect of several substrates and intermediate compounds on foaming in manure digestion system and reported that high content of lipids or proteins could promote foaming. Ganidi et al., (2011) suggested that an organic loading rate (OLR) of 2.5 gVS/L-reactor was the critical threshold for foam initiation in bench scale sewage sludge reactors. In another research, Heard et al., (2008) reported that foaming in sludge digesters was linked to filamentous bacteria, as they produce surface active compounds that contribute in foam proportioning. Dalmau et al., (2010) have developed a knowledge-based model to predict the risk of foaming in activated sludge systems, by selecting as inputs the organic loading rate, the variation in organic loading rate and the presence of filamentous microorganisms. Moreover, Barjenbruch et al., (2000) and Barber (2005) documented that bad mixing, temperature fluctuations, presence of hydrophobic substances and extracellular polymeric materials could enhance foaming in sludge digestion tanks.

An effective strategy to prevent and/or suppress foaming is of a great importance in order to avoid the negative impacts of foaming. However, there is no universal solution for foaming since the cause of foaming can be different from one application to another, which might require different solutions for precise and efficient foam suppression. Among the different antifoam strategies that have been already suggested, the chemical method using antifoam compounds is the most widespread. Nowadays, many antifoaming agent solutions have emerged and abundance of commercial choices is available (Junker, 2007). Though, it is well known that an antifoaming agent may not be suitable for every application (Routledge and Bill, 2012). As a consequence, a number of different compounds should be examined to define which is the most appropriate for a certain treatment.

In full scale biogas plants the antifoaming agents are added in the pre-storage tank, so as to be diluted with the substrate, creating a homogenous mixture. The antifoam efficiency and the economic viability of the antifoaming agents depend on many parameters such as the suggested dosage, the cost of the agents, and influence on the biogas process. It has been previously reported that each agent has a specific concentration where it has its optimum antifoaming effect, below which is less effective, while above the optimum concentration may act as foam stabilizer (Karakashev and Grozdanova, 2012). Most of the antifoaming agents used in AD processes are commercial and often the specific composition of these chemical solutions is not provided by the suppliers. In the cited literature, scientific information and experimental data concerning the efficiency of chemical compounds on AD foam prevention and suppression are very limited.

So far, there has never been thoroughly investigation of foaming problem in manure-based digesters, which is the main anaerobic digestion system applied in Denmark. The purpose of the present study was to investigate the effect of organic loading rate on foam formation and also to evaluate the antifoam efficiency of different chemical compounds on foam suppression.

2. MATERIALS AND METHODS

2.1 Manure characterization

Cattle manure obtained from Snertinge biogas plant, Denmark, was used in the experiment. After arrival, the manure was shredded and sieved (5 mm) to separate large particles and stored at -20 ^{o}C. The frozen manure was thawed at 4 ^{o}C for 2-3 days before use. The characteristics of manure are presented in Table 1.

Table 1. Cattle manure characteristics

		Raw cattle manure
Parameters	**Unit**	**Values**
pH	-	7.43±0.01
Total solids (TS)	g/L	61.6±0.7
Volatile solids (VS)	g/L	47.5±0.6
Total Kjeldahl Nitrogen (TKN)	g/L	3.30±0.17
Ammonium Nitrogen ($N\text{-}NH_4^+$)	g/L	2.11±0.14
Total Volatile fatty acids (VFA)	mg/L	5535±431.6
acetate	mg/L	3151.1±353.4
propionate	mg/L	1288.3±65.7
iso-butyrate	mg/L	138.6±2.0
butyrate	mg/L	608.6±10.4
iso-valerate	mg/L	191.2±0.4
valerate	mg/L	126.8±0.0
n-hexanoate	mg/L	30.6±0.3

2.2. Experimental set up and operation

The experiment was carried out using a CSTR reactor. The total and the working volume of the reactor were 2 and 1.5 L, respectively. The reactor was continuously stirred using a magnetic stirrer. The operating temperature was maintained at 54±1 ^{o}C using thermal jackets. The hydraulic retention time (HRT) was kept constant at 15 days. The whole experiment was divided into four periods. During each period, the OLR of the reactor was stepwised increased by the addition of glucose, in order to investigate the effect of OLR on foaming in the digester. The reactor was automatically fed twice a day using a peristaltic pump, and biogas production was measured by an automated displacement gas metering system with 100 mL cycle (Angelidaki et al., 1992). Biogas production and foam formation were recorded daily, while methane content in biogas, volatile fatty acids concentration, and foaming potential in the liquid sample were measured once or twice per week.

2.3 Antifoaming agents

Five antifoaming agents were tested in this study. The compounds were among the categories of Long Chain Fatty Acids (LCFA), natural oils, esters and commercial antifoams. Two commercial antifoams were tested. One was Struktol SB 2113 Dimethylpolysiloxane, denoted as PDMS, which an emulsion of silicone. The other commercial antifoam was Struktol SB 2080, which is derivative of natural fatty acids. Oleic acid (90%, Sigma-Aldrich) was the

representative of antifoam based on mono-unsaturated LCFA, while a commercial rapeseed oil was used to represent antifoam based on natural oils.

2.4 Foaming test apparatus and methodology

The foaming potential of the solutions was determined by the aeration method modified from Boe et al., (2012). The apparatus was consisted of an acrylic cylinder (inside diameter 4.5 cm, height 40 cm) with a ceramic air diffuser (diameter 1.5 cm, length 2.5 cm) placed at the bottom of the cylinder. The cylinder was closed with a rubber stopper during aeration. The rubber stopper had two openings; one was used for air supply to the diffuser and another one was used for the headspace injection of the antifoam and for air outlet. A 100 mL sample was added in the cylinder and was aerated twice with an air flow rate of 120 mL/min for 10 minutes each time. In the beginning the sample consisted only of manure and after the first aeration period the volume of the formed foam was recorded. Then, without stopping the air supply, the antifoaming agent was injected in the column and subsequently the mixed sample that contained manure and antifoam was aerated for another 10 minutes. Just after the second aeration, the volume of foam in the column was measured again. Thereafter, the air supply was stopped and the sample was kept in the column in order to estimate the foam stability. The foaming potential was defined using three parameters: foaming tendency, foam stability and foam volume inside the reactor. The foaming tendency (mL-foam/(mL-air·min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). The foam stability was determined as percentage of foam remaining in the settling cone at 1 h after aeration compared to the volume of foam right after aeration. The determinations of foaming tendency and stability were carried out in triplicate. The foam formation inside the reactors was recorded daily. The volume of foam produced was determined by the measured average foam height multiplied with the surface area of the reactor. After each measurement, the reactor was rapidly stirred or shaken until all foam disappeared. Hence, the daily amount of foam that was formed inside the reactors was measured.

