Anaerobic Digestion of Fractionated and Non-fractionated Sugar Beet Tops

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Abstract

Continuous stirred tank reactors (CSTR) and batch reactors were used to examine the difference in methane yields and process stability between fractionated and non-fractionated sugar beet leaves.

The hypothesis of this project was that by reducing the amount of juice in the sugar beet leaves the amount of soluble sugars and other easily fermented compounds in the substrate will also decrease, and thereby lead to higher process stability.

Batch reactor experiments were performed with inoculum-substrate ratios (ISR) of 2:1 and 1:1, and with non-fractionated (Total Solids, TS: 13.0%), two different solid fractions (TS: 14.9% and 17.6%) and liquid fraction (TS: 7.1%) sugar beet leaves. The methane yields of non-fractionated substrate for ISR 2:1 and 1:1 was 328 (sd 12) mL gVS^{-1} and 339 (sd 8) mL gVS^{-1}. The methane yields of the solid fraction with TS 14.9% for ISR 2:1 and 1:1 was 302 (sd 14) mL gVS^{-1} and 306 (sd 9) mL gVS^{-1}. The methane yield of the solid fraction with TS 17.6% with ISR 2:1 was 322 (sd 12) mL gVS^{-1}. The methane yield of the liquid fraction for IRS 2:1 and 1:1 was 303 (sd 16) mL gVS^{-1} and 330 (sd 20) mL gVS^{-1}. The liquid fraction with the higher organic load (ISR 1:1) showed signs of inhibition which was not present in other substrates with the same ISR. This could be due to higher concentrations of quickly fermented compounds leading to inhibiting levels of volatile fatty acids, indicating that this substrate more easily can cause disturbances at higher organic loading rates than the other substrates.

The CSTR experiments took place over a period of 80 days and substrates used were non-fractionated (TS: 13.0%) and solid fraction (TS: 14.9%). The methane yields for the two reactors with non-fractionated substrate leaves were 245 and 238 mL gVS^{-1}. The methane yields for the two reactors with fractionated substrate leaves were 205 and 224 mL gVS^{-1}. Process disturbances caused by foam production was common, particularly in reactors with solid fraction. Results from the continuous experiments do not indicate that methane yields or process stability improved for the reactors fed with fractionated substrate, but rather the opposite. However, as the continuous experiments only went on for 80 days and the variations between duplicates were large the results are not conclusive.

Table of Contents

Abbreviations

AMPTS	Automatic Methane Potential Test System
BMP	Biomethane potential
BRS	BioReactor Simulator
CSTR	Continuous Stirred Tank Reactor
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic Retention Time
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometry
ISR	Inoculum Substrate Ratio
LF	Liquid Fraction
OLR	Organic Loading Rate
SBT	Sugar Beet tops
SF	Solid Fraction
TS	Total Solids
VFA	Volatile Fatty Acids
VS	Volatile Solids
ww	wet weight

Introduction

In an attempt to reduce dependency on fossil fuels and to reduce the impact of greenhouse gas emissions, the European parliament and the council of the European Union has set a directive to increase the use of renewable energy sources to 20% by the year 2020 (Directive 2009/28/EC). One such renewable source is the production of biogas from biomass. In order to effectively utilize the substrates required for biogas production, and to determine the appropriate process design parameters, experiments on the lab-scale need to be performed. In this project the use of sugar beet leaves – a by-product from agriculture – is studied. The combination of sugar beet and the sugar beet leaves has been shown to have a high biomass and biogas yield in earlier studies [1]. While there are many studies examining methane production from sugar beets, there are few studies in literature which look specifically on the leaves as a substrate. The Swedish university of agricultural sciences (SLU) investigated the methane yield of fresh sugar beet leaves as well as a co-substrate with straw and corn and concluded that it could be a competitive substrate for biogas production (with regards to the cost per volume methane produced) [2].

Xiao et al [3] found that increased glucose content in the hydrolysate in an aerobic digester inhibited the activity of cellulase and β-glucosidase. Kübler and Schertler [4] found that easily digested monomers tend to be converted to biogas before the more recalcitrant lignocellulosic compounds. As there likely are high amounts [5] of monomeric and dimeric sugars in sugar beet leaves it is possible that hydrolysis inhibition of these lead to lower degradation of difficult-to-digest fiber material in continuous reactors, which might potentially lead to lower methane yields. In addition, since the process of acidogenesis is the fastest stage of anaerobic digestion, a higher amount of monomeric carbohydrates may after substrate feeding lead to higher concentrations of intermediate products such as volatile fatty acids (VFAs) that are known to have an inhibitory effect on methanogens. High VFA concentrations may also reduce surface tension of the digester which can lead to process instability in the form of foam production [5].

This project investigates whether it is possible to increase process stability of biogas production from sugar beet leaves by reducing the amount of quickly fermented compounds – such as glucose – by way of fractionating the beet leaves into a solid and a liquid fraction. Fractionating sugar beet leaves and using the liquid fraction as a substrate for biogas production (and the solid as feed) has been suggested before [5], but no mention could be found in the literature of this having been tested in practice. In addition to biogas production this type of pretreatment might also, for example, be useful in a biorefinery processing many different agricultural residues into products such as proteins and platform chemicals, as suggested by Kamm et al. [6]. The hypothesis of this project is that by reducing the amount of juice in the sugar beet leaves the amount of soluble sugars and other easily fermented compounds in the substrate will also decrease, and thereby lead to higher process stability. Further, this might enable the use of a higher organic loading rate in a process designed for solid substrates when using the solid fraction compared to non-fractionated sugar beet leaves as substrate. It is also hypothesized that the juice can be fermented in a process designed for liquid substrates that can handle higher organic loading rates than processes for solid substrates and overall higher process efficiency can be achieved by digesting the two fractions in separate reactors compared to digesting the entire sugar beet leaves in one reactor. The focus of this thesis work is a comparison of the process stability in anaerobic digestion of the solid fraction of sugar beet leaves and non-fractionated sugar beet leaves in a continuous stirred tank reactor (CSTR). The main goals of

this process were to evaluate the methane production and the stability of the process in a setting more reminiscent of real conditions. Biochemical methane potential (BMP) tests were also performed on fractionated and non-fractionated sugar beet leaves to achieve reference methane potential values. Also, the BMP tests were used as a simple test to see if the beet juice and the non-fractionated sugar beet tops do cause stronger inhibition than the solid fraction at increased loadings. This aspect was explored by running the BMP-test at different substrate loads with constant inoculum amount. Two BMP test series were performed: in the first series solid and liquid fractions were compared against non-fractionated substrate and in the second series the substrate was fractionated further and was also tested at lower inoculum-substrate ratios.

Theoretical background

Anaerobic digestion

Anaerobic digestion can be divided into four distinct phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis [7]. In the hydrolysis, complex polymers such as carbohydrates, lipids and proteins are hydrolysed into smaller units such as long-chain fatty acids (LCFA), sugars and amino acids. In the acidogenesis these compounds are then converted into intermediate products by fast-growing fermentative micro-organisms. The acidogenesis is the fastest stage of anaerobic digestion and it produces some compounds which can inhibit the methanogens, such as VFAs. The acetogenesis produces acetate and hydrogen. In the last step of anaerobic digestion acetotrophic methanogenesis is dominating in many processes but not all. Hydrogenotrophic methanogenesis is the other major pathway. It is worth noting that methanogens are slow-growing microorganisms and thus vulnerable to process disturbances.

Biomethane potential (BMP) test

A BMP-test is a way of determining the methane yield of a specific substrate, commonly named the "specific methane potential" and expressed per mass of volatile solids (VS). There are many different ways of performing a BMP-test (see e.g. Hansen et al. [8]) but the common denominator is that a certain amount of the substrate is placed in a batch reactor together with inoculum. The production of methane is then measured continuously until the daily gas production decreases to a low level or for a pre-determined period of time that is judged to be long enough for all of the substrate to be degraded (e.g. 50 days in Hansen et al.[8]).

Inoculum substrate ratio (ISR)

The organic loads of the reactors in the BMP-tests are measured by the inoculum to substrate ratio (ISR) on a VS basis. Chynoweth et al. [9] found that the maximum conversion rate was achieved with an ISR of 2:1, compared to other ISR between 1:1 and 2:1.

Continuous or semi-continuous?

The feeding pattern of a reactor – sometimes referred to as the 'mode of operation' – can be roughly divided into three distinct types: Batch, fed-batch, and continuous [10]. Batch reactors are fed only once at the start of the experiment and then run until termination of experiment. Fed-batch reactors are fed a small part of the total amount of substrate at the start of the experiment. Then the population of micro-organisms in the reactor is allowed to grow for a certain time in order to increase the yield of product when the rest of the substrate is added in the second feeding. Finally, in

continuous reactors the substrate (or substrates) is fed to the reactor continuously, and the reactor contents are also discharged continuously [10].

In this project, the reactors used are referred to as Continuous Stirred Tank Reactors (CSTR). However, since these reactors are in fact not fed continuously but only at discrete moments (See CSTR - experimental setup below) this term is somewhat misleading. A more accurate term might be 'semi-continuous'. However, CSTR is an accepted term for this type of reactor regardless if it is fed continuously or semi-continuously.

The difference between CSTR experiments and BMP-tests is that BMP-tests give an estimation of the maximum possible methane yield and gives an indication of the time it takes to degrade the substrate. The degradation pattern might however not be the same in a continuous process. CSTR experiments are more reminiscent of conditions in the large-scale and can give a better estimation of the methane yield that the substrate will give in practice. In CSTR experiments the substrate is usually not fully degraded, since there is a continuous inflow and outflow. CSTR experiments can also be used to identify factors which might inhibit the process or cause disturbances in the large-scale [11].

Methods

Overview of experimental setup

This project consisted of two main parts. A rough outline of these follows:

- In the first part, batch tests were performed in an Automatic Methane Potential Test System (AMPTS, Bioprocess control AB, Lund). The produced gas was led through a vial of 3 M NaOH where the CO_2 was separated through diffusion of CO_2 into the liquid. The remaining gas was then led to a unit measuring the volumetric gas flow.
- In the second part, five CSTRs were coupled to a BRS (BioReactor simulator, Bioprocess control AB) unit in order to measure the volumetric gas flow. The composition of the gas was monitored by analyzing samples with gas chromatography.

Substrate and additions

The substrates used for the CSTR processes were the leaves of sugar beets (with the variety name Nexus) harvested (by Thomas Prade, Swedish University of Agricultural Sciences) on the 15th of October, 2013. The leaves were stored in cold room until the 16th to the 19th of October when the leaves were processed (by Emma Kreuger, Department of Biotechnology, LTH) and frozen. The processing consisted of the material first being chopped up (2-3 cm) in a slow-moving coarse mill, and then sliced more finely in a fast-moving mill. Using an apple juice press part of the material was pressed into a liquid fraction (LF) representative of 24% of the wet weight, and a solid fraction (SF) comprising 76% of the wet weight. TS and VS contents are shown in table 1. The solid and liquid fractions, as well as the rest of the chopped unfractionated material, were put into bags in portions of 60 or 200 gram. The LF and SF was prepared on the 16th, 18th and 19th of October, and the non-fractionated beet leaves were chopped finely and put into the freezer on the 19th of October. The substrate was kept in freezer and thawed in the fridge the day before used to feed reactors.

In the AMPTS experiments cellulose (Avicel PH Microcrystalline cellulose (FMC BioPolymer, Philadelphia, USA) was also used as a control substrate.

Some of the SF was fractionated further in order to be used in the second set of AMPTS experiments. This was done in a Fischer press (Fischer Tinkturenpressen HP5M, FISCHER Maschinenfabrik GmbH, Neuss, Germany). In order to do this pressing the substrate was thawed (put into fridge on the night before). The solid fraction from this pressing comprised 57% of the original wet weight and the liquid fraction comprised 43% of the original wet weight. After pressing, the liquid (LF2-SBT) and solid fraction (SF2-SBT) was immediately put into the freezer again. The TS value of the SF2-SBT substrate was 17.6% and the TS value of the LF2-SBT substrate 5.2%.

