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Biogas production from "multi-fuel" substrate

Experimental results and process evaluation

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Problem description

The aim of the work for this MSc-project is to:

1. Charactersize the relevant substrate mixtures

2. Evaluate the relevant substrate mixtures with respect to biogas production and operational performance and stability

3. Propose operational conditions and substrate mixture range based on the experimental results, and recommend future research in order to optimize the biogas production further

Preface

The work of this master thesis was carried out at the biogas lab at UMB/Bioforsk at ÅS, Norway from January-July 2012 as a part of the B2B project. I would like to thank Bioforsk and UMB for letting me use the laboratory and for the help with the experimental design. I would especially thank Roar Linjordet for teaching me how to use the analytical equipment at the laboratory, Pål Jahre Nilsen, Stein Wold Østerhus and Tormod Briseid for the experimental design, Henrik Bjarne Møller at Aarhus University and Åna Kretsfengsel for supplying me with inoculum. Uno Andersen for helping out with everything and Kristian Fjørtoft for helping with the Steam Explosion. I would also thank Biokraft AS for making it possible for me to do this work. Vivekanand and Jane Wittrup Anger for help with the HPLC and so much more. Also thanks to B2B for the funding, and IPM for the C:N analyzes.

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Abstract

A multi fuel biogas plant is under planning at Fiborgtangen, Norway. The plant will utilize biogas from several different sources, including fish silage, animal manures, sludge from the paper factory and straw. In order to make good decisions on how to design the plant, characterization of the substrates in regards to biogas potential and nutrient value was done, and laboratory scale models of a possible plant design was established. The characterization showed that a minimum of 42% of the DM should come from manure in order to meet the micro-nutrient demand, it also showed that some nitrogen rich substrates in addition to the manure needed to be present to avoid nitrogen limitation to balance out the high carbon substrates. A biochemical methane potential study was carried out for all substrates and showed promising results, with the exception of the fiber sludge from the paper factory that had a very poor methane potential. The mixed substrate fed to the reactor models gave a methane yield of 300 mL CH_4/gVS in the biochemical methane potential study. A mix of all the substrates was fed to 4 semi continuous reactors with a HRT of 25 days and OLR of 3 gVS/L. The reactors performance was unstable, and operating with high propionic acid concentrations. The specific methane yield ranged from 170-230 mL CH_4/gVS, but because the reactors did not reach steady state during the experimental period and the propionic acid concentrations were so high, it is not possible to conclude on what yield this design would give. It is recommended that the semi continuous experiments are continued until they reach steady state or collapse because of the high propionic acid concentrations. After this it would be recommended to start experiments with higher proportions of animal manure and to leave the fiber sludge out of the reactor feed as it is has very low methane and nutrient value.

Sammendrag

Et flere-substrats biogassanlegg er under planlegging på Fiborgtangen, Norge. Anlegget vil benytte biogass fra flere forskjellige kilder, inkludert fiskeensilasje, dyregjødsel, slam fra papirfabrikk og halm. For å gjøre gode beslutninger i forhold til å designe anlegget, ble substratene karakterisert med hensyn på biogass potensial og næringsverdi, og laboratoriemodeller av et mulig design av anlegget ble etablert. Karakteriseringen viste at minimum 42 % av TS bør komme fra dyregjødsel for å møte mikronæringsstoffbehovet, den viste også at enkelte nitrogenrike substrater i tillegg til dyregjødselen burde benyttes for å unngå nitrogen begrensning som en motvekt til de karbonrike substratene. En biokjemisk-metanpotensial-studie ble gjennomført for alle substrater og viste lovende resultater, med unntak av fiberslammet fra papirfabrikken som hadde en meget dårlig metan potensial. Blandingen av substrat som ble matet til reaktormodellene ga et metanutbytte på 300 ml CH_4/gVS i den biokjemiske-metanpotensial-studien. En blanding av alle substrat ble gitt til 4 semi-kontinuerlige reaktorer med en HRT på 25 dager og OLR av 3 gVS / L. Reaktorytelsen var ustabil, og driftet med høye propionsyre konsentrasjoner. Det spesifikke metanutbyttet varierte fra 170-230 ml CH_4/gVS, men fordi reaktorene ikke nådde steady state i løpet av forsøksperioden og propionsyre konsentrasjonen var så høy, er det ikke mulig å konkludere på hva gi dette designet vil gi. Det anbefales at de semi-kontinuerlige forsøkene videreføres inntil de når steady state eller sammenbrudd på grunn av de høye propionsyre konsentrasjonene. Etter dette vil anbefalingen være å starte forsøk med høyere andeler av dyregjødsel og utelate fiberslam fra reaktor substratet, siden det har svært lavt metan- og næringsinnholdverdi.

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List of Abrevations

AD *Anaerobic Digestion*

CSTR *Continuous Stirred Tank Reactors*

DM *Dry Matter*

GC *Gas Chromatography*

HPLC *High Precicion Liquid Chromatography*

HRT *Hydraulic Retention Time*

OLR *Organic Loading Rate*

SE *Steam Explosion*

VS *Volatile Solids*

Introduction

Fish byproducts of category 2 and 3 are large waste fractions in Norway. At the same time the awareness of the effect of fossil fuels on climate change the demand for renewable fuels increases. In Trondheim city in Norway, all the public busses run on gas. This makes a good basis for establishing a biogas production facility in the Trondheim area, which produces methane from local waste sources. The wastes made available for the evaluation in this thesis was fish silage with low and high oil content, straw, fiber sludge and biological sludge from a paper factory and manure from poultry and cattle. The question was how could these wastes be used to produce methane with anaerobic digestion technology?

First in this thesis the most basic part of the theory behind AD is presented, then the substrates are described with regards to nutrient content and expected variations. Followed by this is the determination of biological methane potential of the substrates. At last semi continuous experiments are described and final conclusions on substrate mixture ranges and operational parameters are made.

General theory

A very short presentation of the theory of AD is given in this thesis. Most of the work in the thesis is experimental, and only the theory needed to explain the experimental results are therefore presented.

The steps in methane production:

The formation of methane from complex organic matter is commonly divided into four steps referred to as the hydrolysis, acidogenesis, acetogenisis and methanogenesis. The hydrolyzing step is where protein, carbohydrates and lipids are transformed into amino acids, sugars and fatty acids. The hydrolyzing step will also create some VFAs, although this is commonly thought of as the acidogenic step. The acidogenic step is where the monomers from the hydrolysis is transformed into VFAs. These VFAs are then transformed into acetate, hydrogen and carbon dioxide in the acetogenic step. The methaneogenesis is the transformation of acetate and hydrogen into methane. The acetogens are dependent on very low hydrogen concentrations to form acetate, and is therefore dependent on the methanogens to remove this hydrogen.

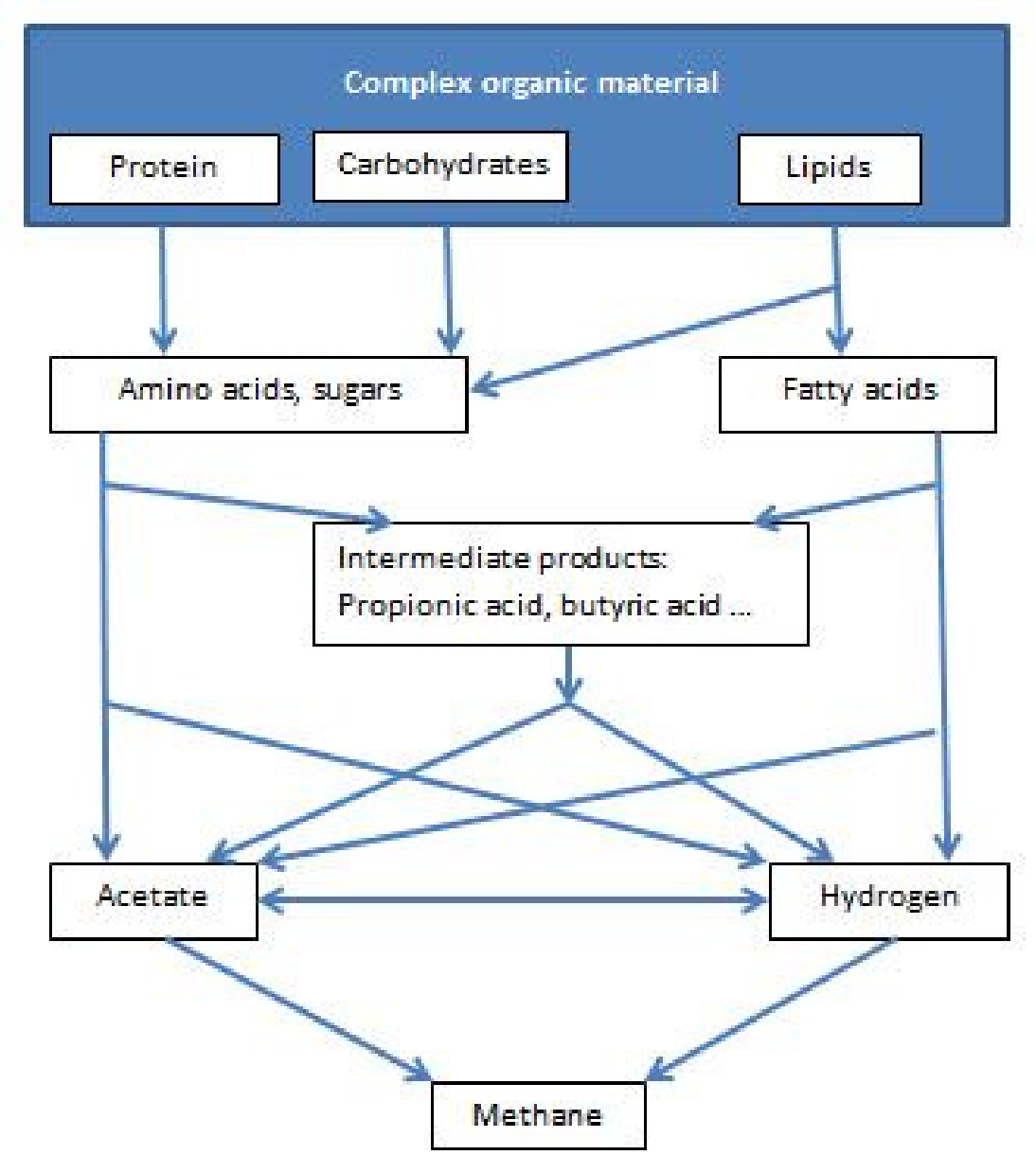

Figure 1: Methane production from complex organic material (Zehnder, 1983)

In addition of being a parameter that indicates inhibition of one or more steps in the methane production process, VFAs themselves can be inhibitory. This is due to the undissociated acids ability to penetrate as lipophilics into the cell, where they denature the cell proteins. Different values for which levels of acids that will disturb the anaerobic fermentation can be found in literature. The values where inhibition occurs ranges from 3g/L for total VFA and 300-900mg/L for propionic acid (Dieter Deublein, 2011). It is therefore important to keep track of this parameter during the upstart of the reactor, especially for substrates that are easily acidified or ensilaged.

One of the slowest growing microbial groups in the AD reactors is those that convert propionate to acetate. Slow growing organisms will lose the competition with faster growing organisms about limited nutrients. These organisms are also sensitive to high partial pressures of hydrogen. When a reactor is overloaded, one can tell by the

propionic acid and acetic acid accumulation which of these steps are inhibited. If propionic acid accumulates first, it is the propionate-assimilating micro-organsims that is inhibited, because the hydrogen-assimilating methanogens and the hydrogen producing organisms are in unbalance. If the acetic acid accumulates first, the acetoclastic methanogens are inhibited. (Björnsson et al., 1997)

Slow growing organisms will lose the competition with faster growing organisms about limited nutrients.