2.5 Statistical method

Descriptive statistics were performed for all variables involving the calculation of mean values, standard deviations and standard errors. “Statistical Package for the Social Science SPSS for Windows” version 20 was used to carry out normality test and subsequently one-way analysis of variance (ANOVA) in order to compare the quantitative variables between the different groups.

3. RESULTS AND DISCUSSION

3.1 Effect of overloading on foaming

From the analysis of the results obtained by the CSTR reactor, it was found that the organic overloading had influence on foam formation (Fig. 1A). Foam initiation occurred when the OLR was higher than 3.5 g VS/L-reactor·day and foam formation increased as the reactor was more overloaded. Specifically, for OLR equal to 4.2, 5.2 and 6.2 g VS/L-reactor·day, the corresponding foam that was created in the reactor was 0.15, 0.18 and 0.37 L/L-reactor·day. This could be explained by the fact that all the surface active compounds in the manure were not fully

degraded due to overload. Ganidi et al., (2009) reported that the digester overloading can result in partial degradation of organic matter and accumulation of surfactants or biosurfactants. The exceeded concentration of these substances can potentially contribute to foaming phenomena. Up to our knowledge in the cited literature, there is not another reference that provides information concerning the correlation of OLR and foaming in manure based digesters, so as to compare our findings. Yet, a recent report investigating foaming in batch sludge digesters concluded that an organic loading of 2.5 kg VS/m^3 was the critical threshold for foam initiation while 5 kg VS/m^3 resulted in persistent foaming (Ganidi et al., 2011).

Additional information on the methane production showed that the methane yield values remained almost constant during the whole experiment (Fig 1B). This indicates that the foam formation slightly inhibited methanogenesis. The methane yield was 231, 271, 241 and 242 mL CH_4/g VS L-reactor·day for the corresponding OLR of 3.5, 4.2, 5.2 and 6.2 g VS/ L-reactor·day, respectively. We could assume that the concentration of VFA formed as intermediate degradation products form glucose, did not affect the methane production, although it was enough to generate foaming. It has also been observed in our physicochemical test that acetic acid significantly increased foaming tendency in manure mixture (Boe et al., 2012). It is also worth mentioning that during the daily foam removal procedure by fast stirring, significant amount of biogas was released into gas phase and the biogas production rate was clearly increased. This observation could explain the decrease of biogas production in the digester with foaming, indicating that the decrease was due to oversaturation of the liquid phase with biogas. Thus a larger portion of methane was leaving the digester with the liquid phase, rather with the gas phase. The lower methane production during foaming periods, could have been mistaken as process inhibition, but was probably rather due to lack of equilibrium between gas and liquid phase and loss of methane with the effluent. It has also been previously reported that the foam covering the liquid surface prevents efficient gas-liquid transfer, thus decreases the biogas production (Ganidi et al., 2009). Moreover, it should be highlighted that during the experimental periods that the OLR was equal to 5.2 g VS/ L-reactor·day or higher, the foam could not be destroyed by increasing the stirring speed of the digester, but only by vigorously manual shaking of the reactor. The foam during these periods was more stable and thick.

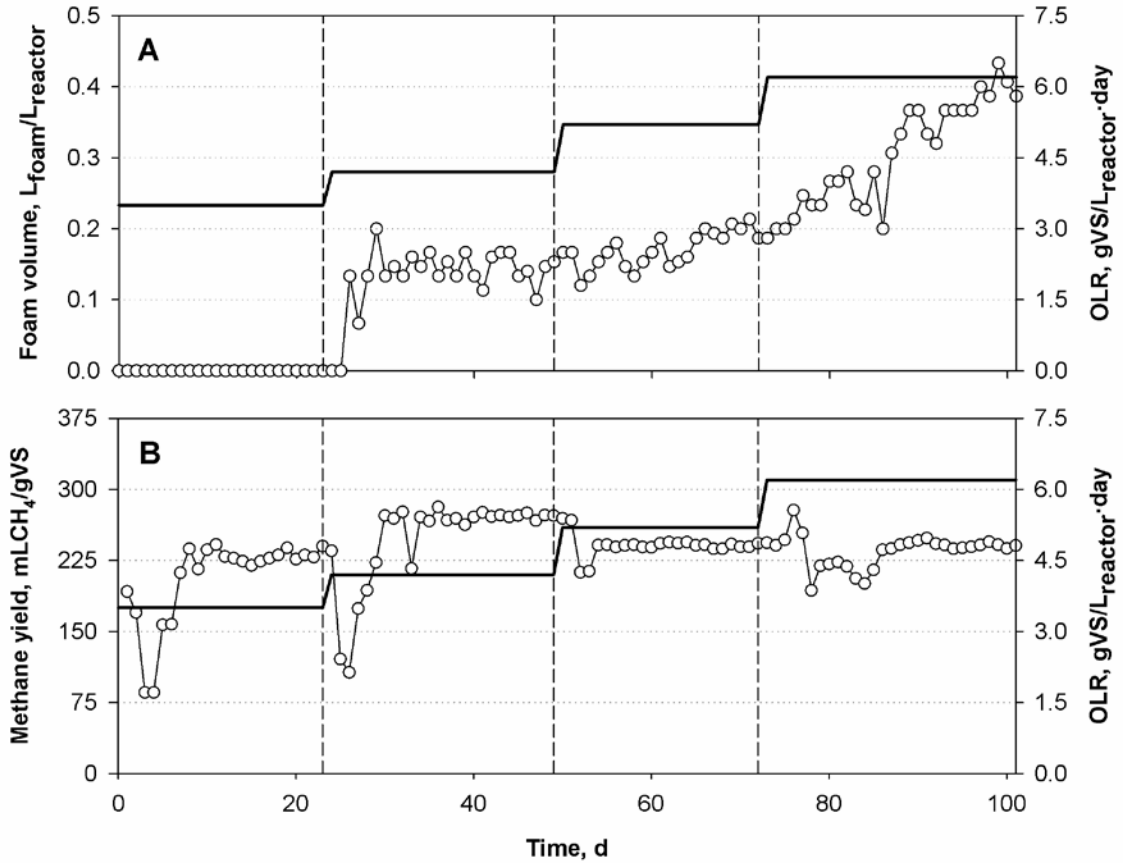

Figure 1. Correlation of OLR with A) foam volume inside the reactor, B) methane yield.

3.2 Effect of antifoam agents on foam suppression

Fig. 2 illustrates the foam reduction efficiency of the tested compounds on digested cattle manure samples obtained from the CSTR reactor. The concentration of the antifoaming dosage was selected according to the specifications of manufacturers that suggest 0.1% v/v as a typical dosage of commercial antifoaming agents suitable for bioprocesses.

