Bioresource Technology 178 (2015) 270–277

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Foam formation in biogas plants caused by anaerobic digestion of sugar beet

Lucie Moeller [a,*], Marcus Lehnig [b], Joachim Schenk [b], Andreas Zehnsdorf [a]

[a] UFZ – Helmholtz Centre for Environmental Research, Centre for Environmental Biotechnology, Permoserstrasse 15, 04318 Leipzig, Germany
[b] Leipzig University of Applied Sciences, Koburger Strasse 62, D-04416 Markkleeberg, Germany

HIGHLIGHTS

- Anaerobic digestion of sugar beet is often accompanied by foam formation.
- Foaming caused by sugar beet is intensified by the presence of divalent ions.
- Foam caused by pectin is stabilized by sucrose and divalent ions.
- Roughly disintegrated sugar beet forms less foam than sugar beet processed to mush.
- Sugar beet-based foaming is reduced by addition of urea and ammonium chloride.

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 18 September 2014
Accepted 19 September 2014
Available online 28 September 2014

Keywords:
Foam formation
Anaerobic digestion
Sugar beet
Pectin

ABSTRACT

The use of sugar beet in anaerobic digestion (AD) during biogas production can lead to process upsets such as excessive foaming in fermenters. In the present study, foam formation in sugar beet-fed digestates was studied in foaming tests. The increasing disintegration grade of sugar beet was observed to have a promoting effect on foaming in the digestate but did not affect the biogas yield. Chemical analysis of foam and digestate from sugar beet silage AD showed high concentrations of pectin, other carbohydrates and N-containing substances in the foam. Both pectin and sucrose showed little foaming in AD. Nevertheless, sucrose and calcium chloride had a promoting effect on foaming for pectin AD. Salts of divalent ions also enhanced the foam intensity in the case of sugar beet silage AD, whereas ammonium chloride and urea had a lessening effect on sugar beet-based foaming.

1. Introduction

The presence of sugar beet in the substrate mix brings many advantages. This substrate is very digestible and has a methane yield of 419 m^3/t VS, which is higher than 360 m^3/t VS in the case of maize (Gissén et al., 2014). On the other hand, the use of sugar beet in biogas production is accompanied by specific problems such as ensuring suitable storage and foam formation in fermenters. Storage and conservation of sugar beet has been extensively discussed and there are diverse approaches such as ensiling in the form of sugar beet pulp in liquid silos (Weiland, 2003) or ensiling of ground beet in large plastic bags (Weißbach et al., 2011).

Foam formation in the course of anaerobic digestion (AD) often represents a serious problem for biogas plant operators because the foam can plug gas pipes and lead to losses in biogas yield (Pagilla et al., 1997). Research into foaming causes had been mainly focused on anaerobic digesters of municipal wastewater sludge until recently (e.g., Ganidi et al., 2009; Westlund et al., 1998; Pagilla et al., 1997). Foam formation in other AD systems for biogas production has only recently begun to attract research attention. Surveys by Moeller et al. (2012b) and Kougias et al. (2014) showed the high percentage of biogas plants that suffered from foam formation: 12 out of 15 waste treating biogas plants in Germany (Moeller et al., 2012b) and 15 out of 16 full-scale biogas plants in Denmark had experienced foaming in fermenters or substrate storage/pre-digesters (Kougias et al., 2014).

Excessive foaming mainly causes operational problems such as plugging of gas pipes, foam binding of recirculation pumps, inversion of digester solids profiles (Pagilla et al., 1997), and structural

* Corresponding author. Tel.: +49 341 235 1841.
E-mail addresses: lucie.moeller@ufz.de (L. Moeller), marcus.lehnig@stud.htwk-leipzig.de (M. Lehnig), joachim.schenk@htwk-leipzig.de (J. Schenk), andreas.zehnsdorf@ufz.de (A. Zehnsdorf).

http://dx.doi.org/10.1016/j.biortech.2014.09.098
0960-8524/

damage to the digester roof in extreme cases (Moeller et al., 2012b).

Formation of foam can also decrease digestion efficiency and digester gas production (Pagilla et al., 1997). As a consequence, excessive foaming causes financial losses due to decreased biogas production (Westlund et al., 1998), increased deployment of staff and costs for foam-suppressing measures such as anti-foaming agents (Moeller et al., 2012b). Sometimes, plant components may have to be replaced after a foaming event (Moeller et al., 2012b).

Foam is a dispersion of a gas in a liquid consisting of a large proportion of gas (Vardar-Sukan, 1998). The prerequisite for foam formation is the presence of surface active substances such as volatile fatty acids (VFA), oil, grease, detergents and proteins (Ganidi et al., 2009). Foam can further be stabilized by proteins, suspended particles (Ganidi et al., 2009) and filamentous microorganisms that occur mainly in waste activated sludge (Pagilla et al., 1997; Lienen et al., 2014). The rumen bloat-causing foam that has many parallels to biogas foam (Moeller et al., 2012b) is formed especially by soluble plant proteins, bacterial slime and fine plant particles (Wang et al., 2012). Foam formation is thus often a result of loading the biogas reactor with specific substrates that contain high concentrations of the above-mentioned compounds.

Sugar beet root consists of 76.8% water, 14% sucrose and 5.5% fiber (pulp) (FAO/EBRD, 1999). The pulp is water insoluble and contains 26–32% hemicellulose, 22–24% cellulose, 21.5–23% uronic acids (pectins), 1–2% lignin, 7–8% protein and 7.5–12% ash (Michel et al., 1988). The chemical composition of sugar beet includes proteins and pectins, which are among the foam-causing compounds mentioned above. Proteins are surface active agents that have both hydrophilic and hydrophobic properties and thus have an impact on the surface tension of a solution (Ganidi et al., 2009). However, the mode of action of pectins lies in their ability to form three-dimensional stable structures and gels and, as a consequence, in the enhancement of the solution viscosity (Clarke and Reid, 1974). Furthermore, they are able to strongly enhance the stability of protein foams (Dickinson, 2003).

Two publications have been published this year on foam formation and control in the AD of sugar beet pulp. Suhartini et al. (2014) compared mesophilic and thermophilic modes of operation in laboratory biogas reactors at two different organic loading rates (OLR) of 4 and 5 g volatile solids (VS) L^{-1} day^{-1}. They found that the foaming potential in mesophilic-operated fermenters rose with ascending OLR. In contrast, thermophilic fermenters showed no foam formation at both organic loading rates (Suhartini et al., 2014). The authors suggested that foam formation in mesophilic fermenters was caused by extracellular polymer substances (EPS).