Table 1. TS and VS measurements for Substrates used in first AMPTS experiment.

Substrate	Average TS [%]	Average VS [%]
SBT 19/10	12.7%	10.8%
LF-SBT 16/10	7.1%	6.1%
SF 18/10	14.7%	12.6%
SBT chopped 17/10	12.7%	11.1%

Table 2. TS and VS measurements for Substrates used in second AMPTS experiment.

Substrate	Average TS [%]	Average VS [%]
SF2-SBT	17.6%	15.2%
LF2-SBT	5.2%	4.2%
Inoculum	4.8%	3.2%
Cellulose		95.2%
19/10 SBT	13.0%	11.1%

Table 3. TS and VS measurements for Substrates used in CSTR experiment.

Substrate	Average TS [%]	Average VS [%]
SF 16/10	14.9%	12.9%
SBT 19/10	13.0%	11.1%

In order to mitigate foaming in the CSTR processes antifoam was occasionally added (see experimental setup below for times and amounts added). The product used was Antifoam Silicone Snapsil RE 20 (VWR international LLC, Radnor, US).

The CSTRs were also supplemented with a solution containing trace nutrients to aid the enzymatic processes of the microorganisms. Trace nutrients added for the CSTR experiments were iron, nickel and cobalt. The chemicals used to supply these nutrients were iron (2) chloride-tetrahydrate, nickel (2) sulphate hexahydrate and cobalt chloride hexahydrate. The nutrient content of the substrates was analyzed by ICP-MS (for cobolt and nickel) and ICP-AES (for iron). The analyses were performed by Lennart Månsson International AB, Box 700, S-25107 Helsingborg, Sweden. The amount of nutrients added each feeding was set so that the concentration for each trace nutrient added was the same as in Nges et al [12]. See appendix for complete results of nutrient analyses. See table 1 for the amounts added. The amount of nutrients added was based on the compositional analyses of the substrates, so that the amount of nutrients already present in the substrate was subtracted when preparing the nutrient solution. Unfortunately, due to a miscalculation, more nutrients were added than was intended. See tables 4 and 5 below for nutrients on a substrate wet weight basis.

Table 4. Nutrient concentrations for SBT reactors.

SBT				
	Nutrients, Nges et al. [mg/kg ww]	**Nutrient content of substrate [mg/kg ww]**	**Nutrient to be added [mg/kg ww]**	**Actual amount added [mg/kg ww]**
Iron	46.00	63.0	0	1070
Nickel	0.50	0.13	0.36	12.1
Cobalt	2.00	0.029	1.97	48.9

Table 5. Nutrient concentrations for SF-SBT reactors.

SF-SBT				
	Nutrients, Nges et al. [mg/kg ww]	Nutrient content of substrate [mg/kg ww]	Nutrient to be added [mg/kg ww]	Actual amount added [mg/kg ww]
Iron	46.00	58.9	0	1070
Nickel	0.50	0.14	0.36	12.1
Cobalt	2.00	0.032	1.97	48.9

Biochemical methane potential test

Experimental setup

First BMP experiment

Anaerobic digester sludge from Källby wastewater treatment plant (collected on the 22nd of January) was used for this process. TS and VS of the sludge was measured on the 23rd of January. TS and VS of the inoculum was 4.28% and 2.63% respectively. The sludge was pre-incubated in a water shaker bath for 5 days in five e-flasks with 4L of sludge in each flask. The flasks were plugged with rubber plugs and fitted with balloons for gas collection during the pre-incubation. On January 28th the batch reactors were filled with pre-incubated inoculum and substrate (see table 6 for amounts). The loads in the reactors are measured as the Inoculum Substrate Ratio (ISR): the ratio between VS content of inoculum and VS content of substrate. Two controls were used: inoculum and inoculum plus cellulose (three replicates each). Reactors were incubated in heated water baths (set to 37°C) and flushed with nitrogen gas for one minute. After 32 days, GC measurements of gas in reactor headspaces were made in preparation for terminating the experiment. The final methane content of the gas in the headspace of the reactors is used as an input value in the AMPTS software for calculation of the total methane yield. Recording of data was discontinued using bioprocess web application on day 34. Heating water bath and stirrers were shut off.

Table 6. Substrates used in first AMPTS experiment, with ISR and weights added.

Substrate	ISR	VS added [g]	ww added [g]	Replicates
SBT shredded 2:1	2:1	3.95	36.80	3
SBT shredded 1:1	1:1	7.89	73.60	3
SBT-SF shredded 2:1	2:1	3.95	29.47	3
SBT-SF shredded 1:1	1:1	7.89	58.95	3
SBT-LF shredded 2:1	2:1	3.95	60.29	3
SBT-LF shredded 1:1	1:1	7.89	120.58	3
SBT chopped 2:1	2:1	3.95	36.80	3

Second BMP experiment

Anaerobic digester sludge from Källby wastewater treatment plant (collected on the 14th of April) was pre-incubated for 6 days in three 5L e-flasks (with 3.3 L of inoculum in each flask) using the same method as for the first BMP experiment. TS and VS of the sludge was measured on the 15th of April. TS and VS of the inoculum was 4.84% and 3.21% respectively. The substrates used were SBT, SF2-SBT and LF2-SBT, see Table 7 and 8. See *substrates and additions* for TS and VS values of the substrates used. Two AMPTS-systems were used: system 83 and system 84. The original planned placement and contents in reactors are shown in table 2 and 3. During initiation of experiment it was discovered that the amount of pre-incubated inoculum was not enough for all experiments, which meant that experiments in reactors 10-15 of system 83 could not be performed. On April 20th the experiment was started. After 28 days measurements of gas in AMPTS reactors were made in preparation for terminating the experiment. Recording of data was discontinued using bioprocess web application day 30.

Table 7. Original planned contents and positions of reactors in system 84.

System 84					
Position	**Substrate**	**ISR**	**VS per 300 g inoculum [g]**	**VS weight added, substrate [g]**	**ww added [g]**
1-3	SF2-SBT	2:1	9.62	4.81	31.67
4-6	SF2-SBT	1:1	9.62	9.62	63.34
7-9	SF2-SBT	1:2	9.62	19.24	126.67
10-12	SBT	2:1	9.62	4.81	43.33
13-14	Control	N/A	9.62	N/A	N/A
15	Cellulose	2:1	9.62	4.81	5.05

Table 8. Original planned contents and positions of reactors in system 83.

System 83					
Position	**Substrate**	**ISR**	**VS per 300 g inoculum [g]**	**VS weight added, substrate [g]**	**ww added [g]**
1	Control	N/A	9.62	N/A	N/A
2-3	cellulose	2:1	9.62	4.81	5.05
4-6	SBT	1:1	9.62	9.62	86.66
7-9	SBT	1:2	9.62	19.24	173.33
10-12	SBT+antifoam	2:1	9.62	4.81	43.33
13-15	LF2-SBT	2:1	9.62	4.81	113.61

Calculations

The methane yields are the average cumulative production of each set of reactors minus the average cumulative production from the control (inoculum). The standard deviation, σ, for the methane yields is calculated according to the rules for linear combination of measured quantities [13]:

$$\sigma_y = \sqrt{(k_a\sigma_a)^2 + (k_b\sigma_b)^2 + \cdots}$$

Where *y* is the combined measurement, *a* and *b* are the measurements combined and *k* is a constant. Which in this case means:

$$\sigma_{methane\ yield} = \sqrt{\left(\frac{\sigma_{average\ meth.\ prod.}}{gVS_{substrate}}\right)^2 + \left(\frac{\sigma_{average\ meth.\ prod.\ from\ control}}{gVS_{substrate}}\right)^2}$$

Experiment log

Second bmp experiment

April 22nd. Severe foaming had developed in several of the AMPTS reactors. In system 84 reactors 4, 6, 7 and 8 had to be terminated. In system 83 reactors 4 to 9 had to be terminated. Sludge had bubbled out of the foaming reactors and into the heated water baths. The water baths needed to be turned off, emptied and cleaned before refilling. This meant that the remaining reactors were without heating for a period of approximately 5-10 minutes.

CSTR design

The setup for the continuous reactors was five 5 L glass reactors with hollow heating mantles. The reactors were heated by a circulating water bath, which kept the reactors at constant temperature during the experiment. The tubes for the heating water were insulated using plastic and Styrofoam. Before start of experiment the temperature of the reactor closest to the water bath and the reactor farthest away from the water bath was measured using a digital thermometer. Difference was negligible (approximately 0.2 °C).

Each reactor had one feed and one discharge port on bottom part of reactor. These were plugged during the duration of the experiment except for when discharging. Rubber plugs were used to seal reactor tops in order to maintain anaerobic conditions in the reactor. Due to difficulties in feeding the substrate through the bottom ports the rubber plugs were cored with a hollow drill and a 22 cm long plastic feeding tube of 18 mm in diameter was inserted through the holes. The length of this tube was adjusted so that it remained 2 centimeters below the liquid surface in the reactor, thereby minimizing risk of gas in feeding tube from coming into contact with the gas in the rest of the reactor. In order to minimize oxygen exposure these feeding tubes were plugged with rubber plugs (loosely placed on the tubes to avoid gas pressure build up in the tubes), except for when feeding reactors.

On each reactor there was a gas port with a tube leading to the gas measuring unit. On this tube a gas sampling coupling in glass was fastened. This was used when sampling gas for measurements in the gas chromatograph. Gas flows are normalized by the BRS web application (1.0 standard atmospheric pressure, 0°C and zero moisture content). For the days in which no GC measurements were made the gas composition was estimated through linear interpolation.

The stirring was done by an electrical stirrer fastened above each reactor. The axle of the stirrer extended through a plastic tube (diameter 0.5 cm) through the rubber plug on top of the reactor. The end of the plastic tube remained below the surface for the duration of the experiment in order to minimize the risk of gas in the stirrer tube coming into contact with gas in the rest of the reactor. The

stirring rate was set to 60 rpm at the start of the experiment, but this was later changed to 80 rpm. Occasionally the stirring rate was temporally increased further in order to break-up foam formation. For full details on changes in stirring rate see experiment log in appendix.

Experimental setup for CSTR

The intended final organic loading rate (OLR) for the continuous reactor tests was 3.5 g VS l^{-1} d^{-1}. The process only reached 75% of this load due to recurrent process disturbances (See full experiment log in appendix for details). See table 9 for wet weight and hydraulic residence times. The processes were planned to start at a lower feeding rate and then slowly ramp-up to the intended rate. On the other hand, the hydraulic residence times (HRT) for the reactors were to start at long residence times and then decrease to 30-40 days when reaching the goal OLR. At the start of the experiment the plan was to feed the reactors every 4th day, then every 3rd day, then every other day and finally each day. Each of these times the reactors were fed the daily amount representing the intended final OLR (see table below) giving an average OLR over time representing a 4th, a 3rd or half of the final OLR. The reasoning behind this feeding pattern was to reduce workload (compared to feeding a lower amount every day) and see if it was possible to reduce the amount of time it took to reach a higher OLR by directly selecting for microorganisms that can stand high substrate concentrations.

Table 9. OLR and corresponding wet weights and hydraulic residence times for CSTR experiments.

Intended final OLR [g VS / l day]	**Reactor volume [L]**	**Total VS added [g]**	**Substrate**	**Wet weight added [g/l]**	**HRT [days]**
3.5	3	10.5	SBT 19/10	31.5	31.7
3.5	3	10.5	SF-SBT 16/10	27.2	36.7
75 % of intended final OLR	**Reactor volume [L]**	**Total VS added [g]**	**Substrate**	**Wet weight added [g/l]**	**HRT [days]**
2.6	3	7.9	SBT 19/10	23.6	42.3
2.6	3	7.9	SF-SBT 16/10	20.4	48.9

Unfortunately, due to problems with foaming (see experiment log below for more details), this manner of feeding could not be maintained. Instead it was decided to keep the reactors at a low OLR for longer and feed more often. In table 1 the feeding schedule for the CSTR experiments can be seen (foaming events are also shown in this table, see superscripts).

Table 10. Feeding schedule for reactors.