Nutrients

Some compounds are essential to microbial growth because they are needed in the construction of the cells. Nutrients are often divided between macro-nutrients and micro-nutrients.

Macro nutrients

The macro nutrients that need to be present for AD are:

1. Carbon dioxide
2. Oxygen
3. Hydrogen
4. Nitrogen
5. Phosphate
6. Sulfide
7. Potassium
8. Calcium
9. Magnesium
10. Iron (1-10mg/L)

A C:N ratio of the substrate of 16:1-25:1 is an indication of what could be an optimal range. For lignin strong substrates, some nitrogen will be bound in the lignin structures, and it will therefore be difficult to predict what is an optimal C:N ratio. The nitrogen is absolutely necessary for the protein formation in the cells, a to high C:N ratio will therefor lead to a lower degradation. A too low C:N ratio, may lead to high ammonia levels, that can inhibit the methanogenisis. (Dieter Deublein, 2011)

Micro nutrients

Micro nutrients or trace minerals are minerals that need to be present in a very small amount for the anaerobic process to run properly. The 15 trace nutrients in anaerobically processes are listed with nutrient demand in parentheses:

1. Manganese (0.005-50 mg/L)
2. Molybdenum (0.005-0.05 mg/L)
3. Zinc
4. Copper
5. Cobalt (0.003-0.06mg/L)
6. Nickel (0.005-0.5mg/L)
7. Vanadium
8. Boron
9. Chlorine
10. Sodium
11. Selenium (0.008 mg/L)
12. Silicon
13. Tungsten
14. Lead (0.02-200 mg/L)
15. Chromium (0.005-50 mg/L)(Dieter Deublein, 2011)

The trace metals that are commonly focused upon in anaerobic digestion are: S, Zn, Cu, Ni, Se, Mo, W and Co (Kelly and Switzenbaum, 1984, Weiland, 2010), deficiency of trace minerals will always be the case in mono-fermentation of energy crops, but can be a problem with any substrate or substrate mix. In which amounts the trace elements need to be present will depend on the substrate feed because the form that the metals will be present in will vary depending on which other compounds are present and in what concentrations. How many mg/L of a compound that is in the reactor is completely irrelevant for the micro-organisms, for them it only matters how many mg/L that is biologically available.

Degradation

Degradation is dependent on the substrates. Lignin is not degradable anaerobically, and lignin strong substrates will therefor never see a full degradation. The degradation will also vary depending on HRT and OLR. 27-76% degradation is observed, with an average degradation of 43.5%. (Dieter Deublein, 2011)

Characterization of substrate mixtures

The plant at Fiborgtangen is thought to be ran on a mix of many different substrates. To say anything about possible substrate ranges, each substrate has to be viewed independently with respect to macronutrients, micronutrients and degradability. In this chapter each substrate type will first be described in regards to expected variations dependent on source. Then ranges for micronutrient and macronutrients from the literature will be listed. For carbon and nitrogen both values from literature and analytic results of the model substrates will be presented. For degradability both values from the literature and results from BMP tests will be presented. Based on this some suggestions of a balanced mix will be made.

The substrates can be divided into five categories:

1. Manures
2. Fish wastes
3. Ligno-cellulosic waste
4. Sludge
5. Fiber mixture

The substrates belonging to each of these groups have some similar characteristics, which will be summarized after the presentation of single substrates in each category.

Manures

The relevant manures for this thesis were cattle manure and poultry manure. As model substrates dairy cattle manure from a mixed hoard of both milk cows and calves standing in a stall barn was used. The poultry manure was collected from an experiment on free range egg-hens.

Cattle manure

Cattle can be kept in various ways depending of the products they produce. In Norway there are three main types of cattle; dairy cattle, beef cattle and organic cattle. From 1. January 2024 all cattle must be free-ranged. Today all organic cattle must be free-ranged. The manure has to be removed daily, with the exception if the cattle are held on built-up litter. Various materials can be used for build-up litter and will affect the composition of the dry manure. For cattle that is not kept on build up litter, where the manure is removed daily, the composition of the manure will not be strongly affected by the material used as bedding material. (matdepartementet, 2004)

Poultry manure

Poultry can be kept either in cages or be free-ranged in big halls. All poultry has to have access to sand that will stimulate them to picking, scratching and sand-bathing. For free range poultry a bedding material of sand and wood chips is commonly used. Under the perch there is no bedding material. (matdepartementet, 2001)

Fish wastes

The relevant fish wastes for this thesis was heated fish silage before or after oil removal. The fish came from salmon fish farms. The composition of the fish silage can vary depending on the age (size) of the fish that is slaughtered. The amount of formic acid, and the temperature and time for heat treatment may also vary from batch to batch. Fish silage from one batch was provided for the experiment, where the fish silage before oil removal was not heated. The fish silage after oil removal was treated in correspondence with Norwegian regulations for treatment of category 2 waste.

Figure 2: Fish silage

Ligno-cellulosic waste

The lingo-cellulosic wastes relevant for this thesis was Straw and Fiber sludge from the paper factory at Skogn, Norway. The model Straw used was wheat straw from Ås, Norway, and the Fiber sludge came from the Norske Skog factory in Skogn, Norway.

Straw

Straw is a waste fraction of a big abundance in Norway, resulting from the production of grains for human consumption and animal feed. Straw is commonly used as bedding material for cattle, but also have a potential as a biogas substrate. Different types of grains are produced in Norway, most abundant are wheat, barley, oat and rye (www.norsklandbruk.no). The characteristics of the straw will depend on the species, growth environment and time of harvest.

Fiber Sludge

Fiber sludge is a large waste fraction at paper factories. Commonly this sludge is burned, but high water content makes it difficult to achieve the full energy potential of the sludge this way. Fiber sludge is in general high in lignin, which is not anaerobically degradable, but has a high incineration value. The fiber sludge comes from both old newspaper and new fiber from spruce that is screened out before further treatment. This sludge should therefore be chemical free. The fiber sludge from Norske Skog is mixed with biological sludge from their activated sludge plant; it is therefore composed by 89% fiber sludge and 11% biological sludge on a DM basis.

Figure 3: Mixed fiber- and bio-sludge from Norske Skog Skogn

Figure 4: Wheat Straw

Sludge

Sludge is one of the most common substrates for biogas production. In this thesis the sludge of relevance was the biological sludge at the paper factory at Norske Skog in Skogn, Norway. Biological sludge from paper factories are by nature different from sludge from municipal wastewater treatment plants. Parameters that will affect the characteristics of the sludge are HRT, nutrient content of the feed, organic loading and storage of the sludge before AD.

Mixed feed

The substrate combination that was given for the semi-continuous experiment had the following composition on a DM and VS basis:

Table 1: Composition of mixed feed

Substrate:	%DM	% VS
Fish silage with oil removed	18 %	18 %
Fish silage	6 %	6 %
Fiber sludge	34 %	32 %
Biological Sludge	4 %	4 %
Straw	24 %	26 %
Egg Hen manure	12 %	11 %
Dairy Cattle manure	3 %	3 %

The manures, sludge and ligno-cellulosic waste was mixed together and then S.E. as a pretreatment. The composition of the Fiber mixture is presented in table 2:

Table 3: Composition of fiber mixture

	Substrate[kg]	VS[%]	VS[kg]	% of VS
Fiber sludge	29.5	25 %	7.4	42 %
Biological sludge	30	3 %	0.9	5 %
Straw	7.5	89 %	6.7	38 %
Poultry manure	9	25 %	2.3	13 %
Cattle manure	3.8	9 %	0.3	2 %

Macronutrient content

C:N-ratio from literature

Table 4: C:N ratios for different substrates found in literature

Substrate	C:N
Cattle manure	14-20[1]
Poultry manure	8-24[1]
Fish silage with oil	2.5-5.5[2]
Fish silage without oil	2.5-5.5[2]
Straw	70[1]
Fiber Sludge	173[1,3]
Biological Sludge	6[1]

Micronutrient content

An example of micronutrient content of manures was found in the literature. It is important to note that the micronutrient content of manures will depend on where the manure comes from. A study from the U.S gives values for micronutrients in different types of Cattle and Poultry manure. For the Cattle manure the animals were given different diets, for the poultry manure one was with wood shavings and one without (Capar et al., 1978).

[1] DIETER DEUBLEIN, A. S. 2011. *Biogas from Waste and Renewable Resources*, WILEY-VCH Verlag GmbH & Co. KGaA, ibid.
[2] http://www.norganics.com/applications/cnratio.pdf
[3] Data for paper

Table 5: Micronutrient content for Cattle and Poultry manure

Element	Demand [mg/L]	Cattle manure low fiber diet [ppm/DM]	Cattle manure high fiber diet [ppm/DM]	Poultry waste with litter [ppm/DM]	Poultry waste without litter [ppm/DM]
Mn	0.005-50	117	161	166	242
Mo	0.005-50	29.9	49.2	5	7.2
Zn		115	86	155	158
Cu		24	21	30.7	20
Co	0.03-0.06	1.7	2.2	2	1.2
V		3.2	8	3.9	4.3
Br		34	29	9	31
Cl		10100	8500	3500	6500
Se	0.008	0.35	0.32	0.38	0.66
Pb	0.02-200	2.1	3.28	2.08	3.45
Cr	0.005-50	20	31	6	4.9
Fe	1-10	2200	5100	730	1800

The numbers from the study is in no means representative for the micro nutrient content in Norwegian manures, but shows that there for instance is a big difference if the poultry manure contains wood shavings or not. It also shows that most nutrients will be more abundant in the manure without wood shavings except from Cr, Cu and Co.

Results

Table 6: C:N content of model substrates

Substrate	DM (% w/w)	C (% w/DMw)	N (% w/DMw)	C:N
Cattle manure[4]	9.8	45.82	2.04	22.5
Poultry manure	16.2	38.05	2.02	18.8
Fish silage with oil	34.9[5]	82.69	8.17	10.1
Fish silage without oil	25.9[5]	53.62	9.75	5.5
Straw	92.1	45.77	0.59	77.6
Fiber Sludge[6]	34.4	42.91	0.99	43.3
Biological Sludge	3.3	44.30	5.31	8.3
Fiber mix	6.9	44.86	1.73	25.9

[4] Data from earlier analysis done at UMB
[5] DW obtained from Karl Fisher titration
[6] The values are calculated assuming that the combined bio sludge and fiber sludge contains 11.1% bio sludge and 88.9% fiber sludge on a DM basis.

Table 7: Fat and protein content in fish silage

Substrate	DM (% w/w)	Protein (% w/w)	Fat (% w/w)
Fish silage with oil	34.9	13.3	16.6
Fish silage without oil	25.9	13.9	6.2

Discussion

Based on the results of the analyses some thoughts about the suitability of the modeled mixture can be made, and of what improvements may be done to it. The C:N ratio of the mixture is 21:1 and is within the recommended range of 16:1-25:1. Since much of the material is lignocellulosic it could be beneficial to get an even lower C:N ratio, this can be done by adding more fish silage, poultry manure or biological sludge to the feed mixture, or by recycling parts of the liquid fractions of the digestate until a desired C:N-ratio is reached. A higher amount of substrates with high C:N-ratio would not be recommended.

