Methane Production Through Anaerobic Digestion of Various Organic Substrates

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Abstract

This Bachelor´s thesis was carried out for Ab Stormossen Oy, a waste treatment company located in Mustasaari with the aim of finding new suitable substrates for anaerobic digestion. It is based on a theoretical part and practical tests with the Automatic Methane Potential Test System II (AMPTS II). The theoretical part consists of a literature research focusing on background information and important parameters of anaerobic digestion as well as properties and usage of biogas and digestate. Different waste types and their required criteria to be used in anaerobic digestion are discussed as well.

With the test system different organic substrates were solely anaerobically digested and tested on their methane potential. The results showed that the different kinds of manure, but also meat, eggs and cucumber plants had a high biogas production. Other substrates on the other hand, like olive oil, did give in one test a high production of biogas and in the other test nothing or nearly nothing. More tests with these substrates would be advisable. For future tests it would be also advisable to test the different substrates as mixtures.

Language: English Key words: Anaerobic digestion, biogas production, biodegradable waste, AMPTS II

EXAMENSARBETE

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Titel: Metanproduktion genom rötning av olika organiska substrat

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Abstrakt

Detta ingenjörsarbete utfördes för avfallshanterings företaget Ab Stormossen Oy i Korsholm, med målet att hitta nya lämpliga substrat för rötning. Arbetet är baserat kring en teoretisk del samt praktiska test som utförts med AMPTS II (Automatic Methane Potential Test System II). Teoridelen är en litteraturgransking som fokuserar på bakgrundsinformation och viktiga parametrar för rötning, samt egenskaper och användningsområden för biogas och rötrester. Även olika avfallstyper samt deras kriterier för rötning diskuteras.

Olika organiska substrat rötades separat och deras metanpotential mättes med hjälp av testsystemet. Resultaten visade att olika sorters gödsel, men också kött, ägg samt gurkväxter hade hög biogasproduktion. Andra substrat som olivolja gav hög produktion av biogas i ett test men producerade inget eller väldigt lite i det andra testet. Flera undersökningar med dessa substrat skulle vara bra att göra. I framtida undersökningar kunde man testa olika substrat som blandningar.

Språk: Engelska Nyckelord: rötning, biogas produktion, biologiskt nedbrytbart avfall, AMPTS II

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Nimike: Metaanin tuotanto mädättämällä erilaisia orgaanisia substraatteja

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Tiivistelmä

Tämä insinöörityö tehtiin Mustasaarelaiselle jätteenhuoltoyritykselle, Ab Stormossen Oy:lle, jonka tavoitteena on löytää uusia sopivia substraatteja mädätykseen. Työ perustuu teoreettiseen osioon sekä käytännön kokeisiin AMPTS II:lla (Automatic Methane Potential Test System II). Teoriaosio on kirjallisuustutkimus, jossa keskitytään taustatietoihin, mädättämisen tärkeisiin parametreihin sekä biokaasun ja mädätteen ominaisuuksiin ja käyttöön. Työssä tarkastellaan myös eri jätetyyppejä sekä niiden kriteerejä mädättämisen näkökulmasta.

Erilaisia orgaanisia substraatteja mädätettiin ja niiden biologinen metaanipotentiaali testattiin koelaitteiston avulla. Tulokset osoittavat että lannalla, lihalla, kananmunilla ja kurkkukasvilla on korkea biokaasun tuotto. Eräät substraatit kuten oliiviöljy, tuottivat yhdessä testissä paljon ja toisessa ei lähes ollenkaan biokaasua, mikä osoittaa että ne eivät ole kovinkaan soveltuvia erillisiksi substraateiksi mädätykseen. Näillä substraateilla olisi hyvä tehdä lisää kokeita. Tulevissa testeissä olisi myös suositeltavaa testata erilaisia substraatteja seoksina.

Kieli: Englanti Avainsanat: anaerobinen mädätys, biokaasun tuotanto, biohajoavat jätteet, AMPTS II

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Abstrakt

Diese Bachelorarbeit wurde für Ab Stormossen Oy ausgeführt, eine Abfallbehandlungsanlage aus Mustasaari, mit dem Ziel neue geeignete Substrate für die anaerobe Vergärung zu finden. Sie basiert auf einem theoretischen Teil und praktischen Tests die mit dem Automatic Methane Potential Test System II (AMPTS II) durchgeführt wurden. Der theoretische Teil besteht aus einer Literaturforschung die Hintergundinformationen und wichtige Parameter der anaeroben Vergärung sowie Eigenschaften und Anwendung von Biogas und Gärgut umfasst. Unterschiedliche Abfallarten und deren erforderliche Kriterien für die anaerobe Vergärung werden ebenfalls diskutiert.

Mit dem AMPTS II wurden verschiedene organische Substrate einzeln anaerobisch verdaut und auf ihr Methanpotential getestet. Die Ergebnisse zeigen, dass Fleich, Eier und Gurkenpflanzen sowie verschiede Arten von Mist eine hohe Biogasproduktion haben. Andere Substrate, wie zum Beispiel Olivenöl, hatten in einem Test eine hohe und im anderen keine oder nur wenig Produktion von Biogas. Für zukünftige Tests wäre es ratsam, die verschiedenen Substrate als Mischung zu testen.

Sprache: Englisch Schlagwörter: Anaerobe Vergärung, Biogasproduktion, biologisch abbaubare Abfälle, AMPTS II

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Terminology definitions and abbreviations

AMPTS	Automatic methane potential test system
Anions	Negatively charged ions
Carbohydrate	Molecule consisting of carbon, hydrogen and oxygen atoms
Cations	Positively charged ions
CNG	Compressed natural gas
COD	Chemical oxygen demand
DS	Dry matter
Facultative	Capable to function under varying environmental conditions
Filamentous	Thin, very small
Forage	Plant material eaten by grazing animals
GGP	Greenhouse gas potential
Heat content	Internal energy of a system plus the products volume and pressure
Hydrolase	Enzyme controlling hydrolysis
Incubate	To maintain optimal environmental conditions
Inhibit	Slow down or prevent
Legume	Specific type of plant (For example beans)
Lignin	Compound used by all land plants to stiffen and support themselves
Lipophilic	Having an affinity for lipids (organic compounds insoluble in water but soluble in alcohol)
LNG	Liquefied natural gas
Monomer	Small molecule capable to react with other molecules
NG	Natural gas

NTP	Normal temperature and pressure
Partial pressure	Pressure that one compound of a mixture would exert if it would occupy the volume of the mixture alone
Phytopathogen	Organism causing disease in plants
Polymer	Large molecule formed of two or more repeated monomers
Siloxane	Compound containing Si-O-Si
Tannin	Plant compound binding to organic compunds
VFA	Volatile fatty acids
VS	Volatile solids

1 Introduction

New challenges are facing the world due to environmental degradation and fossil fuel shortage. To overcome these problems, new environmental friendly ways of producing energy have to be found but also the interest in renewable energy resources has to be raised. One of many new and innovative possibilities is to produce biogas from organic waste. This biogas is a mixture of methane and carbon dioxide and can be used for heat and / or electricity production. Another possible use is to upgrade it into bio methane and use it as transport fuel.

This work is concentrating on the process of biogas production via anaerobic digestion, where bacteria break down organic material under anaerobic conditions. It was carried out for the waste treatment company Ab Stormossen Oy (from here Stormossen) located in Kvevlax. They are looking for new suitable biodegradable substrates from the region for their anaerobic production process. In Chapter 3, more information on the company is found.

To get an overview of the process of anaerobic digestion, information on the theoretical background as well as general information on different anaerobic degradable products, see Chapter 4 and 5 of this report.

In Chapter 6 one can find a detailed description of the practical part of the work including information on how the biogas tests were done as well as a discussion of the obtained results. In the tests were single substrates tested on their biogas potential. Suggestions on what can be done differently in the future are discussed in Chapter 8.

2 Objectives and scope

The main goal of this work was to find new suitable substrates for the anaerobic digestion process at Stormossen. This was done by testing at laboratory scale several different waste types from the region in small 0.5 l bioreactors called AMPTS II (Automatic Methane Potential Test System). The bioreactors are manufactured by bioprocess control and are specially designed for on-line measurements of very small biogas and bio-methane flows.

It is important for a biogas producer to understand the potential of a substrate to produce methane, which is made possible by using this equipment. For more information, see Chapter 6. Also the amount of biogas produced over time gives a good overview on how long different substrates need to stay in the bioreactor to produce the most sufficient amount of methane. It might be that they reach their peak of production after a few days and produce just small amounts after that but for a long time. In that case, it is not economically efficient to keep them in for a longer time but replace them with new substrates sooner.

2.1 **Task**

Stormossen, the waste treatment company who offered this work, is looking for new biodegradable substrates that they can use in their anaerobic digestion process. The task was to find different substrates available in the region of the company and to test them at laboratory scale on their potential to produce biogas. In addition, the DS (dry matter), pH and conductivity of the digestate were needed.

2.2 **Methods**

To meet the goals described above and write this work both theoretical and practical methods were used. The theory part contains general information about anaerobic digestion including the different process steps, important parameters and inhibiting factors, as well as the biogas and the digestate produced. Different types of waste and reactors were shortly researched as well. This theoretical information was obtained by reading different books and reports and summing up the important parts.

The practical part was done by testing different substrates from the region in small-scale bioreactors of the brand bioprocess control to obtain their biogas production and HRT (hydraulic retention time). This was the most time consuming part of the work and was done in close cooperation with Stormossen.

3 Ab Stormossen Oy

Stormossen is a waste treatment plant in Koivulahti (Mustasaari, Finland) that is owned by six municipalities in Ostrobothnia. Amongst other things, extracts the company biogas from different kinds of organic waste via anaerobic digestion. This biogas is then used to produce heat and electricity. In the wintertime is the heat used to warm up buildings like Botniahallen, an athletics-, ball- and activity-hall, while the electricity is all around the year used on the plant itself and the leftovers are sold to the electricity grid. Although for now not all of the produced gas is used, about 1/5 is flared away. For later this year it is planned to upgrade parts of the produced biogas into biofuel. This upgrading means that a higher percentage of the produced gas will be used. The priorities will be: 1) upgrading to vehicle fuel, 2) electricity production and 3) flaring. Due to the lower need for heating during the warm summer time is usually more biogas produced than it is used. This problem is going to be avoided by refining the overproduction into biofuel. The digestive produced as a by-product of the digestion is composted and sold as soil improvement for lawn and garden. (Åkers, 2015, p.8, Interview with Johanna Penttinen-Källroos, 2016)

Stormossen built their first bioreactor in 1990 and the second one followed in 1994 (Åkers, 2013, p. 6). These two bioreactors are still in operation. In the first one, bioreactor 1, sludge from wastewater treatment plants as well as fat from big kitchens are anaerobically digested while in the other one, bioreactor 2, all bio waste as well as the organic fractions from kitchen waste is digested.

STORMOSSEN

3.1.1 Bioreactor 1

The biggest part, 90 – 95% in this reactor is sludge from wastewater treatment plants. The rest is fat from both kitchens and restaurants. Of the total amount of the sludge 70 – 80%

is from Pått wastewater treatment plant in Vasa, the other part is from other smaller treatment plants near Vasa. When the sludge is transported by tanker trucks to Stormossen it has a DS of about 20%. (Öhmann, 2005, p.30, Interview with Penttinen-Källroos, 2016, Interview with Thomas Kalander, 2016)

The organic waste is stored in two different containers. In the first one, LS1, they only store the sludge from Pått wastewater treatment plant, while in the other one, LS2, they store other sludge and fat. The volume of the containers is each about 100 m^3. This amount of waste is enough for two days normal operation of the bioreactor. (Öhmann, 2005, p.31, Interview with Penttinen-Källroos, 2016, Interview with Thomas Kalander, 2016)

From LS1 and LS2 the organic waste is pumped into a homogenising unit where it is mixed with warm water that adjusts the wastes DS and temperature. The main waste comes from LS1, which is about 65 – 75%. In this homogenising unit there are also knives which shred the waste. When leaving this unit the mixture has a DS of 6 – 7%. On the way to the bioreactor 1, the mixture is still heated up to about 55 ˚C. The bioreactor keeps this temperature constantly due to its location in the bedrock. In the reactor the mixing occurs by injecting gas. The size of this reactor is 1500 m^3 and it is fed in intervals Monday to Saturday. The feeding is done by 60 min pumping and 60 min pausing, 24 hours a day. The waste stays in the reactor for about 14 days and the digestive leaving the reactor has a DS of about 5% and is then mixed with polymers. It then goes into a centrifuge to be dewatered to a DS of about 30% and is then composted. (Öhmann, 2005, p.31 - 35, Interview with Penttinen-Källroos, 2016, Interview with Thomas Kalander, 2016)

3.1.2 Bioreactor 2

As already mentioned in Chapter 3.1, are the organic fractions from kitchen waste fed into this reactor. This waste comes from private households, food stores, food processing industries, restaurants, other big kitchens as well as whole sale trade. It has to go through different steps of pre-sorting than the waste for bioreactor 1. This is because it sometimes is mixed with plastic and other waste. (Saarella, 2008, p.12, Interview with Penttinen-Källroos, 2016)

The organic waste is loaded up into a pit and from there it is transported by a slowly moving conveyer into a pre-shredder. It is mixed with hot water (70 ˚C) and pressed through a screw press with 12 mm holes which separates the plastic from the slurry. The reject from here goes to Westenergy, a waste incinerating plant in the same area. The slurry with a DS of 8 to 10% is pumped into a 150 m^3 tank in the mixerhall. From there it is pumped into bioreactor 2. (Interview with Thomas Kalander, 2016)

Bioreactor 2 has similar properties as bioreactor 1. It is placed in the bedrock, which keeps the temperature constantly at 55 ˚C and the mixing is done by injecting gas and three mixer screws. The size of this bioreactor is 1700 m^3 and it is fed Monday to Sunday, three to five tons per hour. The bio waste stays about 22 days in the reactor. The digestive leaving has a DS of about 3% and goes straight to the centrifuge for dewatering and is then composted. (Saarela, 2008, p.21, 25, 28, Interview with Thomas Kalander, 2016)

4 Anaerobic digestion

Anaerobic digestion is a complex biological process in which the chemically bond energy of organic soluble matter is converted into a more easily accessible gaseous form. This process is taking place, as the name suggests, in the absence of oxygen (O) (Murphy & Thamsiriroj, n.y., p.104). As a by-product the nutrient-rich digestate is formed. Due to the many different possibilities of substrates and reactor size that can be used, as well as the varying usage possibilities of biogas produced, anaerobic digestion is considered a very flexible technology. (Pabón Pereira, Slingerland, Van Lier & Rabbinge, n.y., p.167)

4.1 Process steps

In Figure 1 below one can see the four phases of degradation, which will be more detailed discussed in the following sub-Chapters. Depending on the phase, different microorganisms are responsible to carry out the complex process of methane fermentation. The four phases can occur simultaneously but are dependent on each other’s products.

Especially closely linked are the hydrolysis and the acidogenesis as well as the acetogenesis and the methanogenesis. (Deublin & Steinhauser, 2008, p.93)

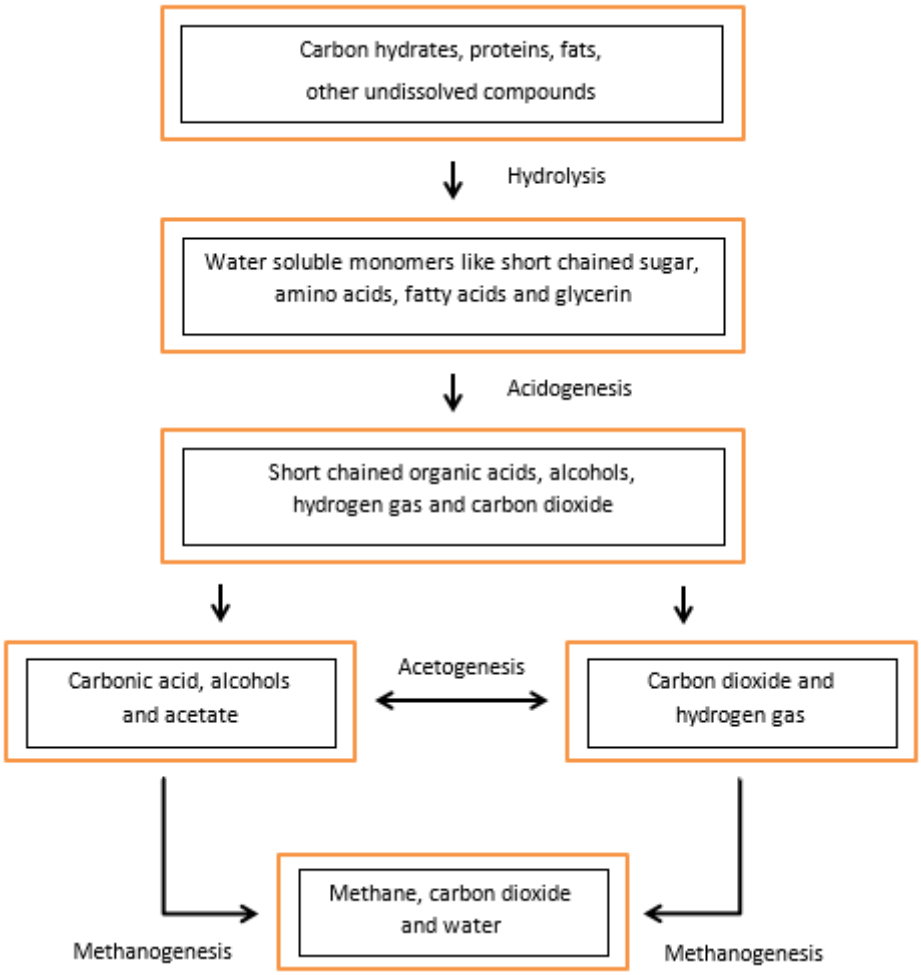

Figure 1: *Process steps of the anaerobic digestion*

4.1.1 Hydrolysis

The hydrolysis is the first phase in the anaerobic digestion, where carbon hydrates, proteins, fats and other undissolved compounds are broken up by hydrolase and anaerobic bacteria into water soluble monomers like short chained sugar and amino acids. This phase depends on how big and easily degradable polymers are in the process and needs between a few hours for carbohydrates and several days for proteins and lipids. Lignocellulose and lignin on the other hand are only slowly and incompletely degraded. This is the most time

consuming step of the anaerobic digestion and can be shortened by the pre-treatment. (Deublin & Steinhauser, 2008, p.94)

Equation 1:

$$C_6H_{10}O_4 + 2H_2O \rightarrow C_6H_{12}O_6 + 2H_2$$

Equation 1 above shows a chemical reaction of the hydrolysis in which the organic waste is broken down into a sugar, in this case glucose under the presence of water (Serena, 2009).