		Reactor 1	**Reactor 2**	**Reactor 3**	**Reactor 4**	
Substrate		SBT	SF-SBT	SBT	SF-SBT	
Date	Day	Wet weight added [g/l]	Wet weight added [g/l]	Wet weight added [g/l]	Wet weight added [g/l]	Comments
2014-03-14	1	40.56	40.54	35.03	35.03	
2014-03-15	2					

2014-03-16	3	[1]		[2]		5 mL antifoam added, foaming event
2014-03-17	4					5 mL antifoam added
2014-03-18	5	31.53	27.27	31.53	27.27	5 mL antifoam added
2014-03-19	6					
2014-03-20	7					
2014-03-21	8	31.53	27.26	31.53	27.26	
2014-03-22	9					
2014-03-23	10					
2014-03-24	11	31.53	27.27	31.53	27.27	
2014-03-25	12					20 ml antifoam added
2014-03-26	13	31.53	27.27	31.53	27.27	5 mL antifoam added
2014-03-27	14					
2014-03-28	15	15.77	13.63	15.77[1]	13.63[1]	5 mL antifoam added, foaming event
2014-03-29	16					
2014-03-30	17	15.77	13.63	15.77	13.63	
2014-03-31	18	15.77	13.63	15.77	13.63	5 mL antifoam added
2014-04-01	19	[1]	[1]	[1]	[1]	Foaming event
2014-04-02	20					
2014-04-03	21					
2014-04-04	22					
2014-04-05	23					
2014-04-06	24					
2014-04-07	25					
2014-04-08	26					
2014-04-09	27					
2014-04-10	28					
2014-04-11	29					
2014-04-12	30					
2014-04-13	31	7.87	6.83	7.87	6.83	
2014-04-14	32	7.87	6.83	7.87	6.83	
2014-04-15	33	7.87	6.83	7.87	6.83	
2014-04-16	34	7.87	6.83	7.87	6.83	
2014-04-17	35	7.87	6.83	7.87	6.83	
2014-04-18	36					
2014-04-19	37	7.87	6.83	7.87	6.83	
2014-04-20	38					
2014-04-21	39					
2014-04-22	40	7.87	6.83	7.87	6.83	
2014-04-23	41	7.87	6.83	7.87	6.83	
2014-04-24	42	7.87	6.83	7.87	6.83	
2014-04-25	43	15.77	13.63	15.77	13.63	5 mL antifoam added
2014-04-26	44	15.77	13.63	15.77	13.63	

2014-04-27	45	15.77	13.63	15.77	13.63	
2014-04-28	46					
2014-04-29	47	15.77	13.63	15.77	13.63	
2014-04-30	48	15.77	13.63	15.77	13.63	
2014-05-01	49	15.77	13.63	15.77	13.63	
2014-05-02	50	15.77	13.63	15.77	13.63	
2014-05-03	51	15.77	13.63	15.77	13.63	
2014-05-04	52	15.77	13.63	15.77	13.63	
2014-05-05	53	15.77	13.63	15.77[1]	13.63	Foaming event
2014-05-06	54	[1]		[1]		Foaming event
2014-05-07	55	15.77	13.63	15.77	13.63	
2014-05-08	56					
2014-05-09	57	15.77	13.63	15.77	13.63	
2014-05-10	58					
2014-05-11	59					
2014-05-12	60	15.77	13.63	15.77	13.63	
2014-05-13	61	15.77	13.63	15.77	13.63	
2014-05-14	62	15.77	13.63	15.77	13.63	
2014-05-15	63	15.77	13.63	15.77	13.63	
2014-05-16	64	15.77	13.63	15.77	13.63	
2014-05-17	65	23.65	20.47	23.65	20.47	
2014-05-18	66	23.65	20.47	23.65	20.47[1]	1 ml antifoam added, Foaming event
2014-05-19	67	23.65	20.47[2]	23.65	20.47	1 ml antifoam added, Foaming event
2014-05-20	68	23.65	20.47	23.65	20.47	1 ml antifoam added
2014-05-21	69	23.65	20.47	23.65	20.47	
2014-05-22	70	23.65	20.47[2]	23.65	20.47	1 ml antifoam added, Foaming event
2014-05-23	71	23.65	20.47	23.65	20.47[2]	1 ml antifoam added
2014-05-24	72	23.65	20.47[2]	23.65	20.47	Foaming event
2014-05-25	73	23.65	20.47	23.65	20.47	
2014-05-26	74	23.65	20.47	23.65	20.47[1]	Foaming event
2014-05-27	75	23.65	20.47	23.65	20.47	
2014-05-28	76	23.65	20.47[2]	23.65	20.47[2]	Foaming event
2014-05-29	77	23.65	20.47	23.65	20.47	
2014-05-30	78	23.65	20.47	23.65	20.47	
2014-05-31	79	23.65	20.47	23.65	20.47	
2014-06-01	80	23.65	20.47	23.65	20.47	

[1)] Foaming event caused some loss of reactor liquid. [2)] Foaming caused sludge to clog gas tube.

Experiment log

This is an excerpt of notable changes in methodology that might have affected experiment results. See the appendix for a more comprehensive experiment log.

April 1st. Extensive foaming occurred in reactor 4. Approximately one liter of reactor liquid was lost. It was decided to discontinue reactor feeding for a period of time, due to the on-going problems with foaming.

April 5th. Added one liter of AD sludge (from Källby 27/2; same inoculum that was used when starting up reactors) to reactor 4 to compensate for liquid lost due to foaming.

April 9th. Discovered malfunction with water bath coupled to reactors, likely due to low water level in bath. Heater and pump failed and the temperature of the water in mantles sunk to ambient levels during a period of approximately 16 hours. The water bath was refilled and reset when this was noticed.

June 2nd. Foaming in reactors 2 and 4 led to loss of reactor liquid.

June 12th. Added water to reactors 2 and 4 to replace liquid lost (Since no inoculum was available). The amount of water added to reactor 2 was 150 mL, and the amount of water added to reactor 4 was 400 mL.

Analyses

TS/VS measurements

Measurements of the total solids (TS) and volatile solids (VS) were done in accordance with the laboratory analysis protocol as laid out by the US environmental protection agency (EPA) [16].

Gas Chromatography measurements

The gas chromatograph used for this project was an Agilent 6890N (Agilent Technologies inc., Santa Clara, United States). The gases analyzed were CO_2 and CH_4. Gas samples were always taken before discharging and feeding the reactors, unless otherwise stated. For the first period of the experiment a 500 µl SGE glass syringe was used for sampling. Later on, a plastic syringe was used instead, see experiment log. See appendix for calibration curves for the different syringes.

Since the gas composition was only taken maximum once per day (and always before feeding) this measurement was assumed to be representative for the entire day, even though it is likely there are variations in gas composition following feeding.

The methane production from the control was subtracted from the methane production of the other CSTRs to remove the contribution from inoculum.

Gas flow measurements

The gas flow was monitored with the BRS unit as mentioned above. The measurements were done through volumetric displacement and the unit registered each incremental 10 mL of gas passing through the unit. The BRS unit was connected to the internet so that the gas flow could be monitored remotely online.

Volatile Fatty Acids (VFA)

Samples for analyses were taken when discharging (i.e. before feeding) reactors. These samples were then stored in freezer. Before analyses were performed the reactor samples stored in freezer were thawed in fridge for 12 hours. The samples were then centrifuged in a Beckman Coulter Spinchron centrifuge (Beckman Coulter inc., Brea, US) for 10 minutes at 3200 rpm. The supernatant from the centrifuged sample was reduced to pH 1-3 using 20% H_2SO_4. The pH was measured using pH indicator paper strips. Afterwards the supernatant was filtered using polyether sulfone 0.2 µm syringe filters. This supernatant was then analyzed for VFAs using HPLC with an Aminex HPX 87H column (Bio-Rad Inc., Hercules, USA) according to the method suggested by Bio-Rad.

Alkalinity and pH measurements

Samples for analyses were taken when discharging reactors. These samples were then stored in freezer. Before analyses were performed the reactor samples stored in freezer were thawed in fridge for 12 hours. Alkalinity and pH was measured with a tim800 Titralab instrument and Abu901 autoburette.

VFA to partial alkalinity ratio

The VFA to alkalinity ratio has been cited as a reliable way of detecting process imbalances that can lead to foaming [15]. A rule of thumb is that this ratio should be below 0.5 for a stable process [15].

Results

BMP

Results

In the figure 1 to 3, the cumulative methane production over the BMP period is displayed. Results from the first BMP test are shown in figures 1 and 2 and results from the second BMP test are shown in figure 3. Accumulated methane yields for 30 days are shown in table 11 and 12. The methane production rates for solid and liquid fractions (SF-SBT and LF-SBT) and non-fractionated (SBT) sugar beet tops are very similar. The one notable exception is the liquid fraction with the higher organic load (figure 2). The methane production in these reactors lags behind the others, which could be a sign of inhibition. In the second BMP test LF2-SBT at ISR 1:1 and 2:1, as well as SF2-SBT at ISR 1:1 were also run. These reactors exhibited severe foaming (see experiment log above) and thus had to be terminated. Therefore there are no results for these tests.

Table 11. Methane yields for first AMPTS experiment.

Substrate	Methane yield [mL gVS^{-1}]	Standard deviation [mL gVS^{-1}]
SBT shredded 2:1	328	12
SBT shredded 1:1	339	8
SF-SBT shredded 2:1	302	14
SF-SBT shredded 1:1	306	9
LF-SBT shredded 2:1	303	16
LF-SBT shredded 1:1	330	20
SBT chopped 2:1	320	20
Cellulose	354	12

Table 12. Methane yields for second AMPTS experiment.

Substrate	Methane yield [mL gVS^{-1}]	Standard deviation [mL gVS^{-1}]
SF2-SBT 2:1	322	12
SBT 2:1	323	9
Cellulose 2:1	369	19

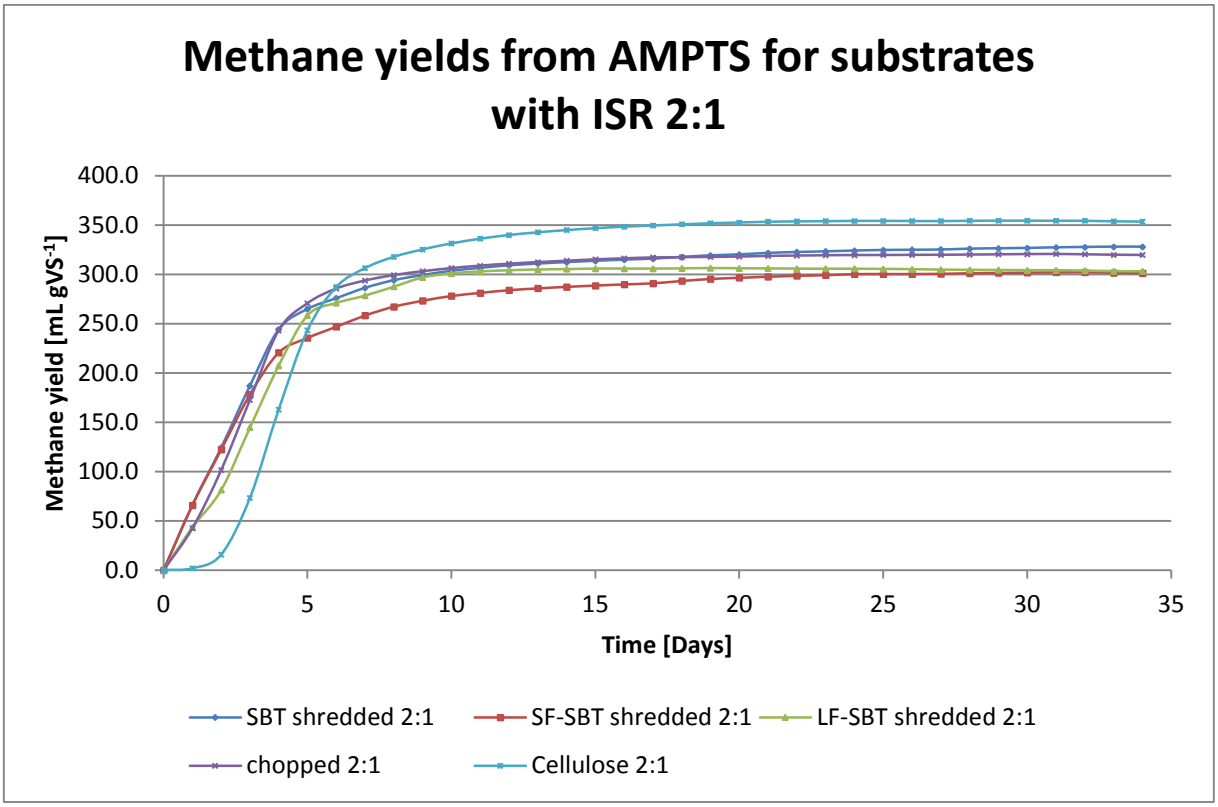

Figure 1. Methane yield averages of three replicates for first BMP test with ISR 2:1.