For the micro-nutrients 15% of the DM comes from manures, 24% of the DM comes from fish silage. Pure fish silage should not be deficient in any micronutrients as long as the fish have been given proper food. If one consider that all the micronutrients from manure must be enough for the degradation of the substrates in the fiber mix, and we have relative manure content in this mix of 20%. Both the manure and the fiber mix have a DM content of 10%. Calculating through the example values given in table ## shows that the mixture is potentially deficient in Cobalt and Selenium. For the other nutrients it is hard to tell, since it is difficult to put any precise number on the trace nutrient demand, however, lead is also in the lower range, while the others are more or less in the middle.

Conclusion

The substrate mixture is in the middle range for the C:N-ratio, but may be suffering from nitrogen deficiency if much of the nitrogen is bound in the lignin structures and therefor is not available for the microorganisms. The substrate mixture is likely to be deficient in one or more trace minerals, with Cobalt, Selenium and Lead as the most likely to be limiting. The proposed solution is to add more manure and preferably both Poultry manure without litter and Cattle manure to both lower the C:N ratio and increase the amount of trace minerals, where the two manures have some different effects. To be on the safe side, increasing the manure content by a factor 3 would be recommended, giving 42 % manure, 24% fish and 30% fiber. An example of a new mixture recipe is given; however, this is only based on highly uncertain numbers and

without taking the energy production into account, and is therefore only meant as a suggestion for further research.

Table 8: Substrate budget suggestion for more optimal macro- and micro-nutrient content.

Substrate	% DM	C:N
Cattle manure	9 %	22.5
Poultry manure	33 %	18.8
Fish silage with oil	6 %	10.1
Fish silage without oil	18 %	5.5
Straw	9 %	77.6
Fiber Sludge	11 %	43.3
Biological Sludge	1 %	8.3
Sum:		21.7

Biochemical methane Potential (BMP)

To evaluate the relevant substrate mixtures with respect to biogas production, a flask test to determine the Biochemical Methane Potential (BMP) of single substrates was conducted. There is no standard protocol for determination of BMP, although there have been many attempts on establishing a common protocol (Angelidaki et al., 2009). The key to determine the BMP is that the microbes does not have any nutrient limitations, or is inhibited in any way. It is also necessary to have enough test substrate so that a representative sample of test substrate can be made. For control, blanks and cellulose controls are used. The average production from the blanks is subtracted from the average of the test substrate analyzed. With the cellulose control it is possible to see if the inoculum performs well on cellulosic materials (like fiber sludge and straw).

SIR

The SIR (Sludge to Inoculum ratio) points out as a parameter that should have a large impact on the results of the experiment. A series of papers that investigate the effect of SIR on BMP have been published (Fernandez et al., 2001, Hashimoto, 1989, Jensen et al., 2011, Neves et al., 2004, Raposo et al., 2006, Raposo et al., 2008). The conclusion of all of them is that a SIR < 1:3 is sufficient to avoid inhibition for the substrates tested.

Microbial community

Only a few studies have examined the variability of results from different inoculums and laboratories (Raposo et al., 2011). Often the inoculum used in the BMP studies comes from an anaerobic wastewater plant nearby(Raposo et al., 2011), while studies of continuous reactors shows an adaption of the microbial community to the feedstock after being feed a different feedstock for a period of time (Bertin et al., 2012). In an inter-laboratory study the influence of inoculum was found to be almost insignificant, but the rate differed significantly (Raposo et al., 2011). However, the substrates tested in this study were cellulose, gelatin, starch and mung beans, all which are easily degradable. In addition the effect of inoculum and experimental conditions was confounded in the experiment. Whether different inoculum will give different results on substrates that are more difficult to degrade, and therefore requires a more adapted microbial community, have not yet been reported in literature.

Temperature

The BMP assay can be conducted at any temperature, and should always give the same end results as long as the necessary microorganisms for methanization are present. Usually either mesophilic or thermophilic conditions is chosen.

Shaking/Mixing

Mixing of the content in the bottles is necessary to accomplish a sufficient flow of substrates and nutrients to the microorganisms. It seems like too much mixing can have a negative effect on the methane yield (Kaparaju et al., 2008), in the end of their experiment the methane yield varied from 190mL/gVS – 320mL/gVS after 73 days, the mixing regimes tested was vigorously continuous mixing (110 rpm), gently continuous mixing (35 rpm) and mixing by hand in one minute before sampling. Gently mixing gave the highest methane yield. It must be noted that this experiment

was done under thermophilic conditions and with cow manure, and is not necessarily relevant for mesophilic conditions and other substrates. Anyway it shows that mixing is an important parameter, and should be considered, especially if the results from the BMP test are far from the theoretical methane potential.

Pre-incubation

Pre-incubation of the inoculum is sometimes used. The reason for pre-incubation is to reduce the production from the inoculum, and thereby get a more precise estimate of the BMP(Raposo et al., 2012). It also helps making the environment in the batch assays anaerobic. This is especially important when the inoculum and substrates are added without continuously flushing with an anaerobic gas, but it can also be important if there is dissolved oxygen in the inoculum as a consequence of the homogenization before it is added to the bottles.

Kinetics of Batch vs. continuous flow CSTRs

To design a study to assess the BMP of substrates that are to be used in a multi-fuel continuous anaerobic digestion plant, it is important to know the difference between the growth kinetics in batch and in continuous CSTRs. One of the main differences between batch and continuous reactors is the occurrence of diauxic growth in batch contra mixotrophic growth in continuous reactors. For instance a study made by (Lee et al., 2008) detected growth and regrowth of archeal populations with biphasic production of methane, corresponding to the diauxic consumption of acetate and propionate.

This will not only apply for the methanogens, but also for the other bacterial groups that will consume the easiest degradable substrate first. In a continuous system however, the consumption will not be diauxic, but mixotrophic, where acetate and propionate will be consumed simultaneously.

Substrates

Substrates relevant to the ones that will be used at the full scale plant at Fiborgtangen were collected from different sources. Samples of manure, straw and fiber materials were steam exploded (SE) at 200^{0}C in 15 minutes and then stored at 5^{0}C. The substrates subject to the test with characteristics is listed in table 9.

Table 10: Substrate Characterization

Substrate	Origin	pH	Solid/Liquid[7]
Biosludge	Norske Skog Skogn	6.9	L
Fish silage with oil	Biokraft AS	3.9	L
Fish silage without oil	Biokraft AS	n.d.	L
Fishery sludge	Biokraft AS	n.d.	L
SE Cattle manure	UMB	8.8	S
Fiber mix	Various	6.2	S
SE Fiber and biosludge	Norske Skog Skogn	6.7	S
SE Poultry manure	UMB (1 y.o.)[8]	7.2	S
SE Straw (Wheat)	UMB	3.8	S

The Fiber-mix is a mix of the solid substrates and the biological sludge and was presented in the substrate characterization chapter.

The straw was cut into approximately 200 mm length before steam explosion. Liquid substrates where vigorously shaken previous of sampling, solid substrates was stirred with a spoon.

Inoculum

Two different inoculums were used, denoted *Inoculum I* and *Inoculum II.* Inoculum I was collected from a biogas plant in Aarhus, Denmark treating manure, waste and horse-manure with high straw content. Inoculum II was collected from the continuous reactors from the continuous experiment. Characterization of the inoculums is presented in table 11 .

Table 12: Inoculum Characterization

Inoculum	Origin	pH	NH4+ [mg/L]
Inoculum I	Aarhus, Denmark	7.9	n.d.
Inoculum II	Reactors	7.5	1695

Inoculum II was filtered before the incubation to remove the fiber, and thereby get a more homogenous inoculum. Both inoculums were constantly stirred with a magnetic stirrer during the addition to the flasks.

Experimental setup

Glass bottles of 1150 mL with rubber septums were used in the experiments. First 600 mL of diluted inoculum was added in randomized order. These were then pre-incubated at a shaker (90 rpm) at 38^{0}C in a dark room. The pressure was measured and released every day, once the daily gas production stared decreasing the bottles

[7] S = Solid, L = Liquid

[8] The Poultry manure had been stored in the hen house at UMB for one year previous of collection, there was no other manure available from egg-hens in the area.

were opened and the substrate was added. For Inoculum I the gas production decreased after 14 days, for inoculum II it decreased after 7 days.

The experiment was done with triplicates, including blanks and cellulose controls. The experiment was run at different times and with different inoculum because the standard deviation in the first experiment was not satisfactory for some of the substrates. The first experiment, denoted *experiment I*, used Inoculum I. The second experiment, denoted *experiment II*, used Inoculum II. The substrates tested in each of these experiments are listed in table 13 and 14, with their respective SIRs. The bottles were standing at the shaker for the first 56 days. After this they were put on the floor, the bottles were then shaken vigorously once a week.

Table 15: Substrate to Inoculum ratio experiment I

Substrate	SIR	Inoculum VS	Substrate VS
Cellulose	0.21	5.3	1.1
Biosludge	0.19	5.3	1.0
Fishery sludge	0.20	5.3	1.0
SE Catlle manure	0.17	5.3	0.9
SE CSTR	0.18	5.3	1.0
SE Fiber and biosludge	0.20	5.3	1.1
SE Poultry manure	0.20	5.3	1.0
SE Straw	0.20	5.3	1.0

Table 16: Substrate to Inoculum ratio experiment II

Substrate	SIR	Inoculum VS	Substrate VS
Cellulose	0.35	5.9	2.1
Fish silage with oil	0.25	5.9	1.5
Fish silage without oil	0.24	5.9	1.5
Fish-oil	0.21	5.9	1.3
Fiber and biosludge	0.26	5.9	1.5
Fiber residual	0.26	5.9	1.5

Measurements

Pressure was checked on a regular basis, and pressure and gas composition was measured when pressure was between 200 mbar and 700 mbar. After measurements the gas was released. Pressure was measured with a barometer, pointing a hospital needle through the rubber septum. The gas composition was measured with a Gas Chromatograph (type, etc##) measuring methane and carbon dioxide concentration.

Controls

Blanks were used as a control to be able to subtract the production caused by the inoculum itself from the production of the substrates. A Cellulose control was used to check if the inoculum was applicable on cellulosic substrates using Cellulose microcrystalline with a known BMP of ##300 mL CH4/gVS.

Expected results

For some of the substrates in this experiment BMP tests have already been conducted, and results been published. For the other substrates an expected result can be calculated based on the amount of carbohydrates, lipids and proteins or the COD value.

The calculation of the methane potential based on Carbohydrates, lipids and proteins is based on caloric value. This gives the following potentials:

Table 17: Factors for calculations of methane potential based on lipid, protein and carbohydrate content(Carlsson, 2009).

Substrate	mL CH4/kg VS
Lipid	960
Protein	510
Carbohydrates	420

According to this, typically carbohydrate substrates like cellulose, straw, fiber, wood should give about 420mL CH4/gVS if all VS are available for anaerobic decomposition. Lignin is however not degradable anaerobically, and lignin strong substrates will therefore have a lower potential. This can be predicted by measuring the lignin content and subtract this from the VS.

Table 18: Lipid, Protein, Carbohydrate content of Fish substrates

Substrate	Lipids [%]	Protein [%]	Potential [mL CH_4/gVS]
Fish silage with oil	16.6	13.3	760
Fish silage without oil	6.2	13.9	649

Theoretical methane potentials for lignin rich substrates were calculated by subtracting the lignin content and assume a degree of degradation.

Previous analyses of steam exploded straw have found a lignin (Klason) content of 38% of dry matter for SE at 210^{0}C and 10 min (Horn, 2011). Assuming all lignin is present in the volatile solids, an estimate of methane potential can be made. All of the remaining VS are considered degradable.