Stoyanova et al. (2014) compared the one- and two-stage mono fermentation of sugar beet press pulp at mesophilic conditions in a continuous stirred tank reactor. It was found that the two-stage AD led to reduction of the overall hydraulic retention time and higher OLRs were possible in this mode of operation with reduced risk of foaming. The authors discussed the effect of substrate composition on the digestate viscosity. They considered the pectin fraction to be one of the factors that influence viscosity in the digestate (Stoyanova et al., 2014). Nevertheless, no conclusions were drawn regarding the causes of foaming in fermenters. Although both studies presented a good overview of the conditions leading to foam formation and suppression by AD of sugar beet pulp, the foam composition and, thus, the real cause of foam formation still remains unclear. For this reason, the aim of this study was to investigate background foam formation and stabilization caused by co-digestion of sugar beet under mesophilic conditions. The problem of foam formation in the course of anaerobic digestion of sugar beet was first considered theoretically by means of two full-scale biogas plants that seasonally utilize sugar beet as substrate. Based on the comparison of the case examples, two main topics for laboratory research on this phenomenon were formulated. Firstly, the effect of sugar beet root disintegration grade on foaming intensity was considered. Secondly, the formation of foam by sugar beet silage AD and its destabilization/stabilization by additives and other chemicals in AD were studied.

2. Methods

2.1. Case examples of sugar beet AD in full-scale biogas reactors

Two biogas plants that co-ferment sugar beet at a high percentage were compared as case examples (their main characteristics are shown in Table 1).

The biogas plant BP A was constructed in 2006, is located on the site of an agricultural cooperative and utilizes the manure of the local cattle. Sugar beet has been seasonally used as a co-substrate since 2007. The daily sugar beet amount accounts for up to 16.5% of the substrate fresh matter. After sugar beet was introduced into the substrate mix, only slight foam formation was observed in the fermenter. Three years later, however, the situation changed after the modification of the manure collecting system. The foam layer was temporarily so high that action was necessary in order to prevent process upsets and damage to equipment. The plant operator tried several empirical methods of combatting foam (e.g., addition of anti-foaming agents, plant oils and acetate, and the prolongation of the stirring cycle). However, the only effective measure was continuous stirring. According to the operator, the foam appeared only when sugar beet mush was added and when cleaning of the cattle barns was carried out more than once a week. The cleaning process included the disinfection of the cattle barn by spreading dolomitic lime on the rubber mats.

The fermenter of BP A was sampled twice. The first sampling was carried out during the period of sugar beet co-digestion and enhanced foaming. The second sampling occurred in the post-foaming period when no sugar beet was digested. The fresh samples were transported to the laboratory and analyzed immediately as described in Section 2.3.

Table 1
Operational data of a foaming biogas plant (BP A) and of a foam-free biogas plant (BP B).

Biogas plant	BP A	BP B
Foam formation in biogas reactor	YES	NO
Agitation cycle	Six minutes per hour	Continuous
Agitation devices	Digester: horizontal paddle agitator and submersible mixer Secondary digester: two submersible mixers	Three digesters: horizontal paddle agitator Three secondary digesters: reeling agitator
Feeding cycle	Once per hour	Continuous feeding
Dry matter content of digestate	7%	13%
Daily substrate composition	30 m^3 cattle manure, 8 t sugar beet, 6 t corn silage, 1 t grass silage, 2 t rest feed, 1.5 t coarse wheat	49 t corn silage, 32 t crop silage, 20–40 t sugar beet, 16 t grass silage, 3 t coarse rye
Additives	None	Urea, iron hydroxide
Sugar beet pre-treatment	Processed to mush once a week using a wood shredder	Coarsely crushed using sugar industry machinery

The biogas plant BP B was commissioned in September 2012. The produced biogas is treated for methane injection into the natural gas grid. Although the proportion of sugar beet represented up to 28% of the daily feed of fresh matter, no foam was formed in this biogas plant.

2.2. Substrates and digestates

The sugar beet silage used in the foaming tests described in 2.5.2 was provided by the Deutsches Biomasseforschungszentrum (DBFZ, Germany). The sugar beet ("Belaner" breed from Syngenta Seeds GmbH) used in the foaming test described in 2.5.1 and in the fermentation batch test was kindly donated by biogas plant BP A. The digestates used in the foaming tests that are described in 2.5.1.1, 2.5.2.2, 2.5.2.4 and 2.5.2.6 originated from an agricultural biogas plant (BP C) close to Grimma, Germany. The digestates used in the foaming tests described in 2.5.2.1 and 2.5.2.3 originated from the research biogas plant of DBFZ (BP D). The digestate used in the fermentation batch tests originated from an agricultural biogas plant close to Leipzig, Germany (BP E). The characteristics of the digestates are given in Table 2.

2.3. Chemical analyses

All samples were analyzed directly after sampling. In the case of foaming tests, the separation of the foam phase and the digestate was carried out mechanically using a laboratory spoon according to Ganidi (2008). The total solid content (TS) and volatile solid content (VS) of the original samples were determined according to DIN 12880 and DIN 12879, respectively.

The samples were pre-treated before further analysis in order to guarantee sufficient homogeneity of the samples. The first step was to pass the mixture through a sieve with a mesh size of 0.75 mm. This material was used for the determination of pectin content as described further as well as of the concentrations of total organic carbon and total nitrogen using a TOC-VCSH/CSN analytical device with a TN unit (Shimadzu, Japan).

An aliquot of sieved sample was centrifuged (20 min, 5,300 rpm and 20 °C, Avanti 30 Centrifuge, Beckman, Brea, USA) and the supernatant was used for the determination of the carbohydrate concentration according to Dubois et al. (1956).

The centrifuged sample was filtered afterwards (pressure filtration device SM 16 249, Sartorius, Göttingen, Germany; nylon membrane filter: pore size 0.45 μm, Whatman, Germany). The filtrate was used for the determination of the concentrations of VFA, with water eluable elements and ammonium-nitrogen (NH_4-N). VFA were analyzed using high performance liquid chromatography (Shimadzu, Japan) with an RID-10A detector, a VA 300/7.8 Nucleogel Ion 300 OA column and 0.01 N H_2SO_4 as the eluent. Elements that are eluable with water were analyzed using inductively coupled plasma optical emission spectrometry (Spectroflame, Spectro Int., Kleve, Germany). The ammonium-nitrogen concentration was determined according to DIN 38406 E5 using the Spectroquant® test kit (measuring range 0.01–3 mg/L NH_4-N, Merck, Germany).