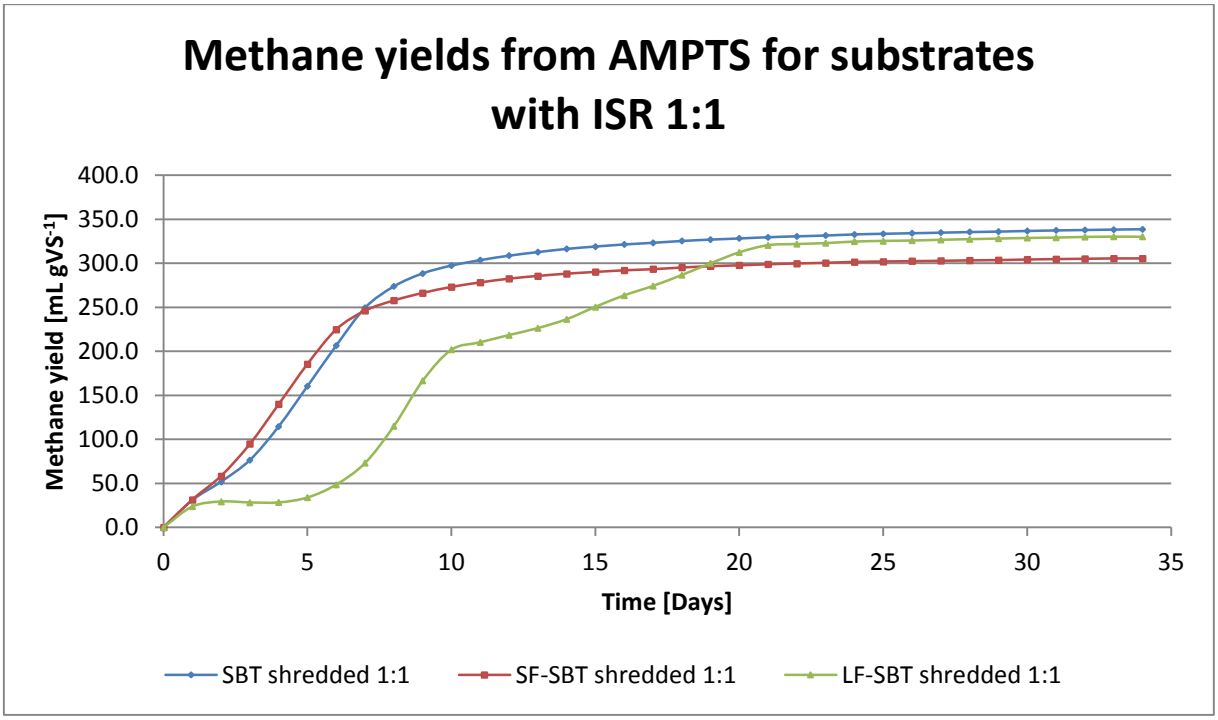

Figure 2. Methane yield averages of three replicates for first BMP test with ISR 1:1.

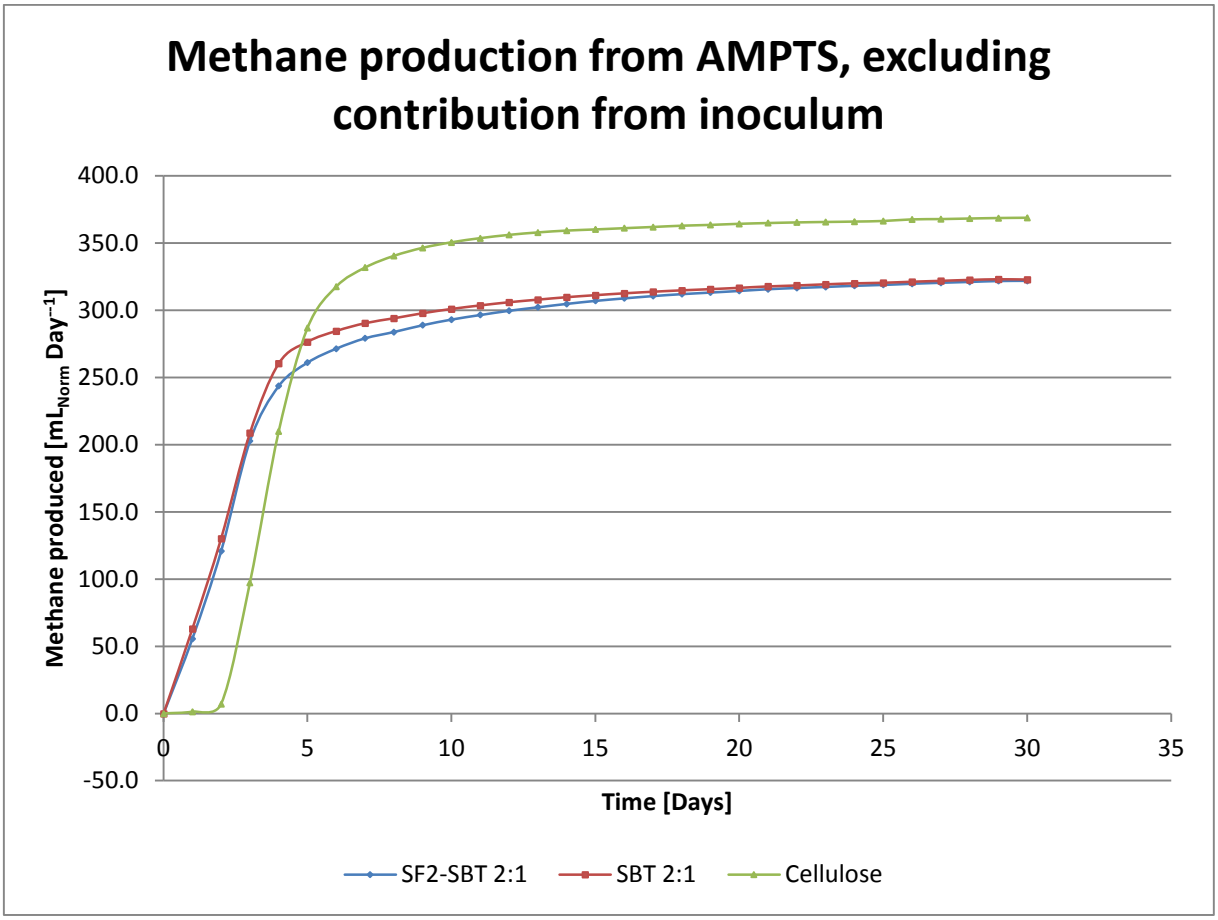

Figure 3. Methane yield averages of three replicates for second AMPTS experiment.

CSTR

Gas production and quality

In figure 4 the flows from all the reactors, excluding control are displayed. Some of the dips in gas production are the results of process disruptions such as foaming/clogged gas tubes, see experimental log for more detail. See figure 2 for CH_4 content of the produced gas.

Organic loading rates (OLR) and hydraulic retention times (HRT) as reported by BRS are displayed in figures 7 and 8, respectively. Finally, in figure 9 and 10 the methane yields for the last 50 days of the experiment are displayed. In these (figure 9 and 10) the average actual OLR for the period is also displayed: starting at an OLR of 0.71 g VS l^{-1}, which is then increased to 1.35 g VS l^{-1} and finally 2.79 g VS l^{-1}. The average methane yield for each of these periods is also displayed in the graph. The average methane yields for SBT are 196, 196 and 245 mL gVS^{-1}, and for SF-SBT they are 201, 216 and 180 mL gVS^{-1}. If these yields are presented as percentages of the BMP yields the SBT reactors range between 60-75% while the SF-SBT reactors range between 60-72%. The total methane yields for each of the reactors (from start of experiment up to and including gas produced up to 15 days after feeding stopped) are shown in table 13 below. However, for reactor 4 the BRS did not register gas flows for the last 15 days (period after final feeding) so the gas produced during that period is not included in the yield for that reactor.

Table 13. Methane yields for the entire CSTR experiment.

	Reactor 1 SBT	Reactor 3 SBT	Reactor 2 SF-SBT	Reactor 4 SF-SBT
Methane yield [mL gVS^{-1}]	245	238	205	224

The points where there are dips in the OLR in figure 7 are due to omitted feeding (either due to foaming having caused a process disruption, see full experiment log, or the time constraints of the project).

During the last three weeks of the CSTR experiment the SF-SBT reactors were markedly more unstable than the SBT reactors: the SF-SBT had multiple critical foaming events where gas tubes were clogged, or reactor liquid was lost. The SBT reactors, on the other hand, ran without problems for the last three weeks of the experiment (minor foam formation but not enough to disrupt the experiment).

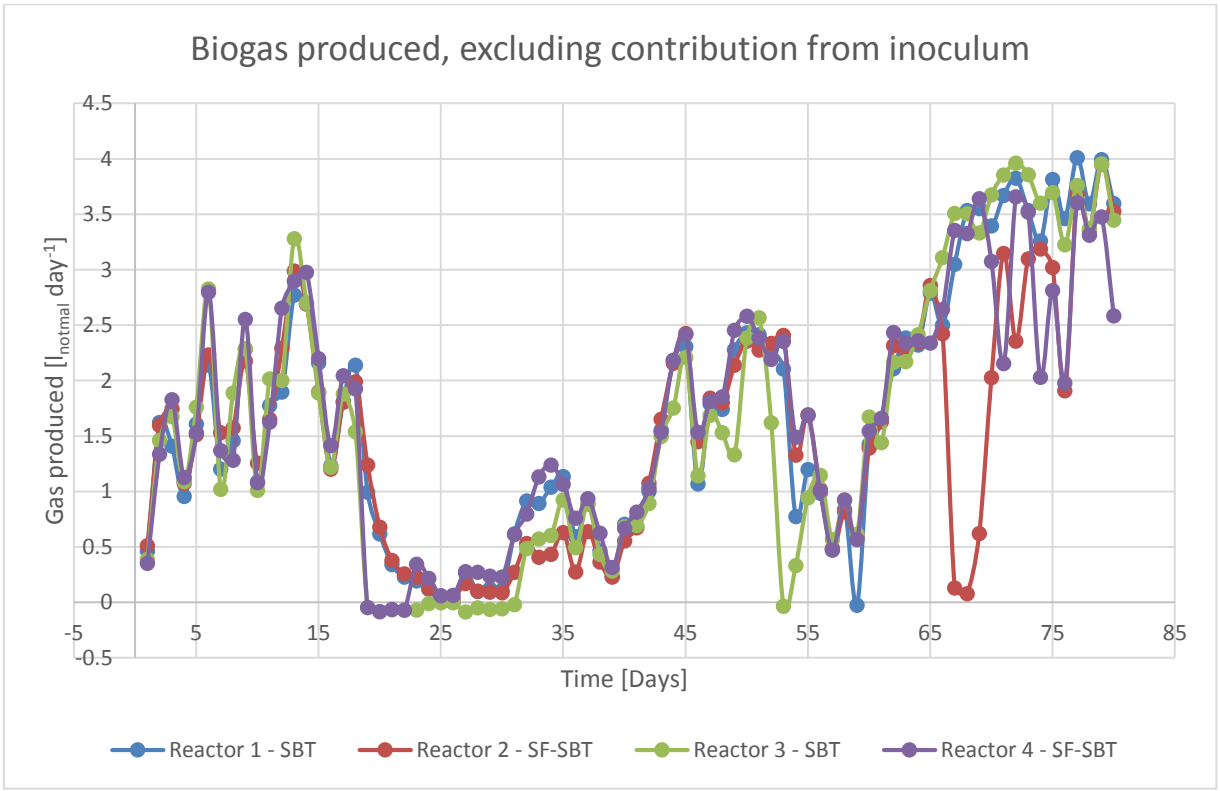

Figure 4. Gas production from CSTR, retrieved using BRS web application on 20140602.