The content of Hemicellulose, cellulose and lignin (Klason) in Norwegian Spruce have been reported to be 24-26%, 45-47% and 27-28% respectively (Bertaud and Holmbom, 2004). Degradation of cellulose in newspaper was found to be 51-58% in a newspaper containing 25% lignin, the degradation was increased to 75-79% by removing lignin by Chlorite treatment(Stinson and Ham, 1995). In this study ball milling increased the degradation to almost 100%.

Assuming that the fiber sludge have approximately 25% lignin before steam explosion and 40% after, would therefore be naturally. By using the 50% degradation found by Stinson and Ham, and assuming that the cellulose content was 75% an estimate of the methane potential can be made. The effect of steam explosion under optimal conditions is expected to give a 50% increase in methane yield.

Table 19: Expected methane potential of lignin rich substrates

Substrate	Degradation	Lignin content of DM	Expected methane potential [mL CH4/gVS]
SE Straw	100%	40%	254
Fiber sludge	50%	25%	154
SE Fiber sludge	75%	40%	185

A study of biogas production potential and limitations of activated sludge from Swedish paper pulp reported a production of 100-200mL CH4/gVS dependent on the sludge age, where the sludge age ranged from 5-20 days (Karlsson, 2011). The activated sludge treatment plant at Skogn is a LSP (Low-Sludge-Production) plant, with a sludge age of 13 days (Odin Krogstad). From this we can expect a production of 100 mL CH4/gVS.

Table 20: Theoretical methane potential Biological sludge (Karlsson, 2011)

Substrate	Expected methane potential [mL CH4/gVS]
Biological sludge	100

Combining the theoretical methane potential from the fiber sludge and the biological sludge, on a dry matter basis, gives a theoretical value of the methane potential from the combined fiber and biological sludge. The combined sludge contains 89% fiber sludge and 11% biological sludge on a dry matter basis.

Table 21: Expected methane potential fiber and biological sludge

Substrate	Degradability	Expected methane potential [mL CH4/gVS]
Fiber and biological sludge	50%	147
SE Fiber and biological sludge	75%	216

Dairy cattle manure collected at UMB have earlier been reported to give 239 mL CH4/gVS (Estevez et al., 2012). With optimal steam explosion conditions it is expected that the yield increases with 50% to 359 mL CH4/gVS.

Poultry manure have been reported to give 270 mL CH4/gVS (Huang and Shih, 1981). With an increase of 50% due to optimal steam explosion conditions this can be increased to 405 mL CH4/gVS. For the manure tested in this experiment lower yields are expected because the manure consists of a large amount of wood chips and the fact that the manure had been stored for 1 year previous of sampling.

Table 22: Expected methane potentials for manures

	Untreated [mL CH4/gVS]	Steam exploded (200^{0}C 15min) [mL CH4/gVS]
Dairy Cattle manure	239	359
Poultry manure	270	405

To predict the methane potential of the SE CSTR-mix, weighting of the potentials for each single substrate was done on a VS basis.

Table 23: Expected methane potential for SE CSTR-mix

Substrate	Expected yield [mL CH4/gVS]
SE CSTR-mix	194 – 252

Calculations

Calculations of the methane concentration were done based on the assumption that the only gas compounds produced were methane and carbon dioxide. Calculations of the volume of gas produced were done using the ideal gas law. The production from the blank assays was subtracted from the methane production. The yield is presented as mL CH4/gVS at standard temperature and pressure. The production of gas in a bottle was calculated according to equation 1.

$$\frac{CH_4[\%]}{CH_4[\%]+CO_2[\%]}\times p[mbar]\times\frac{100000[mL]}{101325[mbar]}\times headspace\times\frac{273}{273+T}$$

Where:

CH_4 is methane measured in percentage

CO_2 is carbon dioxide measured in percentage

p is pressure measured in mbar

Headspace is the volume of the headspace in the assay

T is temperature in ^0C

Results

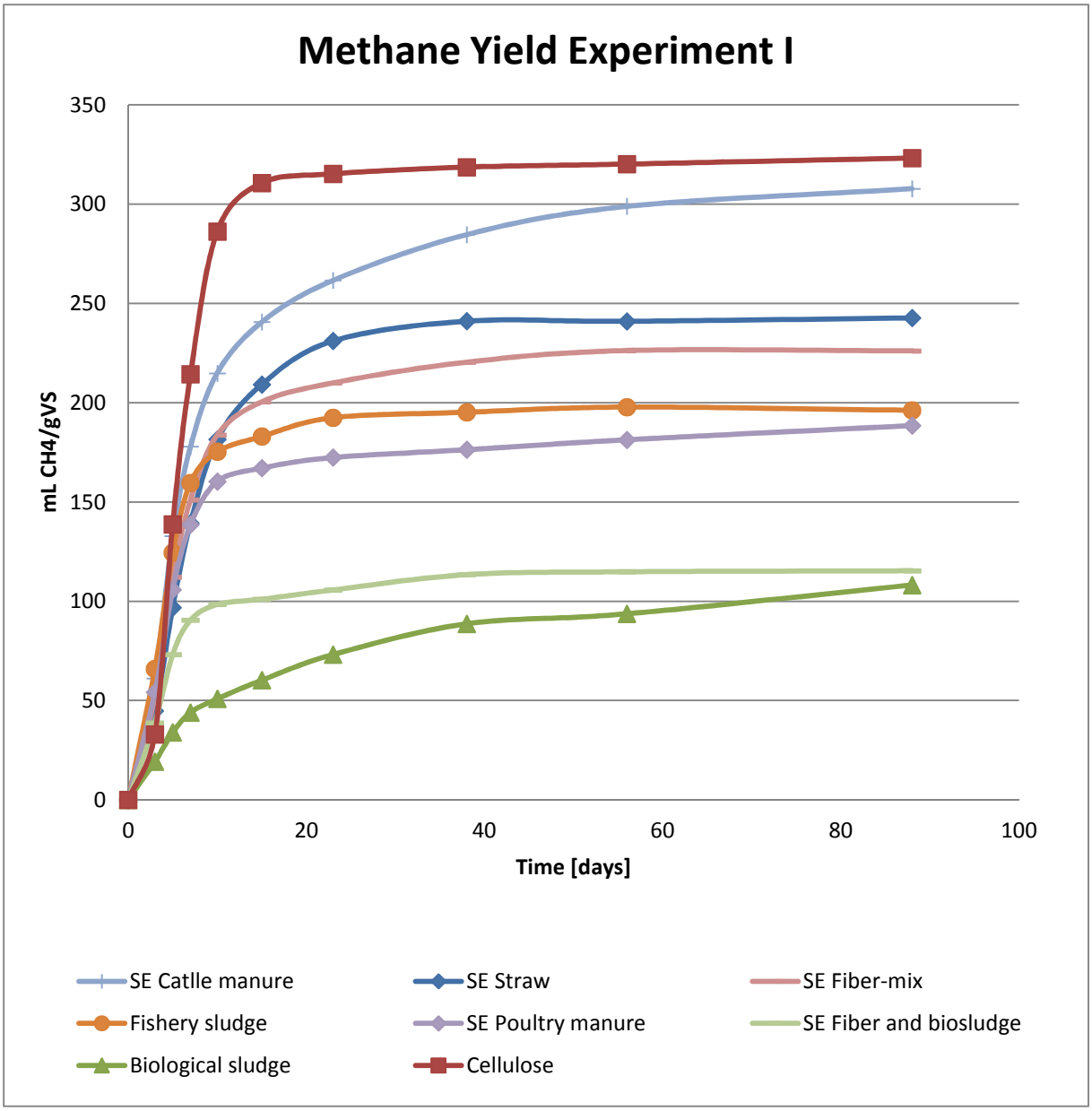

Figure 5: Biochemical methane potential fiber fractions experiment I

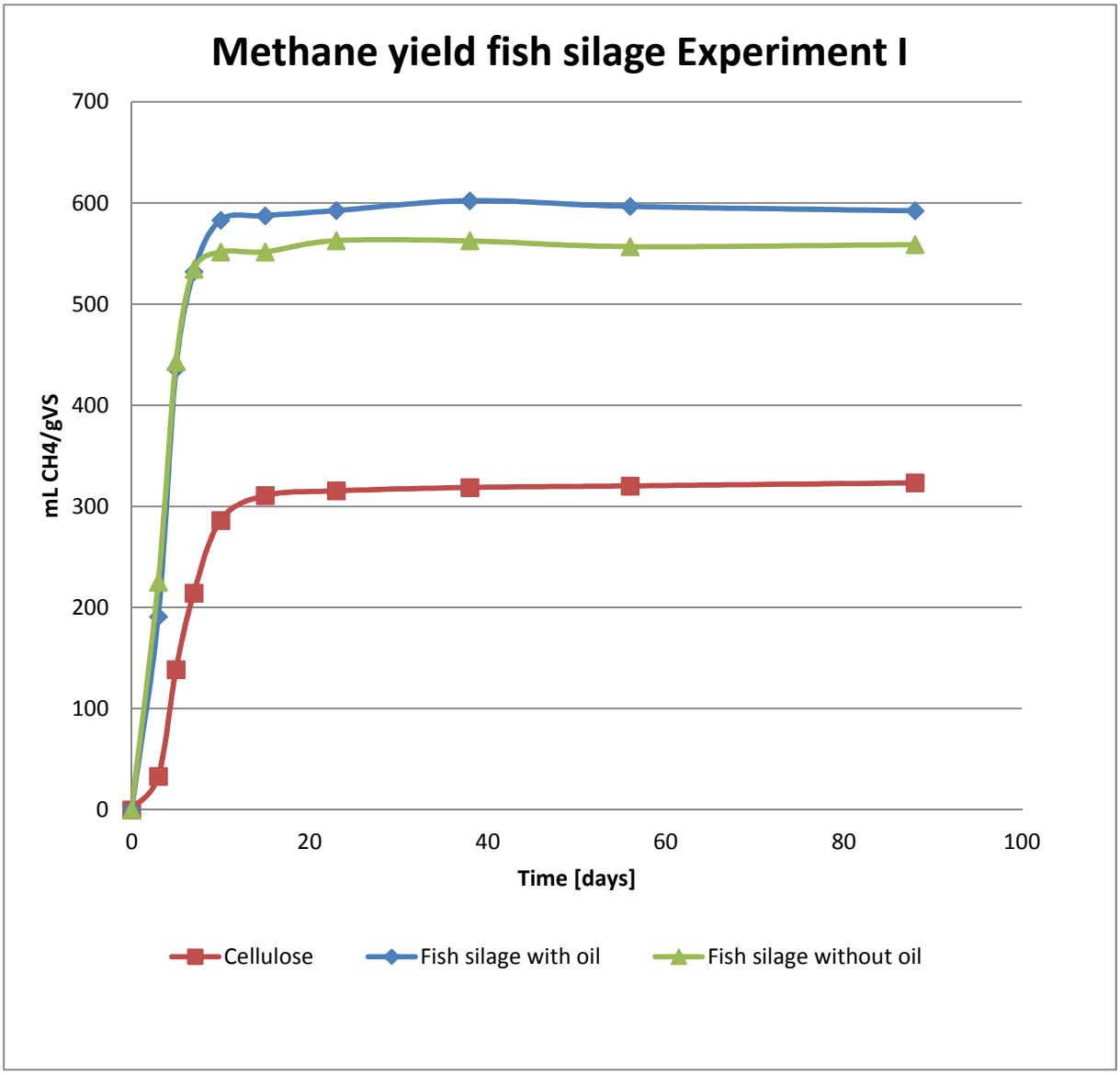

Table 24: Biochemical methane potential Experiment I

Substrate	Yield [mL CH4/gVS][9]	Expected Yield [mL/gVS]
Biological sludge	108 (±33)	100
Fishery sludge	196 (±26)	n.d.
SE Cattle manure	308 (±26)	239 – 359
SE Fiber-mix	226 (±14)	194 – 252
SE Fiber and Biological-sludge	115 (±16)	147 – 216
SE Poultry manure	188 (±18)	270 – 405
SE Straw	242 (±16)	254
Fish silage with oil	558 (±196)	760
Fish silage without oil	542 (±177)	649

[9] Standard deviations among triplicates are listed in parenthesis.