Based on statistical analysis, the commercial antifoaming agents and rapeseed oil were both very efficient to fully suppress the foam. On the other hand, the efficiency of polyaluminium chloride reached only 20%, which was least efficient in suppressing the foam among the tested compounds. A possible explanation could be that polyaluminium chloride might require higher concentration to achieve efficient foam suppression. Westlund et al. (1998) reported that polyaluminium salt at concentrations of 3-6 gAl kg-1 TSS (total suspended solids) exhibited good results on foam suppression. The foam reduction efficiency of oleic acid reached approximately 87%. According to the results obtained from our previous preliminary experiments, the ability of oleic acid to reduce foaming in digested manure was correlated to the unsaturated form of LCFA (data not shown). In the same study we have found that saturated LCFA exhibit higher foam reduction efficiency in raw manure samples. Nevertheless, an

explanation for this correlation is not yet understood, due to the fact that manure contains several compounds that could affect foaming.

The evaluation of the ability of the antifoaming agents to break down the foam was based mainly on physicochemical properties and reactions with the substrate. The present test of the effectiveness of certain antifoaming agents is a common type of foam-related test (Höfer et al., 2000). However, before applying these antifoaming agents in a full scale biogas plant it is very crucial to test them in a continuous digestion system, so as to investigate their long term effect on the bioprocess and also their influence on the microbial ecology of the reactors. This will help in order to avoid a possible deterioration and unstable performance of the biogas reactor.

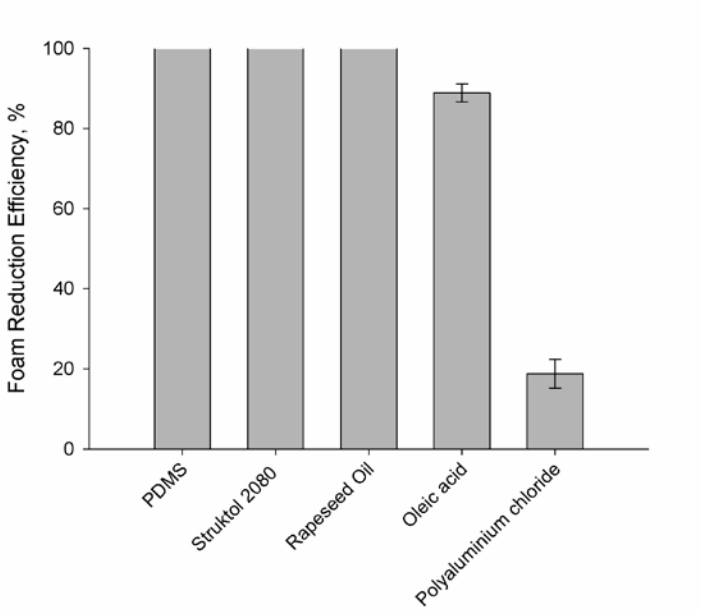

Figure 2. Foam reduction efficiency of chemical agents.

4. CONCLUSIONS

The results obtained in this study clearly underline the influence of OLR on anaerobic digestion foaming in manure-based digesters. An OLR equal to 3.5 g VS/L-reactor·day was identified as the critical threshold for foaming in digesters fed with cattle manure. By determining the defoaming efficiency of five commercial and non commercial substances, it was found that rapeseed oil and the commercial antifoams were the most efficient compounds to suppress foam in digested cattle manure samples obtained by an overloaded digester.

5. ACKNOWLEDGEMENTS

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Influence of microbial composition on foam formation in a manure-based digester

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Foaming is one of the major problems that occasionally occur in the biogas plants, affecting negatively the overall digestion process and results in adverse operational, economical and environmental impacts. The most dominant factors contributing to foaming are organic overloading, feedstock composition and the presence of specific microorganisms. The filamentous microorganisms are known to be the major cause of foaming in sludge digester as they are attached to the gas bubbles and accumulated on the surface of the reactor.

The present case study investigated the microbial composition of one manure-based digester of Lemvig biogas plant that was facing foaming problem, comparing with three non-foaming digesters. The research was focused on the quantitative and qualitative analysis of *Bacteria* and *Archaea* population and on the identification of *Gordonia sp*. The reactor samples were analysed for foaming properties and microbial analysis. The dynamic population of *Bacteria* and *Archaea* were studied by PCR-DGGE method.

The results obtained from this study showed that the composition of *Bacteria* in all reactors was not significantly different indicating that foaming was not caused by *Bacteria*. In contrary, the quantitative and qualitative analysis of *Archaea* revealed significant differences in their population and composition.

Foaming in manure based digesters- Causes and solutions

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Anaerobic digestion foaming is one of the major problems that occasionally occurred in the Danish full-scale biogas plants, affecting negatively the overall digestion process. The foam is typically formatted in the main biogas reactor or in the pre-storage tank and the entrapped solids in the foam cause severe operational problems, such as blockage of mixing devices, and collapse of pumps. Furthermore, the foaming problem is linked with economic consequences for biogas plants, due to income losses derived from the reduced biogas production, extra labour work and additional maintenance costs. Moreover, foaming presents adverse environmental impacts owing to the overflowing of the pre-storage or digester tanks.

So far, there has never been thoroughly investigation of foaming problem in manure-based digester, which is the main anaerobic digestion applied in Denmark. The purpose of the present study was to identify potential causes of foaming in manure based digesters. Moreover, it was also an aim to investigate possible solutions to counteract foam formation with the use of antifoam agents.

Thus, the impact of organic loading rate and content of feeding substrate on anaerobic digestion foaming was studied in continuous mode experiments. Two sets of treatments were examined in duplicate using 5 continuous stirred tank reactors (working volume 1.5L), operating in thermophilic conditions. Two replicate reactors were fed with cattle manure and gelatine, as a representative of proteins, while the other two replicates were fed with cattle manure and Na-oleate, as a representative of lipids. One reactor was kept as a control and was fed only with cattle manure. The experiment was divided in 5 periods. During the 1^{st}, 3^{rd} and 5^{th} period the organic loading rate of all reactors was increased by the addition of glucose in the feeding substrate. During the 2^{nd} and 4^{th} period the organic loading rate was maintained constant, but instead of glucose, higher concentration of Na-oleate or gelatine was added in the feeding substrate.

The results obtained from the above experiment showed that the organic loading rate has a significant impact on foam formation, lowering the methane yield of the reactor. Moreover, it was found that an increase in gelatine concentration does not promote foam, while an increase in Na-oleate concentration enhances stable foam.