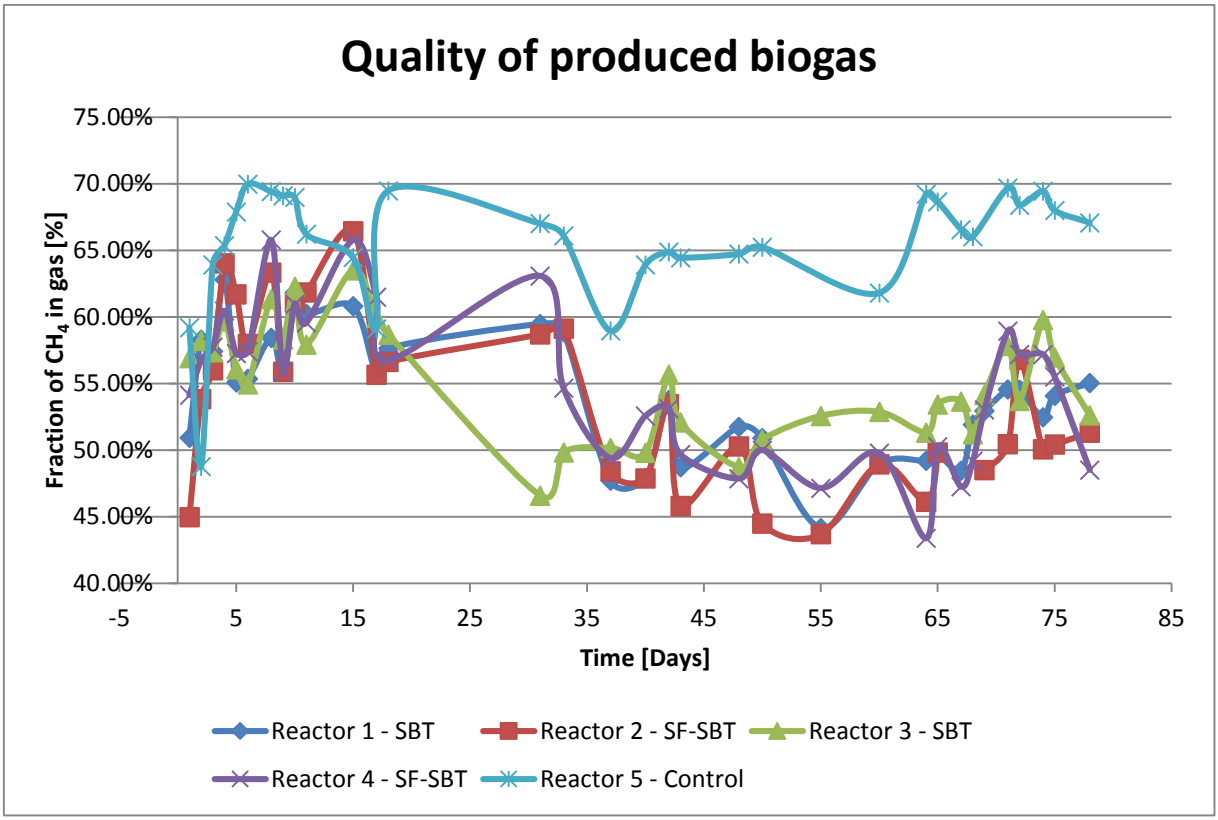

Figure 5. CH_4 content of gas from CSTR, measured in GC.

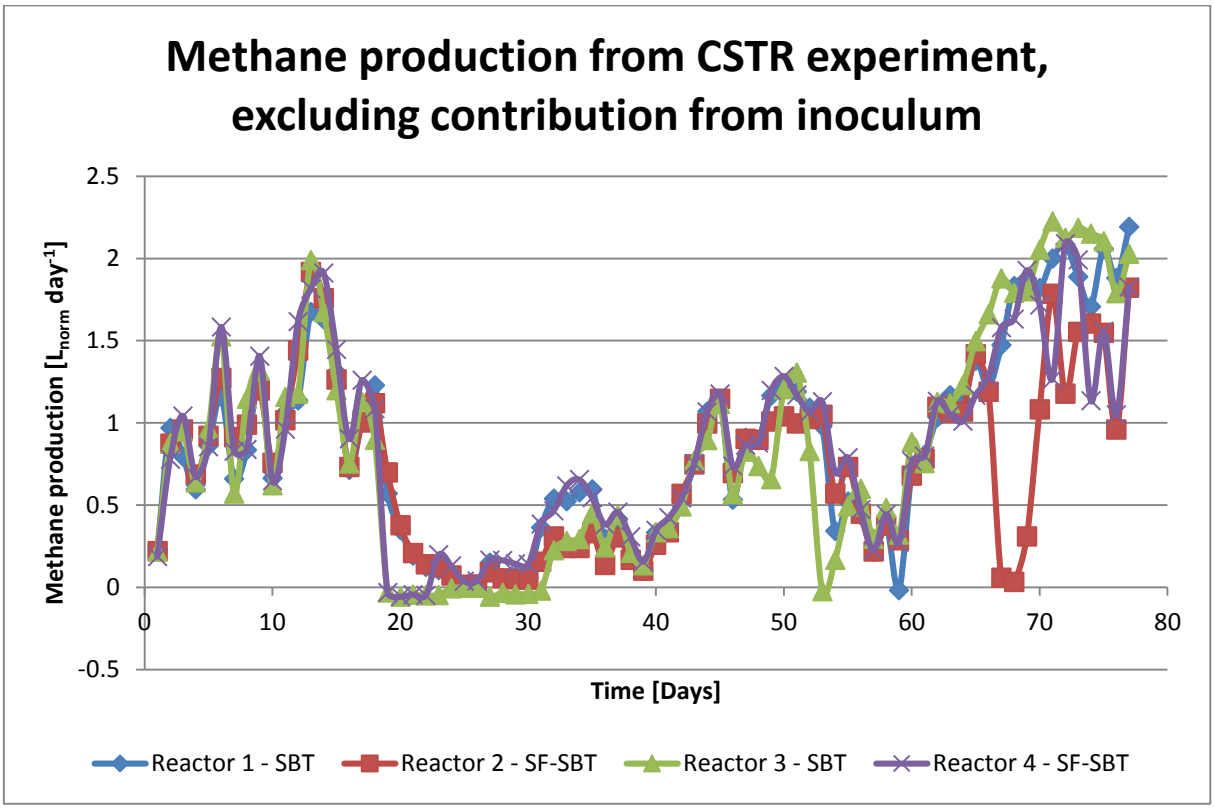

Figure 6. Methane production from CSTR, calculated using the volumetric flow and GC measurements above.

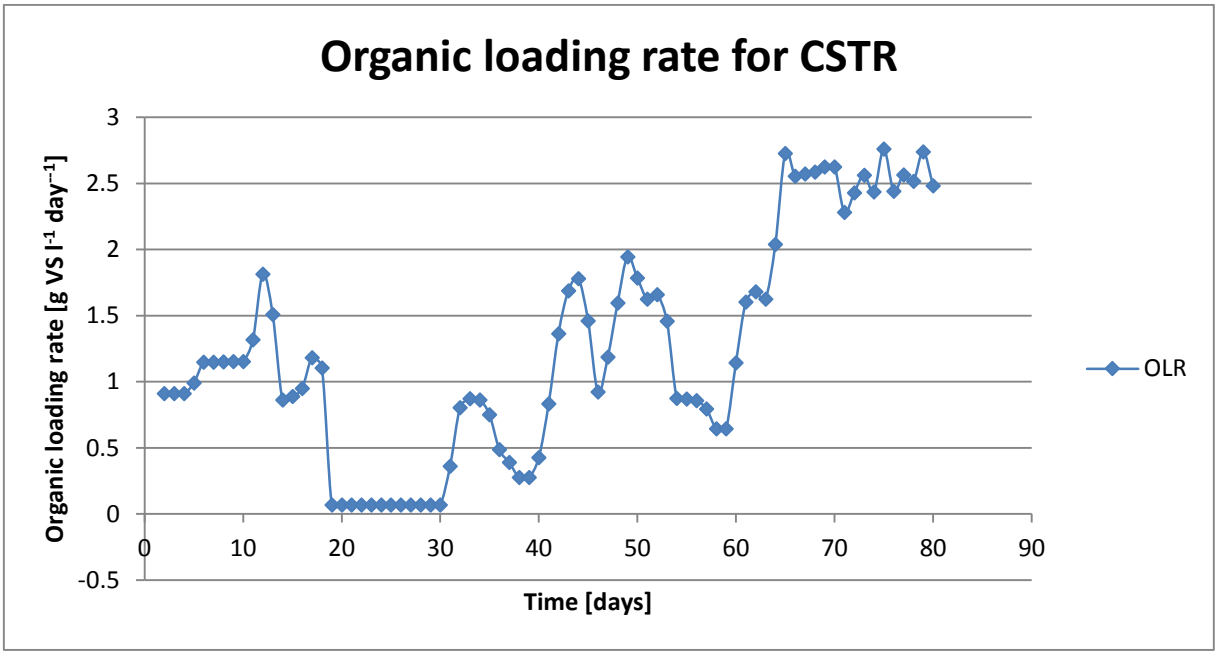

Figure 7. OLR for CSTR as reported by the BRS web application.

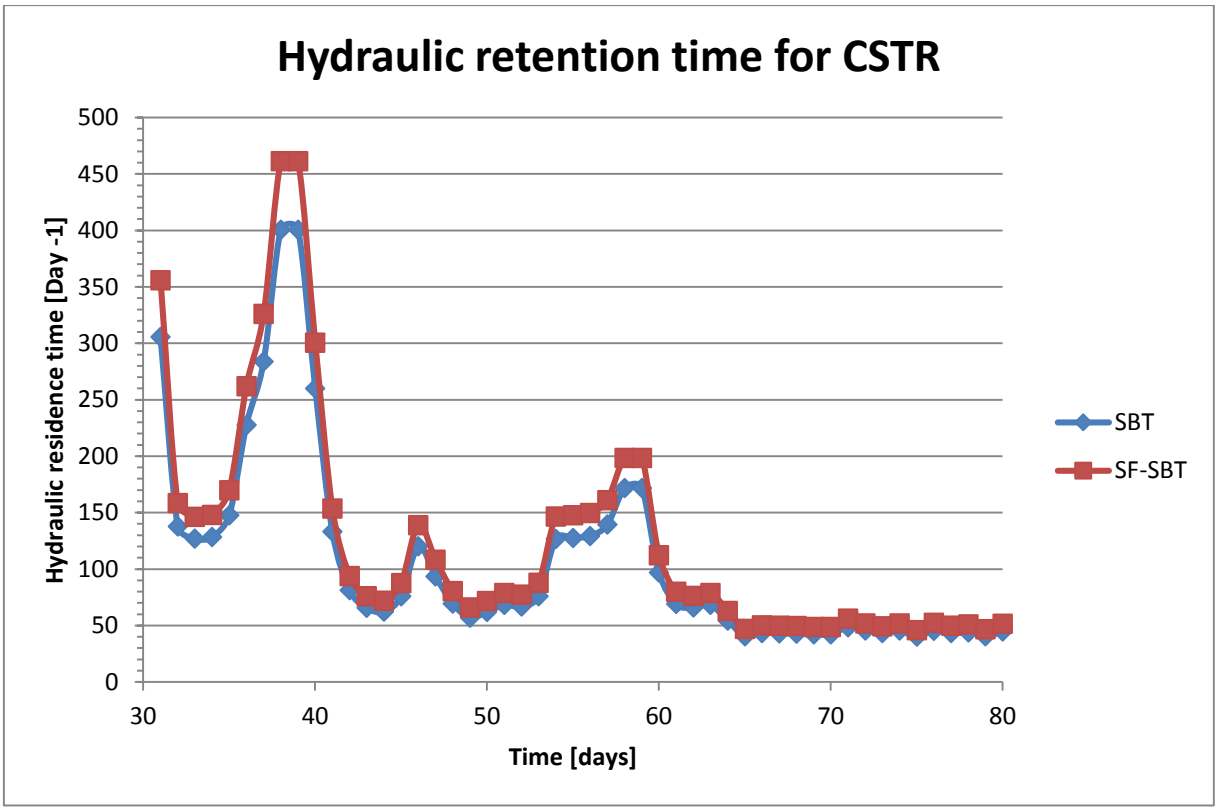

Figure 8. Hydraulic retention times for CSTR. Data was truncated at 31 days to keep reasonable scale.