Table 2
Characteristics of digestates used in experiments.

Biogas plant	BP C	BP D	BP E
TS (g/L)	70.9 ± 1.0	46.8 ± 0.36	48.1 ± 1.60
VS (g/L)	55.82 ± 0.57	32.2 ± 0.23	34.9 ± 1.65
pH	8.15 ± 0.16	8.16 ± 0.02	7.87 ± 0.04
NH_4-N (g/L)	2.01 ± 0.31	1.85 ± 0.11	1.24 ± 0.13
Acetate (mg/L)	210 ± 48	21.9 ± 0.0	<1
Propionate (mg/L)	<1	<1	<1
Butyrate (mg/L)	<1	<1	<1

The NH_4-N concentration was used for the estimation of the crude protein content together with the total nitrogen concentration according to Dumas. The crude protein calculation involves uncertainty because of the presence of other nitrogen-containing compounds in sugar beet that do not contain ammonium-nitrogen such as betaine. Thus, the crude protein concentration only serves as an approximate value.

The pectins were first isolated. 4 g sieved material was diluted with 12 mL distilled water and precipitated with 28 mL 96% ethanol at 85 °C for 10 min. The reaction mixture was centrifuged and decanted afterwards. After removing the supernatant, the precipitation was repeated with 63% ethanol. The pectins were further extracted with 5 mL sodium hydroxide (1 mol/L) and 95 mL distilled water that were added to the pellet. The reaction mixture was shaken thoroughly and filtered after 15 min. The determination of pectins in the filtrate was carried out according to Dische (1947) using the color reaction of galacturonic acid with carbazole.

All photometric measurements were carried out using a MultiLab P5 spectrophotometer (WTW, Weilheim, Germany). All analyses were performed in duplicate.

2.4. Foaming tests

The intensity of foam formation was examined by means of foaming tests that were developed in order to estimate the tendency of substrates to foam in digestates. The foaming tests are deliberately carried out at high OLRs that are not used in practical applications, but which make the foaming behavior of substrates very visible. By using this method, the particular effect of each component in the substrate mix on foam formation in the digestate can be tested.

Fresh digestate originating from a stable-running biogas plant (see also Section 2.2) that utilizes renewables was passed through a sieve with a 10 mm mesh size before the foaming test in order to homogenize the input material. The sieved digestate was put into a 1 L wide-mouth bottle together with the substrate so that the reaction mixture total weight was 500 g. The flask contents were then mixed thoroughly. The test bottles were incubated in a water bath (GFL 1083, GFL, Germany) at a stable temperature of 37 °C overnight. Gas escape was facilitated by only loosely screwing on the lids of the test bottles. Foam formation was evaluated after approximately 20 h. The foam volume and total volume of the reaction mixture were calculated as the height of foam and total height measured by a rule multiplied by the surface area of the reaction mixture (the test bottle inner diameter was 90 mm). The foam content in the reaction mixture was calculated as per formula (I):

$$\text{Foam content} = 100 \times \text{Foam volume} / \text{Total volume of the reaction mixture } [\%] \quad \text{(I)}$$

Each foaming experiment contained foaming tests that were carried out in duplicate or triplicate depending on the limited water bath capacity by simultaneously running the tests. In order to exclude foaming by the digestate itself, a test bottle with 500 g of the appropriate digestate was used without any addition of substrate or additives as a control in each foaming experiment.

2.5. Experimental set-up

2.5.1. Effect of disintegration grade of sugar beet root on AD and foam formation

2.5.1.1. Effect of disintegration grade of sugar beet root on foam formation in AD. The aim of the foaming tests was to find out if the sugar beet disintegration grade plays a role in AD foam formation as stated in Section 3.1. The sugar beet was cut with a knife into

pieces with edge lengths of 1 cm and 0.5 cm, respectively. A further disintegration step was carried out by grating using a kitchen grater. 40 g of sugar beet in each form was mixed with 460 g digestate from BP C that corresponds an OLR of 20 g VS L^{-1} d^{-1} and incubated overnight.

2.5.1.2. Elution of disintegrated sugar beet root. Because foam is formed in the course of the first day of foaming tests, it can be assumed that the foam-forming and stabilizing substrate components are released into the liquid phase during this period and that they are subsequently hydrolyzed by microorganisms. For this reason, an elution method used in the analysis of wastewater and sediments was applied in order to identify the substances released during the foaming tests.

The elution of sugar beet was performed according to the DIN 38414-4:1984-10 guideline for the determination of leachability by water in the context of the examination of water, wastewater and sludge. 25 g of sugar beet in the form of 1 cm cubes, 0.5 cm cubes and grated root, respectively, was eluated with 75 g of tap water at room temperature for 17 h using an overhead-shaker (GFL 3040, Gesellschaft für Labortechnik mbH, Germany) at a rotation frequency of 14 rpm. The elution was carried out in duplicate in order to ensure reproducibility. The eluates were sieved through a sieve with a mesh size of 0.75 mm and analyzed as described in Section 2.3.

2.5.1.3. Fermentation batch tests. Fermentation batch tests for the evaluation of the biogas yield were carried out according to the VDI 4630 guideline. The digestate taken from BP E was first sieved through a sieve with a mesh size of 5 mm and then incubated anaerobically for 1 week at 37 °C in 5 L bottles in a tempered incubator for outgassing prior to being used for batch experiments.

For the fermentation batch tests, 310 g of inoculum was mixed with 11.2 g of sugar beet (cut to a 1 cm edge length in triplicate or roughly grated in duplicate) and 78.8 g of outgassed tap water in a 500 mL test bottle. Moreover, two bottles with 310 g of inoculum and 90 g of outgassed tap water served as zero samples.

The reaction mixtures were incubated at 37 °C in a water bath (GFL 1004, Gesellschaft für Labortechnik mbH, Germany) and the released biogas was entrapped in a gas sampling tube with distilled water as the confining liquid. The bottle contents were shaken every day. The biogas production was read off every working day. The biogas composition (methane, hydrogen, nitrogen and oxygen percentages) was determined twice a week by gas chromatography with an Agilent GC 6850 WLD wavelength detector (Agilent Technologies, USA) using an HP Plot separation column and argon as carrier gas. The fermentation batch tests lasted until the termination criterion (i.e., daily biogas rate equivalent to less than 1% of the total volume of biogas produced up to that time) was achieved. The normalized volume of the fermentation gas was calculated according to VDI 4630 guideline.