4.1.2 Acidogenesis

In the acidogenesis, different facultative and obligatory anaerobic bacteria are present. It is their job to degrade the monomers formed in the hydrolysis into short chained organic acids, alcohols, hydrogen gas (H_2) and carbon dioxide. Also C1 - C5 molecules are formed. (Deublin & Steinhauser, 2008, p.94) Some examples of these are propionic acid (CH_3CH_2COOH), ethanol (C_2H_5OH) and methanol (CH_3OH). Below in equation 2 a typical acidogenesis reaction is shown where glucose is converted into propionic acid. (Serna, 2009)

Equation 2:

$$C_6H_{12}O_6 + 2H_2 \leftrightarrow 2CH_3(CH_2)COOH + 2H_2O$$

4.1.3 Acetogenesis

In this phase the products formed in the acidogenesis are broken down by the bacteria available in the acetogenesis to form carbonic acid, alcohols and acetate as well as carbon dioxide and hydrogen gas. As an example of this, equation 3 below shows the breakdown of propionic acid into acetic acid, carbon dioxide and hydrogen gas. (Deublin & Steinhauser, 2008, p.96)

Equation 3:

$$CH_3(CH_2)COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2$$

The acetogenic bacteria produce hydrogen gas, but for their survival and growth the hydrogen partial pressure has to be very low. To assure this the methanogenic bacteria from the next step have to constantly remove the hydrogen gas. This is possible because the methanogenic bacteria need a higher partial pressure to live. (Deublin & Steinhauser, 2008, p.97)

4.1.4 Methanogenesis

The fourth and last step in the anaerobic digestion is the methanogenesis. This is the most sensitive step and it takes place under strictly anaerobic conditions. Different methanogenic species are available which, depending on the feedstock, degrade the acetogenic products into methane and carbon dioxide. This can be seen by equation 4 in which the acetic acid is degraded. (Deublin & Steinhauser, 2008, p.98, 99)

Equation 4:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

When the hydrogen gas from the acetogenesis is used by other organisms instead of the methanogenesis, less methane can be formed and over acidification in other processes occur (Deublin & Steinhauser, 2008, p.99).

4.2 Substrates

A substrate is an organic material that is suitable to be digested under anaerobic conditions (Deublin & Steinhauser, 2008, p.47). In general can all kind of biomass containing carbohydrates, proteins, fats, cellulose and hemicellulose as their main component be used as a substrate (Deublin & Steinhauser, 2008, p.57). Some examples could be sludge from water treatment plants, food waste as well as agricultural waste. More on different types of substrates can be found in Chapter 5.

4.2.1 Nutrition composition

Carbon, nitrogen, phosphorous, as well as micronutrients, vitamins and other trace elements are needed for the microorganisms of the anaerobic digestion to grow. Therefore should all these be available in a sufficient quantity in the substrate mixture fed into the reactor. (Carlsson & Uldal, 2009, p.9)

A central role plays the so call C/N ratio which describes the ratio of the carbon (C) and nitrogen (N) content in the substrate (Deublin & Steinhauser, 2008, p.116). Since the number of carbons is usually greater than 1 it is often written as a number (4) rather than a ratio (4/1) (House, 2006, p.35).

The exact ratio is dependent on the substrates. According to Deublin and Steinhauser (2008, p.116) the optimum range is between 16 and 25 while House (2006, p.35) says it should be between 25 and 30. These numbers are so called “non-lignin” or “available” carbons. They describe the available carbon rather than the total carbon in a substrate. However, if the carbon is bound, like for example in lignin, the substance resists breakdown and holds on to their carbon atoms. This means that they do not easily or immediately release them and the ratio therefore needs to be higher. A range between 40 and 50 might be needed that the bacteria will have 25-30 available carbon atoms for each nitrogen atom. (House, 2006, p.35, 45) For example do protein rich substrates like sewage sludge have a C/N ratio of 6 while lignin containing substrates like paper have a ratio of 173. (Deublin & Steinhauser, 2008, p.116)

According to House (2006, p.44) is the C/N ratio self-regulating, which means that in case of a too low C/N ratio ammonia (NH_3) is produced and passed off as gas. This leads to a drop in nitrogen (N). A problem with this is that in case of a too high ammonia production the bacteria in the digester are poisoned. If the C/N ratio is too high, more carbon dioxide (CO_2) will be produced which will lower the production of methane (CH_4) and thereby the heat content of the gas. In addition, the pH of the slurry will be acidic. Both too high and too low C/N ratio can slow down the process and eventually stop it. (House, 2006, p.44)

To avoid these problems one could mix different substrates to reach a C/N ratio within the limits.

4.2.2 Dry matter, volatile solids and chemical oxygen demand

The DS gives us the amount of remaining solids in a substrate after letting the water it contains evaporate at 105˚C. (Deublin & Steinhauser, 2008, p.65) The practical way of doing so as well as the calculations needed to find the DS can be found in Chapter 6.6.1. According to Deublin and Steinhauser (2008, p.65) the DS should be below 2-12% in the substrate to assure a proper mixing in the bioreactors as well as the functionality of the pumps. There are some exceptions though. Glycerol for example has a DS of 100% but does not have any problems being pumped. If these exceptions are not the case one can use material with low DS and mix it with high DS material to reach a suitable DS and improve the mechanical characteristics. (Carlsson & Uldal, 2009, p.7)

The DS itself does not say very much about the substrates potential to produce biogas but is more a preparing step for testing the VS (volatile solids). The actual VS is the part of a substrate evaporating while being burned at 550˚C. Other words for VS might also be organic matter or available matter, which describes its purpose very well. It is the "available" part of a substrate for anaerobic digestion. A general rule is that a high percentage of VS gives a lot of biogas, but one should keep in mind that if it contains many substrates like lignin, this rule does not apply. The reason for this is that lignin is burned at these temperatures but not likely to give any biogas. (House, 2006, p.25)

The COD (chemical oxygen demand) gives the amount of oxygen needed to break down a specific amount of organic material. It is used to calculate how much organic material a substrate contains. A high COD concentration gives, similar to a high VS, a high biogas yield. (Carlsson & Uldal, 2009, p.8)

4.2.3 Pre-treatment

The pre-treatment of substrates is becoming more and more common. The aim of it is to ease the digestion as well as to avoid problems with the substrate and digestate. In many cases it is a necessary step that includes separation as well as grinding of waste. For example, metal pieces would disturb the process and therefore need to be removed in beforehand. Some positive effects of this are shorter hydraulic retention times, see Chapter 4.3.6, as well as a lower energy demand for the mixing. However, these steps are in many

cases relatively energy-intense. According to Scholwin and Nelles (n.y., p.214) about 20% of the energy from biogas produced during the digestion is needed for pre-treatment. Many of the pre-treatment technologies on the marked promise a 5-20% increase in biogas yield which can lead to a higher energy demand than increase. This is a factor one should always be aware of and therefore do a careful comparison of the expected energy yield increase and the energy demand of the pre-treatment. If it is absolutely necessary one should try to optimize the energy demand as good as possible. (Scholwin & Nelles, n.y., p.214)

4.3 Important parameters

The living conditions for the organisms in any biological process are dependent on different parameters. They must be taken into consideration and controlled regularly to assure optimal efficiency of the digestion. (Deublin & Steinhauser, 2008, p.100) In this Chapter you can find the most important parameters as well as an explanation why they are important.

4.3.1 Anaerobic environment

Anaerobic stands for “living in the absence of molecular oxygen”. In the anaerobic digestion process, this is important because alternate electron acceptors must be found to replace the missing oxygen. In other words, when the carbon atoms would usually form carbon dioxide and volatile acids, they now will form methane, the actually target product of the anaerobic digestion. (Murphy & Thamsiriroj, n.y., p.109)

4.3.2 Temperature

The acidifying bacteria in the anaerobic digestion can be divided into two main groups, the mesophilic and thermophilic microorganisms. Most of them belong to the mesophilic microorganisms that work best between 32 and 42°C. Only a few are thermophilic microorganisms with an optimum temperature range between 48 and 55°C. (Deublin &

Steinhauser, 2008, p.112) There is also a third group of microorganisms, called psycrophilic, which are active at temperatures between 0 and 5°C. Very little information is known about those but there is a believe that these only digest the material without producing biogas. (House, 2006, p.31)

The optimal operation temperature in the digester is often provided by floor and wall heating systems and has a direct relation to the HRT (see Chapter 4.3.6). A general rule is that the colder the temperature, the longer the retention time. This is though also dependent on the feedstock in the reactor. In most of the modern biogas plants thermophilic temperatures are used due to many advantages: (Al Seadi et al., 2008, p.23-24)

- higher growth rate of methanogenic bacteria at higher temperature
- epidemic and phytopathogenic germs are inactivated at temperatures >55°C and retention time >23h.
- reduced retention time → faster and more efficient process
- improved digestibility and availability of substrates
- solid substrates degrade better & better substrate utilization
- less soluble oxygen in higher thermophilic temperatures → operation conditions reached more quickly
- better possibility for separating liquid and solid fractions
- ca. 50% higher rate of degradation as seen in Figure 2 below

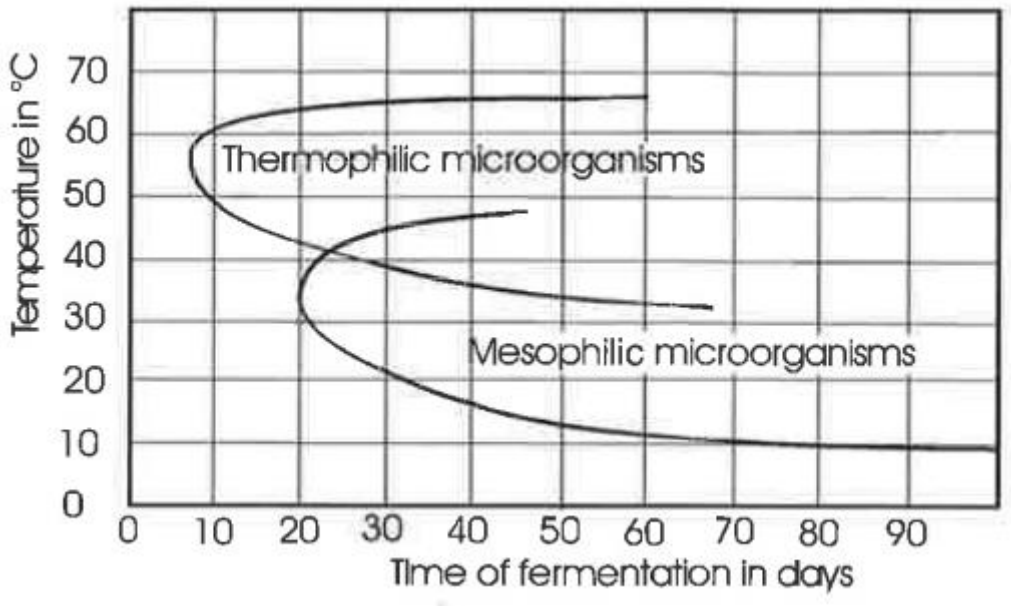

Figure 2: *Time of fermentation depending on temperature (Deublin & Steinhauser, 2008, p. 112)*

Besides those positive sides there are also some disadvantages at this process temperature:

- small variations in temperature lead to significant decrease in activity (gas loss of up to 30% when changes > +/- 2°C)
- high temperatures → high energy demand
- increased risk of ammonia inhabitation
- lower fertilizing value in sludge
- more hydrogen sulfide (H_2S) is produced

Which digestion temperature one uses in the end is decided by the amount of substrate, digestion time as well as the technical conditions. (Deublin & Steinhauser, 2008, p.112-113; Al Seadi, et al., 2008, p.23-24; House, 2006, p.32)

4.3.3 pH

The pH is a numeric value that describes the acidity or alkalinity of a liquid. It gives the negative logarithm of the oxonium-ion (H_3O^+) concentration in a solution; with other words it measures the abundance of hydrogen ions (H^+). If the value is below 7 the liquid is acidic, if it is above 7 it is alkaline. (Fischedick, et al., 2004, p.177, House, 2006, p.26)

In anaerobic reactors the pH can vary depending on the stage it is in but in general too strong or rapid variations as well as too high or low values are damaging to the microorganisms. Especially the methane forming bacteria are sensitive to changes which means if there are problems in the process it affects them first. If the methane forming bacteria die and the other processes continue are different acids formed instead of biogas. Those then accumulate in the reactor and the pH lowers. This explains why the pH is lower in the beginning of the process; the methane forming bacteria still need to stabilize. However it cannot be too low otherwise the bacteria will die. (House, 2006, p.26) According to Deublin & Steinhauser (2008, p.114) is the optimum pH between 6.7 and 7.5. If it is below 6.5 the production of organic acids leads to even further decrease in pH and possible to a stop of the process. Too high pH values are usually not a problem due to the systems self-regulation but it is still preferred to be below 7.5. (Deublin & Steinhauser, 2008, p.114) Different opinions to an ideal pH exist. For example according to Al Seadi (et al., 2008, p.26) the optimum interval is between 6.5 and 8.0 while House (2006, p.26) says it is between 6.8 and 8.5. Since the pH is a logarithmic value is 8.5 ten times more alkaline than 7.5, with other words the difference is large.

Anaerobic digestion has several different buffer systems for the pH. A natural pH regulation is done by the generators ecosystem itself (Deublin & Steinhauser, 2008, p.114).

A too low pH can occur when too much CO_2 is dissolved in the slurry. This is a normal process in the anaerobic digestion but if too much is dissolved the slurry will become more and more acidic and with that the pH will fall. If the pH is too low, CO_2 is giving of via the biogas instead and the pH is rising again. (House, 2006, p.26)

One thing that helps against rising pH is that dissolved CO_2 forms carbonic acid which ionizes. Another buffer system is the so called ammonia-ammonia system which involves that at falling pH ammonium ions (NH_4^+) are formed while releasing hydroxyl ions (OH^-). At rising pH more free ammonia molecules are formed. (Deublin & Steinhauser, 2008, p.114)

Non-natural ways of regulating excessive acidification during anaerobic digestion are for example by adding neutralization substances like sodium carbonate (Na_2CO_3) or adding diluting water. Another option is to stop the substrate supply so that the methanogenic bacteria are able to degrade the acid. (Deublin & Steinhauser, 2008, p.115)

4.3.4 Conductivity

The conductivity is a parameter that is easily measured and gives the salinity of a solution. It can be calculated from the individual contribution of the ions to the electrical conductivity. Different ions contribute to different amounts of conductivity as well as certain ions conductivity are dependent on the liquids pH. It is measured by sending a currency between two electrodes in the liquid and measuring the resulting voltage. In this process, the liquid acts like an electrical conductor for cations that then migrate to the negative electrode and anions that go to the positive electrode. The unit of conductivity is Siemens per meter (S/m). (Levin & Hultman, 2008, p.9-11)

Problem in the anaerobic digestion with a too high conductivity is that it will lead to corrosion and damages the equipment. One can for example use the measured conductivity to determine the need for precipitation chemicals when cleaning the equipment. (Levin & Hultman, 2008, p.13)

In the anaerobic digestion, the conductivity varies with the state of the reactor and the process taking place. It is common that the conductivity is increasing during digestion because the ions are dissolved out of the sludge as well as the decomposition of organic material gives rise to the concentration of for example ammonium and bicarbonate. This means the ion content is increasing. In general, one can say that controlling the conductivity during anaerobic digestion is a good way to check the progress of digestion (Levin & Hultman, 2008, p.19, 32&37)

4.3.5 Volatile fatty acids

VFA (Volatile fatty acids) are defined by having a carbon chain with up to six atoms, like for example acetate or lactate. Those are not available in the process of anaerobic digestion

from the beginning, but produced during the acidogenesis. If there are instabilities during the process of anaerobic digestion it can lead to accumulation of VFA inside the digester which then results in a drop of the pH-value. Due to the buffer capacity of the digester (see Chapter 4.3.3) the accumulation might not be expressed as a drop in the pH. In addition, if the substrates have a surplus of alkalinity the VFA accumulation need to exceed a certain level before it is detected. By the time it is possible to detect it via a significant decrease in pH the concentration of VFA in the digester would be so high that the process would be already severely inhibited. (Al Seadi, et al., 2008, p.26) One way to avoid this is for example to increase the loading rate only very slowly. (Deublin & Steinhauser, 2008, p.121)

4.3.6 Loading rate and hydraulic retention time

If one would like to achieve a complete digestion of the substrates fed into the bioreactor, a long HRT as well as a big digester would be required. This is why in practice a compromise is made between getting the highest possible biogas yield and having a justifiable plant economy. (Al Seadi, et al., 2008, p.28)

If a reactor is continuously fed one needs to consider the loading rate, with other words the amount fed into the reactor as well as the HRT, which is the average time a unit volume of feedstock will stay in the reactor. The loading rate one gets by dividing the weight of VS loaded into the reactor each day with the volume of the reactor. A larger loading rate can only be handled by a well-established population of biogas bacteria as well as a nearly continuous feeding. If this is not the case, the reactors pH falls and it shuts down.