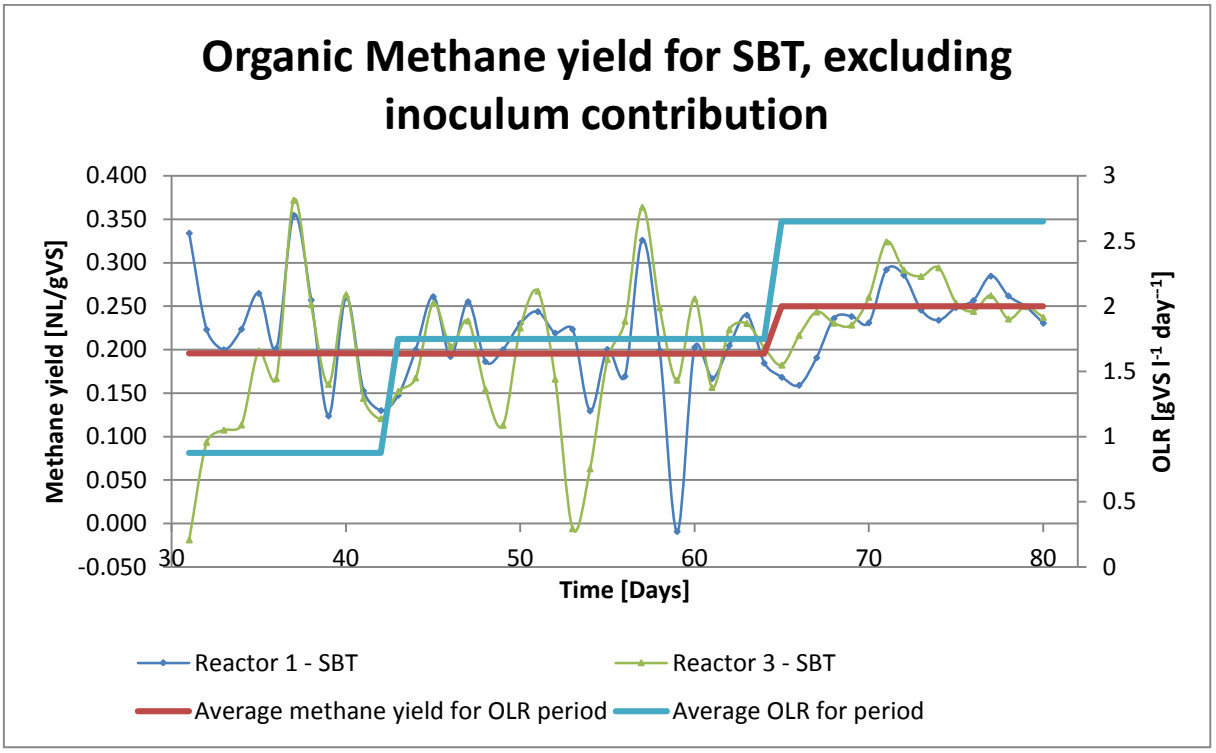

Figure 9. Specific methane yield for SBT, excluding inoculum contribution.

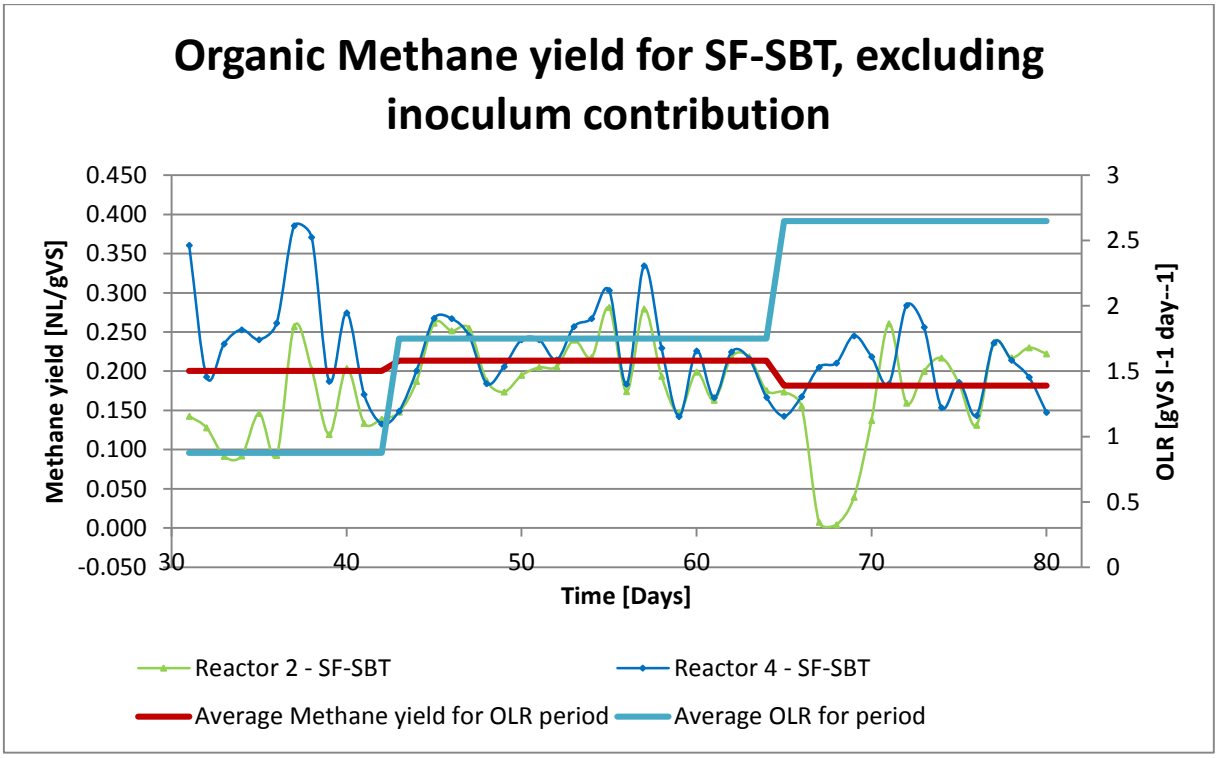

Figure 10. Specific methane yield for SF-SBT, excluding inoculum contribution.

Analyses

pH

pH values are shown below in figure 11.

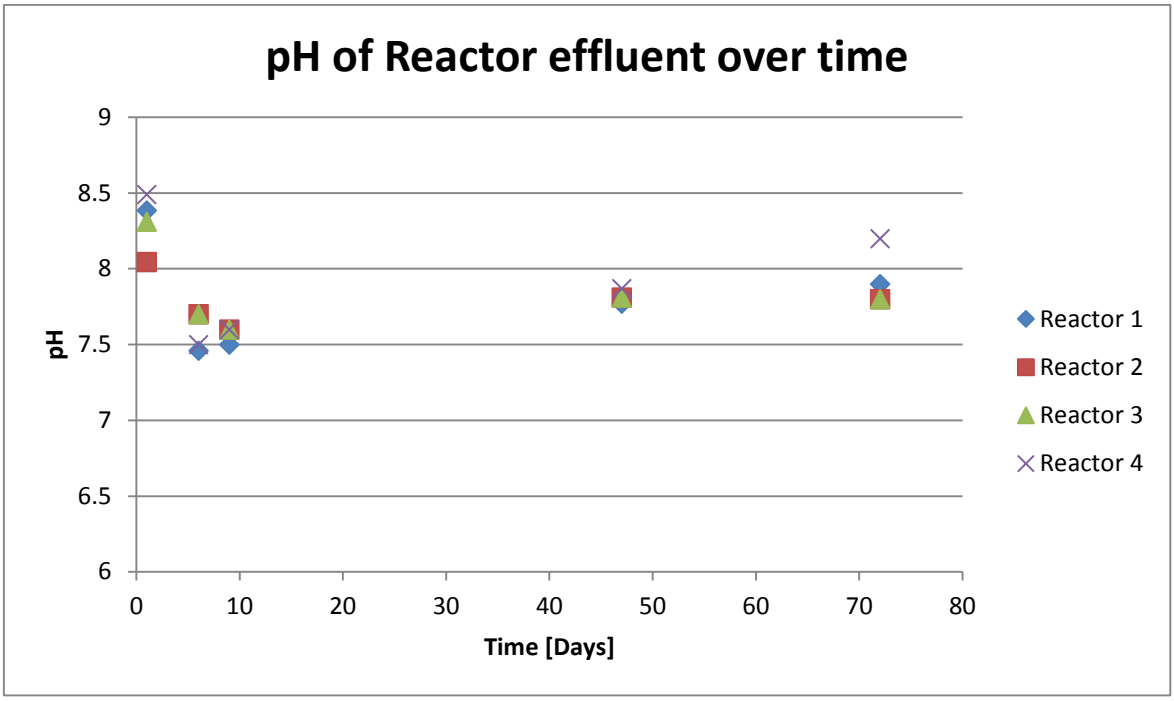

Figure 11. pH measurements for CSTR reactors.

VFA

The total VFA concentrations are shown in figures below. The detected VFA was predominantly propionic acid, with occasional traces of acetic acid. Detected VFA levels for reactors with non-fractionated substrate were, in aggregate, slightly higher than those for the fractionated substrate although there were large differences between individual reactors with the same substrate.

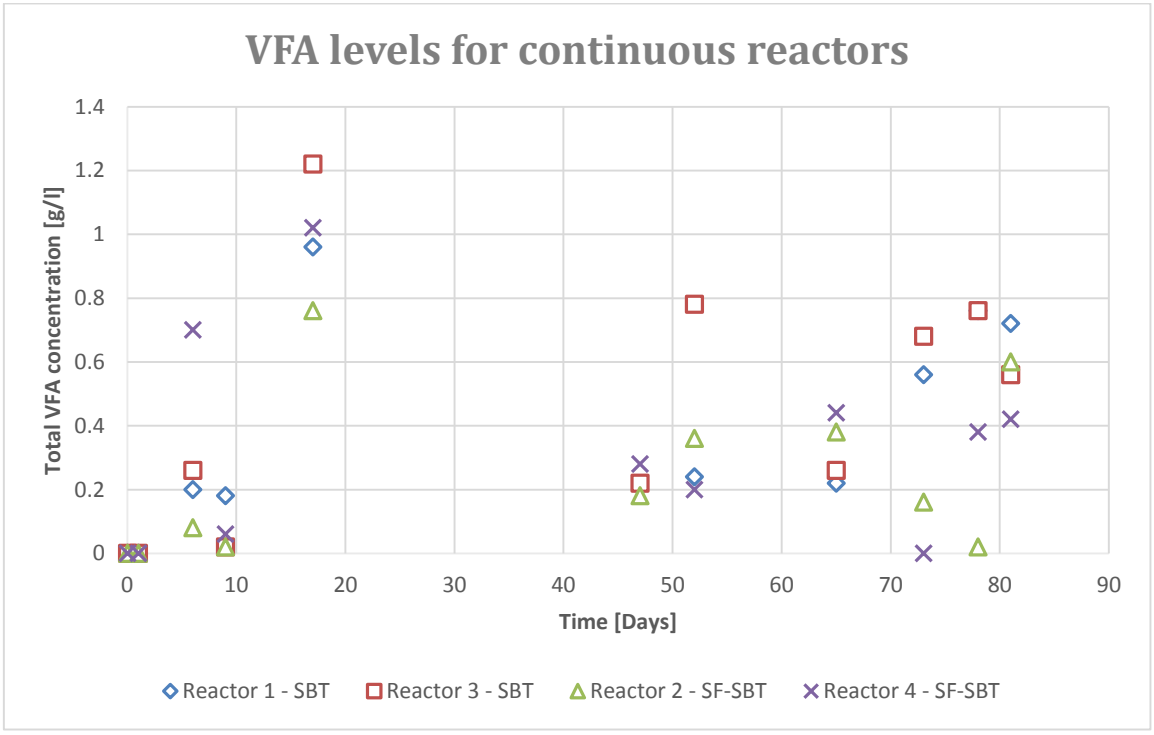

Figure 12. Total VFA concentrations for continuous reactors.