Unfortunately experiment II had to be cancelled before it was finished because of the time frame of the master thesis. A graph showing the results from the first 24 days can be found in appendix B. It is interesting to note that although experiment I gave reasonable results within 24 days, experiment II did not.

By using the results from the single substrates from the experiment, it is possible to calculate the expected methane potential of the SE CSTR-mix also from this, and see how it correlates with the measured value:

Table 25: Expected methane potential for SE CSTR-mix based on results for single substrates from BMP experiment.

	Yield based on single substrate yield [mL CH4/gVS]	Measured Yield [mL CH4/gVS]
SE CSTR	176[10]	226 ±16

Statistic testing of results

A two sample t-test with α=0.05 was performed to assure that the BMP results were significantly different from the blanks.

A Box plot of mL CH4 produced for each substrate was made in MINITAB to illustrate the difference of production and the variation in the data.

The 2-sample t-test gave that all methane potentials are significantly larger than the production of the blanks at α=0.05.

Discussion

Although some of the single substrates fall outside the expected yield, the CSTR-mix falls within and gives a higher yield than when adding the single substrates together. Since the SIR was below 0.2 both for single substrates and the CSTR-mix, inhibition is unlikely. One reason for this difference could be the effect of steam exploding in mix instead of single substrates. A loss of VS during Steam Explosion of both Straw and Poultry manure was observed, and the potential of Straw and Poultry manure was both observed to be less than expected. If it is assumed that all VS lost were Acetic Acid, an estimate of the loss of methane potential can be made. The theoretical methane potential of Acetic acid can be found by stoichiometry:

$$C_2H_4O_2 \leftrightarrow CH_4+CO_2$$

1g acetic acid then gives 0.267g methane. Based on the ideal gas law this equals 374 mL CH_4 under standard temperature and pressure. Loss of VS per g VS can now be used to weight the potential observed and the potential of acetic acid, so that the lost VS is assigned a potential of acetic acid.

[10] Since no data was available for SE biological sludge, it was assumed that the steam explosion effect gave a 50% increase in the methane yield.

Table 26: Correction for VS loss during Steam explosion

Substrate	Loss of VS [gVS/gVS]	Potential [mL CH_4/gVS]	Corrected Potential [mL CH4/gVS]
Straw	0.556	241	289
Poultry manure	0.120	181	202
Fiber-mix	-	226	197

After using the corrected potentials for straw and poultry manure to estimate the potential of the CSTR-mix, the CSTR-mix still gives higher results. This could be because of errors done while preparing the mix. To see if this is a likely reason a calculation of how wrong the mix must have been made was done, assuming the error lies in straw and fiber-sludge.

115*Fiber + 289*straw + 39.29 = 226
Fiber + Straw = 0.80

Solving the equations gives Fiber = 25.6% and Straw =54.4%. In kilos this equals 18 kg Fiber Sludge instead of 29.5 kg and 10.7 kg Straw instead of 7.5 kg. The error introduced while making the mix is more in the range of 100g-500g, hence, most of the deviation between the single substrates and the mix must come from somewhere else.

Possibly there is a synergic effect of steam exploding substrates together. This may be due to several effects:

1. The substrate is wetted before steam explosion
2. The substrate is wetted with ammonia before steam explosion

By wetting the substrate before steam explosion, the time before the substrate is saturated with steam may be shortened. Just like the soaking of rice and beans shortens their cooking time.

Conclusion

Most of the substrates tested gave biochemical methane potential as expected and earlier reported in literature. The mixed fiber and bio-sludge gave lower potential than expected and further research is necessary before this substrate is beneficial to use as a biogas substrate. A biochemical methane potential study to optimize the steam explosion conditions with respect to retention time and pressure should be done both for the fiber sludge, but also for the mixed substrates. The effect of mixing substrate previously of steam explosion should be investigated further, with experiments particularly design for this purpose. It is also necessary to do new experiments on the fish silage to get a more accurate prediction of its methane potential.

Semi continuous experiment

Semi-continuous experiments were carried out to establish a laboratory scale model of the full scale plant. Since the master thesis had a short time frame, only the very beginning of the process could be modeled. Semi continuous lab experiments can be used to optimize different operational parameters like HRT, OLR, mixing, substrate mixtures and recycling before changing these parameters in a full scale plant. Before any of the above can be tested, stabile running reactors must be established. Because of the long HRT in AD, the goal of this experiment was to see if it was possible to establish stabile running reactors for a HRT of 25 days, OLR of 3 gVS/L and the given substrate mixture in table ##. If recycling would have any effect on the establishing of the reactor model was also investigated.

Experimental setup

4 semi continuous reactors were run with identical substrate, OLR, HRT, continuous stirring and feed interval. For two of the reactors the effluent was filtered, and 150 g of liquid digestate recirculated back into the reactors every day. To the two reactors without recirculation, 150 g of tap water was added instead. The reactors where started with two different inoculums, one inoculum came from a mesophilic plant at Åna, Norway, running on cattle manure and fish silage. The other inoculum came from a mesophilic pilot plant at Aalborg University, Denmark, running on horse manure, household waste and sewage sludge.

Figure 6: Sieve

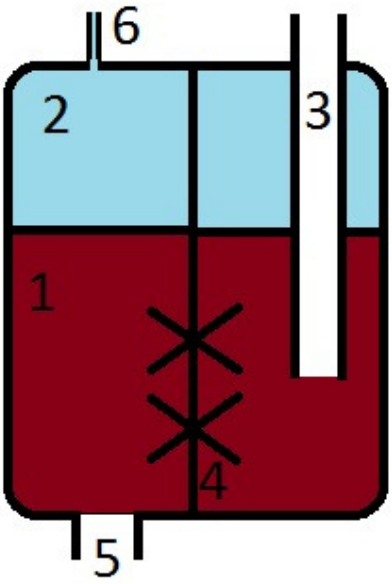

Figure 7: Cross-section semi continuous reactors. 1: Digestate, 2: Gas, 3: Feeding tube, 4: Rotating propellers, 5: Discharge of digestate, 6: Discharge of gas

Experimental matrix

Table 27: Experimental matrix

	Recirculation	No recirculation
Inoculum Åna	R5	R6
Inoculum Denmark	R7	R8

The reactors had a total volume of 20 L, with a 15 L reactor volume and 5 L headspace. The substrate was added through a feeding tube, which was dispersed halfway down into the digestate phase. Gas was lead through a pipe in the top of the reactor and into a tipping bucked gas meter. The effluent was collected from the bottom of the reactor. The bottom of the reactor was flat, hence sediments would be evenly distributed on the bottom, and only some would be removed with the effluent. The mixing was done continuously at 30rpm with 2 propellers connected to the mixer.

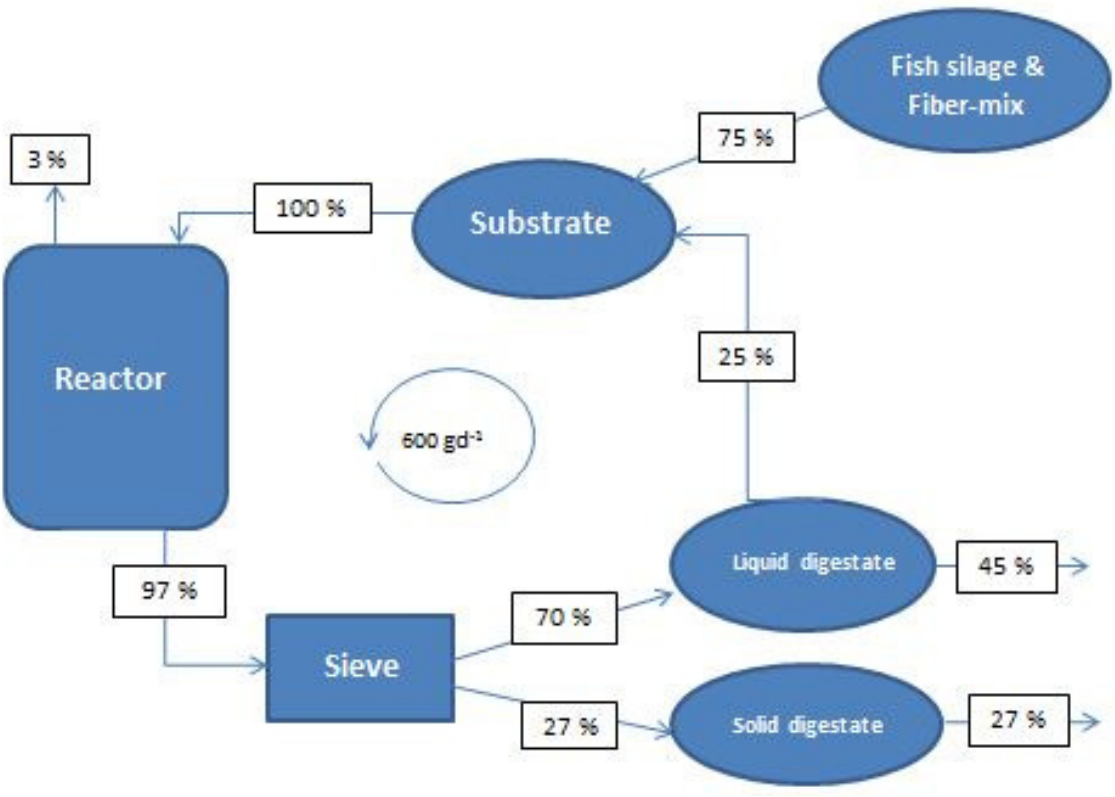

Figure 8: Mass balance reactors with 25% recycling. Assumption of 40% VS degradation.

Preparations

The fish silages were kept at 5 ^{0}C from arrival and all through the experiment. The silage would be transferred to 1L poly ethylene bottles to allow hand shaking of the

silage as a homogenization. The fiber mix was steam exploded and then mixed thoroughly and divided into 3 containers of 70L volume. Each container would last for 1 month, and would be frozen down to avoid decomposition, until it was time to open a new container. The fiber mix was mixed with a cement blender and representative samples were transferred to smaller containers of 10 L, this allowed the fiber mixed to be stirred with a spoon prior to measuring for feeding. The reactor feed was measured every day by weighing on a scale with 1 g accuracy. Because the fiber mix was very difficult to homogenize properly, DM analyses were carried out every time a new 70 L container was opened and in the middle of each of these containers. The feed recipe was adjusted to that the OLR was kept constant.