The HRT is mainly in large-scale reactors important. If one feeds the reactor continuously and the substrates stay longer in it, the reactor needs to be bigger and due to that it will be more expensive. If one removes the digestate form the reactor, one also removes part of the bacteria. This can, if the HRT is too low, lead to an unstable population of bacteria. (House, 2006, p.50) In other words, the amount of microorganisms removed via the digestate from the reactor should not be higher than the reproduced microorganisms. This is why one should keep in mind that the duplication rate of anaerobic bacteria is 10 days or more. (Al Seadi, et al., 2008, p.28)

In general, the idea of continuously fed reactors is to get as much biogas out of the feedstock in as short of a time as possible. If one looks for example at sewage, it produces in the first 15 days about half the amount of what one would get out during 90 days. (House, 2006, p.50)

Figure 3 below shows the influence of temperature and time on the biogas production. It can be clearly seen that depending on the temperature, also the time needed to digest as much as possible changes. With a temperature of 50 °C it takes much less time to produce the maximum amount (about 95%) of biogas, compared with 20 °C where the maximum lies at about 80%. (Al Seadi, et al., 2008, p.24)

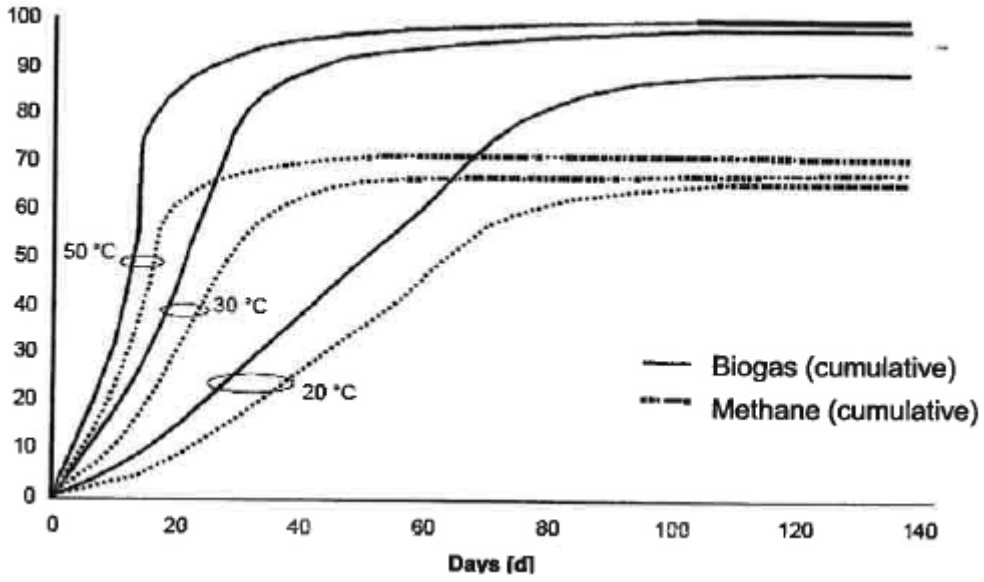

Figure 3: B*iogas* production in percentage according to temperature and time (Al Seadi, et al., 2008, p.24)

4.3.7 Agitation

During anaerobic digestion, some kind of agitation is useful due to several different reasons. This is usually done by mechanical operated devices moving inside of the bioreactor. Other mixing options, like by rising gas bubbles, are usually not sufficient enough for bigger biogas plants. (Deublin & Steinhauser, 2008, p.254) It is important that there is a good contact between the substrates and the bacteria digesting them. Continuous stirring helps with this and supplies the microorganisms evenly with nutrients as well as the products of their metabolism are removed. Also if fresh substrates are mixed in they

need to be put in contact with the digesting bacteria, which is done via stirring. (Deublin & Steinhauser, 2008, p.111)

If the mixture in the reactor is well stirred it can lead to an increase in biogas production of 10 to 15 percent. This is though not the only reason for the agitation. Other positive effects are the control of scum production, the release of CO_2, the maintenance of a proper pH, as well as the temperature is spread more evenly through the reactor. One should keep in mind that the agitation needs extra equipment and energy. The energy needed should exceed the energy gained by the process; otherwise one is producing energy at a loss. (House, 2006, p.52)

4.4 Inhibiting factors

Several different compounds formed as products of the metabolism of anaerobic digestion can slow down the biogas production or even stop it. Also the products of one step can affect the other steps negatively (see Chapter 4.1). However, the different living microorganisms can usually adapt to the inhibitors, even if they exist in toxic concentrations. (Deublin & Steinhauser, 2008, p.118)

One of those is oxygen. For most of the acidifying bacteria is an exclusion of oxygen not absolutely necessary, but the methanogenic bacteria are in the need of anaerobic conditions and start to be inhibited at 0,1 mg/L. They need to take hydrate, carbonate or sulfate instead of oxygen as the hydrogen acceptor. Also sulfur compounds might be bad for the process. If they are present H_2S is formed in the step before methane formation and inhibits the process since the sulfate bacteria dominate the methane forming bacteria. (Deublin & Steinhauser, 2008, p.119)

Organic acids are normally always present in the substrates and are decomposed during the methanogenesis. They can have an inhibiting factor because they penetrate as lipophilics into cells and denaturate the cell proteins. (Deublin & Steinhauser, 2008, p.121)

Usually nitrates are denitrified in the first stage of decomposition. However if the substrates have a too high nitrate content or the denitrification is not working properly they have an inhibiting factor on the methane formation. (Deublin & Steinhauser, 2008, p.122)

Ammonium as well as ammonia are results from the degradation of nitrogen in the process. Ammonia has an inhibiting effect and can with larger concentrations even be toxic, while ammonium is leading to potassium loss of the methanogenic microorganisms. (Deublin & Steinhauser, 2008, p.123)

Heavy metals at higher concentrations can have a toxic effect on the process of anaerobic digestion. While they stimulate the activity of bacteria at lower concentrations, they are poisonous at higher. For example pig slurry can contain high zinc concentrations from feed where often some kind of zinc additive is used as antibiotic. Other inhibiting substances can be disinfectants, herbicides and insecticides as well as other antibiotics. High amounts of tannin which can be found in many legumes can inhibit the methane formation. (Deublin & Steinhauser, 2008, p.125)

Another important factor is foaming. If filamentous microorganisms are poorly degraded they hinder the gas discharge which affects the anaerobic sludge to attain a foamy consistency. (Deublin & Steinhauser, 2008, p.127)

4.5 **Biogas**

The production of biogas is the main reason for anaerobic digestion with the aim to get as much out of the feedstock as possible. The main compounds of raw biogas are varying amounts of methane, which is mainly taking in consideration when determining its properties, and carbon dioxide, but also impurities like H_2S or ammonia (NH_3) are part of it. (Deublin & Steinhauser, 2008, p.49 & 55)

4.5.1 Composition

As already written above biogas is a mixture of several different gases. The most important gas in the mixture is, due to its energy availability, with 55 to 70% methane (Deublin & Steinhauser, 2008, p.50). The only other energy source of biogas is the hydrogen gas, which can be directly compared with the combustion of methane. Since it is only available in small amounts, it is not considered separately. (House, 2006, p.85)

The availability and amount of the different compounds found in raw biogas depend on different factors, mainly the substrates and the plant type. Impurities in the gas consist typically only in small amounts but can still have a negative effect on the gas value. (Petersson, n.y., p.329) Typical compounds and their amounts as well as negative effects on the gas value as well as the reasons for it can be found in Table 1.

Table 1: *Typical compounds found in biogas and their effects on the gas value as well as content and cause*

Component	Content	Effect	Cause
Carbon dioxide	25 – 50 Vol%	Lowers heating value, corrosion, damages alkali fuel cells	High C/N, low pH, O_2 contamination of slurry, in the beginning of digestion process as well as with any disturbances of it
Hydrogen sulfide	0 – 0.5 Vol%	Corrosion, sulfur dioxide (SO_2) emission, spoils catalysts	Low pH, rise in temperature, any disturbances of digestion process, protein or sulfate in substrate, long digestion time
Ammonia	0 – 0.05 Vol%	Nitrogen oxides (NO_x) emission, corrosion	Low C/N, thermophile temperatures
Water vapor	1 – 5 Vol%	Corrosion, condensation, risk of freezing equipment	Increases with temperature increase
Dust	> 5 µm	Blocks nozzles and fuel cells	---
Siloxanes	0 – 50 mg/m^3	Act like abrasive and damages equipment	Siloxanes in substrate

(Deublin & Steinhauser, 2008, p.52; House, 2006, p. 86-87)

The exact composition of the gas depends on several different factors and can be controlled only partly. Carbon dioxide for example is formed in the different steps of anaerobic digestion and its amount depends on many different factors but general one can say that too high or low amounts are typically a sign for flaws in the process. Hydrogen sulfide on the other hand is mainly formed by bacteria available in the digester that reduce sulfate-rich substrates. (Petersson, n.y., p.331) Siloxanes are mainly found in the gas produced of sewage sludge. They are present in cosmetics, detergents, and building materials and hence be found a lot in waste water. (Deublin & Steinhauser, 2008, p.56)

4.5.2 Properties

In general one can say that biogas is similar to NG (natural gas) in its properties since both of the gases contain a high amount of methane. If the methane content in biogas is higher than 45% it is flammable and if the air contains 6-12% biogas there is a risk of explosion. Other exact properties, like the energy content and density are dependent on the purity of the gas. According to Deublin and Steinhauser a biogas with 55-70% methane, 30-45% carbon dioxide and traces of other gases has an energy content of 6,0-6,5 kWh/m^3 and a density of 1,2 kg/m^3 (NTP). (Deublin & Steinhauser, 2008, p.49-50)

The GGP (greenhouse gas potential) of methane is 23 times greater than the one of carbon dioxide, which means that it is very important to avoid emissions of biogas into the air. (Petersson & Wellinger, 2009, p.4.)

To reduce the risk of explosion one must avoid explosive mixtures as well as eliminate sparks. The risks of explosion are much higher when air is leaking in a space filled with biogas than vice versa. Biogas is explosive only when mixed with the right amount of air but not when it is diluted in air. To avoid this one should always keep a higher pressure inside the biogas system and storage tanks so no air can leak into it. (House, 2006, p.176)

Biogas can be stored in different ways. The low-pressure biogas holders are the most common types. Medium- and high-pressure biogas holders are smaller in size but due to the explosion risk specific safety regulations must be followed. (Deublin & Steinhauser, 2008, p.330)

4.5.3 Upgrade

If biogas is upgraded the carbon dioxide it contains will be removed which leaves us with bio methane. This is done to increase the volumetric energy content in the gas. As already mentioned in Chapter 2.4.1 is methane the main energy source in biogas and directly proportional to the gas energy content. When biogas is upgraded new possibilities for its use are generated, however the production costs will rise. Several different techniques of upgrading biogas exist today which are permanently improved as well as new once are developed. (Petersson & Wellinger, 2009, p.4.)

Besides the upgrade of biogas also a cleaning can be done, which separates undesired gas compounds so the gas becomes more pure. This is typically done as a primary step to upgrading and is defined by the composition and the origin of the raw biogas as well as the upgrading technology. In the cleaning step H_2S and water vapor are in the main focus but also other trace components are removed. (Beil & Beyrich, n.y., p.344 - 346)

4.5.4 Usage

Biogas is mainly combusted to produce heat, electricity or both. If the heat is produced on site, it can partly be used to maintain the temperature in the digester but even small plants will have an excess that for example can be used to warm nearby buildings and heat up water. Also to generate electricity from biogas is a good option. It is pretty straightforward and can therefore be the most profitable use. (The Official Information Portal on Anaerobic Digestion, Biogas, 2016)

Combined heat and power (CHP) production is another way of using biogas. Since the process of anaerobic digestion requires some heat is a CHP plants efficiency with 80% a lot higher than a coal power station with an efficiency of 34%. A typical ratio of heat to power is 35-40% electricity and 40-45% heat, while the rest is lost in various stages of the process. At a 60% methane content in the biogas this will give 2 kWh electricity and 2.5 kWh of heat per cubic meter. (The Official Information Portal on Anaerobic Digestion, Biogas, 2016)

If the biogas has been upgraded it can be directly injected into a gas grid. In general, it needs to be cleaned, dried and upgraded to a methane content of around 95%, depending on the country, so it is similar to NG. Another way to use upgraded biogas is as transport fuel. The bio methane can fuel any vehicle designed to run on CNG (compressed natural gas) or LNG (liquefied natural gas). It is considered a renewable transport fuel due to its extremely low emission of local pollutants compared with petrol or diesel. (The Official Information Portal on Anaerobic Digestion, Biogas, 2016)

Even Gas-Otto engines exist that can run on biogas with a minimum of 45% methane. These motors are useful at the start-up of a biogas plant when the heat is used to heat up the digesters.

4.6 Digestate

During the process of anaerobic digestion not only biogas is produced, see Chapter 4.5, but also a nutrient rich substance called digestate. The same kind of nutrients that the substrates fed into the reactor contain can also be found in the digestate. Therefore if one wants to produce high quality digestate, which for example is used as food plant fertilizer, one also needs to use high quality feedstock (Al Seadi, Rutz, Janssen & Drosgn, n.y., p.27). The organic compounds contained in the feedstock are altered by bio-chemical changes during the anaerobic digestion which leads to an increased availability of them to the crops. (Lukehurst, Frost & Al Seadi, 2010, p.8)

4.6.1 Properties

As already mentioned above, the properties of the digestate are dependent on the substrates used in the reactor. It basically consists of dead micro-organisms as well as indigestible material. In general one can say that the nutrients in the digestate are the same as in the feedstock but do exist in a more concentrated form. The nitrogen, phosphorus and potassium present in the feedstock are not present in the biogas and will remain in the digestate. It therefore is very suitable to be used as fertilizer of agricultural soils. (The Official Information Portal on Anaerobic Digestion, Biogas, 2016)

The feedstock can occasionally contain different amounts of heavy metals like lead or cadmium and persistent organic compounds as well as small amounts of micro-nutrients. These are dependent on the substrates used and not biodegradable and thus found in the digestate. For example animal manure contains heavy metal which is introduced though the animals diet. Those must be carefully monitored and are not allowed to exceed the legal limits. (Lukehurst, Frost & Al Seadi, 2010, p.6-7)

Besides the nitrogen can the digestate also contains phosphorous (P), potassium (K) and magnesium (Mg) which are important nutrients for plants. The amount of these and other components found in the digestate can be found in Table 2. There one can see the different amounts contained in the digestate of food waste feedstock vs slurry feedstock. The numbers are derived from two different biogas plants with duplicate measurements,

however the slurry feedstock was derived from a fewer number of measurements. One should keep this in mind when comparing them directly.

Table 2: *Different properties* and c*ompounds found in food waste feedstock vs slurry feedstock*

	food waste feedstock			**slurry feedstock**		
	Mean	**Min**	**Max**	**Mean**	**Min**	**Max**
DS (%)	4,5	2,7	6,8	4,9	3,5	9,3
VS (%)	69,0	68,3	69,6	73,2	73,2	73,2
pH	8,4	8,3	8,4	8	7,6	8,8
Nutrient content in percentage						
Nitrogen, N (%)	15	11,9	20,5	16,1	6,7	24,9
Readily available N (% of total N)	61,9	38,7	86,8	65,4	39,3	85,6
Potassium, K (%)	4,7	1,4	9,3	3,2	1,5	5,9
Phosphorous, P (%)	0,7	0,3	2,0	0,9	0,2	5,0
Calcium, Ca (%)	0,34	0,0	1,70	2,6	0,0	4,8
Magnesium, Mg (%)	0,19	0,0	0,69	0,3	0,0	3,7
Sulfur, S (%)	0,33	0,0	0,57	0,9	0,0	1,7
Heavy metal content in milligram per kilogram						
Copper, Cu (mg/kg)	31,5	18,6	24,6	82,1	20,3	180,7
Zinc, Zn (mg/kg)	105,1	71,0	142,3	240,0	4,4	631,0
Lead, Pb (mg/kg)	46,3	3,6	114,7	1,0	0,0	17,9
Cadmium, Cd (mg/kg)	1,2	0,2	2,2	1,5	0,6	2,3
Mercury, Hg (mg/kg)	1,1	1	1,1	0,1	0,0	0,6
Nickel, Ni (mg/kg)	43,2	5,5	137,3	8,6	0,0	18,8
Chromium, Cr (mg/kg)	50,2	7,8	157,5	12,4	0,3	38,2
Fluorine (F)	209,5	200,0	219,0	118,0	118,0	118,0
Aluminum (Al)	-	-	-	4141	131	11812
Iron (Fe)	-	-	-	14059	1551	37701

(Rigby & Smith, 2011, A1-A9)

In the upper part of Table 2 one can see the DS, VS and pH of the different feedstock. The pH was independent of the feedstock between 7,6 and 8,8 while the DS was more varying from 2,7 (min of food waste) to 9,3% (max slurry). The VS is equivalent to the organic matter content of the digestate and has a content around 70%. This means there is a potential to use it as fertilizer.

In the second part of Table 2 one can see the nutrient content. There is only a slight difference of the average nitrogen content between the food waste (15%) and slurry (16%). Also one can see that between 62 and 65% of the nitrogen was in available form, easily to be taken up by the plants.

When looking at heavy metal one can notice that especially zinc and fluorine are with over 100 mg/kg noticeable high in both feedstocks. In addition, aluminum and iron found in the slurry feedstock are noticeable high, up to 11812 mg/kg and 37701 mg/kg respectively. (Rigby & Smith, 2011, p.6-7)

4.6.2 Usage

In general the digestate is used as fertilizer. Compared with synthetic fertilizer, which is derived from NG, one can save energy, reduce the carbon footprint as well as cut the fossil fuel consumption using digestate instead. Another positive affect is the more available form of the nutrients in the digestate compared with the slurry, which means that it is easier for the plants to make use of them. (The Official Information Portal on Anaerobic Digestion, Digestate, 2016) This is due to the changes the organic compounds undergo during the anaerobic digestion. For example are some parts converted into for the plants more available form of ammonium (NH_4^+) but do not affect the overall nitrogen content. (Lukehurst, Frost & Al Seadi, 2010, p.8)

The fertilizers made of digestate show positive effects on crop yield and soil quality, however when being applied to the field some ammonia volatilization will take place (Lukehurst, Frost & Al Seadi, 2010, p.8). Currently tests are done to investigate the positive sides of using digestate as fertilizer, but also to see how it effects the greenhouse gas emissions from soil and the accumulation of heavy metals in the soil. Different

spreading methods to reduce ammonia volatilization are being tested as well. (Odlare, 2014-2017)

Another way of using the digestate is to market them for home garden use. To achieve the same purpose as the already existing granular multi-purpose fertilizers, as well as to pack them in a more suitable way, one would need to dry them first to produce pellets or granulates. The procedure of drying would also improve the stability of the digestate and reduce the odor. (Rigby & Smith, 2011, p.8)

However, according to the European Biogas Association the drying of digestate reduces its nitrogen content drastically and it thus has a reduced fertilizing effect (2013, p.1). Better ways of separating the solid fraction into liquid would be centrifuges or presses.

Other uses for digestate with an unsuitable quality for agricultural use would be to use it as a landfill cover, for energy production or as raw material for industrial processes to name a few. (Al Seadi, Rutz, Janssen & Drosgn, n.y., p.28)

4.7 Different type of reactors

Today are different kinds of reactors on the market which can be constructed and grouped in different ways. The main difference is between batch wise and continuous feed reactors. When deciding which type of reactor to use one should think about the level of available technology and the availability of substrates.

4.7.1 Continuous digestion

Different types of continuous fed reactors exist, but the principle behind them is the same for all. A digestion process is started and kept going while continuously feeding new substrates to it and removing digestate from it. The theory behind this is that new material is fed into the reactor in the same pace as existing substrates, done producing biogas, are removed as digestate. This leads to a constant volume of digestible material in the reactor. With this technique, one avoids the changes in biogas production, which is typical for batch wise digestion. In addition, the often critical startup, including the time consuming

hydrolysis is avoided. One drawback with this method is that one needs to have a constant access to new substrates. (Appels, et al., 2008, p.760)

4.7.2 Batch wise digestion

The principle of a batch wise digestion is to completely fill the reactor at one single time and then let the bacteria digest the substrates and produce biogas without adding any new substrate or removing any digestate. When one decides that the digestion is ready and wants to stop the process, one removes all the digestate at once. These reactors can handle a lower load compared with continuous fed digesters and therefore need to be bigger in size. They are usually found on farms, where the farmers can take care of the digestion and the reactor by themselves. In this way of digestion, the biogas production will vary on the stage of the process. Starting slowly, it will reach the maximum production at about half time and then start decreasing again. When emptying the reactor a small amount of inoculum is kept to provide the new load with bacteria. (Deublin & Steinhauser, 2008, p.243)

4.7.3 Digestion in several steps

Since the process can be divided into several steps with different requirements on the surrounding, it is also possible to have them happen in different reactors. An example for this is a hybrid reactor in which the acid forming and the methane forming stages are separated. The first stage happens usually in a bigger, colder reactor in which fatty acids are formed. These are then used in a second smaller and heated reactor where methane is formed. The first reactor can be unheated because the bacteria in this step are less sensitive. In addition, it is bigger due to the more time consuming hydrolysis happening there. Both continuous as well as batch wise digestion can be divided into two steps. Positive with this is the smaller energy consumption due to less need for heating. (House, 2006, p.142)

5 Different waste types

In this Chapter different types of waste are discussed. Short definitions as well as typical DS and VS for most of these can be found. Also some tips of using those for biogas production are included.