Based on the above results, a new experiment was designed, where the antifoam efficiency of different commercial and non-commercial compounds, was investigated. The antifoam potential of the compounds was determined by aeration method. The apparatus comprised of a glass cylinder with a diffuser placed at the bottom. A 50 mL sample, derived from a foaming reactor, was aerated in the cylinder with an air flow rate of 60 mL/min for 10 minutes. After that, the aeration was repeated adding different concentrations of antifoam solutions in the sample. The foam height in the cylinder was measured as soon as the aeration was stopped and again 1 hour later. The antifoam potential was defined using two parameters: foaming tendency and foam stability. Foaming tendency (mL-foam/(mL-air·min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). Foam stability was determined as percentage of foam remaining in the cylinder 1 h after aeration compared to the volume of foam right after aeration.

Effect of substrates and intermediate compounds on foaming in manure digestion systems

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Abstract
Manure digestion is a complex matrix containing several compounds that can potentially cause foaming. Understanding the effect of substrates and intermediate compounds on foaming tendency and stability could facilitate strategies for foaming prevention and recovery of the process. In this study, the effect of physicochemical properties of substrates and intermediate compounds on liquid properties such as surface tension, surfactant property, and hydrophobicity were investigated and compared to the effect on foaming tendency and foam stability. Moreover, the effect of these compounds on foaming during batch anaerobic digestion was also tested. The results showed that there was no consistent correlation between foaming potential and hydrophobicity, oil displacement area (ODA) or surface tension of the tested solutions, and the best way to determine foaming property of the solution was to directly measure foaming tendency and foam stability. Na-oleate and acetic acid showed the highest potential to create foam in a manure digester. Moreover, high organic loading of lipids and protein, and high concentration of acetic and butyric acid also showed strong tendency to create foaming during anaerobic digestion. Due to their strong ability to stabilize foam, high organic loading of Na-oleate or gelatine was considered as main potential foaming problem.

Keywords
Anaerobic digestion; manure; foaming tendency; substrates; intermediate compounds

INTRODUCTION

Anaerobic digestion (AD) has gained increased attention in recent years, which has led to the development of many biogas plants worldwide. Many biogas plants, especially in Denmark, are co-digestion plants, where different types of organic industrial wastes are digested together with manure. In these plants, foaming typically occurs in the main reactor or in the pre-storage tank. Foaming often results in operational problems, such as blockage of mixing devices, and collapse of pumps, due to entrapped solids in the foam (Ganidi et al. 2009). The bad mixing leads to inverse solids profile in the digesters with higher solids concentrations at the top of a digester, resulting in the formation of dead zones and thus reducing - the digester active volume. As a result, biogas production is decreased for shorter or longer periods (Nielsen & Angelidaki 2008). Serious economic consequences are linked to foaming, due to income losses, extra labour need, and maintenance costs (Barjenbruch et al. 2000; Barber 2005). Furthermore, foaming often causes environmental problems due to overflowing of pre-storage or digester tanks.

Pure water cannot have foam unless a surface active agent is present as impurity. Surface active agents, such as surfactants or bio-surfactants, generated under metabolic processes, decrease the surface tension of the liquid and thus enhance foaming potential (Barber 2005). Other factors for foam formation include a gaseous phase and the presence of hydrophobic material. Foam in anaerobic digestion systems consists of three-phases, which are gas bubble, liquid (wastewater or soluble microbial products) and solid particles (microorganisms or suspended solids). Foam formation and stabilization require at least the presence of gas bubbles and solid particles in the bulk liquid (Dynarowicz & Paluch 1989). During the last 10 year, many studies have been focused

on foaming problem in sludge digesters from wastewater treatment and several causes for foaming have been suggested. Ross and Ellis (1992) suggested that organic overloading and the accumulation of acetic acid were the cause of foaming in sludge digesters. Pagilla et al. (1997) suggested that Gordonia filamentous bacteria was the cause of foaming, where Gordonia was identified in two full scale sludge digesters. Other factors such as inadequate mixing, temperature fluctuation, shock load, extracellular polymeric substances (EPS) and hydrophobic substances have also been suggested as foaming causes in sludge digesters (Barber 2005; Barjenbruch et al. 2000).

The results from the above mentioned studies are, however, contradictory and the information provided is either site-specific or the supporting experimental information is limited. So far, there has never been thoroughly investigation of foaming problem in manure-based digester, which is the main anaerobic digestion applied in Denmark. Foaming problem is one of the major problems that occasionally occurred and leads to production loss in the Danish full-scale biogas plants. There is a need for investigation of the foaming causes in this system in order to find the method to avoid as well as to resolve the problem. This work aims to identify the potential causes of foaming in manure digesters. The specific compounds commonly present in a manure digester are investigated for their effects on liquid properties and foaming potential in manure.

MATERIALS AND METHODS

The cow manure used in the experiment was obtained from Vegger biogas plant, Denmark. After arrival, the manure was shredded and sieved to separate large particles and stored at -20^{o}C. The characteristics of manure are presented in Table 1.

Table 1. Cow manure characteristics

Parameter	Unit	Value
pH	-	6.98 ± 0.07
Total solids (TS)	g/L	89 ± 1
Volatile solids (VS)	g/L	71 ± 1
Ammonium (N-NH_4^+)	g/L	3.4 ± 0.4
Total Volatile fatty acids (VFA)	g/L	9.1 ± 0.1
Acetic acid	g/L	5.8 ± 0.4
n-butyric acid	g/L	0.78 ± 0.02
Propionic acid	g/L	1.84 ± 0.07
iso-butyric acid	mg/L	222 ± 1
Valeric acid	mg/L	101 ± 4
iso-valeric acid	mg/L	283 ± 3

Physicochemical-effect of substrates and intermediate compounds on liquid properties and foaming potential

The effect of individual substrates and intermediate compounds on liquid properties and foaming potential was investigated in both water and manure. The liquid properties investigated were surface tension, surfactant property and hydrophobicity. The foaming potential was measured as foaming tendency and foam stability. For the preparation of this test, the frozen manure was thawed and diluted to 5% in water to obtain a total solid (TS) content of 0.45%. This solution was kept at 4° C until use for maximum 6 days. Fifteen compounds were chosen, which are proteins (peptone, gelatine), carbohydrates (cellulose, starch, sucrose), lipids (fish oil, sodium oleate, glycerol, sunflower oil), cations (Ca^{2+}, Na^{+} and Mg^{2+}), volatile fatty acids (acetic acid, butyric acid) and ammonia. The concentration of each compound in 50 mL solution was chosen in the range of 0.2-6 g/L, which in accordance with its typical concentration that is commonly found in manure digesters.

The effect of sample matrix was also investigated in manure, by comparing the surface tension and foaming potential of manure at different concentrations corresponding to the total solid (TS) content of 0.45, 0.89, 2.67, 4.45 and 5.34%.