2.5.2. Triggering, increasing and reducing mechanisms in foam formation by sugar beet silage AD

In order to guarantee the reproducibility of further foaming tests, the use of fresh sugar beet root was deliberately avoided. Sugar beet silage was chosen because it was available in large amounts and had a more homogenous character for small-scale experiments than sugar beet roots.

2.5.2.1. Chemical composition of foam caused by sugar beet silage AD. To obtain material for the analysis of foam properties, foaming tests with 40 g sugar beet silage and 460 g digestate from BP D were carried out. The OLR was 14.8 g VS L^{-1} d^{-1}. The formed foam was separated from the digestate and analyzed immediately as described in Section 3.2.

2.5.2.2. Foam formation as a consequence of AD of sucrose and pectin. The effect of pectin and sucrose digestion on foam formation in AD was observed in foaming tests that used 10 g pectin (Roth, Germany) or 10 g sucrose (Applichem, Germany) and 490 g digestate from BP C (20 g L^{-1} d^{-1} OLR). The influence of sucrose and calcium on pectin-based foam was observed in the foaming tests. For this purpose, 5 g sucrose (Applichem, Germany) and 5 g pectin (20 g L^{-1} d^{-1} OLR), and 5 g calcium chloride (Merck, Germany) and 10 g pectin (20 g L^{-1} d^{-1} OLR), respectively, were each mixed with 490 g digestate from BP C. The reaction mixtures were incubated overnight.

2.5.2.3. Effect of cationic valence on foam formation and stabilization by AD of sugar beet silage. The aim of the foaming experiment was to verify the findings gained from the foaming tests described in Section 2.5.2.2 with calcium chloride for sugar beet silage. Moreover, the effect of cation valence on foaming was investigated. Foaming tests were carried out in five combinations by mixing 40 g sugar beet silage with 5 g salt and 460 g digestate from BP D (14.8 g L^{-1} d^{-1} OLR). Two salts that contained divalent cations were calcium chloride (Merck, Germany) and magnesium chloride (J.T. Baker, USA) and three salts that contained monovalent cations were sodium chloride (Merck, Germany), ammonium chloride (Th. Geyer, Germany) and potassium sulfate (Applichem, Germany). In order to quantify the effect of each ion on foaming, the foaming test with 40 g sugar beet silage and 460 g digestate without addition of salts was carried out.

2.5.2.4. Effect of dolomitic lime addition on foam formation by sugar beet silage AD. Based on the foaming experiment described in 2.5.2.3, a set of foaming tests was carried out by including dolomitic lime that is used for disinfection of cattle barns by the agricultural production cooperative that also operates the BP A. 40 g sugar beet silage (15 g L^{-1} d^{-1} OLR) was mixed with 5 g dolomitic lime and 460 g digestate. The foaming propensity of the particular components was tested in foaming tests with 40 g sugar beet silage and 460 g digestate (15 g L^{-1} d^{-1} OLR) and 5 g dolomitic lime and 500 g digestate, respectively.

Elution of dolomitic lime. An elution according to the DIN 38414-4:1984-10 guideline was carried out in order to find out which components of the dolomitic lime enter into solution during the foam forming phase in the digestate. For this purpose, 20 g dolomitic lime was eluated with 20 g tap water as described in 2.5.1.2 for sugar beet.

2.5.2.5. Effect of urea addition on foam formation by sugar beet silage AD. The effect of urea addition that is also used in BP B on sugar beet-based foam formation was tested by mixing 40 g sugar beet silage and 5 g urea (Riedel de Haën, Germany) (26 g L^{-1} d^{-1} OLR) with 460 g digestate from AGP A. The experiment was repeated by enhancement of the urea concentration to 10 g (37 g L^{-1} d^{-1} OLR), which was mixed with 40 g sugar beet silage and 450 g digestate. The foaming propensity of the particular components was tested with 40 g sugar beet silage and 460 g digestate (15 g L^{-1} d^{-1} OLR), 5 g urea and 495 g digestate (10 g L^{-1} d^{-1} OLR) and 10 g urea and 490 g digestate (20 g L^{-1} d^{-1} OLR), respectively.

3. Results and discussion

3.1. Case study of sugar beet-based foaming in full-scale reactors

There are five main aspects that can explain the difference in the tendencies of the digesters of BP A and BP B to foam:

The first aspect is the agitation in the digester. If there is a lot of foam in the fermenter, the only measure that helps is continuous

stirring in the case of biogas plant BP A. In the case of biogas plant BP B, the horizontal paddle agitator stirs continuously at 50% of its normal rating independently of the substrate mixture. The effect of mixing was discussed by Ganidi et al. (2009). While poor mixing can result in solid/liquid phase separation and accumulation of surface active compounds at the air/liquid interface, excessive mixing increases the amount of bubbles in the digestate. Thus, both extremes can lead to foam formation in the fermenter. With regard to the BP A, poor mixing resulted in the development of a foam layer, although this was not the primary cause of foam formation.

Secondly, the digester shape may play a role. A survey of waste disposal biogas plants showed that out of fifteen biogas plants only one had no problems with foaming (Moeller et al., 2012a). This non-foaming biogas reactor had a square horizontal "lying" shape, as in the case of BP B. BP A had a round shape that is typical for agricultural biogas plants in Germany. The influence of digester shape on foam formation was discussed by Ganidi et al. (2009), who compared cylindrical digesters with egg-shaped digesters used in the anaerobic digestion of waste activated sludge. According to the authors, the foam accumulation potential was reduced in egg-shaped digesters due to the limited surface area above the bulk phase of the digester. The surface-to-volume ratio of BP A of 0.175 m^2/m^3 was higher than in BP B (0.169 m^2/m^3), thus confirming the assumption of Ganidi et al. (2009).

The third aspect concerns the feeding cycle. The impact loading in the case of BP A supports overloading mainly due to the easily digestible sucrose in sugar beet. The analysis data of samples from fermenter of BP A in the course of foaming during the sugar beet co-digestion and in the post-foaming period is displayed in Table 3. The concentrations of acetate and propionate were significantly higher during the foaming period than afterwards, and no butyrate was detected in any sample. Due to the increased accumulation of VFA, the pH was 0.2 lower in the foaming digestate than in the non-foaming sample. The ammonium-nitrogen concentration of the digestate of 1.19 g/L was higher during the course of foaming than afterwards (0.77 g/L). On the other hand, the total nitrogen concentration had the opposite tendency so that the calculated crude protein content of 18.4 g/L was higher in the non-foaming sample than in the digestate taken during the foaming period (15 g/L).