5.1 Food Waste

Food waste is usually obtained as “bio waste” from households, restaurants, big kitchens as well as stores. This type of waste needs a pre-treatment including shredding, separating plastics and metals from it as well as mixing with water. In general it has a high biogas production but its quality is dependent on the sorting and pre-treatment. Good sorted food waste has generally a high amount of easily biodegradable organic waste which increases the risk of sinking pH and accumulation of fatty acids (see Chapter 4.3.3 and 4.3.5). A typical DS is between 30 and 35% with a VS of about 85%. (Carlsson & Uldal, 2009, p.11)

5.2 Slaughter waste

In slaughterhouses there are four different types of biodegradable waste. These are sludge from wastewater cleaning, slaughter waste, manure as well as waste from stomach- and bowel-cleaning. The soft waste parts contain much protein and are due to this nitrogen rich. However a pre-treatment including grinding and separation is necessary before going into the bioreactor because it can contain for example stomach-magnets, ropes, metal and other waste from the slaughter process. Animal fat for example has a DS of 9% with a VS of 92%.

This is in general a good waste for anaerobic digestion due to its energy-richness and high biogas potential. However it is less suitable to be used alone due to its high amount of fat and proteins which can affect the biogas process negatively. The fat can for example lead to accumulation of fatty-acids and a sinking pH, while the proteins can lead to a too high amount of ammonia that slows down the methane production (see Chapter 4.4.). A better

way of using this kind of waste is to use it as a mixing agent in a substrate mixture with too low nitrogen content. (Carlsson & Uldal, 2009, p.11, 24)

When dealing with slaughter waste one needs to keep in mind that that waste type is covered by the regulation (EC) of the European parliament and of the council (No 1774/2002, p.53). This regulation is laying down health rules concerning animal by-products not intended for human consumption. It regulates possible uses and processing rules of animal by-products and looks at which once are an increased risk for public health, animals or environment. For example, the intestines of bovine animals of all ages are in category 1, which means they must not be processed in biogas plants.

5.3 **Egg waste**

In the egg industry there are two kinds of waste. One of them are the egg shells (DS = 82% of which VS = 9%) which contain a high amount of DS, some nitrogen, calcium, magnesium and phosphorus. These shells are not really suitable for anaerobic digestion because they go unaffected through the process and can influence it badly by leading to a mechanical stop of the plant. The other part is from eggs which are sorted out as well as unwanted pasteurized egg liquid (DS = 17% of which VS = 94%). This kind of waste is high in protein content and has a high biogas potential. (Carlsson & Uldal, 2009, p.11 - 12)

5.4 **Fish industry waste**

The bi- and waste-products from the fish industry include waste from gutting, discarded fish as well as sludge from cleaning facilities including dirty rinse water. The sludge is the most important one in the biogas production since the other waste is usually used as animal feed and for fish food production. Fish waste contains high amounts of nitrogen which can lead to inhabitation due to its high amounts of ammonia in the process. Other problems can be that the DS-amount varies a lot and to some extent the smell. A general DS is at 42% with a VS of 98%. (Carlsson & Uldal, 2009, p.12, 24)

5.5 Waste grease or fat

Another kind of source is the grease or fat, which can be from private households as well as dripping and chip fat. The fat is not suitable for co-digestion due to high costs for maintenance and cleaning. However it is a good energy source if digested separately. (Deublin & Steinhauser, 2008, p.74) This might be to its high DS of 90% of which 100% is TS. (Carlsson & Uldal, 2009, p.24)

5.6 Bakery waste

The rest products from bakeries can be flour, dough, discarded bread, wrong mixed dough as well as returned bread. The waste is usually a relatively clean product and has generally a high organic fraction that degraded relative quickly. There might be variations in the consistence, size, DS, chemical composition and nutrition value but in general, all the waste has a high biogas potential. For example, bread and dough have a DS of 61 and 67% of which 87 and 90% are their VS respectively. (Carlsson & Uldal, 2009, p.12, 24)

5.7 Dairy industry waste

Separated fat sludge, limit milk and whey with a DS of 7, 0,5 – 2 and 6% respectively are the main by-products of the dairy industry. The whey and limit milk are used as animal feed while the fat sludge from internal cleaning facilities is either spread on fields or used for biogas production. This sludge has a high fat content, which gives it a high biogas potential. Its low nitrogen content and alkalinity makes it difficult to be used as a single substrate in biogas production and is thus often digested with other waste. (Carlsson & Uldal, 2009, p.12)

5.8 Ethanol and starch industry waste

Stillage is produced as a rest product in breweries and distilleries. It has a DS of 8% with a VS of 93%, which makes the storage and transportation expensive and inefficient. The stillage's nutrition composition is dependent on the raw materials used. Regarding the hygiene it is a high quality natural product with a low content of toxics. (Carlsson & Uldal, 2009, p.12)

The by-products in the starch industry are high amounts of juice and pulp with a large organic content and a high biogas potential. Other substrates are glycerol and molasses, which have a high DS and VS content and due to that a high biogas yield per ton wet weight. The glycerol should not be digested alone due to a low nutrient content. (Carlsson & Uldal, 2009, p.12)

5.9 Pulp and paper industry waste

The most common waste product in the pulp and paper industry is fiber sludge, bio sludge and return paper sludge. Because of the high fiber content in the others is only bio sludge suitable for digestion. About 50% of the bio sludge are nowadays burned, 30% composted and 20% recycled or used in other ways. The DS of the bio sludge varies between 2% and 100%. (Carlsson & Uldal, 2009, p.15)

5.10 Crops and crop residues

Basically all kinds of crops can be used to produce biogas. They have a high amount of biodegradable material as well as a high gas potential. If the nutrients in the crops are low they will need to be mixed with other products to work properly in the anaerobic digestion. Commonly all the crops need some kind of pre-treatment, which includes shredding and mixing. For example forage crops contain many fibers which have to be decomposed before the digestion. Crops with high lignin content and crude fiber content are digested slowly and incomplete and need to be shredded before the digestion or need to stay a long time in the reactor to have an as high as possible biogas production. (Carlsson & Uldal,

2009, p.14) When harvesting crops for biogas production one should keep in mind that older crops have an increased cellulosic content and are therefore less digestible and have a lower methane yield. Compared with other annual crops do beet crops have a 30-40% higher biomass yield per hectare.

Besides the normal crop residues there are also crops especially dedicated for energy production. Due to this farmers started to shift from food and feed to energy producers. They now grow on their fields so called energy crops, mainly maize. The environmental sustainability of this behavior is due to high energy input for harvesting and transport as well as the high amount of fertilizers and pesticides spread on the fields negative. (Al Seadi, et al., 2013, p.24-27) The DS of these is very much depending on what it is. Maize for example has a DS of 30% while wheat has about 86% DS with a VS of 90% and 98% respectively. (Carlsson & Uldal, 2009, p.25-26)

Vegetable and fruits, agricultural by-products and harvest residue as well as plant residues are low quality crops due to a DS of 15% with a VS of 95% (Carlsson & Uldal, 2009, p.24). Those substrates are usually only used in co-digestion and need a pre-treatment to break the lingo-cellulose molecules to allow a better access of anaerobic microorganisms. (Al Seadi, et al., 2013, p.24)

5.11 **Manure and slurry**

All in all it is estimated that the yearly production of manure and slurry worldwide is 13 billion tons. Spreading all this on agricultural land will lead to water pollution and air emission, while using it for anaerobic digestion it will produce biogas and digestate instead. The characteristics of manure (10-30% DS) as well as slurry (below 10% DS) are dependent on the species of origin as well as the quality of animal feed. (Al Seadi, et al., 2013, p.22-23) Generally one can say that the feed supplements fed to the animals are still to some part available in the manure and due to this give the anaerobic digestion process important minerals and nutrients (Carlsson & Uldal, 2009, p.14). Manure and slurry contain straw and fiber particles that are high in cellulose and are due to that not economical suitable for mono-digestion (Al Seadi, et al., 2013, p.22-23). Other unwanted contents can be sand, sawdust, soil, skin, bristles, hair, feathers as well as cords, wires plastics and stones (Deublin & Steinhauser, 2008, p.62).

Manure has a high C/N-ratio of about 25 and is rich in different nutrients necessary for the anaerobic microorganisms to grow. In addition, it has a high buffer capacity in case of a significant pH decrease in the digester, see Chapter 4.3.3. (Al Seadi, et al., 2013, p.23) Even though the slurry has a low DS and with this a low methane yield per unit volume, it is according to Deublin and Steinhauser (2008, p.57) one of the main substrates in agricultural co-digestion biogas plant.

5.12 **Sludge**

Sludge can be from different kind of industrial processes from for example breweries, slaughter houses and medication industries but also from wastewater cleaning facilities which will be shortly discussed below. Sludge has in general a low degradability because it is in most cases already partly degrading in earlier cleaning stages. Due to this the VS-reduction only lies at about 50% and a big part of the nitrogen is found as organically bound nitrogen. (Carlsson & Uldal, 2009, p.15)

Two different kind of sewage sludge exist. One is the primary sludge, which is from the pre-purifier and the other one is called excess sludge which is from the final clarification basin. (Deublin & Steinhauser, 2008, p.71) In many modern wastewater treatment plants it is common to have an anaerobic digestion technology installed to treat the produced sewage sludge. This sludge has a similar methane potential as animal slurry but contains, due to its origin, a high amount of biological and chemical pollutants. These pollutants are present in the digestate as well and are the reason why it is in many countries illegal or strictly limited to be used as agricultural fertilizer. Sludge is often used for co-digestion to improve the biogas yield and stability of the process. (Al Seadi, et al., 2013, p.32)

5.13 **Algae**

Algae are another source of substrate. The macro algae are rich in natural sugars and other carbohydrates and known for their high biomass yield. However micro-algae, especially green micro-algae are said to have a higher yield of methane potential than other energy plants. (Al Seadi, et al., 2013, p.32-33)

The positive side of algae is that they can be easily brought up in simple basins due to their ability to use atmospheric CO_2 and sunlight for their photosynthesis. (Deublin & Steinhauser, 2008, p.74)

6 Practical tests

In this Chapter one can find information about the practical tests done. The aim was to test different substrates on their biogas potential to see which once are beneficial in the digestion process. These tests were done with the AMPTS II from bioprocess control, seen in Figure 4 below.

Figure 4: *The AMPTS II*

6.1 Bioprocess Control

Bioprocess Control is a market leader in the biogas industry. It is a Swedish company producing advanced instrumentation and control technology for research and commercial applications in the field of biogas production. It was founded in 2006 and today exports their products to more than 40 countries. The AMPTS II, which was also used in this

practical part of the thesis, is one of the most preferred analytical instruments for analyzing the methane potential around the world. (Bioprocess control, 2015)

6.2 Methane potential test

A methane potential test is generally done to get a preliminary understanding of the biodegradability of a substrate and its methane potential through anaerobic digestion. To test this at laboratory scale the AMPTS II of bioprocess control was used. The AMPTS II is suitable for on-line measurements with very small biogas and bio-methane flows and gives a good understanding of the different substrates methane potential. Also it is a fully automatic methane potential testing device which is less time and labor-intensive than traditional ways of anaerobic digestion. (Bioprocess control AMPTS II, 2014, p.2)

6.3 Principle

The normal procedure to test the amount of any samples gaseous end product (methane) via anaerobic digestion involves some amount of target media as well as inoculum. The inoculum is the starting culture for the process. It contains already working bacteria cultures, which are then incubated at a steady temperature and the volume of the methane produced is regularly checked. (Bioprocess control AMPTS II, 2014, p.2)

6.4 Solutions

The solutions used were all prepared with adequate safety equipment under a fume hood.

6.4.1 3M NaOH

240 g of 3M NaOH was dissolved in distilled water to 2 l.

6.4.2 4% Thymolphthalein

40 g Thymolphthalein was dissolved in 9 ml 99.5% ethanol. 1 ml distilled water was added.

6.4.3 CO_2-fixing solution

In 2L 3M NaOH solution was 10 ml of 4% Thymolphthalein solution added.

6.5 Substrates used

Different substrates were used in the tests obtained. Theoretical values of methane potential as well as their calculated DS% and VS% can be found in Table 3 below (Carlsson & Uldal, 2009, p.24-27). The methane productions given for cucumber and tomato as well as their plants are not specifically for that type of waste but for fruit and vegetable waste in general. The number for olive oil as well as rapeseed oil production is the one of frying fat and the one for hay is the number for straw. These numbers were the closest to the product tested found. For some of the products, like butter, could no value be found.

Table 3: *Substrates tested and their calculated DS and VS; theoretical methane potential of these*

Substrate	**Calculated DS %**	**Calculated VS %**	**Theoretical methane potential (Nm^3 CH_4 / ton VS) (Carlsson & Uldal, 2009, p.24-27)**
Butter	83,93	112,02	
Chicken manure	81,72	60,51	247
Cow manure	17,02	79,83	250
Cow slurry	2,94	99,16	213
Cucumber	3,48	90,66	666
Cucumber plant	11,32	57,80	666
Eggs	31,61	68,91	300 – 520
Fish waste	32,39	76,03	930
Fox manure 1	31,43	62,78	
Fox manure 2	40,06	23,89	
Grain	86,76	80,04	400
Grass	24,54	95,80	250
Hay	92,05	89,82	207
Horse manure	21,24	81,73	170
Meat	27,20	90,96	
Mink manure	33,00	71,99	220
Olive oil	100,65	109,20	757
Pig manure	24,05	78,21	300
Pig slurry	8,83	96,87	268
Rapeseed oil	100,97	118,01	757
Sewage sludge	0,22	413,89	
Tomatoes	4,69	79,15	666
Tomato plant	16,07	73,65	666

In Table 3 above it is visible that olive oil and rapeseed oil have a calculated DS% and VS% over 100 as well as butter and sewage sludge have a calculated VS% over 100. This is theoretically not possible and could be due to too small amounts of fresh and dried substrate as well as other faults in the measurement.

6.6 **Preparation**

To determine the amount of the sample needed in the mixture the moisture content (DS) and ash content (VS) had to be determined. This was done according to the ISO standards for solid biofuels 14774-3 (International organization for standardization, 2010) and 14775 (International organization for standardization, 2009).

6.6.1 Determination of dry matter

A few grams of the different substrates, the bio waste and the inoculum were each weighed into small aluminum pans (Ø =100 mm) with a 0.01g accuracy, see Figure 5. They then were dried in an oven at 105°C. After a minimum of 24h in about 105°C and 40% ventilation the samples were removed from the oven and weighed again.

Figure 5: Chicken manure, grain, fox manure 1 & 2, meat and grass after weighing, placed in the oven, before drying.

With the difference in weight the DS in percentage was calculated according to equation 5 and 6 below where m_1 is the mass of the empty dish, m_2 the dish plus the sample before drying and m_3 the mass of the dish and the sample after drying.

Equation 5: *M_{ad} calculation (International organization for standardization, 2010, p.5)*

$$M_{ad} = \frac{(m_2 - m_3)}{(m_2 - m_1)} x100$$

Equation 6: *DS calculation*

$$DS = 100 - M_{ad}$$

The DS tests were done two times, one time before biogas production and one time after. The results of the calculations can be found in Appendix 1.

6.6.2 Determination of ash content and volatile solids

The samples from the DS measurement were transferred into ceramic pans and placed into another oven. This oven was 10 min heated up to 550°C. After two hours of burning time the samples were placed into a desiccator where they cooled down, see Figure 6 below.

Figure 6: Chicken manure, grain, fox manure 1 & 2, meat and grass in the burning oven (before burning) on the left and in the desiccator on the right.

Later they were weighed again and the ash content was calculated according to equation 7 below where m_1 is the mass of the empty dish, m_2 the dish plus the sample, m_3 the dish plus the ash and M_{ad} the % moisture content of the sample.

Equation 7: *Ash content calculation (International organization for standardization, 2009, p.11)*

$$A_d = \frac{(m_3 - m_1)}{(m_2 - m_1)} x100x \frac{100}{100 - M_{ad}}$$

The VS was calculated by subtracting the ash produced (A_d) by the dried waste (m_4), see equation 8. When calculating the VS% (see equation 9), the VS is divided with the dried substrate transferred into the ceramic cups (m_5) and the result is then multiplied with 100. Results of these calculations can be found in Appendix 2.

Equation 8: *VS calculation*

$$VS = A_d - m_4$$

Equation 9: *VS % calculation*

$$VS\ \% = \frac{VS}{m_5} * 100$$

6.6.3 CO_2-fixing unit

About 80 ml of the CO_2-fixing solution, see 6.4.3, was filled into each glass bottle of the CO_2-fixing unit. The rubber stoppers were lubricated with silicone oil and screwed on with the plastic lid to seal each glass bottle as can be seen in Figure 7. In here are several acid gas fractions like CO_2 and H_2S retained by interaction with NaOH so that only CH_4 is passing through to the methane measuring device.

Figure 7: CO_2 fixing unit while filling up.

6.6.4 Gas volume measuring device

Figure 8 shows the gas volume measuring device that was filled up with water till the level indicator. This device measures the CH_4 content of the gas released from the CO_2 fixing unit (Chapter 6.6.3).

Figure 8: Gas volume measuring device filled up with water.

6.6.5 Substrates

Two reactors per sample were used so that 6 different substrates could be tested simultaneously. Two reactors were used for testing bio waste + inoculum to check the biogas produced by them alone and one to test either water or inoculum. Every substrate sample was mixed with inoculum, bio waste and water. About 11% of starting culture is needed in the reactor to start the biogas production. In our case this is 0,11*450ml=49,5ml, so about 50 ml of Inoculum. The inoculum was taken out of the running process at Stormossen and kept warm till used in the small sample bioreactors. The amount of bio waste and sample were each 200 ml. To get this number in g of substrate with a DS of 5% see equation 10 below where M_{ad} stands for the % moisture content of the sample. The 10 in the numerator is calculated from 200 ml substrate and the 5% DS: 200*0,05=10.

Equation 10: *Calculation of amount of substrate and bio waste in gram*

$$\frac{10}{(100 - M_{ad})/100}$$

The results of these calculations are found in Appendix 3.