Physicochemical-effect of substrates and intermediate compounds in a complex mixture

In a co-digestion system, several substrates and intermediate compounds are present in a digester at the same time, which can affect differently on foaming in the digester. To investigate the effect of each compound in a complex mixture, a fractional factorial design of experiments was carried out, where all the compounds involved were tested at different concentrations. Assuming that the interaction between each compound is insignificant, the design matrix could be reduced to 16 combinations of 15 compounds in manure as shown in Table 2. From each test combination, the liquid properties and foaming potential were measured. The liquid properties measured were surface tension, surfactant property and hydrophobicity. The foaming potential was determined as foaming tendency. The results from each test combination were then analysed using Design-Expert® software (Stat-Ease Inc., USA) to determine the influence of each compound on liquid properties and foaming potential in manure.

Table 2. Concentrations (g/L) of compounds involved in each test combination

Combinations:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Gelatine	-	-	0.8	-	-	-	0.6	0.6	0.7	0.6	-	-	0.6	0.7	0.6	-
Peptone	1.1	-	1.2	-	1.1	-	1.3	1.2	-	-	1.0	-	-	1.2	-	1.4
Fish oil	-	1.4	-	-	1.5	1.3	1.3	-	1.4	1.3	-	-	-	1.6	-	1.1
Sunflower oil	1.1	-	1.2	-	-	1.2	-	-	-	1.1	-	1.2	1.1	1.2	-	1.1
Glycerol	1.6	1.1	-	-	-	1.2	1.8	-	-	-	1.3	-	1.6	1.2	1.0	-
Na-oleate	-	-	2.0	-	2.0	2.0	-	-	2.0	-	2.0	2.0	-	2.0	2.0	-
Starch	2.8	2.8	-	-	3.0	-	-	2.9	-	2.9	-	3.0	-	3.0	2.9	-
Sucrose	-	3.0	-	-	-	-	-	2.9	3.3	-	3.1	2.9	3.0	3.0	-	2.8
Cellulose	2.8	-	-	3.0	3.0	3.0	-	2.9	2.9	-	-	-	2.9	3.1	-	-
Acetic acid	-	3.0	3.0	3.0	-	3.0	3.0	3.0	-	-	-	3.0	-	3.0	-	-
Butyric acid	3.0	-	-	3.0	-	-	3.0	-	3.0	3.0	3.0	3.0	-	3.0	-	-
NH_4^+	-	8.3	7.7	7.6	7.0	-	-	-	-	9.0	8.1	-	7.2	7.1	-	-
Ca^{2+}	-	-	-	11.2	9.8	-	10.5	-	-	-	-	10.7	10.9	11.5	10.4	10.2
Mg^{2+}	3.0	30.4	29.0	30.0	-	-	-	-	30.0	-	-	-	-	29.0	29.0	30.0
Na_+	-	-	-	12.0	-	11.0	-	9.0	-	10.0	11.0	-	-	10.0	10.0	11.0

Analytical methods

Analysis of pH, total solids (TS) and volatile solids (VS) was according to Standard Methods for the Examination of water and Wastewater (1998). All analyses were carried out in triplicate. Volatile Fatty Acids (VFA) were determined by gas chromatograph (GC Shimadzu) with flame ionisation detector (FID). Methane content in biogas was determined using a gas chromatograph (GC Shimadzu) equipped with a Porapak 60/80 molecular sieve column and a flame ionisation detector (FID).

Surface tension measurement was carried out by the Wilhelmy plate method as described by Glinski et al. (2000) and Elmitwalli et al. (2001).

The surfactant property of solution was measure using oil displacement test. By placing 10 µL Murban crude oil on the surface of 50 mL distilled water in a Petri dish (15 cm diameter), a thin oil film formed immediately. Thereafter, 20 µL of sample was dropped onto the centre of the oil film. A clear circle inside the oil film was formed, and the diameter was measured after 30 sec. The area (cm^2) of the clear circle was calculated as oil displacement area (ODA) (Morikawa et al., 2000).

The hydrophobicity was determined by mixing 2 mL of diesel oil with the same amount of sample for 2 min and let it stand for 24 h. The emulsification activity was then calculated as percentage of the height of emulsified layer divided by the total height of the liquid column (Thampayak, 2008).

The foaming potential of solution was determined by aeration method modified from Bikerman method described by Beneventi et al. (2001). The apparatus comprised of an Imhoft settling cone with a diffuser placed at the bottom. A 50 mL sample was aerated in the settling cone with the air flow rate of 60 mL/min. for 10 minutes. The foam height in the settling cone was measured right after aeration was stopped and again at 1 hour later. The foaming potential was defined using two parameters: foaming tendency and foam stability. *The foaming tendency* (mL-foam/(mL-air·min)) was calculated from the volume of foam (mL) right after aeration divided by air flow rate (mL/min). *The foam stability* was determined as percentage of foam remaining in the settling cone at 1 h after aeration compared to the volume of foam right after aeration.

RESULTS AND DISCUSSION

Physicochemical-effect of substrates and intermediate compounds on liquid properties and foaming potential

The liquid properties, which are surface tension, ODA and hydrophobicity, were measured in this experiment for establishing possible correlation between these parameters to foam formation in our tested solutions, in order to find appropriate foaming indicators. Morikawa et al. (1993) introduced ODA measurement as an indicator of surfactant activity, based on the fact that the surface pressure of the surfactant displaces the oil. From our test results, the ODA value of pure water and manure were zero. Figure 1a shows that fish oil and Na-oleate significantly increased ODA in water up to 1.52 and 0.48 cm^2, respectively, while the rests of compounds did not show significant effect. In manure, only Na-oleate significantly increased ODA up to 0.7 cm^2.

Concerning the hydrophobicity, it has been reported that the increased chain length of the hydrophobic part of the surfactants can increase foam formation (Beneventi et al., 2001). From our results, the hydrophobicity of pure water and manure were 0% and 50%, respectively. From Fig.1b, all tested compounds increased hydrophobicity of water to the level similar to manure. On the other hand, their effects were different in manure. The strongest hydrophobic behaviour was observed from sucrose, NH_4^+ and Ca^{2+}, as they have increased the hydrophobicity value by 5.87, 4.85 and 3.58% respectively. In contrast, Na-oleate and cellulose reduced the hydrophobicity of the manure by 3.04 and 2.67%, respectively. The effect of Na-oleate on hydrophobicity could be explained as due to the presence of the free carboxylic end, which was not so hydrophobic compared to neutral oils where the carboxylic end is not available. This contradicts the generalisation that lipids are characterised as hydrophobic organic molecules (Ganidi et al., 2009). Overall, the effects of compounds on hydrophobicity of manure did not demonstrate consistent correlation with the effects on the ODA.