Upstart

The process was started by filling the reactors halfway with inoculum and then running them as batch reactors by slowly increasing the OLR from 1 gVS/L to 3 gVS/L with steps of 0.5 gVS/L and letting them run on the same OLR for 5 days at the time. After this the full reactor volume was reached, and the reactors were run semi-continuously for 1 week before the experiment started. During the upstart pH, methane content of the gas and gas volume was monitored to make sure the reactors did not show any sign of collapse. In R5, a collapse occurred and the reactor content was discarded and the upstart started over again by dividing the content of R6 on the two reactors.

Operation

When feeding the reactors, the gas outlet was connected to a bag filled with anaerobic gas to avoid that oxygen was introduced to the reactor during the collection of effluent. Effluent was always removed first, then the substrate was added and the container for the substrate was flushed with effluent to minimize the amount of substrate sticking to the container and to the walls of the feeding tube. In the beginning of the experiment the gas was collected into bags to measure the gas composition, the gas composition was then measured of the content of the bag on a daily basis, however, the bags were leaking a lot, and this data is therefore not trustworthy. For most of the experiment the gas composition was measured 5 times a day by a GC that automatically sampled gas directly from the outlet. pH was measured Monday-Friday, and samples for VFA, DM and VS were done every week. Total Nitrogen, Ammonium Nitrogen and COD were measured once in the beginning of the experiment and then repeated in the end of the experiment.

Analytical Methods

VFAs were analyzed using HPLC. DM was determined by drying for 24 hours at 105^0C, VS by burning at 550^0C. Gas composition was measured with SRI 8610 C Gas chromatograph. Total nitrogen was attempted measured with Merck cell tests, but the material was to inhomogeneous.

Calculations

The gas production was calculated on a weekly basis, using the average of the gas production during the week. The Yield was calculated using the assumption that all gas produced was methane and carbon dioxide, the mL produced equals the number of tips in the gas meter multiplied with a factor 0.35. It was assumed that the temperature of the gas was 20^{0}C when measured by the gas meter, and this was corrected to 0^{0}C to give gas production under standard conditions.

Degradation was calculated on a VS basis according to mass balance, assuming steady state, although steady state will not be the case in the beginning of the experiment. The formula for the calculations will then be:

$$Degradation[\%] = \frac{VS_{in} - VS_{out}}{VS_{in}} \times 100\%$$

The VS in the recycling liquid was neglected in all calculations.

Results

The results from the experiment will be presented below.

Methane Yield

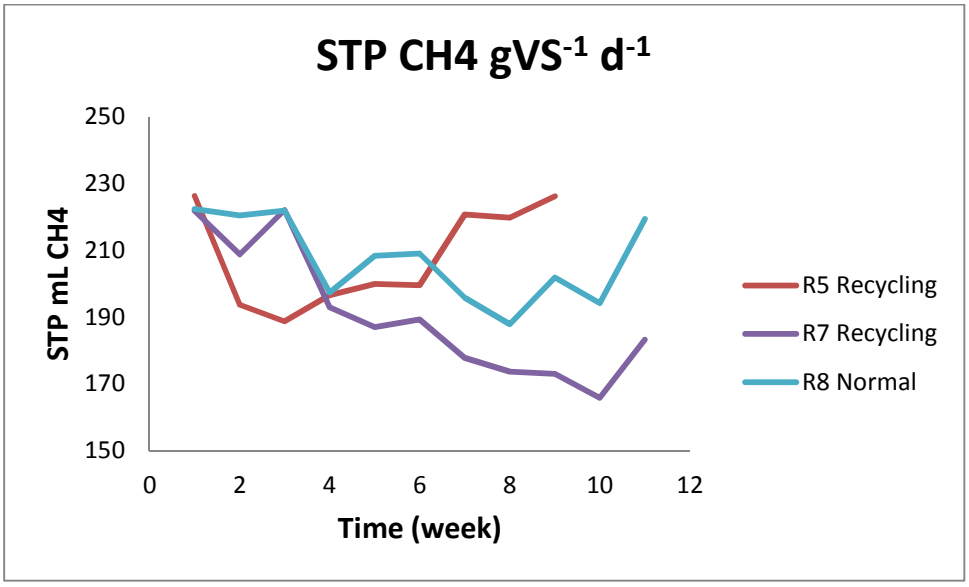

Figure 9: Methane Yield development in semi continuous reactors

The methane yield varied during the experimental period. All reactors started with a specific methane yield of around 220 mL CH_4/gVS to be followed by a drop in the yield, before it in the end of the experiment for all reactors started increasing again.

Degradation

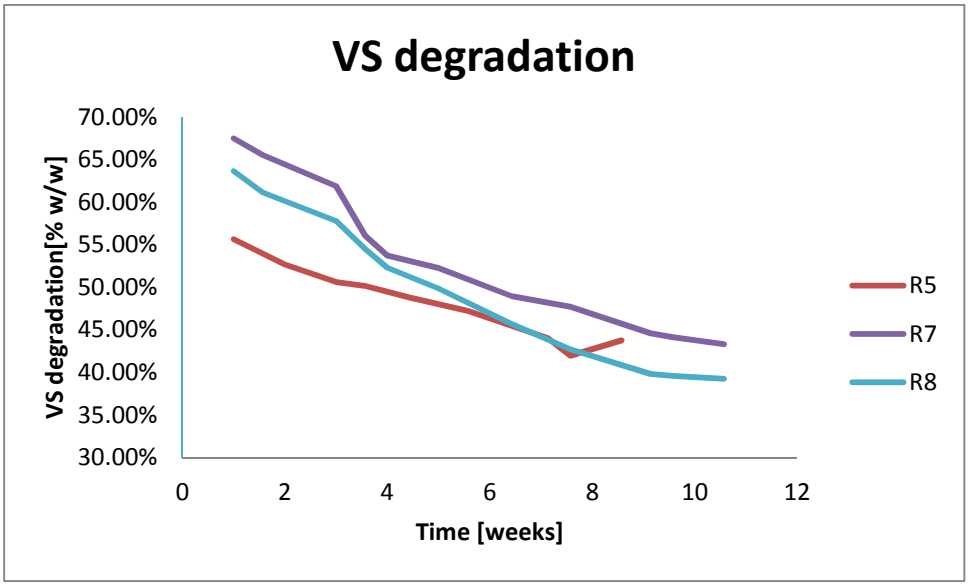

Figure 10: VS degradation in Semi Continuous reactors, development over time

The calculation for degradation is based on the approximation of steady state conditions. The degradation in the beginning of the experiment will therefore be too large. The degradation continuous to decrease, due to that the reactors did not reach steady state during the experimental period. If the degradation is calculated based on biogas production, assuming all gas produced is methane and carbon dioxide, 58% methane content will give the mass of the biogas to be 28g/mol. 1 mol of gas will according to the ideal gas law at standard conditions equal 24L gas . If approximately 17 L of gas is produced each day, this equals 20 g of gas. With 45 g VS/d, this is a degradation of 44%, and seems to correlate well with the results based on the last VS measurement.

pH

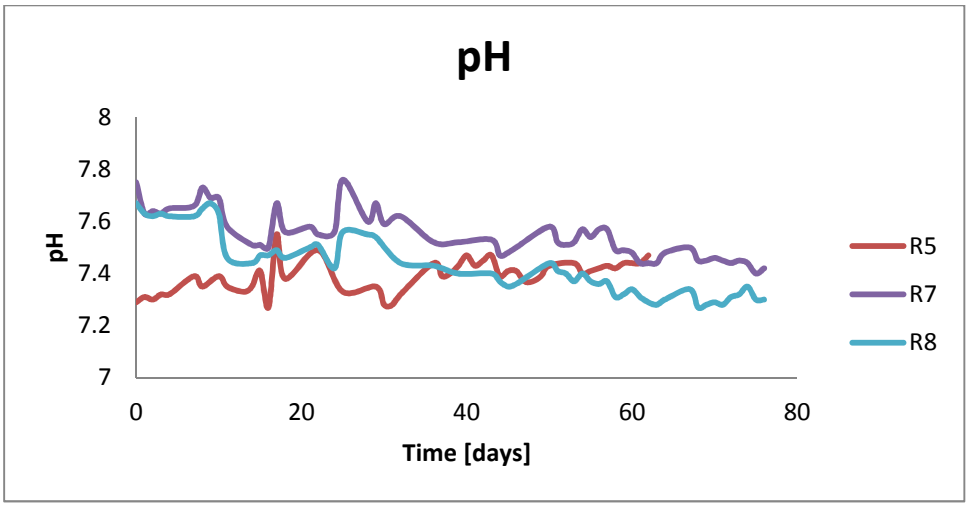

Figure 11: pH in semi continuous reactors, development over time

The pH stayed fairly stable during the experiment, and was always between 7.2 and 7.8.

DM and VS

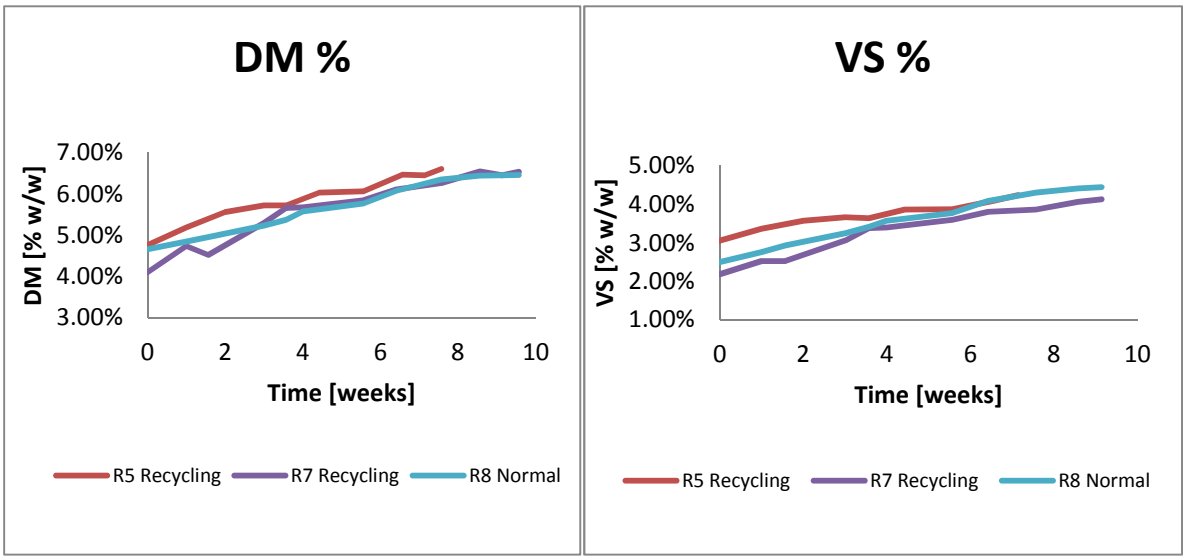

Figure 12: DM and VS in semi continuous reactors, development over time

The reactors did not reach steady state for DM and VS content during the experimental period, but could seem to stabilize in the last measurements on respectively 6.5% and 4.3%.

Nitrogen

The nitrogen content in the reactors did not change during the experiment. Total nitrogen levels were around 2500 mg/L, varying wih 1000 mg/L. The variation in Total nitrogen content was suspected to be due to the measurement method, and the results are therefore not presented. Ammonium nitrogen stayed below 3000 mg/L during all of the experiment.