6.6.6 Reactors

When filling the reactors, first the in 6.6.5 calculated and weighed amount of sample and bio waste were placed into the reactors, as well as 50 ml inoculum. Each reactor was filled up with the required amount of water to reach 450 ml of mixture. The reactors were then sealed with rubber stoppers that were lubricated with silicone oil on the sides. Connected with the rubber stopper also the stirrer was inserted. Figure 9 below shows the reactors filled with substrates and connected to the stirring placed in the thermostatic water bath.

Figure 9: Bioreactors with stirring motors in thermostatic water bath.

6.6.7 Thermostatic water bath

The Thermostatic water bath was filled with warm water so that the samples in the reactors were covered. The water bath was then turned on so that the water temperature is constantly kept at about 55°C.

6.6.8 Motor connection

Each reactor was equipped with a motor to allow the stirring. The first motor was connected to the power adapter and the other motors receive power due to serial connection.

6.6.9 Tubing

For the tubing Tygon® flexible plastic tubes with an inner diameter of 3.2 mm were used. The reactors were connected with the CO_2-fixing unit as well as they each had a second tube that was sealed with a plastic tubing clamp in case samples wants to be taken.

6.7 Start up

To start up the sampling the names of each sample as well as different values seen in the Table 4 below were inserted in the AMPTS II computer program (see Figure 10). These values did stay the same throughout the tests. One might notice that the total volume of reactor was estimated to be 600ml, which is due to the 500 ml bottle size plus 100 ml from the connecting tubes. The I/S ratio is the inoculum to substrate VS ratio which would be 0 if no substrate was added. Since in our case we added both the ratio was estimated to be 0,111. No flushing of the tubes was conducted due to that is the CO_2 in the flush gas 0,039%. After filling in all values, the continuous stirring was started with a speed of 160 revolutions per minute (RPM) on the motors as well as the sample reading with the computer program. All in all 6 tests were conducted.

Table 4: *Values inserted in the AMPTS II computer program*

Total sample amount (g)	450
Inoculum concentration (% w/w)	5
Substrate concentration (% w/w)	5
I/S ratio	0,111
Total volume of reactor (ml)	600
Assumed CH4 content (%)	60
Type of unit (VS/COD)	VS
CO2 in flush gas (%)	0,039
Assumed temperature (˚C)	55

From the filled in values the computer program calculated experimental guidelines for setting up the experiment bottle, see Figure 10 below. The given amounts were not strictly followed but externally calculated for each substrate separately as can be seen in Chapter 6.6.5.

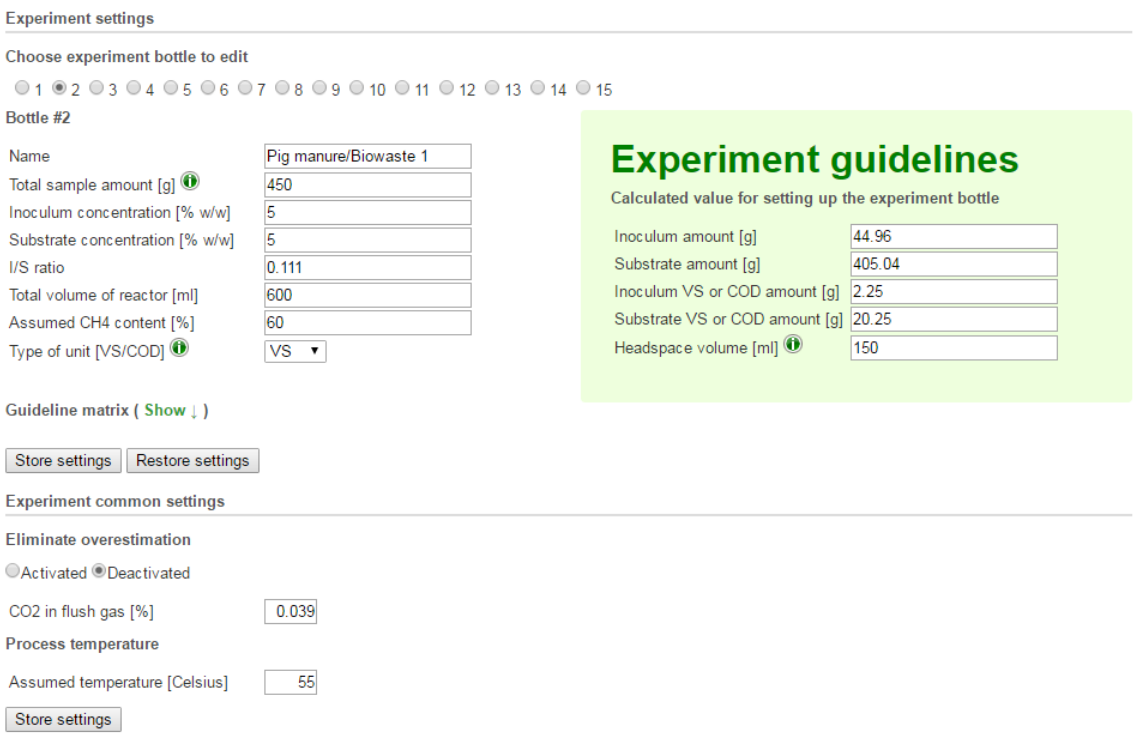

Figure 10: *Filled in values as well as experimental guidelines from the computer program*

6.8 Monitoring

Regular checkup of the tubing, that it is undamaged as well as not sharply bent so that it interferes with the gas flow, was needed. Simultaneously was checked that the stirring works properly as well as a refill of the water in both the thermostatic water bath as well as the gas volume measuring device was required. When the color of the pH indicator turned from blue to colorless the NaOH solution was replaced with a fresh one.

From the computer program one could check the produced amount of gas volume as well as the flow rate. If these stopped giving results the tested sample stopped producing biogas.

6.9 End of operation

When finishing the sampling, a report of the production was generated and downloaded in the computer program. After checking that all data was there, the sample reading, the continuous steering as well as the heating of the thermostatic water bath were stopped. The part of the samples that was not filtered and undergoing the pH and conductivity measurements were refilled in plastic bags and frozen down for later use, in our case being send in to a better equipped laboratory for additional measurements. The bottles were washed for later use.

6.9.1 Filtration

The filtration was done after the samples were removed from the reactors to get rid of oil and particles in the sample which could influence the conductivity testing. A grade 5 Munktell Ahlstrom filter paper, as well as a funnel were used. The first samples were not filtered but due to problems with oily samples manipulating the conductivity testing, all later samples did undergo this step.

6.9.2 pH test

The pH is a numeric value that describes the acidity or alkalinity of a liquid. It is the negative logarithm of the oxonium-ion concentration in a solution. If the value is below 7 the liquid is acidic, if it is above 7 it is alkaline, for more information see Chapter 4.3.3. (Fischedick A. et al., 2004, p.177)

The tests were done according to the ISO Standard 10390 (International organization for standardization, 2007) with a Sentron 2001 pH model, at first with only a still warm sample right after leaving the reactor, and later on also after being filtrated and in room temperature. The electrode of the meter was dipped in the sample, which was continuously stirred, till the result given was stable.

6.9.3 Conductivity test

With the conductivity one measures the ability of a solution to conduct electricity (more information in Chapter 4.3.4). (Fischedick A. et al., 2004, p.119)

The tests were done, like the pH tests, at first right from the reactor and the later once after filtration in room temperature. The meter used was a Metler Toledo conductivity meter and the measurements were conducted according to the ISO standard 27888 (International organization for standardization, 1985). The electrode of the meter was dipped in the sample, which was continuously stirred, till the meter gave the result.

6.10 Results and interpretation

The performed tests took between 0 days until up to 73 days. The 0 days were unsuccessful tests where no biogas was produced. The water was tested in some of the test runs to control that the bioprocess control is not giving any faulty results. The water never produced any biogas, which was as planned. In the Tables one can find the substrates tested, how much biogas they were producing as well as their pH, conductivity and DS after production.

One problem with the tests was the accumulation of a white mass, which might be struvite, ammonium carbonate or something else in the pipes. It completely plugged them so that no biogas produced could be transmitted to the system and be read, see Figure 11. Struvite ($NH_4MgPO_4 \bullet 6H_2O$) contains magnesium, ammonium and phosphate and has a crystal kind of consistency and might be due to a too high pH. The ammonium carbonate ($(NH_4)_2CO_3$) on the other hand has the same kind of appearance but does accumulate due to a high amount of ammonium in the system. (Interview with Kalander and Åkerback, 2016) To be completely sure if it was struvite, ammonium carbonate or something else plugging the pipes, they need to be send in for testing. The tests in which accumulation occurred are marked with one star (*) for small amounts and two stars (**) for bigger amounts with plugged pipes in the Tables of the Chapters 6.10.1. to 6.10.6. In many pipes were very small amounts accumulated, those are not marked in the Tables. As soon as too high amounts were noticed, the pipes were exchanged.

Figure 11: *Accumulation of struvite, ammonium carbonate or some other material plugging the pipes (circled orange).*

6.10.1 Test 1

This was the first test done with a new machine. The test run had to be stopped after 26 days due to travelling. Nearly all the tests had already stopped producing biogas; it was only chicken manure 2 and cow slurry 1 and 2 that still kept producing. The chicken manure tests were redone (seen in Chapter 6.10.6) due to the early stop as well as a big difference in production of biogas. In Table 5 one can see all results of this test run. It shows that the samples with a pH between 7,5 and 8 have the highest production. This is in agreement with the literature research, see Chapter 4.3.3. The only sample not producing anything in this test was inoculum. This is most likely due to the very low DS. Figure 12 shows the changes in production during time of all the samples. It is very visible in the Figure 12 that cow slurry 1 and 2 as well as chicken manure 2 have the highest production. In Figure 13 these were excluded to achieve a better visibility of the smaller productions.

Table 5: *Biogas production of the first test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	24	582,1	5,39	5,90	3,61
Bio waste 2	17	563,9	5,37	5,84	3,21
Bio waste 3	24	127	4,55	6,90	3,69
Water	0	0			
Inoculum	0	0	7,92	15,35	0,17
Pig manure 1 / Bio waste	7	379,8	5,46	8,31	4,27
Pig manure 2 / Bio waste	24	343,8	5,68	8,47	4,04
Cow manure 1 / Bio waste	17	451,8	5,47	6,63	4,38
Cow manure 2 / Bio waste	13	433.8	5,40	7,48	4,28
Chicken manure 1 / Bio waste	23	741,6	5,65	11,77	3,28
Chicken manure 2 / Bio waste	26	4436,3	7,77	10,31	2,61
Cow slurry 1 / Bio waste	26	5117,2	7,85	14,31	2,87
Cow slurry 2 / Bio waste	26	5248,1	7,74	13,23	3,16
Pig slurry 1 / Bio waste	3	117,6	7,33	10,29	3,17
Pig slurry 2 / Bio waste	21	443,4	5,79	11,12	3,44

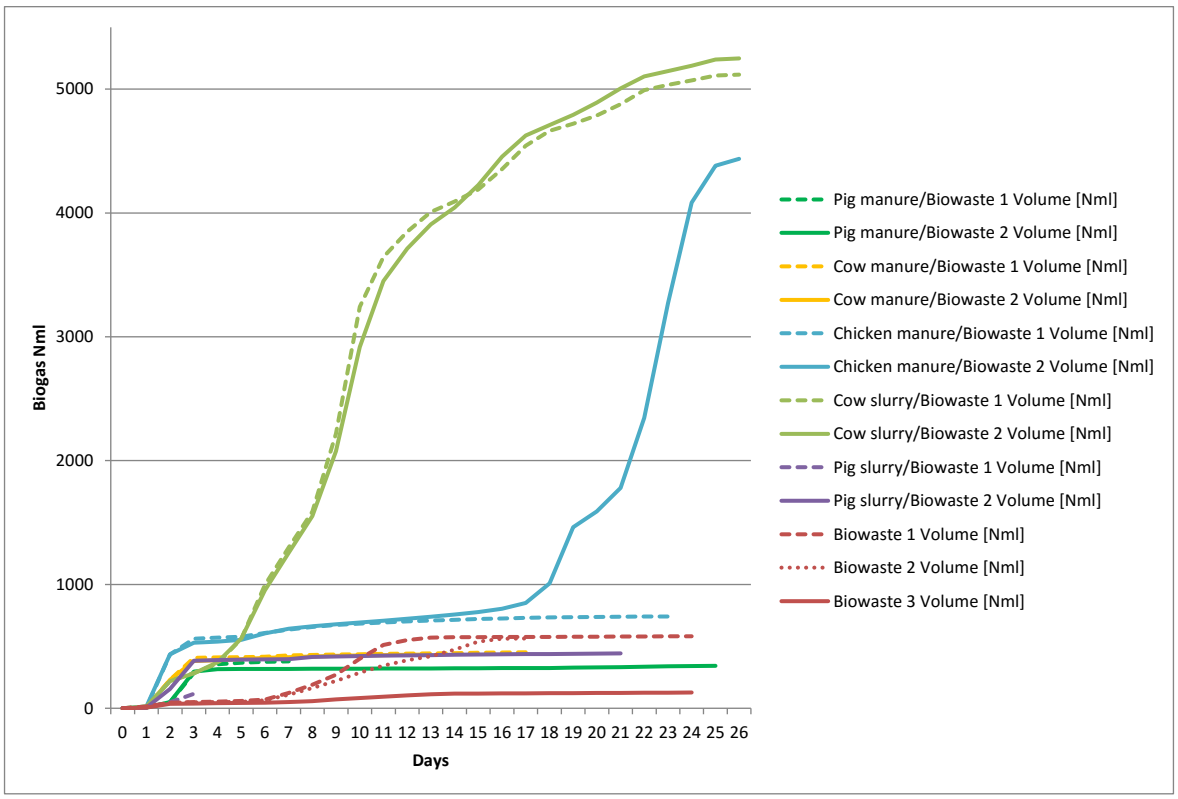

Figure 12: *Biogas production of the first test*

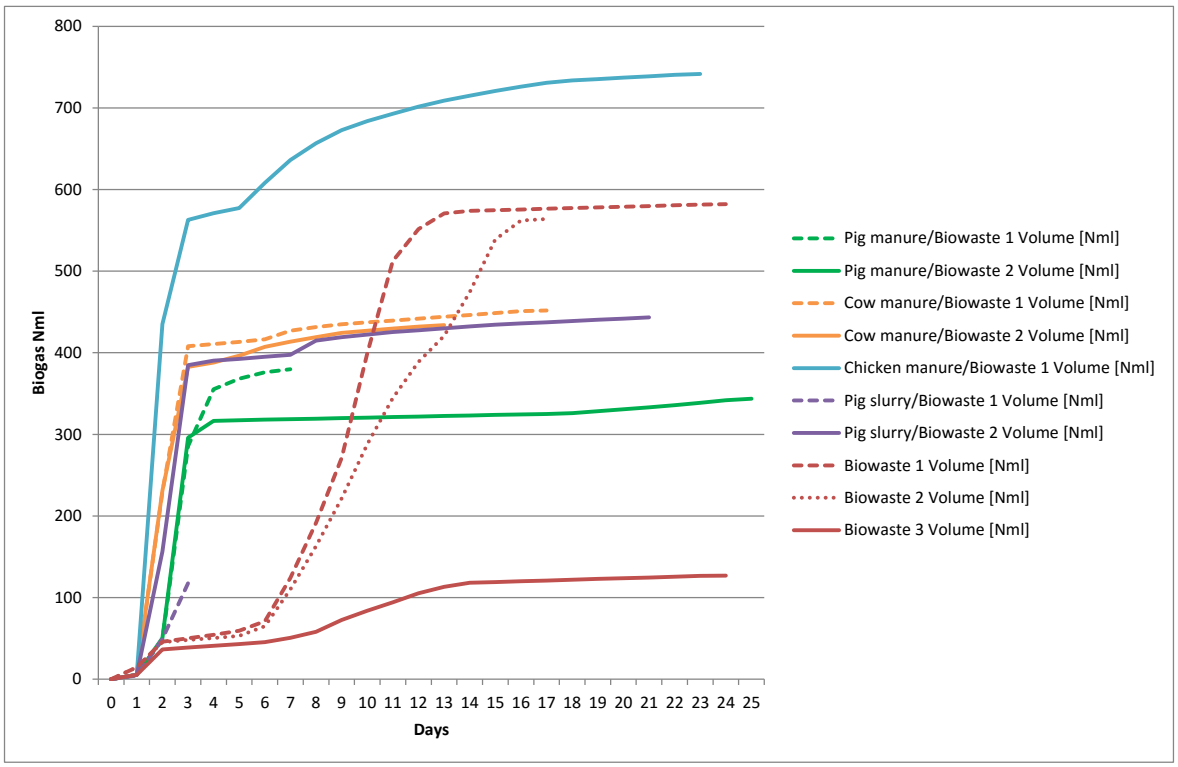

Figure 13: *Biogas production of the first test, smaller productions*

6.10.2 Test 2

The second test was going on for 73 days. Many productions stopped already earlier, just the cucumber plants were producing biogas that long (see Table 6). Also in this test their pH is higher than the others, around 8. The slow start and high production in the end could be due to the high content of lignin, see Chapter 5.10. They had the highest production around day 34±3. Since only one of the grain production tests gave results the tests were redone, see Chapter 6.10.6. Figure 14 shows clearly how much more the cucumber plants were producing. To show in more detail how the other tests were going the cucumber plant was excluded in Figure 15. Bio waste 2, fish waste 1 as well as grain 1 did all produce 18,4 Nml biogas within one day. Their lines are on top of each other in Figure 15 and only fish waste 1 is visible.