Surfactants decrease surface tension and cause foam in the solution (Mulligan, 2005). The surface tension of pure water and manure (0.45%TS) in this test was found to be 72.34 and 60.11 mN/m, respectively. Similar result has been demonstrated by Vardar-Sukan (1998), who reported the surface tension of pure water as 72 mN/m at 20 °C. Fig.1c shows that most of the tested compounds, except cations, decreased surface tension of both water and manure. The effects from proteins and lipids were stronger than carbohydrates. It has also been stated by many researchers, who reported the property of proteins and lipids to behave as surface active compounds capable of

decreasing the surface tension of their solvent and to enhance foaming potential (Ruimi et al., 2005; Foegeding et al., 2006, Glaser et al., 2007; Vardar-Sukan, 1998; Barber, 2005; Junker, 2007).

Na-oleate could also be considered as surfactant, since its effect followed most of the surfactant behaviour reported in literature where a clear correlation between the ability to decrease surface tension and the increase of ODA in the solution was observed. This correlation did not fit with other lipids in this experiment, which is neutral oil, i.e. does not have free polic end (carboxylic end) and therefore doesn't exhibit surfactant properties. Cations, including NH_4^+, clearly increased surface tension of the solution, but had no consistent relationship to the change of both ODA and hydrophobicity of the solution. The increase surface tension from cations did not correspond to the change of foaming tendency either. The effect of cations on foaming tendency could be explained that NH_4^+ and Na^+ were resulting in soluble salts which slightly increased foaming tendency in the solutions, while Ca^{2+} and Mg^{2+} tended to form insoluble salts which could precipitate the surfactants e.g. LCFA, and therefore decreased foaming tendency. Since foaming tendency and foam stability are the most direct measurement of foaming property of the solution, and had been widely used for determination of foaming property of the solution (Desphande and Barigou, 2001; Ganidi et al., 2011), the effect of compounds based on foaming tendency and foam stability would be the most reliable indicator. Moreover, although the liquid properties such as surface tension, ODA, and hydrophobicity had been reported to correlate with foaming potential causing by surfactant, and the similar trend was also observed for Na-oleate in this experiment, the correlation could not generally be applied to other substrates and intermediate compounds tested in this experiment.

Foaming potential was determined by two parameters; foaming tendency and foam stability. From the test results, Na-oleate strongly increased foaming tendency in both water and manure, while other compounds had relatively low effect. This was due to its surfactant properties (Na-oleate is a soap). In contrary, acetic acid significantly increased foaming tendency in manure but had no effect in water. Ganidi et al. (2009) has also reported that accumulation of acetic acid was one of the possible causes of foaming in sludge digester. Although gelatine and NH_4^+ had relatively low foaming tendency, the results from measurement of foam stability (Fig.1d) showed that they could potentially cause foaming problem in the manure digester due to their ability to create foam stability. Na-oleate also created foam stability in both water and manure, which emphasised its strong potential to cause foaming problem in manure digester.

From this study, the effect of compounds on foaming potential could not be correlated to the changes of surface tension, ODA, or hydrophobicity in manure. It has also been previously reported that no consistent relationship was detected between the foaming abilities and the hydrophobicity of mycolata bacteria from activated sludge (Stratton et al., 2002). Other hypothesis has also been reported. Blackall and Marshall (1989) demonstrated that the antifoam cations that adsorb to negative charges on the bacterial cell surface via positive charges on their ends, thus coating the cell, could reduce foam formation in activated sludge. The study results from Stratton et al. (2002) also supported that the mechanisms of these bindings are surface charge-related. Comparing to our study, the strong cations such as Ca^{2+} and Mg^{2+} with heavy molecules tended to decrease foaming tendency. This was due to the decrease availability of surfactant compounds due to the bindings of the polic end. On the contrary, cations such as NH_4^+ and Na^+ did not show this ability. It was also probably due to Na^+ and NH_4^+ can form sodium and ammonium soaps with long-chain fatty acids, i.e. compounds that ionise in water to a compound with both hydrophobic and hydrophilic ends, which could act as surfactant and cause foaming (Palatsi et al. 2009). Another example of charge effect on foaming is the foaming from protein. Foaming from protein is well known and widely applied in food industries. Their foaming effect is probably again based on the formation of

ionisable compounds with both hydrophobic and hydrophilic available ends. Protein foams are formed by a protein film surrounding a gas bubble creating a structure that holds bubbles in place (Foegeding et al., 2006). Proteins consist of different proportions of amino acids and some of them are charged in the solution. At pH 7, basic amino acids such as arginine and lysine carry a net positive charge, while acidic amino acids such as aspartic and glutamic acids have negative charge. The ratio of these amino acids determines the net charge of a protein. A combination of basic and acidic proteins creates very stable foams due to their opposite charges. However, acidic proteins are much more common in nature. It was also known from food industries that fats could destroy the protein foams by displacing the proteins that form the air bubble surface, causing the bubble to collapse (Hart, 1989). The same tendency was observed in this study where gelatine strongly increased foam stability, while lipids such fish oil, sunflower oil and butyric acid tended to decrease foaming.