Methane content

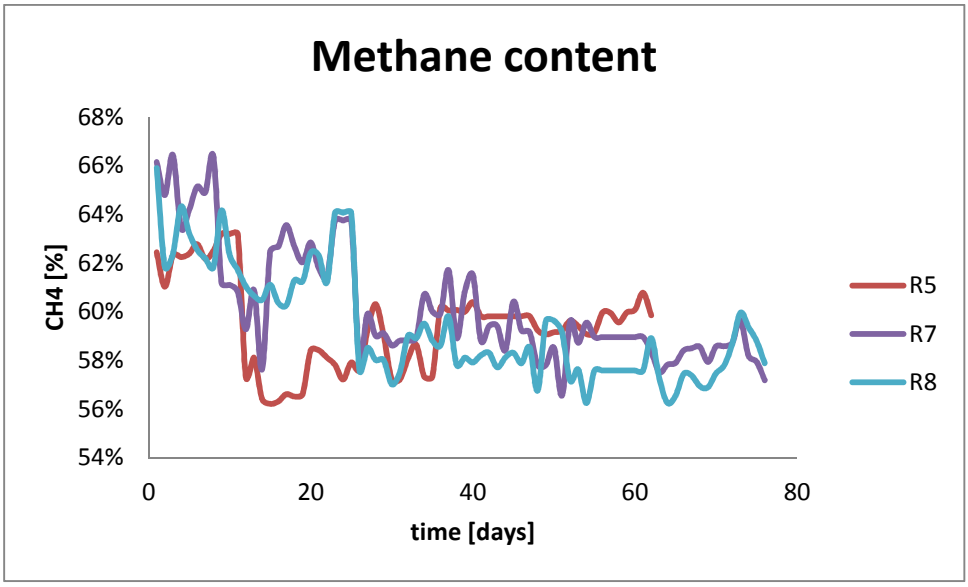

Figure 13: Methane content in semi continuous reactors, development over time

The methane content I the gas was measured to be higher in the beginning. This is possibly because of the method with collecting gas in gas bags that was used in the beginning of the experiment. After the gas was measured directly with a GC the methane content fluctuated with 2-3% for reactor R7 and R8. For reactor R5 it seems like the variation in methane content has decreased after day 40.

VFA

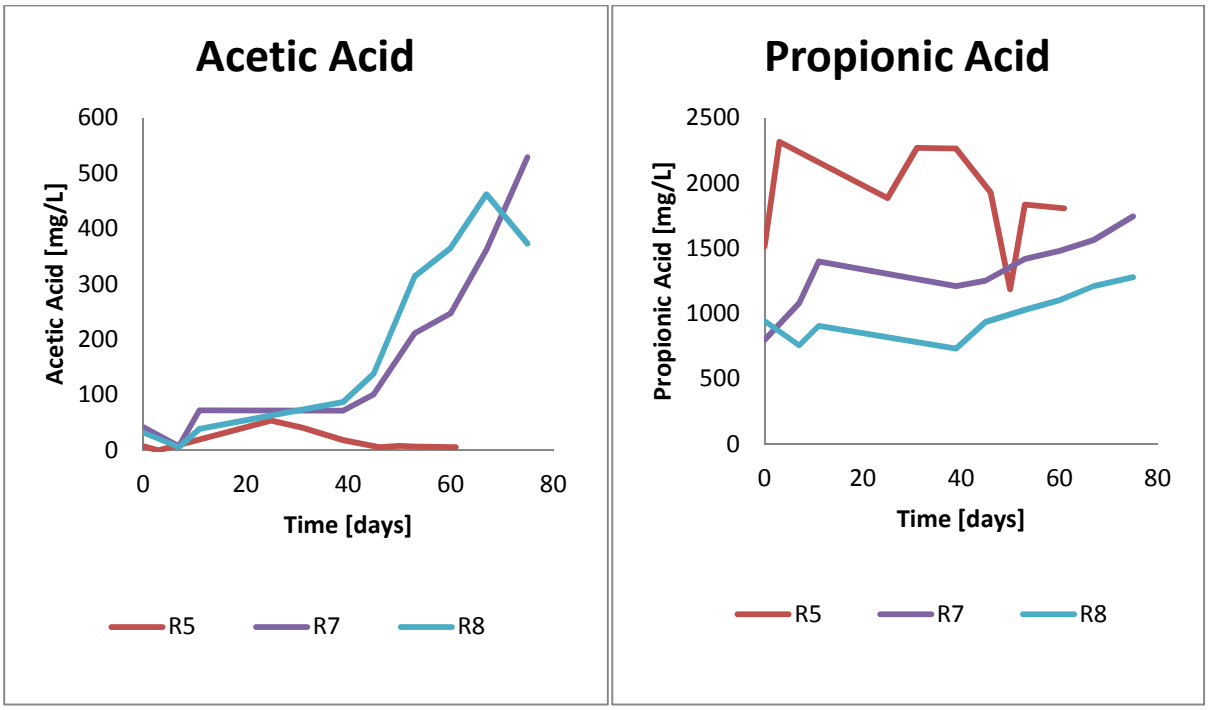

Figure 14: Acetic Acid and Propionic Acid concentrations in semi continuous reactors, development over time

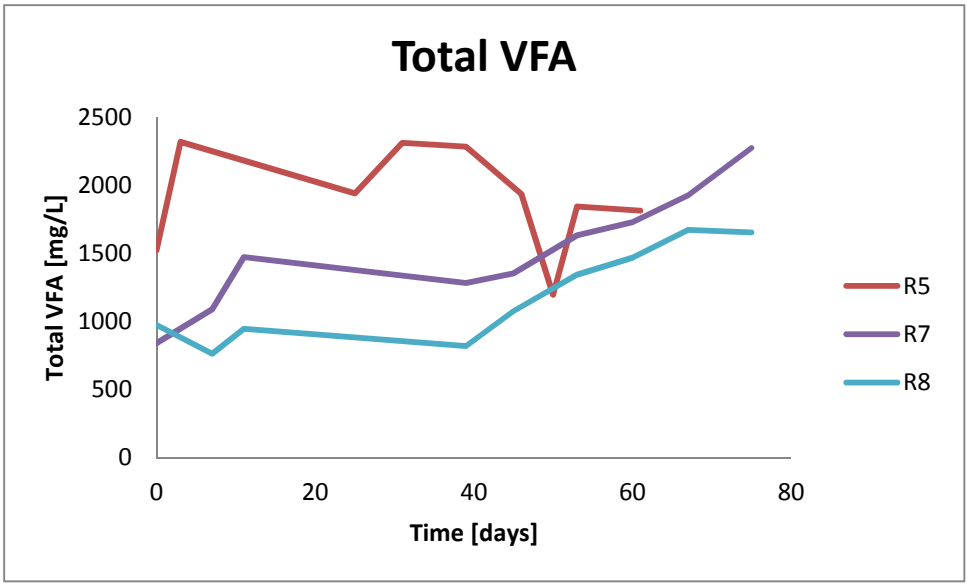

Figure 15: Total VFA in semi continuous reactors, development over time

For R7 and R8 the Acetic Acid accumulates during all of the experiment, except for the last week for R8. For R5 some acetic acid is detected after 3 weeks, but after this the concentration is approximately 0. Propionic Acid concentrations are high. For R7 and R8 they seem to be increasing all through the experiment, for R5 a slight decreasing trend can be seen with a concentration below 2000 mg/L after day 40. For R8 the propionic acid concentration stays below 900 mg/L until approximately day 40. Total VFAs were below 3000 mg/L during the experimental period.

Discussion

All reactors have different methane yields, and different VFA concentrations. After day 40, R5 with the highest concentration of Propionic Acid is actually the reactor with the highest methane yield. Around day 40 is also when the VFAs start accumulating in R7 and R8. The methane yield seems to get higher without any decrease in VFAs in the end of the experiment. With the high Propionic Acid concentrations it is expected that there is inhibition, but for R5 and R8 it seems like the methane yield is more or less stabile on these concentrations.

R5 and R7 were operated with the same conditions, both with recycling of the liquid fraction of the digestate. Still these two reactors are very different. Especially the VFA concentrations and the methane yield is very different. These differences can only have two explanations; random error or the difference in starting conditions. Because the pattern in the development of R7 and R8 are so similar to each other, while R5 is different, random error is viewed as an unlikely cause of the deviations. R5 started up with a much higher propionic acid concentration, and had also a lower methane yield in the beginning; however, the development of the two reactors seemed to be different from here. For R5 the propionic acid concentrations got a little better and the methane yield got higher, for R7 the propionic acid concentration got larger and the methane yield got lower. It could be that since R5 had a longer adaption period before the experiment started, this reactor lays ahead of the others.

The high propionic acid concentration witnesses instability in the process. This could be due to several reasons. One of the reasons causing high propionic acid concentrations is organic overload, if the overload is moderate the propionic acid concentrations may go down again with time as the micro-organisms adapt to the high loading. The high levels could also be caused by nutrient deficiency, if so an increase in propionic acid concentrations should be seen until all of the inoculum material is exchanged by new material, when this has happened, the propionic acid concentrations will either stabilize or accumulate until the reactors collapses. The last reason for the high propionic acid concentrations are toxic compounds, this is considered an unlikely cause in this system because the three reactors develop so differently, although given the same feed.

Conclusions

Recycling of the liquid fraction of the digestate seems to have no effect on the performance of the reactors. All reactors experienced high Propionic Acid concentrations and were unstable. It is recommended that the experiment is continued until the degradation reaches steady state, or the process collapses, to from this state investigate what causes the high propionic acid concentrations.

Future research recommendations

The results from the characterization of the substrates, Biochemical methane potential study and the semi-continuous experiment all points in the same direction. The original substrate mix is not beneficial. Because the fiber sludge from the paper factory is low both in nutrient value and in methane potential there is no reason to include this substrate in any continuous experiments until one knows how to get the full potential from this substrate. Another question that rises from these results is if it is possible to treat the fish silage and the fiber fractions separately? Fiber fractions have generally a slow degradation, while the fish silage is fast. The reject water from a reactor based in fish substrates could be led into the fiber reactor allowing the two reactors to have different HRTs, and at the same time utilize the nitrogen properly. The next thing that should be investigated now is the micronutrient demand, this could be done by running the reactors with different fractions of manure without fish silage at a relatively high HRT and a low OLR. When the process is stable, OLR can be increased slowly to find this limit, and the optimal manure content. When stable reactors are established one can start to change one parameter at the time while keeping one control. The natural next step would be to change HRT. Only after this introduction of fish silage can give useful information.

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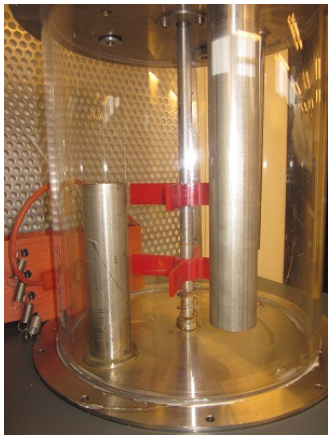
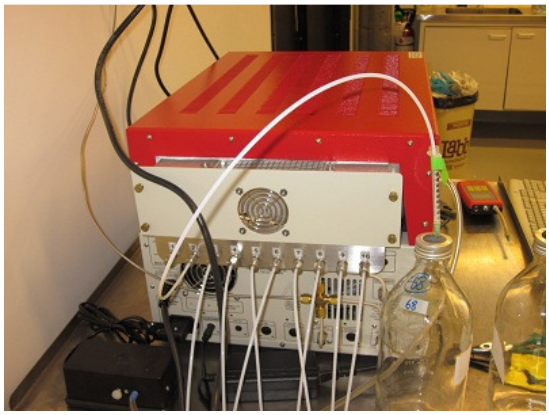
Appendix A: Experimental Plan

Master thesis Kine Svensson Spring 2012





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1. Aim of the experiments

- *Characterize the relevant substrate mixtures*
- *Evaluate the relevant substrate mixtures with respect to biogas production and operational performance and stability*
- *Propose operational conditions and substrate mixture range based on the experimental results, and recommend future research in order to optimize the biogas production further*

2. Characterization of relevant substrate mixtures

A relevant substrate mixture was **defined with background in the expected variations of the substrates available for the full plant.**

Table 28: Recipe CSTR experiment

	Substrate[kg]	TS[%]	TS[kg]
Fish silage without oil	21	50 %	10.42
Fish silage with oil	8.45	65 %	5.48
Fiber sludge	29.5	30 %	10.32
Biological Sludge	30	3 %	2.26
Straw	7.5	85 %	6.37
Poultry manure	9	30 %	2.73
Cattle manure	3.8	11 %	0.41
Sum:	109.25	32 %	38

2.1. List of analyses

A series of analyses must be conducted to characterize the substrate mixes. The characterization important to the operation of the process is organic content, C:N ratio*. In addi*tion, micronutrient content is important, but such analyzes were not available at the laboratory.