Table 6: *Biogas production of the second test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	1	18,4	4,59	7,98	4,01
Bio waste 2	0	0	4,52	7,42	3,97
Water	0	0			
Cucumber 1 / Bio waste	4	82,7	3,90	11,94	4,78
Cucumber 2 / Bio waste	1	82,7	3,89	13,25	4,3
Fish waste 1 / Bio waste	1	18,4	4,5	7,01	3,92
Fish waste 2 / Bio waste	22	82,4	4,5	6,87	3,62
Cucumber plant 1 / Bio waste *	69	6539,6	8,2	11,23	0,93
Cucumber plant 2 / Bio waste *	73	5696,7	8,3	11,01	0,88
Grain 1 / Bio waste *	8	18,4	4,0	5,36	3,06
Grain 2 / Bio waste	0	0	4,0	5,28	3,22
Hay 1 / Bio waste	1	27,5	4,3	14,75	4,91
Hay 2 / Bio waste	1	18,4	4,3	1,68	4,28
Tomato plant 1 / Bio waste	25	36,7	4,1	9,84	3,15
Tomato plant 2 / Bio waste	25	45,9	4,1	9,5	2,72

*) Small struvite, ammonium carbonate or other accumulation

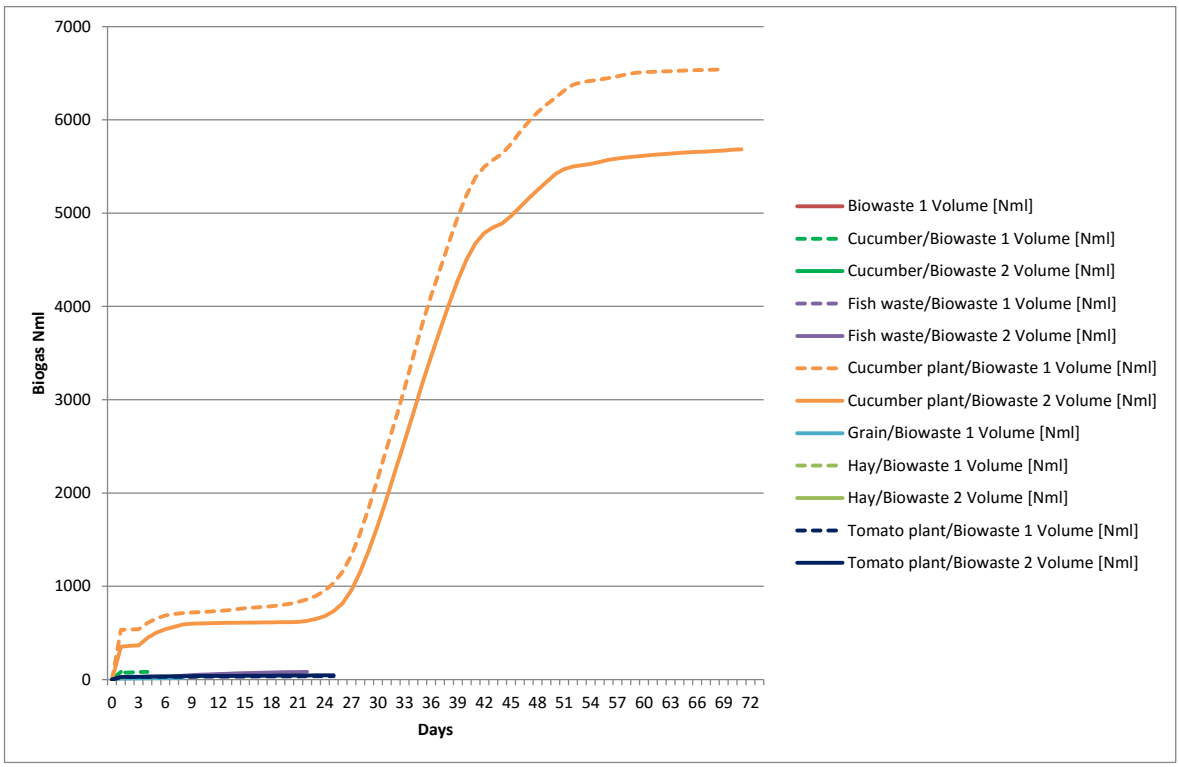

Figure 14: *Biogas production of the second test*

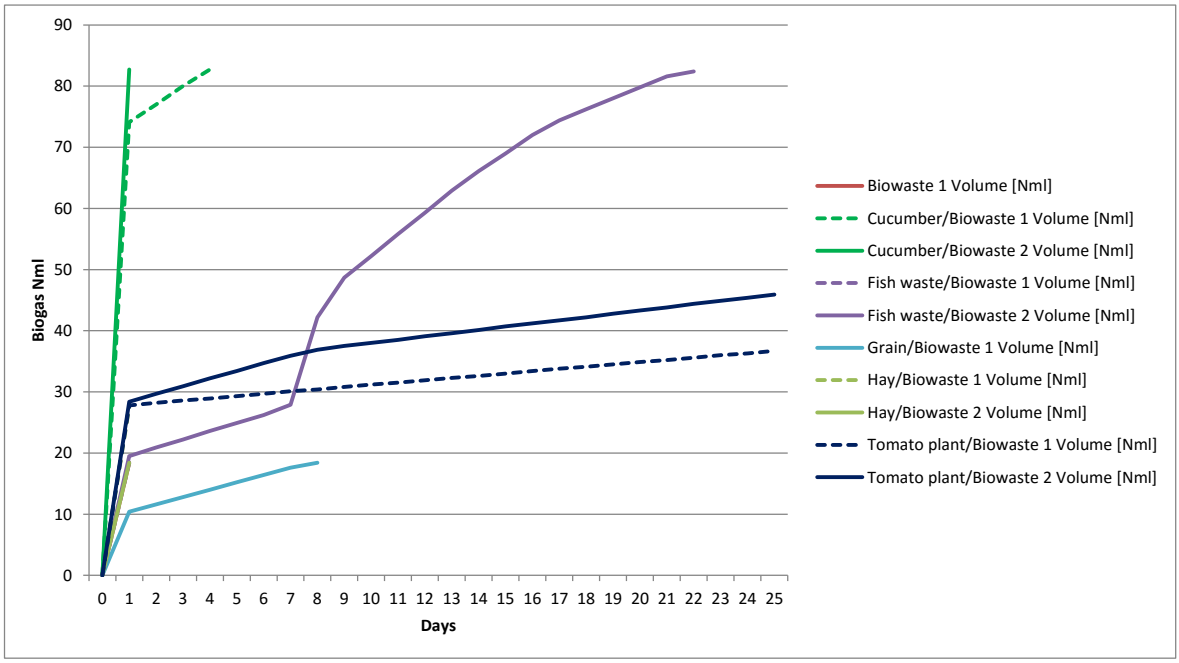

Figure 15: *Biogas production of the second test, smaller productions*

6.10.3 Test 3

This test was going on for 21 days. Only one of the olive oil tests gave results so it has been tested again (see Chapter 6.10.6). For the rapeseed oil the gas production was about the same but the time it took to produce was very different, which could be due to a slight difference in the inoculum. When checking how much they produced every day it was clear that they both had about the end result already on day one and just produced very small amounts after that. Figure 16 shows this very clearly. To see all results of this test see Table 7.

Table 7: *Biogas production of the third test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	16	409,4	5,3	5,57	1,07
Bio waste 2	16	281,8	5,1	6,13	1,02
Water	0	0			
Olive oil 1 / Bio waste	0	0	5,3	5,29	1,74
Olive oil 2 / Bio waste	6	433,6	5,2	5,89	5,33
Rapeseed oil 1 / Bio waste	9	287,8	5,3	5,07	2,05
Rapeseed oil 2 / Bio waste	21	351,3	5,3	5,41	4,59

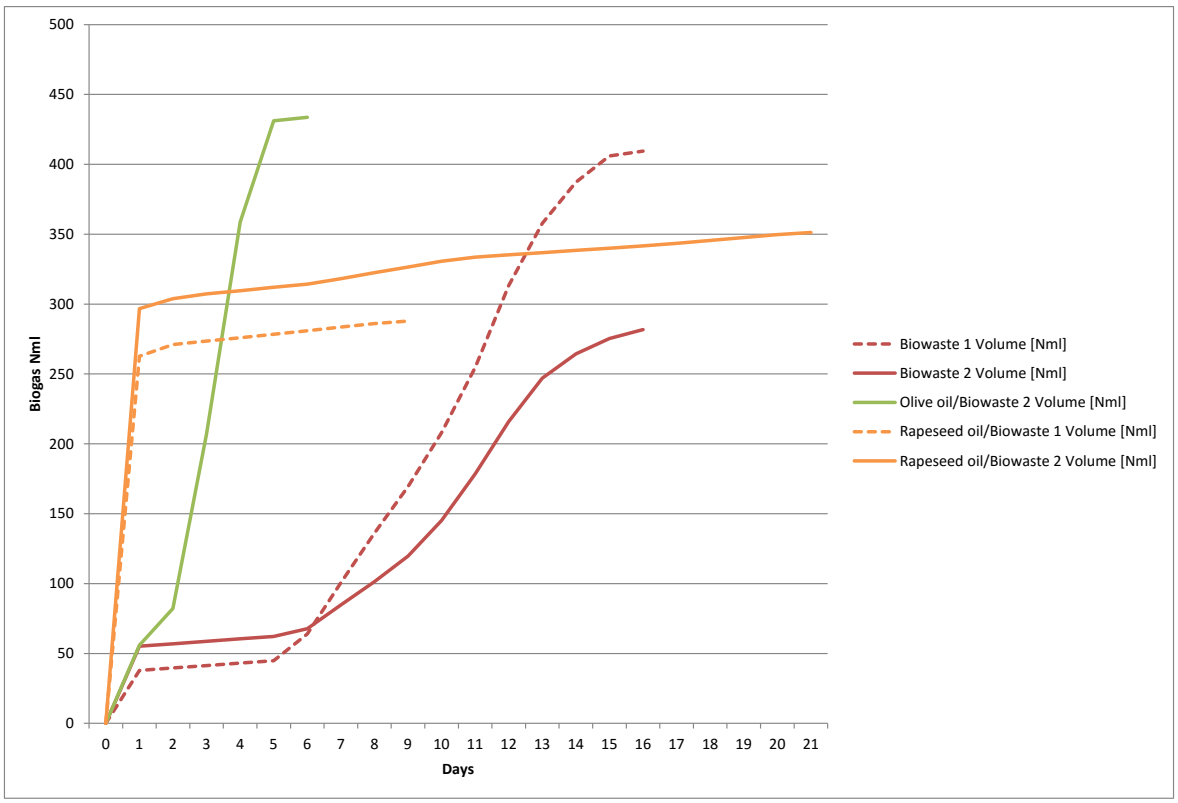

Figure 16: *Biogas production of the third test*

6.10.4 Test 4

The fourth test did go on for 91 days. Only fox manure 1 (fresh) did go on for that long. Their biogas productions were small from about day 26 but fox manure 1.2 did have a high production around day 61 again (see Figure 17). The horse manure is being redone since the results were very different between the two tests (see Chapter 6.10.6). Only one of the tests of fox manure 2 (1 year composted) gave results. This might be because the test not producing had a lower pH and DS. The test could not be redone due to difficulties finding the same kind of fox manure. Other reasons for the horse manure 2 producing less and the fox manure 2.2 not producing any could be due to the plugged the pipes (see Table 8). The meat production had very different production times, the second one did go on for a lot longer (see Figure 18). If one checks the production flow they both produced well the first 11 days, then meat 1 stopped producing and meat 2 produced only very small amount after. Also here one can see that the substrates with the highest production have a pH between 6,5 and 9. All results of this test can be found in Table 8.

Table 8: *Biogas production of the forth test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	1	27,3	4,4	11,52	3,76
Bio waste 2	1	36,4	4,5	13,51	6,79
Bio waste 3 *	1	45,5	4,5	11,48	6,74
Horse manure 1 / Bio waste *	89	6296,8	8,50	9,00	2,05
Horse manure 2 / Bio waste **	33	464,4	5,3	9,57	1,25
Meat 1 / Bio waste *	11	948,9	6,5		0,94
Meat 2 / Bio waste *	44	1095,1	7,5	20,5	0,84
Fox manure 1.1 (fresh) / Bio waste *	73	5186,4	8,90	9,65	4,20
Fox manure 1.2 (fresh) / Bio waste	91	5415,3	8,80	10,59	1,39
Fox manure 2.1 (1 year composted) / Bio waste *	80	3572,3	8,69	5,77	1,22
Fox manure 2.2 (1 year composted) / Bio waste **	0	0	5,6	11,94	0,98
Mink manure 1 / Bio waste **	47	735,5	5,5	21,8	2,06
Mink manure 2 / Bio waste **	48	672,2	6,3	17,67	1,29

*) Small struvite, ammonium carbonate or other accumulation

**) Much struvite, ammonium carbonate or other accumulation

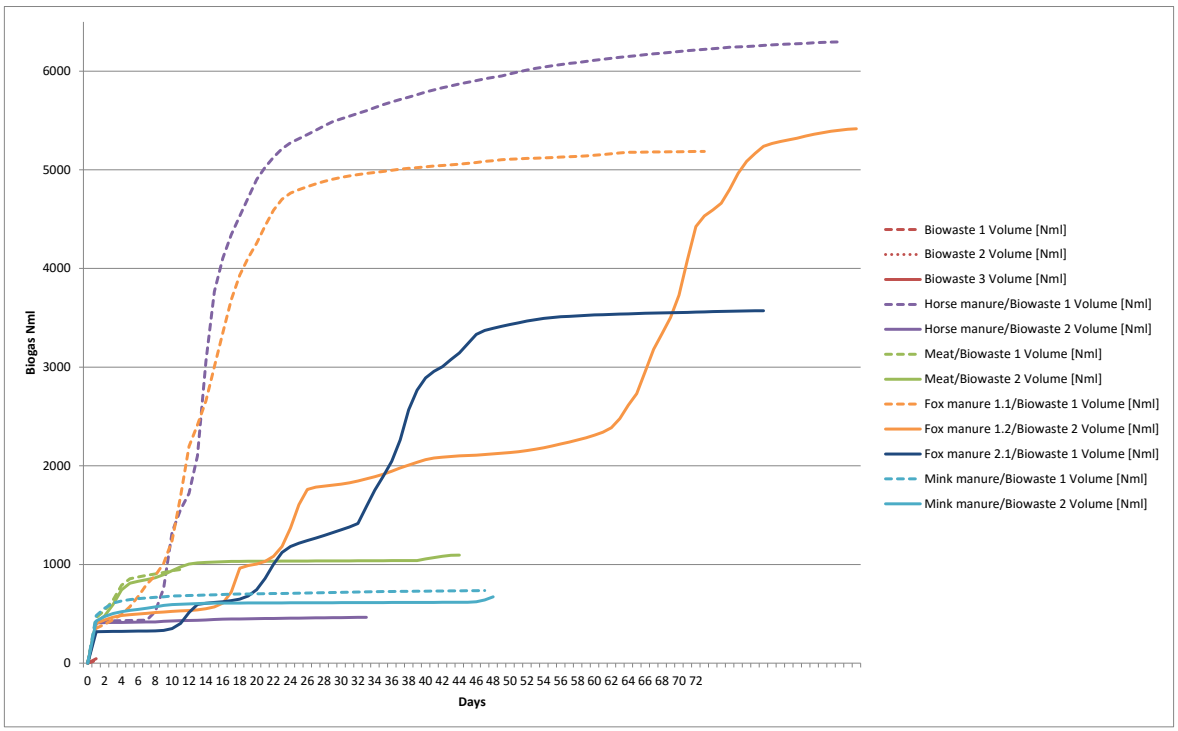

Figure 17: *Biogas production of the fourth test*

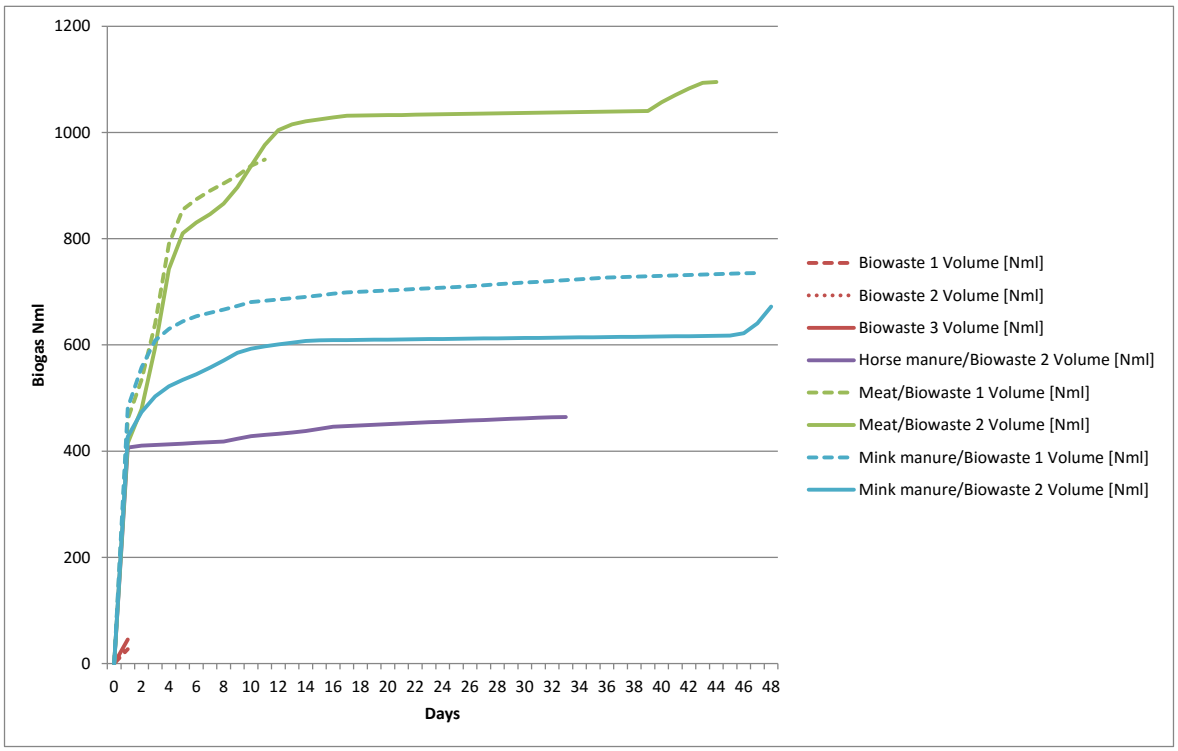

Figure 18: *Biogas production of the fourth test, smaller production*

6.10.5 Test 5

All results of the fifth test can be seen in Table 9 below. The grass is retested in Chapter 6.10.6 due to high difference in production. When checking the produced amount after a few days grass 1 did not show any results at all while now, after day 26 it has produced a very small amount of biogas. In Figure 19 it is shown how much more the eggs (pH about 8,5) were producing compared with everything else in test 5. Those were excluded in Figure 20 to be able to see the other results better. Even though Carlsson and Uldal (2009, p.11 - 12) did point out problems in the production due to egg shells (Chapter 5.3) the tests did go smoothly and they did not lead to a mechanical stop. A reason for this might have been that the process was not continuous.