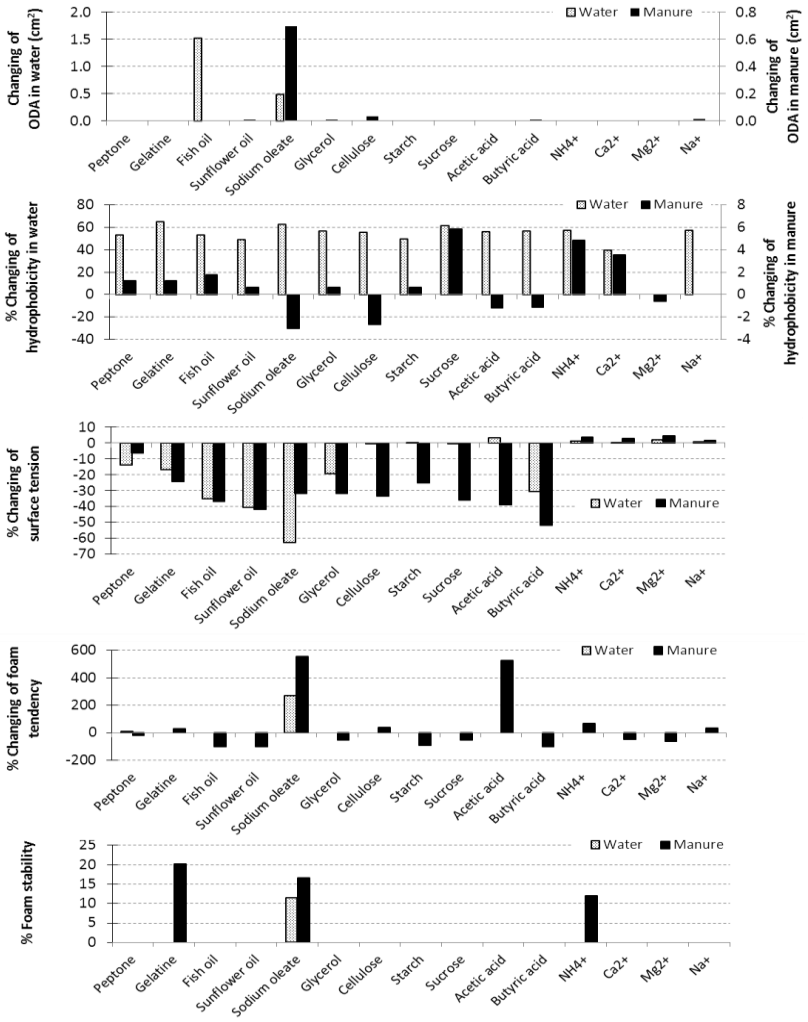

Figure 1. Effect of feedstock compounds on solution properties and foaming in water and manure

Effect of sample matrix on foaming in manure

The quantity, composition, and consistency of cattle manure influence the selection and the design of manure-handling facilities. In its strictest definition, animal manure refers only to feces and urine. However, bedding, feed wastage, rain, soil, milk-house wastes or wash, and more are mixed with the feces and urine on many farms. Manure is complex substrate and showed considerable potential to create foaming. The test results showed that cattle manure had surface tension around 52-59 mN/m at normal feed concentration of 3-6%TS. The low surface tension of manure compared

to water (72 mN/m) could possibly be due to the presence of natural and synthesis surfactants, oils, greases, proteins, lipids and polymers. However, the increase of manure concentration from 1 to 6%TS did not significantly affect the surface tension. In contrast, the increase of manure concentration was corresponding to an increase on foaming tendency, up to more than 2 mL-foam/(mL-air.min) at 6%TS. However, the foaming stability was lowered at high TS concentration (Figure 2). Again, it was obvious in these results that surface tension was a poor foaming indicator in complex slurries such as manure.

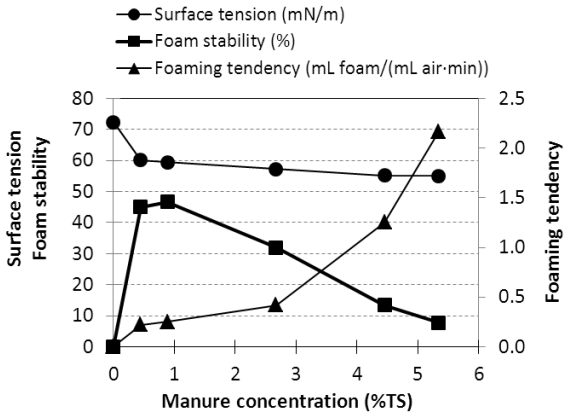

Figure 2. Surface tension and foaming potential of manure at different concentrations

Physicochemical-effect of substrates and intermediate compounds in a complex mixture

Figure 3a shows the change of ODA, hydrophobicity, surface tension and foaming tendency in manure added with combinations of different compounds. From statistical analysis of this data, the factor of influence of each compound on liquid properties and foaming tendency was then calculated and plotted in Figure 3b.

Similar to the results from testing of a single compound, in a complex mixture most of the tested compounds decreased surface tension of manure mixture, except starch and cations. According to literature, carbohydrates and inorganic salts are not surface active substances since they are able to increase surface tension of water when dissolved. Matubayasi and Nishiyama (2006) and Matubayasi and Yoshikawa (2007) showed that aqueous solutions of sodium inorganic salts, glucose, sucrose, nitrate anions, sulphates salts and different ammonium salts, respectively, were not surface active agents since in all cases surface tension within the concentrations ranges studied was higher than the pure water surface tension value of approximately 72 mN/m. From Figure 3a, similarly to the results of a single compound, the combined effect of multi-compound mixtures on surface tension did not show consistent relationship with the change of ODA and hydrophobicity either.

From the overview of the results in Figure 3b, some of the compounds showed similar effects on foaming tendency of manure both when testing as a single compound and when testing in a complex mixture. In both tests, gelatine, Na-oleate, cellulose, acetic acid and NH_4^+ increased foaming tendency in the manure, while fish oil, glycerol, butyric acid and Ca^{2+} decreased foaming tendency. However, some compounds showed opposite effects on foaming tendency when tested in a complex mixture compared to when testing as a single compound, which were sunflower oil, sucrose, Mg^{2+} and Na^+. Moreover, some compounds showed stronger effect on increasing foaming tendency when tested in a complex mixture, for example, gelatine and peptone. Both gelatine and peptone increased foaming tendency strongest in this test, which could be characterized as strong foaming agents in a complex mixture. Similar results had been reported in several studies (Ruimi et

al., 2005; Foegeding et al., 2006, Glaser et al., 2007). It was also noticed that Na-oleate was the only compound which showed consistent relationship between liquid properties and foaming tendency, similarly to when testing as a single compound.