Based on the analysis data, it can be assumed that the digestion process during sugar beet AD was balanced. The increased VFA concentrations were the consequence of the digestion of readily digestable substrate. Nevertheless, according to Hill et al. (1987) only concentrations of acetate higher than 13 mM (i.e., approximately 930 mg/L) indicate process imbalances. Thus, the data does not imply that the foam formation is caused by organic overloading.

The fourth issue is substrate pre-treatment. Both biogas plants differed in terms of sugar beet disintegration prior to loading into the digester. The effect of sugar beet pre-treatment on foam formation in AD was studied in more detail as described in 3.2.

Table 3
Characteristics of digestate taken from fermenter of BP B during and after the foaming period.

	Foaming period	Post-foaming period
TS (g/L)	78.9	79.3
VS (g/L)	63.6	65.0
pH	7.70	7.90
Total nitrogen (g/L)	3.58	3.71
NH_4-N (g/L)	1.19	0.77
Crude protein (g/L)	15.0	18.4
Acetate (mg/L)	491	22
Propionate (mg/L)	98	<1
Butyrate (mg/L)	<1	<1

The fifth aspect is the presence of chemicals in the substrate that influence the foaming propensity of sugar beet. The examination of the effect of dolomitic lime from BP A as well as of urea that is used in BP B on sugar beet-based foam formation is described in 3.3.4 and 3.3.5.

3.2. Effect of disintegration grade of sugar beet root on foam formation

Sugar beet has to be cut in order to allow the substrate to be fed into the fermenter. Several methods of pre-treating sugar beet are used in the biogas sector, two of which are presented in Table 1.

The foaming tests showed that the disintegration of sugar beet root has an impact on foam formation in the digestate. The foam content in test bottles with 1 cm cubes was 51.4 ± 2.0% compared to 56.7 ± 0.0% in the case of 0.5 cm cubes. Grated beet caused over-foaming with a foam content of 66.2 ± 2.7%. The control showed no foaming. The highest intensity of foam formation was achieved during the first day of AD. The foam content in test bottles with 1 cm cubes decreased to 46.0 ± 2.0% and in foaming tests with 0.5 cm cubes to 44.2 ± 2.7% on the second day of AD. The foaming tests with grated sugar beet were stopped on the first day due to over-foaming.

An examination of the effect of disintegration degree on biogas production in batch tests showed no decisive difference in biogas yields between the beet cubes and grated sugar beet root. Batches containing sugar beet cubes with an edge length of 1 cm produced 588 ± 8.6 L biogas/kg VS with 71.9 ± 1.56% methane content, corresponding to a methane yield of 423 L/kg VS. The biogas yield in batches with grated sugar beet was 573 ± 0.4 L/kg with 68.5 ± 1.56% methane content, corresponding to a methane yield of 393 L/kg VS. In addition, the biogas production rates were almost equal during the whole experiment, as shown in Fig. 1. The batch tests ran for 13 days until biogas formation no longer occurred. In the case of grated sugar beet, intensive biogas production occurred for 7 days, slowing down quickly thereafter from 11 L biogas/(kg VS $*$ d) on the eighth day of fermentation to 1 L biogas/(kg VS $*$ d) on the twelfth day. The biogas production rate of 43 L biogas/(kg VS $*$ d) was three times higher in the case of cut sugar beet on the eighth day and the termination criterion was not achieved until the twelfth day. The methane content was 7.2% higher in the test bottles that contained grated beet at the beginning of the fermentation. However, the methane percentage was equal in all test bottles on the eighth day, reaching 73%. The methane content in biogas from grated beet further decreased at the end of fermentation due to the early end of AD at an end value of 68.5%, as described above.

The analysis of eluates of cut and grated sugar beet showed an increase in the concentration of almost all substances with disintegration grade. The carbohydrate concentration in eluates rose with the reduction of the cube size from 1 to 0.5 cm by a factor of two (4.90 g/L vs. 10.2 g/L, respectively) and with grated beet by a factor of four (21.3 g/L). The total nitrogen concentration displayed a similar tendency, rising from 63.2 mg/L in the case of 1 cm cubes up to 352 mg/L for grated sugar beet (Table 4). Consequently, the calculated crude protein concentration also increased from 0.39 g/L (1 cm pieces) to 2.15 g/L (grated sugar beet). Only the calcium content showed the opposite tendency. More calcium was contained in eluates of compact pieces (48.4 mg/L for 1 cm cubes) than for grated beet (24.2 mg/L for grated beet). In contrast, the magnesium concentration rose with the disintegration degree of sugar beet, even if it did so only slightly in comparison with potassium.

In conclusion, the foaming tests showed an increase in foam content in test bottles due to increasing disintegration grade of sugar beet root. The presumption from Chapter 3 that the increased foaming in BP A was caused by differing substrate pretreatment was confirmed.

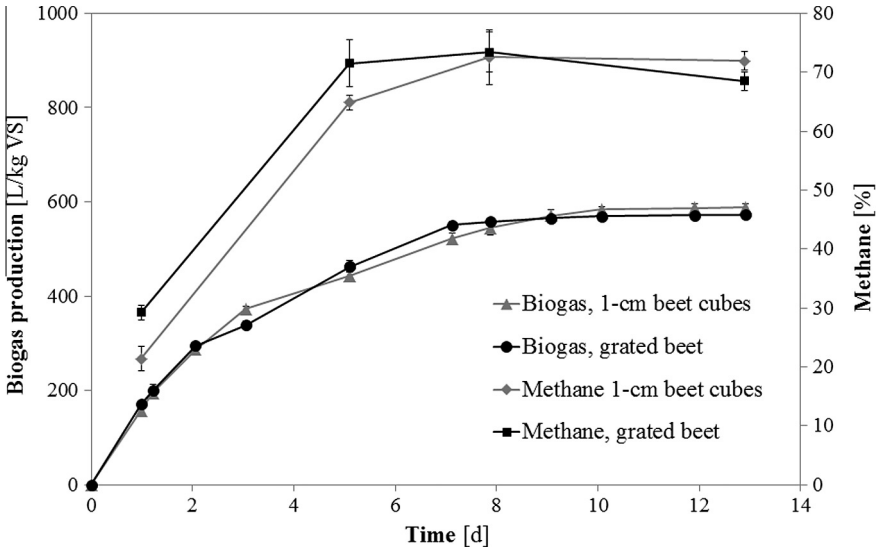

Fig. 1. Biogas production and methane content in the course of fermentation batch tests of 1 cm beet cubes ($n = 3$) and grated beet ($n = 2$).