Table 9: *Biogas production of the fifth test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	5	685,3	5,31	8,70	3,30
Bio waste 2	6	731,1	5,32	8,09	3,1
Inoculum	15	45,1	8,12	3,01	0,25
Tomatoes 1 / Bio waste	1	255,7	3,85	9,41	7,41
Tomatoes 2 / Bio waste	2	182,8	4,43	8,32	4,69
Eggs 1 / Bio waste	41	7441,8	8,38	11,73	0,76
Eggs 2 / Bio waste	40	7403,9	8,62	11,27	1,15
Butter 1 / Bio waste	26	446	5,18	7,75	2,35
Butter 2 / Bio waste	11	409,6	5,18	6,39	3,15
Grass 1 / Bio waste	26	18	5,38	10,9	3,35
Grass 2 / Bio waste	26	700,9	5,22	10,29	4,40

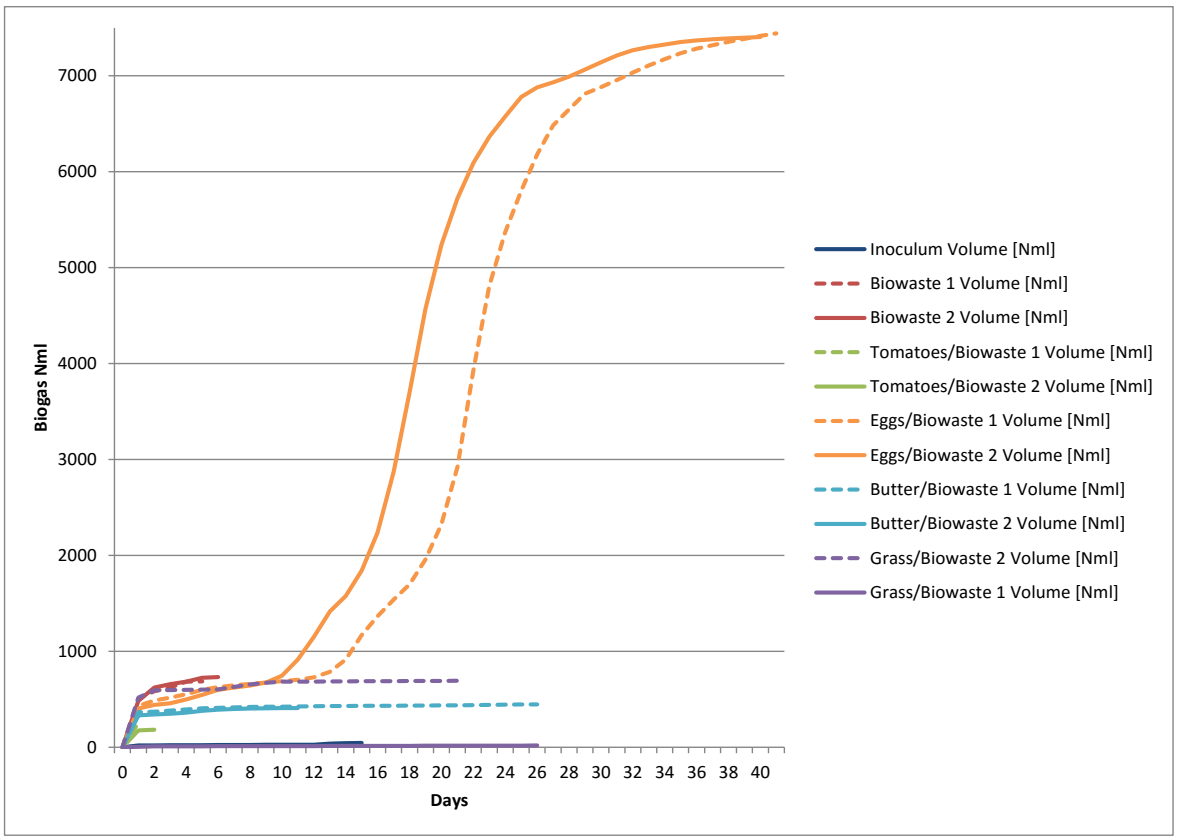

Figure 19: *Biogas production of the fifth test*

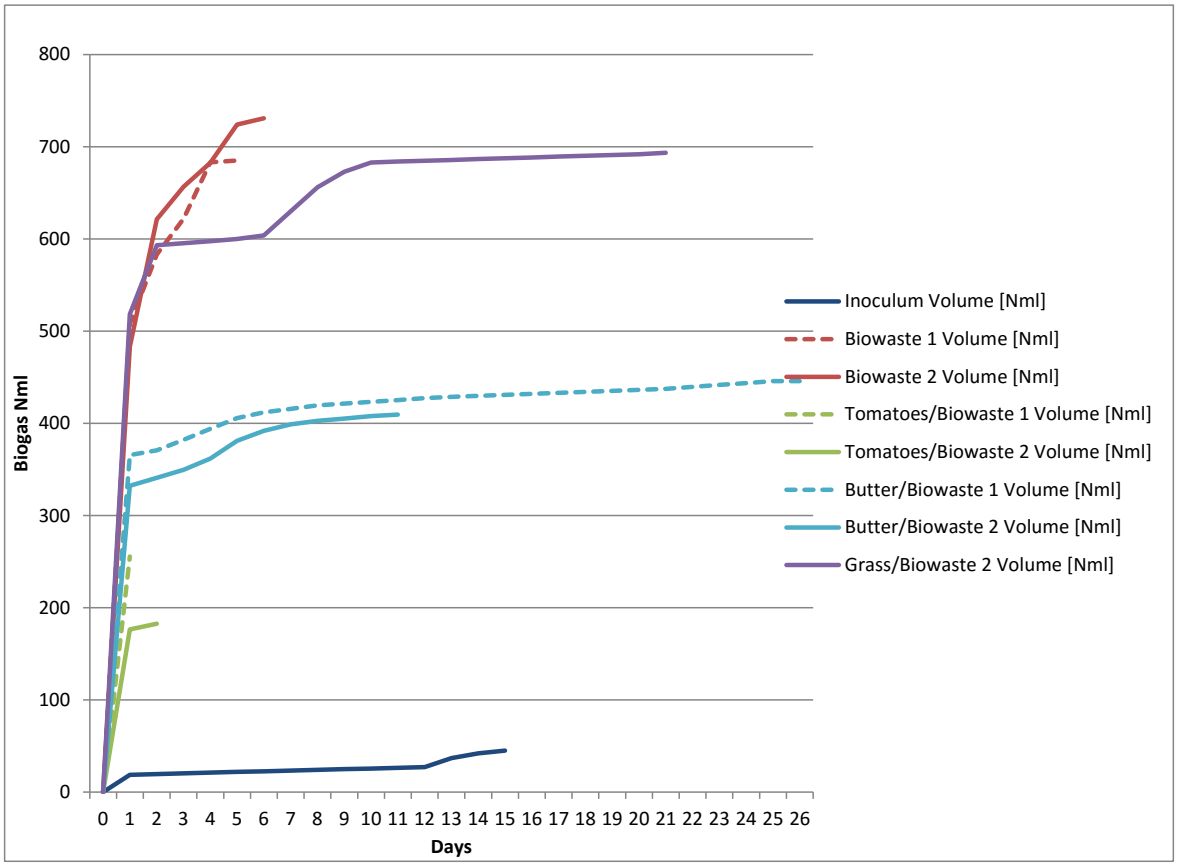

Figure 20: *Biogas production of the fifth test, smaller productions*

6.10.6 Test 6

In this sixth and last test were some of the substrates tested a second time, but also sewage sludge. Some of the samples in which only one of the tests gave a result, but also those with a big difference in biogas production, were chosen for this test. The substrates were mainly collected again, due to problems with the freezer in the laboratory. Due to this one cannot directly compare them with the other tests. Sewage sludge 1 has the highest biogas production with a pH of 9,2 (see Table 10 and Figure 21). Sewage sludge 2 did not produce any, which could be due to a lower pH (6,6). Also grass 1 did not give any results. Those tests could not be redone due to time issues. In Figure 22 one can see the production of the other results excluding sewage sludge 1.

Table 10: *Biogas production of the sixth test, as well as conductivity, pH and DS at the end of operation*

Substrate	**Days**	**Biogas (*Nml*)**	**pH**	**Conductivity (*µS/cm*)**	**DS (*%*)**
Bio waste 1	1	80	4,1	7,28	10,75
Bio waste 2	1	79,9	4,0	7,53	7,84
Bio waste 3	1	80	4,2	7,83	5,13
Inoculum	28	107,7	2,27	7,4	0,24
Sewage sludge 1 / Bio waste	25	6324,1	9,2	9,91	0,90
Sewage sludge 2 / Bio waste	0	0	6,6	7,47	2,52
Olive oil 1 / Bio waste	1	150,9	4,1	6,02	7,00
Olive oil 2 / Bio waste	30	560,8	4,59	6,5	6,85
Horse manure 1 / Bio waste	2	71,1	4,3	6,42	2,86
Horse manure 2 / Bio waste	2	17,8	3,9	5,05	4,04
Chicken manure 1 / Bio waste	19	615,9	6,1	12,59	2,95
Chicken manure 2 / Bio waste	13	749,9	6,3	15,50	2,15
Grain 1 / Bio waste	1	53,3	4,1	6,29	3,19
Grain 2 / Bio waste	1	62,2	4,0	6,80	3,86
Grass 1 / Bio waste	0	0	4,0	7,67	
Grass 2 / Bio waste	7	62,4	4,3	8,05	6,64

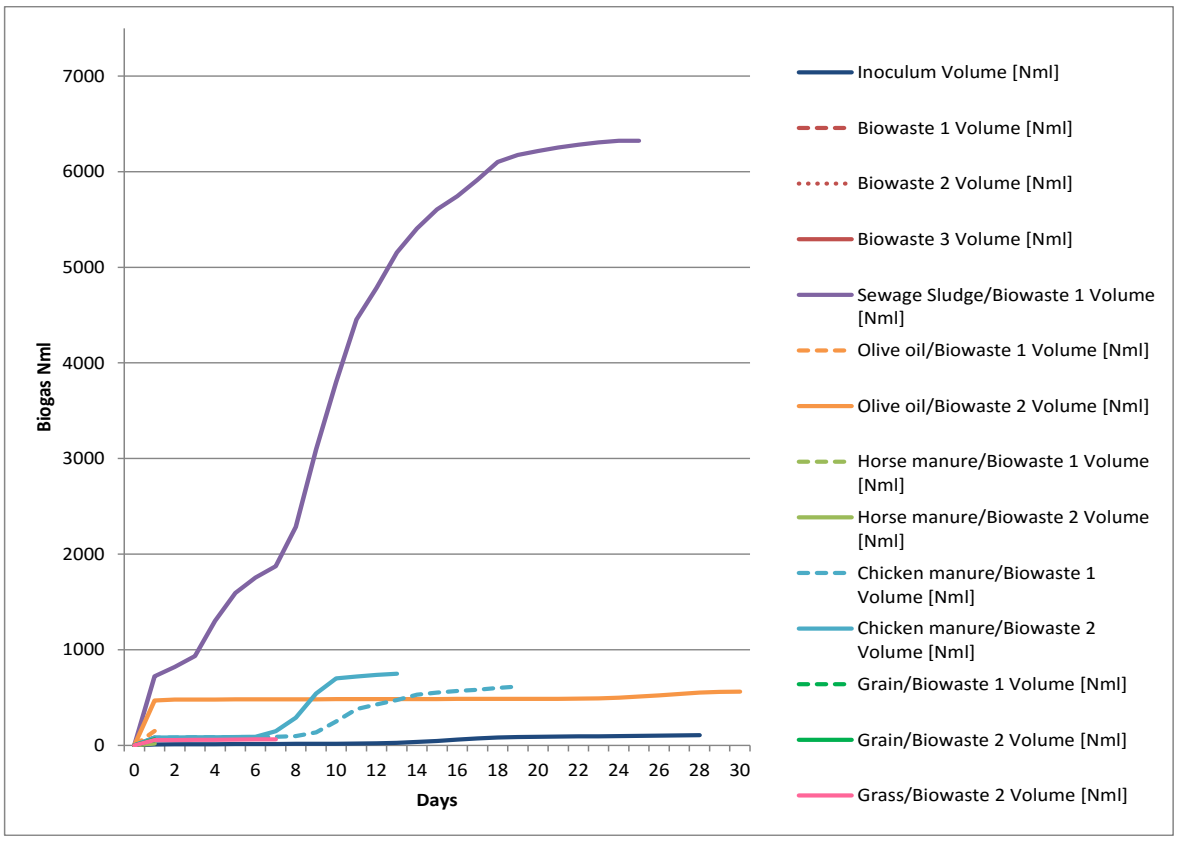

Figure 21: *Biogas production of the sixth test, smaller productions*

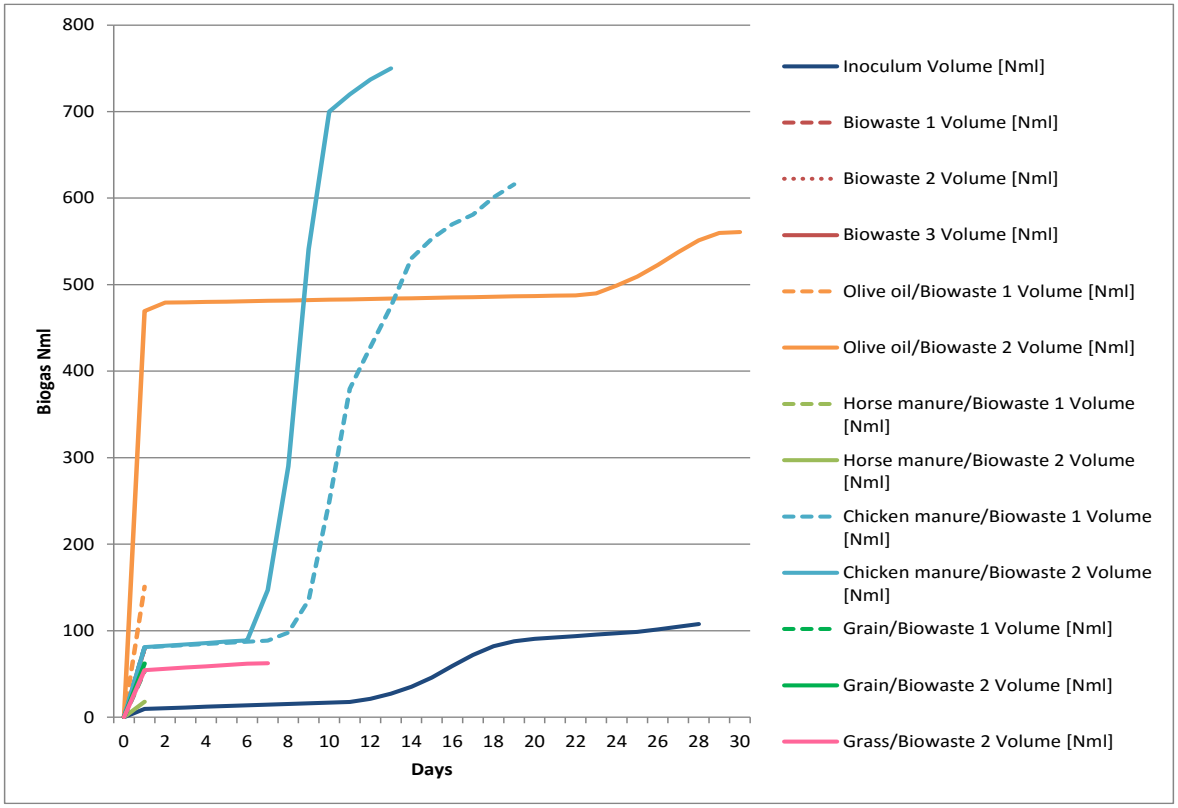

Figure 22: *Biogas production of the sixth test, smaller productions*

7 Conclusions

As in Chapter 2 described was the goal of this work to find and test different substrates on their biogas potential. This goal has been reached by testing several different substrates at laboratory scale. One could have been testing more single substrates, like for example bread, as well as mixtures of substrates to see how the different substrates behave when being mixed. This would also be needed because most of the biogas plants, like Stormossen, use substrate mixtures in their processes. They do not have many small reactors, but one or two big once where they mix everything. Due to the time consuming tests this was sadly not possible, but would be a work that could be carried out in another thesis.

When looking at the results in Chapter 6.10 as well as Appendix 4, where all the biogas productions as well as pH of the different substrates are summarized in one table, one can see that the pH plays an important role in the production of biogas. It would be advisable to test the pH before starting the tests as well as while the tests are going on and maybe adjust it to a more suitable one, see Chapter 4.3.3. In the tests of this thesis, the biogas production was the best with a pH around 8.5. A mixture of different substrates (one with a too high pH and one with a too low pH) could not only be used for pH adjustment but also lead to better nutrition composition and with that to better results in the biogas production (see Chapter 4.2.1).

In Appendix 5, one can find a graph with all the experiments. It is very good visible that eggs as well as different manures and slurries gave the best results within a short time. This is according to literature, see Chapter 5.3 and 5.11. Also the influence of lignin on plants is visible in Appendix 5, where cucumber plants have a slow start and a high production later in the process (see Chapter 5.10 and 6.10.2).

Overall, I would pick out three of the substrates that were interesting (see Appendix 5). One of those are the eggs, which gave a great production within a short time, even though they did include the shells which are leaving the process unaffected and are known to cause mechanical problems. Interestingly I did not have any mechanical problems with the shells, which could be due to the batch wise process. One should consider if it is beneficial to remove the shells or crush them very small when high amounts of eggs are available for a continuous process.

My second pick would be the sewage sludge, which gave such a great difference in production, see Chapter10. One of the productions was having the highest amount of biogas of all tests obtained and the other one did not produce any, which indicates that it is not the easiest substrate to start producing biogas, but if it once starts, it produced high amounts within a short time. It would be interesting to redo that test, which was due to the limited amount of time sadly not possible.

The last pick of substrate is the cow slurry. It did not produce the most of biogas but was still was one of the substrates producing well. It is the only substrate that was producing a good amount of biogas within a reasonable time and did not cause any problems. It seems to be a easily degradable substrate that can be recommended to everyone considering starting biogas production.

8 Suggestions

According to House (2006, p.52) has a continuous agitation (24h a day) a depressing effect on the biogas bacteria and therefore reduces the production of biogas. House (2006, p.52) suggests 15 minutes mixing per hour. In the future, tests with different stirring intervals could be carried out to see the effects of stirring on the biogas production.

I also would suggest to flush the pipes with an inert gas (e.g. nitrogen) to create anaerobic conditions (see Chapter 6.7). The CO_2 fixing liquid as well as the pipes should be changed for every new test to avoid a temporary failure of the CO_2 fixing unit and with that, the impurity of methane gas measured. Also the risk of accumulation of struvite, ammonium carbonate or similar in the pipes (see Chapter 6.6.3 and 6.10) can be reduced by doing so. To be able to calculate the methane potential one should test the same substrate three times instead of only two. With three parallel samples, you also might avoid to redo some of the experiments. In my tests, I had problems with only one sample producing. With three parallel samples and one of those not producing, there would still have been two samples available to be compared with each other.