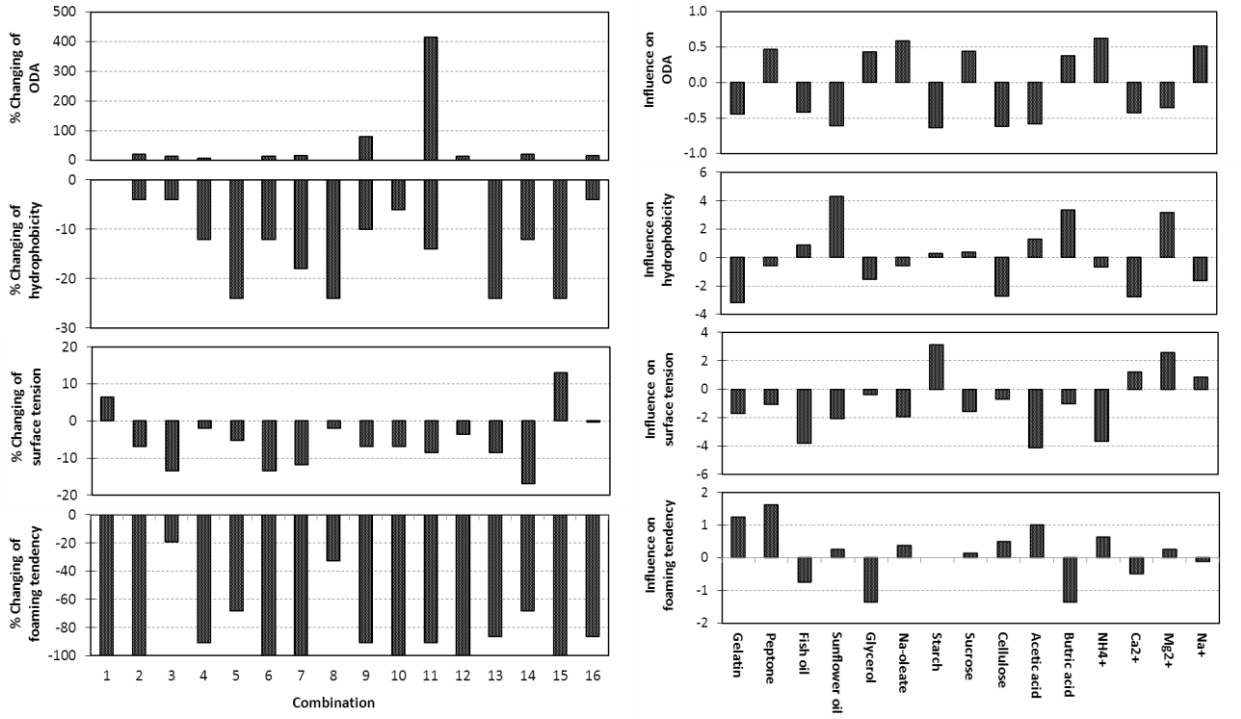

Figure 3. Effect of substrates and intermediate compounds in a complex mixture; a) Change of liquid properties and foaming potential in manure solution with different combinations of compounds, b) Statistical analysis for the influence of compounds on liquid properties and foaming in the manure mixture

CONCLUSION

There was no consistent correlation between the effect on foaming potential of the substrate and the effect on hydrophobicity, ODA or surface tension of the solution. The best way to determine foaming property of the solution was to directly measure foaming tendency and foam stability. Na-oleate and acetic acid showed the tendency to increase foaming in all tests, both for physiochemical effect and during anaerobic digestion. However, acetic acid did not create stable foam. In contrast, gelatine and NH_4^+ had moderate effect on increasing foaming tendency, but had very strong effect on increasing foam stability. Lipids tended to decrease foaming tendency according to physiochemical tests, however, they could also increase foaming during anaerobic digestion due to their high organic load. Na-oleate was the strongest foam forming agent due to its surfactant property and its high organic load. Ca^{2+} and Mg^{2+} tended to decrease foam tendency in the solution. In conclusion, Na-oleate and acetic acid showed the highest potential to create foam in the manure digester. Due to their strong ability to stabilize foam, high organic content of Na-oleate or gelatine was considered as main potential foaming problem.

ACKNOWLEDGEMENTS

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- Procesingeniør Røskva Lill Lindgård Højmark, Krüger A/S
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Rådnetank II - et fortsætter kursus

Den 8. – 9. december 2014

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Medarbejdere, der har arbejdet med området i flere år og har en god basisviden om slam og slamteknologier, svarende til kurset "Rådnetanke".

8. december 2014

09.00 Ankomst og kaffe

09.15 Velkomst og gensidig præsentation
Annette Vesterager og Mette Dam Jensen

09.45 Sikkert arbejdsmiljø ved drift og servicering af rådnetanke
- Fokus på opfyldelse af ATEX krav
Lars Dalum Nielsen

12.00 Frokost

13.00 Opsætning af massebalancer ved drift af rådnetank
John Sørensen

15.00 Kaffepause

15.30 Skumdannelse
- Kortlægning af skumningsproblemer på biogasanlæg
- Udvikling af strategier for at undgå skumning
Irini Angelidaki

16.45 Pause

17.00 Udnyttelse af spildevandets fosforressource
Mette Dam Jensen

18:00 Opsamling og opgave
Mette Dam Jensen

19.00 Middag

21.00 Øl/vand

9. december 2014

08.00 Forøgelse af Energiproduktionen
- Udnyttelse af spildevandets energi indhold
- Tilførsel af andre energikilder
- Udfordringer for renseanlæggets øvrige drift
Mette Dam Jensen

10.00 Pause

10.15 Forøgelse af Energiproduktionen –fortsat inkl. Eksempler

11.15 Pause

11.30 Rejektvandshåndtering (Erfaringer fra Holbæk Renseanlæg)
- Er anammox en primadonna?
Røskva Lill Lindgård Højmark og Henrik Thygesen

13.00 Frokost

14.00 Et eksempel på forøgelse af energiproduktionen:
Co-digestion (slamudrådning) på Grindsted Renseanlæg
Bjarne Bro,

15.00 Opsamling og afslutning
Mette Dam Jensen og Annette Vesterager

15.30 Kurset er slut

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Rådnetank II - et fortsætter kursus

Den 3.- 4. oktober 2013

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Rådnetank II

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Målgruppe

Medarbejdere, der har arbejdet med området i flere år og har en god basisviden om slam og slamteknologier, svarende til kurset "Rådnetanke".

3. oktober 2013

09.30 Velkomst, kaffe og gensidig præsentation
Annette Vesterager og Mette Dam Jensen

9.30 Hvorfor arbejde (mere) med rådnetanke?
- Drift
- Sikkerhed
- Ressourcer
Mette Dam Jensen

10.00 Sikkert arbejdsmiljø ved drift og servicering af rådnetanke.
- Fokus på opfyldelse af ATEX krav
Lars Dalum Nielsen

12.00 Frokost

13.00 Forøgelse af Energiproduktionen
- Udnyttelse af spildevandets energi indhold
- Tilførsel af andre energikilder
- Udfordringer for renseanlæggets øvrige drift
Mette Dam Jensen

15.00 Kaffepause

15.30 Et eksempel på forøgelse af energiproduktionen:
Co-digestion (slamudrådning) på Grindsted Renseanlæg
Bjarne Bro,

17.00 Pause

17.15 Udnyttelse af spildevandets fosforressource
- Præsentation af resultaterne fra fosforpartnerskabet samt EcoInnovation projekt:
Bæredygtig udnyttelse af fosfor fra spildevand
- Gennemgang af teknologityper til genvinding af fosfor
Mette Dam Jensen

19.00 Middag

21.00 Øl/vand

4. oktober 2013

08.00 Opsætning af massebalancer ved drift af rådnetank
John Sørensen

10.00 Pause

10.15 Rejektvandshåndtering (Erfaringer fra Holbæk Renseanlæg)
- Er anammox en primadonna?
Røskva Lill Lindgård Højmark og Henrik Thyesen

12.30 Frokost

13.30 Skumdannelse
- Kortlægning af skumningsproblemer på biogasanlæg
- Udvikling af strategier for at undgå skumning
Irini Angelidaki

14.30 Opsamling og afslutning
Mette Dam Jensen og Annette Vesterager

15.00 Kurset er slut