Table 4
Analysis data of eluates of cut and grated sugar beet.

	Sugar beet disintegration		
	1 cm cubes	0.5 cm cubes	Grated
Total organic carbon (g/L)	1.97 ± 0.06	4.41 ± 0.07	10.6 ± 0.09
Total nitrogen (mg/L)	63.2 ± 9.16	149 ± 19.8	352 ± 10.8
TOC/TN ratio	31.2	29.6	30.1
NH_4-N (mg/L)	0.75 ± 0.26	2.01 ± 0.08	9.03 ± 3.15
Crude protein (mg/L)	0.39 ± 0.06	0.92 ± 0.12	2.15 ± 0.05
Carbohydrates (g/L)	4.90 ± 0.10	10.2 ± 0.76	21.3 ± 1.33
Calcium (mg/L)	48.4 ± 0.49	39.0 ± 1.13	24.2 ± 0.99
Magnesium (mg/L)	22.5 ± 1.70	25.3 ± 0.35	29.1 ± 0.14
Potassium (mg/L)	80.1 ± 15.3	147 ± 26.2	236 ± 10.6

The fermentation batch tests showed the rapid digestibility of sugar beet, which is the reason for its increasing popularity in the biogas sector, on the one hand, but is also the cause of problems during AD such as foaming, on the other hand. The methane yield was in agreement with that of Gissén et al. (2014), who obtained 419 m^3 methane/t VS by digestion of sugar beet roots.

The elution of cut and grated sugar beet roots showed an increase in the concentrations of almost all compounds with disintegration grade, which corresponds with the findings of foaming tests. The opposite tendency of the calcium concentration, which decreased with increasing disintegration grade, was probably due to binding of this element by proteins and/or pectin. These aggregates become water-insoluble and cannot be analyzed in the liquid phase.

3.3. Triggering, increasing and reducing mechanisms in foam formation by sugar beet silage AD

3.3.1. Chemical composition of foam caused by sugar beet silage digestion

A foam content of 37.7 ± 1.5% was detected in foaming test bottles with sugar beet silage AD before sampling. The control showed no foaming. The analysis data of foams and digestates in comparison to the control (digestate without addition of sugar beet silage) is shown in Table 5. Acetate, propionate and butyrate reached high concentrations in foaming tests when compared to the control. The VFA concentrations measured in the digestate were twice as high as those in the foam. As an example, 4.25 g/L acetate was analyzed

Table 5
Analysis data of digestates and foams obtained from three parallel foaming tests using 40 g of sugar beet silage ($n = 3$) and digestate from the control flask without addition of sugar beet silage.

	Control	Foaming tests	
		Digestate	Foam
Carbohydrates (g/L)	0.88	0.97 ± 0.03	4.28 ± 0.37
Pectin (g GA/kg)	0.37	0.50 ± 0.00	0.71 ± 0.09
Total nitrogen (g/L)	2.95	2.82 ± 0.11	3.85 ± 0.20
NH_4-N (g/L)	1.91	1.56 ± 0.36	1.11 ± 0.12
Crude protein (g/L)	6.45	7.86 ± 1.74	17.2 ± 1.73
Acetate (g/L)	0.11	4.25 ± 0.26	2.24 ± 0.66
Propionate (g/L)	0.08	3.27 ± 0.11	1.86 ± 0.46
Butyrate (g/L)	0.03	0.84 ± 0.06	0.45 ± 0.17
Calcium (g/L)	0.08	0.25 ± 0.02	0.20 ± 0.04
Magnesium (g/L)	0.15	0.26 ± 0.01	0.24 ± 0.01
Potassium (g/L)	2.82	2.79 ± 0.08	2.56 ± 0.02

in the digestate and 2.24 g/L acetate was detected in the foam, whereas the control contained only 0.11 g/L acetate. The presence of VFA caused a shift in the pH value from 8.16 in the original digestate down to 7.41 ± 0.04 in the digestate of foaming tests.

The concentrations of nitrogen-containing compounds were significantly higher in the foam than in the digestate (17.2 g/L crude protein in foam versus 7.86 g/L crude protein in digestate). In addition, the carbohydrate concentration of 4.28 g/L was considerably higher in the foam phase than in the digestate (0.97 g/L). As carbohydrate detection was carried out in the supernatant, it was assumed that a significant fraction of the detected carbohydrates was water-soluble sucrose. For this reason, the concentration of pectin was analyzed after extraction from the solid phase. The pectin concentration was also higher in the foam phase (0.71 g/g_{GA}) than in the digestate (0.0.50 g/g_{GA}). In contrast, the concentrations of calcium, magnesium and potassium were lower in the foam phase than in the digestate.

Most analytes had lower concentrations in the control (digestate without addition of sugar beet) than in tests with sugar beet silage AD. Only the concentrations of potassium and ammonium-nitrogen (and total nitrogen, in consequence) were higher in the control than in the foaming tests.

The foam in the foaming tests described here could be initiated by the pH shift into the acid range because the solubility of carbon dioxide in the liquid phase depends on pH. Carbon dioxide was thus released from the digestate into the gaseous phase by forming

foam. However, the increased release of the gas alone is not sufficient to produce foam. Other components that stabilize the foam bubble lamellae may play an essential role. Based on the data in Table 5, it can be concluded that both carbohydrates (soluble as well as insoluble) and proteins formed the foam layer caused by sugar beet loading in foaming tests. Thus, more experiments were required in order to identify the foam-triggering substances.

3.3.2. Foam formation as a consequence of AD of sucrose and pectin

The foaming tests with sucrose and pectin showed that both compounds caused foam formation in AD. The test bottles with sucrose as substrate contained 37.0 ± 0.5% foam as a maximum. This foam was unstable, meaning that only 17.3 ± 3.3% foam content was detected at the end of the foaming test. 35.2 ± 1.1% foam content was formed in the foaming test bottles with pectin AD overnight. The combination of pectin and sucrose caused foaming with 50.9 ± 1.3% foam content in the test bottles. The foam that was produced due to pectin feeding was further intensified by the presence of calcium chloride, leading to 44 ± 0.0% foam content. The control showed no foaming.