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Appendix 1 – Dry matter calculations

		pan (alu) (emprty, g)	fresh waste (g)		dried waste & pan (alu) (g)	dried waste (g)	Moisture content (%)	Dry content (%)
Tomato plant (cut small with scissors, grinded)	1	2,51	15,41		5,38	2,87	81,38	18,62
	2	2,52	20,68		5,76	3,24	84,33	15,67
	3	2,51	23,49		5,78	3,27	86,08	13,92
Cucumber plant (cut small with scissors, grinded)	1	2,51	16,75		4,53	2,02	87,94	12,06
	2	2,51	23,31		4,98	2,47	89,40	10,60
	3	2,53	28,66		5,77	3,24	88,70	11,30
Eggs (including shell, grinded)	1	2,53	13,42		6,94	4,41	67,14	32,86
	2	19,26	16,73		8,36	5,83	65,15	34,85
	3	2,52	14,20		7,02	4,50	68,31	31,69
Cow manure	1	2,52	37,30		8,85	6,33	83,03	16,97
	2	2,52	24,85		6,82	4,30	82,70	17,30
	3	2,53	33,18		8,10	5,57	83,21	16,79
Mink manure	1	2,53	35,36		14,66	12,13	65,70	34,30
	2	2,52	25,08		10,88	8,36	66,67	33,33
	3	2,52	32,41		12,68	10,16	68,65	31,35
Pig manure	1	2,52	28,86		9,23	6,71	76,75	23,25
	2	2,52	34,57		10,99	8,47	75,50	24,50
	3	2,51	21,40		7,73	5,22	75,61	24,39
Horse manure	1	2,52	15,67		5,86	3,34	78,69	21,31
	2	2,53	19,19		6,53	4,00	79,16	20,84
	3	2,53	16,61		6,11	3,58	78,45	21,55
Pig slurry	1	2,52	31,57		5,40	2,88	90,88	9,12
	2	2,53	21,91		4,37	1,84	91,60	8,40
	3	2,51	26,16		4,86	2,35	91,02	8,98
Cow slurry	1	2,51	21,15		3,14	0,63	97,02	2,98
	2	2,51	20,40		3,12	0,61	97,01	2,99
	3	2,50	17,54		3,00	0,50	97,15	2,85
Butter	1	2,53	9,92		10,86	8,33	16,03	83,97
	2	2,54	11,12		11,88	9,34	16,01	83,99
	3	2,52	7,44		8,76	6,24	16,13	83,87
Fish (cut small with scissors, grinded)	1	2,52	24,28		10,37	7,85	67,67	32,33
	2	2,52	34,53		13,74	11,22	67,51	32,49
	3	2,51	34,41		13,64	11,13	67,65	32,35
Chicken manure	1	2,52	8,88		9,61	7,09	20,16	79,84
	2	2,52	10,03		10,70	8,18	18,44	81,56
	3	2,50	14,41		14,57	12,07	16,24	83,76
Grain	1	2,51	15,03		15,54	13,03	13,31	86,69
	2	2,51	19,11		19,08	16,57	13,29	86,71
	3	2,50	12,20		13,10	10,60	13,11	86,89
Fox manue 1 (fresh)	1	2,51	20,62		9,45	6,94	66,34	33,66
	2	2,52	19,92		8,39	5,87	70,53	29,47
	3	2,51	23,22		9,75	7,24	68,82	31,18
Fox manure 2 (one year composting)	1	2,52	25,02		13,23	10,71	57,19	42,81
	2	2,56	18,27		9,25	6,69	63,38	36,62
	3	2,52	16,27		9,15	6,63	59,25	40,75
Meat	1	2,50	24,36		9,16	6,66	72,66	27,34
	2	2,52	22,56		8,66	6,14	72,78	27,22
	3	2,51	16,53		6,98	4,47	72,96	27,04
Grass	1	2,51	4,36		3,63	1,12	74,31	25,69
	2	2,52	4,74		3,67	1,15	75,74	24,26
	3	2,54	3,59		3,39	0,85	76,32	23,68
Hay	1	2,52	0,91		3,36	0,84	7,69	92,31
	2	2,52	0,36		2,85	0,33	8,33	91,67
	3	2,49	1,15		3,55	1,06	7,83	92,17

Oven: 105 Deg. C, 40% Ventilation, 64h

		pan (alu) (emprty, g)	fresh waste (g)		dried waste & pan (alu) (g)	dried waste (g)	Moisture content (%)	Dry content (%)
Olive Oil	1	2,54	8,90		11,50	8,96	-0,67	100,67
	2	2,53	8,29		10,86	8,33	-0,48	100,48
	3	2,52	10,10		12,70	10,18	-0,79	100,79
Rapeseed Oil	1	2,50	6,04		8,57	6,07	-0,50	100,50
	2	2,51	13,52		16,21	13,70	-1,33	101,33
	3	2,53	8,25		10,87	8,34	-1,09	101,09
Tomatoes	1	2,52	24,09		3,65	1,13	95,31	4,69
	2	2,54	15,00		3,24	0,70	95,33	4,67
	3	2,54	19,86		3,47	0,93	95,30	4,70
Cucumber	1	2,52	17,97		3,30	0,78	95,66	4,34
	2	2,50	20,71		3,23	0,73	96,48	3,52
	3	2,52	23,68		3,13	0,61	97,42	2,58
suwage sludge	1	2,51	44,53		2,62	0,11	99,75	0,25
	2	2,51	50,83		2,63	0,12	99,76	0,24
	3	2,52	31,91		2,58	0,06	99,81	0,19
Horse manure 2	1	2,51	24,57		6,58	4,07	83,44	16,56
	2	2,53	13,60		4,80	2,27	83,31	16,69
	3	2,53	8,67		4,00	1,47	83,04	16,96
Chicken manure 2	1	2,53	14,66		6,53	4,00	72,71	27,29
	2	2,53	11,81		5,91	3,38	71,38	28,62
	3	2,52	10,95		5,50	2,98	72,79	27,21
Inoculum 1	1	2,53	23,31		2,94	0,41	98,24	1,76
	2	2,51	14,62		2,62	0,11	99,25	0,75
	3	2,51	15,83		2,66	0,15	99,05	0,95
Inoculum 2	1	2,53	25,64		5,91	3,38	86,82	13,18
	2	2,54	23,38		5,31	2,77	88,15	11,85
	3	2,52	19,83		4,88	2,36	88,10	11,90
Inoculum 3	1	2,53	33,00		2,93	0,40	98,79	1,21
	2	2,53	46,66		3,07	0,54	98,84	1,16
	3	2,52	38,09		2,99	0,47	98,77	1,23
Inoculum 4	1	2,52	49,70		3,21	0,69	98,61	1,39
	2	2,53	35,02		3,02	0,49	98,60	1,40
	3	2,54	29,59		2,96	0,42	98,58	1,42
Inoculum 5	1	2,53	33,56		3,70	1,17	96,51	3,49
	2	2,54	36,43		3,75	1,21	96,68	3,32
	3	2,53	39,39		3,99	1,46	96,29	3,71
Inoculum 6	1	2,51	33,40		3,43	0,92	97,25	2,75
	2	2,51	46,09		3,77	1,26	97,27	2,73
	3	2,49	52,11		4,15	1,66	96,81	3,19
Inoculum 7	1	2,51	36,18		3,02	0,51	98,59	1,41
	2	2,50	69,79		3,50	1,00	98,57	1,43
	3	2,50	34,90		3,00	0,50	98,57	1,43
Biowaste 1	1	2,49	34,12		8,72	6,23	81,74	18,26
	2	2,52	28,38		7,84	5,32	81,25	18,75
	3	2,51	36,46		9,44	6,93	80,99	19,01
Biowaste 2 (used in test 6)	1	2,52	23,13		4,34	1,82	92,13	7,87
	2	2,51	38,94		5,71	3,20	91,78	8,22
	3	2,51	13,17		3,60	1,09	91,72	8,28

Oven: 105 Deg. C, 40% Ventilation, 64h

Appendix 2 – Ash content and volatile solids calculations

	ceramic pan (g)	substrate (g)		Ash & pan (ceramic) (g)	Ash (g)	Ash content	VS	VS %
Tomato plant (cut small with scissors, grinded)	29,45	2,85		30,18	0,73	25,44	2,14	75,09
	26,07	3,23		26,95	0,88	27,16	2,36	73,07
	23,57	3,27		24,46	0,89	27,22	2,38	72,78
Cucumber plant (cut small with scissors, grinded)	26,71	2,02		27,57	0,86	42,57	1,16	57,43
	25,48	2,44		26,51	1,03	41,70	1,44	59,02
	26,36	3,23		27,76	1,40	43,21	1,84	56,97
Eggs (including shell, grinded)	28,35	4,10		29,93	1,58	35,83	2,83	69,02
	26,09	5,37		28,44	2,35	40,31	3,48	64,80
	25,35	4,17		26,81	1,46	32,44	3,04	72,90
Cow manure	26,19	6,26		27,56	1,37	21,64	4,96	79,23
	27,15	4,25		27,96	0,81	18,84	3,49	82,12
	24,10	5,40		25,45	1,35	24,24	4,22	78,15
Mink manure	28,35	12,06		31,96	3,61	29,76	8,52	70,65
	26,91	8,24		29,17	2,26	27,03	6,10	74,03
	26,55	10,00		29,58	3,03	29,82	7,13	71,30
Pig manure	28,94	6,66		30,52	1,58	23,55	5,13	77,03
	24,49	8,43		26,43	1,94	22,90	6,53	77,46
	30,83	5,19		31,89	1,06	20,31	4,16	80,15
Horse manure	28,49	3,32		29,10	0,61	18,26	2,73	82,23
	23,13	3,99		23,86	0,73	18,25	3,27	81,95
	28,76	3,58		29,44	0,68	18,99	2,90	81,01
Pig slurry	16,01	2,67		16,42	0,41	14,24	2,47	92,51
	12,07	1,67		12,24	0,17	9,24	1,67	100,00
	14,10	2,11		14,38	0,28	11,91	2,07	98,10
Cow slurry	21,03	0,46		21,17	0,14	22,22	0,49	106,52
	19,22	0,48		19,34	0,12	19,67	0,49	102,08
	20,99	0,45		21,09	0,10	20,00	0,40	88,89
Butter	26,32	7,18		26,44	0,12	1,44	8,21	114,35
	23,59	8,33		23,70	0,11	1,18	9,23	110,80
	26,36	5,69		26,44	0,08	1,28	6,16	108,26
Fish (cut small with scissors, grinded)	26,73	7,82		28,49	1,76	22,42	6,09	77,88
	26,73	11,23		29,49	2,76	24,60	8,46	75,33
	26,34	19,36		29,15	2,81	25,25	8,32	42,98
Chicken manure	26,74	7,11	Oven: 550 Deg C, 10min warm-up phase, 2h burning time	29,30	2,56	36,11	4,53	63,71
	27,15	8,20		31,88	4,73	57,82	3,45	42,07
	26,17	12,12		29,06	2,89	23,94	9,18	75,74
Grain	16,01	13,04		18,85	2,84	21,80	10,19	78,14
	12,07	16,59		15,22	3,15	19,01	13,42	80,89
	14,14	10,58		16,16	2,02	19,06	8,58	81,10
Fox manue 1 (fresh)	26,91	6,93		29,39	2,48	35,73	4,46	64,36
	26,71	5,87		28,81	2,10	35,78	3,77	64,22
	29,46	7,23		32,38	2,92	40,33	4,32	59,75
Fox manure 2 (one year composting)	28,36	10,72		35,92	7,56	70,59	3,15	29,38
	26,54	6,74		30,38	3,84	57,40	2,85	42,28
	28,97	6,61		35,60	6,63	100,00	0,00	0,00
Meat	23,13	6,57		23,74	0,61	9,16	6,05	92,09
	26,07	6,10		26,66	0,59	9,61	5,55	90,98
	26,38	4,42		26,88	0,50	11,19	3,97	89,82
Grass	29,45	1,07		29,55	0,10	8,93	1,02	95,33
	26,71	1,11		26,81	0,10	8,70	1,05	94,59
	23,13	0,79		23,21	0,08	9,41	0,77	97,47
Hay	23,58	0,84		23,66	0,08	9,52	0,76	90,48
	28,78	0,35		28,82	0,04	12,12	0,29	82,86
	23,12	1,03		23,19	0,07	6,60	0,99	96,12

	ceramic pan (g)	substrate (g)		Ash & pan (ceramic) (g)	Ash (g)	Ash content	VS	VS %
Olive Oil	25,34	8,36		25,34	0,00	0,00	8,96	107,18
	28,49	7,40		28,48	-0,01	-0,12	8,34	112,70
	26,31	9,44		26,32	0,01	0,10	10,17	107,73
Rapeseed Oil	21,75	4,48		21,75	0,00	0,00	6,07	135,49
	21,79	12,96		21,79	0,00	0,00	13,70	105,71
	21,40	7,40		21,39	-0,01	-0,12	8,35	112,84
Tomatoes	28,48	1,00		28,80	0,32	28,32	0,81	81,00
	28,74	0,64		28,95	0,21	30,00	0,49	76,56
	28,94	0,88		29,17	0,23	24,65	0,70	79,89
Cucumber	11,00	0,58		11,09	0,09	11,54	0,69	118,97
	9,70	0,69		9,81	0,11	15,07	0,62	89,86
	13,89	0,76		14,02	0,13	21,31	0,48	63,16
suwage sludge	9,71	0,04		9,71	0,00	0,00	0,11	275,00
	12,71	0,03		12,72	0,01	8,33	0,11	366,67
	12,83	0,01		12,83	0,00	0,00	0,06	600,00
Horse manure 2	25,34	2,73		25,53	0,19	4,67	3,88	142,12
	23,58	2,07		23,72	0,14	6,17	2,13	102,90
	29,44	2,67		29,63	0,19	12,93	1,28	47,94
Chicken manure 2	28,75	4,00		29,77	1,02	25,50	2,98	74,50
	26,05	3,39		26,94	0,89	26,33	2,49	73,45
	25,48	2,97		26,15	0,67	22,48	2,31	77,78
Inoculum 1	12,83	0,38		12,99	0,16	39,02	0,25	65,79
	8,69	0,09		8,70	0,01	9,09	0,10	111,11
	23,37	0,10		23,40	0,03	20,00	0,12	120,00
Inoculum 2	24,48	3,30		25,09	0,61	18,05	2,77	83,94
	30,81	2,71		31,36	0,55	19,86	2,22	81,92
	25,48	2,13		25,89	0,41	17,37	1,95	91,55
Inoculum 3	26,90	0,17		27,01	0,11	27,50	0,29	170,59
	12,69	0,41		12,92	0,23	42,59	0,31	75,61
	26,53	0,36		26,71	0,18	38,30	0,29	80,56
Inoculum 4	9,69	0,51		9,93	0,24	34,78	0,45	88,24
	12,81	0,35		12,99	0,18	36,73	0,31	88,57
	12,03	0,34		12,19	0,16	38,10	0,26	76,47
Inoculum 5	26,33	1,06		26,70	0,37	31,62	0,80	75,47
	28,95	1,10		29,35	0,40	33,06	0,81	73,64
	26,36	1,31		26,84	0,48	32,88	0,98	74,81
Inoculum 6	26,70	0,85		27,01	0,31	33,70	0,61	71,76
	27,15	1,17		27,56	0,41	32,54	0,85	72,65
	24,09	1,49		24,62	0,53	31,93	1,13	75,84
Inoculum 7	26,56	0,33		26,63	0,07	13,73	0,44	133,33
	23,12	0,80		23,40	0,28	28,00	0,72	90,00
	28,49	0,37		28,61	0,12	24,00	0,38	102,70
Biowaste 1	28,34	6,18		29,77	1,43	22,95	4,80	77,67
	28,76	5,27		30,07	1,31	24,62	4,01	76,09
	29,45	6,83		31,20	1,75	25,25	5,18	75,84
Biowaste 2 (used in test 6)	30,82	1,75		31,03	0,21	11,54	1,61	92,00
	24,49	3,11		24,81	0,32	10,00	2,88	92,60
	28,34	1,00		28,46	0,12	11,01	0,97	97,00

Oven: 550 Deg C, 10min warm-up phase, 2h burning time

Appendix 3 – Substrate amount calculation

	5% DS aim:	
	10	
	Average Moisture content:	Waste with 5% DS (g):
Tomato plant (cut small with scissors, grinded)	83,93	62,22
Cucumber plant (cut small with scissors, grinded)	88,68	88,34
Eggs (including shell, grinded)	66,87	30,18
Cow manure	82,98	58,75
Mink manure	67,00	30,31
Pig manure	75,95	41,58
Horse manure	78,76	47,09
Pig slurry	91,17	113,19
Cow slurry	97,06	340,15
Butter	16,07	11,91
Fish (cut small with scissors, grinded)	67,61	30,87
Chicken manure	18,28	12,24
Grain	13,24	11,53
Fox manue 1 (fresh)	68,57	31,81
Fox manure 2 (one year composting)	59,94	24,96
Meat	72,80	36,77
Grass	75,46	40,75
Hay	7,95	10,86

	Average Moisture content:	Waste with 5% DS (g):
Olive Oil	-0,65	9,94
Rapeseed Oil	-0,97	9,90
Tomatoes	95,31	213,44
Cucumber	96,52	287,32
suwage sludge	99,78	4470,04
Horse manure 2	83,26	59,75
Chicken manure 2	72,29	36,09
Inoculum 1	98,85	867,34
Inoculum 2	87,69	81,23
Inoculum 3	98,80	832,56
Inoculum 4	98,60	713,11
Inoculum 5	96,50	285,33
Inoculum 6	97,11	345,87
Inoculum 7	98,57	701,73
Biowaste 1	81,33	53,56
Biowaste 2 (used in test 6)	91,88	123,14

Appendix 4 – All results, table

Substrate	Biogas production test 1 *(Nml)*	pH test 1	Biogas production test 2 *(Nml)*	pH test 2
Butter	446	5,18	409,6	5,18
Chicken manure 1	741,6	5,65	4436,3	7,77
Chicken manure 2	615,9	6,1	749,9	6,3
Cow manure	451,8	5,47	433,8	5,40
Cow slurry	5117,2	7,85	5248,1	7,74
Cucumber	82,7	3,9	82,7	3,89
Cucumber plant	6539,6	8,2	5696,7	8,3
Eggs	7441,8	8,38	7403,9	8,62
Fish waste	18,4	4,5	82,4	4,5
Fox manure 1 (fresh)	5186,4	8,90	5415,3	8,80
Fox manure 2 (1 year composted)	3572,3	8,69	0	5,6
Grain 1	18,4	4,0	0	4,0
Grain 2	53,3	4,1	62,2	4,0
Grass 1	18	5,38	700,9	5,22
Grass 2	0	4,0	62,4	4,3
Hay	27,5	4,3	18,4	4,3
Horse manure 1	6296,8	8,5	464,4	5,3
Horse manure 2	71,1	4,3	17,8	3,9
Meat	948,9	6,5	1095,1	7,5
Mink manure	735,5	5,5	672,2	6,3
Olive oil 1	0	5,3	433,6	5,2
Olive oil 2	150,9	4,1	560,8	4,59
Pig manure	379,8	5,46	343,8	5,68
Pig slurry	117,6	7,33	443,4	5,79
Rapeseed oil	287,8	5,3	351,3	5,3
Sewage sludge	6324,1	9,2	0	6,6
Tomatoes	255,7	3,85	182,8	4,43
Tomato plant	36,7	4,1	45,9	4,1

Appendix 5 – All results, graph