Alkalinity

Partial alkalinity measurements are shown in figure below. Partial alkalinity for the raw inoculum was 5.20 g/l.

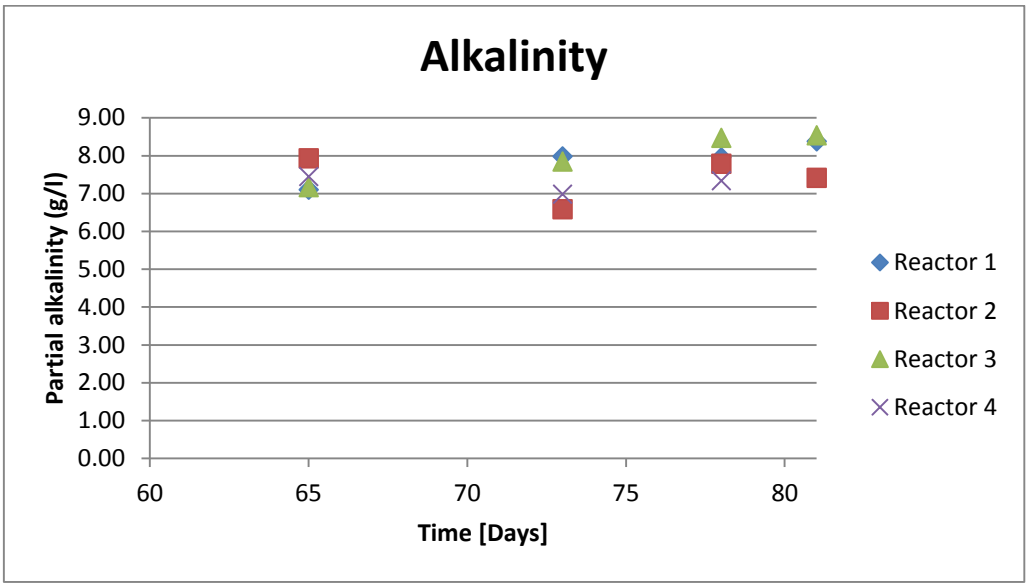

Figure 13. Partial alkalinity measurements for CSTR experiments.

VFA to partial alkalinity ratio

The VFA to partial alkalinity was consistently below 0.5 for the measured points. See table below.

Table 14. VFA to partial alkalinity ratio.

VFA/PA				
	Reactor 1	Reactor 2	Reactor 3	Reactor 4
20140517	0,03	0,05	0,04	0,06
20140525	0,07	0,02	0,09	0,00
20140530	0,10	0,00	0,09	0,05
20140602	0,08	0,08	0,07	0,06

Discussion

Methodological errors

AMPTS

Wang et al investigated the performance of BMP tests performed with AMPTS and found it to be comparable to other conventional BMP tests, but more precise (less variance between replicates of the same substrate) [14].

CSTR

Conducting a continuously fed reactor experiment as the one that has been performed in this project is both time consuming and labor intensive. The time constraints of this project meant that compromises had to be made regarding activities such as taking samples from reactors, measuring gas composition and so on.

In the case of gas composition measurements, the measurements were always done before discharging and feeding the reactor. However, it is quite possible that the composition of the gas changes during the time after feeding. Since the acidogenesis is the fastest stage of anaerobic digestion a heightened concentration of VFAs during the period following a feeding may cause inhibition of the methanogens, which would lead to lower methane production and a higher concentration of CO_2 in the gas. If the process is to be continued in the future, the methods used for determining the composition of the gas should be refined. One way of doing this could be utilizing NaOH to strip the CO_2 from the gas in the same manner used in AMPTS. Since the gas production in the continuous reactors is much higher than in the batch reactors, having a system where multiple NaOH-flasks are coupled in series to each continuous reactor would likely be necessary. Gas flow meters could be placed before the NaOH-flasks in order to get both the total gas flows and only the CH_4 flows (from the BRS unit). Besides the benefit of getting more reliable results, this would also allow for continuous monitoring of the CH_4 flows, instead of just the total gas flows. As can be seen in figure 5, there is quite a large variation in the CH_4 fraction of the gas of the control. This is not reasonable and seems to indicate an error in methodology.

Another problem relating to the CSTR experiment is the manner in which the reactors were fed. As was mentioned in the theoretical background (see above), the feeding was not actually done continuously. As has been mentioned before, methanogenic micro-organisms are slow-growing and susceptible to changes in process conditions. Since the feeding for each day was done in one single event (as opposed to continuous feeding) this could be considered a change in process conditions, and it might have caused inhibition of the methanogens. Therefore, this could have caused a suppression of the methane yield. For the last ten days of the experiment the feeding was instead done twice a day which appeared to mitigate foaming. It is worth mentioning that no good solutions for automating continuous feeding of this type of substrate could be found for the lab-scale and other similar studies have also been performed in a similar manner [12].

In addition, anaerobic digesters are often prone to instability for a period after start-up before stabilizing [7]. In order to get a fuller picture of the effects of fractioning the reactors should be run for a longer period than has been done in this project.

It is possible that the process disturbances in the CSTR reactors are a result of ammonia inhibition since the substrate is rich in nitrogen. Reactor pH was high for the duration of the experiment. High pH leads to higher amounts of free ammonia, which is toxic to methanogens [7]. Methane yields of the continuous reactors were low in comparison to the BMP-results.

The VFA levels in reactors were consistently low in relation to the partial alkalinity, which seems to indicate that high VFA concentrations were not the cause of the process disturbances during the experiment. However, it seems likely that the VFA concentration would peak at some time after feeding. Considering that the samples from the reactors were always taken directly before feeding and that the interval between feeding reactors was long (24 hours), the measured VFA concentrations may not be representative of the concentrations in the reactor prior to actual foaming events. One way of getting better estimates of how the VFA concentrations change between feeding times would be to take samples at consistent intervals (e.g. once every hour). This idea was also considered during the course of the experiment but discarded due to time limitations. In the VFA analysis propionic acid was found but only trace amounts of acetic acid. Unlike acetic acid, propionic acid cannot be utilized directly by methanogens but must first be degraded to acetic acid through a syntrophic metabolic pathway [7]. The presence of propionic acid is also an indication that VFA concentrations may have been higher prior to measurement.

Conclusions

From the CSTR results one cannot say that fractionating the SBT caused a more stable process or gave a higher methane yield. The results indicate the opposite. Considering the methodological errors that arose from the gas chromatography measurements the methods used should be refined by e.g. incorporating a CO_2 sink as suggested above.

The cause of the repeated foaming events in the CSTR experiments should be investigated more closely. An analysis of the ammonia concentrations in the reactor samples would be useful.

Regarding the BMP-tests, the results from the BMP tests show that SBT, SF-SBT and LF-SBT have very similar methane yields, and the time required for full degradation of the substrate does not differ much either. A notable exception is the BMP-test for LF-SBT at the higher organic load (figure 2). This set of reactors shows probable signs of inhibition, which supports the hypothesis that the juice is more inhibiting than the solid fraction at high organic load is true. In future experiments it would interesting to run continuous reactors at a high OLR using the liquid fraction as a substrate to see if this inhibition is present. The cause of the inhibition may be due to high concentrations of monomeric and dimeric sugars in the liquid fraction leading to fast production of VFA, and consequent inhibition from low pH. In order to investigate this more closely, the composition of carbohydrates in the liquid fraction should be analyzed, and pH/VFA-levels should be monitored in future BMP-tests, if possible.

Acknowledgements

Region Skåne for financing the first batch reactor experiments, and providing analyses of the substrate as part of the project “Biogas från Skånsk betblast – potential, teknik och ekonomi”. Thanks to Emma Kreuger for her work as the supervisor of this thesis project. Filip Vrgoc, Ola Wallberg and Christian Roslander Lund University Chemical Engineering contributed by loaning their press for the second fractionation. Thanks to Thomas Prade for harvesting the sugar beets. Stefan Sydoff of the Department of Biology is also thanked for contributing by drilling the feeding holes in the reactor rubber plugs. Finally, the people of the Lund University Department of Biotechnology environmental group: Ivo Achu Nges, Anselm Moshi, Linda Önnby, Marissa Punzi, Bing Wang and Stella Temu are also thanked for their support and advice during the course of the project.

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Appendix

GC measurement curves

Curves for 500µl SGE glass syringe and plastic 1000µl syringe. Calibrated against gas composed of 60% CH_4 and 40% CO_2. The sample gas volume injected into the GC is normally 300 µl. In order to get areas corresponding to different concentrations of CH_4 different volumes of calibration gas were injected.

For example, in a 300 µl sample with a CH_4 concentration of 40% there is effectively 120 µl of CH_4. To get an equivalent amount of CH_4 from the calibration gas (with a CH_4 concentration of 60%) 200 µl should be injected, since 200*0.6 = 120 µl. In this way, a calibration curve between amounts of CH_4 equivalent to different concentrations (in a 300 µl sample) and the area units measured by the GC was created (note: CH_4 equivalent to 10% was not injected for the plastic syringe since the volume was too small).

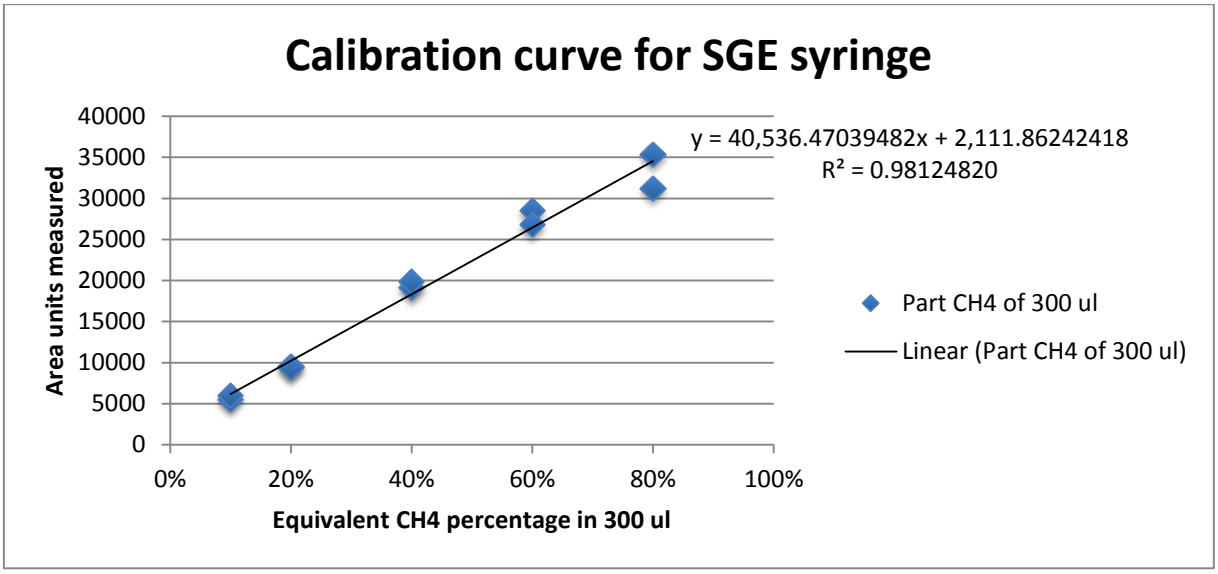

Figure 14. Calibration curve for SGE glass syringe, including equation and R^2-value.