The foam produced by simultaneous addition of pectin and sucrose had larger bubbles and was less stable when compared with the foam caused by the feeding of pectin and calcium chloride.

3.3.3. Effect of cationic valence on foam formation and stabilization by sugar beet silage digestion

The stabilizing effect of calcium chloride was also confirmed for sugar beet silage AD. The foam content reached 52.8 ± 2.5% by addition of calcium chloride to sugar beet silage which was higher than 48.2 ± 0.0% in the case of sole sugar beet silage. Furthermore, magnesium showed an intensifying effect on sugar beet-based foaming as 57.2 ± 1.7% foam content was identified in test bottles of sugar beet silage in combination with magnesium chloride. The presence of potassium and sodium salts had no influence on the foam content in the test bottles. The foam content was 47.9 ± 2.3% in the case of sodium chloride and 47.8 ± 1.8% in the case of potassium sulfate addition to sugar beet silage AD. In contrast, the addition of ammonium chloride caused a decrease in foam content in the test bottles to 40 ± 2%. The digestate in the control test bottle showed no foaming.

The results show the tendency of divalent ions to stabilize foam. By testing the effect of monovalent ions on foam formation, the lessening effect of ammonium chloride on sugar beet-based foaming was evident while other salts of monovalent ions did not influence the foam content in the test bottles.

3.3.4. Effect of dolomitic lime addition on foam formation by sugar beet silage AD

Analysis of the dolomitic lime eluate from BP A showed the presence of calcium, magnesium, potassium and sulfur in a ratio of 2:2:1:3. This implies that divalent ions are present in high amounts. The foaming tests showed that dolomitic lime itself caused no foaming in the digestate. Nevertheless, the combination of dolomitic lime with sugar beet silage enhanced the foam content in AD from 62.3 ± 1.5% in the case of sole sugar beet silage AD to 70.4 ± 4.9% with the addition of dolomitic lime.

In this experiment, the suspicion of the BP A operator that strong foaming is connected with the cleaning of cattle barns was confirmed.

3.3.5. Effect of urea addition on foam formation by sugar beet silage AD

In BP B, urea is added as an additive to the digestate. Urea showed no foaming propensity in pure digestate in foaming tests. Instead, the addition of urea to sugar beet silage AD caused mitigation of the foam content from 79.5 ± 1.9% in the case of sugar beet silage AD to 60.7 ± 7.8% with the addition of 5 g urea and to 56.6 ± 8.8% with the addition of 10 g urea to sugar beet silage AD. The foam in foaming tests with sugar beet silage had an uneven surface with large white stable bubbles. In contrast, the foam arising from use of urea formed a compact layer and had a smooth surface.

3.3.6. Discussion of triggering, increasing and reducing mechanisms in foam formation by sugar beet silage AD

In general, the foaming tests showed that the foam content in test bottles is not only dependent on the substrate itself but also on the digestate used. In each foaming experiment, a different foam content was measured by use of the same sugar beet silage. Thus, parallel foaming tests using the same digestate must be carried out in order to observe the behavior of additives and/or substrate mixtures on foam formation. On the other hand, parallel foaming tests showed good reproducibility as can be seen from the standard deviations (e.g., 37.7 ± 1.5% foam content in triplicate as described in Section 3.3.1). Repeating the foaming tests, the same triggering or reducing effects on foaming can be observed by differing foam contents.

The foaming in the test bottles was undoubtedly caused by organic overloading of digestate as has already been described by several authors (Pagilla et al., 1997; Kougias et al., 2013; Stoyanova et al., 2014; Suhartini et al., 2014). Ganidi et al. (2011) assumed that the compounds are not fully degraded by bacteria within digesters due to organic overloading and this potentially leads to the accumulation of hydrophobic surface active by-products that promote foaming. Suhartini et al. identified extracellular polymer substances with 43.4% protein and 8.3% polysaccharide content as the triggering compounds for foam formation. Brooks et al. (2008) claimed that foam is formed due to the high sugar content in sugar beet press pulp. Other authors looked for the cause of the high digestate viscosity during sugar beet AD. Stoppok and Buchholz (1985) proposed that the high viscosity was the consequence of high concentrations of cellulosic substances in beet pulp, while Stoyanova et al. (2014) considered the pectin fraction to be one of the factors that have a significant influence on the viscosity. According to Norziah et al. (2001), the complex viscosity of pectin solutions decreases with rising temperature. This effect could explain the lower foaming potential of thermophilic digestates in comparison to mesophilic as described in Suhartini et al. (2014), assuming that the viscosity both depends on pectins and directly influences the foaming propensity of digestates.

The foaming tests that are described in Section 3.3.2 and 3.3.3 confirm the role of pectin in sugar beet-based foam formation. As described by Norziah et al. (2001), pectin dispersions are influenced by calcium and sucrose that support their gelation. The behavior of divalent ions in combination with polysaccharides was explained by Grant et al. (1973) by means of the so-called "egg box model". The divalent ions are positioned between two or more polygalacturonate chains, forming a three-dimensional structure that looks like an egg box, where the eggs are the divalent ions. Thus, the requirement for the forming of this structure is the presence of free carboxylic groups in pectin. The sugar beet pulp pectin has a methoxy content of over 60% in terms of degree of methylation (Phatak et al., 1988), so both esterified and non-esterified polygalacturonate strains are present. The influence of sucrose on pectins is of a different nature, as sucrose is bound to highly esterified pectin molecules by a combination of hydrogen bonds and hydrophobic effects (Oakenfull, 1991). The different modes of action of sucrose and divalent ions are reflected in the different foam stability of pectin-sucrose and pectin-calcium chloride formed foams as described in Section 3.3.2.

The abated foaming as a result of the addition of both ammonium chloride and urea to sugar beet silage could be explained by the adjustment of the C/N ratio in AD. Because sugar beet contains a high percentage of carbohydrates, its C/N ratio is high as a consequence. A TOC/TN ratio of 30 was detected in eluates of sugar beet (Table 4). A low C/N ratio leads to ammonia inhibition in AD as described in Chen et al. (2008). In contrast, a high C/N ratio can lead to unbalanced AD. To our knowledge, the impact of a high C/N ratio on foam formation in AD has not been studied until now. Thus more experiments are needed in order to confirm this assumption.