TS

VS

pH

C:N

3. Continuous stirred tank reactors (CSTRs)

Continuous stirred tank reactors will be used to analyze the effect of different inoculums on start-up of a digester. One inoculum will be pre-adapted to fish waste and the other will be pre-adapted to ligno-cellulosic substrates. The

question addressed is which of these inoculums adapts the best to the test-substrate.

The CSTR's will also be used to monitor process stability and the effect of recycling. Questions that are addressed in this part of the experiment are: How high DM can be obtained in the reactors before ammonia levels increases to inhibiting levels? How high DM can be obtained in the reactors before overloading? Is recycling beneficial?

3.1. Equipment

4 CSTR of 15 L reactor volume, 5L headspace
Thermostat
PH-meter
Gas Chromatograph
Inoculum
Test Substrate
Tipping bucket gas meter
Computer
Sieve
Laboratory scale (10 mg accuracy)
Ammonia electrode
COD test kit
TOT-N test kit
HPLC

3.2. *Design of experiment*

The experiment will be testing recirculation vs. no recirculation and two types of inoculum. This gives a need of four reactors. The reactors are named 5, 6, 7 and 8, and will be running the following combinations:

Table 29: Experimental design CSTRs

	Recirculation	***No-Recirculation***	***Inoculum 1***	***Inoculum 2***
Reactor 5	*X*		*X*	
Reactor 6		*X*	*X*	
Reactor 7	*X*			*X*
Reactor 8		*X*		*X*

3.3. Description of reactor system

In this experiment 4 continuous stirred tank reactors will be used. Each reactor has an overflow volume of 15L. The reactors can be fed through a tube on the top which is connected to a pipe that discharges the feed ##mm above the bottom of the

reactor. A whole in the bottom allows for substrate to be discharged, while an overflow makes sure the volume in the reactor remains constant. Each reactor has one automatic mixer with two propellers. Each reactor has a thermostat and a heating belt that is connected to make sure the reactors keeps the wanted temperature. The gas volume in the reactors are 5 L. The gas is lead through a plastic tube to a tipping bucket gas meter.

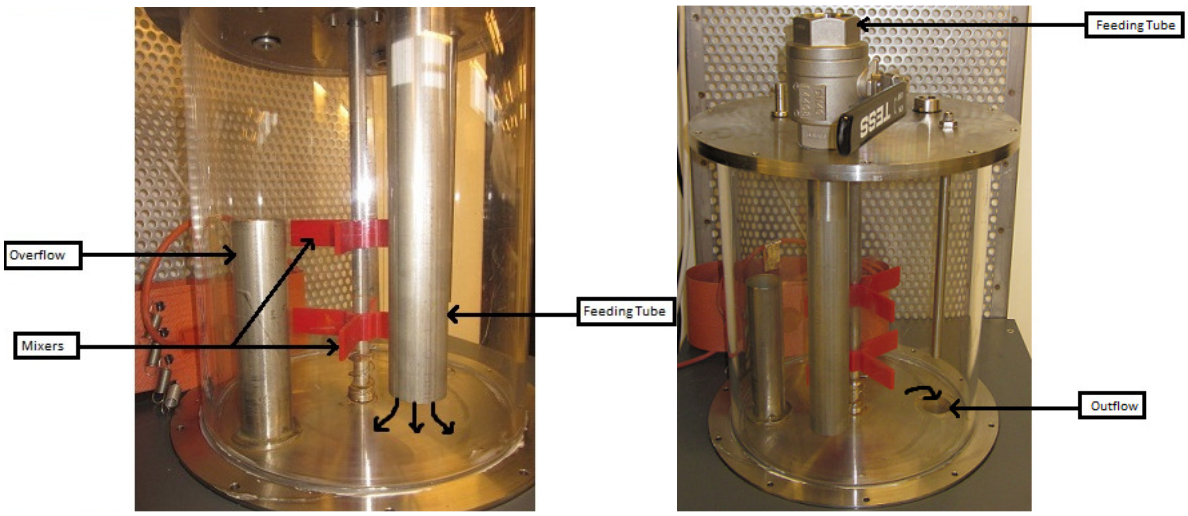

Figure 16: CSTR Reactors

3.4. Description of upstart and operation

3.4.1. Preparation

Homogenization and analyzes of TS, VS and pH of inoculum and test substrate.

3.4.2. Start-up

Reactor 5 and 6 *will be incubated* with inoculum 1 while reactor 7 *and 8 will be incubated with inoculum 2. The reactors will be filled with 7.5L inoculum. The reactors will then be feed with a low organic loading rate (OLR 1gVS/L d) of the substrate mixture. The loading will be increased until an organic loading rate of 3 g VS/L d is reached, the microorganisms are now assumed to be adapted to the specific test-substrate. Estimated start-up time is 3 weeks.*

Monitoring during start-up to avoid overloading and measurements of gas-production and methane content will be done by measuring pH, *gas-production and methane content.*

3.4.3. Operation

Feeding and Discharge

The reactors will be fed once a day. The same premade *substrate will be used in all reactors. The reactors will be fed with 3gVS/L with a hydrau*lic retention time (HRT) of

25 *days. This g*ives a daily feeding volume of 6*00ml. Before the reactors are fed a volume corresponding to the volume that will be added is removed.*

Recirculation

Reactor 5 and 7 *will be operated with recirculation. The amount of liquid recirculated each day will be subtracted from the need of diluting water to reach the desired VS concentration*. Reactor 6 *and 8 will not be operated with recirculation and all the dilution will therefore be with tap-water. The recirculation will be done by sieving the discharge of the day and returning the liquid fraction to the reactor. The sieve that will be* used has a light opening less than 1 *mm.*

3.5. Analyses and monitoring

Analyses and monitoring will be performed to make sure the reactor is operating under the wanted conditions and to assess the process stability and performance. Temperature will be held constant with a heating belt connected to the thermostat. PH and nitrogen levels will be checked to assess the stability of the process and gas measurements to assess the performance and potential of the process. VS of the recirculated liquid digestate will be measured to calculate the OLR and solid retention time in reactor 5 and 6.

Parameter	***Frequency***	***Logged***	***Equipment***	***Operation range***
Temperature	*Continuously*	*Continuously*	*Thermostat, temperature belt*	*36-38*
pH	*Daily*	*Daily*	*pH-meter, buffer solutions,*	*>7.0*
Gas Volume	*Continuously*	*Continuously*	*Gas volume counter*	
Methane content	*Daily*	*Daily*	*Gas chromatograph.*	*~50%*
Tot-N	*14.days*	*14. days*	*Spectrophotometer,*	*<4 gN/L*
NH_4-N	*Weekly*	*Weekly*	*Ammonium electrode, calibration fluids*	*<3 gNH_4/L*
VS and DM	*Fridays*	*Fridays*	*Bowls, drying stuff, laboratory scale*	
VFA	*Fridays*	*Friday*	*Sample directly in tubes.*	
COD	*25 days*	*25 days*	*Spectrophotometer*	

3.6. Estimated time scale

The start-up of the reactors will start approximately the 28th of February. The start of the continous experiment will then be at approximately the 20th of March. The end of the experiment 90 days later, june 18th.

3.6.1. Time use

Monitoring and analyses:

Temperature: 1 minute/day
pH: 1 minute/reactor and day
Gas Volume: 1 minute/day
Methane Content: 7 minutes/reactor and day
TKN: 5 minutes/reactor and week
NH_4-N: 5 minutes/reactor and week
PO_4: 5 minutes/reactor and week

VS: 5 minutes/reactor and week

Sum: 45 minutes/day.
In addition to this feeding and recycling will take at least 1h per day. This gives a minimum use of 2h per day for the CSTR experiment.

4. Batch reactors (Flask reactors)

The experiment will be done after standard operating procedures for bio-methane potential (BMP) at the Norwegian Centre for Bioenergy Research.

4.1. Equipment

Glass bottles of 1150 mL
Rubber septum
Barometer
Inoculum from mesophilic biogas plant
Test substrate
Laboratory scale (10 mg accuracy).
Incubator
Gas Chromatograph

4.2. Preparations

Homogenization and analyzes of TS, VS and pH of inoculum and test substrate.

4.3. Upstart and operation

A mixture of inoculum and water with 4 g VS/ 600 mL are prepared, this mix will be used for all the flasks in the same run. 600 mL of the inoculum and water mixture is added to each flask. The flasks are sealed and inoculated in an incubator in a dark room at mesophilic conditions (37 ^{0}C).

After 3-4 days (at approximately 50 mbar overpressure in the flasks/day) the sealed flasks are opened and 1-2 g VS of test substrate is added with water to a total volume of 700 mL. This gives a headspace of 450 mL. As control, flasks with inoculum is used, water is added to the control flasks until 700 mL of total volume is

reached. Each test substrate and control is carried out as triplicates.

During the experiment overpressure (gas production) in the flasks headspace is measured with a barometer. The overpressure is released by pressing a hospital needle through the rubber septum. The flasks are never opened. Measuring and release of pressure is done once a day at 37 ^{0}C through the entire experiment (60 to 90 days).

Gas concentrations in %, of methane (CH_4) and carbon dioxide (CO_2), are measured when the pressure exceeds 200mbar in all bottles from the start of the experiment to the end of the experiment. To avoid condensation in the GC the flasks are cooled in room temperature in two minutes before measuring the gas concentrations. The gas sample is made before the pressure is released.

The experiment is stopped when the accumulation of gas declines.

If the methane production is much lower than expected, and inhibition is suspected NH_4-N, pH and VFA analyzes may be conducted.

4.4. Experimental Design

All substrates will be analyzed one by one, in addition the fiber mix that is steam exploded will be analyzed.

4.5. Estimated time use:

66 bottles will be used.
*Daily analyses of the batch experiment is methane content and pressure, pressure can be measured while other flasks are analyzed for methane content, and time for this does not have to be accounted for. Time consumption is then 66 bottles*7 minutes =7 h and 42 minutes. (Fast chromatography will probably be used, time consumption is then 66 bottles*3minutes =3 h and 18 minutes).*

4.6. Calculations

Calculations of the methane potential of the test substrate is based on values for mL gas and methane produced per gram VS added. Methane yield is a result of methane production per gram added VS of test substrate adjusted for the controls average methane production per gram VS inoculum. For the calculations of gas concentrations it is assumed that [CH_4](matdepartementet, 2004) + [CO_2] = 100%.

Appendix B: Results from Biochemical Methane Potential Experiment II