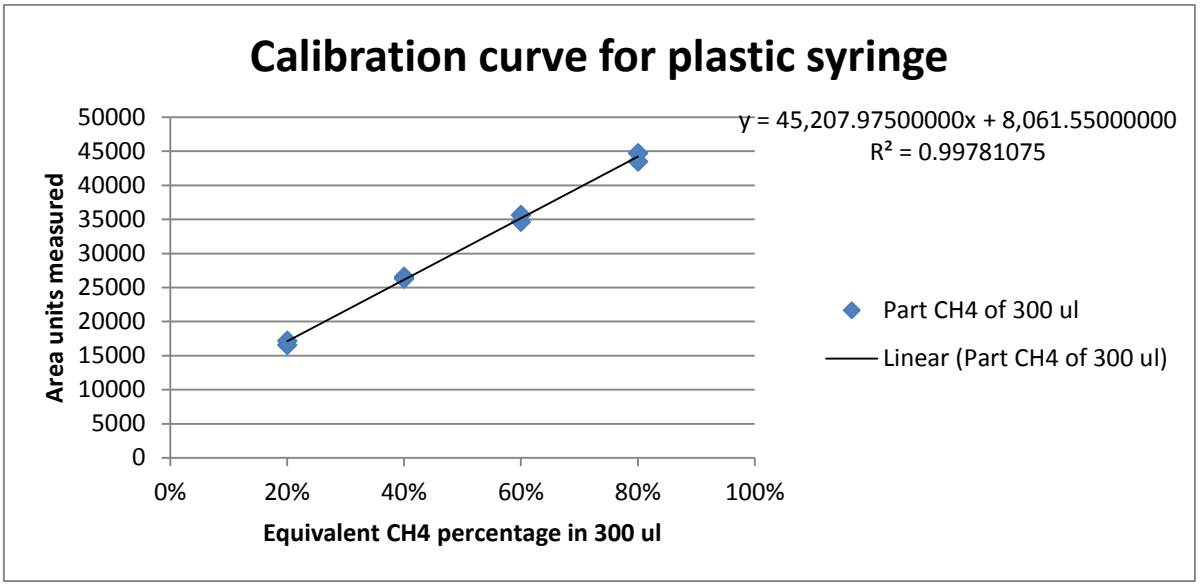

Figure 15. Calibration curve for plastic syringe, including equation and R^2-value.

Full Experiment log for CSTR

On the eighth of March, the reactors were filled with anaerobic digestate sludge which had been collected from Källby wastewater treatment plant on the 27th of February. The stirrer speed was set to 60 rpm and the circulating waterbath was set to 37 °C. On the 10th of March the rpm of the stirrers were set to 130 to mitigate build-up of foam in the reactors. After a preincubation period of six days, the reactors were fed on the 14th of March. The target final OLR for this first feeding was 4.5 g VS L^{-1} day^{-1}.

March, 16th. On this day extensive foaming occurred in reactor 1 and 3. In reactor 1 some of the reactor content had bubbled up through the feeding port. In reactor 3 the reactor sludge had gotten into the gas port and into the tube leading to the BRS. The tube was cleaned and replaced while keeping the reactor sealed. 5 mL of snapsil silicone-based antifoam was added to each reactor, except for control. Stirrer speed was increased to 200 rpm for one minute to break foam and then set to 60 rpm for all reactors. Gas composition was measured in Gas Chromatograph (GC).

March, 17th. Added 5 mL of antifoam to reactors 1,3 and 4 and 10 mL to reactor 2 to mitigate suspected foaming. Gas composition was measured in GC.

March 18th. The reactors were fed substrate for the second time. Reactors 1 and 3 were fed regular sugar beet leaves harvested on 19th of October 2013 (abbreviated to SBT) while reactors 2 and 4 were fed the solid fraction of sugar beet leaves harvested on 16th of October 2013 (abbreviated to SF-SBT). Gas composition was measured in GC. Antifoam was added to all reactors top mitigate foaming.

March 19th. Gas composition was measured in GC.

March 21st. The reactors were fed. Gas composition was measured in GC.

March 22nd. Gas composition was measured in GC.

March 23rd. Gas composition was measured in GC.

March 24th. Reactors were fed. Gas composition was measured in GC.

March 25th. Foam had developed in all reactors. In particular, reactor liquid in reactor 3 had gotten into the gas tube. Cleaned and replaced tube and added 20 mL of antifoam to each of the reactors.

March 26th. Reactors were fed. Added antifoam.

March 28th. Gas composition was measured in GC. Reactors were fed. Some foaming in reactors 3 and 4. Antifoam was added. Stirring was increased to 80 rpm to see if this could mitigate foaming.

March 30th. Gas composition was measured in GC. Reactors were fed.

March 31th. Gas composition was measured in GC. Reactors were fed. Antifoam was added to reactors.

April 1st. Extensive foaming occurred in reactor 4. Approximately one liter of reactor liquid was lost. It was decided to discontinue reactor feeding for a period of time, due to the on-going problems with foaming.

April 5th. Added one liter of AD sludge (from Källby 27/2) to reactor 4 to compensate for liquid lost due to foaming.

April 9th. Discovered malfunction with water bath coupled to reactors, likely due to low water level in bath. Heater and pump failed and the temperature of the water in mantles sunk to ambient levels during a period of approximately 16 hours. The water bath was refilled and reset when this was noticed.

April 13th. Reactor feeding was resumed with lower OLR than before. Gas composition was measured in GC.

April 15th. Reactors were fed. Gas composition was measured in GC.

April 16th. Reactors were fed.

April 17th. Reactors were fed.

April 19th. Reactors were fed. Gas composition was measured in GC. A different syringe was used as it was suspected that the other one was defective.

April 22nd. Reactors were fed. Gas composition was measured in GC.

April 23rd. Reactors were fed.

April 24th. Reactors were fed. Gas composition was measured in GC.

April 25th. Reactors were fed. OLR was increased to approximately 1.75 g VS l^{-1} day^{-1}. 5 mL of antifoam was added to each reactor.

April 26th. Reactors were fed. Gas composition was measured in GC.

April 27th. Reactors were fed.

April 29th. Reactors were fed.

April 30th. Reactors were fed. Gas composition was measured in GC.

May 1st. Reactors were fed. Gas tube from reactor 3 had disconnected sometime during the previous night. The tube was refastened with plastic clamps.

May 2nd. Reactors were fed. Gas composition was measured in GC.

May 3rd. Reactors were fed.

May 4th. Reactors were fed.

May 5th. Reactors were fed. Foaming in reactor 3 had led to some of the reactor content bubbling up through feeding port.

May 6th. Reactors 1 and 3 had foamed during the night, with some liquid lost.

May 7th. Reactors were fed. Gas composition was measured in GC. Reactor liquid had bubbled up through feed port of reactor 1 again, probably due to clogged gas port leading to a build-up of pressure in the reactor.

May 9th. Reactors were fed.

May 12th. Reactors were fed. Gas composition was measured in GC.

May 13th. Reactors were fed. Gas composition was measured in GC. Addition of micronutrients to the reactors was started.

May 14th. Reactors were fed.

May 15th. Reactors were fed.

May 16th. Reactors were fed. Gas composition was measured in GC.

May 17th. Reactors were fed. Gas composition was measured in GC.

May 18th. Reactors were fed. Reactor 4 foamed with loss of some reactor liquid. 1 mL of antifoam was added to each reactor.

May 19th. Reactor 2 had foamed during the night, and sludge had gotten into BRS gas port. Used syringe to suck out most of the sludge in the port then flushed with N_2 gas. This might register as a gas spike in results which should be disregarded. Gas composition was measured in GC. Reactors were fed. 1 mL of antifoam was added to each reactor.

May 20th. Reactors were fed. Gas composition was measured in GC. Antifoam was added to each reactor.

May 21st. Reactors were fed. Gas composition was measured in GC. Glass syringe was used to measure gas composition as comparison to reference gas indicated it was more accurate.

May 22nd. Reactor 2 had foamed again. Sludge had gotten into gas port again. Gas tube was changed. Reactors were fed. Antifoam was added to each reactor.

May 23rd. Reactor 4 foamed during the night and sludge had gotten into the gas tube. Some liquid had bubbled up through feeding port, and liquid level had decreased to approximately 2700 mL. Reactors were fed. Gas composition was measured in GC. Added antifoam.

May 24th. Reactor 2 had foamed again. Sludge had gotten into gas port again. Gas tube was changed. GC measurements were taken. In an attempt to mitigate the repeated foaming events it was decided to feed reactors twice daily instead of just once. Reactors were fed.

May 25th. GC measurements were taken. Gas pen was used to check for leaks, but none were found. Reactors were fed.

May 26th. Reactor 4 had foamed again, possibly due to lower liquid levels from previous foaming events. GC measurements were taken (reactor 4 omitted, since tube had to be changed after foaming). Reactors were fed.

May 27th. GC measurements were taken. Reactors were fed.

May 28th. Reactors 2 and 4 had foamed again. Sludge had gotten into gas tubes again. Reactors were fed.

May 29th. Reactors were fed.

May 30th. GC measurements were taken, with plastic syringe. Reactors were fed.

Nutrient analyses results

ANALYS-RAPPORT nr 11785

Datum 2014-3-21

Uppdragsgivare
LUNDS UNIVERSITET
avd för Bioteknologi
Box 188
221 00 LUND

Provtagningsdat.
Provtagare Emma Kreuger
Ankomstdat. 140307
Resultaten angivna i mg/kg

ÄMNE		Fe	Al	B
Provnamn	id(nr)			
SF-SBT 18/10	439	370	270	41
SBT 19/10	440	440	330	41
SF-eSBT bucket 4	441	320	450	45

ÄMNE		V	Mo	Ti
Provnamn	id(nr)			
SF-SBT 18/10	439	* 0.5	0.8	8.1
SBT 19/10	440	* 0.7	0.7	9.2
SF-eSBT bucket 4	441	* 0.5	0.7	6.8

ÄMNE		Hg	Cu	Cr
Provnamn	id(nr)			
SF-SBT 18/10	439	0.07	43	1.1
SBT 19/10	440	0.06	14	1.1
SF-eSBT bucket 4	441	0.07	11	1.0

ÄMNE		As	Bi	W
Provnamn	id(nr)			
SF-SBT 18/10	439	0.13	< 3	0.01

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Godkänd för F-skatt

forts.

ÄMNE		As	Bi	W
Provnamn	id(nr)			
SBT 19/10	440	0.20	< 2	0.01
SF-eSBT bucket 4	441	0.11	< 2	0.01

ÄMNE		Se	Co	Pb
Provnamn	id(nr)			
SF-SBT 18/10	439	< 0.20	2.3	1.0
SBT 19/10	440	< 0.20	0.2	1.9
SF-eSBT bucket 4	441	0.24	0.6	1.3

ÄMNE		P	S	Zn
Provnamn	id(nr)			
SF-SBT 18/10	439	2300	3000	22
SBT 19/10	440	2600	3000	20
SF-eSBT bucket 4	441	2300	3000	22

ÄMNE		Cd	Mn	Ni
Provnamn	id(nr)			
SF-SBT 18/10	439	0.27	54	3.4
SBT 19/10	440	0.21	51	0.9
SF-eSBT bucket 4	441	0.33	51	0.9

ÄMNE		Sr	Na	Mg
Provnamn	id(nr)			
SF-SBT 18/10	439	15	18000	3200
SBT 19/10	440	12	19000	2800
SF-eSBT bucket 4	441	16	18000	3000

forts.

ÄMNE		Ca	K	NH4-N
Provnamn	id(nr)			
SF-SBT 18/10	439	13000	28000	
SBT 19/10	440	11000	27000	
SF-eSBT bucket 4	441	14000	26000	2640

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