4. Conclusions

The intensity of foam formation in sugar beet AD is influenced by several factors. The particle size of the sugar beet root affected foam content in foaming tests. The sugar beet-based foam was stabilized by divalent ions, while monovalent ions had no effect on foam content. Both ammonium chloride and urea mitigated sugar beet-based foaming in foaming tests. These results are important for practical applications as the research was based on case examples of full-scale reactors. Based on the new knowledge gained in this work, future research on foaming in AD should focus on the C/N ratio in the digestate.

Acknowledgements

This project was funded by the Initiating and Networking Fund of the Helmholtz Association. The authors would like to thank the biogas plant operators who provided them with substrates and digestates. The authors express their thanks to Patrick Sheehan for his helpful discussions and critical reading of the manuscript.

References

Brooks, L., Parravicini, V., Svardal, K., Kroiss, H., Prendl, L., 2008. Biogas from sugar beet press pulp as substitute of fossil fuel in sugar beet factories. Water Sci. Technol. 58 (7), 1497–1504.
Chen, Y., Cheng, J.J., Creamer, K.S., 2008. Inhibition of anaerobic digestion process: a review. Bioresour. Technol. 99, 4044–4064.
Clarke, R.T.J., Reid, C.S.W., 1974. Foamy bloat of cattle. A review. J. Dairy Sci. 57, 753–785.
Dickinson, E., 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocolloids 17, 25–40.
Dische, Z., 1947. A new specific color reaction of hexuronic acids. J. Biol. Chem. 167, 189–198.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
FAO/EBRD, 1999. Agribusiness Handbooks, vol. 4 – Sugar Beets/White Sugar, pp. 35–43.
Ganidi, N., 2008. Anaerobic Digestion Foaming Causes (Dissertation). Cranfield University.
Ganidi, N., Tyrrel, S., Cartmell, E., 2009. Anaerobic digestion foaming causes – a review. Bioresour. Technol. 100, 5546–5554.
Ganidi, N., Tyrrel, S., Cartmell, E., 2011. The effect of organic loading rate on foam initiation during mesophilic anaerobic digestion of municipal wastewater sludge. Bioresour. Technol. 102, 6637–6643.
Gissén, C., Prade, T., Kreuger, E., Nges, I.A., Rosenqvist, H., Svensson, S.-E., Lantz, M., Mattsson, J.E., Börjesson, P., Björnsson, L., 2014. Comparing energy crops for biogas production – yields, energy input and costs in cultivation using digestate and mineral fertilisation. Biomass Bioenergy 64, 199–210.
Grant, G.T., Morris, E.R., Rees, D.A., Schmith, P.J.C., Thom, D., 1973. Biological interactions between polysaccharides and divalent cations: the egg-box model. FEBS Lett. 32 (1), 195–198.
Hill, D.T., Cobb, S.A., Bolte, J.P., 1987. Using volatile fatty acid relationships to predict anaerobic digester failure. Trans. ASAE 30 (2), 496–501.
Kougias, P.G., Boe, K., Angelidaki, I., 2013. Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors. Bioresour. Technol. 144, 1–7.
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 Sci. Technol. 69 (4), 889–895.
Lienen, T., Kleyböcker, A., Verstraete, W., Würdemann, H., 2014. Foam formation in a downstream digester of a cascade running full-scale biogas plant: influence of fat, oil and grease addition and abundance of the filamentous bacterium *Microthrix parvicella*. Bioresour. Technol. 153, 1–7.
Michel, F., Thibault, J.F., Barry, J.L., 1988. Preparation and characterisation of dietary fibre from sugar beet pulp. J. Sci. Food Agric. 42, 77–85.
Moeller, L., Görsch, K., Müller, R.A., Zehnsdorf, A., 2012a. Formation and suppression of foam in biogas plants – practical experiences. Landtechnik (Agricultural Engineering) 67 (2), 110–113.
Moeller, L., Goersch, K., Neuhaus, J., Zehnsdorf, A., Mueller, R.A., 2012b. Comparative review of foam formation in biogas plants and ruminant bloat. Energy Sustain. Soc. 2, 12.
Norziah, M.H., Kong, S.S., Abd, Karim A., Seow, C.C., 2001. Pectin–sucrose–Ca^{2+} interactions: effect on rheological properties. Food Hydrocolloid 15, 491–498.
Oakenfull, D.G., 1991. The chemistry of high-methoxyl pectin gelation. In: Walter, R.H. (Ed.), The Chemistry and Technology of Pectin. Academic Press, New York, p. 87.
Pagilla, K.R., Craney, K.C., Kido, H.K., 1997. Causes and effects of foaming in anaerobic sludge digesters. Water Sci. Technol. 36 (6–7), 463–470.
Phatak, L., Chang, K.C., Brown, G., 1988. Isolation and characterization of pectin in sugar-beet pulp. J. Food Sci. 53 (3), 830–833.
Stoppok, E., Buchholz, K., 1985. Continuous anaerobic conversion of sugar beet pulp to biogas. Biotechnol. Lett. 7 (2), 119–124.
Stoyanova, E., Forsthuber, B., Pohn, S., Schwarz, C., Fuchs, W., Bochmann, G., 2014. Reducing the risk of foaming and decreasing viscosity by two-stage anaerobic digestion of sugar beet pressed pulp. Biodegradation 25, 277–289.
Suhartini, S., Heaven, S., Banks, C.J., 2014. Comparison of mesophilic and thermophilic anaerobic digestion of sugar beet pulp: performance, dewaterability and foam control. Bioresour. Technol. 152, 202–211.
Vardar-Sukan, F., 1998. Foaming: consequences, prevention and destruction. Biotechnol. Adv. 16, 913–948.
Wang, Y., Majak, W., McAllister, T., 2012. Frothy bloat in ruminants: cause, occurrence, and mitigation strategies. Anim. Feed Sci. Technol. 172, 103–114.
Weiland, P., 2003. Production and energetic use of biogas from energy crops and wastes in Germany. Appl. Biochem. Biotechnol. 109, 263–274.
Weißbach, F., Wagner, A., Scholtissek, M., Auerbach, H., Herbes, C., 2011. Conservation losses in the course of ensiling sugar beet for biogas production. Agric. Eng. 4 (2011), 254–258.
Westlund, Å.D., Hagland, E., Rothman, M., 1998. Operational aspects on foaming in digesters caused by *Microthrix parvicella*. Water Sci. Technol. 38, 29–34.